Operant analysis of cognitive behaviours dependent upon prefrontal and hippocampal systems of the brain

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Summary

The hippocampus and the prefrontal cortex are two areas of the brain that are fundamental for a wide range of cognitive processes. Studies of both human patients who have incurred damage to these regions, and animals with circumscribed lesions, have led to a variety of theories concerning their contributions to cognitive behaviours. The hippocampus and the prefrontal cortex are connected anatomically, but the behavioural sequelae of experimental lesions have been shown to be dissociable. This thesis investigates the roles of the hippocampus and the prefrontal cortex in the rat, with a focus on delayed matching working memory tasks.

Chapter 4 reports on a study which demonstrated a delay-dependent deficit on retention of delayed matching to position (DMTP) in the Skinner box following prefrontal lesions; hippocampal lesions left performance intact. Neither lesion impaired the ability to switch between matching and non-matching rules. Chapter 5 describes an experiment which revealed that neither area was involved in postoperative acquisition of DMTP. Next, rats with lesions of the two main hippocampal pathways were assessed on retention of DMTP in Chapter 6. Lesions of the fornix revealed a delay-independent deficit, whereas entorhinal cortex lesions were without effect. Chapter 7 investigated recognition memory using a spontaneous novelty preference task. None of the lesions impaired performance on this task up to a 2 hour delay, however hippocampal lesions showed an impairment when a spatial component was included. Furthermore, there was a suggestion of both prefrontal and hippocampal involvement when memory for relative recency was assessed. Finally, Chapter 8 investigated a novel task in the Skinner box which combined both rules within one session. This task revealed a surprising pattern of results, with hippocampal lesions producing a dramatic impairment, whilst prefrontal lesions were without effect. Additionally, water maze data provided ample support for a hippocampal role in spatial memory.

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If I've forgotten anyone please don't take it personally, it's merely an indication of the degree to which my brain is addled - sorry!

Abbreviations

9HB	nine-hole box
AChE	acetylcholinesterase
CCD	continuous conditional discrimination
CDM/NMTP	conditional delayed matching/ non-matching to position
Cg1	cingulate cortex area 1
Cg2	cingulate cortex area 2
CNM	continuous matching/ non-matching to sample
d	dorsal
DG	dentate gyrus
DMS	delayed matching to sample
DMTP	delayed matching to position
DNMS	delayed non-matching to sample
DNMTP	delayed non-matching to position
ED	extra-dimensional
ERC	entorhinal cortex
ERP	event-related potential
fMRI	functional magnetic resonance imaging
Fr2	frontal area 2
Fx	fornix
HPC	hippocampus
hr	hour
IBO	ibotenic acid
ID	intra-dimensional
IL	infralimbic cortex
ITI	inter-trial interval
m	medial
M2	secondary motor cortex
MD	mediodorsal nucleus of the thalamus
min	minute
MO	medial orbital cortex
MTL	medial temporal lobe
NAC	nucleus accumbens
NMDA	*N*-methyl-D-aspartate
ns	not significant
PBS	phosphate buffered saline
PET	positron emission topography
PFA	paraformaldehyde
PFC	prefrontal cortex
PrL	prelimbic cortex
RAM	radial arm maze
SDT	signal detection theory
sec	second
SEM	standard error of the mean
v	ventral
VI	variable interval
VPC	visual paired comparison
WCST	Wisconsin card sorting test

N.B. The abbreviations listed here are those that are used throughout the text, other abbreviations may occur but will be clearly indicated in the appropriate figure legend.

Table of contents

Chapter 1 Introduction

The hippocampus (HPC) and prefrontal cortex (PFC) are two regions of the brain that are fundamental to cognition. The HPC is implicated in memory and spatial processing (O'Keefe, 1983; Olton, 1983; Squire et al., 2004; Zola-Morgan and Squire, 1992), whilst the PFC contributes to processes involved in a variety of higher cognitive functions, including planning and organising goal-directed behaviour, as well as short-term memory (Fuster, 1997; Goldman-Rakic, 1996; Miller, 2000). As both HPC and PFC are thought to have key roles in a number of diseases, including dementia, a greater understanding of these areas is integral in advancing scientific knowledge with a view to establishing effective treatment strategies for these conditions. The aim of this thesis is to compare the involvement of the HPC and PFC in cognitive behaviours in the rat, by investigating both novel and established tasks that are sensitive to disruption of these areas.

Much can be gleaned from the study of patients with gross lesions of these brain areas, whether caused by natural events, accidents or as treatments for other illnesses. However, these lesions are seldom well controlled in their extent or location, and the remaining brain tissue is often damaged, causing behavioural consequences distinct from pathology in the area of interest (Berman and Weinberger, 1990). This is one of the many reasons why animal models are useful for understanding the functional organisation of the mammalian brain. Both monkeys and rats have been widely used, providing the potential to perform circumscribed lesions of specific brain areas with suitable controls. The advantage of rat models is that group sizes can be significantly larger than those permissible in the study of higher order animals, such as the monkey, conferring a greater potential for validity. Additionally, there is scope for controlling and manipulating experimental parameters to an extent not feasible in the human. This introduction begins with a brief summary of the neuroanatomy of the rat HPC and PFC, and then goes on to provide an overview of the behavioural sequelae of damage to both of these areas, covering human, non-human primates and rodent studies, before finally outlining the specific aims and strategies addressed within this thesis.

1.1 Neuroanatomy

1.1.1 Rat hippocampus

The hippocampal formation lies within the medial temporal lobe (MTL) of the brain and comprises four main regions defined by their connectional, cytoarchitectonic and functional properties. These four main regions are the dentate gyrus (DG), the hippocampus proper (HPC), the subicular complex and the entorhinal cortex (ERC) (Amaral and Witter, 1989; Amaral and Witter, 1995). The HPC can be divided into three sub-regions based on differences in pyramidal cell size and connectivity, termed CA1, CA2 and CA3; these regions are named after *Ammon's Horn* (Latin: *Cornu Ammonis)*, as they were thought to resemble the horns of this Egyptian deity (Fitzgerald, 1996). The subicular complex consists of the subiculum, presubiculum and the parasubiculum, whilst the ERC can be divided into two or more subdivisions (Amaral and Witter, 1989; Amaral and Witter, 1995). The hippocampal formation is a C-shaped structure that extends longitudinally from the septal nuclei of the basal forebrain, over the thalamus, to the temporal lobe, and is thus often discussed in terms of its septotemporal axis, with the septal pole being the most rostral (Amaral and Witter, 1995).

These areas are grouped together under the term "hippocampal formation", due to their unique intrinsic connections (see figure 1.1). The DG receives its major input from the ERC by way of the perforant path; the granule cells of the DG then project their mossy fibres to the CA3 subfield of the HPC, which in turn projects to the CA1 field via Schaffer collaterals (Amaral and Witter, 1995). Finally the CA1 field projects back to the ERC, both directly and indirectly via the subiculum (Witter et al., 2000). Adjacent cortices such as the perirhinal cortex are not included under the term hippocampal formation due to connections with the ERC being reciprocal in nature (Amaral and Witter, 1995; Burwell and Amaral, 1998b), although there are direct projections from the perirhinal cortex to the CA1 region (Liu and Bilkey, 1996).

In addition to its intrinsic connectivity, the HPC has dense reciprocal connections to the rest of the brain by way of two main routes, the ERC and the fibres of the fornix (Fx). The ERC projects to the entire cortical mantle including motor, auditory, visual and somatosensory areas (Insausti et al., 1997; Swanson and Kohler, 1986), as well as being

the principal relay for this information to the HPC (Witter et al., 1989). The Fx is a fibre tract which links the subiculum and HPC with various cortical and subcortical sites, including the mamillary bodies, hypothalamic and thalamic nuclei, basal forebrain, nucleus accumbens (NAC) and PFC (Aggleton et al., 1992; Swanson et al., 1987), and provides the majority of cholinergic inputs to the HPC (Swanson et al., 1987). Fibres originate from the pyramidal cells of the HPC and subiculum, and collect in a thicker fibre bundle called the fimbria, before leaving the HPC as the Fx (Amaral and Witter, 1995).

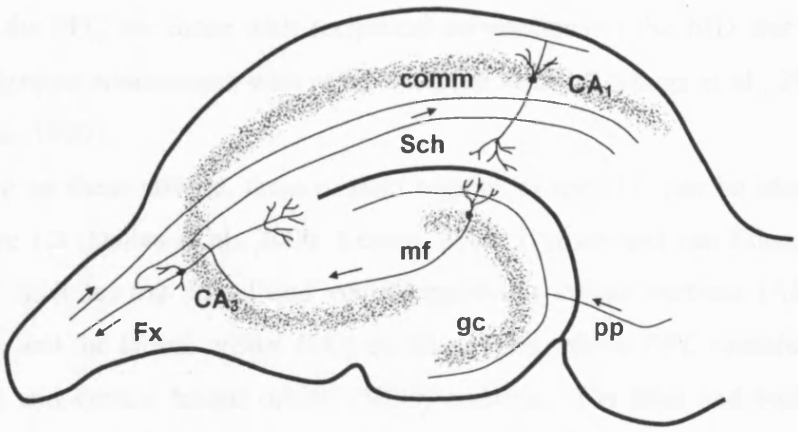

Figure 1.1 Schematic diagram of hippocampal circuitry in the rat, adapted from Bliss (1979). Commissural fibres from contralateral hemisphere (comm), granule cells of dentate gyrus (gc), mossy fibres (mf), perforant path (pp), Schaffer collaterals (Sch).

1.1.2 Rat prefrontal cortex

The cortex of the anterior part of the frontal lobe of the brain is commonly designated the PFC (Fuster, 1997). There is ongoing speculation as to whether or not there is an area in the rat cortex that is equivalent to the PFC of primates (Kolb, 1990b; Preuss, 1995). The definition of PFC originally relied on cytoarchitecture, namely the presence of a granular cell layer in layer IV of the cortex. However this presents an obstacle in the rat as the prefrontal area is in fact agranular (Preuss, 1995). To define the PFC by means of cytoarchitecture alone is not practical, due to variability both within and between species(Fuster, 1997) and, as such, alternative criteria for inferring homologies between brain regions in different species must be taken into consideration. These criteria include

the pattern and relative density of nerve connections and functional correlates resulting from lesions and electrical stimulation studies (Uylings and van Eden, 1990).

The most widely accepted definition of the rat PFC is that it is the area innervated by the mediodorsal nucleus (MD) of the thalamus (Conde et al., 1990; Divac et al., 1978; Groenewegen et al., 1990; Kolb, 1990b; Rose and Woolsey, 1948). However, this definition requires further qualification, because not only does the MD project to cortical areas outwith the PFC, but the PFC also receives inputs from other thalamic nuclei (e.g. the midline and intralaminar nuclei (Groenewegen and Berendse, 1994; Uylings et al., 2003). As a final refinement, it has been suggested that the only areas which can be included in the PFC are those with reciprocal connections to the MD that are stronger than the reciprocal connections with other thalamic nuclei (Uylings et al., 2003; Uylings and van Eden, 1990).

Based on these criteria, three distinct regions of the PFC can be identified in the rat, see figure 1.2 (Dalley et al., 2004; Kesner, 2000; Uylings and van Eden, 1990). The *lateral PFC* includes the dorsal and ventral agranular insular cortices (AID and AIV, respectively) and the lateral orbital (LO) cortices. The *ventral PFC* contains the ventral orbital (VO) and ventral lateral orbital (VLO) cortices. The final and most significant region in this thesis is the *medial PFC* which includes a dorsal portion consisting of frontal area 2 (Fr2)* and cingulate areas 1 and 2 (Cg1, Cg2), and a ventral portion comprising the prelimbic (PrL), infralimbic (IL) and medial orbital (MO) cortices. However, some authors dispute the inclusion of the rat Fr2 and Cg areas in the PFC (see (Conde et al., 1990; Conde et al., 1995; Preuss, 1995). It is the *ventral medial PFC* that will be targeted in the lesion studies discussed within this thesis, and therefore connections primarily with these areas will be discussed below.

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* Terminology for prefrontal areas varies between authors, with some regions having multiple synonyms. The Fr2 region, based on Zilles' (1995) nomenclature, is frequently referred to as the precentral medial area (PrCm). Cg1 is often dorsal anterior cingulate, with Cg2 being ventral anterior cingulate, additionally PrL is sometimes termed Cg3. In an attempt to provide some level of consistency, the terminology of Paxinos and Watson (1998) will be adopted wherever possible; although these authors use secondary motor cortex (M2) in lieu of Fr2 or PrCm.

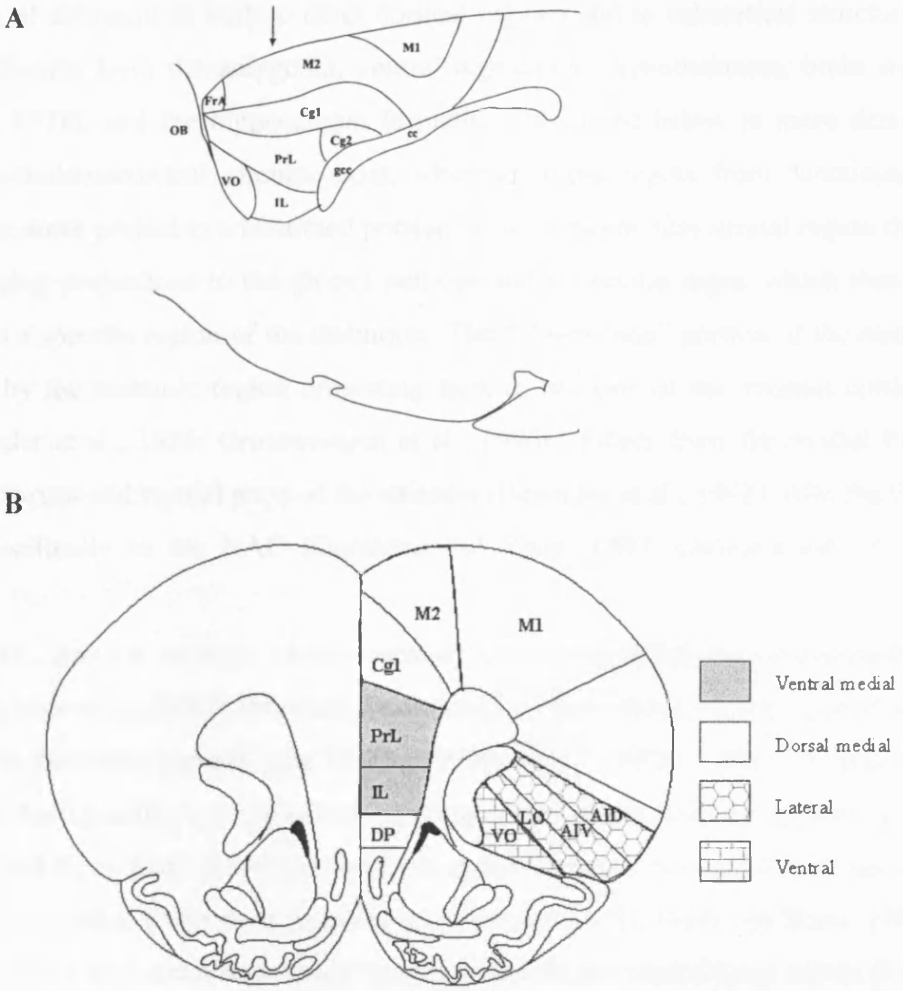


Figure 1.2 Illustrative diagrams of the rat PFC, schematics taken from Paxinos and Watson (1998), based on Dalley et al (2004). Lateral view, 0.9 mm from the midline (A). Coronal section, approximately 2.7 mm anterior to bregma, indicated by arrow in A (B). Shadings represent the major subdivisions, not all components are present on this section. Dorsal and ventral agranular insular cortices (AID, AIV), corpus callosum (cc), genu of corpus callosum (gcc), cingulate area 1 (Cg1), cingulate area 2 (Cg2), dorsal peduncular cortex (DP), frontal association area (FrA), infralimbic cortex (IL), lateral orbital cortex (LO), primary and secondary motor cortices (M1 and M2), olfactory bulb (OB), prelimbic cortex (PrL), ventral orbital cortrex (VO).

The MD nucleus itself can be subdivided based on its cytoarchitecture and its reciprocal connections with the PFC; the rostral part of the medial segment projects mainly to the PrL and Cg1 cortices, while the IL projects to the most medial part of the nucleus

(Groenewegen, 1988). In addition to the connections with the MD nucleus, the PFC has an abundance of connections both to other cortical regions and to subcortical structures. It receives afferents from the amygdala, ventral tegmentum, hypothalamus, brain stem (Divac et al., 1978), and the hippocampal formation (discussed below in more detail). Basal ganglia-thalamocortical circuits exist, where multiple inputs from functionally related cortical areas project to a restricted portion of the striatum; this striatal region then sends converging projections to the globus pallidus and substantia nigra, which then in turn project to a specific region of the thalamus. The "closed loop" portion of the circuit is completed by the thalamic region projecting back to just one of the original cortical areas (Alexander et al., 1986; Groenewegen et al., 1990). Fibres from the medial PFC project to the rostral and medial parts of the striatum (Berendse et al., 1992), with the PrL projecting specifically to the NAC (Gorelova and Yang, 1997; Groenewegen et al., 1990).

The PFC also has multiple cortico-cortical connections which are predominantly ipsilateral (Uylings et al., 2003); these can be divided into those from sensory regions and those from the posterior parietal area (Kolb, 1990a; Kolb, 1990b). The PrL and the rostral part of the Cg cortex are extensively connected, but fewer direct projections exist between PrL and IL cortices. However there are sparse afferents from the PrL to the IL, with slightly more dense projections from the IL to the ventral PrL (Fisk and Wyss, 1999; Hurley et al., 1991); both areas have dense connections with the contralateral cortex (Fisk and Wyss, 1999). The PrL and IL cortices share a number of common afferents, including projections from the agranular insular cortex and the entorhinal and piriform cortices (Conde et al., 1995).

When comparing the cortical and subcortical connections between monkey and rat a very similar pattern emerges, providing justification for using the rat as a model for understanding this brain region (Kolb, 1990a). Most authors are in agreement that, despite the relative undifferentiation of the rat PFC useful comparisons can still be made (Brown and Bowman, 2002; Uylings et al., 2003). The PrL cortex in particular provides the most likely homologue to the dorsolateral PFC of humans and non-human primates (Granon and Poucet, 2000; Preuss, 1995).

Swanson (1981) was the first to report a direct projection from the HPC to the PFC, specifically from CA1 to the IL (Swanson, 1981). Since then numerous studies have replicated this finding with direct projections being shown to exist between the CA1 region and subiculum and the PrL and MO cortices (Carr and Sesack, 1996; Ferino et al., 1987; Ishikawa and Nakamura, 2003; Jay et al., 1989; Jay and Witter, 1991; Thierry et al., 2000; Verwer et al., 1997). A well established projection also exists between the ERC and PrL and IL cortices (Burwell and Amaral, 1998a; Swanson and Kohler, 1986), with more lateral regions of ERC projecting to the PrL and more medial regions projecting to the IL cortex (Insausti et al., 1997).

1.2 Hippocampus: behavioural findings

1.2.1 Introduction to memory

Memory is the storage of information acquired as a result of specific learning experiences (Eichenbaum, 2002; Longstaff, 2000). Rather than being a unitary system, there are thought to be multiple memory systems that operate in parallel (Moscovitch, 1992). The most unambiguous distinction is between the broad categories of declarative and nondeclarative memory (Milner et al., 1998; Squire, 1986; Zola-Morgan and Squire, 1993). Declarative memory (often called explicit memory) affords the capacity to consciously recall facts and events (Eichenbaum, 1997). Declarative learning is fast and requires few trials, the memories can be consciously recalled and are easily forgotten (Longstaff, 2000). Nondeclarative memory (often called procedural or implicit memory) includes the learning of skills and habits, such as riding a bike, or playing a musical instrument (Zola-Morgan and Squire, 1993). In contrast to declarative memory, procedural memory is acquired gradually and requires a lot of rehearsal; critically, it does not involve conscious recall and once established this type of memory can be retained for many years (Eichenbaum, 2000; Milner et al., 1998).

Declarative memory can be further subdivided into episodic and semantic components (Squire and Zola, 1998). Episodic memory is defined as the capacity to recollect individual events which are specific in time and space (Baddeley, 2001; Tulving, 1983), whereas semantic memory is general knowledge of factual information

that can be acquired and recalled in isolation (Manns et al., 2003b). For example, remembering where you dined and what you ate the previous evening requires episodic memory, whereas the knowledge that there are sixty seconds in a minute is purely semantic. Another important form of declarative memory is recognition memory, which will be discussed in detail later in this introduction.

These different facets of memory were originally delineated through the study of amnesic patients (Cohen and Squire, 1980) and the observation that performance on tasks that taxed certain types of memory remained intact. Thus declarative and nondeclarative memory systems are thought to rely on different neural substrates; for example, the HPC and related areas are implicated in declarative memory (Eichenbaum, 1997; Squire, 1992) whilst the striatum is implicated in nondeclarative, procedural memory (Da Cunha et al., 2003; White, 1997). The emergence of novel non-invasive functional imaging methods has also advanced our understanding of the different brain areas involved in the various aspects of learning and memory (Zola-Morgan and Squire, 1993). The most common techniques include measuring blood oxygenation by functional magnetic resonance imaging (fMRI) and task-related differences in regional blood flow using positron emission topography (PET) (Fletcher et al., 1997; Zola-Morgan and Squire, 1993). These techniques supplement findings from studies of lesioned individuals and provide valuable insight into the brain activity that supports memory in normal healthy subjects.

1.2.2 Human data

It is well established that damage to the MTL in humans can result in severe memory impairment (Gaffan and Gaffan, 1991; Scoville and Milner, 1957; Squire et al., 2004; Zola-Morgan and Squire, 1992). Patient H.M. was the first, and continues to be the most thoroughly studied, of such cases; he underwent bilateral surgical removal of the MTL to treat severe epilepsy which resulted in a distinctive memory impairment (Scoville and Milner, 1957). H.M. suffers from profound memory loss for day to day events, whilst retaining normal intellectual ability and intact short-term memory (Corkin, 2002; Scoville and Milner, 1957). He suffers from severe anterograde amnesia (loss of memory for information acquired after the onset of amnesia), resulting in impaired acquisition for events that have a specific spatial and temporal context (episodic memory), and for

general knowledge and factual information (semantic memory) (Corkin, 2002). However, recent evidence suggests that his ability to acquire semantic knowledge about famous people has not been lost entirely (O'Kane et al., 2004). These impairments occur regardless of the sensory modality of the information (Corkin, 2002); this holds true for other subjects with damage to the medial temporal lobes (Levy et al., 2003; Levy et al., 2004; Squire et al., 2001b), in accordance with the MTL receiving inputs from all the sensory modalities (Lavenex and Amaral, 2000). H.M. maintains intact nondeclarative memory; for example, his performance improves on a mirror-tracing task despite having no explicit memory of having experienced the task before (Corkin, 2002). He is also impaired on recall of the spatial location of objects in an array (Smith, 1988) - a theme that will be revisited later in this introduction.

In recent years many more cases with similar damage have been described, including patient R.B., who provided the first detailed post mortem neuropathological examination of a patient with severe anterograde amnesia (Zola-Morgan et al., 1986). R.B. became amnesic after an ischemic episode, and was shown to have damage restricted bilaterally to the entire CA1 field of the HPC (Zola-Morgan et al., 1986). What is clear is that patients with damage limited to the HPC region (Rempel-Clower et al., 1996; Zola-Morgan et al., 1986) have a memory impairment that is less severe than that seen in patients with larger lesions that include adjacent cortical areas (Corkin et al., 1997; Stefanacci et al., 2000). A common feature in all these cases is intact short term, or immediate, memory; for example patients with HPC damage are unimpaired on a standard test of digit span (Cave and Squire, 1992), and patient H.M. is able to retain two or three digit numbers over several minutes, provided rehearsal is allowed during the delay (Milner et al., 1998).

There is little doubt that the MTL, and the structures therein, are involved in declarative memory (Eichenbaum, 1997; Hasselmo and McClelland, 1999; Squire et al., 2004; Squire and Zola-Morgan, 1991), as distinct from nondeclarative abilities such as habits and skills that are expressed through performance as opposed to recollection (Manns et al., 2003a; Squire, 1986). However, there is some debate over whether the integrity of these structures is required for both the semantic and episodic types of declarative memory. Some studies point to a role for the HPC in both types of memory

(Bayley and Squire, 2005; Manns et al., 2003a; Manns et al., 2003b; Squire and Zola, 1998), while others implicate the HPC to a greater extent in episodic as opposed to the semantic acquisition of factual knowledge (Schmolck et al., 2002; Tulving and Markowitsch, 1998; Vargha-Khadem et al., 1997).

Recognition memory is a type of declarative memory that provides the capacity to judge recently encountered information as familiar. This can be further subdivided into an episodic component (the ability to remember the specific episode in which an item was encountered, often referred to as "remembering", e.g ability to remember someone's name and the context of meeting) and a familiarity component (the ability to recognise the item, often referred to as "knowing", e.g. knowing you've met someone before, but being unable to remember who they are or under what circumstances you met them) (Brown and Aggleton, 2001; Manns et al., 2003a; Yonelinas et al., 2002). Dissociating these two facets of recognition memory is a complicated matter, but there is evidence to suggest that HPC damage affects both "knowing" and "remembering" components (Manns et al., 2003a; Reed and Squire, 1997).

Functional neuroimaging studies have been crucial in furthering our understanding about the various functions of the HPC in the intact brain. These studies have confirmed the involvement of the HPC in the encoding and retrieval of episodic memory (Cabeza and Nyberg, 2000; Yancey and Phelps, 2001), with the HPC thought to be critically involved in representing relationships amongst various objects and events (Cohen et al., 1999). For example, a PET study demonstrated greater HPC activation during a task that required learning an association between two pictures than when these two items had to be encoded separately (Henke et al., 1997). Dissociations have also been observed between the activity of different brain regions within the MTL depending on the type of episodic memory being retrieved e.g. autobiographical events versus general knowledge and public events (Maguire et al., 2000).

Imaging studies also suggest that the detection of novelty depends on the HPC to some extent (Tulving and Markowitsch, 1997; Yancey and Phelps, 2001). In a matching task using complex colour scenes the HPC shows a greater signal change when subjects are required to match and maintain novel as opposed to familiar stimuli (Stern et al., 2001). A separate fMRI study reports HPC activation that is maximal where the overall

degree of novelty is largest when learning category-exemplar word pairs (e.g. 1st pair- *dog...boxer*, 2nd pair- *stone...granite* (new category and exemplar); this contrasts with maximal PFC activation when there is a change in a category-exemplar pairing (e.g. 1st set- *dog...boxer*, 2nd set- *sportsman...boxer* (new category, same exemplar)) (Dolan and Fletcher, 1997). Furthermore, in humans HPC damage is shown to reduce characteristic intracranial event-related potentials (ERPs) upon presentation of novel stimuli (Knight, 1996). These studies all provide strong evidence for the involvement of the HPC in registering stimulus novelty in the human.

An important factor in many patients with damage to the MTL is the presence of a temporally graded retrograde amnesia (Bayley et al., 2003; Corkin, 2002; Kapur and Brooks, 1999; Manns et al., 2003b; Rempel-Clower et al., 1996; Stefanacci et al., 2000), that is more profound loss of recent as opposed to remote memories. This phenomenon has been instrumental in the development of theories involving the HPC in consolidation of memory processes (Squire and Alvarez, 1995). These theories suggest that those memories that were acquired nearer the amnesic episode are more likely to be susceptible to disruption, with the HPC having a time-limited role in information storage, and that reorganisation, or "consolidation" of memories occurs with the neocortex gradually coming to support stable long-term storage (Murray and Bussey, 2001). Indeed, these findings have been replicated in animal studies where animals were trained at two or more distinct times prior to bilateral damage of the HPC system before being assessed on postoperative retention (Squire et al., 2001a; Sutherland et al., 2001; Wiig et al., 1996; Winocur, 1990; Winocur et al., 2001; Zola-Morgan and Squire, 1990). However, the water maze has yet to reveal such a distinct temporal gradient of retrograde amnesia in the rat (Clark et al., 2005a; Mumby et al., 1999).

Other authors propose an alternative theory for the involvement of the HPC in memory consolidation, namely the multiple-trace model (Moscovitch and Nadel, 1998; Nadel et al., 2000). In this view the HPC is always involved in the storage and retrieval of episodic memory, and does not have a time-limited role. This view is substantiated by neuroimaging studies which suggest that retrieval of remote memories elicit just as much HPC activation as retrieval of recent memories (Nadel and Moscovitch, 2001; Ryan et al., 2001). Furthermore, in this model a clear distinction is made between episodic and

semantic memory, with the latter becoming independent of the HPC and stabilised within the cortex (Nadel et al., 2000).

1.2.3 Monkey data

Monkey models of amnesia have been successfully developed in which behavioural impairments are comparable with those seen in amnesic patients (Zola-Morgan and Squire, 1985). The prototypical recognition memory task in the monkey is that of delayed non-matching to sample (DNMS). DNMS and its variants are typically carried out in an apparatus called the Wisconsin General Test Apparatus (WGTA), which is a chamber that has an opaque shutter which provides the monkey with access to food wells when in the raised position (Ridley and Baker, 1993). This task involves the monkey being presented with a baited object followed by a delay, and then choosing between the previously encountered object and a novel object to receive a reward: choosing the novel object is rewarded in non-matching, choosing the previously encountered object is rewarded in the matching version (DMS). The side of the correct object is randomised across trials and stimuli can be either trial unique or repetitive, with repetitive stimuli providing a greater challenge to the animal and thus requiring more training (Mishkin and Delacour, 1975).

Zola-Morgan and Squire (1985) demonstrated that monkeys with aspirative MTL lesions involving HPC and the amygdala were impaired on four different tasks which are known to be sensitive to human amnesia. Monkeys were impaired on delayed retention of object discriminations, concurrent discrimination learning (eight separate pairs of objects presented five times each randomly across a session with the same object in each pair always being rewarded, therefore all eight discriminations must be learnt simultaneously), DNMS and delayed-response tests, which had previously been suggested to be sensitive to MTL damage (Mahut et al., 1982; Moss et al., 1981). Zola-Morgan and Squire (1985) elaborated on these findings by demonstrating that impairments were exacerbated by an increase in the delay, or by distraction during the delay. Monkeys with these lesions had previously been shown to be unimpaired on learning motor and skill based tasks, such as complicated manipulation of food rewards and pattern discrimination learning (Zola-Morgan and Squire, 1984). This corresponds

nicely with the human literature (Corkin, 2002; Squire et al., 2004) and serves to support the viability of this animal model of the human amnesic syndrome. Indeed when amnesic patients are tested on tasks explicitly designed to replicate the animal models of DNMS and object discrimination, they are impaired to a similar degree as the monkeys (Owen et al., 1995; Squire et al., 1988). Further support for involvement of the human HPC in the DNMS task comes from an fMRI study which confirms that during the encoding phase of this task a greater activation is elicited in the right HPC than in a control perceptuomotor task (Monk et al., 2002). In another study long (15 sec) delays in DNMS were associated with greater activity in the HPC than short (5 sec) delays (Elliott and Dolan, 1999), which correlates with the delay-dependent deficit seen in both monkeys (Alvarez et al., 1995) and humans (Owen et al., 1995). In addition, metabolic rate, as measured by a local cerebral glucose utilisation technique, was shown to increase in the HPC of monkeys performing a delayed object alternation task (Friedman and Goldman-Rakic, 1988).

There is substantial evidence to corroborate the findings reported above, with monkeys showing delay-dependent impairments in DNMS following circumscribed HPC damage induced by a variety of techniques (aspirative: Zola-Morgan and Squire, (1986); ischemic damage: Zola et al., (2000); Zola-Morgan et al., (1992); radio-frequency lesions: Alvarez et al., (1995); Zola et al., (2000); ibotenic acid (IBO) lesions: Beason-Held et al., (1999); Zola et al., (2000)). In contrast to this body of work is the study by Murray and Mishkin (1998) which reported no deficit on DNMS following excitotoxic lesions of the HPC and amygdala. However, this discrepancy might be accounted for by the fact that the monkeys received extensive preoperative training on the task, in contrast to the studies discussed above. This training may have provided the animals with sufficient practise at retaining novel objects in memory to overcome any postoperative impairment, additionally the IBO lesions were performed in two sequential surgery phases which may add to the inconsistencies (Murray and Mishkin, 1998; Zola et al., 2000). As in humans, the degree of memory impairment in monkeys has been shown to be related to both the locus and extent of damage within the MTL, with the most severe deficits arising from damage that encompasses the HPC, adjacent ERC and perirhinal cortex (Zola-Morgan et al., 1994).

Monkeys with damage limited to the HPC have also been shown to be impaired in a delay-dependent manner on the visual paired-comparison task (Zola et al., 2000), a task known to be disrupted in amnesic patients (McKee and Squire, 1993). This task was adapted for the monkey by Bachevalier et al (1993), and involved the monkey being presented with one new picture along-side one recently presented picture, with the tendency to look at the new picture being measured. Monkeys have also been shown to be impaired on a delayed recognition span test in which the animal is required to identify the novel stimulus in an increasing array of previously presented stimuli (Beason-Held et al., 1999). The animals were equally impaired on spatial, colour and object conditions of the test, with the number of correct responses before an error constituting the span score (Beason-Held et al., 1999). Monkeys with Fx transection are impaired on conditional visuomotor associations using a touch-screen (Brasted et al., 2002) and conditional object-choice discriminations using a non-spatial paradigm (Ridley et al., 2002). These results suggest that, in the monkey at least, the HPC can be regarded as having an important and widespread role in memory processes, and significantly, a role that is not limited to the spatial element.

1.2.4 Assessing cognitive behaviour in the rat

Before reviewing the behavioural literature concerning the rat, it is necessary to provide a brief introduction to the most common methods employed in assessing cognitive behaviour. In cognitive testing one can only make inferences regarding mnemonic capabilities based on overt behaviours displayed by the animal (Sarter, 2004; Steckler and Muir, 1996). This usually comprises of either learning to repeat a behaviour that results in a palatable reward, or avoiding a behaviour that results in an aversive situation for that animal, e.g. receiving a footshock, or even just receiving slightly less food!

1.2.4.1 Mazes

A number of maze paradigms exist for the study of spatial working memory in the rat. These are centred around mazes of different shapes and comprising of different numbers of arms, for example the T-maze (or Y-maze) and the radial arm maze (RAM). These mazes generally require the rat to "navigate" through the maze to locate a hidden food

reward positioned at the far end of an arm, based on a variety of different rules. One of the most common procedures in the T-maze is the delayed alternation task, which involves a free series of trials with the opposite arm to the previously visited arm being baited. A variant of this is the forced alternation or pair-trial delayed alternation procedure, in which the rat is permitted entry to only one arm on the first run, followed by a delay, and then must choose the opposite arm in the choice run to receive the reward (Dudchenko, 2004). It should be noted that this task is sometimes called DNMTP; however, authors often refer to it as DNMS. For the purposes of this discussion, the task will always be referred to as DNMTP, because the animal knows the location of the required response before the delay, in comparison with a true DNMTS task where the response position is not predictable until after the delay. These tasks are thought to rely on representation of extra-maze cues, although Dudchenko et al (2001) have shown that rats are still able to perform this task in the absence of cues, which suggests a variety of strategies could be in operation.

The standard RAM (Olton, 1987) procedure uses a maze with eight arms radiating out from a central platform, each of which has a baited food well at its distal end. Working memory, as applied to animal cognition, is defined as a short term memory for an object, stimulus or location that is used within a testing session and which is readily forgotten (Dudchenko, 2004). It is distinct from reference memory which is typically acquired with repeated training and is memory for events that happen in all sessions, for example remembering the general rule that food pellets are present at the end of a maze arm (Dudchenko, 2004; Olton, 1983). Working memory is assessed in the RAM by measuring the rats' ability to avoid re-visiting arms from which it has already received a reward within a session. Jarrard et al (1983) developed a version of the RAM which allows working and reference memory to be assessed simultaneously. In this version the same four arms are baited daily and entry into a never-baited arm constitutes a reference memory error; re-entry to one of the four baited arms within a session would constitute a working memory error.

The Morris water maze (Morris, 1984; Stewart and Morris, 1993) has been used extensively as a means for testing spatial learning and memory in the rat. This task was designed to eliminate the possibility of intra-maze cues, such as scent-marking. The task

requires rats to find a submerged platform in a large circular pool of opaque water. In a typical reference memory version, the platform remains in the same location in the pool across days, and rats learn the position of the platform to a high degree of accuracy based on the spatial relationship between the platform and extra-maze landmarks. This task can also be modified to assess working memory by moving the platform to a new location each day and observing any reduction in latency to find the platform across trials (Cassel et al., 1998; Stewart and Morris, 1993). This task will be discussed in further detail within the relevant experimental chapters.

1.2.4.2 Automated operant testing

Despite the value of such maze tasks, they tend to be time-consuming to run and labour intensive, which naturally imposes limits on the number of animals that can be run and the number of trials attempted (Rawlins and Deacon, 1993). Automated operant tasks assess an animal's performance on a task, which usually consists of a learned response to a stimulus, reinforced by a food or liquid reward or avoidance of an aversive stimulus. Operant testing is typically conducted in one of two pieces of apparatus, the Skinner box and the nine-hole box (9HB) (Robbins et al., 1993), in which every aspect of the test is under computer control. Both boxes are enclosed in sound attenuating chambers and require rats to make lever presses (Skinner box) or nose-pokes into holes fitted with infra-red beams (9HB). Multiple boxes can be controlled simultaneously by one computer allowing many animals to be run at the same time, and computer control means the speed of each trial is significantly shorter than that taken in a traditional maze test. The greater number of animals that can be tested and the greater number of trials that can be assessed all serve to increase the statistical power of the results obtained (Dunnett, 1993).

1.2.5 Rat data

Performance on DNMTP in the T-maze is shown to be indisputably impaired following HPC damage, whether caused by excitotoxic lesion (Bannerman et al., 1999; Bannerman et al., 2002a; Deacon et al., 2001), aspiration (Aggleton et al., 1986) or transection of the Fx (Aggleton et al., 1995; Bannerman et al., 2001b; Bussey et al., 2000; Rawlins and Olton, 1982; Shaw and Aggleton, 1993). A similar pattern emerges when considering the

RAM, with HPC damage resulting in impairment in spatial working memory tasks (Cassel et al., 1998; Jarrard et al., 2004; Liu and Bilkey, 2001; Mair et al., 1998; Winters et al., 2004). In a four-arm plus shaped maze, inactivation of the HPC with lidocaine, an anaesthetic, resulted in an impairment in a place learning task, where the position of the rewarded arm remained constant throughout testing (Chang and Gold, 2003). However, in a response version, where the relationship between the start and goal arms was always the same (e.g. 90°), these same animals actually showed an enhancement of performance when compared with controls (Chang and Gold, 2003). This effect was also shown using a plus maze located in a water maze pool, where again HPC inactivation actually promoted performance of a response task (Schroeder et al., 2002). Thus it appears that by removing the contribution of the HPC it is possible to enhance learning in a response task, perhaps by preventing the ineffective application of spatial strategies.

Rats with HPC damage have also been assessed on a variety of non-spatial DNMS tasks. For example, rats with HPC lesions were not impaired on a version of the Y-maze that required the rat to choose the arm based on distinctive goal boxes (Aggleton et al., 1986). Similarly, no impairment was seen in HPC-lesioned rats on a version of the RAM where the baited arms were indicated by inserts of different materials (Jarrard et al., 2004); although Olton and Feustle (1981) demonstrated a significant impairment in this non-spatial version of the RAM following Fx lesions. A rat version of the DNMS task has been developed using trial-unique objects in a runway consisting of two identical goal areas (Mumby et al., 1990). This task has been used extensively in an attempt to establish the involvement of the HPC in DNMS, with results ranging from little or no impairments following HPC damage (Mumby et al., 1992; Mumby et al., 1995) to significant delay-dependent deficits (Clark et al., 2001). However, ischemia-induced HPC damage results in severe DNMS impairments (Mumby et al., 1996; Wood et al., 1993), despite this damage being restricted to the CA1 region of the HPC. The paradoxical effect of restricted damage exposing a greater impairment than total HPC ablation (Mumby et al., 1996) is thought to be due to some extra-HPC damage induced by the ischemia; indeed focal cytotoxic injections in the CA1 region leave DNMS performance intact (Duva et al., 1997). Evidence implicates the rhinal cortex (lateral ERC and perirhinal cortex) as being critical for performance on DNMS (Mumby and

Pinel, 1994). Furthermore, an alternative DNMS test has been developed which relies on the rats' innate preference for exploring novel over familiar objects (Ennaceur and Delacour, 1988). Thus object recognition memory can be assessed without the need for extensive training (Ennaceur and Delacour, 1988); this spontaneous novelty preference task is discussed in detail in Chapter 8, where experimental results are also reported.

Recognition memory can also be assessed in a task in which the rat is required to dig in cups of artificially scented sand and remember the odours. Animals are introduced to a sequence of different odours by digging for food rewards buried deep in the cups, once they have been exposed to the sequence they are then tested for either recognition of odour or sequential order. The recognition test is a single-choice test in which the rat is presented with two cups, one containing a novel odour and the other containing one of the odours from the sequence, and the rat is rewarded for choosing the novel odour. The sequential order test is also a single-choice test where the rat is presented with two odours from the original sequence and is rewarded for selecting the odour that appeared earlier in the sequence. Using these tests it has been shown that rats with HPC lesions are able to discriminate and remember the odours, as illustrated by the recognition test, but they are impaired upon remembering the order of the sequence (Fortin et al., 2002; Kesner et al., 2002). Dudchenko et al (2000) reported a similar test where they trained rats in an odour span task, rats were trained to sequentially non-match an increasing number of odours; when tested postoperatively, rats with neurotoxic lesions of the HPC performed as controls, even up to 24 odours. However a separate group of rats were trained on a spatial span task, where they had to remember an increasing number of spatial locations, HPC lesioned rats were able only to recall one spatial location before performance dropped to chance (Dudchenko et al., 2000). These results clearly implicate the HPC in separating sensory events in time and suggest a role for mediating associations between events that constitute elements of episodic memory.

Further support for involvement of the rat HPC in episodic-type memory comes from a study designed to test this memory explicitly. Ergorul and Eichenbaum (2004) trained rats to remember single training episodes, each composed of a series of odours presented in different locations on an open field. Rats were then presented with a choice between an arbitrarily selected pair of the previous odours in their original positions and

were rewarded for choosing the stimulus that occurred earlier in the sequence; normal rats were shown to use a combination of spatial and olfactory cues to distinguish between the stimuli. However, rats with HPC lesions performed at chance and were impaired at integrating the various components of the information ("what", "where" and "when"), despite being able to perceive the different spatial and odour cues (Ergorul and Eichenbaum, 2004). This result may also reflect an impairment in the ability to remember the order of the sequence, providing further impetus to the HPC being critical for episodic memory.

1.2.6 The hippocampus and spatial memory

The involvement of the HPC in tasks that require memory for spatial information is one of the most universally accepted doctrines regarding this structure, whether it has an exclusive role in this type of memory is worthy of considerable debate. The most compelling evidence for this involvement arises from the discovery of so-called place cells in the rat HPC. Unit activity recordings of cells in the HPC of freely moving rats demonstrated that there were certain units that fired maximally only when the rat was in a specific position on the testing platform (O'Keefe and Dostrovsky, 1971). The firing activity in these units, or place cells, has been shown to be independent of sensory stimuli, by virtue of maintained firing following modifications such as rotating the platform, changing the surrounding environment, or turning off the room lights (O'Keefe, 1976; O'Keefe and Dostrovsky, 1971; Quirk et al., 1990). Place cell activity has even been recorded in blind rats giving further support to the suggestion that this firing is independent of any visual environmental cues (Save et al., 1998). These findings led to the cognitive map theory which postulates that the HPC is the neural substrate for an allocentric cognitive representation of environmental space (Nadel, 1991; O'Keefe, 1991; O'Keefe and Nadel, 1978). There has been some investigation of place cells in the HPC of the monkey, providing evidence for these location-specific cells (Ono et al., 1993) and also cells whose firing is dependent on the direction of auditory and visual stimuli (Tamura et al., 1992). In contrast, a separate study in monkeys found no evidence for place cells, and instead located cells that were maximally activated in response to spatial views (Rolls, 1999).

Some authors argue that this spatial mapping theory is too narrow and that spatial memory is merely an example of a broader category of tasks that require the HPC (Squire and Cave, 1991). Unit activity was recorded in rats performing an odour-guided non-matching to sample task using different locations on an open platform (Wood et al., 1999). Over half the recorded cells were associated with nonspatial variables, such as the odour or the rule in question (matching or non-matching), furthering the idea that the HPC has a broader role in information processing than is allowed by the cognitive map theory (Wood et al., 1999).

Impairment of allocentric spatial memory is a hallmark of HPC damage in the human (Holdstock et al., 2000; Smith, 1988). When patients with unilateral HPC removal were tested on a virtual version of the water maze task, they were found to be severely impaired compared with both age-matched controls and patients with damage outside the temporal lobes. This impairment was irrespective of which hemisphere had been damaged (Astur et al., 2002). The HPC has also been implicated in learning and reversing associations between stimuli and spatial locations; subjects were observed under fMRI whilst performing a stimulus-compatability task, where buttons (1,2,3) corresponded to different digits (1,2,3). When an incompatible rule was applied, e.g. press button 3 for digit 1, HPC activity was observed (Casey et al., 2002). However, humans with HPC damage are capable of remembering spatial information provided they have extensive premorbid experience of the environment in question, for example the neighbourhood they grew up in, or their family home (Corkin, 2002; Rosenbaum et al., 2000; Teng and Squire, 1999). This preservation of spatial information is similarly true in rats which are exposed to a complex environment prior to excitotoxic lesioning of the HPC (Winocur et al., 2005). However, rats that received extensive training early in life on the water maze were impaired on remote memory for this task following HPC lesions (Clark et al., 2005b).

One of the most characteristic impairments following HPC damage in the rat is that of the reference memory task in the water maze (Bannerman et al., 1999; Broersen, 2000; Cassel et al., 1998; Duva et al., 1997; Galani et al., 1998; Good and Honey, 1997; Gould et al., 2002; Liu and Bilkey, 2001; Morris et al., 1982; Richmond et al., 1999; Wright et al., 2004). Indeed this task is used in this thesis as a behavioural screen to

validate the integrity of the HPC lesions. The HPC does not function in isolation; rather it is thought to act in concert with adjacent cortical areas such as the ERC and postrhinal cortex, with multiple routes proposed for the relay of spatial information to both the HPC and subiculum. However lesion studies of these discrete cortical areas fail to confirm their direct involvement in spatial processing, although this may merely reflect the fact that these lesions only partial disconnect the HPC, due to the presence of alternative pathways (Aggleton et al., 2000).

1.2.7 Functional differentiation

There is a widely held opinion that the HPC is functionally differentiated along its septotemporal axis (Moser and Moser, 1998b); however, this thesis does not attempt to address this topic and as such only a brief summary follows. The majority of evidence comes from studies in the rat, with dorsal and ventral HPC lesions showing functional heterogeneity in a delayed alternation task in an operant chamber (Maruki et al., 2001) and in spatial learning in the water maze (Moser et al., 1993). Further evidence for differentiation along this axis comes from studies comparing dorsal, ventral and complete HPC lesions on a variety of tests including the water maze, elevated T-maze, locomotor activity and the differential reinforcement of low rates task, which requires the rat to suppress responding in an operant chamber until some minimum time has elapsed (Bannerman et al., 1999; Bannerman et al., 2002a). A study on recognition memory in the monkey failed to reveal any correlation between lesion size and performance, but, as the authors highlight, the study was not designed to test this supposition, and as such group sizes were relatively small (Zola et al., 2000). Of significance is that the lesions were intended to remove the entire HPC uniformly, so there were only three monkeys with minimal lesions; if this minimal lesion was sufficient to induce the maximal deficit then no correlation could emerge (Zola et al., 2000; Zola and Squire, 2001).

In addition to the debate over functional differentiation in the HPC, there is the issue of whether the neurons that are responsible for encoding and retrieving spatial memories are localised or distributed diffusely throughout the HPC. Moser and Moser (1998a) investigated this issue by inactivating small regions of the HPC in pretrained rats prior to a retention test in the water maze. By systematically varying the volume of

dorsal and ventral HPC lesions they were able to show that successful retrieval required the integrity of the entire dorsal 70% of the HPC. This suggests that normal encoding and retrieval of memories relies on a widely distributed HPC network and not on a specific localised ensemble of neurons.

Many studies have been explicitly designed to test the contribution of adjacent hippocampal cortical areas to certain types of memory. It is generally agreed that there is at least some dissociation between the effects of the perirhinal cortex and the HPC, with these lesions having little or no effect on spatial memory (Ennaceur et al., 1996; Machin et al., 2002; Winters et al., 2004; Winters and Bussey, 2005), whilst being implicated in certain types of recognition memory (Wan et al., 1999). The role of the ERC in spatial and other types of memory is less clear-cut (Bannerman et al., 2001b; Eijkenboom et al., 2000; Good and Honey, 1997) and this area will be further investigated and discussed within this thesis.

1.3 Prefrontal cortex: behavioural findings

1.3.1 Human data

One of the earliest and best-known cases of prefrontal damage is that of Phineas Gage, a railroad constructor foreman, who was the victim of a horrific accident in 1848. The accident arose from an explosion, which resulted in a pointed tamping iron penetrating Gage's face and exiting through his skull, inflicting massive damage to the frontal lobes of his brain. Remarkably, Gage survived this ordeal and lived for a further 14 years, which allowed the considerable changes in his personality to be well documented. Before the accident, Gage was responsible and socially well-adapted, but following it he exhibited profanity, poor planning, impulsivity and irreverence, whilst remaining able-bodied and of sound intelligence (Harlow, 1848, as cited in Fuster, 1997 and Damasio et al, 1994). The damage is thought to have involved both left and right PFC, and provided the first insight into the function of this area in human cognitive and emotive processes (Damasio et al., 1994). It follows that one of the most important roles of the frontal lobes is often believed to be for social and personality development and self-awareness (Stuss and Alexander, 2000). For example, impaired social and moral reasoning (Anderson et

al., 1999) and insensitivity to future consequences (Bechara et al., 1994) are often common in patients with frontal damage.

"Executive function" is the umbrella term used to describe the complex coordination of cognitive processes thought to engage the PFC and which is necessary for higher cognitive functions such as language, planning and problem-solving (Baddeley, 1992; Duncan, 2001; Duncan and Owen, 2000; Fuster, 1997; Miller, 2000; Stuss and Alexander, 2000). Working memory is an example of this type of process and is the ability to hold, manipulate and monitor information "on-line" whilst constantly updating and discarding information that is no longer relevant (Goldman-Rakic, 1990). There is abundant evidence to suggest that this type of memory is supported by the PFC (Smith and Jonides, 1999), for example, patients with frontal lobe damage are impaired on both delayed alternation and delayed response paradigms (Freedman and Oscar-Berman, 1986). Neuroimaging methods in healthy individuals have also been instrumental in establishing the involvement of the PFC in working memory (Cabeza and Nyberg, 2000), with fMRI studies confirming the activation of PFC during tasks such as the sequential-letter (*n*-back) memory task (Cohen et al., 1997; Smith and Jonides, 1999). In this task the subject is presented with a sequence of consonants on a computer screen; in the 0-back condition they must respond to a single pre-specified target letter. In the 1-back condition the target is any letter identical to the one preceding it, and then in the 2-back condition the target is any letter identical to the one presented two trials previously, and so on (Cohen et al., 1997).

fMRI has also revealed the activation of the PFC when subjects are required to perform two tasks concurrently, but not when each task is performed in isolation (D'Esposito et al., 1995). For example, activation of the PFC is detected when subjects perform an auditory and a visual choice reaction task concurrently (Szameitat et al., 2002). This dual-task performance requires an enhanced level of executive control to coordinate the competing streams of information, and the imaging data suggest that this executive function is localised in the PFC (Szameitat et al., 2002). However, there is ongoing debate regarding functional specificity of the different subdivisions of the PFC and working memory, with a number of alternative models being proposed (Müller et al., 2002). Briefly, the "type of information" theory argues for fractionation based on

different stimulus categories, for example spatial v. object memory (Goldman-Rakic, 1996), whilst the "type of processing" model assumes fractionation based on the degree to which memory content must be manipulated or monitored (D'Esposito et al., 1998; Owen, 1997; Owen et al., 1999).

The symptoms of disorganisation and problems with planning and decision making are common in patients with frontal lobe damage (Burgess, 2000; Gershberg and Shimamura, 1995; Shallice and Burgess, 1991). Deficits in attention are also commonly reported following PFC damage, for example in a task requiring stimuli to be counted patients are only impaired when stimuli are presented at a slower rate, suggesting a lack of voluntary attentional mechanisms (Wilkins et al., 1987). Subjects with frontal lesions are also known to be more susceptible to distractors, for example in an auditory DMS task, revealing a failure in the inhibitory control of sensory processing (Chao and Knight, 1995; Chao and Knight, 1998).

Like the HPC, the PFC is thought to have a role in episodic memory (Yancey and Phelps, 2001), although the PFC may only be implicated when highly elaborate retrieval strategies are required (Fletcher et al., 1997). In patients with frontal lobe lesions, memory for the exact source of information is impaired; patients can remember facts that have been presented to them in experimental sessions, but are unable to remember where and when those facts were learnt, often attributing it incorrectly to another source, e.g. seen in a magazine (Janowsky et al., 1989b). These same patients showed intact word recognition and cued recall, but were impaired on free recall (Janowsky et al., 1989a). This impairment is of particular interest as it is thought to result from a deficit in subjective organisational strategies that usually serve to aid this type of free recall (Gershberg and Shimamura, 1995); for example, grouping words into categories based on semantic meaning, such as fruit versus animals.

The human PFC has been shown to have a role in the temporal ordering of memory; frontal lobe damage is known to cause impairments in judging the relative recency of items (Milner et al., 1985; Milner et al., 1991; Milner and Petrides, 1984). However, this impairment can be overcome by providing suitably salient associations with the item, like including an action such as "*squeeze the sponge*" (McAndrews and Milner, 1991). In further support of this idea, frontal-damage patients were unable to

reproduce the correct order of a list of 15 words, despite being able to recognise the words themselves (Shimamura et al., 1990). Additionally, an fMRI study of healthy individuals confirmed that in a verbal recency judgment task the PFC is more active than in a control non-mnemonic task (Zorrilla et al., 1996).

1.3.2 Monkey data

The behavioural deficit induced by frontal ablation in the monkey was first described by Jacobsen (1935; 1936). This deficit was shown in the delayed-response test, which requires the short-term retention of sensory cues, and differs from the DNMS test discussed above in that it requires the position of a baited object to be retained over the delay. Each trial involves the monkey being shown food being placed under one of two identical objects, then follows a delay where the objects are out of reach or sight, finally the monkey is presented with both objects and must choose the correctly baited object in order to receive the food reward (Fuster, 1997; Jacobsen, 1936).

Deficits on this delayed response task have been replicated consistently in a wealth of studies on monkeys with frontal damage (Mishkin and Pribram, 1956; Pribram et al., 1952; Pribram, 1961). Monkeys have also been shown to be impaired on delayed alternation following lesions of the PFC (Goldman et al., 1971; Mishkin, 1957; Mishkin and Pribram, 1955), along with impairments on delayed matching using colours (Passingham, 1975) and DNMS (Kolb, 1990a; Mishkin and Appenzeller, 1987). Deficits are also reported in a spatial working memory task that involved monkeys having to locate peanuts from behind 25 different doors, the lesioned monkeys would revisit already visited doors (Passingham, 1985). Thus PFC damage induces impairments in tasks where information must be retained across a delay interval, and deficits are apparent under both spatial and non-spatial conditions.

