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Several of the experiments reported in this thesis have been published.

Experiment 1 has been published as : Neave, N., Sahgal, A., & Aggleton, J.P. (1993). Lack of effect of dorsomedial thalamic lesions on automated tests of spatial memory in the rat. *Behavioural Brain Research*, **55**: 39 - 49.

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ABSTRACT.

The human anterograde amnesic syndrome is a condition whereby the person can no longer learn new facts or pieces of information, and yet retains short-term memory processing, and older memories learnt before the onset of the syndrome. Both human case studies, and experimental animal models of this condition, have strongly indicated that damage to certain closely interrelated structures and cortical areas may be responsible for this failure to learn new information. Particular emphasis has been placed on the hippocampus, the mammillary bodies, certain thalamic nuclei, and those regions of cortex (particularly rhinal cortex and parts of prefrontal and cingulate cortices) that receive strong connections from the hippocampus and selective thalamic nuclei.

While the evidence for the role of the hippocampus in mnemonic processing (especially regarding rats performing spatial tasks) is strong, the evidence is less certain concerning the involvement of the other structures and regions. This thesis has directly attempted to ascertain the relative contributions of certain thalamic nuclei, one region of cortex (the cingulate region), and a fibre pathway (the cingulum bundle) which connects the hippocampus and thalamus with cingulate cortex. The contribution of this fibre bundle received particularly close experimental scrutiny in this thesis as it's possible role in the neuroanatomical circuitry governing certain forms of mnemonic processing may have been underestimated.

A series of five related experiments are described, each involving the DA pigmented strain of rat, whose spatial working memory processing was evaluated using a range of automated and maze-type tasks. These animals received a variety of lesions to the hippocampus, thalamus, cingulate cortex or the cingulum bundle, created by either neurotoxic or radiofrequency methods. Two forms of spatial memory processing were assessed; these consisted of egocentric and allocentric processing, and evidence is presented that they may be mediated by dissociable neuroanatomical circuits. Lesions of the mediodorsal thalamic nucleus and cingulate cortex had no effect on tasks assessing egocentric or allocentric processing. However, bilateral lesions of the cingulum bundle, whilst having no effect upon egocentric tasks, caused a severe impairment on tasks assessing allocentric processing. Lesions of the hippocampal system (the fornix) caused a severe impairment on both types of tasks. The nature of the putative neuroanatomical circuitry governing both allocentric and egocentric memory processing is discussed.

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CHAPTER 1. THE AREA OF STUDY AND SOME DEFINITIONS.

1.1 THE STUDY OF MEMORY.

Over the years, the study of the mnemonic impairments demonstrated by human patients suffering from anterograde amnesia due to a variety of causes has raised many questions concerning those regions of the brain that are assumed to be important in the storage and implementation of memories. It is the aim of this thesis to provide a review of the evidence concerning anterograde amnesia in both human patients and experimental animals, and to present a set of experiments that it is hoped will further clarify the interrelationships between a set of structures and pathways which have been strongly implicated in such amnesia's.

Throughout this thesis, two questions will be addressed. The first will consider: "*What are the specific brain regions that are involved in the mnemonic process* ?" .The second will focus on: "*Is there just one, inter-connected anatomical system of memory, or are there multiple anatomies, which reflect multiple types of learning and memory ?*".

The remainder of this introductory chapter will outline the evidence concerning the anterograde amnesic syndrome in both humans and experimental animals, and will also present further detail concerning the brain regions and structures that are currently regarded as being those neural substrates most critical for learning and memory. It will first be necessary to outline some important definitions concerning the types of memory which will form the prime focus of this thesis.

1.1.1 Procedural and declarative memory.

Over the years, the study of memory deficits in both human patients and experimental animals has provided evidence for distinguishing between what Squire (1986) referred to as, a capacity - limited (short-term) memory, and a more long lasting (long-term) memory. Recent findings using data from human amnesics and from other sources has suggested a further distinction within the domain of long-term memory (Schacter, 1987). This is because the memory deficit in amnesia is not so profound as previously thought, as not all types of learning and memory are similarly affected.