1.3.3 Rat data

Prefrontal damage affects the way that rats respond in the T-maze, with a reduction seen in spontaneous alternation following PrL lesions (Delatour and Gisquet-Verrier, 1996). Delay-dependent deficits have been routinely shown in the DNMTP task (or forced alternation), in the T-maze, thus indicating an impairment in spatial working memory

(Brito and Brito, 1990; Delatour and Gisquet-Verrier, 1996). Rats with aspirative PFC lesions were impaired on a NMTP in the Y-maze, but the same rats were not impaired on a non-spatial Y-maze task using goal boxes to indicate the correct arm, suggesting that PFC damage affects performance only under certain circumstances (Shaw and Aggleton, 1993). A NMTP T-maze task that allowed reference memory to be assessed in addition to working memory, revealed effects of PrL lesions on the working memory component only (Granon et al., 1994). There is also evidence for greater impairment on MTP relative to NMTP, which is thought to reflect the more effortful nature of the MTP rule, given that it uses a rule that is counter to the animals' innate tendency to alternate between the two arms (Dias and Aggleton, 2000; Granon et al., 1994). However, Aggleton et al (1995) report no such deficit in a forced alternation task even when the delay was 60 sec, and a group of rats with lesions of the PrL/IL areas were able to overcome an initial deficit when trained on DNMTP in a plus maze (Delatour and Gisquet-Verrier, 2000). These discrepancies may reflect subtle differences in the task parameters and differences in lesion techniques and locations.

Reports of PFC lesion effects on the standard eight arm RAM test vary, ranging from impairments during acquisition which are transient (Fritts et al., 1998; Joel et al., 1997a), to no discernable impairment whatsoever (Delatour and Gisquet-Verrier, 1996). However, this task is thought to rely predominantly on unlearned, spontaneous strategies which may make it less sensitive to PFC damage (Granon and Poucet, 2000). Rats with medial PFC lesions were not impaired on a two choice DNMP task in the RAM, provided the arms were selected at random; alternatively, if the same two arms were used in every trial then the rats did show an impairment, suggesting an increased susceptibility to interference between trials (Porter and Mair, 1997). Impairments have also been shown following inactivation of the PrL in the delayed win-shift task in the RAM, where four arms are baited in the first phase, followed by a delay, and then the remaining four arms must be chosen (Di Pietro et al., 2004). An impairment in a similar experimental set-up was only apparent when the PrL was inactivated between the training and test phases and not when inactivated before training (Seamans et al., 1995), implicating the region specifically in retrieval or use of information acquired during the delay.

Additionally, studies have suggested that the rat PFC is involved in the ability to make effort-based decisions. For example, rats were trained preoperatively in a cost-benefit T-maze, where a choice was made between climbing a barrier to access an arm containing a high reward, or choosing the arm with no barrier for a low reward (Walton et al., 2002). Prior to surgery rats consistently chose the high reward, but following excitotoxic lesions to the PFC they were much more likely to select the arm with the low reward. This suggests that the PFC is involved in making decisions which are motivated by cost-benefit. Cardinal et al (2001) assessed rats on a related task, where "impulsivity" can be measured in an operant chamber; choosing a small but immediately available reward in preference to a larger but delayed reward is indicative of impulsive choice behaviour. Lesions of the NAC, of which PrL is an afferent, induced impulsive choice behaviour in this task, however direct lesions of the mPFC were without effect (Cardinal et al., 2001). Thus in this task the cost is in terms of the delay in reward delivery, which may not depend on the integrity of the PFC in the same way that climbing a barrier in the T-maze task does (Walton et al., 2002).

Hanneson et al (2004b) reported a study that assessed rats' ability to judge the temporal order of arms that had been visited in the RAM; whereas a normal rat will direct more exploration at the least recently explored familiar arm, a rat with lidocaine inactivation of the PFC will not, however these rats are still able to perform the recognition component. A similar deficit had been noted in animals with aspirative lesions of the PFC (Kesner and Holbrook, 1987), helping to verify that it is likely that the rat PFC is involved in some way in the temporal ordering of memory, at least as far as memory for spatial locations is concerned.

Evidence for the involvement of PFC in working memory for egocentric responses comes from a study by Ragozzino and Kesner (2001), where rats with excitotoxic PFC lesions were impaired on a DMTP task on a plus shaped maze which required a solution based entirely on a body turn. This deficit had also been seen in rats with aspirative PFC lesions in an eight arm RAM using an adjacent arm task, whilst performance on an allocentric cheeseboard task remained intact (Kesner et al., 1989). The exact role of the PFC in spatial working memory is unclear, although performance on navigation-based tasks is commonly intact (Broersen, 2000; de Bruin et al., 1994; de

Bruin et al., 2001; Kesner et al., 1989; Lacroix et al., 2002; Sullivan and Gratton, 2002). Efforts to establish the existence of place cells in the rat PFC have located cells that are activated according to the exact type of behaviour being performed, and not necessarily the spatial location of the animal (Jung et al., 1998; Poucet, 1997); although recent evidence suggests that there may be some cells which mediate a similar function to the place cells of the HPC (Hok et al., 2005). It has been shown that in the rat there are spatio-selective units in the medial PFC that fire differentially according to the side of the cue in a delayed performance U-shaped maze. Different units also respond at different stages in the trial (Batuev et al., 1990), thus providing further evidence for the involvement of this area in short-term memory function in the rat.

PFC lesions also result in impairments on a variety of tasks performed in operant chambers; these include GO/NO-GO tasks, where the rat must alternate between responding and withholding a response (Sakurai and Sugimoto, 1985), conditional discrimination (Delatour and Gisquet-Verrier, 1999), delayed alternation between two levers (Dunnett et al., 1999; Izaki et al., 2001; van Haaren et al., 1985) and tasks which assess attention in the 9HB (Chudasama et al., 2003; Muir et al., 1996). Temporary inactivation of either the PFC or HPC has also been shown to disrupt lever responding in the Skinner box (Izaki et al., 2000).

1.3.4 Attentional set shifting

One critical role of the PFC is thought to be in providing behavioural flexibility, which is the ability to adapt and respond to changes in circumstance. The Wisconsin Card Sorting Task (WCST) (see figure 1.3) is a classic test of PFC damage in humans. Subjects are required to sort cards according to the shape, colour or number of symbols on the card. The test starts with one rule (e.g. sort by colour) and then the experimenter changes the rule without informing the subject. Normal subjects have very little difficulty in switching between the rules, but those with PFC damage or schizophrenia tend to learn the first rule and are then unable to shift to the new one (Janowsky et al., 1989a; Milner and Petrides, 1984; Shimamura et al., 1992). They tend to perseverate with the original incorrect rule, indicating an inability to "set-shift", i.e. an inability to inhibit a previously

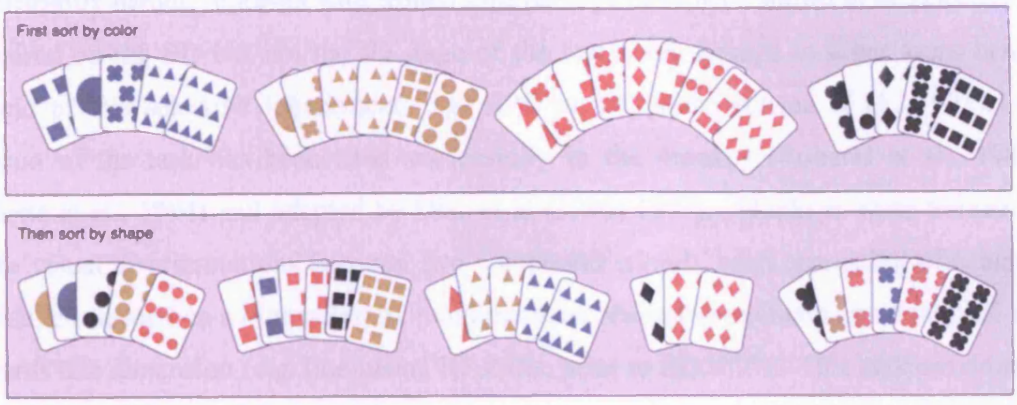


Figure 1.3 The Wisconsin Card Sorting Test from Bear et al (1996).

acquired cognitive set (Bear et al., 1996; Miller, 2000; Stuss et al., 2000). Additionally, this type of set-shifting is related to increased activity of the PFC during neuroimaging studies (Konishi et al., 2003; Nagahama et al., 1996; Rogers et al., 2000; Rushworth et al., 2002). However, Anderson et al (1991) were unable to reveal any deficit in the WCST in patients with PFC lesions, and caution against relying on it as a sole indicator of frontal lobe damage. Another example of a task which reveals a prefrontal deficit of this nature is an odd-man-out task, where cues indicating the relevant dimension to be attended to can be presented or withheld to vary task complexity (Ravizza and Ciranni, 2002). In this task, the "odd-man-out" is one of four stimuli comprised of letters within shapes, with either of these two attributes being the relevant dimension; when the relevant dimension was switched, patients with PFC damage were impaired at choosing the odd-man-out, even with the presence of a cue e.g. LETTER.

The WCST and its related tasks are useful tools in the field of animal models of disease, and various analogues have been developed to allow the investigation of behavioural flexibility and strategy switching. One common paradigm involves subjects performing a visual compound discrimination involving either a shift within the previously relevant dimension (intra-dimensional (ID) shift) or a shift of attentional set from one dimension to another (extra-dimensional (ED) shift) (Roberts and Sahakian, 1993; Rushworth et al., 2002). If the performance of the ID shift is superior to the ED shift this is taken as evidence that the subject has developed an attentional set (e.g. attend to colour only, ignore shape), because shifting to the previously irrelevant dimension is

significantly harder. Patients with frontal lobe damage have been shown to be selectively impaired on the ED but not the ID stage of the test, with damage to other areas being devoid of this selective impairment (e.g. HPC or amygdala) (Owen et al., 1991). A version of the task has been used successfully in the monkey (Roberts et al., 1988; Roberts et al., 1994) and adapted by Dias et al (1996; 1997). Monkeys were trained to make visual discriminations between two compound stimuli, each consisting of a black line superimposed on a blue polygon; monkeys were trained to maintain an attentional set towards one dimension (e.g. line) using ID shifts, prior to ED shifts. This task provided a double dissociation within the PFC with orbital lesions disrupting ID but not ED shifts, and lateral lesions disrupting ED but not ID shifts (Dias et al., 1996). These findings provided evidence for yet another theory of PFC working memory fractionation, based on a hierarchy of the complexity of rules required to solve a task (Wise et al., 1996).

Various attempts have been made to develop models of this attentional set-shifting paradigm in the rodent. One example investigated the involvement of rodent prefrontal cortex subregions in strategy switching using a task known as the cheeseboard task (Ragozzino et al., 1999). This is a dry-land version of the water maze, using a circular platform containing small round holes in which food rewards can be hidden. Rats were tested first on spatial and then visual-cued versions of the task and vice-versa. It was shown that inactivation of the PrL and IL areas, but not the dorsal anterior cingulate (Cg1) area, impaired the rats performance when they switched from one version of the task to the other. This suggests that an intact PrL/IL area is crucial for this type of strategy switching. Birrel and Brown (2000) developed an elegant model for testing attentional set-shifting in the rat, utilising food-rewarded bowls that differed in either their odours, the medium that filled the bowls or the covering texture. Rats were required to make a series of discriminations between two bowls, one of which contained a food reward, based on these different dimensions. Rats with lesions encompassing PrL, IL and often encroaching on Cg1 and Cg2, were seen to have no deficit in simple reversals or ID shifts, but did show a significant impairment on ED shifts (Birrel and Brown, 2000), which correlates with the effects seen following lateral PFC lesions in the monkey (Dias et al., 1996; Dias et al., 1997). Similarly, rats with PrL/IL damage were unimpaired on learning odour discriminations, but when the task was switched such that the position of

the bowl rather than the odour became the relevant dimension, these same rats were impaired (Ragozzino et al., 2003).

Further evidence for deficits in rule switching following PrL inactivation exists in tasks involving the RAM. PrL rats are impaired on a delayed spatial win-shift paradigm, but not a non-delayed random foraging task (Seamans et al., 1995). However, when switched unexpectedly between the two different tasks the PrL rats were unable to adapt to the new foraging strategy, providing further impetus to the idea that the PrL is involved in strategy switching (Seamans et al., 1995). Also, rats with PrL and IL lesions were shown to be impaired when switched between a fixed-goal task (same goal-arm every day) and a variable-goal task (same goal-arm on each trial, but differed across days) (Delatour and Gisquet-Verrier, 1996). However, rats with either discrete or combined lesions of Cg1 or PrL were not impaired compared with controls, when the four baited arms on a RAM were reversed (Joel et al., 1997a). Finally, rats with excitotoxic lesions of the PrL/IL were able to learn to locate the one baited box out of thirteen located in a circular arena, provided just one start position is used (Delatour and Gisquet-Verrier, 2000). These same rats were severely disrupted if four different start positions were used in this task, suggesting an impairment in behavioural flexibility.

The T-maze has also been used to investigate involvement of the PFC in behavioural flexibility and strategy switching, with rats being exposed to rule switches between MTP and NMTP paradigms. Rats with excitotoxic lesions of the PrL were impaired on this type of switching, tending to perseverate with the incorrect rule for longer than control rats (Dias and Aggleton, 2000). This impairment mirrors that seen in an identical task following lesions of the MD, which is densely connected with the PFC (Hunt and Aggleton, 1998). However, these tasks cannot be interpreted as providing deficits in switching attentional set *per se*, as clearly both rules exploit the same stimuli of allocentric spatial cues; rather, they might be interpreted as evidence of a failure to shift response rules (Dias and Aggleton, 2000; Hunt and Aggleton, 1998). Similar types of switching deficits are being assessed in the Skinner Box, using the switch from DNMTP to DMTP, for example, to investigate effects of lesions of the mPFC in rats (Joel et al., 1997b). One of the aims of this thesis is to investigate these types of tasks

further, and therefore a more detailed discussion of this topic will follow in the appropriate experimental chapters.

1.4 Hippocampus and prefrontal cortex compared

So far in this introduction, involvements of the HPC and PFC in cognitive behaviour have been considered in isolation. However, one of the aims of this thesis is to try to establish a dissociation between the effects of lesions of these two areas within the same task. In order to provide a rationale for predicting any such dissociation it is necessary to review studies in which these two lesions have been directly compared, utilising both lesion and disconnection paradigms.

Dissociations have been observed between the effects of HPC and PFC lesions on complex maze learning (Winocur and Moscovitch, 1990). Winocur and Moscovitch (1990) trained rats which had received either HPC and PFC lesions on one of two complex mazes (Maze A) from the Hebb-Williams series; these mazes are arenas in which the rat must learn the route from a start box to a goal box containing food, barriers are inserted to create different mazes of comparable difficulty. A separate cohort of lesioned rats did not receive any training prior to the test period, which consisted of both trained and non-trained animals being tested on Maze A and Maze B. Trained HPC rats showed sparing for the general skill of maze learning but poor recall for the specific maze they had been trained on (as demonstrated by an equal number of errors in Maze A and B but enhanced performance compared with their non-trained counterparts). The opposite pattern was observed in the trained PFC group, with no sparing of the general skills required for maze learning but evidence for recall of the specific maze (as demonstrated by similar performance to non-trained counterparts on unfamiliar Maze B, but enhanced performance on Maze A). These findings implicate the HPC in transferring relevant information to similar problems and remembering non-specific skill-related information; for example, the general rule for not re-entering blind alleys is not applied to the new maze problem by PFC-lesioned rats, with rats showing an increased tendency for repeating incorrect responses. In contrast, the PFC is implicated in remembering highly

specific information rather than retention of the general skills required for learning the maze problems (Winocur and Moscovitch, 1990).

Dissociations have also been found between the effects of these lesions in operant chambers. In a conditional-discrimination learning paradigm, HPC-lesioned rats exhibited a delay-dependent deficit compared with an impairment at all delays following PFC lesions (Winocur, 1991). This paradigm involved rats having to respond to the left lever in response to being presented with any combination of three lights on the left side of the chamber, and similarly responding to the right lever on presentation of lights on the right side. PFC-lesioned rats were thought to be impaired on organising the available information to perform the conditional discrimination, whereas HPC-lesioned rats showed performance that deteriorated with increasing delay, suggesting a compromised capacity for working memory. This pattern of impairments was also demonstrated in another study by Winocur (1992a), in which rats were trained on an MTS task in an operant chamber. This task required rats to respond on a lever when two lights of identical brightness were presented in succession, but to withhold responding if the two lights differed. Rats with HPC lesions were impaired in a delay-dependent manner, whereas rats with PFC lesions were impaired across all delay intervals. A final example of this dissociation is that HPC and PFC-lesioned rats exhibit this impairment pattern on a delayed alternation task in the Skinner box utilising the GO/ NO-GO regime (Winocur, 1992b).

Fundamental to these dissociations is the idea that multiple memory systems exist within the brain (Kim and Baxter, 2001; Squire, 1992). Kesner and Rogers (2004) propose that memory is organised into three main systems, namely event-based (akin to episodic memory), knowledge-based (akin to semantic memory) and rule-based (receives and integrates information from the event-based and knowledge-based systems and applies rules for subsequent actions). They also propose that dissociations between the HPC and PFC may arise from the relative contributions of these two areas to the event-based and rule-based memory systems respectively (Kesner and Rogers, 2004). Support for this theory has already been provided by the maze learning study discussed above (Winocur and Moscovitch, 1990), but studies which examine the dynamic interaction

between the PFC and HPC can also be of value in determining the functions of these two areas.

Asymmetric disconnection procedures involve blocking the transmission of information within specific pathways in each hemisphere (Floresco et al., 1997). This technique has been used successfully to determine that different pathways are involved in different foraging strategies in the rat. Unilateral lidocaine inactivation of the vCA1/ subicular region combined with contralateral inactivation of the PrL resulted in impaired performance on a delayed spatial win-shift paradigm in the RAM, but left performance on a non-delayed random foraging version intact. Conversely, a similar procedure inactivating the vCA1/ subicular region and the NAC resulted in the opposite pattern of results, with impairment on the non-delayed random foraging version. These results indicate that different aspects of spatial foraging behaviour are served by separate networks, and that the neural circuit linking the PFC and HPC is an essential pathway for the integration of spatial information (Floresco et al., 1997).

Another study which investigated the relationship between the PFC and HPC in the rat utilised a DNMTP task in the RAM with short-term (10 sec) or long-term (5 min) delays (Lee and Kesner, 2003). In this study bilateral cytotoxic lesions of either dorsal HPC or mPFC (PrL and IL) were assessed alone and in combination with bilateral reversible inactivation of the opposite structure e.g. mPFC lesion + inactivation of the dHPC or vice versa. A dramatic set of results was obtained; both bilateral lesions resulted in an initial impairment in working memory at the short-term delay (5 sec), with performance recovering to control levels by the second testing block. This suggests that some sort of compensatory adjustment took place that allowed performance to be maintained at this delay. However, if both structures were inactivated performance at this delay was severely impaired, indicating that at least one of these structures must be intact for successful short-term spatial memory function. The final important finding was that the dHPC lesion impaired performance at the longer delay interval, whereas the PFC lesion was without effect. Therefore, from these data it would appear that the dHPC and PFC process spatial memory in parallel within the short-term range, but once a critical time period is exceeded the dHPC becomes crucial (Lee and Kesner, 2003). These results suggest that the delay interval is an essential factor in the dissociation of the

functions of the HPC and the PFC in this type of delayed-choice task (Kesner and Rogers, 2004).

1.5 Delayed matching to position

1.5.1 The task

The predominant task used in this study is the Delayed Matching to Position (DMTP) task in the Skinner box (Dunnett, 1985; Dunnett et al., 1988). This task will be discussed in full before reviewing the relevant literature. DMTP was designed as an analogue of the monkey DMS tests, with the response levers of the box providing spatially distinct stimuli (Dunnett, 1985). Some similar paradigms exist that involve using "ports" as opposed to levers in which the rat must nose-poke and break the infra-red beam (Harrison and Mair, 1996; Young et al., 1996). DMTP has proven successful in assessing the effects of aging (Dunnett et al., 1988; Kolb, 1990b), lesions (Döbrössy et al., 1996; Mair et al., 1998; Porter et al., 2001) and drugs (Han et al., 2000; Iversen, 1997). Each trial involves the rat remembering the side of the sample lever over a variable delay interval and then responding to this lever (matching) in the choice phase to receive a food reward. The fundamental aspect of this task that allows working memory to be assessed is the inclusion of the variable delay interval; this also allows a distinction to be made between mnemonic impairments and non-specific impairments. Rats can show intact performance at the shortest delays, thereby demonstrating knowledge of the specific rule, but progressively impaired performance as the delay increases, and thus the load on working memory is increased (Dunnett, 1993); this would be considered a delay-dependent mnemonic deficit. In contrast, non-specific impairments are manifest at all delay intervals and may involve factors such as impaired retention of the matching rule, diminished attentiveness to the task and general motivational, motor or sensory impairments (Dunnett et al., 1990; van Hest and Steckler, 2001). This distinction can be particularly useful in teasing out differences between related brain areas (Dunnett, 1985; Dunnett, 1990; Young et al., 1996).

DMTP has an obvious variant, namely delayed non-matching to position (DNMTP), where responding to the opposite lever in the choice phase (non-matching) is

rewarded. Although believed to be logically symmetrical to DMTP, there is evidence to suggest that there may be differences between the two variants. Dunnett (1988) has shown that aged rats show lower asymptotic variance on DNMTP in comparison with DMTP. Lesions of the basal forebrain had dissociable effects on reversing between the two rules, depending on which rule had been learnt first, i.e. an impairment was seen on reversing from DMTP to DNMTP, but not in the opposite scenario (Dunnett et al., 1989). Additionally, in these animals there was a deficit in acquiring DNMTP but not DMTP (Dunnett et al., 1989). The two rules are also learnt in different manners, with the DMTP rule requiring a correction procedure in order to prevent a position bias being adopted, whereas DNMTP is learnt in a more gradual fashion and does not require the use of a correction procedure (Dunnett, 1993). These findings highlight the need for caution when comparing results between the two contingencies and suggest that they may involve separate strategies at least at the acquisition stages (Blokland and Dunnett, 1995).

1.5.2 Effective parameter manipulation

The selection of appropriate delays between the sample and choice presentation is essential in providing meaningful data (van Hest and Steckler, 2001). Delay intervals must be chosen with the expected outcome of the lesion or drug manipulation in mind. If a manipulation is expected to enhance performance, suitably long delays must be chosen to prevent any ceiling effects which might act to mask any enhancement. Similarly, if a manipulation is expected to reduce accuracy, control performance must be such that floor effects are avoided in order for the genuine effect to be apparent (Dunnett, 1993). Alternatively, delay intervals can be adjusted for each animal as the session progresses, in a so called titration paradigm (Han et al., 2000). This allows performance to be maintained across a chosen accuracy for every animal, however this means delays will differ between subjects and memory load will not be consistent (van Hest and Steckler, 2001).

Other procedural variables which may be manipulated include the inter-trial interval (ITI), and the reinforcement contingency (van Hest and Steckler, 2001). Proactive interference is a phenomenon whereby the stimulus and response on the previous trial can interfere and effect choice accuracy on the current trial; the shorter the

ITI the greater the chance of proactive interference (Dunnett and Martel, 1990). For a task to be regarded as taxing working memory, the performance of the animal should be affected by the manipulation of these parameters, for example if a particular response no longer elicited a reward (devaluation) the animal would be expected to cease performing that response (Corbit and Balleine, 2000).

Tasks need to be designed with regard for the instinctive behaviour of the species in question, such as natural exploratory tendencies (Thorpe et al., 2004). For example, in a study by Wilkie et al (1999) rats were trained to press one lever in a four-lever Skinner box to receive food in a morning session, whilst pressing a different lever resulted in food in the afternoon sessions. Responses from the start of each session were recorded, prior to food reinforcement; these responses were taken as indicative of which lever the rat expected to result in food presentation. However, rats were seen to "patrol" all four levers in the box equally, i.e. they were not discriminating between the two rewarded levers, and were pressing levers which never resulted in food. With the simple introduction of a 10-sec time-out procedure at the start of the session, performance was improved to well above chance levels. Thus, instinctive tendencies can conflict with experimental demands; it should be noted that in the Skinner box tests employed in this thesis, rats were always allowed at least 30 sec in the boxes before the session began.

1.5.3 Delayed matching to position: effects of hippocampal and prefrontal damage

The DMTP task was originally designed as an analogue of the DMS test (Dunnett, 1985). DM/NMS has been shown to be sensitive to HPC damage in humans (Owen et al., 1995; Squire et al., 1988) and monkeys (Alvarez et al., 1995; Beason-Held et al., 1999; Zola et al., 2000; Zola-Morgan and Squire, 1986). Similarly damage to the PFC has also been shown to impair performance on this task in humans (Fuster, 1997) and monkeys (Fuster, 1997; Kolb, 1990a; Mishkin and Appenzeller, 1987; Passingham, 1975). Tables 1.1, 1.2 and 1.3 provide summaries of the literature concerning operant DM/NMTP tasks in the rat, they summarise the effects of HPC, Fx and PFC lesions respectively. These studies will be discussed in full in the relevant experimental chapters, but it is necessary to briefly review their findings in order to highlight the disparities that exist.

Damage to the HPC provides contradictory results on DM/NMTP (Table 1.1), with evidence for delay-dependent and delay-independent deficits, whilst other studies report intact performance. Additionally, the only known study to have investigated cytotoxic lesions of the HPC is confounded by the lack of control group (Hampson et al., 1999), thus providing grounds for investigating this type of fibre-sparing lesion technique within the present thesis. There have been numerous studies investigating the effect of Fx damage on DM/NMTP (Table 1.2) and these have proved to be slightly more consistent than studies of specific HPC lesions. A significant number of these studies report delay-dependent deficits, although again there is some evidence for delay-independent impairments. In contrast to the HPC lesions, rats with Fx damage always incurred impairments in performance of this task, rendering this lesion suitable for use as a "control" lesion, i.e. one likely to provide a deficit. Finally, studies on the consequences of PFC lesions (Table 1.3) tend to be in agreement that delay-independent impairments are incurred, although there are some exceptions to this general supposition. Two studies have investigated cytotoxic lesions of the PrL and both resulted in delay-independent deficits on this task (DNMTP: Aggleton et al., 1995; DMTP: Chudasama and Muir, 1997), however in one of these studies the lesions encompassed the cingulate cortex (Aggleton et al., 1995) and therefore further investigation of more discrete lesions is warranted. Based on this literature it would appear that a dissociation between the effects of HPC and PFC lesions would not be unexpected, and one might speculate that HPC lesions would result in deficits that were more dependent upon delay interval than the PFC group, which might exhibit a more non-specific impairment. The hope is that the studies presented within this thesis might make some inroads towards a reconciliation of the inconsistencies in the data reviewed above, and provide a clearer view of the involvement of the HPC and PFC in this particular task.

Target area	Type of lesion or manipulation	Task	Impairment	Reference
HPC	Aspiration	DNMTP in Skinner box	Delay-dependent	(Aggleton et al., 1992)
	N/A*	DNMTP in Skinner box	Delay-dependent	(Broersen, 2000)
	Scopolamine	DMTP in Skinner box	Delay-dependent	(Dunnett et al., 1990)
	IBO	DMTP and DNMTP in Skinner box	Delay-dependent †	(Hampson et al., 1999)
	Radiofrequency	DNMTP in Skinner box	Delay-independent	(Porter et al., 2000)
	Radiofrequency	DMTP in Skinner box	None	(Mair et al., 1998)
	Cholinergic	DNMTP and switch to DMTP in Skinner box	None	(Winters and Dunnett, 2004)
	Radiofrequency	DMTP and DNMTP in operant chamber equipped with "ports"	None	(Young et al., 1996)

Table 1.1 Summary of rat studies of HPC involvement on working memory DM/NMTP tasks performed in operant chambers. All studies assess postoperative retention of the rule. * These results were presented within a review paper and lesion details were not made available (Broersen, 2000). † There was no control group within this study; instead postoperative and preoperative performance were compared (Hampson et al., 1999).

Target area	Type of lesion or manipulation	Task	Impairment	Reference
Fx	Radiofrequency	DNMTP in Skinner box	Delay-dependent	(Aggleton et al., 1991)
	Radiofrequency	DNMTP in Skinner box	Delay-dependent	(Aggleton et al., 1992)
	Radiofrequency	DNMTP in Skinner box	Delay-dependent	(Aggleton et al., 1995)
	Radiofrequency	DMTP in Skinner box	Delay-dependent	(Chudasama and Muir, 1997)
	Aspiration	DMTP in Skinner box	Delay-dependent	(Dunnett, 1985)
	Aspiration	DMTP in Skinner box	Delay-dependent	(Dunnett, 1990)
	Radiofrequency	DNMTP in Skinner box	Delay-dependent	(Ennaceur et al., 1996)
	Aspiration	DMTP and DNMTP in 9HB	Delay-dependent (although see discussion in Ch.6)	(Etherington et al., 1987)
	Knife-cut	DNMTP in Skinner box	Delay-dependent	(Weiner et al., 1998)
	Aspiration	DNMTP and switch to DMTP in Skinner box	Evidence for both delay-dependent and independent impairments	(Winters and Dunnett, 2004)
	Radiofrequency	DMTP and DNMTP in operant chamber equipped with "ports"	Delay-independent	(Young et al., 1996)

Table 1.2 Summary of rat studies of Fx transection on working memory DM/NMTP tasks performed in operant chambers. All studies assess postoperative retention of the rule.

Target area	Type of lesion or manipulation and specific target area	Task	Impairment	Reference
PFC	Scopolamine-Fr2	DMTP in Skinner box	Delay-dependent	(Broersen et al., 1994)
	Aspiration-M2,MO,Cg1,PrL	DMTP in Skinner box	Delay-dependent	(Dunnett, 1990)
	NMDA-mPFC, inc. Cg1	DNMTP in Skinner box	Delay-independent	(Aggleton et al., 1995)
	N/A*	DNMTP in Skinner box	Delay-independent	(Broersen, 2000)
	Aspiration- PrL, Cg1 and Cg2	DMTP in Skinner box	Delay-independent	(Dunnett, 1990)
	Scopolamine-PrL and IL	DMTP in Skinner box	Delay-independent	(Dunnett et al., 1990)
	NMDA- PrL	DMTP in Skinner box	Delay-independent	(Chudasama and Muir, 1997)
	Scopolamine- (Cg1 and PrL)	DMTP in Skinner box	Delay-independent	(Herremans et al., 1996)
	Radiofrequency- Cg1, Cg2 and Fr2	DMTP in Skinner box	Delay-independent	(Mair et al., 1998)
	Radiofrequency- Cg1, Cg2, Fr2 and PrL	DNMTP in Skinner box	Delay-independent	(Porter et al., 2000)
	Radiofrequency -mPFC	DMTP and DNMTP in operant chamber equipped with "ports"	Delay-independent	(Young et al., 1996)
	Radiofrequency - mPFC	DNMTP in operant chamber equipped with "ports"	Impaired at 3 sec fixed delay	(Harrison and Mair, 1996)
	Electrolytic- Cg1, Fr2 and PrL	Acquired DNMTP postoperatively, then switched to DMTP: Skinner box	Impaired on switch between rules, but not on initial acquisition	(Joel et al., 1997b)

Table 1.3 Summary of rat studies of PFC involvement on working memory DM/NMTP tasks performed in operant chambers. All studies assess postoperative retention of the rule unless stated otherwise. *These results were presented within a review paper and lesion details were not made available (Broersen, 2000)

1.6 Hippocampus and prefrontal cortex in disease

Finally, it is worth considering that both the HPC and the PFC are thought to be involved in a range of diseases in the human; abnormalities in these areas are thought to underlie the cognitive deficits that underpin many of these disease states. For example, PFC dysfunction is widely held to be involved in the cognitive aspects of schizophrenia (Goldberg et al., 1987; Joel et al., 1997b; Li et al., 2002; Weinberger and Berman, 1998). Upregulation of D_1 dopamine receptors in the PFC of schizophrenic patients has been shown to be predictive of working memory deficits (Abi-Dargham et al., 2002) and schizophrenic patients exhibit reduced activity in the PFC when performing tasks such as the WCST, as measured by reduced regional cortical blood flow (Berman and Weinberger, 1990; Meyer-Lindenberg et al., 2002). The HPC is also implicated in this disease; rats with ventral HPC lesions made on day 7 of life exhibited behavioural changes indicative of schizophrenic like symptoms (Daenen et al., 2002). Ventral HPC lesions have also been shown to produce differential changes in cortical and limbic dopamine activity that may also be of significance in the search for a viable animal model of schizophrenia (Lipska et al., 1992). Neuroimaging techniques have shown that schizophrenics demonstrate abnormal levels of hippocampal activity at rest, during the experience of auditory hallucinations and during the performance of memory retrieval tasks (Heckers, 2001). It has also been observed that the HPC of schizophrenic patients is reduced in volume compared with controls (Harrison, 2004; Heckers, 2001) and synaptic circuitry is altered both within the HPC and in its extrinsic connections, particularly with the PFC (Harrison, 2004).

In addition to the undisputable involvement of these areas in schizophrenia, there is also evidence for involvement in both Parkinson's and Alzheimer's diseases. Parkinson's disease is accompanied by deficits in executive functioning and attentional set-shifting, resembling those seen in patients with frontal lobe damage (Owen et al., 1992; Owen, 2004; Tsuchiya et al., 2000), suggesting that there might be some underlying prefrontal pathology associated with this disease. Dysfunction of the PFC is thought to underlie the cognitive deficits seen in Alzheimer's disease (Akbarian et al., 1995; Berman and Weinberger, 1990), with abnormal nicotinic binding in the HPC also

thought to be of significance (Levin et al., 1999; London et al., 1989; Newhouse and Kelton, 2000). Furthermore, the earliest pathological changes are evident in the ERC and the HPC, before progressing on to other cortical areas (Braak et al., 1993; Panegyres, 2004).

The brief summary above clearly illustrates the fundamental need to understand these areas further in order to implement successful treatment strategies, and the rat is widely regarded as a viable model from which one can make valid assumptions about the human mind (Davis, 1996; Kolb, 1990a). Obviously, experiments must be designed carefully (D'Mello and Steckler, 1996) and the review of the literature presented in this chapter hopefully serves to illustrate the many ways in which subtle aspects of cognition can be assessed in the rodent. Experimental work in rats will continue to provide a framework from which insight into the workings of the human brain can be made.

1.7 Aims of thesis

The aim of this thesis is to provide a clearer understanding of the involvement of the PFC and HPC in cognitive behaviours in the rat, by investigating both novel and established tasks that are sensitive to disruption of these areas. It is hoped that investigation of these two regions in parallel may be effective in the advancement of animal models of human brain dysfunction, with both areas implicated in an array of diseases which result in complex cognitive impairments. In the future, strategies for brain repair may be assessed in these models, either through neuroprotective drug treatment or cell transplantation therapies. In particular, as reviewed above, many studies have investigated the effects of either PFC or HPC lesions on a range of tasks, but seldom have the two lesions been explicitly compared in the same study.

This thesis employs discrete excitotoxic lesions of PFC, HPC and ERC, with aspirative lesions of the Fx providing a further comparison group. Chapter 4 compares PFC and HPC lesions on retention of DMTP in the Skinner box, with Chapter 5 discussing the effects of these lesions on acquisition of the task. Chapter 6 describes lesions of other components of the MTL, namely the ERC and Fx, and assesses their effects on retention of DMTP, providing a comparison with the HPC lesions. In Chapter

7, an object recognition task is described which provides a distinct method of assessing the rats' behaviour on a delayed non-matching task. This task differs significantly from the Skinner box tasks in many ways, with the most prominent being that rather than relying on a learned response that requires extensive training, it utilises the rats' spontaneous tendency to explore novel objects. The effects of lesions of PFC, HPC, ERC and Fx are assessed on variants of this task which explore recognition memory across different retention intervals and incorporate both spatial and recency components. Finally, Chapter 8 details a novel task in the Skinner box (originally developed by Mátè Döbrössy and described in his thesis (1997)), on which the effects of PFC and HPC lesions are assessed.

Chapter 2 Behavioural Methods

2.1 Introduction

This chapter contains descriptions of the various behavioural apparatus and paradigms used throughout this thesis. Tests involving the Skinner box, water maze, spontaneous locomotor activity and spontaneous novelty preference are described in detail. Any deviations from these procedures are noted in the appropriate section.

2.2 Subjects

Male Lister Hooded adult rats (Harlan, UK) were used throughout; all rats weighed at least 350 g before testing. Rats were housed four per cage, in a holding room with a 12-hr light/ dark cycle (lights on at 07:00), maintained at 23 ± 1°C. All procedures adhered to Home Office regulations and complied with the Scientific Procedures Act (1986). The rats had *ad libitum* access to food and water when not being tested, except for when they were undergoing Skinner box testing. At least three days prior to Skinner box testing, rats were food restricted in order to maintain their weight at no less than 90% of their free feeding weight for the duration of the testing period.

2.3 Skinner Box

2.3.1 Apparatus

Behavioural testing was carried out in either 8 or 12 Skinner boxes (Paul Fray Ltd., Cambridge) enclosed in sound attenuating, ventilated chambers (figure 2.1). Each box was equipped with two retractable levers, 7.5 cm on either side of a central food hopper; access to the food hopper was gained by nose poking a perspex hinged panel which was subsequently registered as a panel press. The food hopper could be illuminated from behind the perspex panel and a house light permitted illumination of the chamber. A pellet dispenser delivered 45 mg sucrose pellets (NOYES precision pellets: Research

Diets, Inc., NJ) to the food hopper. Cameras were positioned in the ceiling of each box to allow observation of the animals' behaviour on monitors outside the testing room.

A

B

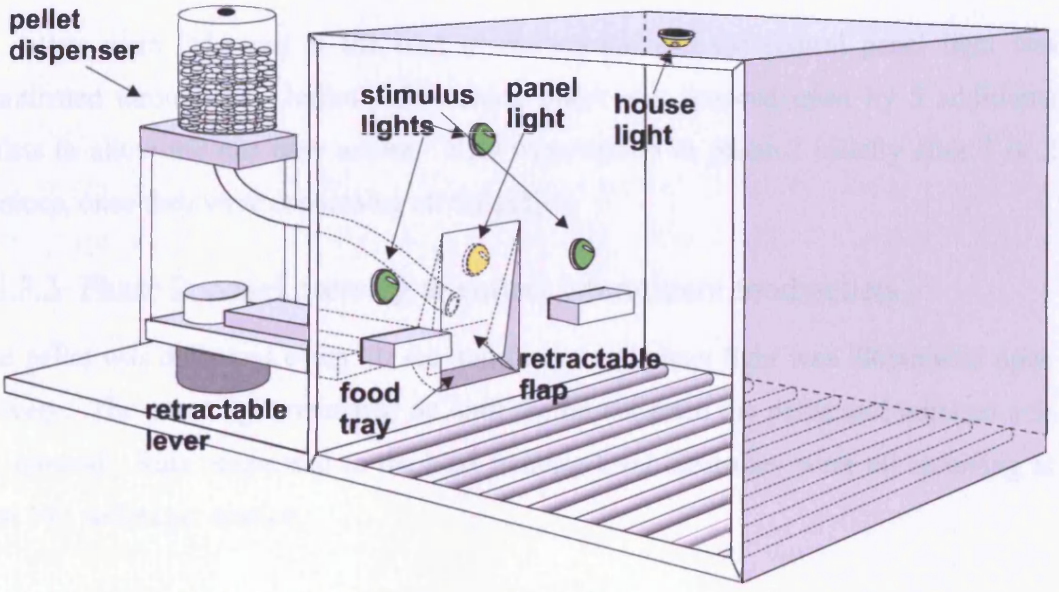


Figure 2.1 Photo of rat in Skinner box with perspex door open (A), schematic diagram of Skinner box (B).

2.3.2 Software

The Skinner boxes were controlled by an Acorn A500 computer programmed in Basic, modified with the Arachnid control language (Fray, 1993). The Arachnid/Basic programming language is an event-based, real-time language which allows the performance of several animals to be monitored and acted upon simultaneously. The programmes for the operant behaviour paradigms were written by Prof. Stephen Dunnett and modified by Hazel Sloan.

2.3.3 Training

2.3.3.1 Habituation to pellets

Rats were given a handful of sugar pellets in their home cages for three days prior to Skinner box testing. This procedure habituated the rats to the smell and taste of the pellets in order to facilitate Skinner box training in which these pellets serve as the positive reinforcer. Training in the Skinner boxes consisted of 4 phases; rats were tested once a day during the light phase for a 30-min session duration, the house light remained illuminated throughout.

2.3.3.2 Phase 1: habituation to boxes

25 pellets were delivered at the start of the session and the central panel light was illuminated throughout. Initially the central panel was propped open by 5 additional pellets to allow the rats easy access. Rats were moved to phase 2 usually after 1 or 2 sessions, once they were consuming all the pellets.

2.3.3.3 Phase 2: panel pressing to collect intermittent food pellets

One pellet was delivered every 10 sec and the central panel light was illuminated upon delivery. The panel light remained on until the rat collected the pellet and initiated a 5-sec interval. Rats progressed to the next training level once they were all receiving at least 100 pellets per session.

2.3.3.4 Phase 3: continuous reinforcement

Both levers were extended throughout and a response on either lever was reinforced with delivery of a pellet. Rats can become biased to responding to one lever if kept at this training stage for too long, so as soon as they were achieving 100 lever responses per session they were moved to the final training level.

2.3.3.5 Phase 4: alternating continuous reinforcement

Rats were presented with one lever at a time, alternating between trials, and a press on the lever resulted in delivery of a pellet. Three days on this training programme was sufficient for all rats to be completing at least 100 presses per session

2.3.4 Delayed matching to position task (DMTP)

A DMTP trial consists of three phases, the sample, delay and choice, as illustrated in figure 2.2. DMTP sessions were of either 30 or 40-min durations depending on the experiment, with the houselight illuminated throughout except during time-out periods. The side of the sample lever was chosen pseudorandomly by the computer before the start of the trial, such that left and right levers were presented approximately the same number of times in each session. Once the rat had pressed the sample lever, it was retracted and the central panel light illuminated. The first nose poke made in the central panel after the delay interval had elapsed initiated the choice phase. This insured a high rate of nose poking during the delay and prevented the rats from waiting at the sample lever for the duration of the delay. Both levers were extended for the choice phase and a correct response (to the sample lever) was rewarded with delivery of a food pellet, upon collection of the pellet the 5-sec intertrial interval began prior to the next trial. An incorrect response was punished by a time-out period of 5 sec, during which the houselight was extinguished and no reward was delivered.

2.3.4.1 DMTP0

Training began with a programme that had no delay interval between the sample and choice phases. A correction procedure was introduced to prevent rats from always responding to the same lever during the choice phase, so that the incorrect trial was

repeated (i.e. sample presented on the same side) until a correct response was made. Any rats that stopped responding completely due to this correction procedure were returned to phase 4 of training until they had made ~100 lever presses and then switched straight onto DMTP0. Performance of over 80% correct on two consecutive days resulted in progression onto the next programme.

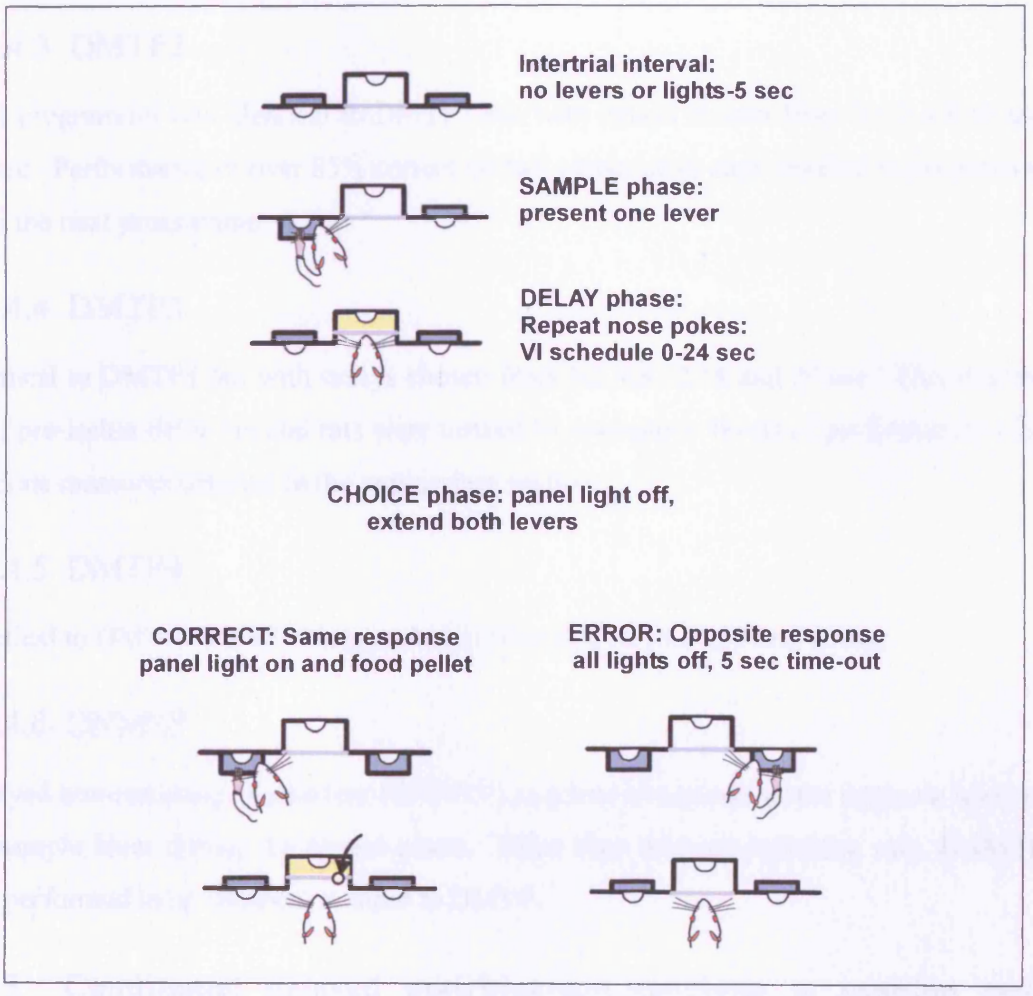


Figure 2.2 Schematic diagram of one trial of DMTP, variable interval (VI).

2.3.4.2 DMTP1

This programme introduced the delay component to the task; the computer chose the delay at random from a set of 7 possible durations, again such that each delay appeared equally throughout the session. In DMTP1 the delays were 0,1,2,3,4,5 and 6 sec. Performance of over 85% correct on two consecutive days resulted in progression onto the next programme.

2.3.4.3 DMTP2

This programme was identical to DMTP1 but with delays chosen from 0,1,2,4,8,12 and 16 sec. Performance of over 85% correct on two consecutive days resulted in progression onto the next programme.

2.3.4.4 DMTP3

Identical to DMTP1 but with delays chosen from 0,2,4,8,12,18 and 24 sec. This was the final pre-lesion delay set and rats were trained to asymptotic levels of performance, with baseline measures detailed in the appropriate section.

2.3.4.5 DMTP4

Identical to DMTP1 but with delays chosen from 0,6,12,18,24,32 and 40 sec.

2.3.4.6 DNMTP

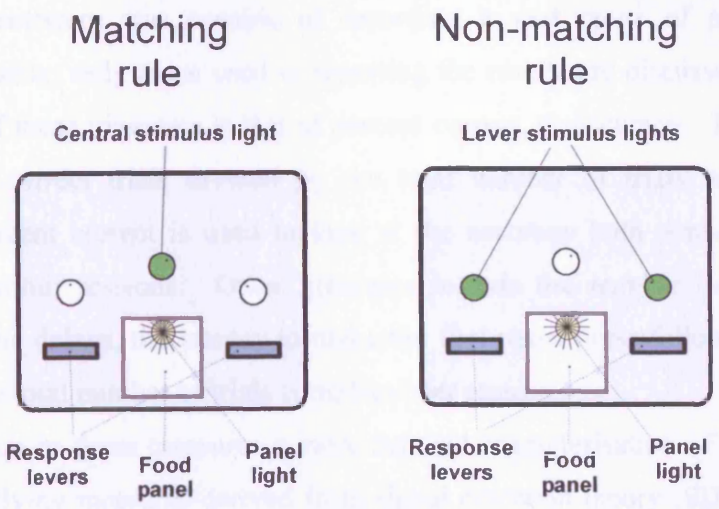
Delayed non-matching to position (DNMTP) required a response to the opposite lever to the sample lever during the choice phase. Other than this non-matching rule, DNMTP was performed in an identical manner to DMTP.

2.3.5 Conditional delayed matching/non-matching to position task (CDM/NMTP)

Habituation and lever press training were exactly as outlined in section 2.3.3. This task combined both the matching and non-matching rules within one session, using distinct visual cues to distinguish which rule was needed to perform the trial correctly. Rats were first trained on DMTP with no delays for 1 hr sessions; the stimulus light above the

central panel was illuminated on presentation of the sample lever, and remained on until a choice response was made (see figure 2.3 A). In addition, the houselight remained off for the duration of the session, and was switched on only to signal a time-out period; this served to increase the likelihood of the rat attending to the stimulus lights, and did not impair acquisition of the task relative to those animals trained with the houselight on. Upon reaching asymptotic performance on this task, rats were switched to the non-matching version of the task. In this version of the task, the stimulus lights above both levers were illuminated throughout the trial until a choice response had been achieved. Once rats reached asymptotic performance on the non-matching version they were then subject to subsequent switches between the rules, with the number of sessions required to reach asymptotic performance on each task becoming progressively fewer, until they required only one session on each. The next stage of training involved integrating the two rules within one session; initially the rats were exposed to one rule for 30 min, followed by the alternate rule for the remaining 30 min. Finally, both rules were presented randomly within the same session. Extensive training was required for the rats to master this combined task, on reaching asymptotic performance at this zero-delay version, delays of 0, 1, 2, 4, 8, 12 and 16 sec were gradually introduced, and session time was reduced to 40 min. The paradigm for the final task is described in figure 2.3 B.

A



B

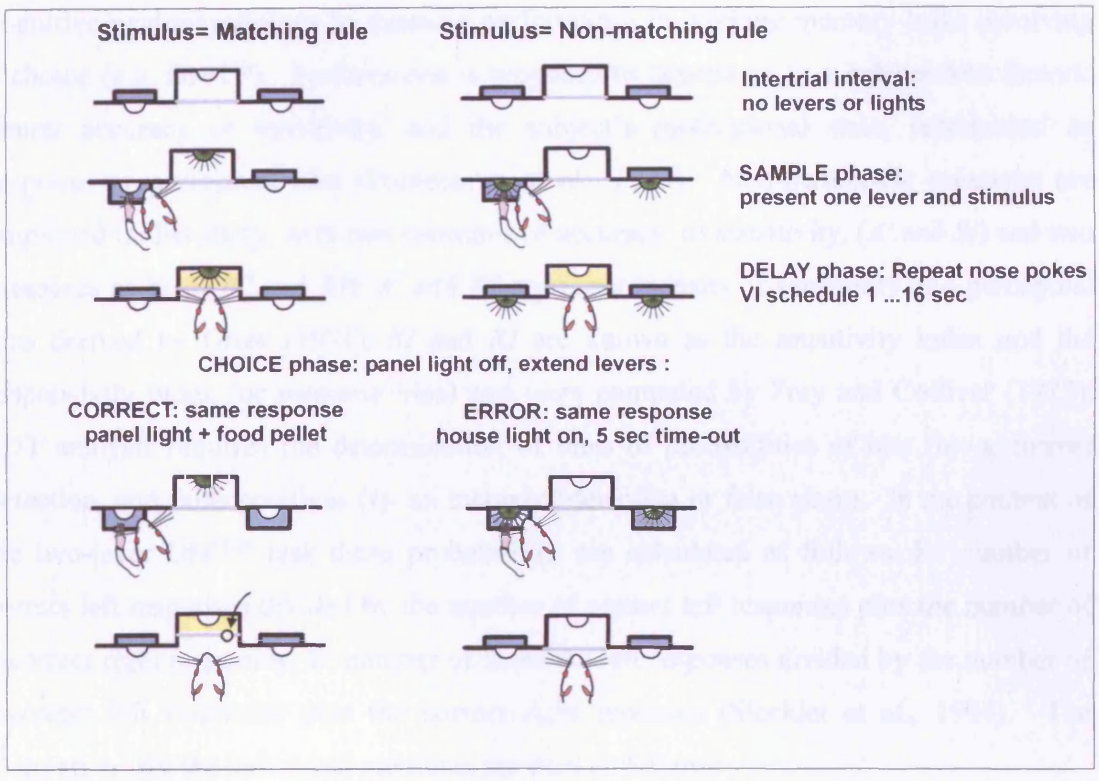


Figure 2.3 Schematics of the CDM/NMTP task. Schematic of the front wall of the Skinner box, indicating the visual cues for each rule (A), these stimulus lights were illuminated from the start of the sample phase until a choice response was made. Schematic diagram of one trial of the CDM/NMTP task (B).

2.3.6 Data collection and analysis

The computer software was capable of recording a vast range of parameters and measures, for clarity, only those used in reporting the results are discussed below. The most intuitive of these measures is that of percent correct, or accuracy. This measure is the number of correct trials divided by the total number of trials, expressed as a percentage. Percent correct is used to look at the accuracy both across sessions and across delays within sessions. Other measures include the number or rate of panel presses during the delays, the latency to make the first panel press following the end of the delay and the total number of trials completed per session.

In addition to these measures a more detailed characterisation of deficits can be achieved by applying measures derived from signal detection theory (SDT) (Marston et al., 1993; Marston, 1996). Briefly, SDT applies procedures derived from human cognitive neuropsychology to examine performance in working memory tasks involving a choice (e.g. DMTP). Performance is proposed to depend on two independent factors: neural accuracy or sensitivity, and the subject's motivational state, represented as response or perceptual bias (Pontecorvo et al., 1996). Non-parametric measures are employed in this study, with two measures of accuracy, or sensitivity, (*A'* and *SI*) and two measures of bias (*B"* and *RI*); *A'* and *B"* represent indexes of sensitivity and perceptual bias derived by Grier (1971), *SI* and *RI* are known as the sensitivity index and the responsivity index (or response bias) and were computed by Frey and Colliver (1973). SDT analysis requires the determination of rates of probabilities of hits (h)- a correct detection, and false positives (f)- an incorrect detection or false alarm. In the context of the two-lever DMTP task these probabilities are calculated as follows: h= number of correct left responses divided by the number of correct left responses plus the number of incorrect right responses, f= number of incorrect left responses divided by the number of incorrect left responses plus the correct right reponses (Steckler et al., 1994). The calculations for the individual measures are then as follows:

$$A' = \frac{0.5 + (h-f)(1+h-f)}{4h\,(1-f)}$$

$$B'' = \frac{h\,(1-h) - f\,(1-f)}{h\,(1-h) + f\,(1-f)}$$

$$SI = \frac{h\text{-}f}{2(h\text{-}f)\text{-}(h+f)^2}$$

$$RI = \frac{h+f\text{-}1}{1\text{-}(f\text{-}h)^2}$$

An additional measure that does not derive from SDT is the "cognitive" bias measure Index Y (I_y) (Sahgal, 1987). I_y contrasts accuracy between the two levers and is defined as the absolute value of left minus right lever correct responses, divided by the total number of correct responses. The absolute values of both B'' and RI will also be used as magnitude rather than side of bias is of interest. Both B'' and RI will be shown averaged across all delays, because the paucity of incorrect responses at the shortest delays can lead to misrepresentative results. This type of analysis has proved useful in numerous papers in which operant matching or non-matching procedures have been employed (Aggleton et al., 1992; Ennaceur et al., 1996; Estapé and Steckler, 2001; Herremans et al., 1996; Pouzet et al., 1999b; Reading and Dunnett, 1991).

2.4 Water maze

2.4.1 Apparatus

Spatial reference memory was assessed in an open-field water maze consisting of a large circular fibreglass tank (diameter: 200 cm, height: 60 cm) containing water at a temperature of ~25°C and a depth of 32 cm. The maze was located in a room that contained various visual extra-maze cues including posters and abstract shapes which remained constant throughout the testing period. The room was illuminated by four floodlights and one light directly above the centre of the tank, illumination was kept constant throughout testing. A stable circular platform (10 cm diameter) was submerged 2 cm below the surface of the water to serve as the escape platform. The platform was hidden from the rat's view by the addition of 500 ml of non-toxic white paint to the water to render it opaque. A separate platform with a 28 cm high black pole screwed into its base served as a visual cue, a circular piece of white card was attached to the top of the pole to prevent the tracking system from visualising it.