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Typically, amnesic patients demonstrate intact learning and retention of a variety of perceptual and motor skills, for example, on mirror tracing and serial reaction time tasks, and also exhibit intact priming effects (Corkin, 1968; Milner et al., 1968). The performance of amnesic subjects on such tasks, like that of normal subjects, can be influenced by recent exposure to stimulus material (Nissen et al., 1989). These results have suggested a distinction between information based on skills or procedures, and information based on specific facts or data.

The terms '*procedural*' and '*declarative*' have been proposed to describe the kinds of information that amnesic patients can and cannot learn; the distinction reflecting the operation of two kinds of memory processes or systems. As described by Cohen (1984), declarative memory is explicit, and accessible to conscious awareness, and includes such as facts, episodes, and lists. It can be declared, i.e. brought to mind, either verbally or non-verbally, and includes both episodic memory (specific time and place events), as well as semantic memory (facts and general information). By way of contrast, procedural memory is defined as being implicit, and accessible only by engaging in the skills or operations in which that knowledge is fixed. It has been found in humans that procedural memory is principally affected by striatal damage, whilst declarative memory is affected by diencephalic, medial temporal or basal forebrain damage (Mishkin & Appenzeller, 1987; Squire et al., 1993).

1.1.2 Spatial and non-spatial memory.

Further distinctions have been proposed regarding the spatial nature of memory processing. With regards the work discussed in this thesis, the medial temporal / diencephalic memory systems, and the hippocampal formation in particular, have been considered important for spatial memory (O'Keefe & Nadel, 1978). While the functions of the hippocampus are not exclusively spatial (Squire et al. 1993), there is more evidence for the greater importance of this structure in spatial, rather than non-spatial memory (at least where rodents are concerned, though it is much less clear whether there is any difference in primates). For example, the '*cognitive mapping*' theory of O'Keefe & Nadel (1978) proposed that the hippocampus is the neural substrate for a memory system based upon an '*allocentric*' spatial framework.

Allocentric memory is based upon the memory for specific stimuli representing places, or relations between places, that are independent of the orientation of one's body in space. Thus, information about objects, places in the environment, and the relationship between an object and its position is stored in this system. The converse memory system is referred to as '*egocentric*',' and is based upon the memory for responses that depend upon the accurate assessment of the orientation of one's body in space , and is ascribed to other parts of the brain (Kesner et al., 1989).

1.1.3 Working and reference memory.

A final distinction is that between so-called '*working*' and '*reference*' memory. Working memory refers to the mnemonic processes by which information is temporarily maintained and modified, or reprocessed through the succession of trials that comprise a testing session (Baddeley, 1986). A working memory theory of hippocampal function has been proposed by Olton (1983) who argued that the hippocampus is the substrate for a memory system that "*emphasizes the temporal/personal context that separates one instance from another*". The working memory component of a task is trial specific and the temporal span of the memory may be brief or extended. The content of working memory may be spatial or non-spatial. He further argued that reference memory could be said to " *...contain general information about the rules and procedures that is applicable to many different instances of the same class of events, and thus, does not require the current instance of the class to be distinguished from any other instance of the same class*" (Olton, 1983).

This thesis and the experimental work reported in it is primarily concerned with spatial working memory processing in rats with particular regard to the egocentric versus allocentric nature of this mnemonic processing, and the neural circuitry that may underlie such a system.

1.2 THE ANTEROGRADE AMNESIC SYNDROME.

Much of our knowledge concerning the anatomy of human memory comes from studies involving patients who are seemingly unable to learn new information, such as peoples' names, telephone numbers, places visited etc. and is generally referred to as '*anterograde amnesia*'.

Such a syndrome has been defined as: " *...a permanent, stable, and global disorder of memory due to organic brain dysfunction which occurs in the absence of any other extensive perceptual or cognitive disturbance* " (Parkin & Leng, 1993).

According to the above authors, patients suffering from an anterograde amnesic syndrome typically exhibit five general symptoms:

1. Pre-morbid levels of intellectual functioning are maintained.

2. Immediate, '*working memory*' often appears to remain intact with patients showing, for example, normal scores on memory span tasks. Semantic memory, and other intellectual functions are also often spared.

3. Some degree of retrograde amnesia (loss of memory for the period immediately before the onset of the neurological dysfunction).

4. A severe and mostly permanent anterograde amnesia (impaired memory for new information) with performance on conventional long-term memory tests at least two standard deviations below the norm.