Eight points of equal spacing along the circumference of the pool were arbitrarily assigned as the cardinal points: North (N), Northeast (NE), East (E), Southeast (SE), South (S), Southwest (SW), West (W) and Northwest (NW). These points were used as the starting positions for the trials, at which the rats were carefully lowered into the water facing the wall of the tank. The tank was conceptually divided into four quadrants of equal size (NE, SE, SW, NW) and initially platform locations were in the centre of these quadrants 50 cm from the tank wall (figure 2.4). The most proximal start position to the platform was never used, e.g. start position NW was never used with the platform in the NW quadrant, giving a total of seven different start positions per platform.

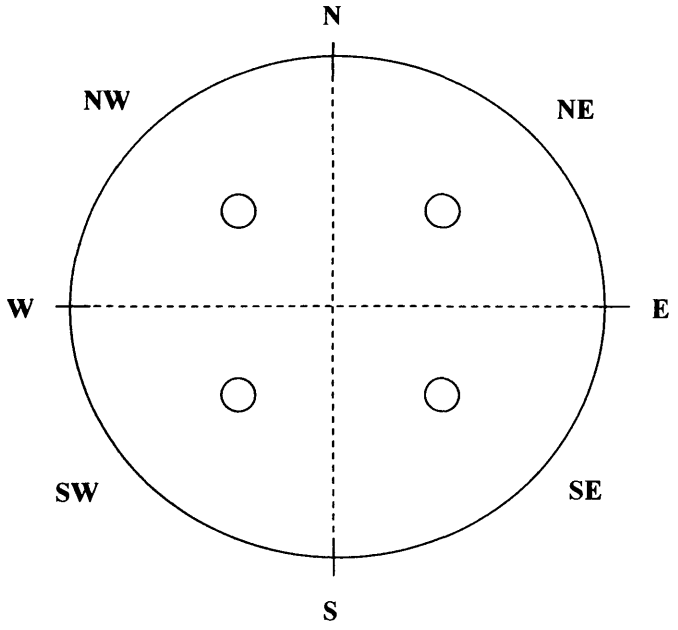


Figure 2.4 Schematic representation of water maze, the four small circles indicate the platform positions.

2.4.2 Software

A video camera was mounted above the centre of the water maze; this camera was connected to an image analysis system (HVS Image, Hampton, UK) which in turn was connected to a PC running the HVS maze software. The swim path of the animal was tracked and stored for subsequent behavioural analysis using the same software. The experimenter observed the rats' swim paths on a monitor located behind a screen; escape

latency was measured manually by operating a remote switch connected to the PC which was also used to signal the start and end of the trial.

2.4.3 Reference memory task

2.4.3.1 Training to platform

Rats were assigned to one of four groups, each trained to a different platform position; groups were counterbalanced such that equal numbers of rats from each lesion group were trained to each quadrant. The order of testing was also counterbalanced so that time of day could not contribute to any potential differences in performance. Rats were tested for 6 days, receiving 4 consecutive training trials per day with a gap of approximately 15 sec between each trial. Platform position for each rat was kept the same across trials and days but the start position varied pseudorandomly across trials and days. Trials ended either when the rat found the platform, or, if the rat had failed to locate the platform within the maximum time of 120 sec, when they were guided to it. Once on the platform, rats were given 30 sec before commencing the next trial. Following their final trial, rats were hand dried with a towel and placed in a heated recovery cage before being returned to their home cages.

2.4.3.2 Probe Trial

Twenty-four hours after the final training trial, rats were exposed to a probe trial that involved the rats being placed into the tank for 60 sec in the absence of the platform. Start position was always from the quadrant opposite the training quadrant to allow accurate comparison of rats' swim paths.

2.4.3.3 Reversal

Rats were trained for 4 days with the platform position moved to the opposite quadrant to the one that they had been trained to, e.g. initial position NW, moved to SE. All testing was conducted as described previously, with 4 consecutive trials per day with the start position varying across trials and days.

2.4.3.4 Visual cue

The visual cue platform was used for these trials, therefore the rats should have been able to use the clear visual cue of the black pole to locate the platform. This served to assess the rats' levels of motivation to escape and whether motor and sensory skills were intact. Rats received 4 consecutive trials with the platform position and start position being moved pseudorandomly between trials, this order was the same for all rats. Platform positions were chosen from a total of eight positions that were 25 or 75 cm from the edge of the tank, thus no rat had to swim to the exact position it had originally been trained to.

2.4.4 Data collection

The main parameters that were collected during training sessions were the latency to find the platform and the distance travelled before locating the platform. These data were averaged across the 4 trials of each day's training. In the probe trial the percentage of time and the percentage of the path length in the training quadrant were collected. Swim paths were also collected to give representative patterns for each lesion group.

2.5 Spontaneous locomotor activity

2.5.1 Apparatus and assessment

Spontaneous locomotor activity was assessed in a set of eight wire cages (36 cm long x 22 cm high x 24 cm wide) in which two horizontal photocell beams transected the long axis of each cage (approximately 12 cm from both ends and 2 cm above the floor). Activity data were collected by computer (Acorn A500) using specialized software (Arachnid activity monitor: Paul Fray Ltd., Cambridge, UK). Rats were placed individually into the cages for session durations of either 60 or 120 min at the same time of day as Skinner box testing took place. Rats were always on food restriction when activity testing took place. The total number of beam breaks were recorded across the session in 5 or 10-min blocks.

2.6 Object recognition: spontaneous novelty preference

2.6.1 Apparatus

The apparatus consisted of an open-field arena (1 m^2 base, 60 cm high) made of wood painted matt grey. The floor of the arena was covered with sawdust which was moved around between trials to prevent odours from building up in any particular location. The arena was situated in a well lit room with various posters and shapes on the walls to serve as cues. A video camera was mounted directly above the arena to enable the rats' behaviour to be analysed and recorded. The objects to be discriminated were made of glass, plastic, metal or ceramic (figure 2.5) and, if needed, were filled with sand to prevent displacement by the rats. Objects were available in triplicate copies to prevent scent-marking between familiarisation and test phases, all objects were cleaned thoroughly with alcohol between trials. Figure 2.6 shows the positions of the objects within the arenas for each test.

Figure 2.5 Representative objects used in the spontaneous novelty preference test.

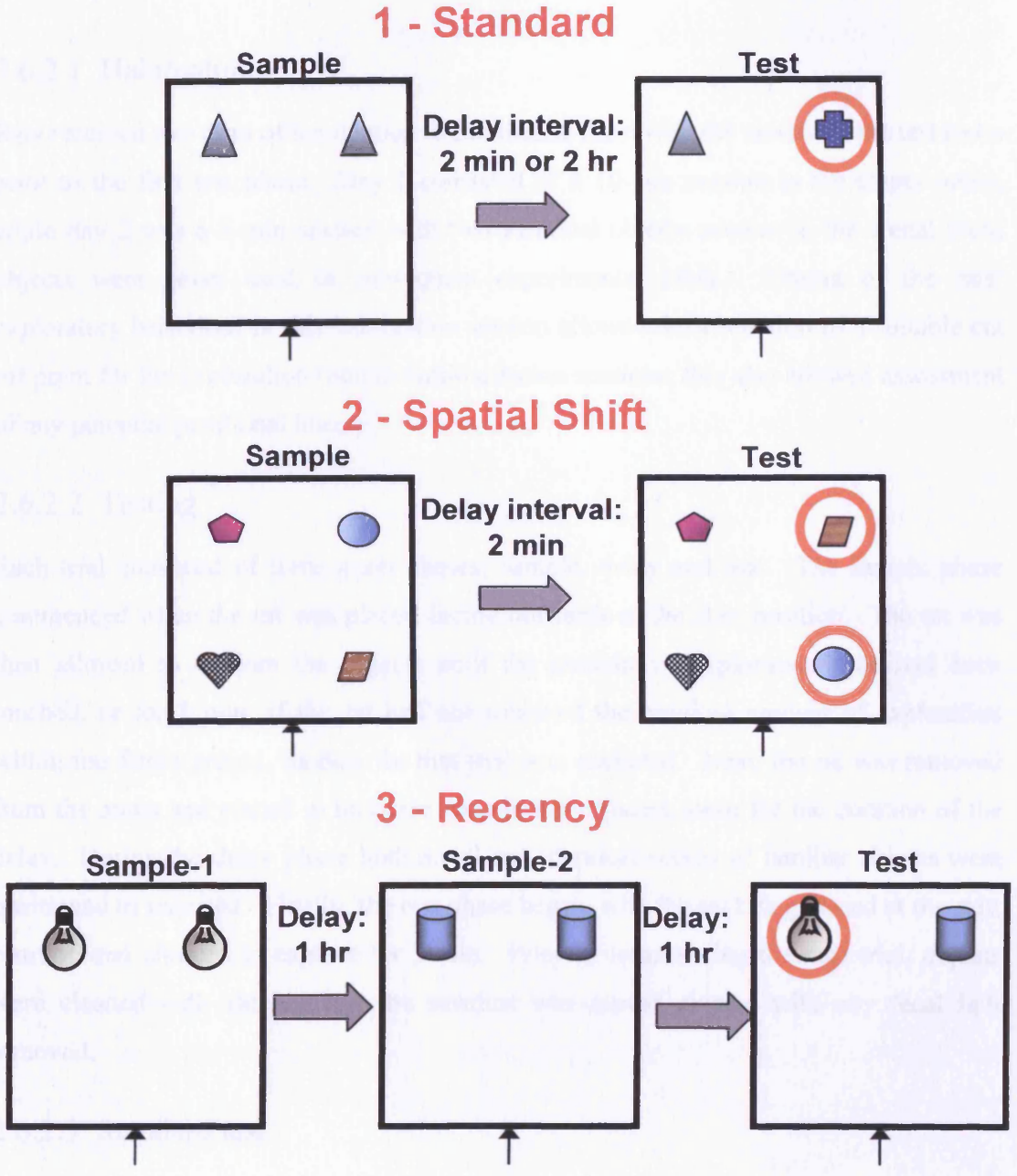


Figure 2.6 Schematic diagrams of the three spontaneous novelty preference tasks. "Novel" objects indicated by orange circle, arrow indicates start position of rat. The standard object positions were in two adjacent corners (27 cm from the opposing corners). For the spatial shift trials two additional positions (also 27 cm from opposing corners) were used.

2.6.2 Behavioural procedures

2.6.2.1 Habituation

Rats received two days of habituation to familiarise them with the arena and the test room prior to the first test phase. Day 1 consisted of a 10-min session in the empty arena, while day 2 was a 5-min session with two identical objects present in the arena; these objects were never used in subsequent experimental trials. Timing of the rats' exploratory behaviour in this habituation session allowed determination of a suitable cut off point for the exploration time in familiarisation sessions; this also allowed assessment of any potential positional bias.

2.6.2.2 Testing

Each trial consisted of three main phases; sample, delay and test. The sample phase commenced when the rat was placed facing outwards at the start position. The rat was then allowed to explore the objects until the cumulative exploration time had been reached, or for 5 min; if the rat had not achieved the required amount of exploration within the 5-min period, its data for that trial was excluded. Next, the rat was removed from the arena and placed in its home cage in the adjacent room for the duration of the delay. During the delay phase both novel and identical copies of familiar objects were positioned as required. Finally, the test phase began, with the rat being placed at the start position and allowed to explore for 3 min. Prior to commencing the next trial, objects were cleaned with alcohol and the sawdust was moved around with any fecal boli removed.

2.6.2.3 Standard test

The standard test is shown schematically in figure 2.6. Two identical objects were explored in the sample phase, followed by a delay of either 2 min or 2 hr. In the test phase an identical copy of one of these "familiar" objects and one totally novel object were positioned in the arena and the rats' exploration was recorded.

2.6.2.4 Spatial shift

The spatial shift test is shown schematically in figure 2.6. Four different objects were explored in the sample phase, followed by a delay of 2 min. In the test phase identical copies of all four objects were placed in the arena, but the locations of two of the objects were switched; the rats' exploration of the four objects was recorded. In this test, the objects that had been displaced were now the "novel" objects and the objects that had remained in their original positions constituted the "familiar" objects.

2.6.2.5 Recency

The recency test is shown schematically in figure 2.6; this test differed from the other two tests in that it involved two separate sample phases. In sample phase 1, two identical objects were explored, followed by a 1-hr delay. Sample phase 2 allowed the rats to explore two more identical objects different from those presented in sample phase 1. Following the final delay phase of 1 hr, the rat was presented with one copy of each of the objects from the two sample phases and exploration was recorded. In this test the object explored in sample 1 was the "novel" object as it was encountered least recently; the object from sample 2 was the "familiar" object, as it was relatively more familiar than the sample 1 object.

2.6.3 Experimental design

Each rat received three trials of each test, although the order of tests was not randomised, object and side of novel object were fully counterbalanced across and between groups. Objects were presented to each rat for one trial only, i.e. no object was reused at any point for any given rat. The testing schedule was governed by the availability of the Ethovision® system, with no rat receiving trials on consecutive days. Blocks of testing ranged from 6 to 8 days, with rats always receiving a 5-min habituation session before each new block commenced. Prior to the 1st spatial shift trial rats were subject to a 5-min habituation session with four objects present in the arena; this allowed the determination of a suitable cut-off point for this task.

2.6.4 Scoring and analyses

All phases of the trial were analysed "on-line" using Ethovision® (Noldus) software; trials were also videoed to provide a back-up. Keyboard buttons were assigned to the objects to allow exploratory behaviour to be recorded manually; this was defined as when the rat was directly attending to the object at a distance of no more than 2 cm and did not include using the object as a platform, sitting on the object or touching the object with the body but heading in another direction. In addition to this manual score, the software recorded time spent in a defined zone around the objects (2 cm from perimeter of object). A wealth of behavioural measures could be analysed following data collection, for clarity, only the exploration time of the objects and a discrimination ratio are presented. The calculation for the discrimination ratio is shown below:

$$\frac{\text{(time exploring novel object- time exploring familiar object)}}{\text{total exploration time}}$$

Therefore the ratio ranged from -1 (if the rat explored only the familiar object) to +1 (if the rat explored only the novel object), with a score of 0 indicating no discrimination between the two objects.

2.7 Statistical analysis

Data were manipulated using Microsoft Excel 2003, plotted using SigmaPlot 8.0 (SPSS, Erkrath, Germany) and analysed using the Statistica package (STATISTICA for Windows (version 5.0), Statsoft, Inc., Tulsa, USA). Statistical analyses were carried out using repeated-measures analyses of variance (ANOVA) with lesion group as the between-subject factor and sessions, delays etc. treated as the within-subject factors as appropriate, unless stated otherwise. Newman-Keuls' *post hoc* test was used to determine the locus of significant main effects and any interactions. A significance level of $p<0.05$ was set for all statistical analyses, with p values greater than 0.05 reported as not significant (ns), unless particularly noteworthy.

Chapter 3 Surgical and histological methods

3.1 General

3.1.1 Excitotoxins

Excitotoxins are glutametergic compounds that are capable of producing neuronal degeneration (Meldrum, 1990). Their mechanism of action is exclusively post synaptic, with excessive activation of N-methyl-D-aspartate (NMDA)-type glutamate receptors leading to increased intracellular calcium concentrations and the subsequent cascade of enzyme activation that ultimately results in cell death (Lynch and Guttmann, 2002; Meldrum, 1990). Although NMDA itself was piloted, ibotenic acid (IBO) was used for all experimental excitotoxic lesions.

IBO is a glutamate analogue derived from the poisonous fungus *Amanita muscarina* (Michelot and Melendez-Howell, 2003); its neurotoxic properties were first described by Johnston et al (1968). When injected intracerebrally into rats, IBO produces a marked degeneration of nerve cells, with axons of passage and nerve terminals remaining undamaged (Schwarcz et al., 1979). Advantages to using this toxin, as opposed to other similar toxins (e.g. kainic acid), are its lower toxicity to the animal, and its ability to produce more discrete lesions (Schwarcz et al., 1979). Decarboxylation of IBO gives rise to muscimol which is a widely used gamma-aminobutyric acid (GABA)-analogue (Johnston et al., 1968). It is this metabolism to muscimol that is thought to account for the anaesthesia-potentiating effects sometimes observed after intracerebral injection of IBO (Schwarcz et al., 1979).

Jarrard (1989) described an elegant approach for lesioning the HPC of the rat using multiple stereotactically guided injections of IBO. These lesions are more selective than those made with conventional lesion techniques such as aspiration, electrolytic or thermocoagulation (Jarrard, 1989). Determination of the exact concentrations and volumes of IBO is crucial to prevent spread to adjacent structures (Jarrard, 2002). In addition to lesioning the HPC, IBO has been successfully used in lesioning the PFC

(Delatour and Gisquet-Verrier, 1996; Sullivan and Gratton, 2002), the striatum (Isacson et al., 1984) and components of the hippocampal system such as the ERC (Eijkenboom et al., 2000; Hagan et al., 1992).

3.1.2 Surgical techniques

All surgical procedures were conducted under Isoflurane (Abbott, UK) anaesthesia, with either Oxygen and Nitrous Oxide, or Oxygen alone as the carrier gas. Rats were secured in a stereotaxic frame (Kopf instruments) using atraumatic ear-bars. Stereotaxic coordinates are given with dorsal-ventral (DV) relative to dura and anterior-posterior (AP) and medial-lateral (ML) measured relative to bregma, except in those prefrontal lesions mentioned where the ML coordinate is taken as the midline of the exposed sagittal sinus. All lesions were induced by injection of IBO (Biosearch Technologies, Inc., CA, USA) dissolved in 0.1 M phosphate buffered saline (PBS) and adjusted to pH 7.4 with NaOH. Following surgery, wounds were cleaned and sutured and covered with topical antibiotic powder (Aureomycin). Rats received 5 ml of glucose-saline subcutaneously to reduce any postoperative dehydration and recovered in a heated cage. All animals received soluble paracetamol in their home cages for at least two days post surgery.

3.2 Pilot Surgery

3.2.1 Parameter manipulation

Prior to and throughout these experiments numerous pilot surgeries were performed to minimise problems with variability in lesion extent and size. These pilots involved systematic manipulation of the many parameters involved in the surgery to try to optimise lesion reproducibility; these manipulations are summarised in table 3.1.

Table 3.1 Parameters manipulated during pilot surgeries.

Parameter	Modifications
Toxin	Both HPC and PFC lesions were piloted using NMDA and IBO.
Concentration and volume of toxin	Concentrations of toxin were adjusted for PFC lesions, as were volumes of toxin injected at each site.
Anaesthetic and post-surgery care	Surgeries were conducted with and without nitrous oxide delivery and post-surgery diazepam injections.
Delivery Method	A variety of injection methods were tested, these comprised: (1) a 30-guage stainless steel cannula connected by polyethylene tubing to a 10 µl Hamilton glass syringe mounted on a Harvard microdrive pump, (2) a 25-guage bevelled stainless steel needle on a 2 µl Hamilton syringe mounted directly on the frame with a manual microdriver system (Kopf), (3) a 33-guage blunt ended stainless steel needle on a 5 µl Hamilton syringe mounted directly on the frame with a digital microdriver system (kd Scientific, PA, USA), and (4) a 26-guage bevelled stainless steel needle on a 2 µl Hamilton syringe mounted directly on the frame with a digital microdriver system.
Bone flap vs. drill holes	Bone flap removal was compared with discrete drill holes at every injection site for both HPC and PFC lesions.
Coordinates	Number and position of injection sites were varied according to both lesion results and fluorescent bead injection pilots.

3.2.2 Fluorescent bead injections

Fluorescent latex microspheres or "beads" (Lumafluor Corp., FL, USA) were injected to establish exact location of injection sites. Beads were injected using a 26-guage bevelled stainless steel needle on a 2 µl Hamilton syringe mounted directly on the frame. A digital microdriver system (kd Scientific, PA, USA) was used to inject the beads at the same rate as the corresponding lesion (i.e. 0.03 µl/min for entorhinal and hippocampal placement, 0.25 µl/min for prefrontal placement). The bead solution was injected neat without any dilution; 0.1 µl was injected at each site, with a 2-min diffusion time. Rats were perfused as normal with 4% Paraformaldehdye (PFA), brains were left in PFA overnight before being transferred to 25% sucrose until they sunk. 60 µm-sections were taken on a microtome and every 6^{th} section was mounted, dehydrated in ascending alcohols and coverslipped with DPX. Another 1:6 series of sections was stained with Cresyl Violet before coverslipping. Representative photomicrographs are shown in figure 3.1.

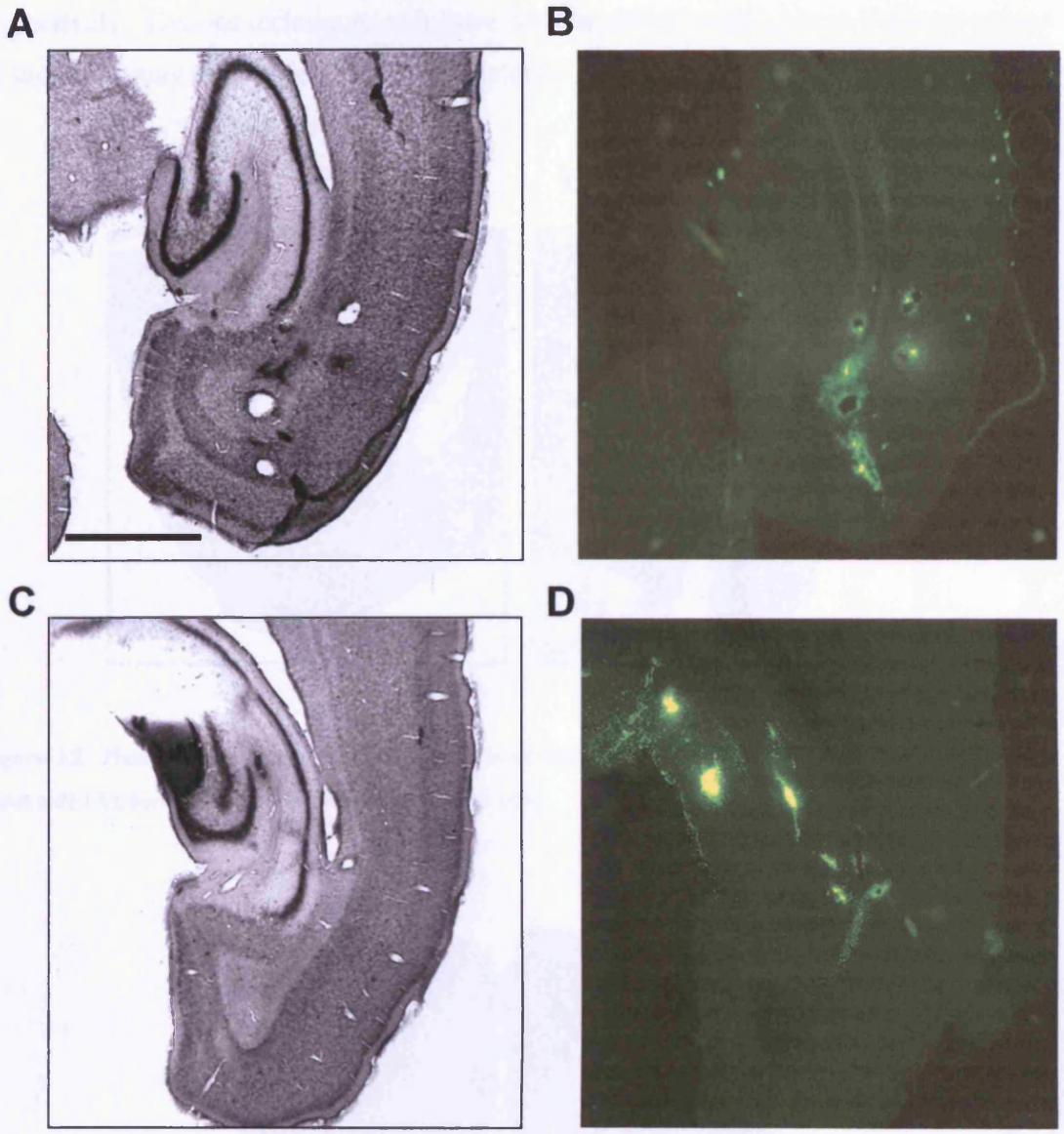


Figure 3.1 Representative photomicrographs of horizontal sections of pilot fluorescent bead injections, note coordinates will have been modified based on these results. Cresyl Violet stained ERC pilot (A), ERC pilot section, adjacent to 'A', with no staining viewed under fluorescence (B), Cresyl Violet stained HPC pilot (C), HPC pilot section, adjacent to 'C', with no staining viewed under fluorescence (D). Scale bar in A =1 mm.

3.2.3 Pilot results

Figures 3.2, 3.3 and 3.4 show representative pilot lesions of ERC, PFC and HPC lesions respectively. Lesions techniques may have been modified resulting from these pilots and as such they may not be identical to experimental lesions.

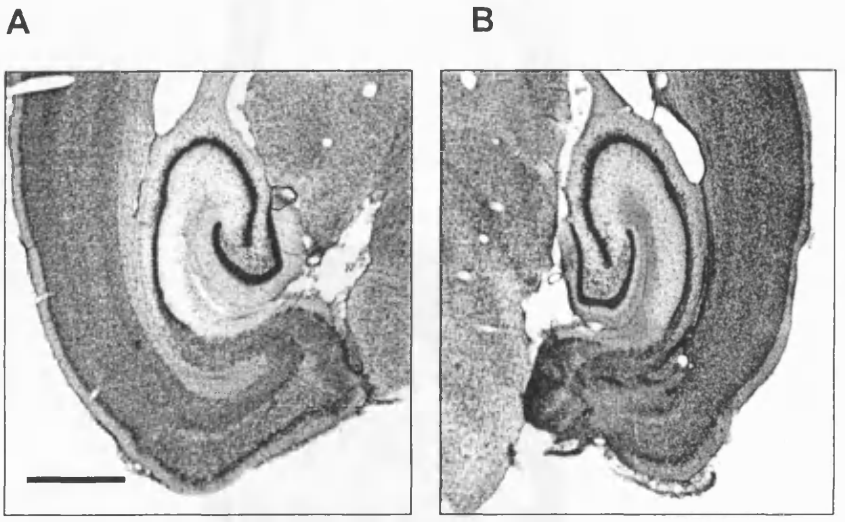


Figure 3.2 Photomicrographs of Cresyl Violet stained horizontal sections through a unilateral ERC pilot, intact side (A), lesioned side (B). Scale bar in A =1 mm.

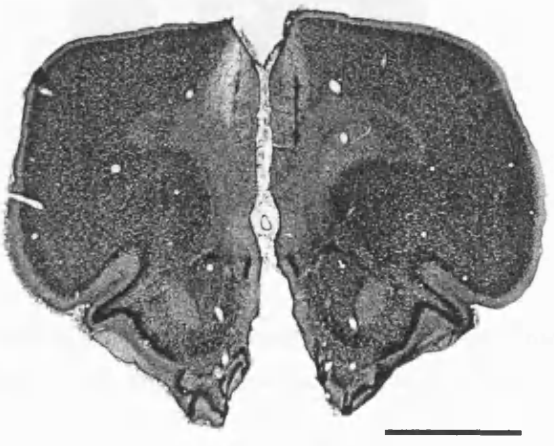


Figure 3.3 Photomicrograph of a Cresyl Violet stained coronal section through a bilateral PFC pilot. Scale bar =2 mm.

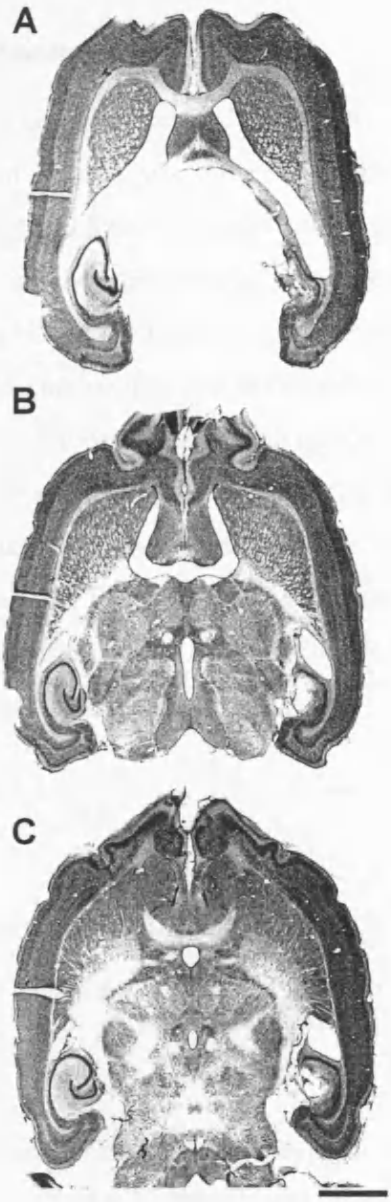


Figure 3.4 Photomicrographs of Cresyl Violet stained horizontal sections through a unilateral HPC pilot; with A the most dorsal and C the most ventral section. Scale bar in C =2 mm.

3.3 Experimental Surgery

3.3.1 Bilateral PFC lesions

Bilateral IBO (0.05 M) lesions were centred on the PrL and IL areas of the PFC with injection coordinates shown in table 3.2. All PFC lesions were performed using nitrous oxide (0.4 L/min) and oxygen (0.8 L/min) as the carrier gases. Injections were made via a 30-guage stainless steel cannula connected by polyethylene tubing to a 10 µl Hamilton glass syringe mounted on a Harvard microdrive pump; rate of injection was maintained at 0.25 µl/min. Rats used in Experiment 1 had discrete drill holes at each of the four sites, rats in Experiments 2, 4 and 6 received bone flap removals. For bone flap removals the skull was exposed, and a small piece of bone overlying the site was carefully removed using a hand-held mechanical drill (Foredom), the dura was incised with a syringe tip at each site. Removal of the bone flap exposed the sagittal sinus which was taken as the midline coordinate, because pilot lesions had shown this to be a more accurate measure than taking the midline from bregma.

AP	ML	DV	Vol (µl)
+2.2	±0.6	-2.5	0.2
+2.2	±0.6	-4.3	0.2
+3.7	±0.6	-3.0	0.3

Table 3.2 Stereotaxic coordinates for bilateral cytotoxic lesion of the PFC using IBO (0.05 M) with an infusion rate of 0.25 µl/min. Nose bar set to 2.3 mm below the interaural line. Cannula left in situ for 3 min after each injection to allow diffusion.

In experiment 1, sham PFC lesions were performed exactly as the toxin lesions, except PBS was injected instead of IBO. In experiments 2, 4 and 6, sham lesions were performed by removing the bone flap and incising the dura, no needle was lowered into

the brain. The decision to change the sham protocol was taken following the findings discussed in Chapter 4, whereby non-specific damage could not be ruled out.

3.3.2 Bilateral HPC lesions

Bilateral hippocampal lesions were achieved by injection of IBO (0.063 M) at the coordinates shown in table 3.3 (Experiment 1) and table 3.4 (Experiments 2, 4 and 6). All experimental HPC lesions were performed using a bone flap technique; a piece of bone was removed bilaterally from above the injection areas and the dura was then incised with a syringe tip at the individual sites of injection. Both nitrous oxide and oxygen were used as the carrier gases for experiment 1, the surgery for all remaining experiments was performed with oxygen alone. Sham lesions in Experiment 1 were performed exactly as toxin injections but with PBS injected instead of IBO. Sham lesions in Experiments 2, 4 and 6 had bone flap removal and dura incision but no injection or lowering of the needle. Again this decision to modify the sham protocol was taken following the findings discussed in Chapter 4, where two of the HPC sham rats had received non-specific damage resulting in behavioural impairments in the water maze. Due to the length of these surgeries rats required special post operative care, this included hand feeding with porridge and extra glucose-saline injections. Rats were kept in a heated cage until they had recovered fully which often took at least 24 hr.

AP	ML	DV	Vol (µl)
-2.4	±1.0	-3.0	0.05
-3.1	±1.4	-2.1	0.10
-3.1	±1.4	-3.0	0.10
-3.1	±3.0	-2.7	0.10
-3.9	±2.2	-1.8	0.10
-3.9	± 2.2	-3.0	0.10
-3.9	±3.5	-2.7	0.10
-4.7	**±4.0**	-3.5	0.05
-4.7	±4.0	-7.2	0.10
-4.7	±5.0	-6.6	0.05
-4.7	±5.0	-3.7	0.05
-5.4	±4.2	-3.1	0.10
-5.4	±5.0	-4.5	0.09
-5.4	±5.0	-5.3	0.08
-5.4	±5.0	-6.1	0.08

Table 3.3 Experiment 1: Stereotaxic coordinates for bilateral cytotoxic lesions of the HPC using IBO (0.063 M). The nose bar was adjusted so that the head was level between bregma and lambda. Injection was via a 25-guage bevelled stainless steel needle on a 2 µl Hamilton syringe mounted directly on the frame with a manual microdriver system (Kopf). The needle was left in situ for 2 min after each injection to allow diffusion. Height of dura was taken from the highlighted coordinate. Coordinates adapted from those received by personal communication from Dr. Mark Good (Cardiff University, Psychology Department).

AP	ML	DV	Vol (µl)
-2.4	±1.0	-3.0	0.05
-3.1	±1.4	-2.1	0.10
-3.1	±1.4	-3.0	0.10
-3.1	±3.0	-2.7	0.10
-3.9	±2.2	-1.8	0.10
-3.9	± 2.2	-3.0	0.10
-3.9	±3.5	-2.7	0.10
-4.7	**±4.0**	-3.5	0.05
-4.7	±4.0	-7.2	0.10
-4.7	±4.5	-6.5	0.05
-5.4	±4.2	-3.1	0.10
-5.4	±5.0	-4.5	0.09
-5.4	±5.0	-5.3	0.08
-5.4	±5.0	-6.1	0.08

Table 3.4 Experiments 2, 4 and 6: stereotaxic coordinates for bilateral cytotoxic lesions of the HPC using IBO (0.063 M). The nose bar was adjusted so that the head was level between bregma and lambda. Injection was via a 26-guage bevelled stainless steel needle on a 2µl Hamilton syringe mounted directly on the frame with a digital microdriver system, injection rate of 0.03 µl/min. Needle was left in situ for 2 min after each injection to allow diffusion. Height of dura was taken from the coordinate in bold. Coordinates received by personal communication from Dr. Mark Good (Cardiff University, Psychology Department).

3.3.3 Bilateral ERC lesions

Rats used in experiments 3 and 5 received bilateral IBO (0.063 M) lesions of the ERC using the coordinates in table 3.5. Bilateral bone flaps overlying the injection sites were removed as detailed in section 3.3.2, but for these lesions this involved removing a large

portion of the lateral ridge of the skull; a new DV reading was taken at each injection site. Sham ERC lesions received bone flap removal and piercing of the dura, but no injection or lowering of the needle.

AP	ML	DV	Vol (µl)
-6.5	±5.8	-6.4	0.08
-7.0	± 4.5	-6.1	0.08
-7.0	±5.8	-5.3	0.08
-7.5	±4.6	-5.3	0.1
-8.0	±4.7	-4.8	0.1
-8.5	±4.8	-3.1	0.1

Table 3.5 Experiments 3 and 5: stereotaxic coordinates for bilateral cytotoxic lesions of the ERC using IBO (0.063 M). The nose bar was set to 3.3 mm below the interaural line. Toxin injection was via a 26-guage bevelled stainless steel needle on a 2 µl Hamilton syringe mounted directly on the frame with a digital microdriver system, injection rate of 0.03 µl/min. Needle was left in situ for 2 min after each injection to allow diffusion.

3.3.4 Bilateral Fx transections

Rats in experiments 3 and 5 received bilateral aspirative lesions of the Fx. Lesions were made under visual guidance through an operating microscope. A small bone flap was removed just posterior and lateral to bregma on either side of the midline. An 18-guage stainless steel aspiration cannula was attached via polyethylene tubing to an aspirator (Aesculap, Portable 80; Germany) and was used to aspirate a small hole bilaterally through the cortex and corpus callosum to reveal the fibres of the Fx which was transected by suction. Upon completion of aspiration of the Fx each hole was packed with gelfoam (Spongostan® standard, Johnson & Johnson Medical Limited, UK) and closed. Sham animals received aspiration of the overlying cortex alone followed by packing with gelfoam.

3.4 Histology

3.4.1 Perfusion and preparation of tissue

Rats under deep Euthatal (Rhône Merieux, Essex, UK) anaesthesia were transcardially perfused with prewash buffer (pH 7.3) followed by 4% PFA (pH 7.3). The brains were removed and postfixed overnight in PFA and then transferred to 25% sucrose until they sank. Brains were sectioned on a freezing sledge microtome (Bright) at 60-μm thickness. Adjacent 1:6 series were mounted on slides prior to staining, following which they were dehydrated in ascending alcohols to xylene and then coverslipped using DPX mountant. For staining protocols and fixative preparation see Appendix A.

3.4.2 Staining methods

3.4.2.1 Cresyl Violet

The Cresyl Violet stain is the most commonly used standard staining method; the stain labels ribosomes, referred to as Nissl bodies. It is a non-specific marker, staining cell bodies independent of their phenotype. In the central nervous system it is used to mark both neurons and glial cells. Staining was carried out using a conventional Cresyl Violet protocol given in full in Appendix B.

3.4.2.2 Acetylcholinesterase

Acetylcholinesterase (AChE) is the enzyme responsible for hydrolysing acetylcholine upon its release into the synaptic cleft. The enzyme is localised in the presynaptic terminal and in the cell body. Staining of AChE is therefore used as a marker of cholinergic neurons and terminals within the CNS. The staining protocol is given in Appendix C.

3.4.3 Methods of assessment of lesions

Lesions were assessed using either a Leica DMRBE microscope or a Wild Makroskop. Lesion extent was then mapped onto serial sections taken from the atlas of Paxinos and Watson (1998).

Chapter 4 Retention of delayed matching to position: effect of hippocampal and prefrontal lesions

Experiment 1

4.1 Introduction

HPC damage has been shown to have varied effects on performance in delayed matching to position tasks (DMTP) in the Skinner box, however, the majority of studies conclude that damage results in delay-dependent impairments in this task (Aggleton et al., 1992; Broersen, 2000; Dunnett et al., 1990; Hampson et al., 1999). Conversely, the water maze provides much more conclusive results after HPC lesions, with lesions routinely causing severe and robust impairments in spatial learning (Bannerman et al., 1999; Duva et al., 1997; Good and Honey, 1997; Gould et al., 2002; Liu and Bilkey, 2001; Morris et al., 1982; Morris et al., 1990; Richmond et al., 1999; Wright et al., 2004).

PFC lesions have primarily been shown to produce delay-independent deficits in delayed matching tasks (Broersen, 2000; Chudasama and Muir, 1997; Dunnett et al., 1990; Herremans et al., 1996; Joel et al., 1997b; Mair et al., 1998), indicative of a non-mnemonic effect, such as attentional or motivational problems. In the water maze, PFC lesions are shown to have little if any effect on performance (de Bruin et al., 1994; Lacroix et al., 2002; Mogensen et al., 2004; Sullivan and Gratton, 2002), although the area may be implicated if complexity of the task is increased sufficiently (de Bruin et al., 1997; Granon and Poucet, 1995; Lacroix et al., 2002).

There have been few direct comparisons of the effects of these lesions within the same experimental group, on either the Skinner box task or the water maze. Therefore the present experiment aims to provide such a direct comparison, with excitotoxic lesions of HPC or PFC being assessed in DMTP in the Skinner box, and on a reference memory task in the water maze. Rats were trained preoperatively on DMTP with postoperative

retention of the task being assessed; water maze performance and general locomotor activity were assessed following surgery but prior to retesting on DMTP. Following testing for retention of DMTP rats were exposed to a switch in the task rule (from matching to non-matching); this was to serve as an analogue of switching between decision rules in the human WCST, in which human patients with PFC damage are impaired (Berman and Weinberger, 1990; Goldberg et al., 1987; Stuss et al., 2000; Tsuchiya et al., 2000). Experiments have provided support for the hypothesis that the PFC is also engaged in rule switching in the rat (Dias and Aggleton, 2000; Joel et al., 1997b). It is therefore postulated that there may be a dissociation in performance on the DMTP task with the two lesions, and furthermore a difference between the way that the lesions affect the rats' ability to switch between the rules. The water maze is expected to reveal a clear HPC deficit, with PFC performance remaining intact, although reversal of the platform position could provide a deficit unique to the PFC group.

4.2 Materials and methods

4.2.1 Subjects

In this experiment a group of 32 rats were used. In the course of training 2 rats were sacrificed due to repeated seizures and 1 rat, which was unable to learn the task, was used for a pilot lesion, resulting in a final group size of 29. All other subject details are covered in section 2.2.

4.2.2 Behavioural testing

Rats were trained on 40-min sessions of DMTP in the Skinner box as detailed in section 2.3. Upon reaching asymptotic performance at the final delay set (0-24 sec), rats were tested for two 5-session blocks to provide a baseline. Following baseline testing rats were assigned to one of four surgical treatment groups using a random matching procedure based on accuracy. Treatments were HPC lesions (n=10), sham HPC (n=4), PFC lesions (n=10) and sham PFC (n=5), with all surgical details in section 3.3. Rats were given two weeks to recover from surgery before they were tested in the water maze where they received the testing schedule outlined in table 4.1, all details in section 2.4.

Days	Water maze procedure
D1-6	Place learning-1
D7	Probe-1
	Two week break
D1-4	Place learning-2
D5	Probe-2
D6-9	Reversal of platform position
D10	Visual Cue

Table 4.1 Water maze testing procedure

In the first place learning and probe tests two animals from the sham HPC group were shown to be clear outliers in their performance; a two week break before retesting with the same platform positions was given to see if this behaviour was due to surgery-related complications that might be overcome. However, these two rats remained outlying performers; both consistently failed to locate the platform within the 120 sec maximum trial time and were thus excluded from all analyses.

After water maze testing rats had their locomotor activity assessed over a 2-hr period. Rats were then tested for postoperative performance in the Skinner boxes as outlined in table 4.2.

5-session block	Skinner box task
1	DMTP3
2	DMTP3
3	DMTP4
4	DNMTP3 (Non-matching)
5	DNMTP3
6	DNMTP3

Table 4.2 Postoperative Skinner box testing procedure, dashed line indicates rule switch from matching to non-matching. D(N)MTP3 indicates delay set of 0-24 sec; DMTP4 indicates delay set of 0-40 sec.

Rats received three blocks of DMTP testing before the rule switch to DNMTP for another three blocks. Upon completion of block six, rats were run on a 30-min DNMTP programme with a fixed 2-sec delay to minimise any deficits. They were run on this programme for a total of 13 days, with the programme modified such that data could be analysed trial by trial. This trial by trial analysis allowed the assessment of whether there

were group differences across the length of the session. Rats were then finally switched to DMTP with a 2-sec delay for 5 days and data were assessed in a trial by trial manner. No group differences were seen at any point in this phase of testing and as such results are not reported. Following completion of Skinner box testing all rats were sacrificed and histology dealt with as detailed in section 3.4.

4.3 Results

4.3.1 Histology

4.3.1.1 Bilateral PFC lesions

Figure 4.1 shows photomicrographs of a representative lesion and figure 4.2 illustrates the minimum and maximum lesion extent. In all cases there was substantial cell loss within the PrL and IL cortices, with complete neuron loss within these regions in most animals. One animal had damage extending to the most rostral MO cortex and two animals showed damage encompassing the Cg1, Cg2 and DP cortices. Out of the ten rats in this surgery group, four did not incur sufficient bilateral damage and were therefore excluded from all analyses. These excluded animals all showed unilateral damage; this prompted the thorough piloting of the bone flap technique to allow the medial-lateral coordinate to be taken from the sagittal sinus, thereby providing more consistency in lesions.

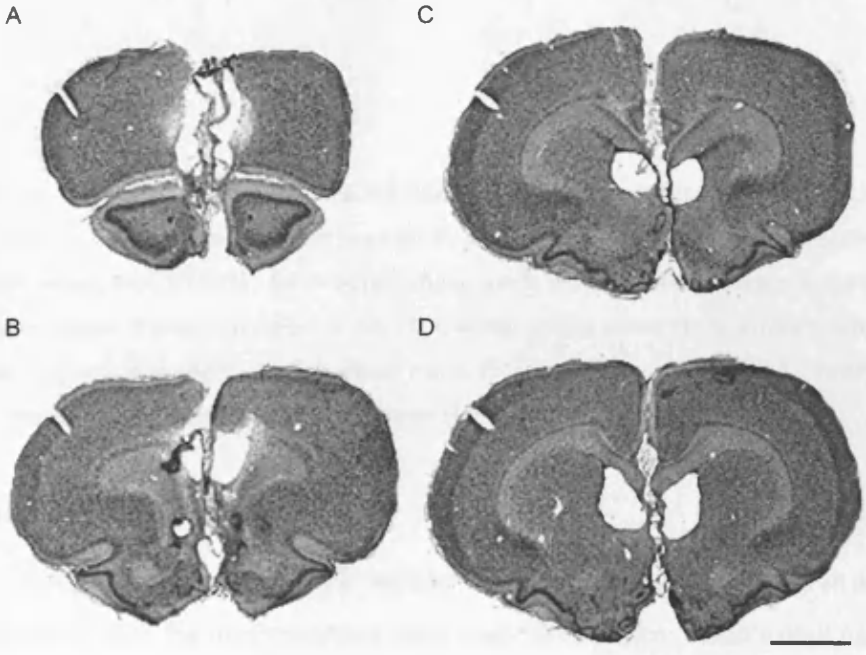


Figure 4.1 Photomicrographs of a representative bilateral PFC lesion, coronal sections stained with Cresyl Violet, with A being the most anterior. Scale bar =2 mm.

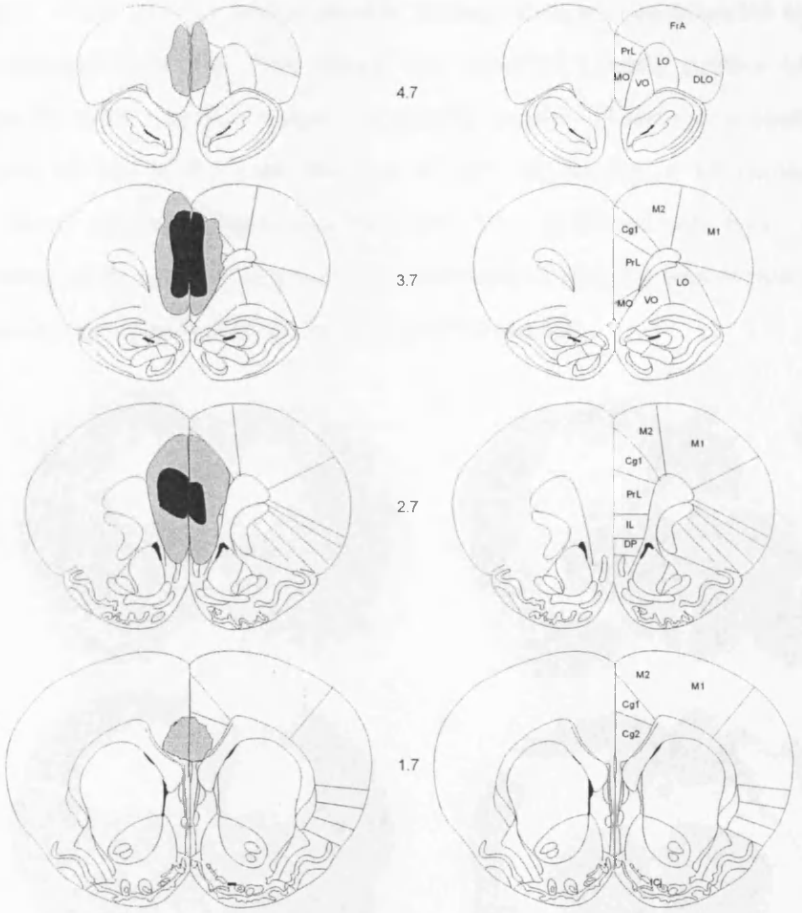


Figure 4.2 Extent of the largest (grey) and smallest (black) PFC lesions, with duplicate sections indicating the specific regions. Numbers indicate mm anterior to bregma, abbreviations: cingulate cortex area 1 (Cg1), cingulate cortex area 2 (Cg2), dorsolateral orbital cortex (DLO), dorsal peduncular cortex (DP), frontal association cortex (FrA), infralimbic cortex (IL), lateral orbital cortex (LO), primary motor cortex (M1), secondary motor cortex (M2), medial orbital cortex (MO), prelimbic cortex (PrL), ventral orbital cortex (VO). Drawings taken from Paxinos and Watson (1998).

4.3.1.2 Bilateral HPC lesions

Figure 4.3 shows photomicrographs of a representative case that was included in analyses and figure 4.4 illustrates the minimum and maximum lesion extent. Successful cases had extensive cell loss throughout the dorso-ventral extent of the hippocampal formation. The case with the most extensive cortical damage showed damage in the trunk region of the primary somatosensory cortex, the parietal association cortex and the medial areas of

the secondary visual cortex, whilst another animal showed considerable sparing of the dorsal hippocampal formation (see figure 4.4, minimal extent); neither of these cases differed from the others in their behavioural performance. Histological analysis revealed a large degree of variability with five out of ten rats having to be excluded from all analyses. Those rats that were excluded had very minimal cell loss, perhaps from injections being made into the ventricle or from toxin loss at the seal between the syringe barrel and the needle, again prompting further pilot studies.

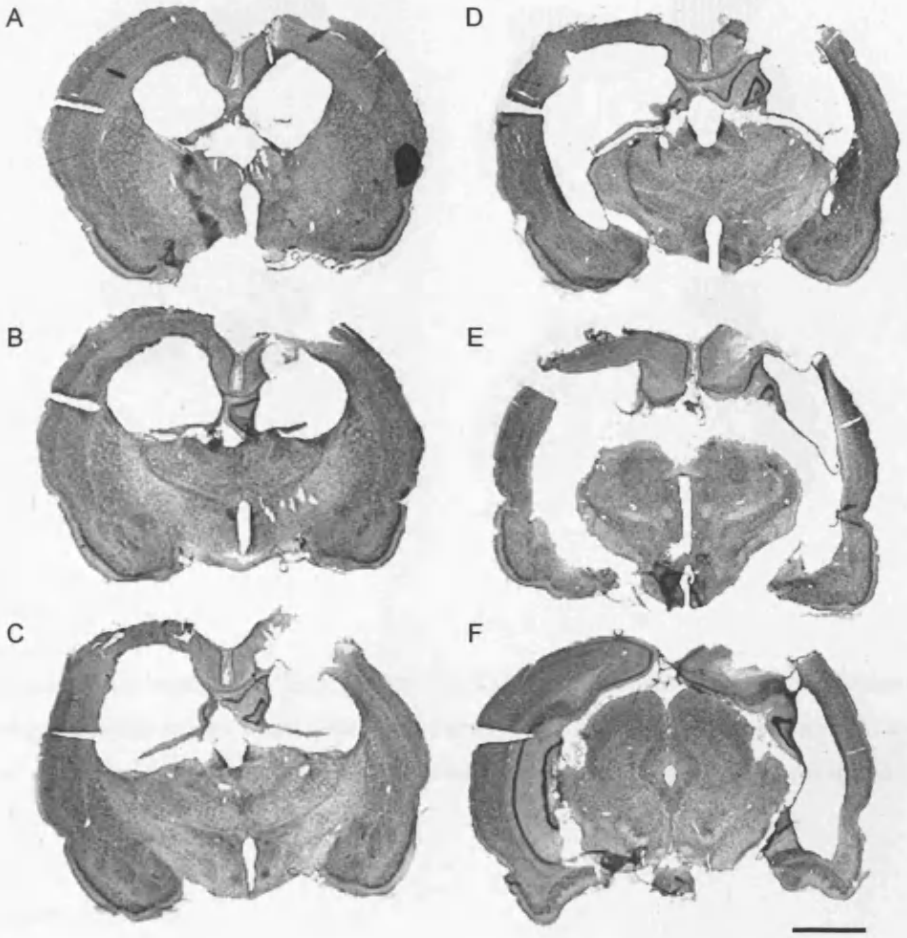


Figure 4.3 Photomicrographs of a representative bilateral HPC lesion, coronal sections stained with Cresyl Violet, with A being the most anterior. Scale bar =2 mm.

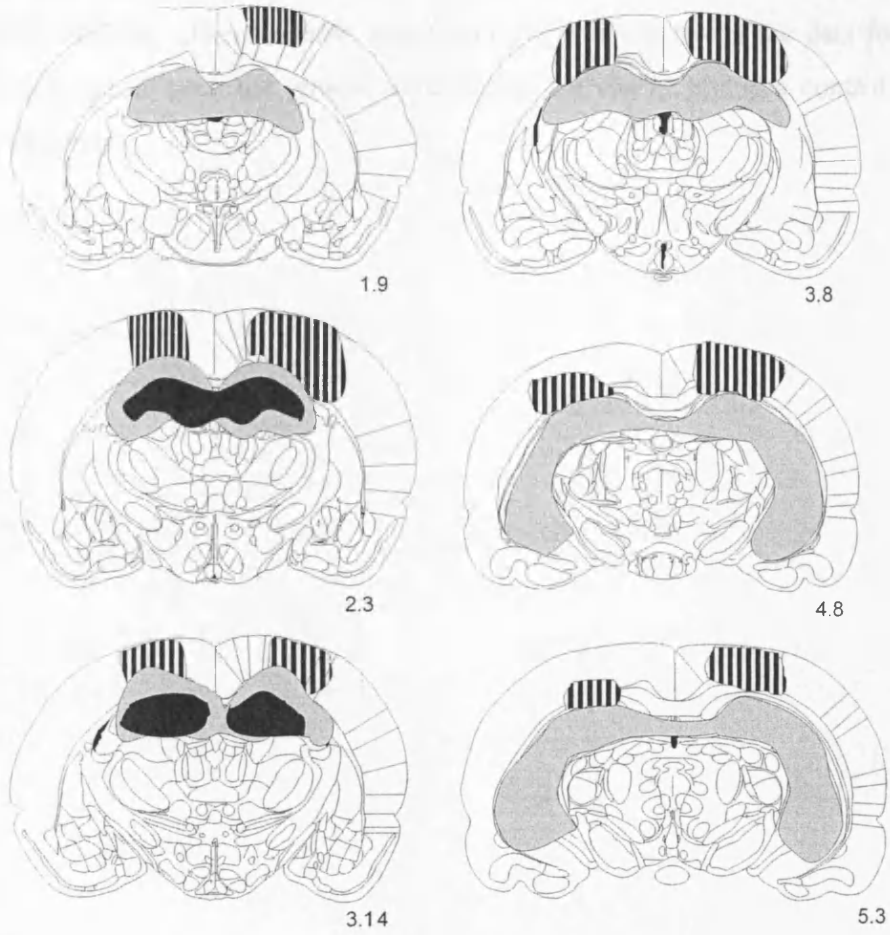


Figure 4.4 Extent of the largest (grey) and smallest (black) HPC lesions, the extent of the greatest cortical damage is indicated by the striped areas, note that the greatest cortical damage did not occur in the animal with the most complete HPC lesion. Numbers indicate mm posterior to bregma. Drawings taken from Paxinos and Watson (1998).

4.3.1.3 Sham lesions

Sham lesion cases were not found to have any cellular loss or any obvious damage as a result of the needle penetration. However, the possibility remains that there may have been some underlying damage that went undetected, which will be considered further within the discussion. HPC shams had a small amount of cortical swelling resulting from the bone flap removal. The two HPC sham rats that were later excluded from all analyses

based on their water maze performance did not show any obvious distinction in their histological analysis. There was no significant difference between the data for the two sham groups and as such the groups were pooled for clarity giving a control group of seven individuals.

4.3.2 Behaviour

4.3.2.1 DMTP

4.3.2.1.1 Baseline

After reaching asymptotic performance, where rats were consistently performing at around 90% accuracy, rats were tested for two additional baseline blocks of 5 sessions to allow allocation into performance-matched groups. As can be seen from figure 4.5, there was no difference between the groups preoperatively in measures of accuracy, total trials performed or rate of panel pressing (accuracy: Group ($F_{(2,15)}$=0.36, ns), total trials: Group ($F_{(2,15)}$=2.76, ns), rate of panel pressing: Group ($F_{(2,15)}$=0.59, ns)).

Data were also analysed across delays (figure 4.6) which showed that performance decreased as delay interval increased and that there was no difference between the groups preoperatively (Block 1: Group ($F_{(2,15)}$=1.07, ns), Delay ($F_{(6,90)}$=45.07, p<0.01), Group x Delay ($F_{(12,90)}$=0.54, ns). Block 2: Group ($F_{(2,15)}$=1.14, ns), Delay ($F_{(6,90)}$=27.94, p<0.01), Group x Delay ($F_{(12,90)}$=0.47, ns)).

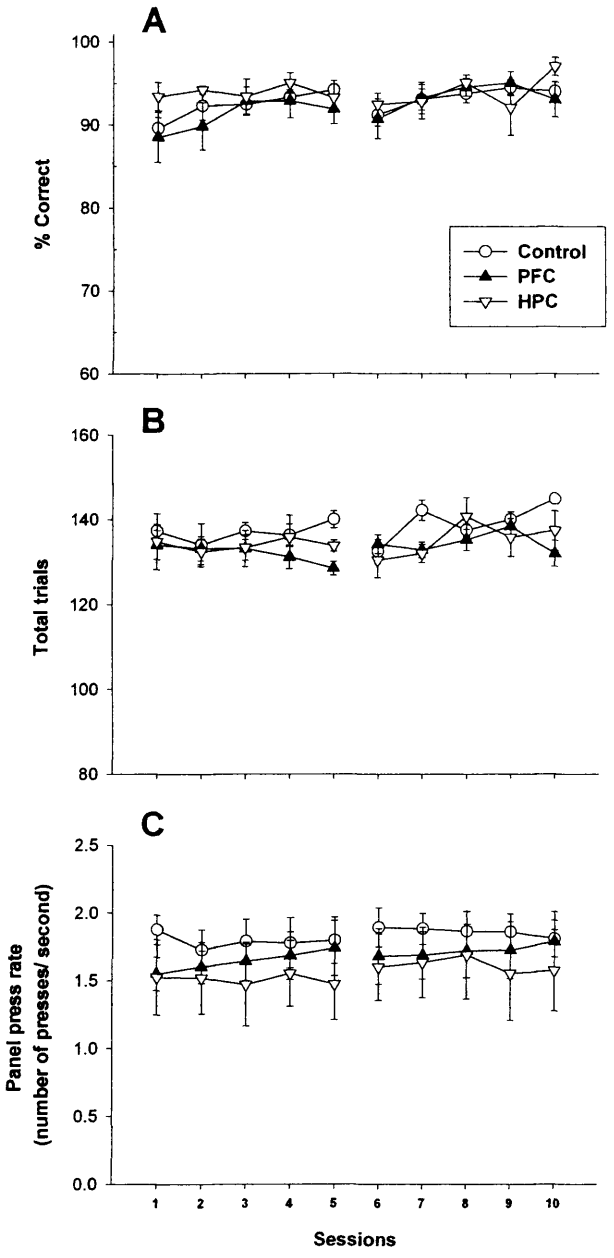


Figure 4.5 DMTP3 baseline pre-surgery data for animals used in each group. % Correct against sessions (A), total trials per session against sessions (B), panel press rate against sessions (C). Data expressed as mean ± standard error of the mean (SEM) (Control n=7, PFC n=6, HPC n=5).

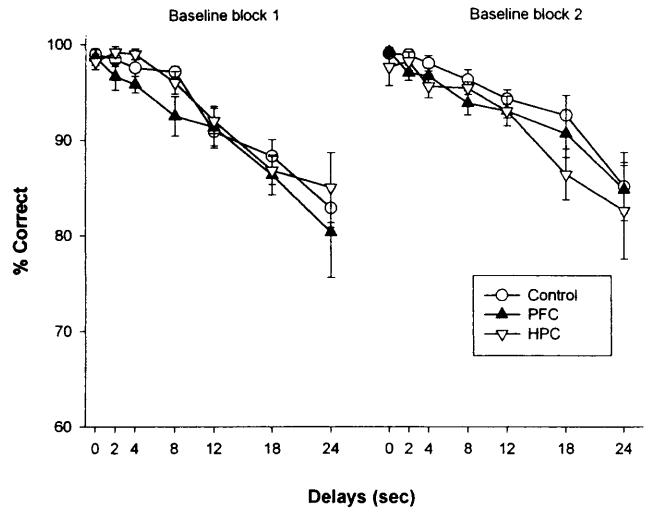


Figure 4.6 % Correct against delays for blocks 1 and 2 of baseline DMTP3. Data expressed as mean ± SEM (Control n=7, PFC n=6, HPC n=5).

4.3.2.1.2 Postoperative testing

Figure 4.7 shows accuracy across sessions both before and after surgery. Data were analysed across all the postoperative sessions, i.e. 30 sessions in total. There was a significant effect of group and session (Group ($F_{(2,15)}$=7.90, p<0.01), Session ($F_{(29,435)}$=103.20, p<0.01)), with *post hoc* analysis revealing that this group difference was due to the significant impairment of the PFC group relative to both the HPC and control groups (Newman-Keuls: PFC v. HPC p<0.01, PFC v. Control p<0.05). However, there was no significant interaction between the lesion groups and the session (Group x Session ($F_{(58,435)}$=0.75, ns)). Analysis was also performed comparing the baseline block with the first postoperative block and additionally the final DMTP block with the first DNMTP block, with block as a within-subject factor. The baseline v. first postoperative block analysis revealed a significant effect of group ($F_{(2,15)}$=5.11, p<0.05) and block ($F_{(1,15)}$=40.72, p<0.01) and a significant group by block interaction ($F_{(2,15)}$=6.07, p<0.05). The PFC group were significantly impaired compared with the HPC group, however the PFC group failed to differ significantly on comparison with the controls (Newman-Keuls: p=0.07). The interaction revealed that the performance of the HPC group was the only group which did not differ significantly between blocks. The analysis of the final DMTP block v. the first DNMTP block revealed significant effects of group ($F_{(2,15)}$=7.96, p<0.01) and block ($F_{(1,15)}$=360.66, p<0.01), but no interaction between group and block

($F_{(2,15)}$=0.77, ns). Post hoc analysis indicated that the PFC group were impaired compared with both control and HPC groups (Newman-Keuls: PFC v Control (p<0.05), v HPC (p<0.01). This shows that the session manipulations did not affect the groups differentially, i.e. they were all affected by the increase in delays and the rule switch to the same extent. Following surgery all animals were impaired compared with pre-surgery figures; the increase in the delay set in the third block of DMTP testing reduced the performance of all groups. Switching the rule from DMTP to DNMTP caused a marked reduction in performance in all groups, with all groups performing below chance for the first block.

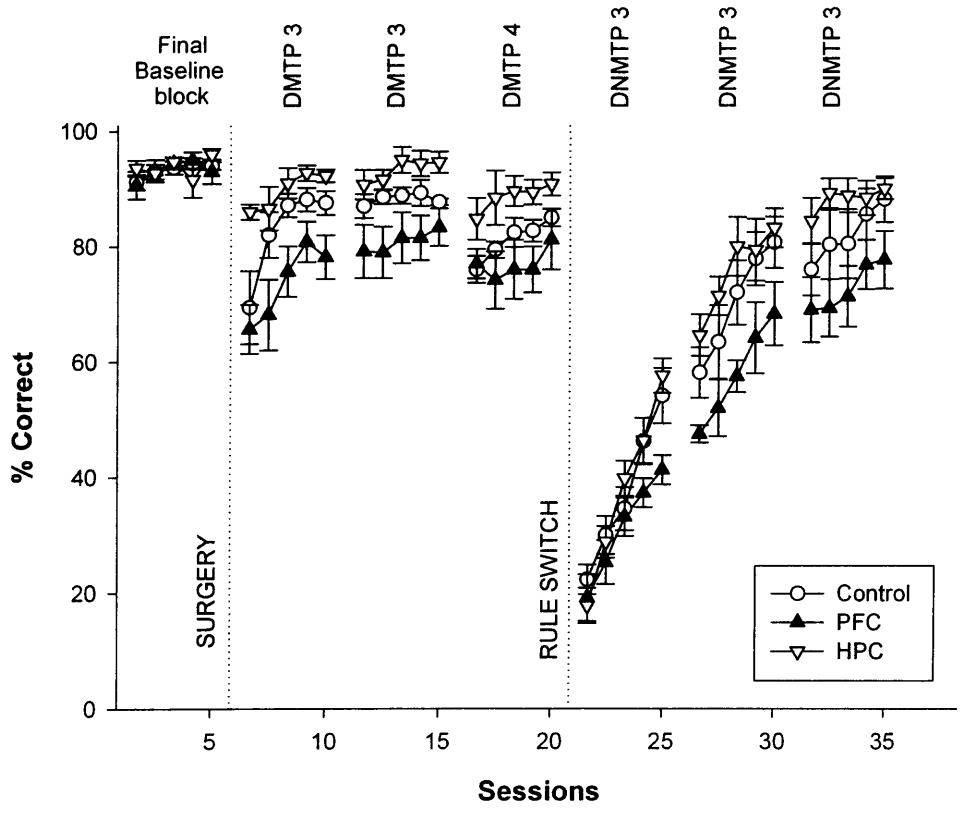


Figure 4.7 % Correct against sessions for 1 block of baseline and 6 blocks of postoperative testing. The rule switch indicates the switch from DMTP to DNMTP. D(N)MTP3 indicates a delay set of 0-24 sec; DMTP4 indicates a delay set of 0-40 sec. Data expressed as mean ± SEM (Control n=7, PFC n=6, HPC n=5).

Data were analysed across delays to assess the nature of the deficit. Figure 4.8 shows data for the average of blocks 1 and 2 which utilised the same delay set (0-24 sec) and figure 4.9 shows the data across all 3 blocks of DMTP. The averaged data for blocks 1 and 2 showed that there were main effects of lesion group and delay, and a significant interaction between group and delay (Group ($F_{(2,15)}$=6.45, p<0.01), Delay ($F_{(6,90)}$=64.65, p<0.01), Group x Delay ($F_{(12,90)}$=5.14, p<0.01)). *Post hoc* analysis revealed that the PFC group were significantly impaired compared with the HPC group (Newman-Keuls: PFC v. HPC (p<0.01), and just failed to reach significance for the comparison with controls (Newman-Keuls: PFC v. Control (p=0.054, ns). Analysis of the interaction between group and delay revealed that the PFC group were impaired compared with the HPC group at all but the shortest 2 delays.

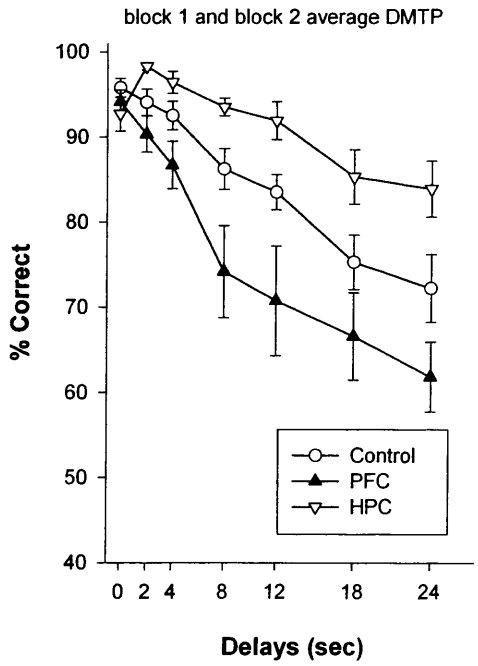


Figure 4.8 % Correct against delays for the average across blocks 1 and 2 of DMTP3. Data expressed as mean ± SEM (Control n=7, PFC n=6, HPC n=5).

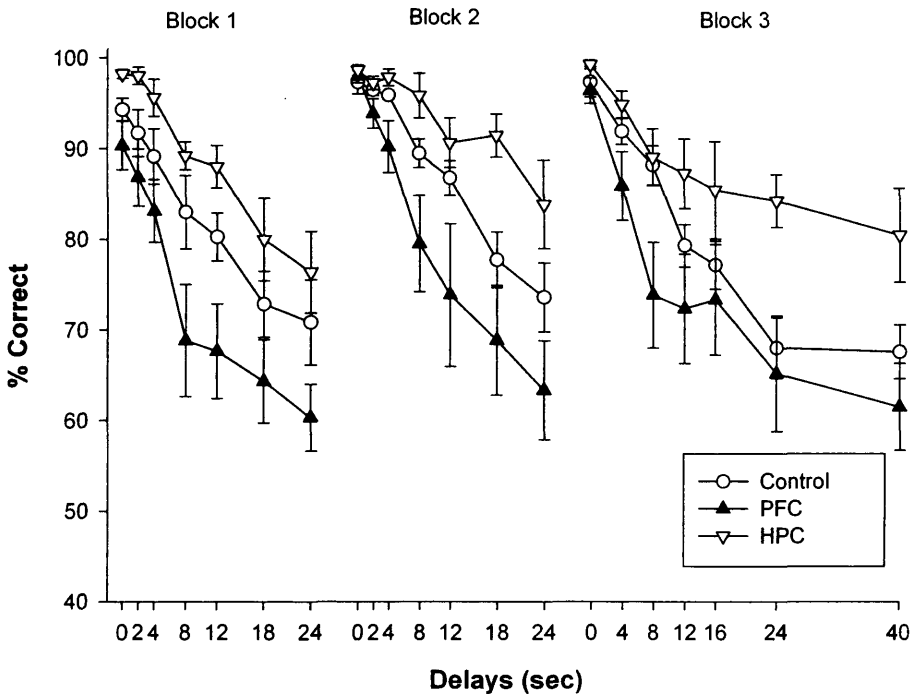


Figure 4.9 % Correct against delays for the 3 blocks of DMTP testing. Data expressed as mean ± SEM (Control n=7, PFC n=6, HPC n=5).

Despite there not being a significant main effect between the PFC and control groups, they were shown to differ significantly on all but the shortest 3 delays as determined by *post hoc* analysis of the interaction. Indeed it is noteworthy that the HPC group were even shown to be significantly more accurate than the control group at the 3 longest delay intervals. Therefore the PFC group were impaired, with a tendency for the deficit to be greater at longer delays. In block 3 of testing the delays were increased up to 40 sec, in this block there were main effects of group and delay, and a group by delay interaction (Group ($F_{(2,15)}$=4.67, p<0.05), Delay ($F_{(6,90)}$=39.32, p<0.01), Group x Delay ($F_{(12,90)}$=1.87, p<0.05)). *Post hoc* analysis revealed that the group difference was due solely to the significant difference between the PFC and HPC groups (Newman-Keuls: PFC v. HPC (p<0.05). *Post hoc* analysis of the interaction between group and delay indicated that the PFC group differed from the HPC group at every delay except for 0 and 4 sec. The controls were significantly different from the HPC group, but only at the two longest delays of 24 and 40 sec. Finally, the PFC group did not differ from the controls on this block of testing.

Rats with PFC lesions therefore exhibited a significant deficit in the first two blocks of retesting; following the introduction of longer delays this deficit failed to reach significance when they were compared to controls, however performance was significantly poorer than the HPC group in a delay-dependent manner. The final three blocks of testing involved the rule switch from DMTP to DNMTP, figure 4.10 illustrates that all groups were severely impaired in the first block of DNMTP testing, with recovery evident over the next two blocks. The data illustrate that animals experienced an enduring deficit at the shortest delay; this is probably due to the rats being unable to withhold their previously learned response of matching at this shortest delay. In blocks 1, 2 and 3 there was a significant effect of the delay, but no interaction between the group and delay (block 1: Delay ($F_{(6,90)}$=42.68, p<0.01), Group x Delay ($F_{(12,90)}$=0.75, ns), block 2: Delay ($F_{(6,90)}$=12.60, p<0.01), Group x Delay ($F_{(12,90)}$=0.71, ns), block 3 : Delay ($F_{(6,90)}$=6.70, p<0.01), Group x Delay ($F_{(12,90)}$=0.47, ns)). An effect of group was seen only in block 2, where the PFC group where shown to be significantly impaired compared with the HPC group (block 1: Group ($F_{(2,15)}$=1.12, ns), block 2: Group ($F_{(2,15)}$=3.77, p<0.05), Newman-Keuls: PFC v. HPC, p<0.05), block 3: Group ($F_{(2,15)}$=2.67, ns)).

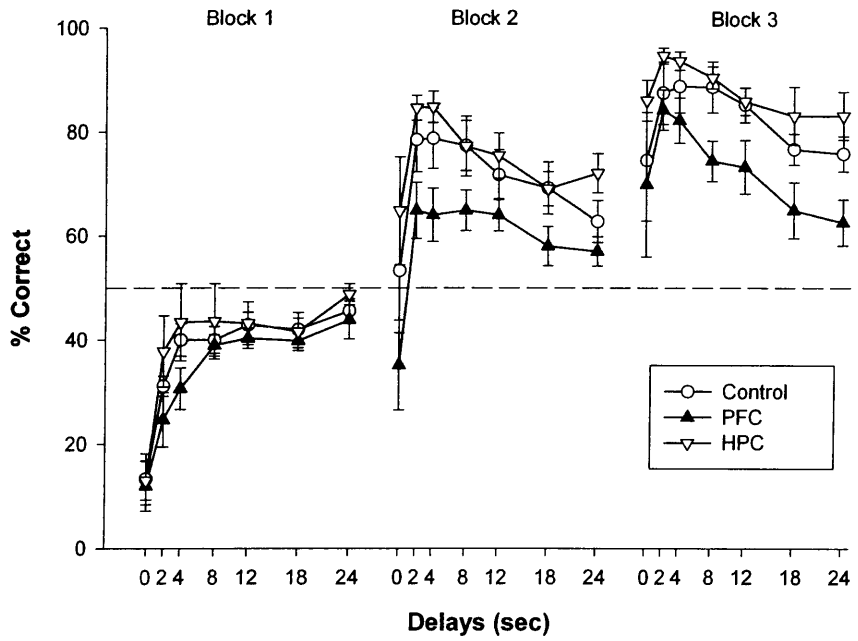


Figure 4.10 % Correct against delays for blocks 1, 2 and 3 of DNMTP. Dashed line indicates chance level. Data expressed as mean ± SEM (Control n=7, PFC n=6, HPC n=5).

In an effort to clarify the deficit further, two measures which might reveal motivational factors were investigated, namely number of panel presses and the latency to the 1st panel press after the delay (figure 4.11). Panel press data did not reveal any effects of group ($F_{(2,15)}$=0.47, ns) or any group by delay interaction ($F_{(12,90)}$=0.90, ns); predictably it did reveal an effect of delay, with more panel presses being achieved the longer the delay duration ($F_{(6,90)}$=295.20, p<0.01). However, latency data revealed main effects of group ($F_{(2,15)}$=5.99, p<0.05) and delay ($F_{(6,90)}$=13.88, p<0.01), and a group by delay interaction ($F_{(12,90)}$=2.71, p<0.01). *Post hoc* analysis revealed that the PFC group exhibited a longer latency than the controls, the interaction revealed that this was due to a significant difference between the latencies of these two groups at the two longest delays.

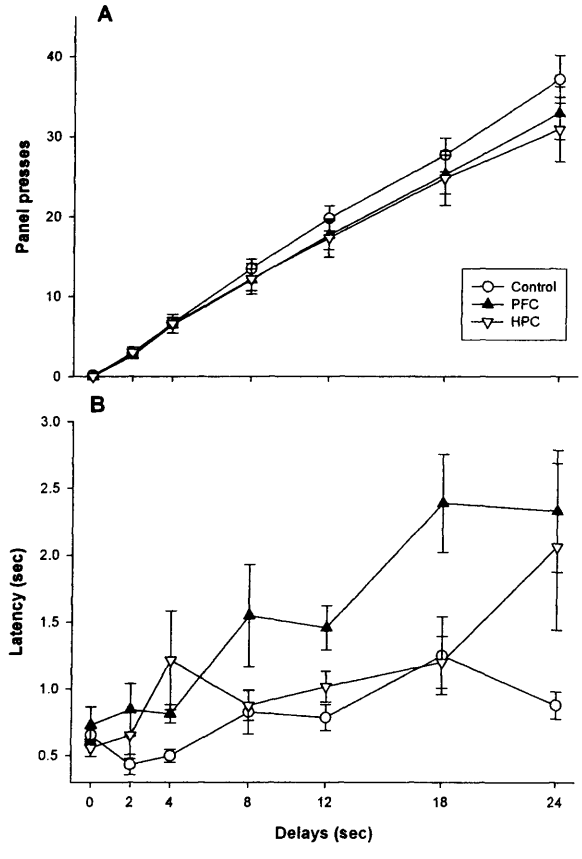


Figure 4.11 Graphs of panel presses (A) and latency (B) against delays averaged across the first two blocks of postoperative testing. Data expressed as mean ± SEM (Control n=7, PFC n=6, HPC n=5).

In addition, measures derived from signal detection theory (SDT) were also analysed; figure 4.12 shows measures corresponding to accuracy and figure 4.13 shows measures corresponding to bias. Results for both the accuracy measures were essentially identical, with main effects of group (*A'*: $F_{(2,15)}=3.93$, $p<0.05$. *SI*: $F_{(2,15)}=6.44$, $p<0.01$), delay (*A'*: $F_{(6,90)}=31.21$, $p<0.01$. *SI*: $F_{(6,90)}=72.09$, $p<0.01$) and group by delay interactions (*A'*: $F_{(12,90)}=2.05$, $p<0.05$. *SI*: $F_{(12,90)}=2.66$, $p<0.01$). *Post hoc* analysis revealed that the PFC group were significantly impaired compared with the HPC and control groups at the four longest delays. For the *SI* measure only, the HPC group achieved a significantly higher score than the control group for the longest two delays. The bias indice I_y, which contrasts accuracy across levers, failed to reveal any effect of group, or any group by delay interaction. Similarly, the bias indices *RI* and *B"* failed to reveal any effect of group when assessed over all delays

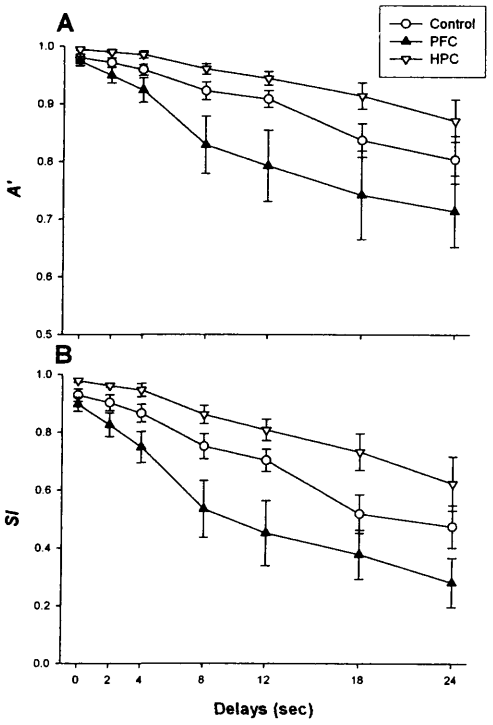


Figure 4.12 Accuracy measures against delays based on SDT, for average of blocks 1 and 2. *A'* (A), *SI* (B). Data expressed as mean ± SEM (Control $n=7$, PFC $n=6$, HPC $n=5$).

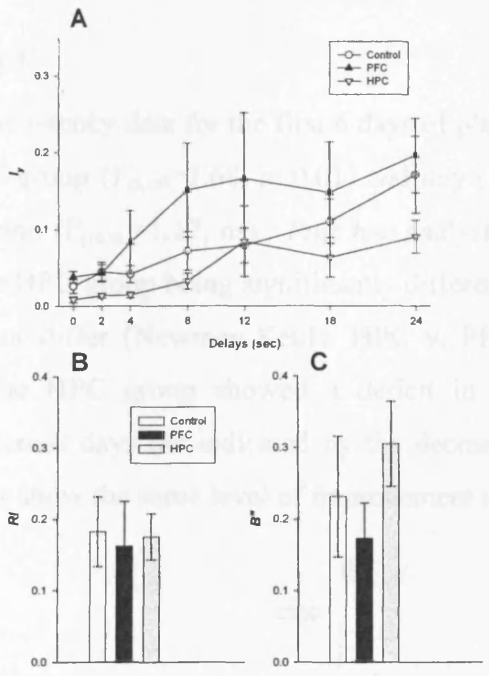


Figure 4.13 Bias indices for blocks 1 and 2. I_y across delays (A), *RI* averaged across all delays (B) and *B"* averaged across all delays (C). Data expressed as mean ± SEM (Control n=7, PFC n=6, HPC n=5).

4.3.2.2 Water maze

4.3.2.2.1 Place learning-1

Figure 4.14 (A) shows the latency data for the first 6 days of place learning. There was a significant effect of both group ($F_{(2,15)}$=7.68, p<0.01) and day ($F_{(5,75)}$=36.99, p<0.01), but no group by day interaction ($F_{(10,75)}$=1.17, ns). *Post hoc* analysis revealed that the group difference was due to the HPC group being significantly different from both the PFC and the controls, who did not differ (Newman-Keuls: HPC v. PFC (p<0.01) and Control (p<0.05)). Therefore the HPC group showed a deficit in learning this task, their performance improved across days (as indicated by the decrease in latency to find the platform) but they did not show the same level of improvement as the other two groups.

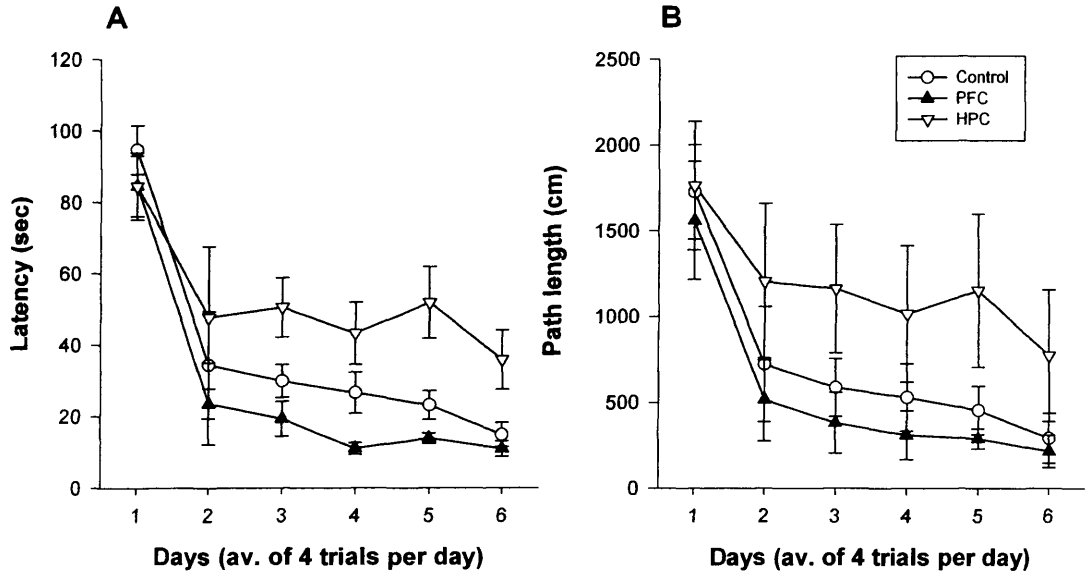


Figure 4.14 Place learning-1: Latency against days (A), path length against days (B). Data expressed as mean ± SEM (Control n=7, PFC n=6, HPC n=5).

The PFC lesion group was shown to be without effect on this task using the measure of latency. To establish if these effects were measure-specific, the path length of each trial was also investigated; these data are shown in figure 4.14 (B). The path length data show a very similar pattern to the latency data, thus ruling out the possibly of swimming speed being a factor in the results. There was a significant effect of group and day, but no group by day interaction (Group ($F_{(2,15)}$=10.82, p<0.01), Day ($F_{(5,75)}$=28.74, p<0.01), Group

x Day ($F_{(10,75)}$=0.89, ns)). *Post hoc* analysis revealed that this group effect was again due to the difference between the HPC group and the PFC and control groups (Newman-Keuls: HPC v. PFC and Control, p<0.01).

On day 7 of testing a 60-sec probe trial was undertaken in which the platform was absent. Figure 4.15 shows the probe data for the percentage of time and path length spent in the training quadrant. The HPC group spent significantly less time in the training quadrant than the other two groups (Group ($F_{(2,15)}$= 9.35, p<0.01), Newman-Keuls: HPC v. PFC and Control, p<0.05). The same pattern was seen for the percentage of path length in the training quadrant, with the HPC group shown to have significantly less of their path length within the correct quadrant compared with PFC and control animals (Group ($F_{(2,15)}$=6.92, p<0.01), Newman-Keuls: HPC v. PFC and Control, p<0.05). This suggests that the HPC rats had not learnt the platform position as well as the other two groups and were in fact performing near chance for these two measures in the probe trial. Figure 4.16 illustrates representative swim paths for each lesion group on day 1 and day 6 of training and on the 60-sec probe session, the traces for days 1 and 6 are taken from the 4th trial. The representative for each group was chosen as the animal which showed the nearest score to the group average for "% time in the training quadrant" on the probe day.

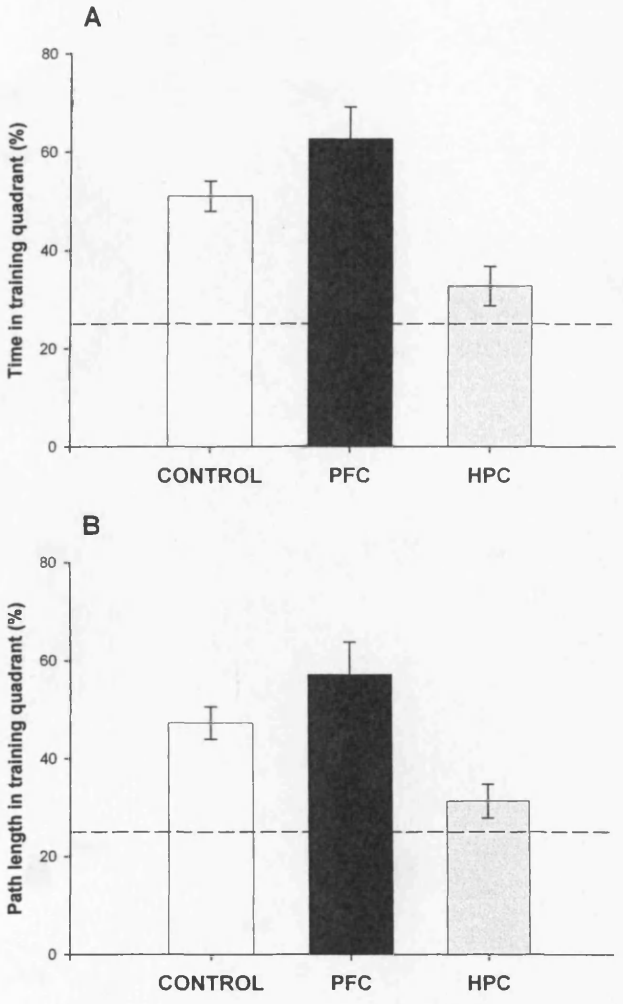


Figure 4.15 Place learning-1 Probe. % Time in training quadrant against group (A), % path length in training quadrant against group (B). Dashed line indicates chance level. Data expressed as mean ± SEM (Control n=7, PFC n=6, HPC n=5).

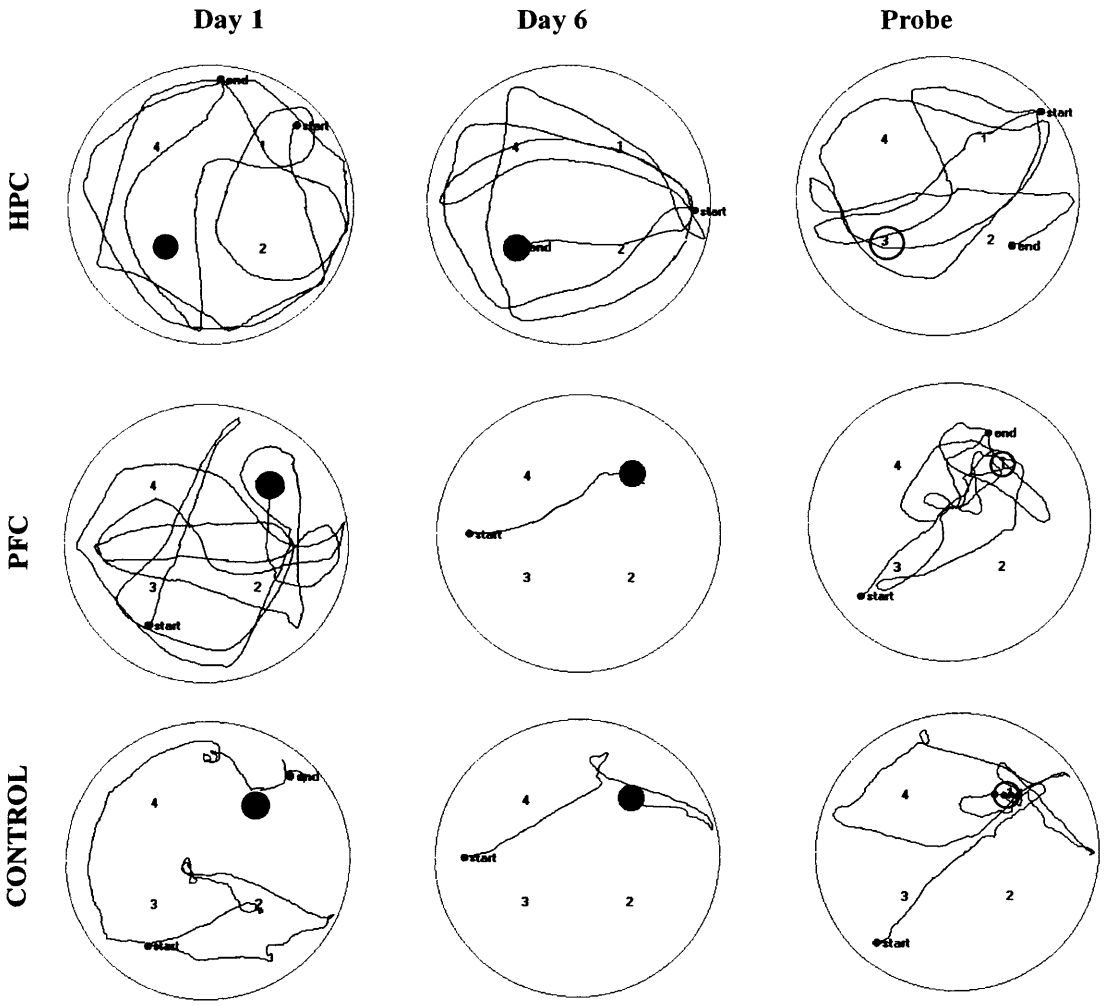


Figure 4.16 Representative swim paths for hippocampal (HPC), prefrontal (PFC) and control rats on days 1 and 6 of place learning and the 60-sec probe trial. Filled circles indicate the platform position, empty circles in the probe trials indicate the position of the platform in training trials and hence the "target" quadrant. Numbers refer to the four different platform positions used.

4.3.2.2.2 Place learning-2

The second place learning training occurred two weeks after the probe trial, animals were retested primarily to see if the deficit in the two sham HPC rats had diminished. These two animals still performed poorly, consistently failing to find the platform within the maximum time of 120 sec, and were therefore excluded from all analyses. Figure 4.17 shows the latency data for the second block of place learning, path length data are not presented as the same pattern as latency was revealed. Group and day both had a significant effect, but there was no group by day interaction (Group ($F_{(2,15)}$=5.03, p<0.05), Day ($F_{(3,45)}$=7.93, p<0.01), Group x Day ($F_{(6,45)}$=1.52, ns). The HPC group were significantly slower at finding the platform than the PFC and control groups (Newman-Keuls: HPC v. PFC and Control, p<0.05). A second probe was carried out following the 4 days of training with data shown in figure 4.18. Despite the significant effect on latency, neither measure on this probe reached significance for a group effect (% Time: Group ($F_{(2,15)}$=1.96, ns), % Path Length: Group ($F_{(2,15)}$=1.34, ns)).

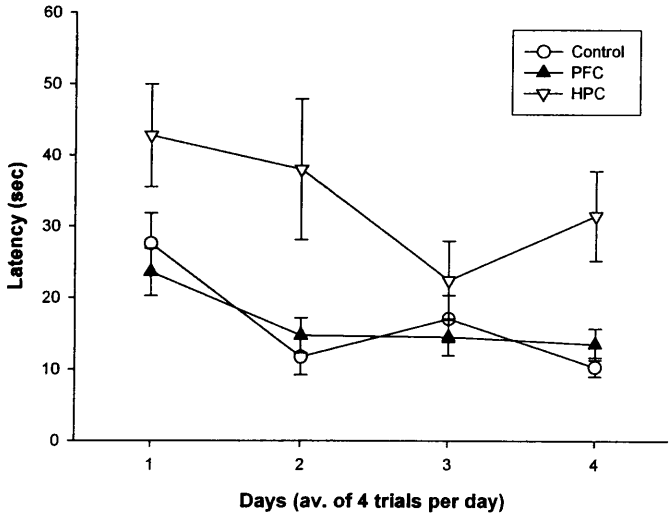


Figure 4.17 Latency against days for place learning-2. Data expressed as mean ± SEM (Control n=7, PFC n=6, HPC n=5).

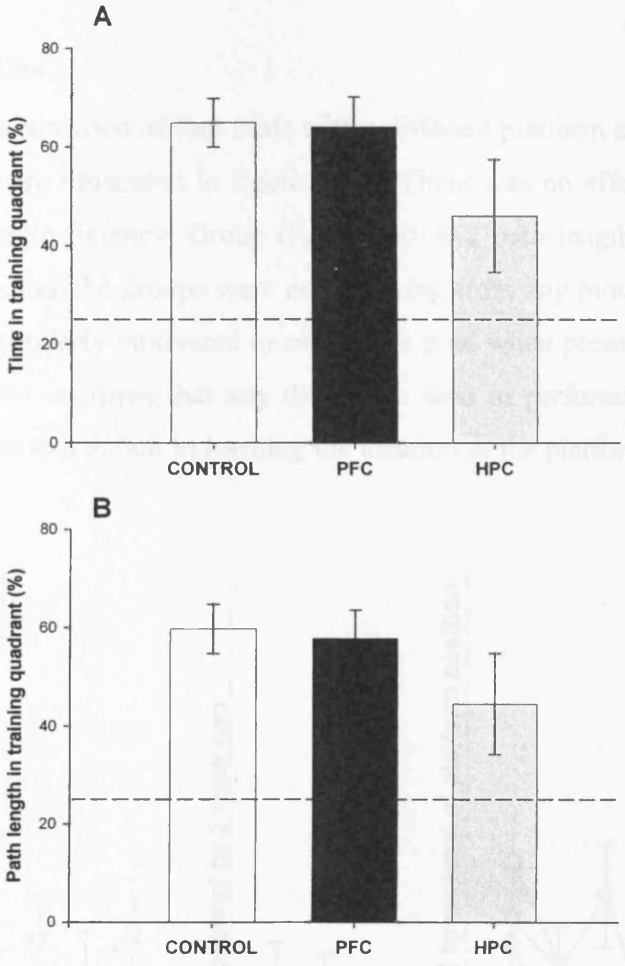


Figure 4.18 Place learning-2 probe. % Time in training quadrant against group (A), % path length in training quadrant (B). Dashed line indicates chance level. Data expressed as mean ± SEM (Control n=7, PFC n=6, HPC n=5).

4.3.2.2.3 Place learning- Reversal

Rats were exposed to a reversal of the platform position so that it was in the opposite quadrant to the one that they had been trained to. All water maze latency data are summarised in figure 4.19, to illustrate the increase in latency following platform reversal. There was no effect of group on this reversal, but there was an effect of day and a group by day interaction (Group ($F_{(2,15)}$=2.19, ns), Day ($F_{(3,45)}$=21.17, $p<0.01$), Group x Day ($F_{(6,45)}$=4.11, $p<0.01$)). This shows that all the groups exhibited the same increase in the amount of time taken to find the platform when it was relocated. However the HPC group again showed that they were less effective at learning the platform position than the other groups.

4.3.2.2.4 Visual Cue

The visual cue test consisted of four trials with a different platform and start position used for each, the data are illustrated in figure 4.19. There was no effect of group in either latency or path length (latency: Group ($F_{(2,15)}$=1.00, ns), path length: Group ($F_{(2,15)}$=0.74, ns)). This verifies that the groups were not suffering from any motor or sensory deficits and that all were similarly motivated to escape the pool when presented with this visible platform. This also confirms that any difference seen in performance between groups was likely to be due to a deficit in learning the location of the platform.

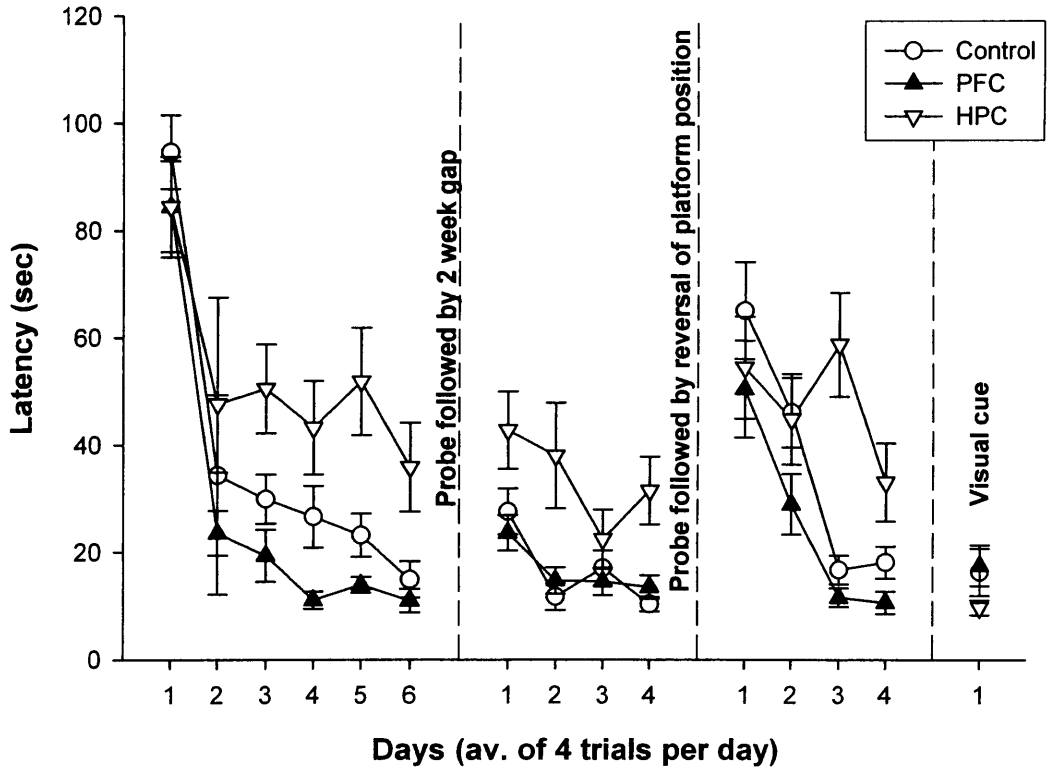


Figure 4.19 Latency against days for place learning-1, place learning-2, reversal and visual cue. Data expressed as mean ± SEM (Control *n*=7, PFC *n*=6, HPC *n*=5).

4.3.2.3 Spontaneous locomotor activity

Rats were assessed on locomotor activity over a 120-min session, the results are shown in figure 4.20. There was a main effect of group ($F_{(2,15)}$=4.65, p<0.05), and block ($F_{(11,165)}$=17.23, p<0.01), but no group by block interaction ($F_{(22,165)}$=1.15, ns). Activity decreased across all groups as the session progressed; both lesion groups were more active than controls, but whereas this was significant in the HPC group (Newman-Keuls, p<0.05), it just failed to reach significance for the PFC group (Newman-Keuls, p=0.0528). HPC and PFC groups did not differ from each other.

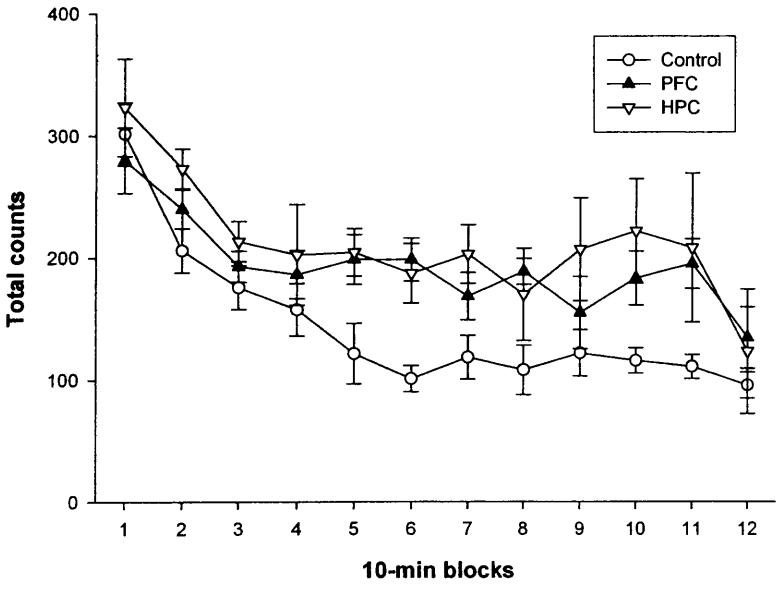


Figure 4.20 Total activity counts against 10-min blocks. Data expressed as mean ± SEM (Control n=7, PFC n=6, HPC n=5).

4.4 Discussion

This experiment sought to examine the effects of HPC or PFC lesions on DMTP and a reference memory task in the water maze, in order to clarify the roles of these two areas within these tasks. The two lesions revealed a double dissociation of behavioural consequences. HPC lesions resulted in a robust impairment in the water maze task, whilst performance on the DMTP task in the Skinner box was not compromised. Conversely, PFC lesions induced a delay-dependent deficit in the DMTP task whilst allowing control levels of performance in the water maze. The DMTP results are contrary to many published findings and are therefore of particular interest with respect to understanding the extent of involvement of these two areas in the task.

Retention of DMTP: HPC lesions

HPC rats displayed absolutely no impairment at any point during testing. They performed as well as controls on the rule switch and when assessed across delays they showed no deficit even at the longest durations; indeed hippocampal rats had significantly enhanced performance compared with the controls at the longest two delay intervals in the first two weeks of testing. These rats were also unimpaired on all measures of motivational performance. These findings are not consistent with Aggleton and colleagues' work (1992), which showed that HPC lesions produced delay-dependent deficits on DNMTP. However, their lesions were aspirative and additionally the task was non-matching rather than matching (Aggleton et al., 1992). The rule is unlikely to have resulted in this discrepancy because the experiment described in this chapter also shows that HPC rats were not impaired on DNMTP, albeit after switching rule from DMTP. Another study in support of delay-dependent HPC involvement in DNMTP is that of Broersen (2000). However, it is unfortunate that Broersen's results are mentioned only as part of a review and therefore lesion details, group sizes and exact training protocol are absent, providing little information with which to compare the present results. Despite these difficulties this paper will be cited frequently, as it provides one of the few examples of HPC and PFC lesion comparisons within both DNMTP and the water maze (Broersen, 2000). Additionally, scopolamine (a muscarinic receptor antagonist)

injections in the dorsal HPC have also been seen to induce delay-dependent impairments in DMTP (Dunnett et al., 1990).

However, the most pertinent findings come from a study in which rats were trained on either DMTP or DNMTP, then retested following IBO lesioning of the HPC (Hampson et al., 1999). These rats were shown to have a delay-dependent impairment on both tasks. A fundamental problem with this study was the absence of a control group; lesioned rats were compared to their own preoperative performance and as such the potential effects of training and surgical procedures have not been taken into consideration. This study required rats to nose-poke in a device on the back wall of the box throughout the delay period (Hampson et al., 1999). This served to preclude the rats from using mediation strategies (see Chapter 9), and therefore may suggest the use of these strategies for intact task performance in this present experiment.

Evidence in support of the present findings also exists, with cholinergic specific lesions of the HPC shown to have no effect on rats pretrained and retested on DNMTP, then switched to DMTP in the Skinner box (Winters and Dunnett, 2004). Radiofrequency lesions of the HPC elicited no deficit in DMTP, when rats were trained in a chamber that required a response on an additional lever on the back wall to initiate the choice phase (Mair et al., 1998). However similar lesions in an identical apparatus were seen to produce delay-independent deficits on DNMTP (Porter et al., 2000), suggesting that the rule may be a significant factor. Young et al (1996) have also demonstrated a lack of impairment in delayed matching and non-matching tasks in an operant box, in rats that received radiofrequency lesions of the dorsal HPC. The apparatus required nose-poking in ports equipped with infra-red photocells, but was essentially equivalent to DMTP/DNMTP, except that it also required a response on the back wall to initiate the choice phase (Young et al., 1996).

Therefore it would appear that the present finding of a lack of effect of HPC lesions on DMTP is not outwith the scope of the existing work on this subject. Discrepancies may arise from differing lesion techniques and specificities, and also variability in task details and training requirements. The possibility of intact performance by means of a mediating strategy alone cannot be entirely dismissed. However, as mentioned above, other studies have shown that in tasks where such strategies should at

least in part be eliminated, HPC damage still failed to cause any deficit (Mair et al., 1998; Young et al., 1996) .

Retention of DMTP: PFC lesions

In contrast to the preserved performance seen following surgery in the HPC group, PFC rats showed a distinct impairment in this task. They were significantly impaired compared with both control and HPC rats; this deficit was shown to be delay-dependent. Specifically, performance did not differ from either of the other two groups at the shortest two delays. Panel pressing was not affected by the lesion; however, latency to make the first panel press after the delay was shown to increase in a delay-dependent manner. This effect on latency could be due to the PFC rats being more distractible and less able to sustain attention over these longer delay periods, or as a consequence of the increased difficulty in processing the task with increasing delay length. Measures derived from signal detection theory corroborate the % correct data, with prefrontal rats being impaired on the accuracy and sensitivity measures in a delay-dependent manner. The bias measures failed to reveal any significant effects of this group, suggesting that their deficit in performance was more likely to be due to some mnemonic impairment rather than the adoption of positional biases which could reduce accuracy.

The delay-dependent effect on accuracy is of great interest in light of numerous reports that damage to the PFC in operant tests of delayed matching and non-matching results in delay-independent deficits (Broersen, 2000; Dunnett et al., 1990; Harrison and Mair, 1996; Herremans et al., 1996; Mair et al., 1998; Porter et al., 2000; Young et al., 1996). One study has also shown delay-independent deficits following excitotoxic lesions of the medial striatum (innervated by dorsal PrL, amongst other areas) and the NAC and ventral striatum (innervated by ventral PrL and IL) (Burk and Mair, 2001). These studies suggest that an impairment in performance that is equal across all delays is indicative of an effect on more non-specific factors such as reduced attention, motivation or planning. However, the majority of these studies use alternative lesion techniques such as aspiration, radiofrequency or neurotransmitter specific lesions, with lesions often being less selective than in the present study.

Studies that utilised comparable lesion techniques to the one used in this study tend to replicate the delay-independent deficits mentioned above. Chudasama and Muir's

work (1997) showed that NMDA-induced lesions centred on the PrL resulted in delay-independent deficits in DMTP; which they suggested was due to an impairment in effective utilisation of mediation strategies to solve the task. NMDA lesions of the PFC have also been shown to induce delay-independent deficits on DNMTP, although lesions were larger than in this experiment, encompassing Cg1 (Aggleton et al., 1995). Of particular interest is that this study showed increases in the SDT measures of bias, *RI* and *B''*, in addition to deficits in the accuracy measures (Aggleton et al., 1995). This may be a reflection of the larger lesion size, as in the present study no effect was seen on any of the bias measures. The only prior evidence for PFC lesions resulting in delay-dependent deficits in DMTP comes from a paper by Dunnett (1990). This study used an identical procedure to the one used in the present experiment, except that PFC lesions were induced by aspiration; rats were seen to have delay-dependent deficits when PFC lesions were more rostral, but delay-independent deficits when lesions were more caudal and extensive (Dunnett, 1990). Additionally, a study has suggested delay-dependent impairments in DMTP following scopolamine injections into the dorsal medial PFC (Broersen et al., 1994). However, these injections appear to have been rather lateral and the authors indicate that they were in the Fr2 and dorsal anterior cingulate (Cg1) areas; yet on comparison with a more current brain atlas (Paxinos and Watson, 1998), they appear to be in the M2 (or Fr2) area alone and as such cannot be compared directly with the results of the present experiment (Broersen et al., 1994).

The present experiment also shows that PFC rats were not differentially affected by the rule switch from matching to non-matching; this result is surprising and is in contrast to a study which suggests that the PFC is involved in exactly this type of switching situation (Joel et al., 1997b). However, Joel et al (1997b) used electrolytic rather than excitotoxic lesions and rats acquired DNMTP following surgery before being switched to DMTP. PFC rats were reported to take longer than controls to reach criterion performance, and performance across all blocks was worse in the PFC group. It should be noted that the authors do concede that there was no difference between the rate of improvement in the PFC group versus the controls (Joel et al., 1997b); this therefore suggests that their impairment might not be specific to the rule switch *per se*, but rather that the PFC group exhibited a more general impairment in task performance.

Impairments have also been demonstrated in a paradigm involving switching from a matching to a non-matching rule using an aversively motivated visual discrimination task in a novel rotating T-maze (Li and Shao, 1998). However, this task does not provide a valuable comparison with the present study, due to both the aversive nature of the task and the use of mechanically-induced lesions within the PFC (Li and Shao, 1998). The lack of PFC effect on the rule switch in the present study is interesting as it contrasts with the rat literature implicating both the PrL and IL in behavioural flexibility and tasks which involve switching between the use of different attributes (see Chapter 1 for more detail) (Birrel and Brown, 2000; Ragozzino et al., 1999; Ragozzino et al., 2003). However, it should be made clear that the present study does not involve a switch in attention to a new dimensional attribute, but rather a reversal of the rule; with rule reversals within the same dimension typically being unaffected by PFC lesions (Birrel and Brown, 2000; Ragozzino et al., 2003). Nevertheless, there is evidence for PFC involvement in even the simplest forms of spatial reversal learning (Salazar et al., 2004).

Thus the present finding of PFC-lesioned rats performing as controls on switching between the matching and non-matching rules, and therefore successfully inhibiting a previously learned response, is clearly quite distinctive. This finding may in part be due to the specificity of the lesions and the excitotoxic lesion technique, although to my knowledge this is the first investigation of adaptation to a rule switch of this nature that was trained preoperatively.

Retention of DMTP: Sham lesions

One finding that must be addressed is the issue of whether or not there may have been an effect of the sham lesions on performance of the DMTP task. Despite there being no difference between the HPC group and controls when assessed across sessions, there was a tendency for the HPC group to perform above controls, as exemplified by the significant difference between these two groups at the longer delays. Thus it might be that rather than the HPC group showing enhanced performance, the sham PFC group may instead have incurred sufficient non-specific damage from the lowering of the needle, and/or the PBS injection, to impair performance. Unfortunately the small group sizes precluded any statistical difference between the two sham groups from being established.

A variety of techniques for performing sham PFC lesions have been used in other studies that investigated PFC lesions on D(N)MTP tasks. In those studies that employed electrolytic, radiofrequency or aspirative lesions, controls were either unoperated or had small pieces of bone removed from the skull above the corresponding site to the lesion (Dunnett, 1990; Harrison and Mair, 1996; Joel et al., 1997b; Young et al., 1996). In studies where excitotoxic lesions were used shams were unoperated (Chudasama and Muir, 1997) or had incisions made in the dura with no lowering of the needle (Aggleton et al., 1995). Finally, the study by Broersen (2000) does not provide details of lesion techniques or sham surgeries. Therefore none of these studies used sham techniques that were directly comparable with those of the present study, where the needle was lowered and an equivalent volume of PBS injected. Thus one can only speculate as to whether or not there was an effect of the sham PFC lesions on performance, which in turn led to the apparent enhancement of the HPC group at the longest delays. However, it does seem a likely explanation given that previously there has been no report of enhancement of HPC lesions on performance on this task, and that the control group was comprised of five PFC shams and just two HPC shams.