5. Some degree of residual learning capability is exhibited, but this is often restricted to memory tasks which do not require the patient to access the memory of a specific event; for example, patients can show improvements on a mirror drawing task whilst showing no knowledge of having performed such a task in the past.

The main causes of such a syndrome are as follows:

1. Herpes Simplex Encephalitis (HSE), a virus which results in a rapid necrotising process which first attacks temporal lobe structures followed by other cortical regions, notably the orbito-frontal cortex. The result of this viral attack is a severe anterograde amnesia that is generally extensive, but which varies considerably. Associated problems such as anomia are also common (Damasio & van Hoesen, 1985).

2. Traumatic head injuries such as those described by Teuber et al., (1968), Dusoir et al., (1990), for example.

3. Anoxia, giving rise to cerebral ischemia, arising typically from cardiac arrest, carbon monoxide poisoning, and near-drowning. Instances of cerebral ischemia resulting in anterograde amnesia have been reported in a number of studies involving humans (Zola-Morgan et al., 1986), and rodents (Auer et al., 1989). The key region involved in such episodes appears to be the temporal lobes, and in particular the hippocampus. In rodents at least, the most vulnerable portion of which appears to be the anterior-dorsal CA1 sub-field (Olsen et al., 1994).

4. Aneurysms (localised dilation's of the walls of a blood vessel which lead to rupturing and then haemorrhage). Various types of aneurysm can occur in the cerebral arterial system and they often cause lasting impairments of memory, either as a direct result of the rupture, or as a result of the surgery performed to repair the aneurysm. One particular form of aneurysm that commonly leads to an amnesic state is that of an aneurysm involving the anterior communicating artery (ACoA), the effects of such damage and the resulting surgical repairs have been described by Vilkki (1985), Phillips et al. (1987), and Bondi et al., (1993). However, this syndrome does not always lead to an amnesic state, as resultant damage to the basal forebrain seems to be a crucial factor (DeLuca, 1993). A second type involving the paramedian thalamic arteries, will be discussed more fully in a later section.

5. Chronic alcohol abuse which results in a condition described as the Wernicke - Korsakoff syndrome, affecting primarily the diencephalic regions with the mammillary bodies and the medial dorsal thalamic nucleus (MD) being the worst damaged (Victor et al., 1971).

6. Temporal lobe surgery carried out principally to alleviate chronic and intractable epilepsy. Such surgery typically involved the removal of the rostral parts of the medial temporal lobes and included the hippocampus, uncus, amygdala, and parts of the entorhinal cortex and parahippocampal gyrus (Scoville & Milner, 1957).

As will be appreciated, such a diversity of clinical conditions, operating alone, or in tandem with other disorders, can affect a good deal of the cortex, and underlying cortical structures. The important question here is whether the amnesic syndrome results from the widespread destruction of a variety of cortical regions; or whether it can be produced by damage to distinct and separate cortical components.

If the form of the amnesic syndrome can be seen to vary in relation to differences in the brain regions affected, then the nature of this variation may give valuable insight on the nature of the components of the memory process. Neuropathological studies have in fact shown that the human amnesic syndrome can be assessed in two broadly similar ways, depending on the areas of the brain that are damaged. The amnesia's in question are generally defined as having either a medial temporal, or a diencephalic origin.

1.3 THE MEDIAL TEMPORAL LOBE SYSTEM AND MEMORY.

The temporal lobes comprise all the tissue below the sylvian fissure, anterior to an imaginary line running roughly from the end of that fissure to the boundary of area 37 with area 19, and the boundary of areas 22 and 37 with the parietal association areas 39 and 40. The region enclosed by these boundaries includes neocortical areas such as Heschl's gyrus, superior temporal, middle temporal, and inferior temporal gyri; and phylogenetically older cortex consisting principally of the fusiform gyrus, parahippocampal gyrus, uncus, amygdala, and the hippocampus (Kolb & Whishaw, 1990). The temporal lobe is rich in internal connections comprising both afferent projections from the sensory systems, and efferent projections to parietal and frontal association cortices, the limbic system, and the basal ganglia. The left and right temporal lobes are connected via the corpus callosum and anterior commissure.

1.3.1 The Hippocampus.

The principal subcortical structures of the temporal lobes are the hippocampus and the amygdala. Both of these structures receive dense cortical inputs channelling an array of sensory information from different sensory modalities, to these structures. The hippocampus is a clearly defined structure forming the medial margin of the cortical hemisphere and located on the medial wall of the lateral ventricle.

The term '*hippocampal formation*' generally includes the subiculum, along with the hippocampus proper , the hippocampus being divided into the sub-regions CA1- CA4 (Lorente de Nó, 1934). The hippocampus receives inputs from numerous limbic, cortical, and subcortical regions, primarily via the adjacent entorhinal / perirhinal cortices (now strongly implicated in the anatomy of amnesia - see later section), and the subiculum.

In primates, the primary pathway of neural activity entering the hippocampus is from entorhinal cortex via the perforant path to the dentate granule cells, with collateral's to CA1 and CA3 pyramidal cells, whence they pass to CA1, CA2, and the subiculum (van Hoesen, 1982).

The principal direct output from the hippocampus is via the fornix, which serves to connect the hippocampus with the mammillary bodies, anterior medial (AM) and anterior ventral (AV) thalamic nuclei, septum, basal forebrain, and hypothalamus. These thalamic and septal projections of the hippocampus further provide it with indirect links to the anterior cingulate and retrosplenial cortices (Powell & Hines, 1975).

In the rat, non-fornical outputs principally consist of internal projections to entorhinal cortex thus providing the hippocampus with indirect pathways to numerous temporal, sensory and associational neocortical regions, as well as to the amygdala, thalamus, and mammillary bodies, and retrosplenial cortex (Swanson et al., 1987; van Groen & Wyss, 1992).

1.3.2 The fornix

The fornix is an arch-shaped major white-matter tract consisting of a large bundle of fibres formed as the anterior and dorsal aspects of the hippocampus merge, at this level the total fornix bundle is composed of fibres of the fimbria, but from the point of convergence, the fibres arch downwards and separate into two components (pre- and post-commissural), one descending into the septum, and the second descending to the mammillary bodies and other regions. This is true in both humans and rats (Hamilton, 1976; Mark et al., 1993). As such, the fornix is the primary efferent system of the hippocampus, allowing this structure to potentially influence a wide range of both cortical and subcortical structures.

Given the anatomical importance of the fornix, the surgical destruction of it has been assumed to mimic the effects of the total destruction of the hippocampus, thus this method is thought to be a useful model of hippocampal dysfunction. The main problem with this approach is the fact that total fornix destruction by whatever method, spares some important hippocampal efferents, principally those via entorhinal and perirhinal regions (Jarrard, 1993).

1.4 MEDIAL TEMPORAL LOBE AMNESIA.

1.4.1 Human case studies involving medial temporal lobe amnesia.

Perhaps the best known case study involving the neuropsychology of memory concerns the story of patient H.M. In 1953, at the age of 27, H.M. received a bilateral resection of the medial temporal lobe in an attempt to cure his intractable epilepsy. Though a precise description of the full extent of the surgery is unavailable as yet, it has been assumed that the surgery involved the prepiriform cortex, uncus, amygdala, hippocampus, entorhinal cortex, and parahippocampal gyrus (Scoville & Milner, 1957).

Following surgery, H.M. displayed no obvious deficits in perception, reasoning, or short term memory, and in fact scored above average on the '*Wechsler-Bellvue Intelligence Scale*' but did however demonstrate a deep and profound anterograde amnesia. H.M's memory prior to the surgery was good, as was his capacity to recall remote events from childhood, yet he couldn't recall how old he was, where he lived, or what he did several minutes previous (Scoville & Milner, 1957). Over the last 30 years or so, H.M's cognitive abilities have been extensively studied, the key finding being that he is impaired at many kinds of learning tasks in which there is a delay between presentation and recall. He is severely limited in the ability to acquire new verbal, or non - verbal information, regardless of which sensory modality is used in the acquisition phase (Milner et al., 1968; Milner, 1972).