Reference memory water maze task: HPC lesions

HPC rats displayed a severe impairment in this task, with longer latencies to find the platform and significantly less time spent in the training quadrant than the other groups; this is in full accordance with the literature (Bannerman et al., 1999; Broersen, 2000; Cassel et al., 1998; Duva et al., 1997; Galani et al., 1998; Good and Honey, 1997; Gould et al., 2002; Liu and Bilkey, 2001; Morris et al., 1982; Richmond et al., 1999; Wright et al., 2004). It should be noted that HPC-lesioned rats have been shown to learn a platform position provided that the start position remained fixed (Compton et al., 1997), suggesting that flexible use of cue relationships is the crucial faculty lost following these lesions. HPC rats were not affected by the positional reversal, although they still took longer to find the platform than the other groups. Finally, the visual cue test clarified that the hippocampal rats were not experiencing any motor or motivational problems which might lead to this reduced performance. The water maze results serve to substantiate the Skinner box data, namely HPC lesions that are without behavioural effects on DMTP induce a characteristic deficit in the water maze.

Reference memory water maze task: PFC lesions

PFC rats did not show any impairment in learning the position of the platform and their latencies decreased across days. This is consistent with many reports where PFC lesions were without effect on learning a reference memory task in the water maze (Broersen, 2000; de Bruin et al., 1994; de Bruin et al., 2001; Lacroix et al., 2002; Sullivan and Gratton, 2002), although some early reports give evidence for PFC deficits (Kolb et al., 1983; Sutherland et al., 1982). Furthermore, PFC rats were not differentially affected by the reversal of the platform position. This is in general accordance with previous work in which mild impairments following reversal (de Bruin et al., 1994; Lacroix et al., 2002), or no impairment at all (Broersen, 2000; de Bruin et al., 2001; Sullivan and Gratton, 2002; Sutherland et al., 1982), are reported. Granon and Poucet (1995) demonstrated that PFC rats were capable of learning the position of a platform after it was reversed, provided start positions remained fixed at just two locations. But when two more start positions were introduced, PFC rats took longer to find the platform with the impairment specific to the two new start locations (Granon and Poucet, 1995). This suggests an impairment due to the contingency being switched, i.e. from using just two start positions to using four, because the present study utilised all seven start positions at random and there was no impairment in the PFC group.

The PFC is thought to be involved in egocentric tasks in the water maze (Broersen, 2000; de Bruin et al., 2001; Mogensen et al., 2005; Nieto-Escámez et al., 2002), where a particular body movement is required to find the platform irrespective of external cues (e.g. turn to the right). However it is unlikely that this type of task would have provided further dissociation between the effects of these two lesions, as the HPC is also thought to have a role in egocentric water maze tasks (Broersen, 2000; Mogensen et al., 2005). From this experiment there is no evidence to suggest a role for the PFC in spatial memory. Furthermore, the lack of effect on the reversal does not provide additional evidence for a role in behavioural flexibility.

Spontaneous locomotor activity

Spontaneous locomotor activity was assessed in the rats over a 120-min session. HPC rats exhibited hyperactivity compared with controls, in line with numerous reports of HPC damage resulting in increased locomotor activity (Bannerman et al., 2002a; Cassel

et al., 1998; Coutureau et al., 2000; Galani et al., 1998; Good and Honey, 1997; Higgs et al., 2001). This characteristic of HPC-lesioned animals has been attributed to the HPC serving as an inhibitor of general activation; upon its removal, this level of inhibitory control is eliminated and subsequently levels of activity are amplified (Tracy et al., 2001).

PFC lesions did not induce such a clear-cut effect on locomotor activity. The difference between the PFC group and controls just failed to reach significance when their total activity counts were compared across 10-min blocks. This result could therefore be interpreted as a tendency for increased locomotor activity. This result is not unexpected as both NMDA lesions (Salazar et al., 2004) and dopamine depletion (Sokolowski and Salamone, 1994) in the PFC have been shown to have no effect on locomotor activity. However Yee's paper (2000) presents some evidence in support of PFC rats showing some hyperactivity; namely NMDA-lesioned rats showed increased locomotor activity, although only over the first two 5-min blocks.

Conclusions

The aim of this experiment was to provide a direct comparison between lesions of the HPC or PFC on DMTP in the Skinner box, and a reference memory task in the water maze. A double dissociation has been revealed, with HPC lesions impairing performance in the water maze and not DMTP, and PFC lesions impairing DMTP and not water maze performance. This suggests that the HPC is not critical to performance of DMTP, at least using the selective lesion techniques described herein. However, PFC lesions reveal a delay-dependent deficit, implicating this task as a potentially viable tool for assessing novel therapeutic strategies for ameliorating cognitive impairments associated with frontal damage.

Chapter 5 Acquisition of delayed matching to position: effect of hippocampal and prefrontal lesions

Experiment 2

5.1 Introduction

As discussed in Chapter 4, both the HPC and the PFC have been implicated in the DMTP task in the Skinner box. Experiment 1 tested retention of DMTP following preoperative training on the rule and revealed a significant effect of the PFC lesion on this task, with a delay-dependent deficit being evident; HPC lesioned rats showed intact performance. However, often different areas can be targeted when acquisition of the rule is tested instead, where learning the rule and task contingencies are in question. It is possible that learning of a rule recruits different neural substrates in comparison with performance of a rule attained prior to surgical intervention, and it is this hypothesis which will be tested in the experiment described within this chapter.

There is evidence to suggest that acquisition and retention of certain tasks do indeed show dissociations, and that preoperative training can have significant effects on retention performance (Wilcott, 1986). PFC lesions have even been shown to facilitate learning in some operant procedures such as the acquisition of a conditional association, despite impairment on operant delayed alternation (van Haaren et al., 1988). Furthermore, preoperative training can affect performance on tasks such as MTP and NMTP in the T-maze (Granon et al., 1994). Preoperatively-trained rats with medial PFC lesions showed a permanent deficit on MTP, but NMTP performance recovered to control levels; in contrast, postoperatively-trained rats displayed similar deficits in both MTP and NMTP (Granon et al., 1994). Rats can also be impaired on acquisition of a task and not retention (MTP in T-maze with MD nucleus lesions, Hunt et al (1998)), or conversely impaired on retention and not acquisition (object discrimination in Y-maze with angular

bundle lesions which disrupt the perforant pathway, Vnek et al (1995)). In the monkey, preoperative training is thought to account for the lack of effect of HPC lesions on a DNMTS task, perhaps by providing practice at holding information over a delay (Murray and Mishkin, 1998). Further evidence of practice effects come from a reference memory task in the water maze, where preoperative training in Fx-lesioned rats speeded postoperative attainment of asymptotic performance levels, when compared with rats that received only postoperative training (Hannesson and Skelton, 1998). These pretrained animals did not show any retention of the platform position *per se*, but it is thought that the procedural aspects of the task may have been retained and thus facilitated relearning of the position. Thus it appears that pretraining may have a significant influence on the outcome of an experiment, either by masking any potential impairment, or by involving different brain areas to those required for the initial learning of task contingencies.

This experiment investigated the effects of lesions of the HPC or the PFC on the acquisition of DMTP in the Skinner box. Rats received no training prior to surgery and were therefore experimentally naïve upon introduction into the Skinner boxes. Following DMTP acquisition, rats were exposed to a switch in the task rule (from matching to non-matching), this was to serve as an analogue of the switching between decision rules in the human WCST, in which human patients with prefrontal damage are impaired. Rats were also subjected to a reference memory task in the Morris water maze and to general locomotor activity evaluation.

Therefore the aim of this experiment was to provide data on the effects of HPC and PFC lesions on acquisition of DMTP that were directly comparable with the results from Experiment 1, where retention of the task was investigated. This would hopefully identify any potential dissociation in performance based on whether or not the rats had received preoperative training.

5.2 Materials and methods

5.2.1 Subjects

32 rats were used in this experiment; all other subject details are covered in section 2.2. Rats were assigned to one of four surgical treatment groups with treatments being HPC

lesions (n=10), sham HPC (n=6), PFC lesions (n=10) and sham PFC (n=6), with all surgical details in section 3.3. Rats were given two weeks to recover from surgery before testing began.

5.2.2 Behavioural testing

Locomotor activity was assessed over a 60-min session, one day prior to commencing training in the Skinner boxes. All Skinner box training procedures are described in detail in section 2.3, briefly, rats were habituated to the pellets in their home cages, then trained to collect pellets from the central panel and finally to press the levers to obtain the pellets as reward. In the course of training one rat had repeated seizures prior to being placed in the Skinner box and was therefore removed from the experimental group and used for pilot lesioning; this rat had a PFC lesion and therefore the PFC group size was reduced to nine. Upon reaching asymptotic levels of performance at alternating continuous reinforcement, rats were trained on 30-min sessions of DMTP in the Skinner box as detailed in section 2.3. Rats received training as outlined in table 5.1.

Skinner box task	Days training
DMTP0	4
DMTP0 +CP	11
DMTP2	10
DMTP3	10
DNTMP3	20

Table 5.1 Training procedure for Skinner box. Dashed line indicates switch from matching to non-matching, DMTP0 indicates no delay, DMTP2 indicates delay set of 0-16 sec, D(N)MTP3 indicates delay set of 0-24 sec, CP indicates the inclusion of a correction procedure.

One month after completion of Skinner box testing rats were tested in the water maze. They received 6 days of place learning testing followed by a probe day where the platform was not present. Following completion of water maze testing, all rats went on to be tested in the spontaneous novelty preference task reported in Chapter 8.

5.3 Results

5.3.1 Histology

5.3.1.1 Bilateral PFC lesions

Figure 5.1 shows photomicrographs of a representative lesion and figure 5.2 illustrates the minimum and maximum lesion extent. In all cases there was substantial cell loss within the PrL and IL cortices, with complete neuron loss within these regions in most animals. Two animals had damage that encompassed the Cg1 cortex, one of these animals also incurred damage to the MO and DP cortices. Out of the nine rats in this surgery group, three did not incur suitable damage and were therefore excluded from all analyses, leaving a group size of six. One of these excluded rats showed only unilateral damage, whilst the other rats had lesions that were too extensive, with damage in the FrA, Cg1 and M2 cortices.

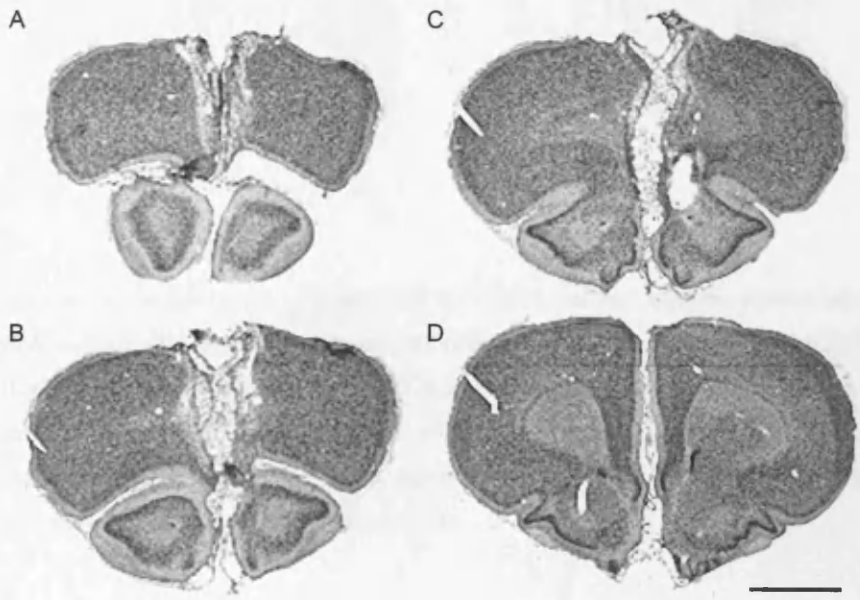


Figure 5.1 Photomicrographs of a representative bilateral PFC lesion, coronal sections stained with Cresyl Violet, with A being the most anterior. Scale bar =2 mm.

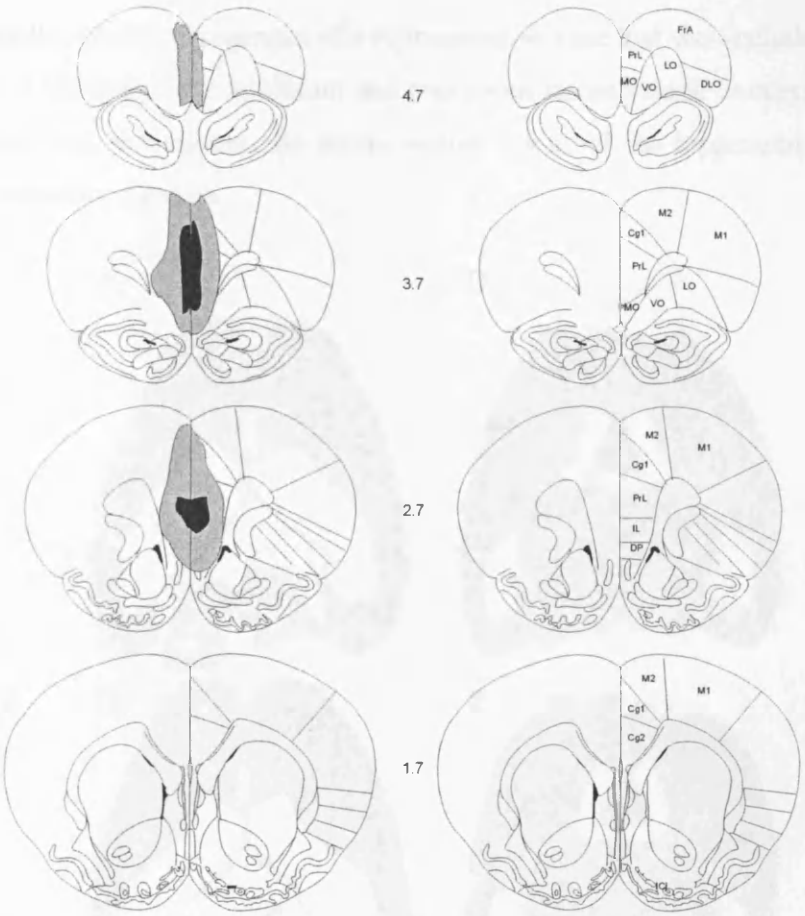


Figure 5.2 Extent of the largest (grey) and smallest (black) PFC lesions, adjacent sections show specific regions. Numbers indicate mm anterior to bregma, abbreviations: cingulate cortex area 1 (Cg1), cingulate cortex area 2 (Cg2), dorsolateral orbital cortex (DLO), dorsal peduncular cortex (DP), frontal association cortex (FrA), infralimbic cortex (IL), lateral orbital cortex (LO), primary motor cortex (M1), secondary motor cortex (M2), medial orbital cortex (MO), prelimbic cortex (PrL), ventral orbital cortex (VO). Coronal drawings taken from Paxinos and Watson (1998).

5.3.1.2 Bilateral HPC lesions

Brains with HPC lesions were sectioned horizontally to allow clearer examination of lesion extent, and to prevent sections from becoming detached. Histological analysis of HPC lesions revealed that all but one out of the ten animals had sufficient bilateral damage for inclusion in analyses. This excluded rat showed only unilateral damage.

Figure 5.3 shows photomicrographs of a representative case that was included in analyses and figure 5.4 illustrates the minimum and maximum lesion extent. Successful cases had extensive cell loss throughout the dorso-ventral extent of the hippocampal formation, with some subicular damage.

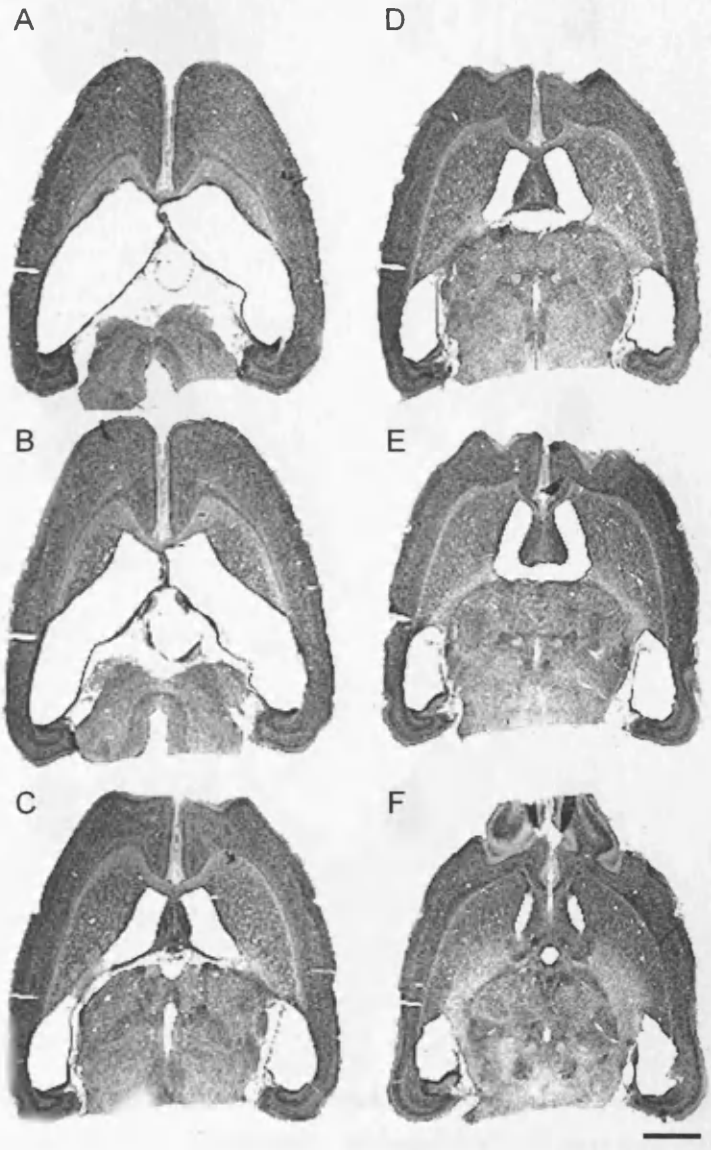


Figure 5.3 Photomicrographs of a representative bilateral HPC lesion, horizontal sections stained with Cresyl Violet, with A being the most dorsal. Scale bar =2 mm.

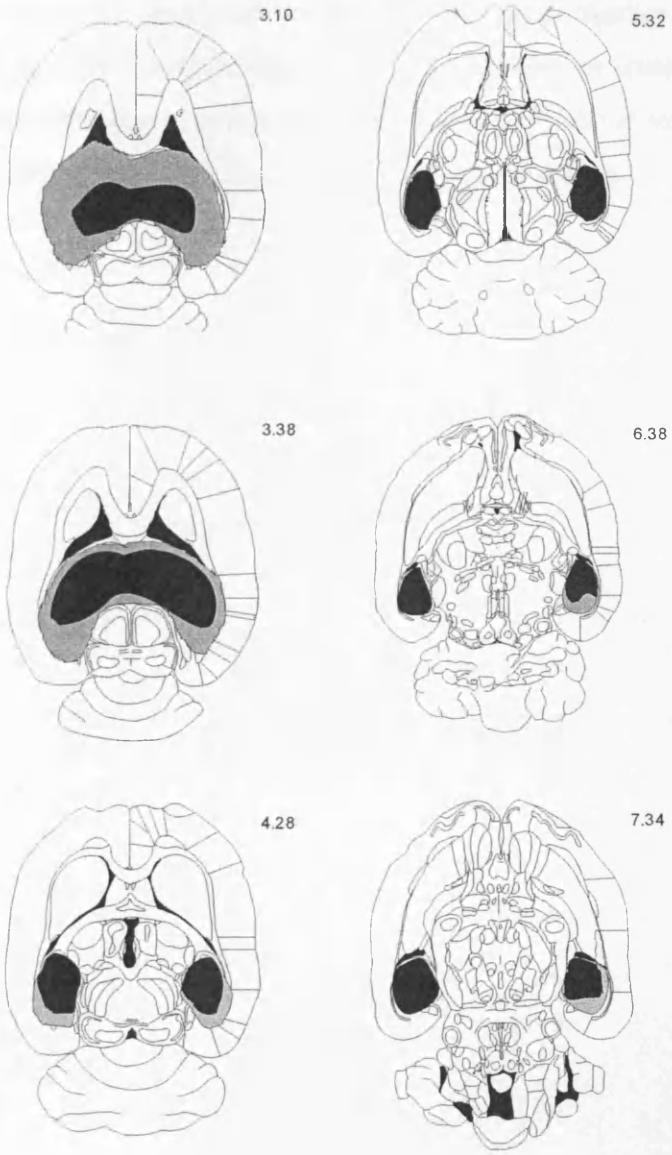


Figure 5.4 Extent of the largest (grey) and smallest (black) HPC lesions. Numbers indicate mm below the horizontal plane passing through bregma and lambda. Horizontal drawings taken from Paxinos and Watson (1998).

5.3.1.3 Sham lesions

Sham lesion cases were not shown to have any cellular loss or damage. A small amount of swelling resulting from the bone flap removal was evident in some cases. The data from the two different sham groups did not differ significantly and as such the two groups were pooled for clarity giving a control group of twelve individuals.

5..3.2 Behaviour

5.3.2.1 Delayed matching to position (DMTP)

There were no group differences for the initial stages of learning such as lever press acquisition, therefore these data are not reported. Figure 5.5 shows the data for the subsequent training phases of DMTP, which clearly illustrates that there were no group differences at any point in acquisition. In the first four days of DMTP training there was no effect of group or any group by session interaction, effect of session also failed to reach significance (DMTP0; Group ($F_{(2,24)}$=0.95, ns), Group x Session ($F_{(6,72)}$=0.05, ns), Session ($F_{(3,72)}$=2.42, ns (p=0.07)).

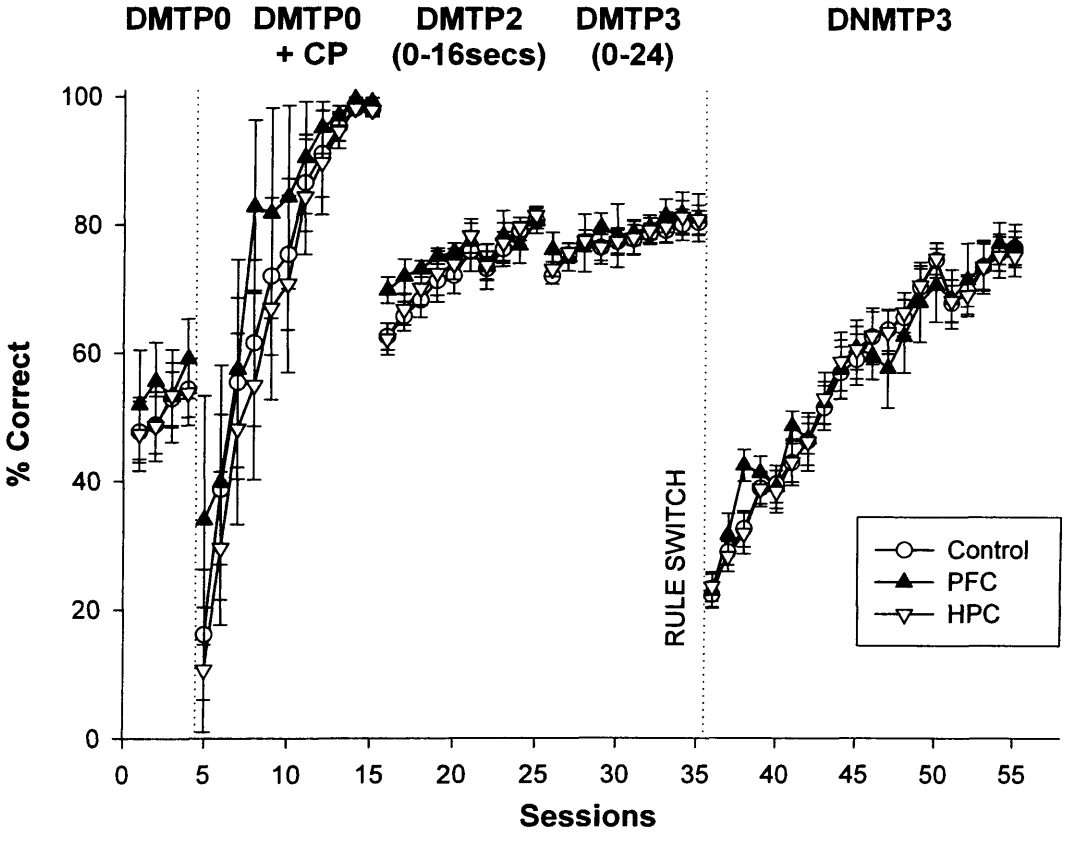


Figure 5.5 Accuracy against sessions for rats trained following surgery. DMTP0 indicates no delay, DMTP2 indicates delay set of 0-16 sec, DNM/MTP3 indicates delay set of 0-24 sec, CP indicates the inclusion of a correction procedure. Data expressed as mean ± SEM (Control n=12, PFC n=6, HPC n=9).

Following these first 4 days of training at DMTP0 a correction procedure was introduced; this fixed the side of the sample lever if the previous trial had been incorrect, randomisation of sample side was only reinstigated once a trial was performed correctly. This manipulation again did not show any group differences, although all rats improved their accuracy across sessions (DMTP0+CP; Group ($F_{(2,24)}$=0.24, ns), Group x Session ($F_{(20,240)}$=0.69, ns), Session ($F_{(10,240)}$=23.20, p<0.01)). Longer delay sets were subsequently introduced, neither delay set caused any group differences (DMTP2; Group ($F_{(2,24)}$=2.90, ns), Group x Session ($F_{(18,216)}$=1.35, ns), Session ($F_{(9,216)}$=17.31, p<0.01). DMTP3; Group ($F_{(2,24)}$=0.35, ns), Group x Session ($F_{(18,216)}$=0.53, ns), Session ($F_{(9,216)}$=7.53, p<0.01)). The final intervention was the rule switch from matching to non-matching. Again there were no significant differences between the groups (DNMTP3; Group ($F_{(2,24)}$=0.01, ns), Group x Session ($F_{(38,456)}$=0.81, ns), Session ($F_{(19,456)}$=90.54, p<0.01)).

Days to criterion performance were also analysed to provide a measure of rate of acquisition. This was taken as the number of days required to achieve a performance of > 85 % correct on the "DMTP0 + correction procedure" phase of task. This measure failed to reveal any difference between groups ($F_{(2,24)}$=0.39, ns), with a high level of variation between individual animals (mean± SEM: Control 4.42± 0.73, PFC 3.83± 1.19, HPC 5.22± 1.27)).

5.3.2.2 Water maze

Figure 5.6 illustrates the latency data across the 6 days of place learning testing. There was a significant effect of group ($F_{(2,24)}$=11.44, p<0.01) and day ($F_{(5,120)}$=45.92, p<0.01) but no group by day interaction ($F_{(10,120)}$=0.84, ns). *Post hoc* analysis revealed that the group difference was due to the HPC group being significantly different from both the PFC and the controls, who did not differ (Newman-Keuls: HPC v. PFC and Control (p<0.01). Therefore the HPC group showed a clear deficit in learning this water maze task whilst PFC lesions were without effect.

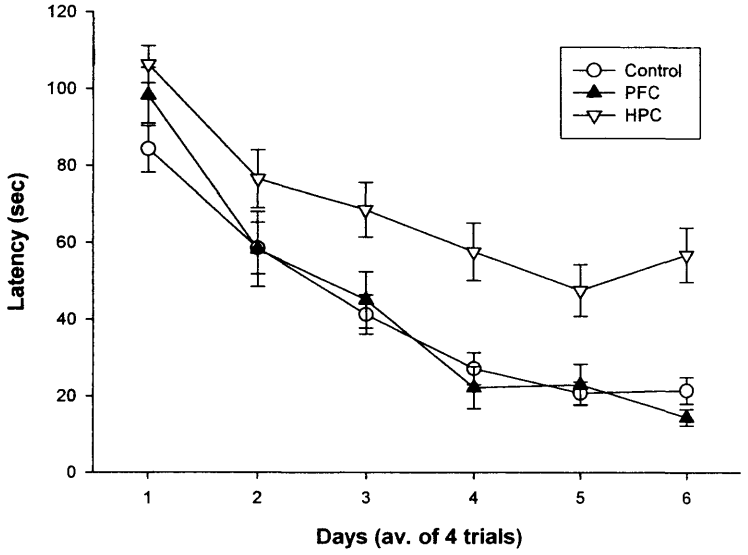


Figure 5.6 Latency against days for place learning. Data expressed as mean ± SEM (Control n=12, PFC n=6, HPC n=9).

Figure 5.7 illustrates the data for the probe trial undertaken on the final day of testing. The HPC group spent significantly less time in the training quadrant than the other two groups (Group ($F_{(2,24)}$=8.57, p<0.01), Newman-Keuls: HPC v. PFC and Control (p<0.01)). This pattern of results was replicated in the percentage of path length spent in the training quadrant (Group ($F_{(2,24)}$=7.18, p<0.01), Newman-Keuls: HPC v. PFC (p<0.05) and control (p<0.01)). This suggests that the HPC rats had not learnt the platform position as well as the other two groups and were performing near chance for both measures in the probe trial. Finally, figure 5.8 illustrates representative swim paths for each lesion group on day 1 and day 6 of training and also on the 60-sec probe session, the traces for days 1 and 6 are taken from the 4th trial. The representative for each group was chosen as the animal which showed the nearest score to the group average for % time in the training quadrant on the probe day.

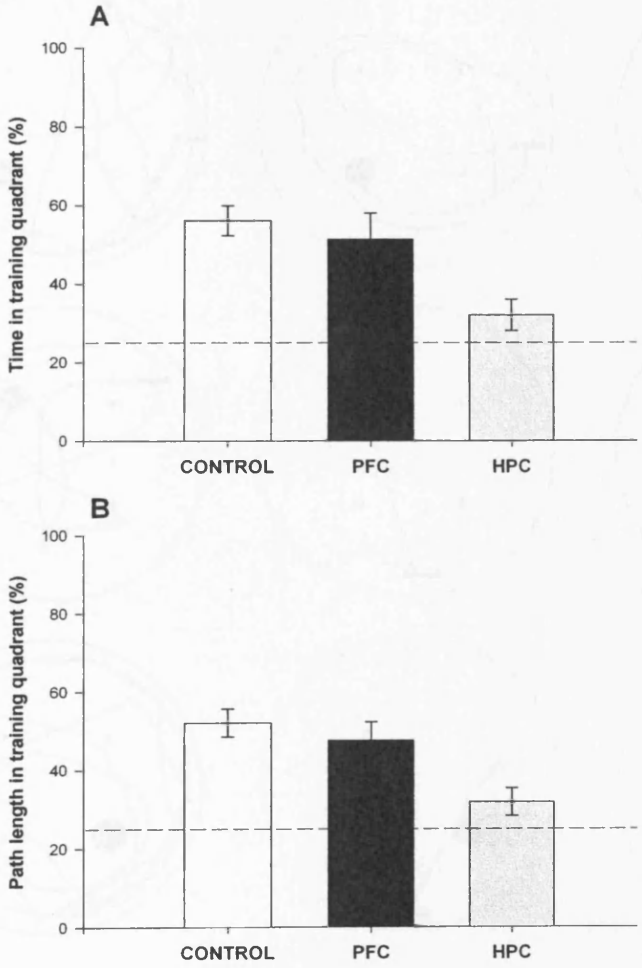


Figure 5.7 Probe data. % Time in training quadrant against group (A), % path length in training quadrant against group (B). Dashed line indicates chance level. Data expressed as mean ± SEM (Control *n*=12, PFC *n*=6, HPC *n*=9).

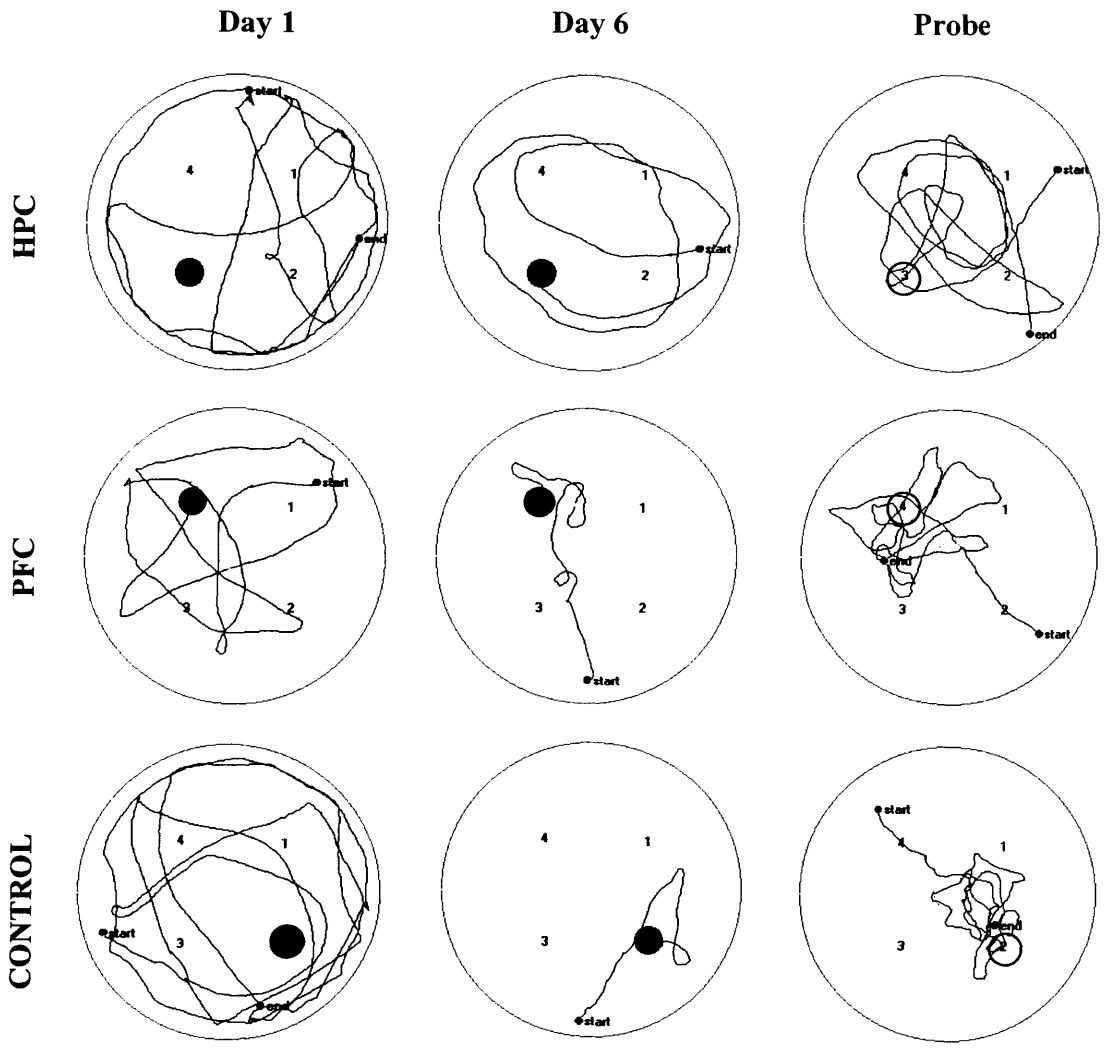


Figure 5.8 Representative swim paths for hippocampal (HPC), prefrontal (PFC) and control rats on days 1 and 6 of place learning and the 60-sec probe trial. Filled circles indicate the platform position, empty circles in the probe trials indicate the position of the platform in training trials and hence the "target" quadrant. Numbers refer to the four different platform positions used.

5.3.2.3 Spontaneous Locomotor Activity

Rats were assessed over a 60-min session, just prior to DMTP training; the results are shown in figure 5.9. There was a main effect of group ($F_{(2,24)}$=5.82, p<0.01) and block ($F_{(11,264)}$=20.59, p<0.01) but no group by block interaction ($F_{(22,264)}$=1.44, ns). *Post hoc* analysis revealed that the HPC rats were more active than the PFC and control rats, who did not differ (Newman-Keuls: HPC v. PFC and Control (p<0.05)).

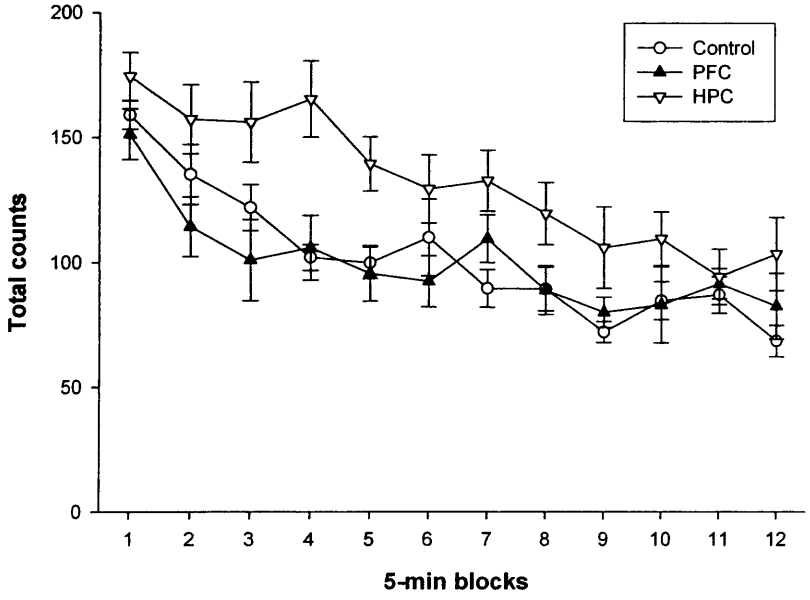


Figure 5.9 Total activity counts against 5-min blocks for a 60-min session. Data expressed as mean ± SEM (Control n=12, PFC n=6, HPC n=9).

5.4 Discussion

This experiment examined the effects of HPC and PFC lesions on acquisition of DMTP in the Skinner box. Neither of these two lesions resulted in an impairment on learning or performing this task when compared with control animals. The rats were also exposed to a switch in the rule from matching to non-matching, all three groups of rats were able to adapt to the new rule in a similar fashion, with no significant differences in the rate of acquisition. The rats were also tested in a standard reference memory task in the water maze, which revealed a characteristic HPC lesion-induced impairment.

Acquisition of DMTP: HPC lesions

HPC lesions were without effect on acquisition of DMTP, additionally, the present experiment also demonstrated that the rats were able to adapt to the switch in rule, in accordance with Experiment 1. The "days to criterion" measure also failed to reveal any differences; this might be due to the high degree of variability in this measure resulting from the tendency for rats to acquire the task in an all or nothing fashion, i.e. rats often acquired the task to near perfect performance within the space of one session. Few studies have assessed the effects of HPC lesions on acquisition of operant D(N)MTP, possibly because preoperative training provides the additional benefit of allowing surgical groups to be behaviourally matched. The review by Broersen (2000), mentioned previously in Chapter 4, discusses the effects of HPC lesions on acquisition of DNMTP in the Skinner box. Although the review does not provide precise experimental details, it does serve to substantiate the results of this experiment, with HPC lesions having no effect on acquisition of DNMTP. In the case of the Broersen study, this finding was of particular significance given that they had also shown delay-dependent deficits on retention of DNMTP.

Another study, which aimed to address the effects of IBO-induced HPC lesions on acquisition of this type of task, is that of Hampson et al (1999). However, their group of naïve animals with HPC specific lesions was comprised of only two individuals. Furthermore, there were no control animals with which to compare performance, rendering the results somewhat difficult to interpret. Nonetheless, on a DNMTP task learnt after surgery these two rats performed better than pretrained HPC-lesioned rats, but

worse than those rats' prelesion performance. In further support of this finding, an additional group of rats with lesions that included ERC and subiculum showed a similar pattern of behaviour (Hampson et al., 1999). These studies suggest that where an HPC lesion results in impairment on retention of DNMTP, the same lesion may have little or no detrimental effect on acquisition of the task.

Acquisition of DMTP: PFC lesions

PFC lesions did not result in any impairment on acquisition of DMTP, in stark contrast to the delay-dependent deficit that was evident when postoperative retention of the rule was assessed in Experiment 1. The ability to switch between the rules was not affected differentially by the PFC lesion, again concurring with Experiment 1. In addition to HPC lesions, Broersen's study (2000) also investigated the effects of PFC lesions on acquisition of DNMTP; it demonstrated that, like HPC lesions, PFC lesions did not impede learning of the task. Another study investigated the effects of electrolytic lesions of the medial PFC of the rat on acquisition of DNMTP in the Skinner box and its subsequent reversal to DMTP (Joel et al., 1997b). These lesions did not impair the acquisition of NMTP in the absence of delays, which concurs with the present study's finding on DMTP; these same animals were then impaired upon introduction of delays in a delay-independent fashion, a finding which was not replicated by the present study. When the rats in Joel et al's study (1997b) were switched from DNMTP to NMTP the PFC group were slower to acquire the new rule as revealed by significantly greater days to criterion. It should be noted that this study differed from the present study in a number of ways, which may have contributed to the discrepancies between the two findings. In addition to the different lesion technique used, the Joel et al study (1997b) tested the rats on the original rule with no delays, prior to making the switch to MTP also initially without delays. However, in the present study the rule was switched with both tasks on the maximal delay; one might assume that rather than masking any apparent deficits this would in fact exaggerate any impairment in learning the new rule.

Broersen (2000) and Joel at al (1997b) both used a non-matching procedure rather than the matching procedure used in this study. It is possible that the choice of the initial rule may determine the outcome of the experiment, as it has been shown that the two variants are not learnt in exactly the same manner (Blokland and Dunnett, 1995).

However the present study replicates the Broersen study (2000), with no effect on acquisition even when delays were introduced, suggesting that in these two cases at least, PFC lesions did not impair acquisition regardless of the rule.

Reference memory water maze task: HPC and PFC lesions

HPC lesions resulted in a significant impairment in this task, with longer latencies to find the platform and less time spent in the training quadrant than the other two groups. The PFC group did not differ from the control group at any point. The swim paths indicate that the HPC group may have employed a strategy in which a certain distance from the pool edge was maintained; this resulted in a decrease in latency over days, however, the probe day suggests that these rats were not using an allocentric representation of extra-maze cues to guide their behaviour. These findings replicate those found in Experiment 1, and correspond with the literature on this topic (see Chapter 4 discussion). This verifies that the HPC lesions were sufficient to impair performance on this HPC-sensitive task, despite being without effect on the Skinner box paradigm.

Spontaneous locomotor activity: HPC and PFC lesions

Spontaneous locomotor activity was assessed over a 60-min session. HPC rats were significantly hyperactive compared with the PFC group and the controls, which did not differ from each other. This again corroborates the results from Experiment 1 and the literature discussed in Chapter 4, and provides further validation of the HPC lesions.

Conclusions

The aim of this experiment was to investigate whether rats trained on DMTP following surgery would exhibit a dissociation in performance compared with those that had received training prior to surgery. A considerable dissociation was evident in rats that had received PFC lesions, with no effect on acquisition in comparison with the deficit on retention that had been shown in Experiment 1. Thus it would appear that the PFC may be involved in working memory processes only when it is functionally involved in the acquisition of the task in question. However, the HPC lesions were without effect on acquisition, which corroborates the lack of effect on retention demonstrated in Experiment 1. Therefore, these two experiments do not provide support for the

hypothesis that the HPC might be integral for successful performance on D(N)MTP. It can be concluded that the processes involved in learning a rule compared with retention of a rule are clearly very distinct, and should be taken into consideration when comparing across species and tasks.

Chapter 6 Retention of delayed matching to position: effect of entorhinal cortex and fornix lesions

Experiment 3

6.1 Introduction

Experiments 1 and 2 demonstrated that excitotoxic lesions of the HPC were without effect on the acquisition or retention of the DMTP task. This finding is at variance with the majority of the literature that reports hippocampal deficits in this type of task (Aggleton et al., 1992; Broersen, 2000; Dunnett et al., 1990; Hampson et al., 1999). One possible explanation for the lack of effect could be the specificity of the lesion, which left the adjacent cortical areas intact. The hippocampal formation comprises four main regions, namely the DG, HPC proper, subicular complex and the ERC (Amaral and Witter, 1989; Amaral and Witter, 1995). Of these four regions, only the ERC had been spared in the HPC lesions described in experiments 1 and 2. Thus the possibility arose that this cortical area might be the critical locus for performance in the DMTP task.

The ERC has a pivotal role within the hippocampal formation, providing the major input to the DG via the perforant path and being the principal relay for reciprocal connections with the cortex (Amaral and Witter, 1989; Swanson and Kohler, 1986; Witter et al., 1989; Witter et al., 2000). The ERC also has efferent projections to the CA1 and CA3 fields of the HPC (Burwell et al., 1995; Swanson et al., 1987; Witter et al., 1989). Thus there follows a natural assumption that the ERC might be critical for normal hippocampal functioning(O'Keefe and Nadel, 1978). However, studies involving lesions of the ERC on hippocampal-dependent learning have yet to provide conclusive results. For example, although there are reports of ERC lesions resulting in reference memory deficits in the water maze (Eijkenboom et al., 2000; Good and Honey, 1997; Oswald and Good, 2000; Parron et al., 2004; Schenk and Morris, 1985; Spowart-Manning and van der

Staay, 2005), there are also studies which show intact performance on this task following these lesions (Bannerman et al., 2001b; Galani et al., 1998 (impaired on working memory but not reference memory version); Hagan et al., 1992; Pouzet et al., 1999b). These discrepancies cannot be explained solely on the basis of lesion technique as studies within both factions have employed comparable methods; however, the studies that showed no impairments generally had less subicular damage, implicating this area in performance of the task. Contextual fear conditioning, which is impaired following HPC damage (Good and Honey, 1997), is not impaired following ERC damage (Bannerman et al., 2001a; Good and Honey, 1997), lending support to the hypothesis that the ERC is not necessarily crucial to HPC-dependent learning.

Further motivation for studying the involvement of the ERC in cognitive behaviour comes from the observation that damage occurs in this area in the initial stage of Alzheimer's disease. The damage correlates with memory deficits that are present during this stage of the disease (Braak et al., 1993; Hyman et al., 1986; Hyman et al., 1987). It has therefore been proposed that animals with lesions of the ERC might serve as a model for investigating the cognitive deficits that accompany Alzheimer's disease (Eijkenboom et al., 2000; Miwa and Ueki, 1996; Spowart-Manning and van der Staay, 2005).

The other major route by which the HPC has reciprocal connections with the rest of the brain is the fornix (Fx). This fibre tract provides the majority of cholinergic inputs to the HPC, and links the HPC with a multitude of cortical and subcortical sites (Amaral and Witter, 1995; Swanson et al., 1987). Debate exists over whether or not transection of the Fx results in a similar pattern of behavioural impairments to those of specific lesions of the HPC (Aggleton et al., 1992; Whishaw and Jarrard, 1995b). The reference memory task in the water maze is routinely impaired following disruption of the Fx (Bannerman et al., 2001b; Cassel et al., 1998; de Bruin et al., 2001; Pouzet et al., 1999a; Whishaw and Jarrard, 1995a; Whishaw and Jarrard, 1995b), although performance may eventually recover (Hannesson and Skelton, 1998; Mogensen et al., 2004); this suggests that, in this task at least, Fx transection can provide equivalent behavioural consequences to HPC lesions. Fx lesions have also been shown to induce delay-dependent deficits on D(N)MTP (Aggleton et al., 1991; Aggleton et al., 1992; Aggleton et al., 1995;

Chudasama and Muir, 1997; Dunnett, 1985; Dunnett, 1990; Ennaceur et al., 1996; Weiner et al., 1998), and it is for this reason that they are included within the current experiment.

The aim of this experiment was to explore the involvement of the ERC in the DMTP task, with a view to establishing if this area has a role in this working memory task. Rats with excitotoxic lesions of the ERC or aspirative lesions of the Fx were assessed on postoperative retention of DMTP performance in the Skinner box. In addition they were exposed to a switch in the task contingency from matching to non-matching, before being subjected to general locomotor activity evaluation. Of particular interest was whether the ERC lesions would induce a deficit in DMTP, given that experiment 1 found no deficit following HPC lesions. The Fx group were included with the hope that a deficit might be revealed in this experiment, providing validation that such a deficit is attainable using the present experimental conditions and providing a comparison with the effects of the ERC lesions. Also of interest would be the comparison between Fx lesions and the HPC lesions from Experiment 1, i.e. would it be possible to show a behavioural dissociation between damage of the target structure, and damage to one of its major pathways?

6.2 Materials and methods

6.2.1 Subjects

In this experiment a group of 32 rats were used. In the course of training one rat was sacrificed due to illness whilst another rat, which was unable to learn the task, was used for a pilot lesion; this resulted in a presurgery group size of 30. All other subject details are covered in section 2.2.

6.2.2 Behavioural testing

Rats were trained on 30-min sessions of DMTP in the Skinner box as detailed in section 2.3. Upon reaching asymptotic levels of performance at the final delay set (0-24 sec) rats were tested for two 5-session blocks to provide a baseline. Following baseline testing rats were assigned to one of four surgical treatment groups using a random matching

procedure based on accuracy. Treatments were bilateral ERC lesions (n=10), sham ERC lesions (n=5), bilateral aspirative Fx lesions (n=10) and sham Fx lesions (n=5), with all surgical details in section 3.3. Rats were given two weeks to recover from surgery before they received the testing schedule outlined in table 6.1, all details in section 2.3.

5-session block	Skinner box task
1-4	DMTP3
5	DMTP4
6	DMTP1
7-9	DNMTP1 (Non-matching)
10	DNMTP3

Table 6.1 Testing procedure for Skinner box. Dashed line indicates switch from matching to non-matching, D(N)MTP1 indicates delay set of 0-6 sec, D(N)MTP3 indicates delay set of 0-24 sec, DMTP4 indicates delay set of 0-40 sec.

Rats were retested on DMTP3 before increasing the delay set up to 40 sec in block five. Prior to switching the rule, rats received one week of testing on DMTP1 to minimise any pre-switch group differences, they were then tested on DNMTP. Following completion of Skinner box testing, all rats went on to be tested in the spontaneous novelty preference task reported in Chapter 8. After testing on that task was complete, rats had their locomotor activity assessed over a 60-min session. Due to technical reasons the water maze task could not be assessed during this experiment. Finally, all rats were sacrificed and histology was dealt with as detailed in section 3.4.

6.3 Results

6.3.1 Histology

6.3.1.1 Bilateral ERC lesions

Out of the ten rats in this surgery group, one incurred substantial damage to the CA1 region of the HPC and was therefore excluded from all analyses, leaving a group size of nine. Figure 6.1 shows photomicrographs of a representative lesion and figure 6.2 illustrates the minimum and maximum lesion extent. In all cases there was significant cell loss in the presubiculum and parasubiculum, with the majority of the medial ERC being lesioned. The lateral ERC was completely spared as was the subiculum proper, therefore rather than being complete ERC lesions, these lesions were restricted to the medial region of this cortex.

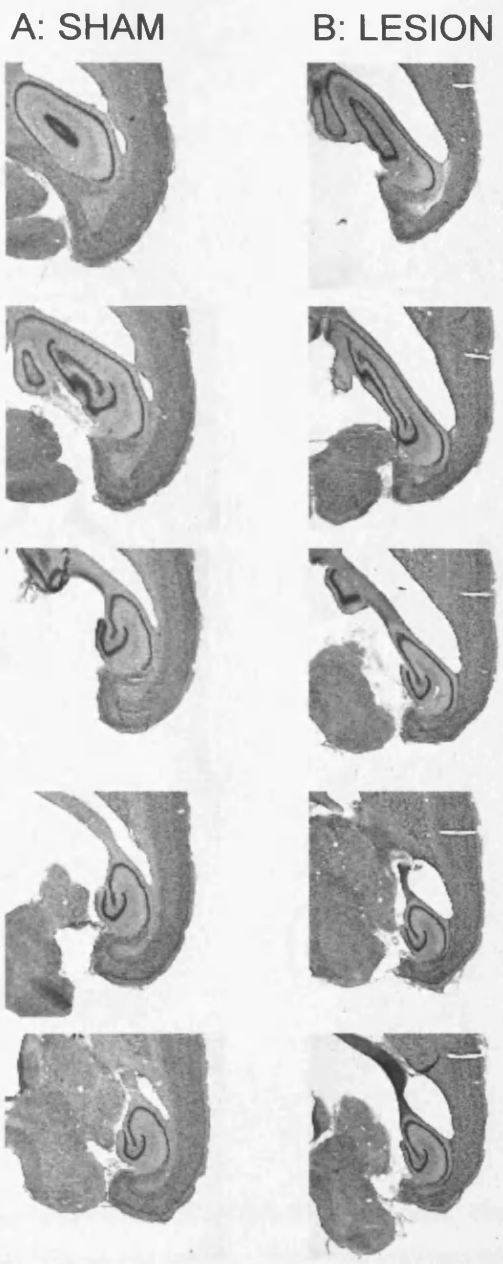


Figure 6.1 Photomicrographs of horizontal sections stained with Cresyl Violet. Sham ERC lesion (A), excitotoxic ERC lesion (B), from most dorsal to ventral. Scale bar =1 mm.

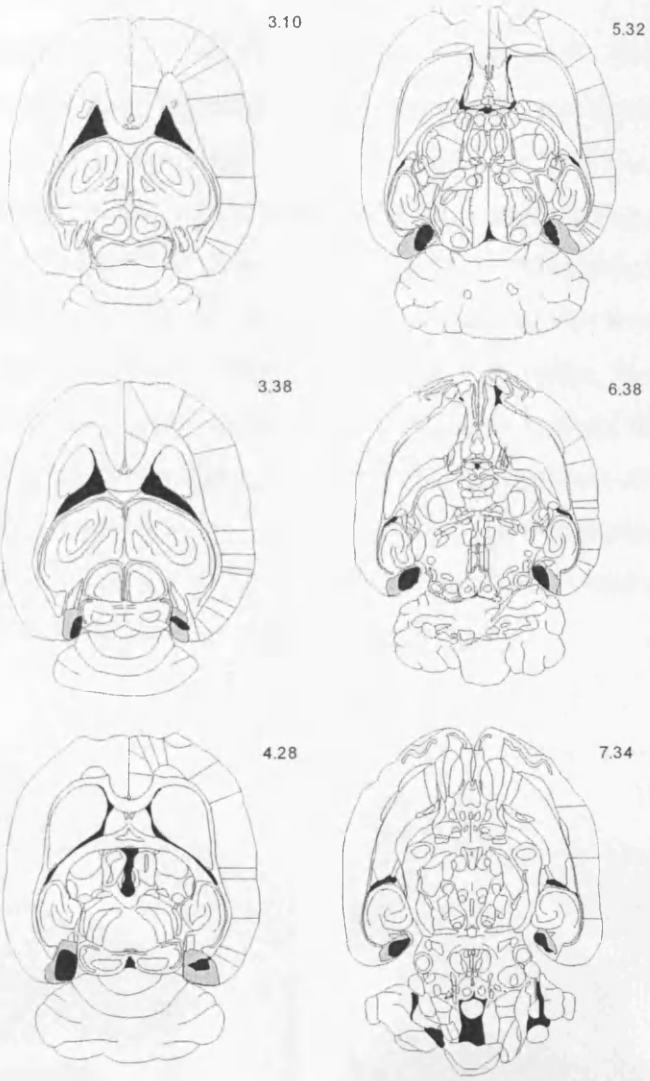


Figure 6.2 Extent of the largest (grey) and smallest (black) ERC lesions. Numbers indicate mm below the horizontal plane passing through bregma and lambda. Horizontal drawings taken from Paxinos and Watson (1998).

6.3.1.2 Bilateral Fx lesions

Figure 6.3 illustrates the AChE staining, figure 6.4 shows representative photomicrographs of a sham (A) and a lesion animal (B) and figure 6.5 illustrates the minimum and maximum lesion extent. All lesions involved cell loss in the Fx, although in most cases there was sparing of the lateral tips; AChE staining was reduced in all cases although it was not completely abolished. These two characteristics suggest that the aspirative lesion did not descend far enough down ventrally, thus leaving some of the Fx tract intact, and as such must be described as a partial, rather than complete, lesion. There was also substantial aspirative damage to cingulate, M1 and M2 cortices. One of the ten Fx rats died soon after surgery and two were excluded from all data analysis based on histology, leaving a group size of seven. One of these excluded rats had received substantial damage to the dorsal HPC, whilst the other showed intact acetylcholinesterase (AChE) staining in the HPC and minimal cell loss in the Fx.