Some interesting findings regarding H.M's abilities have shed light on the nature of his memory deficit. Although H.M has a severe memory defect covering a wide range of memory related tasks, he is surprisingly competent at motor learning and other cognitive tasks. For example, when tested across several days on mirror tracing, rotary pursuit, and bimanual tracking tasks, H.M. showed a normal learning curve and yet demonstrated no sign of having remembered performing the task on previous days (Corkin, 1968). This dissociation between the impairments in motor learning and other types of memory tasks, indicates that certain perceptual motor skills are acquired independently, and may be processed by different structures to those damaged in H.M's operation.

From the data gathered from H.M. and other temporal lobectomy patients, Scoville and Milner (1957) argued that the most severe memory impairments were found in those cases in which the hippocampus was principally involved. The greater the degree of hippocampal damage, the more profound the amnesia.

The key role of the hippocampus in temporal lobe amnesia has been further strengthened by the recent case study of R.B. (Zola-Morgan et al., 1986). The authors of this case study made the point that clinical studies have often linked memory impairments to damage of the hippocampus, yet the damage in these cases has not been confined solely to the hippocampus itself. In contrast, their patient R.B, developed a severe anterograde amnesia following an ischemic episode. A thorough histological examination revealed a circumscribed bilateral lesion involving the entire CA1 field of the hippocampus, but no other damage that could be associated with the memory deficit was discovered.

It is interesting to note that evidence from animal studies also indicates that limited damage to the CA1 sector of the hippocampus can impair some forms of learning and memory. Auer et al. (1989) reported that ischemic brain damage in rats limited to the CA1 sector resulted in deficits in spatial navigation.

A case which at first sight appeared to contradict this '*hippocampal hypothesis*' was reported by Dimsdale et al., (1964). Their patient (N.T), underwent a right temporal lobectomy for intractable epilepsy, the excision extending into the temporal horn removing Ammon's horn, uncus and the amygdala. This resulted in a severe and persistent amnesic state which was rather unexpected as the surgery was unilateral rather than bilateral. If this was indeed the case, then this would have cast serious doubt on a large number of other reports which emphasised that only a bilateral temporal lobectomy would result in an anterograde amnesic syndrome.

Following the patient's death, Warrington and Duchen (1992) discovered a sclerotic lesion of the unoperated left hippocampal formation thus proving that N.T. was not in fact an exception to the orthodox view that a bilateral hippocampal lesion was an invariant contributor to a severe temporal lobe amnesia. A similar case had been earlier described by Penfield and Mathieson (1974).

1.4.2 The production of temporal lobe amnesia in experimental animals.

In view of the neuropathological findings related to anterograde amnesia in human patients, attempts to mimic temporal lobe amnesia in experimental animals have focused on the effects of bilateral temporal lobe lesions involving the hippocampus. Squire & Zola-Morgan (1983) reviewed a wide range of studies comprising some eighty monkeys that described the behavioural effects of selective damage to the hippocampus. The findings were somewhat disappointing as three main difficulties became apparent.

Firstly, different studies using the same kind of task reported contradictory findings. For example, of seven studies investigating the effect of hippocampal damage on the learning of pattern discriminations, three found an impairment whereas four did not (e.g. Moss et al., 1981).

Secondly, among three tasks that have been shown to be particularly sensitive to medial temporal damage in humans, some revealed an impairment (delayed non-matching to sample (DNMS), Mishkin, 1978), whereas others did not (delayed matching to sample - DMS) and delayed response, Mahut, 1971).

Thirdly, reversal and alternation tasks, did not yield similar results. For example, animals were impaired on a task of spatial reversal but not on one utilising object reversals (Mahut, 1971). Such findings indicated that lesions of the hippocampus may have a greater effect upon spatial rather than non-spatial memory, but did not produce a profound global amnesia, as that found in some human patients.

From such a review, it seemed possible that several fundamental problems could be inherent in the then animal model of temporal lobe amnesia.

Firstly, it could be possible that the hippocampus serves a different role in rats and monkeys than in humans; such an assertion is difficult to disprove fully as it is highly likely that some cross-species differences do indeed exist, one example of course is the key contribution of language to human memory processing. It must be borne in mind however that the basic cytoarchitectonic structure of the hippocampus in monkeys and humans is remarkably similar and indeed, the major connections of the hippocampus among a wide range of mammalian species share a great many similarities.

Given this degree of cross - species similarity, it seems doubtful (though of course not impossible), that the hippocampus could perform qualitatively different functions among mammals.