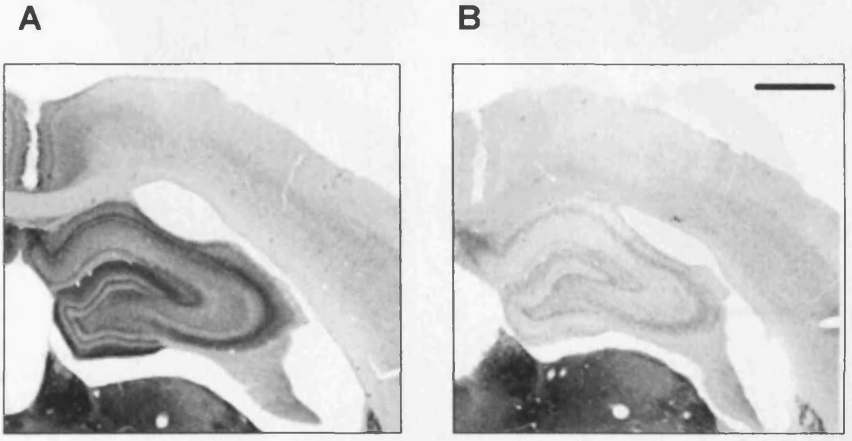


Figure 6.3 AChE staining in coronal sections of a sham Fx lesion (A) and a Fx lesion (B). Scale bar =1 mm.

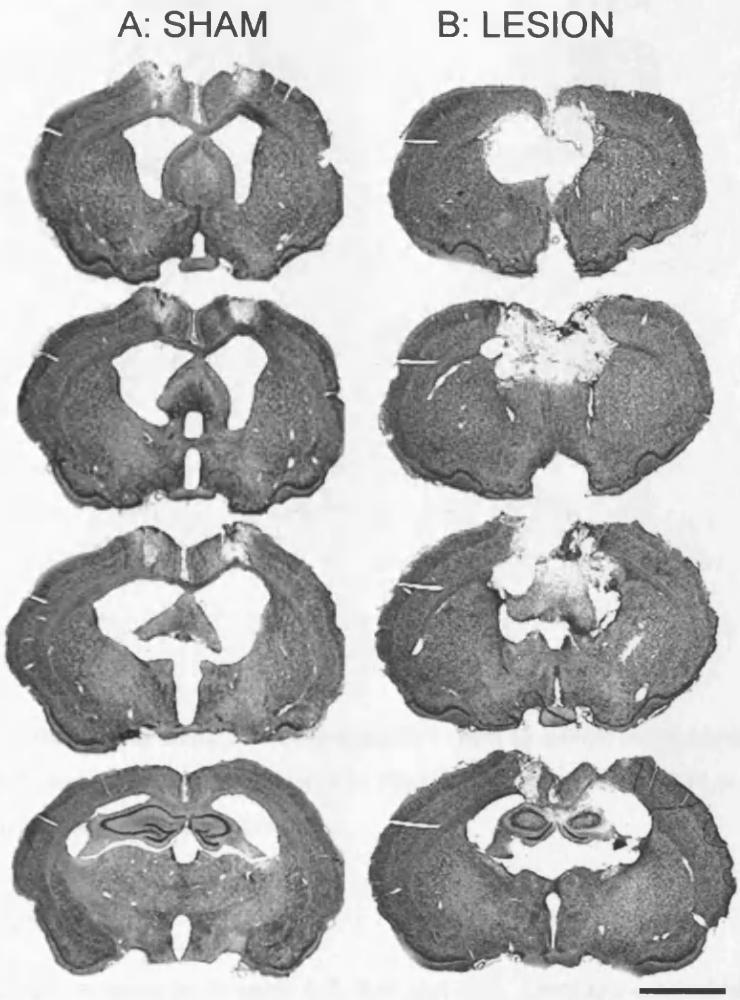


Figure 6.4 Photomicrographs of coronal sections stained with Cresyl Violet. Sham Fx lesion ~0.92 mm posterior to bregma (A). Aspirative Fx lesion ~0.92 mm posterior to bregma (B). Scale bar =2 mm.

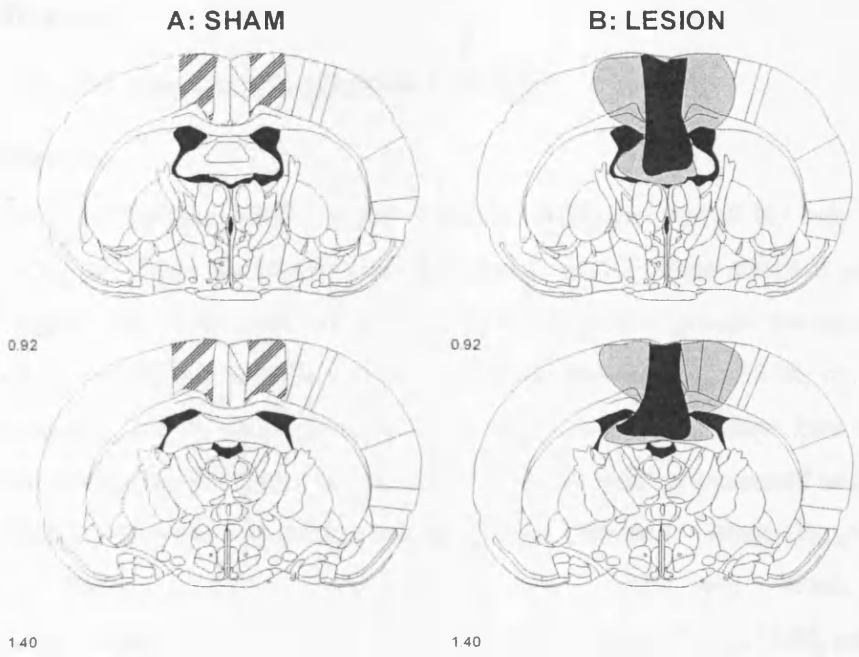


Figure 6.5 Extent of the cortical damage in a representative sham Fx animal (striped areas) (A), extent of the largest (grey) and smallest (black) Fx lesion (B). Numbers indicate mm posterior to bregma, coronal drawings taken from Paxinos and Watson (1998).

6.3.1.3 Sham lesions

Sham Fx lesions can be seen in figures 6.3, 6.4 and 6.5. Damage extended to the corpus callosum and cortical damage was similar to that in the Fx lesions except that in most cases the most medial portion of the cingulate cortex was spared. ERC shams incurred no cortical damage; however one of these animals had to be sacrificed due to poor health. The two sham groups were not seen to differ significantly from each other in the behavioural assays and as such the two groups were pooled for clarity giving a control group of nine.

6.3.2 Behaviour

6.3.2.1 Delayed matching to position (DMTP)

6.3.2.1.1 Baseline

After reaching asymptotic levels of performance, rats were tested for two additional baseline blocks of 5 sessions to allow allocation into performance-matched groups. As shown in figure 6.6, there was no difference between the groups preoperatively in measures of % correct ($F_{(2,22)}$=0.37, ns), total trials performed ($F_{(2,22)}$=0.78, ns) or rate of panel pressing ($F_{(2,22)}$=0.19, ns). Data were also analysed across delays (see figure 6.7) which demonstrated that performance decreased as delay interval increased and that there was no difference between the groups preoperatively (Week 1: Group ($F_{(2,22)}$=0.66, ns), Delay ($F_{(6,132)}$=103.11, p<0.01), Group x Delay ($F_{(12,132)}$=0.83, ns). Week 2: Group ($F_{(2,22)}$=0.47, ns), Delay ($F_{(6,132)}$=102.44, p<0.01), Group x Delay ($F_{(12,132)}$=1.00, ns)).

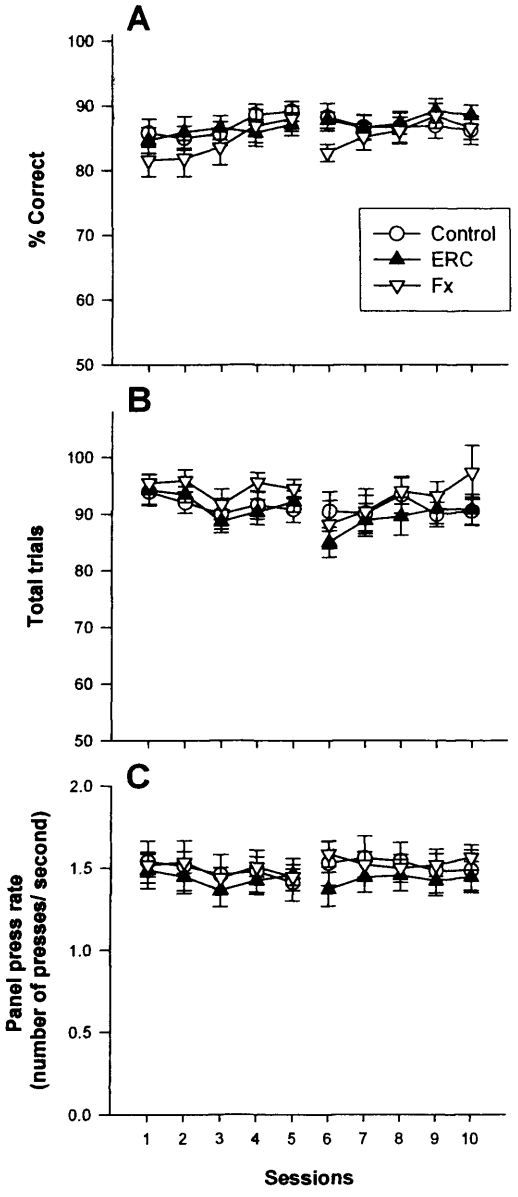


Figure 6.6 DMTP3 baseline data for animals used in each group. % Correct against sessions (A), total trials against sessions (B), panel press rate against sessions (C). Data expressed as mean ± SEM (Control n=9, ERC n=9, Fx n=7).

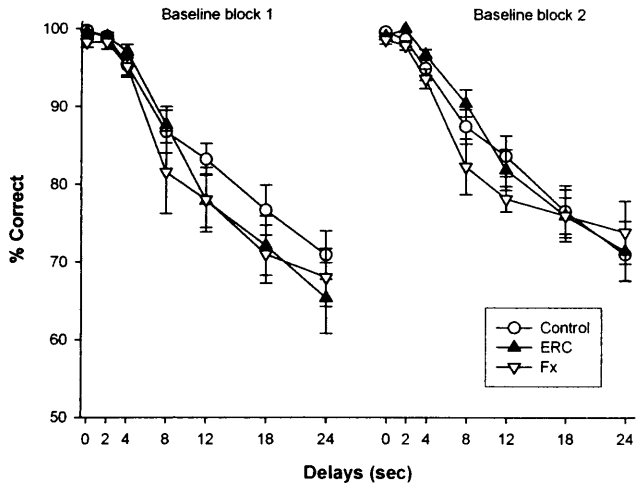


Figure 6.7 % Correct against delays for blocks 1 and 2 of baseline DMTP3. Data expressed as mean ± SEM (Control n=9, ERC n=9, Fx n=7).

6.3.2.1.2 Postoperative testing

Figure 6.8 shows % correct across sessions both before and after surgery. Analysing the first 20 sessions of DMTP3 following surgery revealed a significant effect of group ($F_{(2,22)}$=7.53, p<0.01), and session ($F_{(19,418)}$=15.01, p<0.01), but no group by session interaction ($F_{(38,418)}$=1.18, ns). *Post hoc* analysis revealed that this group difference was due to the significant impairment of the Fx group relative to both the ERC and control groups (Newman-Keuls: Fx vs. ERC and Control p<0.01), which did not differ; all groups improved their performance with session progression. Data were also assessed by comparing across the baseline block and the first postoperative block, with block as a within subject factor. This analysis revealed significant main effects of group ($F_{(2,22)}$=7.07, p<0.01) and block ($F_{(1,22)}$=118.02, p<0.01), with the Fx group being impaired compared with controls and ERC. In addition there was an interaction between block and group ($F_{(2,22)}$=14.70, p<0.01) with the Fx group showing the most significant drop in performance after surgery. The 5-session block of DMTP4 failed to show any group differences ($F_{(2,22)}$=2.19, ns) or any group by session interaction ($F_{(8,88)}$=0.89, ns), however there was an effect of session ($F_{(4,88)}$=3.88, p<0.01). Therefore the Fx group were impaired compared with the ERC and control groups, which did not differ, and the introduction of the longer delay set did not reveal any further deficits.

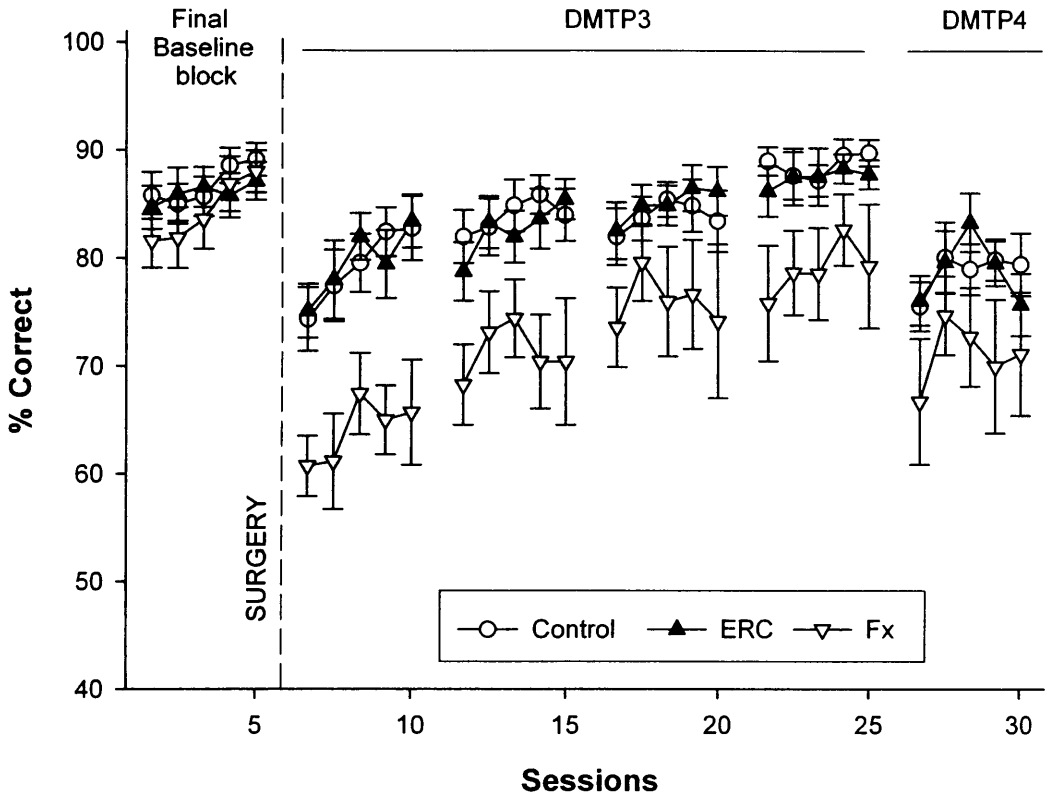


Figure 6.8 % Correct against sessions. DMTP3 indicates a delay set of 0-24 sec, DMTP4 indicates a delay set of 0-40 sec. Data expressed as mean ± SEM (Control n=9, ERC n=9, Fx n=7).

Data were analysed across delays to allow further assessment of the nature of the deficit seen in the Fx group. Figure 6.9 shows the data averaged across all four blocks of DMTP3 testing. There was a clear effect of lesion group ($F_{(2,22)}$=7.17, p<0.01) and delay ($F_{(6,132)}$=91.05, p<0.01), but no group by delay interaction ($F_{(12,132)}$=0.97, ns). *Post hoc* analysis verifies that this group effect was due to the impairment of the Fx group compared with both the ERC and control groups (Newman-Keuls: Fx v. ERC and Control p<0.01). Therefore the Fx group was significantly impaired at all delays, suggestive of a non-mnemonic deficit.

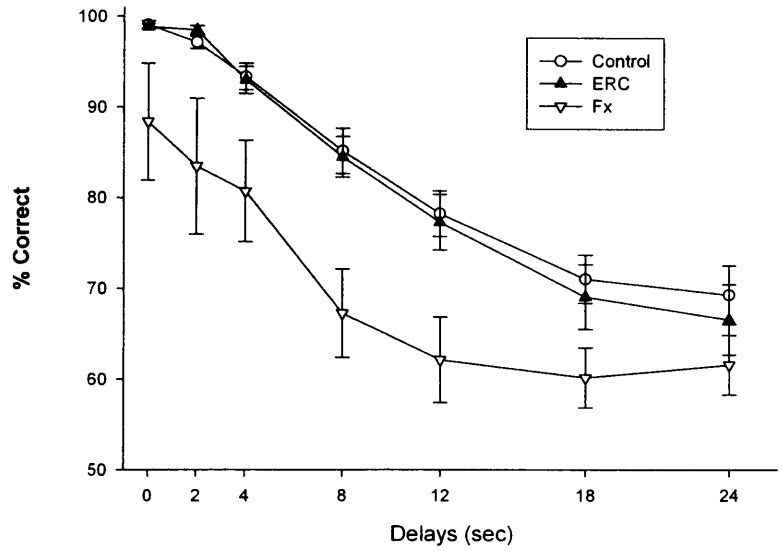


Figure 6.9 % Correct against delays averaged across all four blocks of DMTP3 testing. Data expressed as mean ± SEM (Control n=9, ERC n=9, Fx n=7).

Figure 6.10 shows the data across sessions for the final block of DMTP testing where delays were reduced to between 0 and 6 sec, the three subsequent DNTMP1 blocks and then the final DNMTP3 block. The block of DMTP1 testing was performed to minimise any differences between the three groups before performing the rule switch, this was demonstrated by the lack of any group effect ($F_{(2,22)}$=2.30, ns) or any group by session interaction ($F_{(8,88)}$=11.21, ns), with only an effect of delay ($F_{(4,88)}$=3.87, p<0.01) apparent in this block. The implementation of the rule switch did not effect any of the groups differentially; analysing the first 15 sessions of DNMTP1 testing showed no effect of group ($F_{(2,22)}$=1.16, ns) or any group by session interaction ($F_{(28,308)}$=1.03, ns), with a clear main effect of session ($F_{(14,308)}$=147.80, p<0.01). Furthermore, the final DMTP block was compared with the first DNMTP block, with a significant effect of block being revealed ($F_{(1,22)}$=339.09, p<0.01). Crucially, this analysis did not reveal any effect of group ($F_{(2,22)}$=1.23, ns) or interactions between group, block and session (Group x Block ($F_{(2,22)}$=0.68, ns); Group x Session ($F_{(8,88)}$=0.50, ns); Group x Session x Block ($F_{(8,88)}$=1.10, ns)). Therefore all groups were impaired by the switch but learnt and adapted to the new rule at a similar rate.

Finally, the fourth block of DNMTP was analysed; in this block the original delay set of 0-24 sec was reinstated. In this final block of DNTMP there was no effect of group

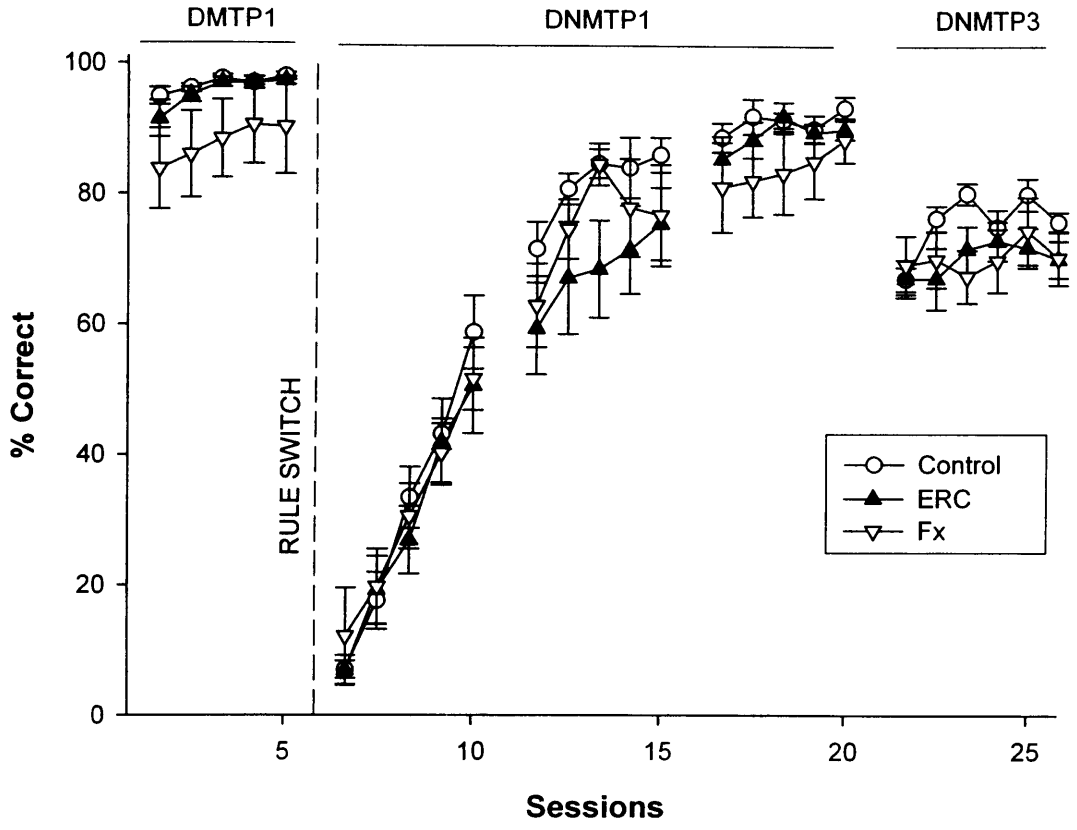


Figure 6.10 % Correct against sessions for DMTP1 (0-6 sec delays), DNMTP1 and DNMTP3 (0-24 sec delays). Data expressed as mean ± SEM (Control n=9, ERC n=9, Fx n=7).

($F_{(2,22)}$=1.33, ns), suggesting that the lesion-induced deficit in the Fx group was transient in nature. Panel presses and latency to the 1st panel press after the delay were both investigated, in order to provide further clarification of the deficit. These two measures were assessed collapsed across the four blocks of DMTP3 testing (figure 6.11). Panel press data just failed to reveal any effect of group ($F_{(2,22)}$=2.88, ns), however there was both a significant effect of delay ($F_{(6,132)}$= 348.42, p<0.01) and a group by delay interaction ($F_{(12,132)}$=2.40, p<0.01). *Post hoc* tests revealed that the Fx group performed significantly fewer panel presses than the control and ERC groups at the 3 longest delays (Newman-Keuls: Fx v. Control and ERC, p<0.05). Latency data did reveal a group effect ($F_{(2,22)}$=7.50, p<0.01) and an effect of delay ($F_{(6,132)}$=26.50, p<0.01), but no group by delay interaction ($F_{(12,132)}$=0.76, ns). *Post hoc* analysis revealed that the Fx group exhibited

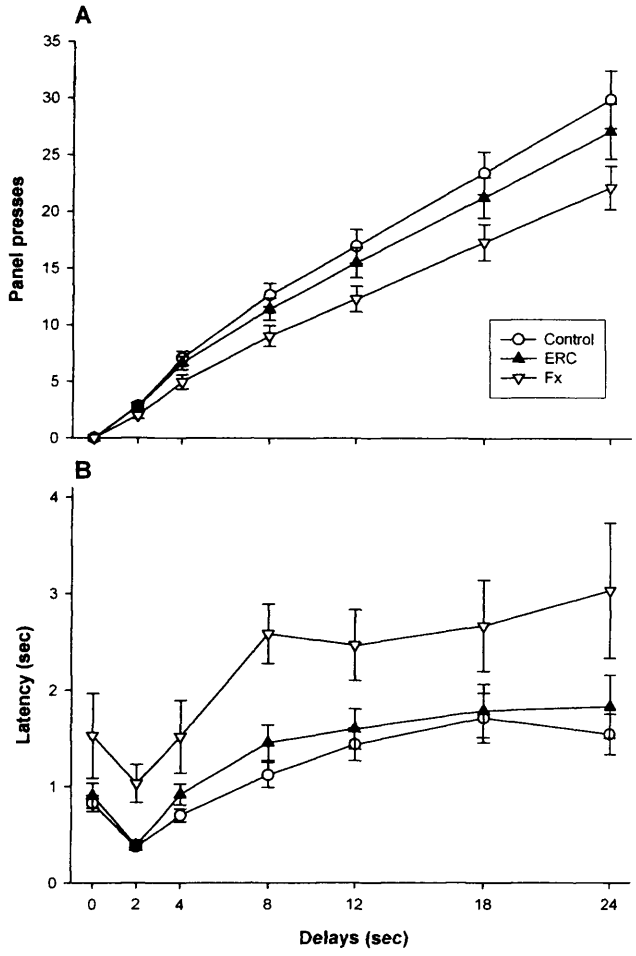


Figure 6.11 Panel presses (A) and latency (B) against delays, averaged across the four blocks of DMTP3 testing. Data expressed as mean ± SEM (Control n=9, ERC n=9, Fx n=7).

significantly longer latencies than the control and ERC groups (Newman-Keuls: Fx v. Control and ERC, $p<0.01$).

In addition, measures derived from SDT were also analysed, figure 6.12 shows measures corresponding to accuracy and figure 6.13 shows measures corresponding to bias. The accuracy measure *A'* revealed effects of group, delay and a group by delay interaction (Group: ($F_{(2,22)}=6.02$, $p<0.01$), Delay: ($F_{(6,126)}=98.67$, $p<0.01$), Group x Delay ($F_{(12,132)}=2.07$, $p<0.01$)). The group effect was due to the difference between the Fx group and the other two groups (Newman-Keuls: Fx v. Control and ERC, $p<0.01$), while the interaction resulted from the Fx group differing from the ERC and controls only at the four longest delays. SI revealed similar results, except that there was no interaction

between group and delay (Group: ($F_{(2,22)}$=4.59, p<0.05), Delay: ($F_{(6,132)}$=191.58, p<0.01), Group x Delay ($F_{(12,132)}$=0.87, ns)). The bias measure, I_y, revealed a significant effect of group ($F_{(2,22)}$=8.57, p<0.01) and delay ($F_{(6,132)}$=15.02, p<0.01) but no group by delay interaction ($F_{(12,132)}$=0.48, ns), with *post hoc* tests confirming that the Fx group differed from both the control and ERC groups (Newman-Keuls: Fx v. Control and ERC, p<0.01). *RI* revealed an effect of group ($F_{(2,22)}$=4.40, p<0.05), with the Fx group differing from the control and ERC groups (Newman-Keuls: Fx v. Control and ERC, p<0.05). This group difference did not reach significance for the B'' measure (Group ($F_{(2,22)}$=2.69, ns).

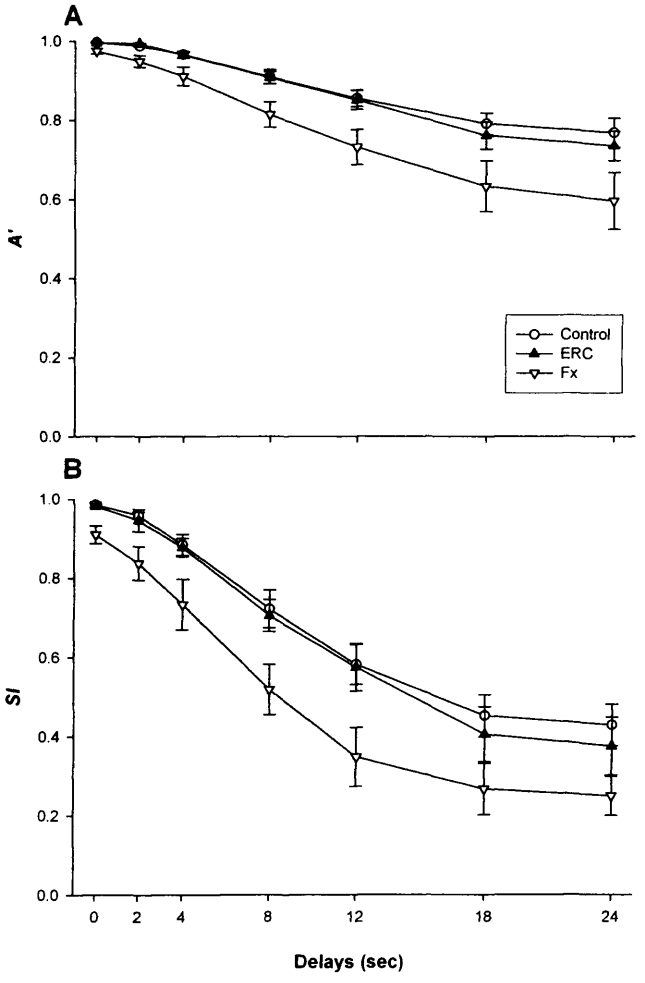


Figure 6.12 Accuracy measures based on SDT for average across all four blocks of DMTP3, A' (A), *SI* (B). Data expressed as mean ± SEM (Control n=9, ERC n=9, Fx n=7).

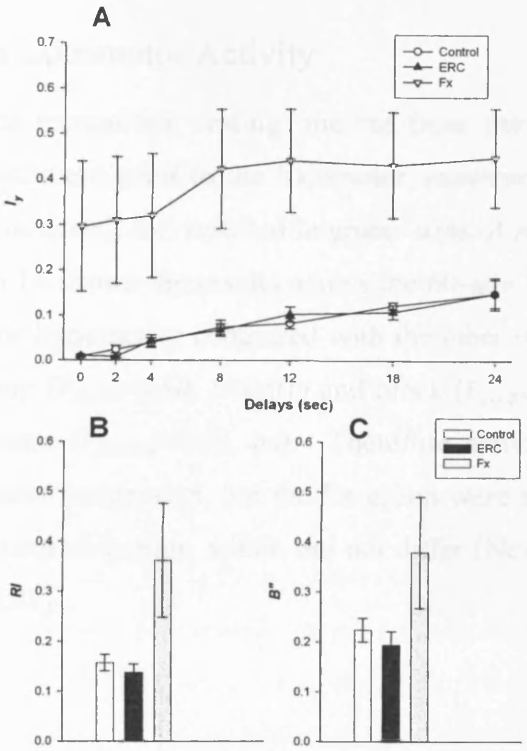


Figure 6.13 Bias indices for average across all four blocks of DMTP3, I_y (A), *RI* (B) and *B"* (C). Data expressed as mean ± SEM (Control *n*=9, ERC *n*=9, Fx *n*=7)

6.3.2.2 Spontaneous Locomotor Activity

In the course of object recognition testing one rat from the ERC group had to be sacrificed due to ill health and prior to the locomotor assessment another ERC rat had become ill and was not assessed; this resulted in group sizes of n=9 (control), n=7 (ERC) and n=7 (Fx). Figure 6.14 shows the results across the 60-min session in 5-min blocks. Fx rats were shown to be hyperactive compared with the other two groups. There was a significant effect of group ($F_{(2,20)}$=5.50, p<0.01) and block ($F_{(11,220)}$=18.40, p<0.01) but no group by block interaction ($F_{(22,220)}$=0.62, ns). Therefore activity decreased uniformly across all groups as session progressed, but the Fx group were significantly more active than both the ERC and control groups, which did not differ (Newman-Keuls: Fx v. ERC p<0.05 and Control p<0.01).

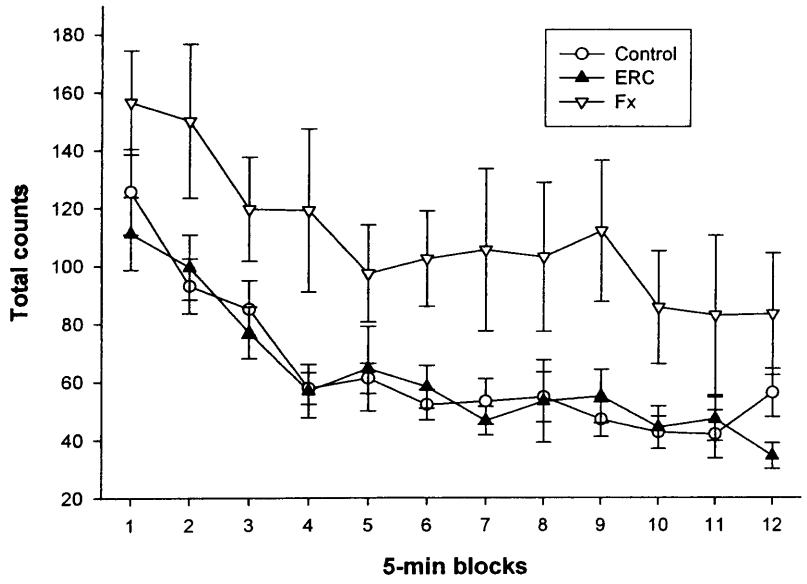


Figure 6.14 Total activity counts against 5-min blocks. Data expressed as mean ± SEM (Control n=9, ERC n=7, Fx n=7)

6.4 Discussion

This experiment sought to examine the effects of ERC and Fx lesions on retention of the DMTP task and its subsequent switch to DNMTP. The ERC lesion was completely without effect on this task and the Fx lesion induced a delay-independent deficit, suggesting a non-mnemonic impairment.

Retention of DMTP: ERC lesions

Excitotoxic lesions that damaged the medial ERC were wholly without effect on retention of DMTP; they were also without effect on the ability to adapt to the switch in the rule from matching to non-matching. These results suggest that the medial ERC does not play a key role in the type of working memory assessed in this task, with performance paralleling control levels even at the longest delay intervals. To my knowledge, the only study to have addressed the specific issue of involvement of the ERC in DMTP is that of Pouzet et al (1999b). This study provides a good comparison with the present experiment, with excitotoxic lesions being utilised (NMDA-induced), although rats were tested for acquisition of DMTP rather than retention. This study found no effect of the lesion group at any point even up to delay intervals of forty seconds, in agreement with the findings in the present experiment on retention of DMTP. Pouzet et al (1999b) also subjected their rats to a rule switch from matching to non-matching, which also failed to reveal any effect of the ERC lesion.

The ERC does not have a uniform cytoarchitecture; instead it is comprised of two distinct subdivisions known as the lateral and medial ERC (Witter et al., 2000). One potential explanation for the lack of effect of the ERC lesions in the present study might be that the lateral ERC was completely spared in almost all cases. These two subdivisions not only have different projection fields within the HPC (Witter et al., 2000) but they also receive distinct afferent projections from the perirhinal cortex (projects to lateral ERC) and the postrhinal cortex (projects to medial ERC) (Burwell and Amaral, 1998b; Suzuki, 1996; Witter et al., 1989). Therefore, it is thought that at least two parallel pathways exist mediated via the perirhinal and postrhinal cortices, although the presence of a functional dissociation between them has yet to be confirmed (Aggleton and Pearce, 2001; Burwell and Amaral, 1998a; Witter et al., 2000). Furthermore, this

anatomical distinction in connectivity extends to the entorhinal projections to the PFC, with the medial ERC projecting to the IL, and the lateral area projecting to the PrL (Insausti et al., 1997). One could therefore argue that if it were damage to the PrL rather than the IL that contributed to the deficit seen in the PFC group from Experiment 1, then the integrity of this pathway might spare performance on this task. The counter to this argument would be that lesion of the lateral area would be expected to impair performance; however Pouzet et al's (1999b) lesions encompassed the lateral area and were still without effect. Finally there is evidence from studies in monkeys (Leonard et al., 1995) and mice (Cho and Jaffard, 1994) that lesions of the ERC might effect acquisition rather than retention of delayed matching tasks. However, the Pouzet et al study (1999b) indicates that ERC lesions are similarly without effect on acquisition of DMTP. Thus, from the present results, there is no evidence in support of the ERC being involved in working memory of this nature.

Retention of DMTP: Fx lesions

Aspirative lesions of the Fx induced a deficit on retention of DMTP and were without effect on the switch between the task contingencies. The deficit was apparent in % correct data and the two SDT derived accuracy measures *A'* and *SI*, with % correct and *SI* showing a delay-independent deficit. The accuracy measure *A'* did however reveal some evidence for delay-dependency, with a significant group by delay interaction due to the Fx group differing from the ERC and control groups at the 4 longest delay intervals. The Fx group also exhibited significant differences compared with the other two groups in the side bias measures I_y and *RI*, but not the perceptual bias measure *B"*; they also displayed a reduction in panel presses that was only apparent at the 4 longest delay intervals. These findings concur with previous reports of Fx-induced deficits on D(N)MTP tasks, however the majority of these have shown delay-dependent deficits (Aggleton et al., 1991; Aggleton et al., 1995; Chudasama and Muir, 1997; Dunnett, 1985; Dunnett, 1990; Ennaceur et al., 1996; Weiner et al., 1998). The increase in bias observed in the present study has also been reported previously in Fx-lesioned rats (Aggleton et al., 1992; Aggleton et al., 1995; Ennaceur et al., 1996; Winters and Dunnett, 2004).

The predominantly delay-independent deficit described in the present experiment is not completely uncharacteristic, with Fx lesions resulting in a similar deficit in an

operant chamber equipped with ports and not levers (Young et al., 1996). Moreover, Aggleton and colleagues (1992) report a deficit on DNMTP that is "consistent with mnemonic impairment", however this is not corroborated by a delay by group interaction. The most significant study for comparison with the present findings is that of Winters and Dunnett (2004), where aspirative Fx lesions were assessed on retention of DNMTP and a subsequent switch to DMTP. Interestingly this study failed to reveal any Fx-induced deficit in DNMTP until the third postoperative 5-session testing block, at which point the deficit was not significantly delay-dependent. Rats were not impaired on acquiring the switched rule, which mirrors the results of the present study in which the opposite order of rules was applied. This provides confirmation that the intact acquisition performance in both the present study and Winters and Dunnett's (2004) cannot be accounted for by the specific nature of the rule in question. Finally, after these rats had acquired the new task contingency (DMTP) delays were introduced; after two blocks of testing the Fx deficit was again apparent, but on this occasion it was shown to be only at the longer delay intervals (Winters and Dunnett, 2004).

A complementary version of the D(N)MTP task has been investigated using the 9-hole box apparatus (Etherington et al., 1987). In this task, aspirative Fx lesions induced a deficit that the authors described as delay-dependent. However, it would appear that this "delay-dependent" effect may in fact have resulted from the Fx group showing a distinctive improvement in performance at the longest delay interval. Furthermore, although the Fx group were undoubtedly impaired compared with the controls, there is evidence for impairment at the shortest delays from the graphical data (Etherington et al., 1987); this suggests that the deficit may be comparable with that revealed in the present study.

The present results are indicative of a non-mnemonic impairment in the rats with Fx damage, however, the ability to acquire the new rule at a rate akin to the control animals suggests that general levels of motivation and sensory and learning abilities must be intact. The deficit on accuracy might be secondary to the development of the side bias (I_y), as both are apparent at all delay intervals. This significant impairment is clearly in stark contrast with the intact performance shown in the HPC-lesioned rats in Experiment 1. One study has shown that Fx lesions can have more pronounced behavioural

consequences than selective lesions of the HPC in a water maze task (Whishaw and Jarrard, 1995b). Thus it is a possibility that damage to the Fx disrupts fibres that are associated with extra-hippocampal structures which may be important for performance on this task.

Spontaneous locomotor activity

Spontaneous locomotor activity was assessed over a 60-min session and provided unambiguous results. ERC lesions did not result in an increase in locomotor activity when compared with controls; this is in line with previous findings (Coutureau et al., 2000; Hagan et al., 1992), except for one study which did demonstrate hyperactivity but this was when activity was assessed in the home cage and not a novel environment (Galani et al., 1998). Fx lesions induced hyperactivity compared with controls, this activity decreased across the session but remained significantly above both the controls and the ERC group throughout. This is in agreement with previous work (Bussey et al., 2000; Coutureau et al., 2000; Dunnett, 1990; Pouzet et al., 1999a; Weiner et al., 1998; Whishaw and Jarrard, 1995b) and suggests that severing the Fx pathway might be assumed to remove the level of inhibitory control normally provided by the HPC (Tracy et al., 2001).

Conclusions

The primary aim of this experiment was to investigate the involvement of the ERC in the DMTP task. The results are unambiguous and suggest that the medial ERC does not have a role in this working memory task. Moreover, it is unlikely that lesions encompassing the lateral ERC would induce a deficit (Pouzet et al., 1999b). The lack of dissociation between the ERC group and controls indicates that this particular model could not be a candidate for investigating the cognitive deficits that accompany Alzheimer's disease.

Lesions of the Fx were also investigated and an impairment in the DMTP task was revealed. This provided not only validation of the task, but also a comparison with the HPC-lesioned group in Experiment 1. The dissociation between the effects of Fx lesions and the ERC or HPC lesions is consistent with the idea that the separate components of the hippocampal formation may have independent and complementary roles within the memory system. It is likely that performance in a task of this nature

involves a complex interplay between a number of different neural structures and perhaps disconnection studies might be able to provide greater insight into these relationships.

Chapter 7 Object recognition - spontaneous novelty preference task: effect of prefrontal and hippocampal system lesions.

Experiments 4 and 5

7.1 Introduction

The aim of the experiments presented within this chapter is to investigate the involvement of the PFC and the hippocampal system in a test of object recognition memory known as the spontaneous novelty preference task. In the standard version of this task a rat is placed into an open-field arena and allowed to explore two identical sample objects, usually until a certain amount of exploration has been accumulated (Ennaceur and Delacour, 1988). The rat is then removed from the arena for a delay period, following which it is returned to the arena which contains one identical copy of the object from the sample phase and one completely novel object; the amount of time spent exploring each object is subsequently recorded over a fixed period of time. This task exploits the rat's natural tendency towards exploring novel objects over familiar objects, thus a normal rat will spontaneously explore the novel object in preference to the object it has encountered previously, provided it retains some memory of the sample object. The difference between the time spent exploring the novel versus the familiar object is taken as an index of recognition memory, with normal rats typically being able to discriminate between the two objects up to delays of 24 hours (Ennaceur et al., 2005; Ennaceur and Delacour, 1988; Mumby, 2001). Rats can display intact recognition memory for objects encountered as long as 5 weeks before retest (Gaskin et al., 2003).

This task benefits from being free from a reference memory component, thus rats are not required to learn and remember a particular contingency. Therefore any impairment resulting from a particular treatment can be regarded as an indication that the rat cannot discriminate between the two objects (Ennaceur and Delacour, 1988).

However, an alternative interpretation of such a deficit might be that the treatment has simply disrupted the rat's natural bias for the novel object; this hypothesis could only be rejected categorically if discrimination remained intact at minimal delays (Mumby, 2001).

The spontaneous novelty preference task was originally developed to serve as a rat alternative to the trial-unique DNMS task of the monkey; however it is more strictly analogous to the visual paired-comparison (VPC) task, in which subjects are presented with two pictures, one novel and one familiar, and the tendency to look at the novel picture is recorded (Bachevalier et al., 1993; Mumby, 2001). However, in the spontaneous novelty preference task rats may also utilise tactual properties of the objects in addition to the visual properties provided in the VPC task. Amnesic patients (McKee and Squire, 1993) and monkeys with HPC damage (Bachevalier et al., 1993; Nemanic et al., 2004; Zola et al., 2000) have both been shown to be impaired on the VPC task, implicating the HPC in this type of novelty detection.

Further evidence for a hippocampal role in novelty detection comes from a study in which HPC damaged patients exhibited reduced characteristic intracranial ERPs upon presentation of novel stimuli (Knight, 1996). However, it is thought that the role of the HPC in recognition memory may have been overemphasised (Mumby, 2001), with considerable evidence pointing to a more significant involvement for the adjacent rhinal cortical areas (Aggleton et al., 1997; Bussey et al., 1999; Meunier et al., 1993; Mishkin and Murray, 1994; Mumby and Pinel, 1994; Winters et al., 2004; Winters and Bussey, 2005). Indeed, in the rat, HPC lesions tend to leave spontaneous object recognition performance intact (Galani et al., 1998; Gaskin et al., 2003; Mumby et al., 2002; Winters et al., 2004), although there has been an exception to this finding (Clark et al., 2000).

Despite PFC involvement in the DNMS (Fuster, 1997; Kolb, 1990a) and DNMTP tasks (Broersen, 2000; Chudasama and Muir, 1997; Dunnett et al., 1990; Herremans et al., 1996; Joel et al., 1997b; Mair et al., 1998), evidence suggests that rats with PFC lesions are not impaired on discriminating between the novel and familiar objects in the spontaneous novelty preference task (Ennaceur et al., 1997; Granon et al., 1996; Hannesson et al., 2004a; Mitchell and Laiacona, 1998; Mogensen et al., 2004; Yee, 2000).

The spontaneous novelty preference paradigm has been successfully adapted to investigate different facets of recognition memory, such as the ability to remember the position of an object, and the ability to judge the relative recency of previously encountered objects. Two main versions exist for assessing the memory for location of objects; in the first, the sample phase is identical to the standard configuration but in the test phase two identical copies of the original objects are present, but one is positioned in a novel location. Normal rats will discriminate between the two objects based on their locations, and therefore spend more time exploring the displaced object (Dix and Aggleton, 1999). The second version requires memory for both object and position, in the previous version recognition of the object *per se* need not be intact to allow discrimination based on the location. Therefore, in this second "spatial shift" version rats explore four different objects in the sample phase, following a delay they then explore these same four objects but two of the objects have their locations switched. Normal rats are able to discriminate between those objects that have been displaced and those objects that have remained in their original position (Dix and Aggleton, 1999). This task assesses memory for the objects and their location within the environment, an ability often attributed to the HPC (especially the right side) in the human (Crane and Milner, 2005; Milner et al., 1997; Pigott and Milner, 1993; Spiers et al., 2001).

The version that assesses memory for the relative familiarity of objects, or "recency" consists of three phases and was first employed by Mitchell and Laiacona (1998). There are two sample phases, separated by a one hour delay, in which two different pairs of identical objects are explored. Following a delay the test phase occurs, in which one copy of each of the previously explored objects is present in the arena. These two objects now differ in their relative familiarity and a normal rat will direct more exploration towards the object that was seen least recently, i.e. the object from the 1st sample phase. This task has been validated by Hannesson et al (2004a), who demonstrated that rats had not merely forgotten the first object (thus equating the task to the standard version), as they would reliably explore a completely novel object in preference to the first object in the test phase. The PFC has been implicated in temporal memory in the human, with frontal lobe damage routinely impairing the ability to make recency judgements (Butters et al., 1994; McAndrews and Milner, 1991; Milner et al.,

1985; Milner et al., 1991; Milner and Petrides, 1984; Shimamura et al., 1990; Zorrilla et al., 1996). There is also some evidence to suggest that the HPC might be involved in the capacity to judge recency under certain circumstances (Charles et al., 2004; Fortin et al., 2002; Kesner et al., 2002).

The experiments described in this chapter assess the effects of PFC and HPC lesions (Experiment 4) and ERC and Fx lesions (Experiment 5) on the spontaneous novelty preference task. Three separate versions are investigated; these are the standard configuration (at two separate retention delays), the spatial shift configuration and finally the recency version. The aim is to establish the relative involvements of the lesioned areas within these tasks. It is hypothesised that damage to the HPC, by way of cytotoxic lesion, Fx transection or ERC lesion, might disrupt discrimination in the spatial shift task, with this area being responsible for this type of object-location specific memory in the human. Conversely, the PFC may play a more significant role in the recency task, although the HPC has also been implicated in temporal memory.

7.2 Materials and methods

7.2.1 Experiment 4:PFC and HPC lesions

The rats used in this experiment had previously been used in experiment 2, described in Chapter 5; they had therefore been tested on acquisition of DMTP in the Skinner box and had also received water maze testing. All subject details are outlined in section 5.2 with histological results discussed in section 5.3.1. Following histology, group sizes were control (n=12), HPC lesions (n=9) and PFC lesions (n=6). All details of the spontaneous novelty preference task are discussed in detail in section 2.6. Rats received testing on a total of three different versions, namely the standard configuration (at delay intervals of 2 min or 2 hr), spatial shift and recency tasks. Each rat was exposed to each task for 3 repetitions, with the averaged data from these repetitions being used in analysis. Each rat was exposed to each object only once, objects used and the side of the novel object, or side of the spatial shift, were fully counterbalanced within and between groups.

Due to the exclusion of those rats that did not explore the sample objects for at least 40 seconds in the standard and recency tests, or 80 seconds in the spatial shift, group

sizes varied between tests and are noted in the figure legends. During the course of this experiment one rat had to be sacrificed due to illness, this will be similarly detailed in the appropriate figure legends.

7.2.2 Experiment 5: ERC and Fx lesions

The rats used in this experiment had previously been used in experiment 3, reported in Chapter 6; they had therefore been tested on retention of the DMTP task in the Skinner box. All subject details are outlined in section 6.2 with histological results discussed in section 6.3.1. Following histology, group sizes were control (n=9), ERC lesions (n=9) and Fx lesions (n=7). All details of the spontaneous novelty preference task are discussed in detail in section 2.6 and above in section 7.2.1. This experiment commenced following the completion of experiment 4, therefore methods and objects used were identical between the two experiments. During the course of this experiment three rats had to be sacrificed, this will be detailed in the appropriate figure legends.

7.3 Results

7.3.1 Experiment 4: PFC and HPC lesions

7.3.1.1 Habituation

Data collected during the habituation sessions were used to validate the manually recorded exploration time and also to judge a suitable cut-off point for exploration in the sample phase of the tasks. Figure 7.1 shows the exploration time for the manually recorded exploration time measure and the software recorded "time in zone" measure, over a 5-min habituation session in which two identical objects were present in the arena.

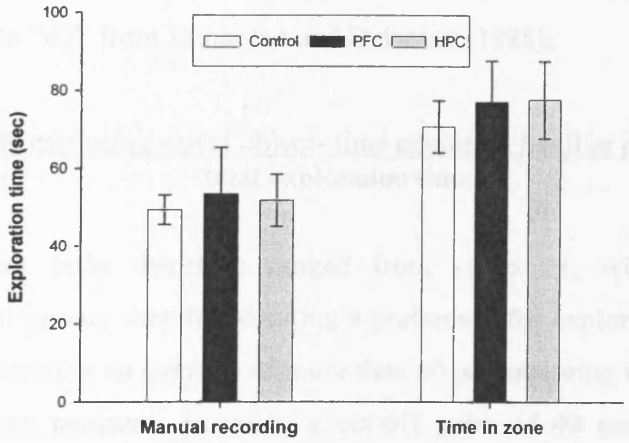


Figure 7.1 Experiment 4: PFC and HPC lesions. Exploration time against two different measures during a 5-min habituation session where two identical novel objects were present. Data expressed as mean ± SEM (Control n=12, PFC n=6, HPC n=8 (1 rat did not explore the objects)).

An ANOVA, with group as the between subject factor and measure as the within subject factor, revealed that there was no effect of group ($F_{(2,23)}$=0.59, ns) or any group by measure interaction ($F_{(2,23)}$=1.87, ns), however there was a clear effect of measure ($F_{(1,23)}$=51.68, p<0.01), with all groups showing a greater exploration time in the "time in zone" measure. The Pearson product moment correlation coefficient (r) was calculated to assess the correlation between these two measures (r =0.83); this shows that there was a highly significant correlation to a confidence level of 0.05%. Thus the same pattern

between groups was seen in each measure, but a greater overall exploratory time was recorded by the software. This greater time was most likely to be due to the software recording the amount of time spent within a defined zone around the object, irrespective of the rats' behaviour within that zone, e.g. this included passes through the zone where no object exploration occurred, sitting in the zone but heading in the opposite direction and grooming within the zone. Recording these behaviours as "exploratory time" is clearly not valid in this task, in which actual exploration of the object is the crucial factor.

Therefore the "time in zone" measure was used only for computing the cut-off point for each rat in the sample phase, thus providing a similar object exposure time for each rat. The manually recorded time spent exploring the objects was therefore used in all test data, as this was deemed to be more accurate than the "time in zone " measure for the reasons discussed above. This measure was also used to calculate the "discrimination ratio", equivalent to "d2" from Ennaceur and Delacour (1988):

$$\frac{\text{(time exploring novel object- time exploring familiar object)}}{\text{total exploration time}}$$

This discrimination ratio therefore ranged from -1 to +1, with 0 indicating no discrimination, and greater than 0 indicating a preference for exploring the novel object. All groups were spending an average of more than 60 sec exploring the objects according to the time in zone measure; as such, a cut-off point of 40 sec was chosen to be conservative enough to allow the inclusion of the majority of animals in each test.

Figure 7.2 shows exploration time against the side of the object in the arena, to assess if the rats were showing any side bias. There was no effect of group ($F_{(2,23)}$=0.09, ns), side ($F_{(1,23)}$=1.34, ns) or any group by side interaction ($F_{(2,23)}$=0.10, ns). Despite this demonstration of equivalent exploration of the objects regardless of side, the side of the "novel" object in each test was always fully counterbalanced to ensure validity of the test.

Habituation to four objects prior to the spatial shift test gave the following mean manually recorded exploration times over a 5-min session: Control (117.65± 6.90), PFC (123.07± 9.56) and HPC (137.87± 6.11), with no group difference ($F_{(2,23)}$=2.05, ns). The cut-off point was therefore doubled to 80 sec for the spatial shift test to allow all four objects to be explored.

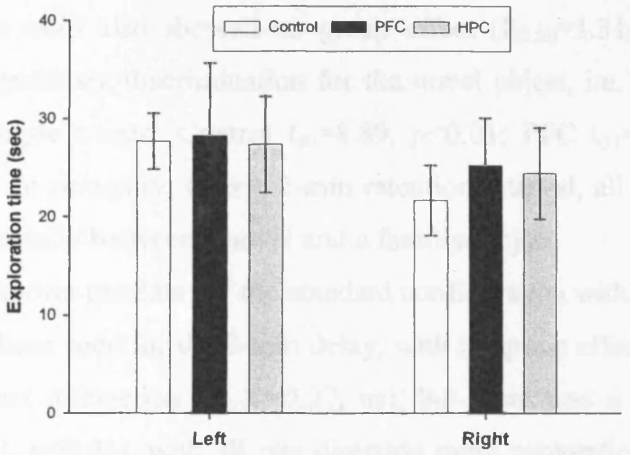


Figure 7.2 Experiment 4: PFC and HPC lesions. Exploration time against side of object for 5-min habituation session. Data expressed as mean ± SEM (Control n=12, PFC n=6, HPC n=8 (1 rat did not explore the objects)).

7.3.1.2 Standard Configuration

Figure 7.3 illustrates the data for the standard configuration test with a 2-min retention delay. Exploration time data showed no group effect ($F_{(2,20)}$=2.62, ns) or any group by object interaction ($F_{(2,20)}$=1.98, ns), however there was a significant effect of object ($F_{(1,20)}$=69.29, p<0.01), with all rats directing more exploration towards the novel object.

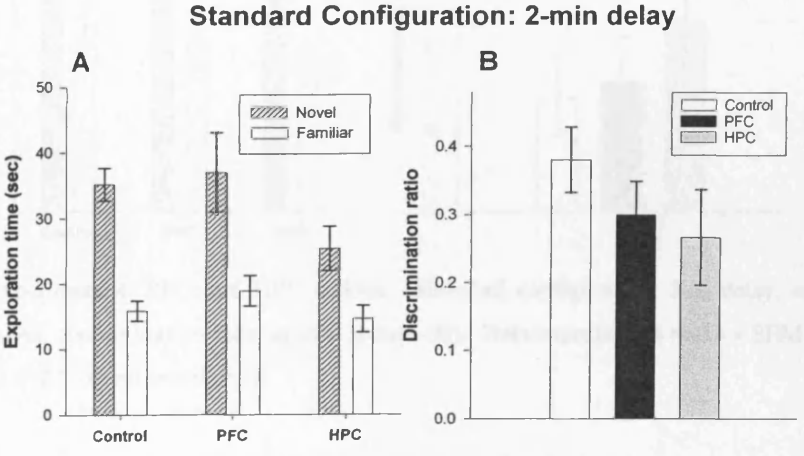


Figure 7.3 Experiment 4: PFC and HPC lesions. Standard configuration: 2-min delay, exploration time against groups (A), discrimination ratio against groups (B). Data expressed as mean ± SEM (Control n=10 (2 rats excluded for failing to reach cut-off in sample), PFC n=6, HPC n=7 (1 rat did not reach cut-off in sample, 1 ill rat sacrificed)).