Secondly, if it is assumed that the hippocampus serves a similar role among mammals, then it is more likely that the assessment of this structures possible roles in learning and memory is faulty. This is essentially the conclusion drawn by Squire & Zola-Morgan (1983) who recommended that four standard tests: DNMS, delayed response, concurrent learning, and object discrimination, should form the benchmark of future assessment.

Such problems aside, early research soon revealed intriguing information. While it became accepted that damage limited to the hippocampus, and its principal efferent - the fornix, resulted in mild impairments, Mishkin (1978) reported that conjoint damage to both the hippocampus and the amygdala resulted in a severe impairment in a one-trial object recognition task, whereas damage restricted to either the hippocampus or the amygdala alone had little effect.

This raised the possibility that the hippocampus was indeed involved in learning and memory in animals, but the extent of any deficit depended crucially on there being additional damage to other structures. Such a proposal was consistent at the time with the finding in human temporal lobe patients that a clear case of amnesia with damage limited to the hippocampus had not been reported, and that lesions restricted to the amygdala did not lead to a severe memory impairment (Anderson, 1978). The severity of the amnesia in both monkey and man could well be related to the degree of damage caused to these structures.

Zola-Morgan & Squire (1989) reviewed a range of studies that tested this hypothesis by comparing monkeys with either lesions of hippocampus, the amygdala, or bilateral amygdala plus unilateral hippocampus, or bilateral hippocampus plus unilateral amygdala damage. Animals in the latter two groups displayed similar impairments on a DNMS task, impairments that were greater than those found in animals with just hippocampal or amygdala damage, but less severe than the deficits found in animals with bilateral damage to both structures. Such a gradation of impairment strongly suggested at that time that total damage to both structures was essential for a full blown amnesic syndrome, comparable to that seen in humans after temporal lobe surgery - such as patient H.M.

Squire & Zola-Morgan (1983) provided a cautionary note concerning the relative degree of damage suffered by these structures under the various types of surgical methods that were typically employed. Before any useful comparisons can be made, there must be equivalence between the extent of lesioning involving the hippocampus and amygdala, or there remained the possibility that other, (unreported) damage is contributing to the amnesic state.

This point had been noted earlier by Correll and Scoville (1965). They reported that the surgical technique involved in selective hippocampal lesioning spared the anterior one-third of the structure, total hippocampal lesions also invariably included surrounding cortical regions such as entorhinal cortex as well. The possibility that various regions of the hippocampus may be spared, or conversely that surrounding cortices may be damaged, is of vital importance when interpreting data from monkey studies, especially when comparisons are made between such results and data from human studies. For example, the surgery carried out on patient H.M. was described as having focused on the anterior two-thirds of the hippocampus, evidence from monkey studies in which this portion consistently remained undamaged was hardly a valid comparison.

Jarrard (1993) pointed out that the difficulty in pinning down the exact nature and function of the hippocampus, (as seen in the many theories put forward over the years, such as those by O'Keefe & Nadel (1978), Eichenbaum et al., (1992), Olton et al. (1979), Rawlins (1985) and Sutherland & Rudy (1989), lies in the nature of the evidence presented to back up such theories. The evidence has been typically obtained from lesion experiments in which the nature and extent of the damage varied widely as a result of the differing surgical techniques used. As pointed out, conventional surgical methods result in extra-hippocampal damage; Jarrard (1993) presented a review of a series of experiments in the rat, in which the hippocampus was removed with injections of ibotenic acid which selectively removed the cells of the hippocampus and which caused no other extra-hippocampal damage. The general finding was that rats without a hippocampus were impaired on those tasks that required the utilisation of spatial and contextual information.

1.4.3 The role of adjacent hippocampal cortices.

Recent studies have focused upon the areas of cortex lying adjacent to the hippocampus and amygdala.

Using a set of standardised tests that are known to be sensitive to anterograde amnesia, the severity of impairment between groups of monkeys with different brain lesions can be compared. Zola-Morgan et al., (1989a; 1989b) reported that monkeys with hippocampal formation lesions (including hippocampus, entorhinal/perirhinal cortices and parahippocampal gyrus) were severely impaired, in a comparable manner to H.M., i.e. the deficits were multimodal, enduring, and yet skill learning remained intact.

Much less severe deficits were found with lesions of the amygdala, fornix, or mammillary bodies. In the first instance, monkeys with lesions of the amygdaloid complex that spared the surrounding perirhinal and entorhinal cortices, performed normally on a set of memory tasks. A second group of animals with conjoint lesions including both the amygdaloid complex and the hippocampal formation were impaired on the same tasks. Interestingly, the severity of the impairment in this second group was no greater than in the group with hippocampal formation lesions alone. Such results suggested that the amygdala may not be as crucial as was previously supposed.

Even more recently, Zola-Morgan et al., (1993) have extended the above study to include an analysis of the effects of hippocampal plus perirhinal lesions on the DNMS and object recognition tasks. Monkeys with such lesions were severely impaired on both tasks, an impairment that was greater than in animals with hippocampal plus parahippocampal cortex damage. That report emphasised the role of the perirhinal cortex in the medial temporal lobe memory system, a finding echoed by Suzuki et al., (1993), and Meunier et al., (1993).

In the Meunier et al., (1993) study, the performance on a visual DNMS task was assessed in monkeys with combined and separate ablations of perirhinal and entorhinal cortices. Lesions which destroyed both of these cortical areas (a rhinal lesion) led to a severe impairment on this task, a deficit that was almost as severe as that seen after combined amygdala plus hippocampal removal. Ablations of the perirhinal cortex alone also produced a severe impairment, but damage to entorhinal cortex alone only yielded a mild deficit. The authors concluded that damage limited to rhinal cortex is sufficient to produce a severe deficit on visual recognition, a deficit that is more severe than that produced by damage to other single structures in the medial temporal region.

Neuroanatomical evidence suggests why the cortical regions adjacent to the amygdala and hippocampus should be important in learning and memory. Perirhinal cortex, along with the parahippocampal gyrus provide the major source of cortical input to the entorhinal cortex, which, in turn, is the source of the principal efferent connection to the hippocampus.

The rhinal cortex itself projects directly to the mediodorsal nucleus of the thalamus (MD) via the ventral amygdalofugal pathway (Aggleton et al., 1986), to the anterior thalamic nuclei, and to the mammillary bodies via the fornix (Rosene & Saunders, 1987). Thus, these regions of medial temporal lobe cortex collectively provide the main route by which information is exchanged between medial frontal cortex and hippocampus (Zola-Morgan & Squire, 1989).

This viewpoint was echoed by von Cramon et al., (1989), these authors focused upon the potential role of the parahippocampal gyrus in human case studies of anterograde amnesia. They reported that a lesion in the white matter of this region would disrupt fibres travelling via the collateral isthmus and the cingulum, such a '*strategic*' lesion would have the effect of depriving the hippocampus of its main afferent projection sources. The effects of lesions of this nature have been reported in patients with left - sided posterior infarctions, in which severe and possibly long-lasting deficits of learning and memory were noted. In contrast, patients with such infarctions that spared the white matter showed no appreciable mnemonic impairments.

In an extensive review, Squire (1992) considered the precise role of the hippocampal formation in learning and memory, and made the important point that evidence from humans, monkeys and rodents appeared to be largely in agreement. That the hippocampus itself is the key structure involved, is in little doubt, as many studies have addressed its importance.

For example, reports have indicated that damage limited solely to the hippocampus can result in a significant mnemonic impairment in humans (Zola-Morgan et al., 1986; Victor & Agamonolis, 1990); and advances in Magnetic Resonance Imaging techniques, have shown that living patients with memory impairments display abnormal hippocampal functioning (Press et al., 1989). However, the argument concerning the role of the hippocampus in spatial rather than nonspatial memory (in rodents at least), remains controversial (Rawlins et al., 1993).

1.4.4 The role of the fornix.

Delay & Brion (1969) proposed that the hippocampus, fornix, and the mammillary bodies constituted a single functional memory system, with the same functional consequence - anterograde amnesia, resulting from an interruption of this system at any stage. With regards the fornix, evidence from both human and animal studies have served to provide some support for this hypothesis.