The discrimination ratio also showed no group effect ($F_{(2,20)}$=1.31, ns), with all three groups showing significant discrimination for the novel object, i.e. significantly greater than zero (one-sample t-tests: Control $t_{(9)}$=8.89, p<0.01; PFC $t_{(5)}$=6.02, p<0.01; HPC $t_{(6)}$=3.77, p<0.01). In summary, after a 2-min retention interval, all groups were able to discriminate successfully between a novel and a familiar object.

Figure 7.4 shows the data for the standard configuration with a 2-hr delay. These results replicated those seen for the 2-min delay, with no group effect ($F_{(2,23)}$=2.02, ns) or any group by object interaction ($F_{(2,23)}$=0.27, ns), but there was a significant effect of object ($F_{(1,23)}$=19.23, p<0.01), with all rats directing more exploration towards the novel object. The discrimination ratio also showed no group effect ($F_{(2,23)}$=0.83, ns) with all three groups showing significant discrimination for the novel object (Control $t_{(11)}$=2.59, p<0.05; PFC $t_{(5)}$=2.87, p<0.05; HPC $t_{(7)}$=3.83, p<0.01). Therefore, after a 2-hr retention interval, all groups were able to discriminate successfully between a novel and a familiar object.

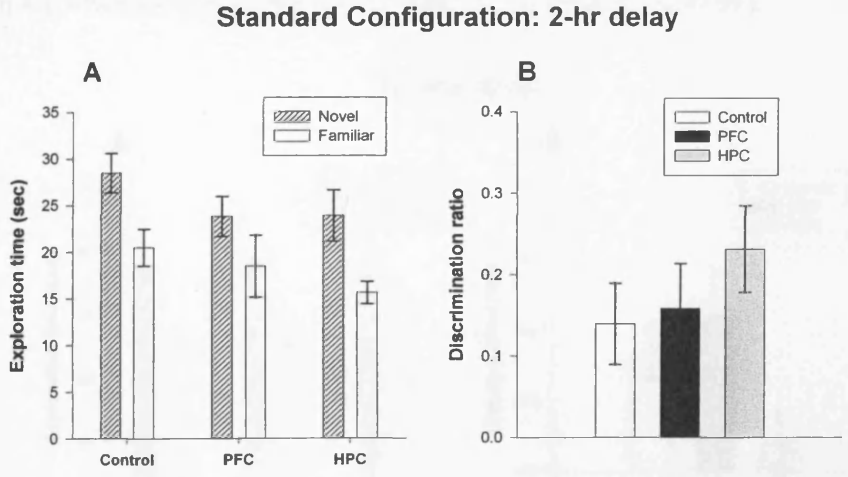


Figure 7.4 Experiment 4: PFC and HPC lesions. Standard configuration: 2-hr delay, exploration time against groups (A), discrimination ratio against groups (B). Data expressed as mean ± SEM (Control n=12, PFC n=6, HPC n=8 (1 ill rat sacrificed)).

In order to determine the effect of delay on the discrimination ratio, an ANOVA was performed with delay as the within subject factor, those rats that were excluded from the 2-min delay, due to lack of exploration in the sample phase, were also excluded from the 2-hr data for this analysis. This revealed no effect of group ($F_{(2,20)}$=0.01, ns) or group by

delay interaction ($F_{(2,20)}$=2.70, ns), but there was a significant effect of delay ($F_{(1,20)}$=15.61, p<0.01), with all groups showing less clear discrimination with the longer delay.

7.3.1.3 Spatial Shift

Figure 7.5 shows the data for the spatial shift task. In this task the rat samples four different objects, following a 2-min delay it is presented with copies of these same four objects, but two have had their positions switched i.e. they are no longer in the same spatial configuration. The results from this test for the exploration time show no main effect of group ($F_{(2,23)}$=0.84, ns) and the group by object interaction just failed to reach significance ($F_{(2,23)}$=3.25, ns, p=0.057), although there was a main effect of object ($F_{(1,23)}$=32.44, p<0.01). However, analysis of the discrimination ratio revealed a main effect of group ($F_{(2,23)}$=3.73, p<0.05). *Post hoc* analysis revealed that the HPC group showed significantly less discrimination than the PFC group (Newman-Keuls: HPC v. PFC p<0.05), but the comparison between HPC and control groups approached but failed to reach significance (Newman-Keuls: HPC v. Control, ns, p=0.07).

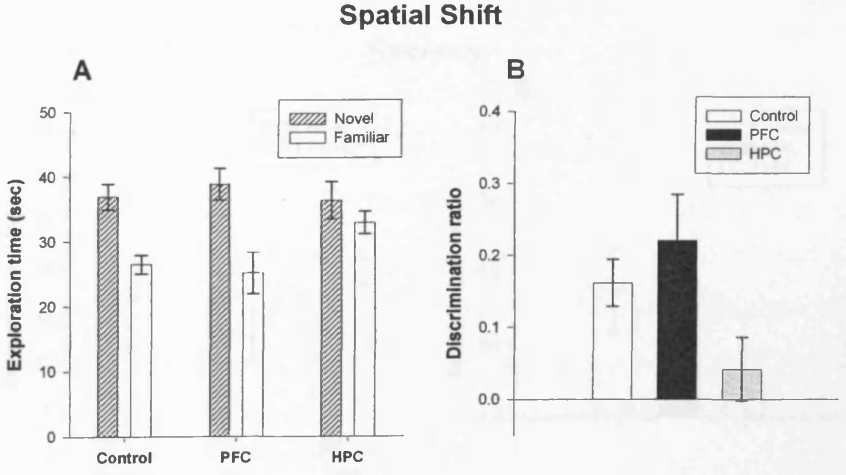


Figure 7.5 Experiment 4: PFC and HPC lesion. Spatial shift, exploration time against groups (A), discrimination ratio against groups (B). "Novel" now refers to the displaced objects, i.e. the cumulative time spent exploring the two objects that underwent the spatial shift, whilst "familiar" indicates the two non-displaced objects. Data expressed as mean ± SEM (Control n=12, PFC n=6, HPC n=8 (1 ill rat had been sacrificed)).

Upon analysis of the discrimination ratio, both the control and PFC groups showed discrimination that was significantly above chance (Control $t_{(11)}$=4.98, p<0.01; PFC $t_{(5)}$=3.42, p<0.05). However, the HPC group were unable to discriminate between displaced and non-displaced objects (HPC $t_{(7)}$=0.94, ns).

7.3.1.4 Recency

Figure 7.6 shows the data from the recency test. This test consists of two sampling phases with two different objects separated by 1 hr; the test occurs 1 hr after the second sample, with one object from each of the two sample phases present. Analysis of the exploration time data revealed only an effect of object ($F_{(1,23)}$=7.61, p<0.05), with no difference between group ($F_{(2,23)}$=2.85, ns) or group by object interaction ($F_{(2,23)}$=0.45, ns). The discrimination ratio data failed to reveal any effect of group ($F_{(2,23)}$=0.80, ns), suggesting that there was no significant difference between the groups. However, separate t-tests revealed that only the control group showed a significant discrimination (Control $t_{(11)}$=2.92, p<0.05; PFC $t_{(5)}$=1.99, ns; HPC $t_{(7)}$=0.81, ns).

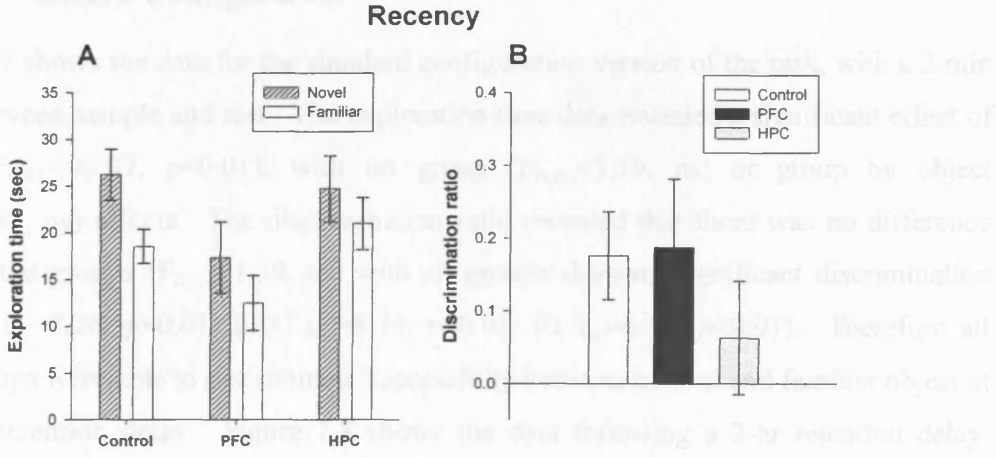


Figure 7.6 Experiment 4: PFC and HPC lesions. Recency, exploration times against groups (A), discrimination ratio against groups (B). "Novel" now refers to the object from sample-1, i.e. the least recently sampled, whilst "familiar" indicates the object from sample-2, i.e. the more recently sampled. Data expressed as mean ± SEM (Control n=12, PFC n=6, HPC n=8 (one ill rat had been sacrificed)).

7.3.2 Experiment 5: ERC and Fx lesions

7.3.2.1. Habituation

All three groups in this experiment showed similar exploration times of two novel identical objects over a 5-min habituation session; means for manually recorded exploration time were control (53.33± 5.70), ERC (55.31± 13.60) and Fx (50.94± 6.33), with no group difference ($F_{(2,22)}$=0.05, ns). This mirrored the times seen in the previous experiment (Control mean 49.38± 3.78), and a 40 sec cut-off point for exploration in the sample phase was therefore maintained.

Habituation to four novel objects over a 5-min session also demonstrated that all groups displayed similar exploration times; means were control (118.64± 15.24), ERC (116.17± 8.00) and Fx (96.17± 8.30), with no group difference ($F_{(2,20)}$=1.01, ns). Again, these results were equivalent to those from the previous experiment (Control mean 117.65± 6.90), therefore the 80 sec cut-off point for the spatial shift version was also maintained.

7.3.2.2 Standard Configuration

Figure 7.7 shows the data for the standard configuration version of the task, with a 2-min delay between sample and test. The exploration time data revealed a significant effect of object ($F_{(1,21)}$=93.27, p<0.01), with no group ($F_{(2,21)}$=3.19, ns) or group by object ($F_{(2,21)}$=0.85, ns) effects. The discrimination ratio revealed that there was no difference between the groups ($F_{(2,21)}$=1.39, ns) with all groups showing significant discrimination (Control $t_{(8)}$=7.26, p<0.01; ERC $t_{(7)}$=8.75, p<0.01; Fx $t_{(6)}$=6.71, p<0.01). Therefore all three groups were able to discriminate successfully between a novel and familiar object at a 2-min retention delay. Figure 7.8 shows the data following a 2-hr retention delay. Exploration data showed no effect of group ($F_{(2,19)}$=1.20, ns) or any group by object interaction ($F_{(2,19)}$=1.92, ns), but again there was a significant effect of object ($F_{(1,19)}$=59.75, p<0.01), with all groups directing more exploration towards the novel object. However, the discrimination ratio data revealed a significant main effect of group ($F_{(2,19)}$=3.88, p<0.05), with *post hoc* testing indicating this effect was due to a significant difference

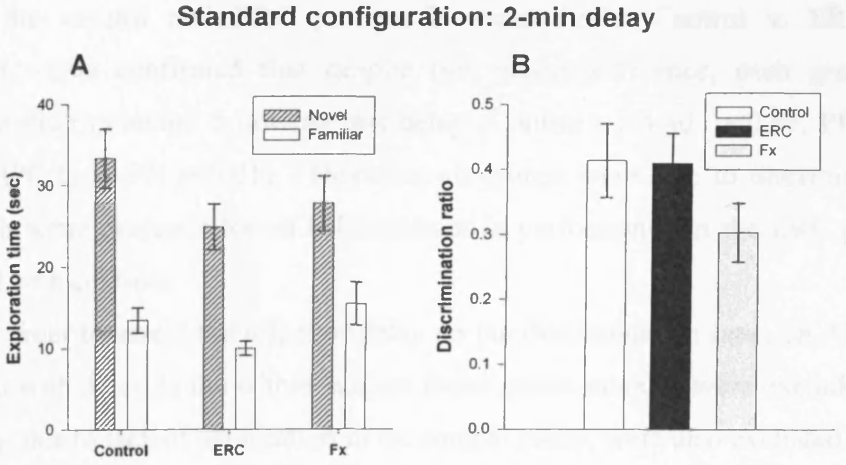


Figure 7.7 Experiment 5: ERC and Fx lesions. Standard configuration: 2-min delay, exploration time against groups (A), discrimination ratio against groups (B). Data expressed as mean ± SEM (Control n=9, ERC n=8 (1 ill rat had been sacrificed), Fx n=7).

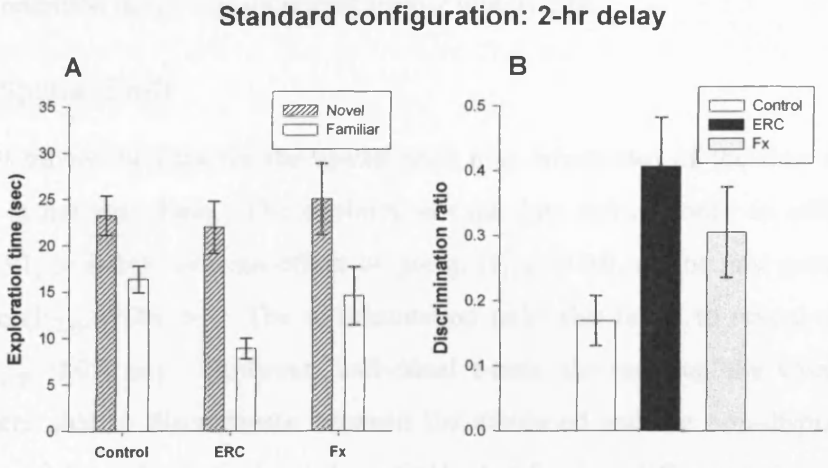


Figure 7.8 Experiment 5: ERC and Fx lesions. Standard configuration: 2-hr delay, exploration time against groups (A), discrimination ratio against groups (B). Data expressed as mean ± SEM (Control n=8 (1 rat not exploring), ERC n=7 (1 rat not exploring, 1 ill rat had been sacrificed), Fx n=7).

between the control and ERC groups (Newman-Keuls: Control v. ERC $p<0.05$). Individual t-tests confirmed that despite this group difference, each group showed significant discrimination following this delay (Control $t_{(7)}=4.40$, $p<0.01$, PFC $t_{(6)}=5.42$, $p<0.01$, HPC $t_{(6)}=4.37$, $p<0.01$). Therefore all groups were able to discriminate at this delay with some evidence for an enhancement in performance in the ERC group when compared with controls.

In order to assess the effect of delay on the discrimination ratio, an ANOVA was performed with delay as the within subject factor, those rats that were excluded from the 2-hr delay, due to lack of exploration in the sample phase, were also excluded from the 2-min data for this analysis. This did not reveal an effect of group ($F_{(2,19)}=2.55$, ns), but did reveal a significant effect of delay ($F_{(1,19)}=4.64$, $p<0.05$), and a significant delay by group interaction ($F_{(2,19)}=3.78$, $p<0.05$). *Post hoc* testing revealed that this interaction was due to the significant difference between the discrimination ratios of the control group at either delay (Newman-Keuls: Control 2 min v. Control 2 hr $p<0.05$). Therefore, in this experiment, only the control group showed a reduction in their ability to discriminate when the retention delay was increased from 2 min to 2 hr.

7.3.2.3 Spatial Shift

Figure 7.9 shows the data for the spatial shift test, where two of the four objects were displaced in the test phase. The exploration time data showed only an effect of object ($F_{(1,20)}=20.91$, $p<0.01$), with no effect of group ($F_{(2,20)}=1.16$, ns) or any group by object interaction ($F_{(2,20)}=0.25$, ns). The discrimination ratio also failed to reveal any effect of group ($F_{(2,20)}=0.01$, ns). However, individual t-tests showed that the Control and Fx groups were able to discriminate between the displaced and the non-displaced objects (Control $t_{(8)}=2.48$, $p<0.05$, Fx $t_{(6)}=3.71$, $p<0.01$), but for the ERC group this just failed to reach significance (ERC $t_{(6)}=2.39$, $p=0.054$).

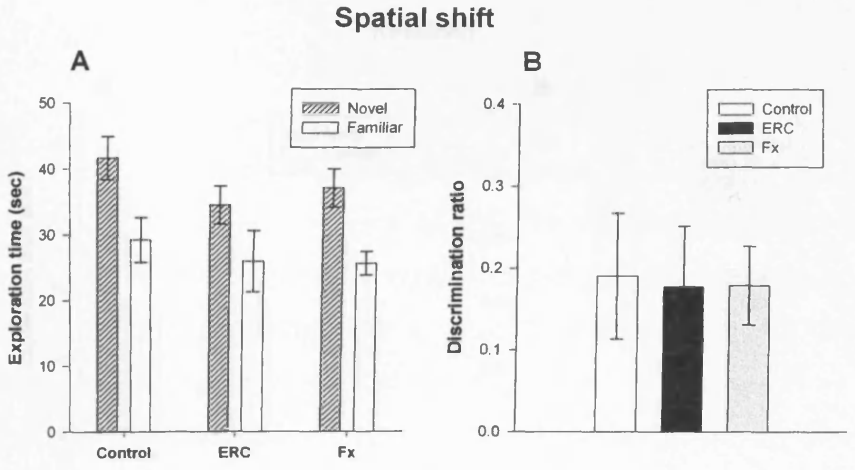


Figure 7.9 Experiment 5: ERC and Fx lesions. Spatial shift, exploration time against groups (A), discrimination ratio against groups (B). "Novel" now refers to the displaced objects, i.e. the cumulative time spent exploring the two objects that underwent the spatial shift, whilst "familiar" indicates the two non-displaced objects Data expressed as mean ± SEM (Control n=9, ERC n=7 (2 ill rats had been sacrificed), Fx n=7).

7.3.2.4 Recency

The results from the recency test are shown in figure 7.10. Exploration time data revealed that there was no effect of group ($F_{(2,16)}$=0.19, ns) or any group by object interaction ($F_{(2,16)}$=2.57, ns), there was however an effect of object ($F_{(1,16)}$=26.47, p<0.01), indicating that all rats were spending more time exploring the least recently seen object in preference to the object presented more recently. The discrimination ratio data also failed to reveal a group effect ($F_{(2,16)}$=1.20, ns), with the control and Fx group showing discrimination significantly above chance (Control $t_{(6)}$=5.36, p<0.01, Fx $t_{(5)}$=4.36, p<0.01), but on this version the ERC group did not show significant discrimination (ERC $t_{(5)}$=1.89, ns).

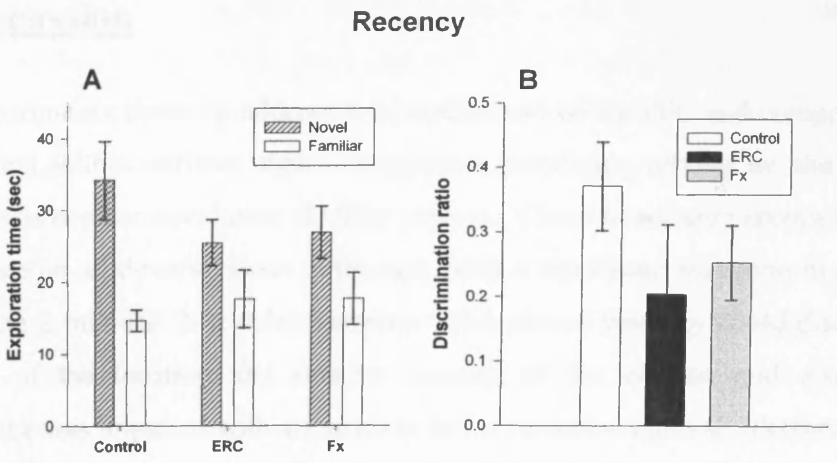


Figure 7.10 Experiment 5: ERC and Fx lesions. Recency, exploration time against groups (A), discrimination ratio against groups (B). "Novel" now refers to the object from sample-1, i.e. the least recently sampled, whilst "familiar" indicates the object from sample-2, i.e. the more recently sampled. Data expressed as mean ± SEM (Control n=7 (1 rat not exploring, 1 ill rat had been sacrificed), ERC n=6 (1 rat not exploring, 2 ill rats had been sacrificed), Fx n=6 (1 rat not exploring)).

7.4 Discussion

These experiments aimed to address the involvement of the PFC and components of the HPC system within various object recognition paradigms relying on the rats' innate preference to explore novel over familiar objects. Control rats were shown to be able to discriminate on all three versions of the task, with a significant reduction in performance between the 2-min and 2-hr delay versions. This shows that they could discriminate on the basis of the location and relative recency of the objects, and also that their performance was impaired with an increase in the retention interval. Performance in the recency task was not probed explicitly by a test phase, for example by including the 1st sample object and an entirely novel object (see Hannesson et al., 2004a). However, intact discrimination at the 2-hr delay in the standard version serves to validate the recency task by verifying that the rats can remember object information up to at least a 2-hr retention interval - with 2 hr being the time elapsed since exploring the 1st object. The results from the lesion groups will be discussed in detail below, but briefly, none of the lesion groups impaired performance on the standard version of the task; the spatial shift version may have shown a slight impairment following HPC and ERC lesions. Finally, all lesion groups except for the Fx group exhibited a modest disruption in performance on the recency task.

It is worth mentioning that there is another commonly used paradigm for investigating spontaneous novelty preference in the rat. This alternative paradigm uses a circular arena containing usually four or five objects, and typically consists of seven successive 6-min sessions separated by 3-min delays. The normal testing procedure, as described by Save et al (1992), is as follows: session 1; habituation session in empty arena, session 2 to 4; exploration of five objects which remain in the same position, session 5 and 6; spatial change - exploration of five objects, one of which replaces the position of another object, which in turn is displaced to a new location within the arena, session 7; novel object - one of the objects that was not displaced previously is replaced by a new object.

Variations on this paradigm exist, but what is clear is that in all versions there is likely to be some level of interference between the sessions (Granon et al., 1996). For

example, an object may acquire an intermediary status when it has recently moved position, making it more salient than other objects, but less so than a novel object. The novel object substitution occurs at the final stage of testing, by which point rats may no longer be attending to the task, or might still be preferentially exploring those objects that were displaced two sessions previously. Therefore there is some question over the validity of this task. However, it has been effective in establishing lesion-induced differences and will therefore be included within this discussion. Hereafter, it shall be described as the "continuous spontaneous novelty preference task" to differentiate it from the paradigm used in the present experiments where discrete trials were employed.

Finally, it should be noted that there is a chance that these data may have been confounded by the sequential presentation of the tasks. Unfortunately, the lack of counterbalancing was necessary in the first instance to allow assessment of whether or not rats could discriminate up to the 2 hr point, as this was crucial in order to rationalise the recency task. Experiment 5 was then conducted in the same order as Experiment 4 in order to allow a valid comparison between the two. However, it is assumed that any interference or additive effects would be minimal due to sessions being conducted on separate days; furthermore, there was consistency in the amount of exploration across all four versions.

Experiment 4: PFC and HPC lesions

PFC: standard configuration

The PFC group showed intact discrimination performance at both the 2-min and the 2-hr delays. Their level of discrimination was attenuated at the longer delay, which implies a temporal decay for the memory of the object. This finding of intact spontaneous novelty preference following PFC damage in the rat is in line with many studies where varying delay intervals have been used (15-min delay: Ennaceur et al., 1997; 6-min delay: Granon et al., 1996; 105-min delay: Hannesson et al., 2004a; 24-hr delay: Mitchell and Laiacona, 1998; 15-min delay: Mogensen et al., 2004; 15-min delay: Yee, 2000). Therefore, it is likely that the rat PFC does not play a significant role in the detection of novelty in a paradigm of this nature.

PFC: spatial shift

The PFC group were not impaired in discriminating between objects that were displaced and those that remained in the same position. This concurs with a study where NMDA lesions of the PrL left discrimination intact when two objects were explored and then one was subsequently displaced to a novel location (Ennaceur et al., 1997). This has also been demonstrated using the continuous spontaneous novelty preference task, where rats with radiofrequency lesions of the mPFC were shown to explore the displaced objects exclusively (Poucet, 1989). Another study which used the continuous version of the task suggested that rats with radiofrequency lesions of the mPFC actually reacted more strongly to the spatial change than control rats, although once the 6-min session was broken down into smaller time bins the control rats were shown to preferentially explore the displaced objects. This highlights the notion that discrimination will be maximal at the beginning of a test session, because as the novel object is explored it becomes progressively more "familiar", ultimately abolishing its novel status (Dix and Aggleton, 1999; Ennaceur et al., 2005). This provides a further confound to the continuous version where sessions are usually of 6-min duration, and thus differences may be masked once data are pooled across the session. There is therefore no evidence to suggest that the rat PFC is involved in remembering the spatial attributes of an object.

PFC: recency

The PFC group did not show a significant difference from the control group on the recency version of the task; both the exploration time and the discrimination ratio data were concurrent, with no main effect of group. However, upon analysis of the discrimination ratio it was revealed that the PFC group did not show a discrimination that was significantly above zero. These results are therefore somewhat inconclusive, as it would appear that the PFC group were able to discriminate based on relative recency, but the lack of a significant discrimination ratio clearly argues against this. The overall discrimination ratio for the PFC group is actually slightly greater than in the control group, but the amount of variability ensuing from the smaller group size may be a contributory factor in the non-significant result.

One might expect that damage to the PFC would result in a deficit on this version of the task, due to the considerable evidence for the involvement of this area in temporal memory in the human (McAndrews and Milner, 1991; Milner et al., 1985; Milner et al., 1991; Milner and Petrides, 1984; Shimamura et al., 1990; Zorrilla et al., 1996). Deficits on judgements of recency in a RAM task have also been observed in rats following both lidocaine inactivation (Hannesson et al., 2004b) and aspirative lesions (Kesner and Holbrook, 1987) of the PFC.

There have been at least two studies examining the effects of PFC damage on this recency version of the spontaneous novelty preference task. The first examined radiofrequency lesions of the PFC and demonstrated that rats maintained intact discrimination for novel objects up to 24 hr delays, but that discrimination was abolished on the recency version of this task (Mitchell and Laiacona, 1998). This provides evidence for a PFC involvement on this task; however it should be noted that rats were compared to their own prelesion performance, and as such surgical procedures were not controlled for. The second study showed that lidocaine inactivation of the PFC impaired the recency version (1 hr gap between sample phases, 45 min before test) but not the standard familiarity version (Hannesson et al., 2004a). These studies substantiate the hypothesised involvement of the PFC in recency judgement, but unfortunately the experiment discussed in this chapter provides only mild support for this theory based on the discrimination ratio data. It would be unwise to attempt to draw any definitive conclusions from these data as one can only postulate whether a larger group size might have confirmed the presence of a deficit or in fact revealed intact performance.

HPC: standard configuration

The HPC group showed intact discrimination on the standard version of the spontaneous novelty preference task at both the 2-min and the 2-hr delays. Their level of discrimination was lower for the longer delay interval, paralleling the result seen in the PFC group, and demonstrating that memory for the object decayed with time. This finding concurs with numerous studies which confirm intact novelty preference after HPC damage following a range of delay intervals between sample and test (48-hr delay: Forwood et al., 2005; continuous version, 6-min delay: Galani et al., 1998; 24-hr delay:

Gaskin et al., 2003; continuous version, 6-min delay: Lee et al., 2005; 5-min delay: Mumby et al., 2002; 24-hr delay: Winters et al., 2004).

A study by Liu and Bilkey (2001) failed to show significant discrimination following IBO-induced lesions of the HPC, however this result may have been confounded by the fact that this group had accumulated less exploration of the objects in the sample phase. The only remaining case for impaired recognition memory following HPC damage comes from a study by Clark et al (2000), which revealed a deficit in discrimination at longer delay intervals following IBO or radiofrequency induced lesions of the HPC. However, on closer inspection of the data this interpretation is somewhat questionable, as the radiofrequency-lesioned rats were not in fact shown to be impaired compared with the control group at the longest delay of 24 hours: indeed at this delay these rats were in fact performing marginally above chance. Therefore it would appear that the present finding of intact performance following HPC damage is in agreement with the majority of the literature.

HPC: spatial shift

There is some evidence to suggest that the HPC group were impaired on discriminating between the objects based on the spatial displacement. Although there was no overall effect of group in the exploration time measure, the interaction between the groups and the objects only just fails to reach significance (p=0.057). The discrimination ratio does reveal a main effect of group, however this is attributed to the fact that the HPC group are significantly impaired compared with the PFC group, but this comparison just fails to reach significance with the control group (p=0.07). Of greatest significance is the finding that the HPC rats do not show discrimination between the displaced and non-displaced objects based on the ratio. This suggests that despite failing to demonstrate an overall difference from the control group, it would appear that the HPC-lesioned rats are unable to discriminate based on the spatial shift.

This finding is in agreement with previous studies which utilised either the continuous version of the task (Galani et al., 1998; Lee et al., 2005; Liu and Bilkey, 2001; Save et al., 1992) or the paradigm where two objects are explored and one is relocated to

a new position (Mumby et al., 2002). It is also fits well with the concept that the HPC is critical in those tasks that contain a spatial component.

HPC: recency

The HPC group were shown to be unable to discriminate between objects based on their relative familiarity according to the discrimination ratio. Disappointingly, no main effect of group was revealed due to the high degree of variability within the groups. However, the ratio data indicate that there was at least some evidence for an impairment on judging the recency of the objects. To my knowledge there have been no previous studies investigating lesions of the HPC on this recency version of the spontaneous novelty preference task in the rat. Of interest are studies that have shown that lesions of the HPC can impair the capacity to recall the correct sequence of a series of odours, despite being without effect on recognition memory (Fortin et al., 2002; Kesner et al., 2002). These findings sit well with the current demonstration of intact performance on the standard version of the novelty preference task, but deficits when the recency component is incorporated.

Experiment 5: ERC and Fx lesions

ERC: standard configuration

Rats with lesions of the ERC were not impaired on discriminating at the 2-min delay on the standard version of this task. At the 2-hr delay discriminatory performance remained intact, to the extent that there was a significant difference between the ERC group and the control group, with the ERC group spending a significantly greater proportion of time exploring the novel object. This finding also meant that the ability to recall the sample object had not declined following the 2-hr retention interval.

Two studies have reported the effects of ERC damage on the spontaneous novelty preference task, although both utilised the continuous version and therefore comparisons are difficult. Galani et al (1998) used aspirative ERC lesions on a paradigm involving 7 successive sessions, the novel object substitution occurred in the final session and therefore suffered from the interference effects discussed earlier. These rats did not demonstrate any preferential exploration of the novel object; however variability was

large within the group which might have resulted from the relatively small group size (n=6) or the fact that the test was performed only once. Of interest is the observation that their lesions encroached upon the perirhinal cortex, which is known to be critical for exactly this type of task (Aggleton et al., 1997; Bussey et al., 2000; Winters et al., 2004; Winters and Bussey, 2005). Therefore the apparent lack of discrimination may have been due to the perirhinal rather than entorhinal cortical damage. The other study investigated radiofrequency lesions on a continuous version of the task where the novel object substitution occurred in the 10th and final session (Parron and Save, 2004). A deficit in reaction to the novel object was reported, based on a significant difference between the ERC and control groups, however, it is unclear whether or not the ERC group were in fact showing a significant discrimination between the novel and familiar objects (Parron and Save, 2004). From the present study there is no evidence to suggest that the medial ERC is involved in recognition memory for objects.

ERC: spatial shift

The ERC group did not display any difference between their performance and the performance of the control group. However, upon analysis of their discrimination ratio it became apparent that they just failed (p=0.054) to reach a significant level of discrimination between the displaced and the non-displaced objects. Again the two studies mentioned previously (Galani et al., 1998; Parron and Save, 2004) both reported a deficit in reacting to a spatial change in the objects, although obviously these studies utilised the continuous paradigm and are not directly comparable with the present study. However Galani et al (1998) did demonstrate a slight increase in exploration for the displaced objects, but in the absence of an index of discrimination taking into account the total exploration time, it is impossible to tell if this exploration was greater than for the non-displaced objects. Therefore the present study suggests that the ERC might have some involvement in remembering the spatial attributes of an object, although this result is marginal and is based only on the lack of intact discrimination, with no difference seen between groups overall.

ERC: recency

There was no difference between the ERC and control groups on performance in the recency version of the test. However, on analysis of the discrimination ratio the ERC group did not show significant discrimination based on the relative familiarity of the objects. As in the PFC group, small group size may have been a contributory factor to the amount of variation that was apparent in this group. To my knowledge there have been no previous studies to substantiate this finding.

Fx: standard configuration

The group that received Fx lesions were not impaired on discriminating at either the 2-min or 2-hr standard versions of the task. This group did not show an attenuation of discrimination with the longer delay interval; however their performance did not differ significantly from the control group at the longer 2-hr delay. There have been numerous studies which confirm that disruption of the fornical pathway leaves object recognition performance on this task intact after delays of 15 min (Bussey et al., 2000; Ennaceur et al., 1996; Ennaceur et al., 1997; Ennaceur and Aggleton, 1994; Mogensen et al., 2004), and even up to 24 hours (Clark et al., 2000). It would appear that the current finding of intact object recognition up to the 2-hr delay period corresponds with most of the literature and substantiates the idea that the hippocampal formation is not essential for this type of memory, at least up to a 2-hr retention interval.

Fx: spatial shift

The Fx group did not reveal any impairment in discriminating between the objects based on their spatial location. This finding contrasts with the impairment in the capacity to remember the spatial attributes of the objects following from the specific HPC lesion, and further suggests that transection of the Fx need not induce the same behavioural sequelae as lesions of the HPC itself. Previous studies are in agreement that lesions of the Fx result in impaired discrimination between familiar objects that have been moved to novel locations (Bussey et al., 2000; Ennaceur et al., 1997).

However, previous work using the same spatial shift paradigm as that employed in the present study is less conclusive. Rats with Fx lesions did not show any overall group difference from controls, and in fact, when analysed over the first minute of

testing, they were actually achieving significantly higher discrimination scores than the controls (Bussey et al., 2000). Although when analysed across the full 3-min testing block the Fx group were not significantly above chance for the d2 measure- equivalent to the discrimination ratio in the present study (Bussey et al., 2000). However in Bussey et al's paradigm rats were not matched for exploration time in the sample phase, in fact the Fx rats displayed significantly higher levels of exploration than the other groups, which may complicate interpretation of the results. In the present study there was clearly no evidence for an impairment on discriminating between objects in rats that had received lesions of the Fx.

Fx: recency

The Fx group showed intact performance on the task that relied on judging the relative familiarity of the objects. They were the only lesion group to do so, with the discrimination ratio indicating that they were spending significantly more time exploring the object that was presented least recently. There is little information available on whether or not the Fx has a role in this type of temporal memory. A study in monkeys using a rewarded version of a recency task suggests that damage to the Fx does impair this type of memory (Charles et al., 2004).

Conclusions

The aim of experiments 4 and 5 was to investigate the involvement of the PFC and the hippocampal system in the spontaneous novelty preference task. There was no evidence to implicate the PFC, HPC, ERC or Fx in this particular form of recognition memory, even up to retention delays of 2 hours. This finding validates that all lesion groups retained the capacity to appreciate novelty. In the spatial shift task there was an indication that the HPC group were impaired on remembering the spatial attributes of the objects, with this group failing to achieve significant discrimination. Finally, the recency test resulted in a high degree of variability, with the Fx group being the only lesion group to show significant discrimination. Therefore little can be gleaned from the recency data, although it does provide anecdotal support to the idea that the PFC and the hippocampal system are involved in the temporal organisation of memory.

Chapter 8 Retention of conditional delayed matching/ non-matching to position task: effect of hippocampal and prefrontal lesions

Experiment 6

8.1 Introduction

The work described in this thesis so far has not revealed an effect of specific excitotoxic lesions of the HPC on the DMTP task in the Skinner box. This finding is significant for two main reasons, firstly it is contradictory to a number of studies which implicate the HPC in this type of task (Aggleton et al., 1992; Broersen, 2000; Dunnett et al., 1990; Hampson et al., 1999), and secondly it questions the involvement of the HPC in working memory in the rat. Thus far, the only behavioural sequelae of this type of lesion have been impairment in the allocentric spatial reference memory task in the water maze, an increase in spontaneous locomotor activity and a suggestion of an impairment in the spatial and recency versions of the spontaneous novelty preference task. These findings provide validation that the HPC lesions were comprehensive enough to induce deficits characteristic of this lesion, and provide further proof that the lack of effect on DMTP was not just an anomalous finding.

In an effort to establish if there might be some facet of the DMTP task that renders it insensitive to HPC lesions, a novel working memory task of considerably greater complexity was employed. The task used was the conditional delayed matching/non-matching to position task (CDM/NMTP) in the Skinner box. CDM/NMTP uses the presence of specific cues (stimulus light above levers or central panel) to indicate whether a particular trial is matching or non-matching in nature. Thus it incorporates both the task contingencies within one session, and supplements the requirement for remembering the side of the lever in the sample phase with attending to the stimulus lights and remembering the conditional discrimination for the rule. The increased

complexity of this task naturally requires a much more extensive training phase than DMTP, with each component of the task being introduced gradually. The rats must learn each of the rules separately, then learn the conditional visual discrimination that signals each rule by having both occurring randomly throughout the session, and finally maintain their performance over delay intervals. This task was first described in Döbrössy's thesis (1997), where bilateral dorsal striatal lesions resulted in a general decline on both the matching and non-matching trials, with performance being reduced to chance levels. This work proved an invaluable reference point with which to compare the current study, particularly as far as training procedures were concerned.

The aim of this experiment was to assess the effects of HPC and PFC lesions on retention of CDM/NMTP. Rats were trained thoroughly prior to surgery, with postoperative lesion effects being assessed. It was hoped that this more complex task might be sensitive to damage of the HPC. It was also hypothesised that the PFC group would be impaired on this task, given the demonstration in Experiment 1 that these lesions produced a delay-dependent deficit in DMTP alone. Rats were also subjected to general locomotor evaluation.

8.2 Materials and methods

Forty rats were used in this experiment; all other subject details are covered in section 2.2. Rats were trained on the CDM/NMTP task described in detail in section 2.3.5. Briefly, this task involved combining the matching and non-matching rules in one session, using visual cues to indicate which rule was needed for correct performance of each trial. The illumination of the central stimulus light indicated a matching trial, whilst the illumination of the two stimulus lights above the levers indicated a non-matching trial. Rats were initially trained on DMTP then switched to DNMTP upon reaching asymptotic performance. Subsequent rule switches were performed until both types of trial were presented randomly throughout the session. Following this stage, one rat had to be sacrificed due to recurring seizures before being placed in the box. Delays were introduced gradually and after reaching asymptotic performance at a delay set of 0-16 sec, rats were tested for a 5-session baseline block. Following baseline testing, rats were

assigned to one of four surgical treatment groups using a random matching procedure based on accuracy. Treatments were HPC lesions (n=12), sham HPC (n=7), PFC lesions (n=12) and sham PFC (n=8), with all surgical details in section 3.3. One rat died during HPC surgery and was therefore substituted with an animal that would have had sham PFC surgery, therefore resulting in a final group size of seven for the sham PFC group.

Rats were given two weeks to recover from surgery before having general locomotor activity assessed over a 60-min session. They were then retested on CDM/NMTP with a delay set of 0-16 sec; rats received four blocks of five sessions. A measure of bias was analysed to indicate any preference for either the matching or non-matching contingencies. Bias was calculated as the number of correct matching trials plus the number of incorrect non-matching trials divided by the total number of trials; scores therefore ranged from 0 to 1, with 0.5 indicating no preference, scores above this figure indicated preference for matching and below it indicated preference for non-matching. Finally rats were sacrificed and histology was dealt with as detailed in section 3.4.

8.3 Results

8.3.1 Histology

8.3.1.1 Bilateral PFC lesions

Figure 8.1 shows photomicrographs of a representative lesion and figure 8.2 illustrates the minimum and maximum lesion extent. In all cases there was substantial cell loss within the PrL and IL cortices, with complete neuron loss within these regions in most animals. One animal had damage that encompassed the most rostral MO cortex and two animals showed damage to Cg1 and Cg2, the largest lesion extended ventrally into the DP cortex. Out of the twelve rats in this surgery group, four did not incur sufficient bilateral damage and where therefore excluded from all analyses.

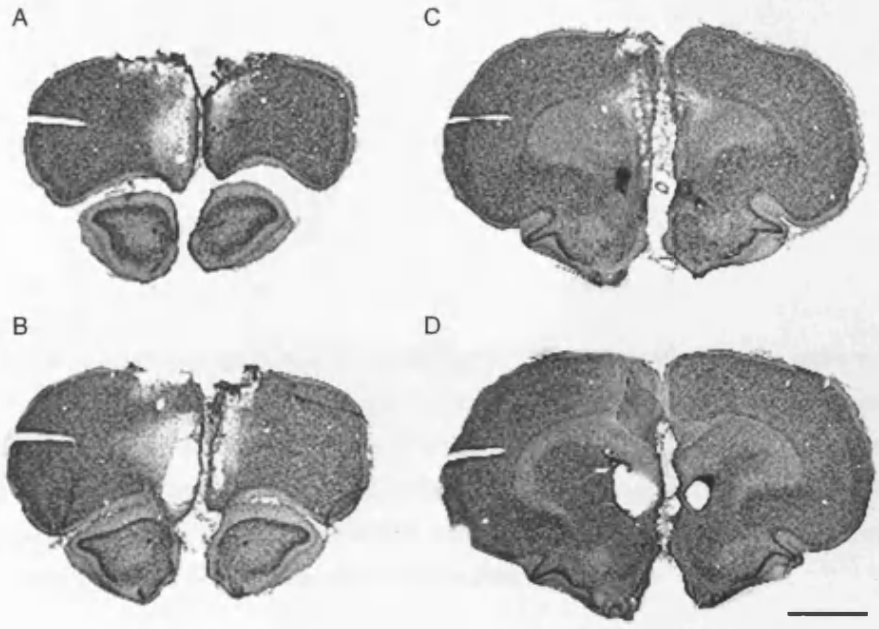


Figure 8.1 Photomicrographs of a representative bilateral PFC lesion, coronal sections stained with Cresyl Violet, with A being the most anterior. Scale bar =2 mm.

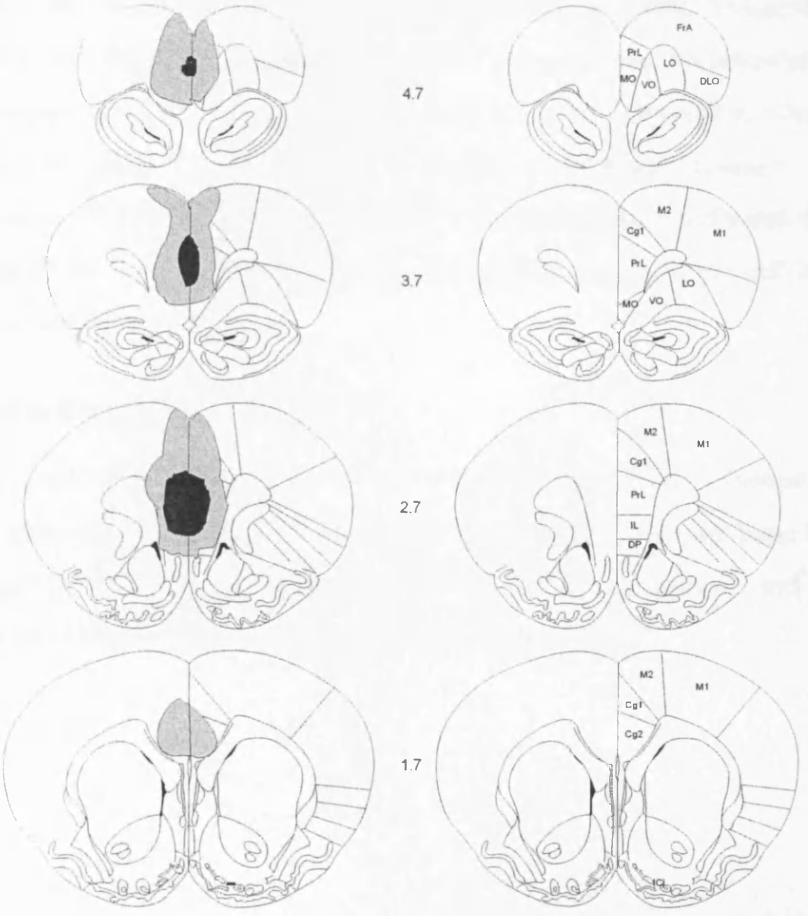


Figure 8.2 Extent of the largest (grey) and smallest (black) PFC lesions, with duplicate sections indicating the specific regions. Numbers indicate mm anterior to bregma, abbreviations: cingulate cortex area 1 (Cg1), cingulate cortex area 2 (Cg2), dorsolateral orbital cortex (DLO), dorsal peduncular cortex (DP), frontal association cortex (FrA), infralimbic cortex (IL), lateral orbital cortex (LO), primary motor cortex (M1), secondary motor cortex (M2), medial orbital cortex (MO), prelimbic cortex (PrL), ventral orbital cortex (VO). Drawings taken from Paxinos and Watson (1998).

8.3.1.2 Bilateral HPC lesions

Figure 8.3 shows photomicrographs of a representative case that was included in analyses and figure 8.4 illustrates the minimum and maximum lesion extent. Successful cases had extensive cell loss throughout the dorso-ventral extent of the hippocampal formation. One case showed some sparing of the CA1 unilaterally (see figure 8.4, minimal extent); this case did not differ from the others in its behavioural performance. Histological analysis revealed that three out of the total of twelve rats had insufficient damage to be included in analyses. Those rats that were excluded had very minimal cell loss, with two having only unilateral damage.

8.3.1.3 Sham lesions

Sham lesion cases were not shown to have any cellular loss or any obvious cell damage. HPC shams had a small amount of cortical swelling resulting from the bone flap removal. The data from the two groups were not shown to differ significantly, and as such they were pooled for clarity giving a control group of 14 individuals.

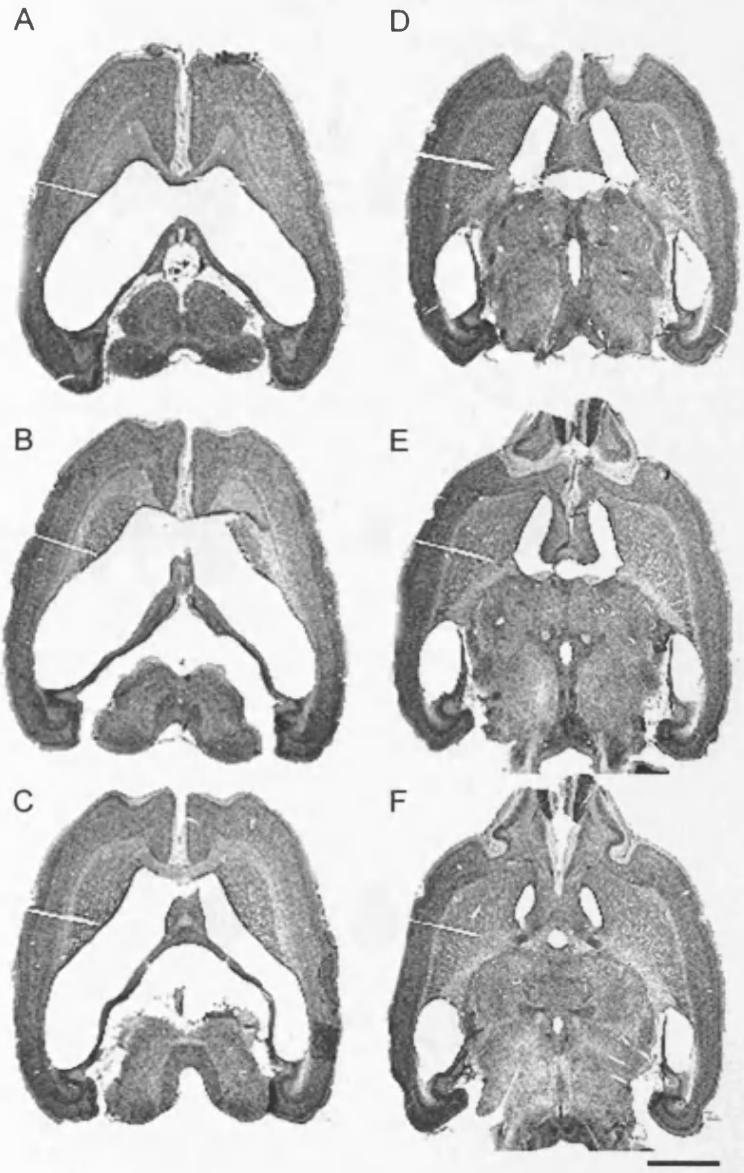


Figure 8.3 Photomicrographs of a representative bilateral HPC lesion, horizontal sections stained with Cresyl Violet. ~3.86 mm below the horizontal plane passing through bregma and lambda (A). ~6.1mm below the horizontal plane passing through bregma and lambda (B). Scale bar =2mm.

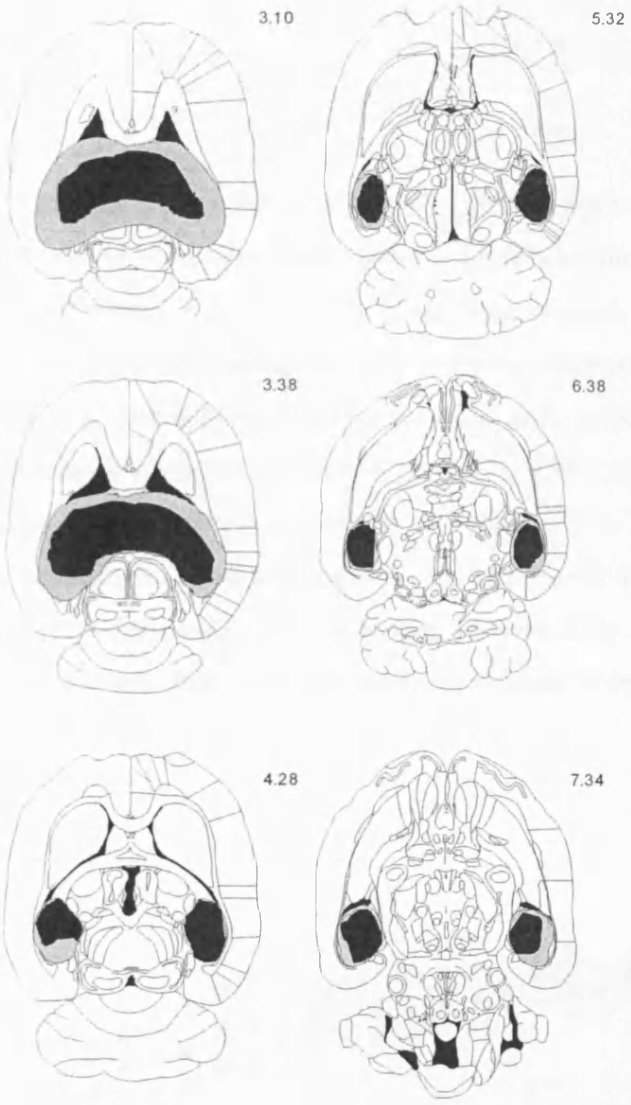


Figure 8.4 Extent of the largest (grey) and smallest (black) HPC lesions. Numbers indicate mm below the horizontal plane passing through bregma and lambda. Horizontal drawings taken from Paxinos and Watson (1998).

8.3.2 Behaviour

8.3.2.1 CDM/NMTP

8.3.2.1.1 Training

Rats required approximately four months of training before both matching and non-matching could be introduced within the same session. Figure 8.5 shows the training data across sessions for the combined rule task, at the zero delay interval. % Correct data are shown for each trial type and as an average between the two versions. An ANOVA, with trial type and sessions as within-subjects factors, revealed main effects of both trial type ($F_{(1,39)}$=145.24, p<0.01) and sessions ($F_{(20,780)}$=82.38, p<0.01), with a significant interaction between the two ($F_{(20,780)}$=3.73, p<0.01) (N.B. all 40 trained rats were used in these analyses). *Post hoc* tests showed that performance improved over sessions in both trial types, but that rats were significantly more accurate on matching as opposed to non-matching trials at every session. Rats were gradually introduced to delays before baseline measures were taken.

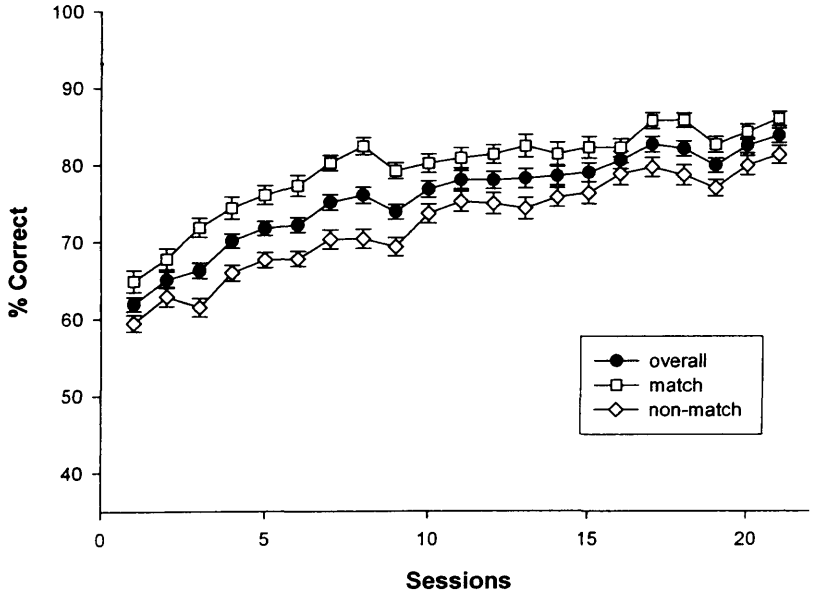


Figure 8.5 % Correct against sessions for zero-delay CDM/NMTP task. Data expressed as mean ± SEM (n=40).

8.3.2.1.2 Baseline

Rats were tested for five sessions after reaching asymptotic performance on CDM/NMTP with 0-16 sec delays. Figure 8.6 shows these baseline data, with no differences seen between the groups on measures of % correct ($F_{(2,28)}$=0.96, ns), total trials completed ($F_{(2,28)}$=1.29, ns) or rate of panel pressing ($F_{(2,28)}$=0.15, ns).

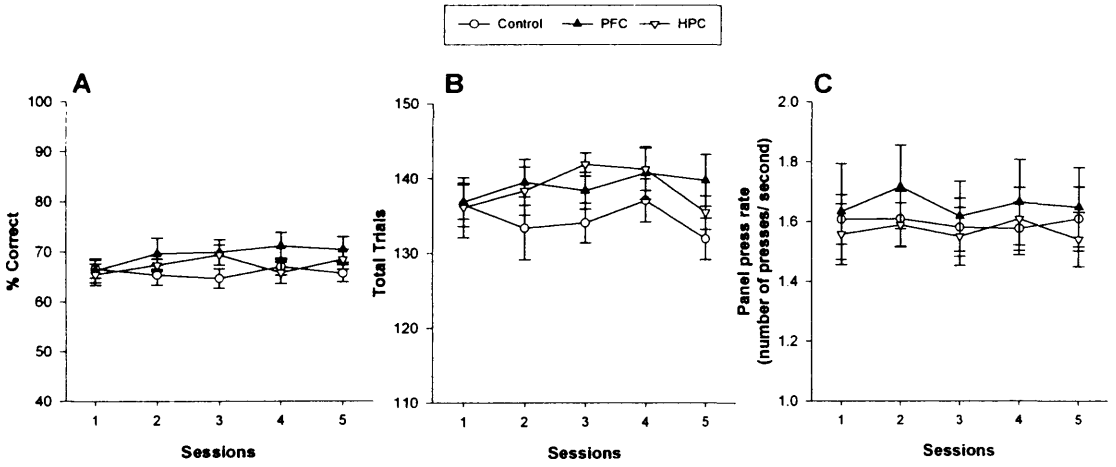


Figure 8.6 CDM/NMTP (0-16 sec delays) baseline data, % correct (A), total trials (B) and panel press rate (C) against sessions. Data expressed as mean ± SEM (Control n=14, PFC n=8, HPC n=9).

Data were also analysed across delays (see figure 8.7) for % correct; no difference between groups was seen ($F_{(2,28)}$=0.85, ns), although there was a significant effect of delays ($F_{(6,168)}$=67.93, p<0.01). This confirmed that performance levels decreased as the delay interval increased, verifying the mnemonic element of the task. The measure of bias between matching and non-matching (matching > 0.5 > non-matching) was also assessed over the baseline week. None of the groups displayed a significant bias towards applying one rule over the other, and no difference was seen between the groups ($F_{(2,28)}$=1.87, ns); means for each group were controls (0.506± 0.008), PFC (0.490± 0.012) and HPC (0.515.± 0.005).

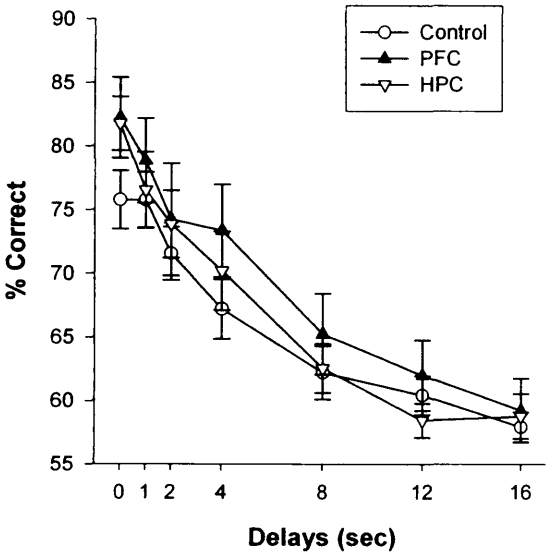


Figure 8.7 CDM/NMTP (0-16 sec delays) baseline data, % correct against sessions. Data expressed as mean ± SEM (Control n=14, PFC n=8, HPC n=9).

8.3.2.1.3 Postoperative testing

Figure 8.8 illustrates % correct data against sessions following surgery, with significant effects of group ($F_{(2,28)}$=11.32, p<0.01), session ($F_{(19,532)}$=12.16, p<0.01) and a group by session interaction ($F_{(38,532)}$=1.77, p<0.01). *Post hoc* analysis revealed that the group effect was due to the impairment in the HPC group relative to both the PFC group and controls (Newman-Keuls: HPC v. PFC and Control, p<0.01). Overall performance increased across sessions, although the interaction showed that whereas both PFC and control rats showed an improvement over sessions, the HPC group failed to show a similar increase in accuracy across sessions. Figure 8.9 illustrates these data across delays collapsed across all 20 postoperative sessions. The percent correct measure revealed effects of group ($F_{(2,28)}$=11.53, p<0.01), delay ($F_{(6,168)}$=38.87, p<0.01) and a group by delay interaction ($F_{(12,168)}$=2.89, p<0.01). The group difference was due again to the HPC group showing significantly poorer performance than both PFC and control rats (Newman-Keuls: HPC v. PFC and Control, p<0.01).

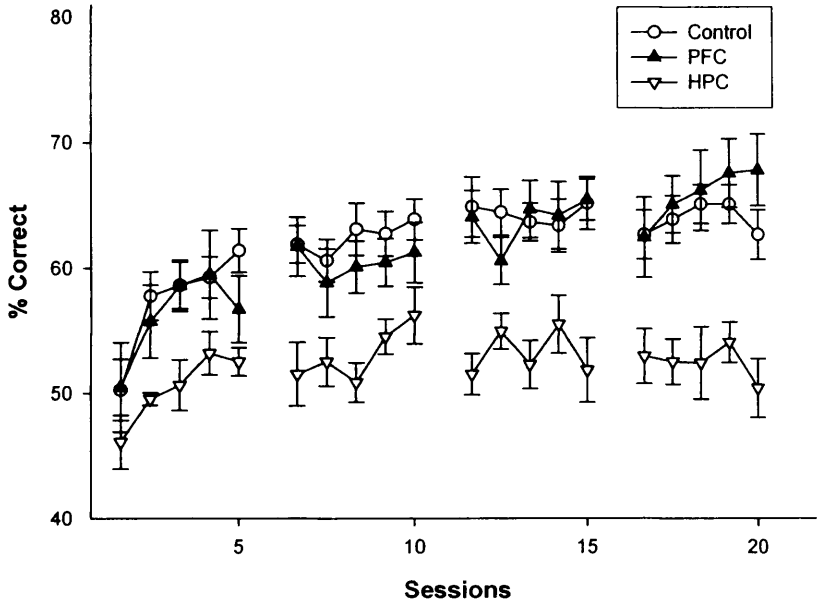


Figure 8.8 CDM/NMTP (0-16 sec delays) postoperative data, % correct against sessions. Data expressed as mean ± SEM (Control *n*=14, PFC *n*=8, HPC *n*=9).

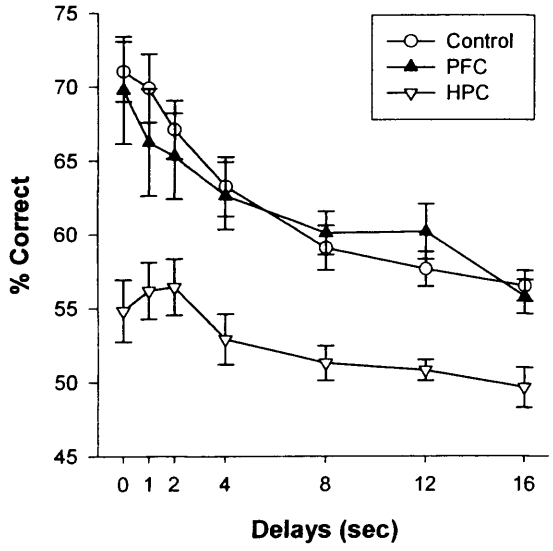


Figure 8.9 CDM/NMTP (0-16 sec delays) postoperative data averaged across all 20 sessions, % correct against delays. Data expressed as mean ± SEM (Control *n*=14, PFC *n*=8, HPC *n*=9).

The HPC group differed from both the PFC and control groups at each delay interval, thus showing that the deficit was apparent even at the shortest delay. There was a significant effect of delay on all groups, although in the HPC group this was accounted for by only the first three delay intervals, with the performance at zero delay differing only from the longest delay, and delays of 1 and 2 sec giving significantly higher accuracy than at 8, 12 and 16 sec. The measure of bias between matching and non-matching (matching > 0.5 > non-matching) was also assessed averaged across all four blocks of DMTP3. None of the groups displayed a significant bias towards applying one rule over the other, and no difference was seen between the groups ($F_{(2,28)}$=0.63, ns); means for each group were controls (0.495 ± 0.005), PFC (0.502± 0.006) and HPC (0.502.± 0.003).

For further characterisation of the deficit resulting from the HPC lesion, numbers of panel presses across delays and latency to the first panel press after the delay were investigated. These data are not presented as neither measure revealed any differences between groups, although both increased with increasing delays as expected (Panel presses: Group ($F_{(2,28)}$=0.25, ns), Delay ($F_{(6,168)}$=589.46, p<0.01), Group x Delay ($F_{(12,168)}$=0.76, ns). Latency: Group ($F_{(2,28)}$=0.78, ns), Delay ($F_{(6,168)}$=16.37, p<0.01), Group x Delay ($F_{(12,168)}$=0.90, ns)).

Measures from SDT were also analysed, and are presented in figures 8.10 and 8.11. The two accuracy measures, *A'* and *SI*, provided very similar results with significant effects of group (*A'*: $F_{(2,28)}$=18.44, p<01. *SI*: $F_{(2,28)}$=10.46, p<0.01), delay (*A'*: $F_{(6,168)}$=7.20, p<0.01. *SI*: $F_{(6,168)}$=35.37, p<0.01) and group by delay interactions (*A'*: $F_{(12,168)}$=1.93, p<0.05. *SI*: $F_{(12,168)}$=4.08, p<0.01). *Post hoc* analysis showed that for *A'* the HPC group differed from the PFC and control groups at each delay except for 1 and 2 sec, and that there was a significant effect of delay on the HPC group, with higher performance at the shorter delays. *SI* data showed that the HPC group differed from PFC and controls at each delay, and that performance at 1 and 2 sec delays was greater than at the 12-sec delay. The three measures of bias did not reveal any effect of group (I_y: ($F_{(2,28)}$=1.24, ns), *RI*: ($F_{(2,28)}$=2.00, ns) and *B''*: ($F_{(2,28)}$=0.80, ns), or any effect of delay or interactions for I_y (Delay ($F_{(6,168)}$=0.52, ns), Group x Delay ($F_{(12,168)}$=0.70, ns)).

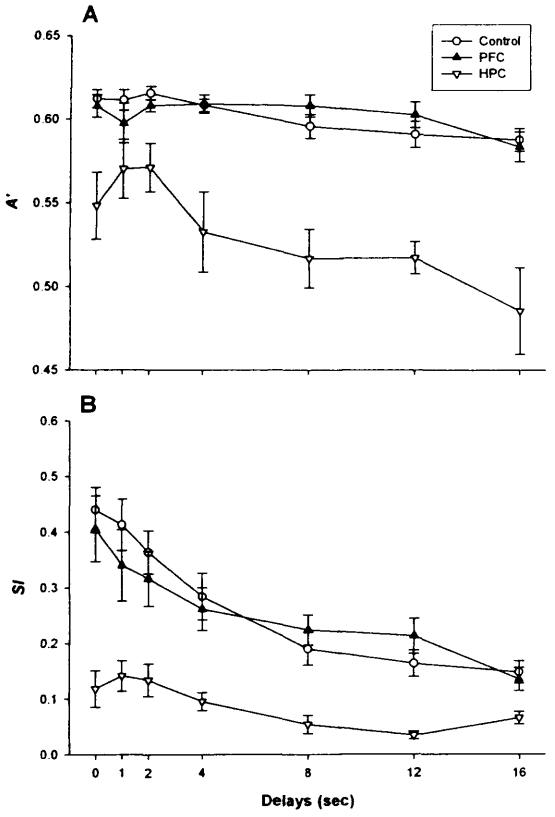


Figure 8.10 CDM/NMTP (0-16 sec delays) postoperative data averaged across all 20 sessions. *A'* (A) and *SI* (B) against delays. Data expressed as mean ± SEM (Control *n*=14, PFC *n*=8, HPC *n*=9).

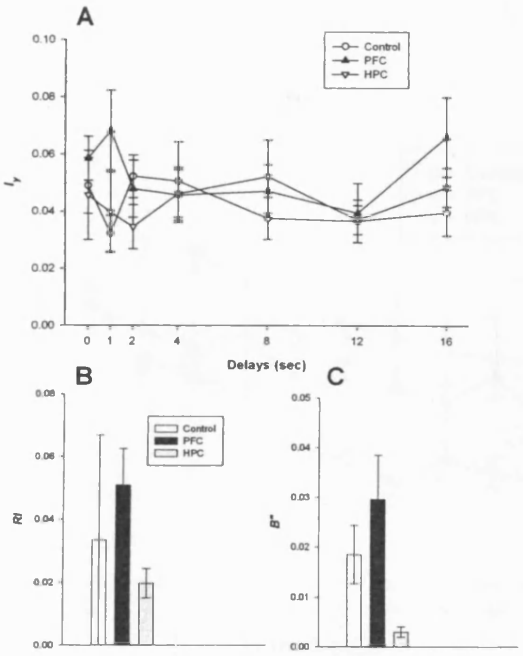


Figure 8.11 CDM/NMTP (0-16 sec delays) postoperative data averaged across all 20 sessions. I_y (A), *RI* (B) and *B''* (C). Data expressed as mean ± SEM (Control n=14, PFC n=8, HPC n=9).

8.3.2.2 Spontaneous locomotor activity

Rats were assessed before being retested on CDM/NMTP and results across the duration of the 60-min session are shown in figure 8.12. There was a main effect of group ($F_{(2,28)}$=17.12, p<0.01) and block ($F_{(11,308)}$=27.90, p<0.01), but no group by block interaction ($F_{(22,308)}$=1.08, ns). Activity decreased as the session progressed, with all three groups significantly different from each other; controls were the least active, and the HPC group were the most active (Newman-Keuls: HPC v. PFC and control, p<0.01, PFC v. Control, p<0.05). Therefore HPC and PFC rats were hyperactive compared with controls, although this effect was significantly greater in the HPC group.

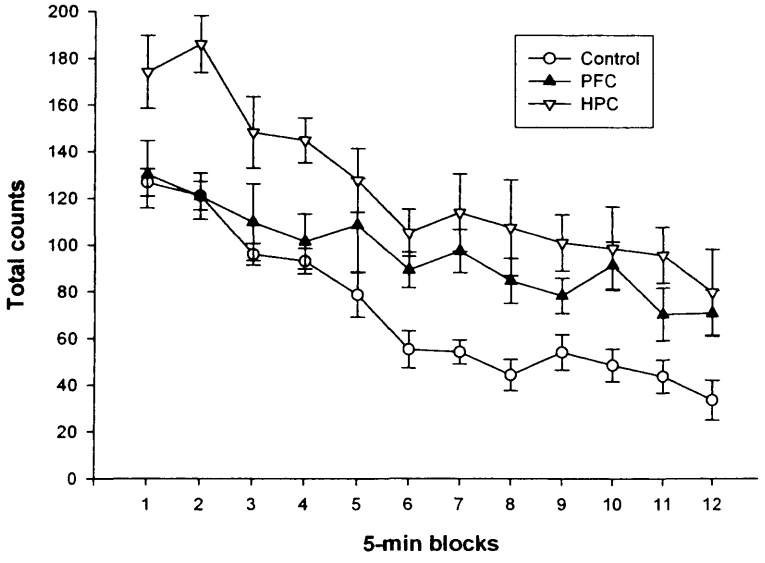


Figure 8.12 Total activity counts against 5-min blocks. Data expressed as mean ± SEM (Control n=14, PFC n=8, HPC n=9).

8.4 Discussion

This experiment sought to examine the effects of HPC and PFC lesions on CDM/NMTP, a more complex working memory task in the Skinner box that combined the elements of matching and non-matching within a session. There was a clear dissociation in the effects of these two lesions, the nature of which was entirely unanticipated. The HPC lesions were seen to induce a deficit in performance that was apparent at all delays, whereas the PFC lesions did not result in any deviation from the performance of the control group. The more surprising result is the intact performance in the PFC group. Rats with these lesions were impaired on the DMTP task in Experiment 1, which was a considerably less complex task comprised of just one of the components involved in the CDM/NMTP.

Training and baseline performance

Döbrössy (1997) has previously demonstrated that when the visual signals for the conditional discrimination were not present during the delay stage, performance dropped to chance levels. It was for this reason that the stimulus lights remained on throughout the entire delay phase, despite this compromising the mnemonic load on the rats. The animals took approximately four months of training before being introduced to the zero delay interval version of the CDM/NMTP task. Rats then required 21 sessions to achieve asymptotic performance, this is considerably fewer than were needed in Döbrössy's thesis (1997), where 38 were required. This difference may have arisen from the fact that the present study used slightly longer sessions in this training phase (60 min v. 50 min) and also that rats were exposed to more switches between the two contingencies. Rats in the present study also received an additional training phase where the rule was switched half way through a session; this may have facilitated the transition to the final phase where both rules were presented randomly throughout the session.

Over the 21 sessions, rats were shown to be consistently more accurate on matching as opposed to non-matching trials; this is corroborated by Döbrössy's work (1997) where a similar result was seen. This may reflect the inherent difference between the two variants, as discussed in Chapter 1 (Blokland and Dunnett, 1995; Dunnett et al., 1988; Dunnett et al., 1989; Dunnett, 1993; Sahgal et al., 1990). Alternatively, it might be

an indication that the matching visual cue discrimination is easier to acquire than the non-matching discrimination, perhaps because of interference for remembering the side of the lever when the two lever stimulus lights are illuminated in the latter. Baseline measures confirmed a delay-dependent decrease in performance, validating that the task probes working memory (Dunnett, 1993). Bias data did not reveal any preference towards either rule, which suggests the initial preference for the matching rule observed during zero-delay training had diminished completely by the point of baseline testing.

Previous attempts have been made to combine both task contingencies within one session. For example, the continuous matching/ non-matching to sample test (CNM), of Pontecorvo et al (1988; 1991), relies on trial stimuli that are not paired, but instead are continuously related to each other. In each trial one stimulus (e.g. light or tone) is presented along with both levers, if the stimulus matches the stimulus presented in the previous trial then one of the levers is reinforced, if they do not match the other lever is reinforced. Thus the rat must remember the most recent stimulus in order to compare it with the current one and then select a lever based on whether these two stimuli match; therefore the task is a true matching task in the sense that the position of the response cannot be determined until after the interval between trials.

Another task, designed explicitly to preclude rats from using postural mediating strategies, is the combined D(N)MTP task (Pache et al., 1999; Pache et al., 2003). This task is similar to the present DMTP task in that it combines matching and non-matching within a session and uses distinct sample, delay and choice phases. However, in the combined D(N)MTP task, the side of the correct choice lever is indicated by the illumination of the stimulus light above this same lever in the sample phase. Therefore for example, if the left light is illuminated in the sample, irrespective of the side of the sample lever, then a response to the left lever is rewarded in the choice phase (Pache et al., 1999)[†]. The authors then designate each trial as matching or non-matching, depending upon whether the correct response matches the sample lever. However, one should be cautious regarding the implication that the rat is applying a matching or non-

[†] An identical procedure is adopted in the later study by Pache et al (2003); except that rather than signalling the side of the correct response using the stimulus lights located above the levers, this study uses darkness or flashing lights.

matching rule, because the side of the sample lever is in fact irrelevant to the solution of the trial; rather the rat is matching its response to the side of the stimulus light in all trials. The assertion that this task design may preclude mediation strategies is obviously confounded by the position of the response being determined before the delay begins. In defence of the design, the authors were able to identify drug-induced effects that were dissociable depending on the trial type (Pache et al., 1999; Pache et al., 2003). However it is possible that this may merely reflect a greater susceptibility to interference between the side of the sample lever and the choice response in one "rule" over the other.

Therefore, it would appear that although the present CDM/NMTP task does not rule out mediation strategies, it does provide a valid means for testing both types of rule within a session. It requires that the rat switches response strategies according to the stimulus presented in that trial, and therefore introduces a significantly greater degree of complexity to the original DMTP task.

Retention of CDM/NMTP: effect of HPC lesions

Excitotoxic lesions of the HPC were shown to induce a substantial deficit in performance on CDM/NMTP that was apparent at all delays. In contrast to the control and PFC groups, the HPC group failed to show an increase in accuracy across sessions. There was however a significant effect of delay on the HPC group, suggesting that some element of the task contingencies had been retained at the shortest delays, but if delays were greater than 2 sec performance dropped to near chance levels. The accuracy measures derived from SDT substantiated these data, with both *A'* and *SI* revealing a significant impairment in the HPC group compared with the control and PFC groups. *A'* revealed a sizeable effect of delay in the HPC group, with higher scores at the shorter delays. There was no evidence for bias towards either of the rules, and additionally no effect on the SDT measures of side bias.

It is necessary to address the nature of this deficit in order to postulate what the role of the HPC might be in this task; an impairment in working memory cannot be assumed unless all other interpretations can be discounted. Firstly, one can eliminate bias as being a contributory factor towards the impairment, as the rats showed neither bias towards either lever, nor preference for either rule. Secondly, it is highly unlikely that the

effect is due to a non-specific impairment such as motor, sensory or motivational difficulties (Dunnett, 1985) due to the intact performance of rats with identical lesions in the DMTP task in Experiment 1. However, one cannot rule out a general decline in attentiveness that may have arisen from the inherent complexity of the task. The most significant difference between the CDM/NMTP and the DMTP task is the requirement to attend to the conditional visual stimuli and respond accordingly. The rats might thus be impaired on remembering the conditional discrimination, or alternatively they might be unable to distinguish between the two separate stimuli.

There is some evidence to implicate the HPC in conditional discrimination tasks, with humans with MTL damage being impaired on eye-blink conditioning, e.g. red light + tone= puff, green light+ tone= no puff (Fortier et al., 2003). Some authors implicate the HPC in the performance of conditional discriminations in the rat (Gray and McNaughton, 1983; Gray and McNaughton, 2003). A study by Ross et al (1984) demonstrated a dissociation in the performance of HPC-lesioned rats, with impairment on a conditional discrimination and intact performance on a nonconditional discrimination. In this task the conditional discrimination was of the form (B- A+, A-), with B being a 5-sec light and A being a 5-sec tone which followed B after a 5-sec delay; reinforcement was only delivered when A was presented following B, and not if presented alone. The nonconditional discrimination was of the form (C+, D-), where C and D were two novel stimuli. However, this finding has not been replicated in other studies, where rats with IBO-induced HPC lesions exhibited intact performance on retention (Davidson and Jarrard, 1989) and acquisition (Jarrard and Davidson, 1990) of a similar conditional discrimination. Interestingly, when lesions were aspiratively induced, as in the Ross et al (1984) study, performance was selectively impaired on the conditional but not the nonconditional task (Jarrard and Davidson, 1991). Thus it would seem that extra-hippocampal damage may account for the impairment in this task, with aspirative lesions resulting in damage to adjacent areas such as the subiculum and presubiculum in addition to disruption of fibres of passage (Jarrard and Davidson, 1991).

Another study supporting the idea that the HPC itself might not be crucial for conditional discrimination tasks comes from work by Deacon et al (2001). These authors assessed rats with IBO-induced HPC lesions on a variety of tests; these included

conditional object discriminations cued by internal states (if hungry, A+B- = food: if thirsty, A-B+ = water) or visuospatially (if two copies of object A, always choose lefthand object: if two copies of object B always choose righthand object). The lesioned rats were not impaired on either of these tasks (Deacon et al., 2001), which suggests that the HPC is not essential for the capacity to make conditional discriminations of this sort. It is unlikely that the deficit that was apparent in the present study can be accounted for by an impairment on the conditional discrimination. The most significant factor is the delay-dependency that was observed; this verifies that performance was better at the shorter delays; indicating that the rats had retained the conditional rule and were able to apply it to allow correct performance.

An alternative explanation might be that the HPC-lesioned rats were more susceptible to interference than the other groups. Working memory is susceptible to interference (Olton et al., 1979) and, specifically, proactive interference occurs when responses on previous trials interfere with choice accuracy on the current trial (Dunnett and Martel, 1990). This interference can be reduced by increasing the ITI and in the DMTP task it is most influenced by the previous response i.e. a greater accuracy is achieved on those trials in which the previous response was to the same side as the current trial (Dunnett and Martel, 1990). There is evidence to suggest that interference may be an important consideration in tasks involving the HPC. The effect of HPC lesions were assessed in a MTS procedure using delayed object recognition; an impairment was only apparent when a single pair of objects was used throughout the session, and not when stimuli were trial-unique stimuli (Rawlins et al., 1993). Moreover, this susceptibility to interference has also been demonstrated in the operant chamber. Interference was specifically manipulated in the CNM task (Pontecorvo et al., 1988; 1991) mentioned earlier in this discussion, and HPC-lesioned rats were shown to have a deficit that increased with both increasing delay and interference (Wan et al., 1994). Interference was highest when stimuli changed from trial to trial (i.e. high proportion of non-match trials) and it was therefore the ratio of non-matching to matching trials that was altered to increase interference (Wan et al., 1994). One can therefore hypothesise that the HPC group may have been subject to greater levels of interference than the other groups in the CDM/NMTP task; this interference may have arisen from the presentation

of the different stimuli, in conjunction with interference from the side of the previous responses. This possibility obviously warrants further investigation and could be clarified by a more in depth analysis of performance. This would necessitate modifications in the programme which would allow collection of supplementary parameters, including percent correct by previous rule being the same or opposite to the current trial, and by previous side of response being the same or opposite.

The final possibility for the deficit seen in the HPC rats would be an impairment in working memory. There is evidence to implicate the HPC in working memory that is non-spatial in nature (Aggleton et al., 1992; Broersen, 2000; Clark et al., 2001; Wan et al., 1994; Winocur, 1991), however one of the hallmarks of this type of deficit is intact performance at minimal delays. Clearly the HPC group had a significant impairment that was apparent at even the shortest delay, which indicates that although a working memory deficit cannot be ruled out, it is unlikely to account for the substantial deficit that was observed.

Retention of CDM/NMTP: effect of PFC lesions

The PFC-lesioned rats performed exactly as controls throughout testing; their performance mirrored the controls with a decrease across delays and no evidence of bias towards either rule or side of lever. This result was unexpected in light of the impaired performance of the PFC group in Experiment 1 which involved the DMTP task. The CDM/NMTP task is essentially a more complicated version of DMTP, involving both matching and non-matching rules signalled by different stimuli; thus the idea that performance is intact on CDM/NMTP but impaired on DMTP is paradoxical in the extreme.

Any suppositions to explain this intact performance are usually belied by the impaired performance on DMTP in Experiment 1. For example, upon considering whether the PFC-lesioned rats might have employed a mediating strategy in order to circumvent the complexity of the CDM/NMTP task, it becomes clear that this is unlikely given that no such strategy was employed successfully in DMTP. The CDM/NMTP task utilised a conditional discrimination component to indicate the pertinent rule, so the role of the PFC on this class of task must be considered. Unfortunately the literature on the

involvement of the rat PFC in conditional discrimination is somewhat inconclusive. Winocur (1991) demonstrated an impairment across all delays in PFC-lesioned rats using a simple conditional discrimination learning paradigm. This paradigm was discussed in Chapter 1 and used an operant chamber, however a tone-light conditional discrimination in another study revealed a deficit that was only apparent at longer delays (Delatour and Gisquet-Verrier, 1999). However, there is also evidence for intact performance on conditional tasks, with rats with cytotoxic lesions of the PrL/IL showing no impairment on acquisition of a conditional discrimination performed using a touch-screen apparatus (Chudasama et al., 2001). This task required the rats to learn a discrimination of the type "if stimulus A go left, if stimulus B go right", although crucially presentation of the two choice stimuli occurred immediately upon responding to the sample stimuli. Therefore in this task the lack of effect may simply have been due to the proximity of the sample and choice phases (Chudasama et al., 2001).

There is ample evidence to suggest that the present finding is uncharacteristic. Not only is the PFC implicated in operant DM/NMTP tasks (Aggleton et al., 1995; Broersen, 2000; Chudasama and Muir, 1997; Dunnett et al., 1990; Dunnett, 1990; Herremans et al., 1996; Mair et al., 1998; Porter et al., 2000; Young et al., 1996) and conditional discrimination (see above), but it is also implicated in switching between matching and non-matching rules (Dias and Aggleton, 2000; Joel et al., 1997b). This switching deficit is observed when rules were switched between sessions, so one might speculate that this impairment should actually be exacerbated when switching occurs from trial to trial as in CDM/NMTP. However, Experiment 1 failed to reveal a PFC-induced deficit on switching between the two rules in the Skinner box. Histological analysis did not reveal any differences between the lesions in the present experiment and those in Experiment 1. If anything, the present lesions encroached slightly further into the more posterior cingulate areas, which certainly argues against the lack of impairment resulting from an insufficient lesion.

Spontaneous locomotor activity

Spontaneous locomotor activity was assessed in the rats over a 60-min session. HPC rats demonstrated significantly higher levels of activity than both the controls and the PFC

group, this is in line with the many previous reports of HPC-induced hyperactivity (Bannerman et al., 2002a; Cassel et al., 1998; Coutureau et al., 2000; Galani et al., 1998; Good and Honey, 1997; Higgs et al., 2001). However, of particular significance was the observation that the PFC group were significantly more active than the controls. Therefore HPC and PFC rats were hyperactive compared with controls, although this effect was significantly greater in the HPC group. The PFC effect is not totally uncharacteristic (Yee, 2000), and indeed in Experiment 1 the PFC group only just failed to attain a significant difference from the control group (p=0.0528).

Conclusions

The aim of this experiment was to establish the effects of HPC and PFC lesions on CDM/NMTP. The hypothesis was that an HPC-related deficit might be revealed, whilst the PFC lesions were anticipated to result in a significant impairment. However, a dissociation was revealed between the two lesions, with HPC lesions inducing a deficit that was apparent at all delays, and PFC lesions leaving performance entirely intact. The most convincing explanation for the HPC deficit is likely to be a combination of reduced attentiveness, increased susceptibility to interference and perhaps some impairment in working memory performance. Conversely, the intact performance in the PFC group is wholly counterintuitive in light of the impairment observed in Experiment 1. Nonetheless, this result is unlikely to be an anomalous finding because the spontaneous locomotor activity concurs with that observed in Experiment 1. Additionally there is no ambiguity regarding the statistical analysis, and no suggestion that the lesions are more minimal in the present experiment than in Experiment 1. Further investigation is obviously required in order to substantiate this result.

Chapter 9 General Discussion

This thesis has sought to investigate the involvement of the PFC and HPC in cognitive behaviours in the rat. Specifically, working memory was assessed using an operant delayed matching task with a view to establishing the extent of the roles of the PFC and HPC in this task. Rats were also assessed for their capacity to recall objects in the spontaneous novelty preference test. Finally, a novel task was used to expand on the DMTP data, and to investigate the viability of including both matching and non-matching trials within one session. The findings presented in this thesis have been both revealing and contradictory. This general discussion will synthesise the findings from the individual experimental chapters, and discuss them in terms of the themes that were introduced in Chapter 1. For example, is there sufficient evidence to implicate the HPC in cognitive behaviours that are distinct from spatial memory processes, and what might be the key role of the rodent mPFC? Methods of improving and expanding upon the current results will also be considered.

Delayed matching tasks and the PFC

Experiments 1 and 2 revealed that the PFC is critical for maintaining performance of the preoperatively-learned DMTP task: however, it is not implicated when the task is acquired following surgery. It is necessary first to establish how these findings relate to the existing data in the field (see Table 1.3), before discussing what the broader implications of these results might be. Previous studies are all in agreement that damage to the mPFC results in some degree of impairment on postoperative retention of this task. These results vary with respect to the nature of the impairment, with the majority indicating a deficit that is independent of delay. Two studies that indicated a delay-dependent deficit similar to Experiment 1 targeted more discrete (Fr2: Broersen et al., 1994) or more rostral and extensive areas of the PFC (M2, MO, Cg1 and PrL: Dunnett, 1990). However, the two most closely comparable studies in terms of lesion technique have both demonstrated delay-independent impairments (Aggleton et al., 1995; Chudasama and Muir, 1997), although lesions in Chudasama and Muir's (1997) study

were more discrete than in Experiment 1. Furthermore, the intact acquisition observed in Experiment 2 replicates the findings of both Broersen (2000) and Joel et al (1997b), where PFC lesions were similarly without effect.

Of particular interest are studies which investigate the involvement of the NAC in DMTP. The NAC is intimately connected with both the PFC (Gorelova and Yang, 1997; Groenewegen et al., 1990) and the HPC (Kelley and Domesick, 1982; Powell and Leman, 1976), making it a worthwhile comparison between the lesions examined within this thesis. Lesions of the NAC have been shown to result in a similar spectrum of impairments to HPC lesions in the rat, for example in the T-maze and water maze (Annett et al., 1989). However, these lesions have been shown to induce delay-dependent deficits on DMTP (Dunnett, 1990), similar to the effect of PFC lesions in Experiment 1. This finding has been replicated, with further analysis revealing that the deficit may have resulted from a side-dependent response bias, as indicated by the SDT bias measure I_y (Reading and Dunnett, 1991). Despite a slight indication of an increase in I_y in the PFC group compared to other groups in Experiment 1 this was not statistically significant, suggesting that the nature of this deficit was not identical to the NAC-lesion-induced impairment. Further evidence arguing against a direct correlation between the effects of the two lesions is that NAC lesions were shown to impair switching between the matching and non-matching rules, in either direction (Reading and Dunnett, 1991). This finding is clearly at odds with the intact switching observed in the PFC group.

It is clear that direct comparisons between studies that have utilised different lesion techniques, and also targeted different areas, should be made with caution. In the light of the proposed functional heterogeneity within the rodent PFC (Fuster, 1997; Gisquet-Verrier et al., 2000; Kesner, 2000; Kolb, 1990b), it is perhaps no surprise that the current findings do not precisely mirror those from other studies. Experiments 1 and 2 destroyed cells within the PrL and IL regions of the PFC; the results obtained in these experiments suggest that these areas contribute to the maintenance of working memory across delays in the DMTP task, but not to the learning of this rule if acquired postoperatively. The relative contributions of these areas cannot be determined until more specific lesion studies are undertaken; it is therefore conceivable that one area

might be wholly responsible for the observed deficit, or indeed that both areas are similarly implicated.

A putative role for the PFC in working memory has received wide acceptance (Fuster, 1997; Granon et al., 1994; Kesner, 2000), and the findings reported above seem to concur with this proposition. However, the intact performance observed in Experiment 6 is much harder to reconcile. This experiment investigated the CDM/NMTP task, where one of the main premises was that the task would certainly recruit the PFC, given its greater degree of complexity in comparison to DMTP. Indeed the PrL is thought to be critical when task demands are increased, such that information processing is made more difficult (Granon and Poucet, 2000). However, what transpired was retention of performance that was on a par with control rats. This finding is not only remarkable because of the numerous reports of prefrontal involvement in DMTP (see table 1.3), but also with respect to the impaired performance seen in the PFC-group in DMTP in this thesis. CDM/NMTP is essentially comprised of DMTP and DNMTP within one session thus the idea that rats with similar lesions can be impaired on DMTP alone is perplexing. Obviously, these two experiments involved different groups of rats so a direct comparison is not entirely defensible; however, apparatus and surgical techniques were identical, in addition to the similar effect on locomotor activity, all of which suggests that these two results have direct relevance. We cannot assume a result is anomalous purely because it does not conform to the expected pattern and does not merit a satisfactory explanation. Only with further investigation and replication (or otherwise) of this finding might this matter be resolved.

PFC: no evidence for involvement in behavioural flexibility

Another remarkable finding obtained in this thesis is the lack of any corroboration of the notion that the PFC – especially the PrL and IL areas - is involved in behavioural flexibility. In fact the data seem to argue against any such involvement. PFC-lesioned rats were not differentially affected by the shift between rules in the DMTP task (Experiment 1 and 2), similarly they were not affected by the reversal of the platform position in the water maze task (Experiment 1). The most surprising result comes from the intact performance on the CDM/NMTP task, which has already been discussed above. This task requires the rats to switch between different strategies from trial to trial; which

confers a degree of complexity that one would assume would implicate any region proposed to mediate behavioural flexibility. Although there have been reports of PFC lesions impairing switching between matching and non-matching rules (Dias and Aggleton, 2000; Joel et al., 1997b), more commonly, impairments in behavioural flexibility attributed to the PFC result from more significant shifts in strategy. For example, PFC-lesioned rats were impaired when switching between an odour and a place based strategy in an odour discrimination task (Ragozzino et al., 2003). Thus a possible explanation for the intact performance on the DMTP switch might be due to the nature of the task, which does not require rats to shift their attention to a new dimension (as in the ED/ID shift tasks discussed in Chapter 1). Rats did not exhibit the perseverative tendencies that were apparent on tasks that explicitly tested ED shifts (Birrel and Brown, 2000; Ragozzino et al., 1999; Ragozzino et al., 2003).

Instrumental conditioning: PFC involvement

The concept of instrumental conditioning merits a brief consideration in this discussion, by virtue of the increasing evidence implicating the PFC in this type of learning. Instrumental conditioning is the learning that takes place when a rat is required to acquire a seemingly arbitrary action, such as pressing a lever, to gain access to food. This type of learning is thought to comprise two distinct processes which may depend on different neural structures (Dickinson and Balleine, 1994). The initial acquisition of an instrumental action is based on goal-directed action-outcome (A-O) associations which are thought to be encoded in declarative memory. As training proceeds, performance is thought to become more habitual and ultimately independent of the value of the goal, such associations are termed stimulus-response (S-R), and are assumed to be encoded in procedural memory (Corbit and Balleine, 2000; Killcross and Coutureau, 2003). The distinction between processes that are under either A-O or S-R control can be ascertained experimentally using goal devaluation procedures. For example, a specific satiety procedure involves rats being trained to press one lever to receive pellets and another to receive a sucrose solution; then, before being given a choice between both levers, rats are pre-fed on one of these reinforcers which serves to devalue that specific outcome (Corbit and Balleine, 2003). If performance is under goal-directed A-O control then rats should reduce the number of responses on the lever that is associated with the devalued outcome.

However, if performance is S-R guided, rats should be insensitive to this devaluation procedure. The PrL has been strongly implicated in goal-directed control of conditioning. Lesions of the PrL render rats insensitive to reward devaluation suggesting that these animals are habit-driven (Balleine and Dickinson, 1998; Corbit and Balleine, 2003; Killcross and Coutureau, 2003), furthermore the IL is thought to mediate the ability to inhibit goal-directed responding in order to permit an S-R response (Coutureau and Killcross, 2003; Killcross and Coutureau, 2003). Similarly, the A-O contingency can be degraded such that reward is delivered irrespective of the action. Whereas control animals reduce performance on the non-contingent lever, rats with either PrL (Balleine and Dickinson, 1998) or dHPC (Corbit and Balleine, 2000) lesions have shown insensitivity to this degradation. This thesis has not attempted to address the issue of whether or not performance on the operant tasks was under A-O or S-R control. Nevertheless, it would seem from the evidence above that both the PFC and HPC may be involved in contingency learning and representing the causal relationship between an action and its consequences. Thus deficits arising from inabilities to make A-O associations would be expected to affect both lesion groups, and therefore this would not provide a satisfactory explanation for the double dissociation seen between the lesions on DMTP and CDM/NMTP.

Delayed matching tasks and the hippocampal system

Lesions of the HPC were without effect on retention and acquisition of DMTP. This impairment concurs with a lack of effect on this task after radiofrequency or cholinergic manipulations of the HPC (Mair et al., 1998; Winters and Dunnett, 2004; Young et al., 1996), however other authors have reported deficits (Aggleton et al., 1992; Broersen, 2000; Dunnett et al., 1990; Hampson et al., 1999; Porter et al., 2000). This finding does not provide any evidence towards a role for the HPC in non-spatial working memory as suggested by some authors (Clark et al., 2001; Winocur, 1991; Winocur, 1992a). This result is in stark contrast to the substantial impairment observed in the CDM/NMTP task. In this experiment rats with cytotoxic lesions of the HPC were severely impaired across all delays, although they showed some evidence of retention of the conditional rule by way of a significant delay effect. This finding is postulated to result from a combination of reduced attentiveness, increased susceptibility to interference and possibly some

impairment in working memory performance (see Chapter 8 discussion). Although the presence of such a dramatic deficit does not lend itself well to the notion of a pure working memory deficit and a more global impairment, such as interference, seems to be the more plausible argument.

The HPC has two major routes of communication with the rest of the brain, namely the Fx and the ERC. Lesions of these two areas have been shown to result in behavioural consequences that can be dissociated from each other, and additionally also from selective lesions of the HPC (Bannerman et al., 2001b; Bannerman et al., 2002b; Cassel et al., 1998; Coutureau et al., 2000; Galani et al., 2002). Thus cytotoxic lesions of the ERC and aspirative lesions of the Fx were compared on the DMTP task. Lesions of the ERC left retention of DMTP intact, in line with a previous study (Pouzet et al., 1999b). Conversely, the Fx lesions resulted in an impairment that was independent of delay. Deficits in DMTP are routinely reported following Fx damage. However, the majority of studies have demonstrated delay-dependent deficits (see table 1.2). The dissociation between lesions of the HPC itself and the Fx suggest that some extra-HPC structure may be involved in the performance of this task, and that perhaps inadvertent damage outwith the HPC may account for the numerous HPC-dependent deficits reported on this task.

Further investigation of the proximal cortical areas is warranted on this task. The perirhinal and postrhinal areas are also intimately connected with the HPC both directly (Kosel et al., 1983; Liu and Bilkey, 1996) and indirectly via the ERC (Burwell and Amaral, 1998b; Witter et al., 2000). These two areas are also connected with the PFC via two parallel pathways which may serve separate learning and memory processes (Delatour and Witter, 2002). Furthermore, they have reciprocal connections with widespread cortical sensory areas (Suzuki, 1996), which make them suitable candidates for involvement in mnemonic functions. However, the behavioural significance of these areas has yet to be fully established. There is evidence to suggest that lesions of peri/postrhinal cortices have little or no effect on spatial tasks in the rat (Aggleton et al., 2000; Bussey et al., 1999; Ennaceur et al., 1996; Machin et al., 2002), thus distinguishing them from lesions of the HPC. A proposed key role for the peri/postrhinal cortices in recognition memory is gaining increasing credence (Brown and Aggleton, 2001; Bussey

et al., 1999; Winters et al., 2004; Winters and Bussey, 2005). There is evidence to suggest that the perirhinal cortex is involved in processing information essential for recognition memory of individual items, whereas the HPC is more implicated in recalling arrangement of items, such as complex scenes (Wan et al., 1999). Moreover, interactions between the PFC and the perirhinal cortex are thought to be necessary for long-term retrieval of temporal object information (Hannesson et al., 2004a).

Some studies have investigated the involvement of the perirhinal cortex in delayed matching paradigms in the rat, but yet again, the results are inconclusive. Lesions that involved the perirhinal cortex and the lateral ERC induced a delay-dependent impairment on DNMS using objects (Mumby and Pinel, 1994). Similarly, a delay-dependent deficit was demonstrated in DNMTP in the Skinner box following lesions that were confined to the perirhinal cortex (Wiig and Burwell, 1998). However, in another study these lesions left DNMTP performance intact (Ennaceur et al., 1996), suggesting that this issue is far from resolved.

Allocentric spatial memory and the HPC

One of the clearest findings in this thesis is the impairment in the reference memory water maze task following HPC lesions. This impairment was first demonstrated in Experiment 1 and was subsequently replicated in Experiment 2. This finding is completely in line with the existing literature (Bannerman et al., 1999; Broersen, 2000; Cassel et al., 1998; Duva et al., 1997; Galani et al., 1998; Good and Honey, 1997; Gould et al., 2002; Liu and Bilkey, 2001; Morris et al., 1982; Richmond et al., 1999; Wright et al., 2004), and serves to corroborate the idea that the HPC is implicated in allocentric spatial information processing. Further support for this idea is provided by the spontaneous novelty preference data reported in Chapter 7. Despite no overall group difference in the discrimination ratio, rats with HPC lesions failed to show a significant discrimination on the spatial shift version of this task. This version required the rats to discriminate between objects based on their spatial displacement, and thus taxed the ability to recall not only the object but also its location within the arena with respect to the other objects and the environmental cues. Therefore this thesis provides a convincing endorsement of the belief that the HPC is vital for accurate performance in allocentric spatial memory tasks (Aggleton et al., 2000). However, the CDM/NMTP task reveals

that the HPC may also have a much wider role in information processing, and that perhaps it is only recruited in non-spatial tasks under certain conditions.

Task design

Statements attributing overt behaviours displayed by animals to memory function should always be made with caution (Sarter, 2004; Steckler and Muir, 1996). Indeed many authors question the validity of DMTP type tasks with respect to working memory deficits, suggesting that rather than retaining the relevant information over the delay, they are in fact using mediating strategies to facilitate performance (Chudasama and Muir, 1997; Dudchenko, 2004; Dudchenko and Sarter, 1992; Herremans et al., 1996; Melia et al., 1990). These strategies arise from the fact that the correct response can be determined before the delay phase, i.e. the rat knows which lever it must respond to, and the rat can then perform behaviours to "bridge" the gap between sample and choice (Steckler and Muir, 1996). These behaviours can take the form of the assumption of body postures, licking or even biting towards the correct lever for the duration of the delay, thereby negating any memory component. In the paradigm employed within this thesis the rat was forced to nose-poke in the central panel to initiate the choice phase of the task in order to limit the use of such strategies (Dunnett, 1985). Delay intervals were variable, and therefore the most effective strategy was to nose-poke as frequently as possible throughout the delay period; this high consistent rate of responding is what is normally seen and suggests that the rats typically remain centralised between the two choice levers (Dunnett, 1993). However, there is increasing evidence to suggest that this is not always the case.

Herremans et al (1996) have shown that rats will display behaviour such as looking at, or moving towards, the correct lever, with subsequent responding on the correct lever, for approximately 50% of all trials. Chudasama and Muir (1997) provided a more detailed analysis of these mediating behaviours using systematic analysis of video clips restricted to the delay periods in DNMTP. Two independent observers were able to predict the animals' response to a high degree of accuracy, based on behaviours such as head turns, nose pokes and paw pushes performed during the retention interval. These authors report delay-independent (scopolamine and PrL lesions) and delay-dependent (Fx lesions) deficits on this task, but suggest that these impairments may not be fully

accounted for by mnemonic failure, but may instead reflect a disruption in effective use of mediating strategies (Chudasama and Muir, 1997). Furthermore, rats were shown to maintain biased body positions in a spatial working memory task in a modified 9HB (Gutnikov et al., 1994); this study also indicated that drug-induced deficits could result from simple locomotor effects, for example disrupting the ability to maintain a particular body alignment, rather than true amnesic impairments. This hypothesis was echoed in a study employing DMTP in the Skinner box, where concerns over the potential motor and motivational effects of certain drugs were raised (Stanhope et al., 1995). Additional strategies to preclude animals from using these facilitatory behaviours include the insertion of dividers between the central panel and the levers (Stanhope et al., 1995), and the addition of admission levers or responses, such that the animal must make a nose poke or lever press on the back wall of the chamber to initiate the choice phase of the task (Hampson et al., 1999; Mair et al., 1998). One task explicitly designed to remove mediation strategies is the delayed conditional discrimination (DCD) task designed by Herremans et al (1994). The DCD task involves the rat being presented with a stimulus (light or tone) which signals whether that trial's correct response will be to the left or right lever. Following a delay, an admission lever is presented (always the same side for each rat) which must be pressed before the choice phase commences; this task has been proven to be effective in reducing mediation strategies (Herremans et al., 1994; Herremans and Hijzen, 1997). Another such task is the combined delayed matching and non-matching to position task of Pache et al (1999; 2003), this task is discussed in full in chapter 8; however, it does not appear to preclude the use of mediating strategies as the side of response is determined in the sample phase, and the choice phase is only initiated after the first panel press following the delay (as in Dunnett's (1993) task). The CNM task also discussed in chapter 8 provides a more viable alternative for reducing the probability of mediating strategies (Pontecorvo et al., 1988; Pontecorvo et al., 1991).

It would appear that the only definitive way of preventing mediating behaviours would be to have a true matching to stimulus task in which the response cannot be anticipated before the choice presentation (Herremans and Hijzen, 1997). Operant DNMS tasks have been investigated, for example responding to the opposite lever to the one under the sample stimulus (e.g. high frequency flashing stimulus light) (Döbrössy,

1997). However, accuracy has not been suitably high enough to permit the introduction of delay schedules (Döbrössy, 1997; Pontecorvo et al., 1996). With the advent of touchscreen technology the potential scope of behavioural paradigms will be increased dramatically (Bussey et al., 1997). For example, this technology could be used to allow computer generation of stimuli and randomisation of the location of the correct response (Sahgal and Steckler, 1994).

Nonetheless, despite the potential confound of mediation strategies, DMTP has routinely been shown to result in dissociable lesion effects (e.g. Aggleton et al., 1995; Broersen, 2000; Dunnett, 1985; Winters and Dunnett, 2004). Indeed this thesis provides further justification towards the use of this paradigm. The impairment exhibited by the PFC group in DMTP is highly unlikely to be a result of an inability to successfully apply a mediation strategy; if such a strategy was the sole means of solving the task then the PFC-group would be expected to be at least as impaired on the CDM/NMTP task. However, although casual observation of the rats' behaviour did not reveal any obvious postural biases or strategy use, this matter warrants more formal investigation. This would have to take the form of employing an unbiased observer to predict the side of the response based on the behaviour during the delay (Chudasama and Muir, 1997), and only once this analysis had been performed could the integrity of the task be established.

The various tasks employed within this thesis have served to highlight some of the pitfalls encountered in behavioural testing. The object recognition task used in Experiments 4 and 5 has been advocated as a pure test of recognition memory that involves no training due to relying on an innate exploratory response displayed by the rat. However, in the hands of this experimenter at least, these data were subject to a large degree of variability even when trials were repeated on three separate occasions. This variability is an inevitable consequence of measuring behaviours that have an inherent degree of variation and serves to highlight the value of operant tasks in eliminating these problems. Operant tasks would appear to provide a more reliable and robust means of assessing cognitive behaviours, allowing more animals to be tested in shorter daily sessions and with greater consistency across procedures. However, greater task complexity demands an increase in the required training time; for example CDM/NMTP required 7 months of training compared with 4 months for the less complex DMTP task.

Clearly, when designing a task, a balance must be reached between the amount of training time and a sufficient degree of difficulty to avoid ceiling effects.

Evidence for multiple memory systems

It is generally assumed that memory can be divided into multiple systems that are served by different neural substrates (Kim and Baxter, 2001). There are numerous accounts of studies which provide evidence for the existence of such systems (e.g. McDonald and White, 1993; Packard et al., 1989). Moreover, it is also widely considered that the PFC and HPC may be dissociable based on their relative contributions to these systems (Kesner and Rogers, 2004). There are three basic classes of interaction; these are competition, synergism and independence (Kim and Baxter, 2001). This thesis has certainly provided evidence of independent memory systems, for example with HPC lesions impairing water maze performance, whilst PFC lesions left performance intact. Similarly the suggestion of an impairment in the spatial shift version of the spontaneous novelty preference task indicates that again the HPC is involved in a system that is concerned with spatial information processing. There is also a suggestion that there might be a degree of synergistic interaction, indicated by the probable involvement of both the PFC and HPC in the recency version of the spontaneous novelty preference task. The assertion that these structures might have a role in temporal memory correlates with a number of studies (e.g. Fortin et al., 2002; Kesner et al., 2002; Milner et al., 1985; Zorrilla et al., 1996). It would be of great interest to probe this synergistic interaction by examining crossed-unilateral inactivation lesions on these two areas. If the two areas are indeed acting synergistically then inactivation of both would be expected to completely abolish performance on this task (Kim and Baxter, 2001).

The most significant and novel finding reported in this thesis is the distinct double dissociation between the effects of PFC and HPC lesions on the DMTP and CDM/NMTP tasks respectively. This suggests that these two tasks are being governed by different memory systems, which in turn are mediated by different neural substrates. Undoubtedly, such an incongruous result requires further validation and clarification before one can speculate as to the grounds for the dissociation. Manipulation of the relevant task parameters might serve to elucidate the exact nature of the deficits; for example, increasing the ITI in the CDM/NMTP task would be expected to minimise any

interference effects between trials and would therefore test the hypothesis that the HPC-deficit was related to these effects. Furthermore, it would be of interest to delineate the exact contributions of the PrL, IL and Cg1 cortices to the DMTP task by performing restricted cytotoxic lesions of these areas. Finally, as mentioned above, disconnection studies might provide a more lucid account of the underlying mechanisms involved in the performance of these tasks.

Conclusions

The aim of this thesis was to provide a clearer understanding of the involvement of the PFC and HPC in cognitive behaviours in the rat; although this aim has not been met resolutely, what has been established is that the behaviours that control performance on delayed matching tasks are certainly complex and multifaceted. A variety of interesting results have been obtained which highlight the crucial functions in which both the PFC and HPC participate. The HPC has been implicated unambiguously in spatial information processing, whereas the PFC has been shown to mediate no such role. Both areas are implicated in the temporal organisation of memory, a capacity which is vital for performance on numerous tasks and confers the ability to coordinate information to a high degree of sophistication. Finally, the DMTP task provided evidence that the PFC is involved in working memory, and the CDM/NMTP task suggested that HPC involvement in this function cannot be discounted. Evidently, the significance of these two brain areas should not be underestimated, this is attested to by their reputed roles in a wide array of diseases that bear impairments of a cognitive nature. Ultimately, further investigation of these matters should go some way towards providing the rationale for effective treatment strategies for amelioration of a wealth of cognitive deficits.

Appendix

Appendix A

Solutions used in perfusion and fixation protocols, and general buffers.

1. PREWASH for perfusions

Di-sodium hydrogen phosphate (dihydrate)	18 g
Sodium chloride	9 g

Make up to 1 litre with distilled water. Adjust to pH 7.4 with orthophosphoric acid (store in cold room).

2. 20% PARAFORMALDEHYDE stock solution

Paraformaldehyde	1 kg
Distilled water	5 litres

Dissolve with heat on the stirrer without boiling. Add 5 ml of 10 M sodium hydroxide to make the solution alkaline, thus promoting the paraformaldehyde to go into solution.

3. 4% PARAFORMALDEHYDE perfusate

Di-sodium hydrogen phosphate (dehydrate)	90 g
Sodium chloride	45 g
20% paraformaldehyde stock solution	1 litre

Make up to 5 litres with distilled water. Adjust to pH 7.3 with orthophosphoric acid.

4. 25% SUCROSE solution (used for cryoprotection of brains)

Di-sodium hydrogen phosphate (dihydrate)	18 g
Sodium chloride	9 g
Sucrose	250 g

Make up to 1 litre with distilled water.

5. PHOSPHATE BUFFERED SALINE (PBS)

Basic 0.1 M solution used as vehicle for Ibotenic acid.

Distilled water	1 litre
Disodium hydrogen orthophosphate (dihydrate)	11.46 g

Sodium dihydrogen orthophosphate (dihydrate)	2.96 g
Sodium chloride	9.00 g

Heat the distilled water gently whilst adding the salts until all are dissolved. Adjust to pH 7.4 with hydrochloric acid (store at +4°C).

Appendix B

Cresyl fast violet (nissl) stain

Solutions:

1. Cresyl violet working solution

Cresyl violet acetate	7.04 g
Sodium acetate (anhydrous)	5.00 g
Distilled water	600 ml

Mix on stirrer overnight. Adjust to pH 3.5 with glacial acetic acid, make up to final volume of 1 litre with distilled water and filter (store at room temp.).

2. Acid alcohol

Add 5 ml of acetic acid to 200 ml of 95% alcohol.

Method:

1. STAINING using a Shandon processing machine

70% alcohol	5 min
95% alcohol	5 min
100% alcohol	5 min
50/50 chloroform alcohol	20 min
95% alcohol	5 min
70% alcohol	5 min
Distilled water	5 min
Cresyl violet	5 min
Distilled water	STOP

Racks are then removed from the machine and sections allowed to sit in distilled water for 10-30 min before differentiation and dehydration.

2 DIFFERENTIATION AND DEHYDRATION

70% alcohol	5 min
Acid alcohol	as necessary to achieve desired degree of staining
95% alcohol	5 min
100% alcohol	5 min
Xylene	5 min

Coverslip using DPX.

Appendix C

Acetylcholinesterase (AChE) histochemistry.

(method based on that of Koelle (1955))

Solutions:

1. Stock incubation solution

Copper sulphate ($5H_2O$)	781 mg
Glycine	750 mg
Sodium acetate	2.88 g

Make up to 1 litre with Distilled water and adjust to pH 5.0 with acetic acid (store at +4°C).

2. Incubation medium (made up just prior to use)

Stock incubation solution	200 ml
Acetylcholine iodide	230 mg
Ethopropazine	10 mg

Heat solution to 40-50°C to dissolve substrates; allow to cool to 37°C or less before use.

3. Sulphide solution (mix and use in fume cupboard)

Sodium sulphide	2 g
Distilled water	200 ml

Adjust to pH 7.5 with acetic acid.

Method:

This is a metal precipitation method and glass Coplin jars and plastic forceps are used at all times.

1. Sections are mounted on gelatinised slides and allowed to dry at 37°C overnight.
2. Leave slides in incubation medium for minimum of 3 hr at 37°C (or overnight at room temp.).
3. Wash 4 x 3 min in distilled water.
4. Develop in the sulphide solution, for approximately 30 sec to 2 min, until golden brown.
5. Wash 4 x 3 min in distilled water.
6. Dehydrate in ascending alcohols to xylene and mount in DPX.

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