1.4.5 The effects of fornix damage in human patients.

There is controversy as to whether fornix transection has any long-term effect on memory in human patients. Parkin (1984) for example, has argued that damage to the fornix does not reliably produce a resultant amnesia, though he did stress that most of the key studies have not involved detailed psychological assessment. Gaffan & Gaffan (1991) reviewed the evidence for amnesia in man following fornix transection. They focused upon the two surgical procedures that typically involved damage to the fornix - namely the removal of colloid cysts, and temporal lobe surgery for intractable epilepsy. The authors concluded that although the evidence from the temporal lobe surgeries was controversial, that from the colloid cyst patients gave strong support to the hypothesis of Delay & Brion (1969).

Gaffan et al., (1991) reported 2 patients who developed memory problems following the removal of a colloid cyst, which also resulted in unilateral damage to the fornix. The pattern of impairment showed by the patients was examined on a series of visual memory tests modelled on those used to study the effects of fornix transection in monkeys. The fornix patients were particularly impaired at concurrent object and pattern discriminations, and in scene recognition, deficits which although not severe, were moderate and disabling.

Further evidence from human patients have come from studies which have focused upon the effects of splenial tumours, and of direct surgery to the corpus callosum. In the first instance, Rudge & Warrington (1991), described the neuropsychological abnormalities found in 9 patients with tumours involving the splenium of the corpus callosum. The outstanding feature of their deficits was a severe memory impairment, which the authors argued was the result of disruption to the fornix, which is invariably damaged in such cases, (the point must be made however, that such splenial tumours also tend to involve the retrosplenial cortex, and also possibly the cingulum bundle: see later sections).

In the second instance, Clark & Geffen (1989) reviewed a number of clinical cases involving the surgical sectioning of the corpus callosum in which severe and persistent memory problems had resulted. The authors pointed out that such memory deficits only occurred where there had been concurrent damage to the fornix and its connections.

1.4.6 The effects of fornix damage in experimental animals.

The severe effects on learning and memory in both monkeys and rats following transection of the fornix has been well documented, though the precise form of memory affected remains contentious. Hippocampal damage has been typically found to interfere with spatial learning and memory (Jernigan et el., 1993; Morris et al., 1982; Olton & Papas 1979), and it would be predicted that fornical damage would result in similar impairments.

Where rats are concerned, that prediction has received support. For example, Sutherland & Rodriguez (1989) reported that fornix/fimbria transection produced significant impairments in place learning and memory in the Morris water task. Such lesioned animals did not learn to navigate to the location of the hidden platform, nor did they retain place navigation that had been acquired preoperatively. The authors concluded that the performance of the fornix group was very similar to animals with extensive damage to the hippocampal formation.

Olton et al. (1978) described a series of experiments using the radial-arm maze (a spatial test) in which rats were trained preoperatively to a criterion. Following this training period, the animals received lesions of the fornix and were tested again in the maze, their post-operative performance dropping to chance levels, and they showed no recovery of function.

On a variety of tasks focusing upon spatial working memory in the T-maze, rats with fornix damage have again demonstrated significant impairments (Gaffan 1972; Markowska et al., 1989).

With regards automated tasks of spatial learning and memory, the delayed matching / non matching-to-position (DMP / DNMP) tasks have consistently revealed delay-dependant deficits following transection of the fornix.

Dunnett (1985, 1989) used the matching-to-position version of the task and reported no effects at short delays, but progressively greater impairments as the delays lengthened. Similarly, Aggleton et al., (1991a; 1992) have shown that fornix transection severely impairs the performance of rats on the non matching-to-position (DNMP) version of this automated task, a deficit which was again delay-dependant, and which was comparable in severity to that found after extensive hippocampal damage.

With regards other tests that are known to be sensitive to human anterograde amnesia, rats with lesions of the fornix have also been found to be impaired on the acquisition of object discriminations (Aggleton et al., 1991b).

In monkeys however, the effects of fornix transection upon spatial memory are not so clear cut. Monkeys with fornix transection have been found to be impaired in spatial reversal learning, memory for visuospatial configurations, delayed nonmatching-to-sample in the T-maze, and conditional learning tasks using spatial positions (Mahut 1972; Gaffan & Saunders 1985; Murray et al., 1989; Gaffan & Harrison 1988).

However, fornix damage does not appear to disrupt all forms of place discrimination learning (Murray et al., 1989). Gaffan & Harrison (1989) attempted to resolve some of these discrepancies by investigating the effects of fornix transection on the memory for the spatial arrangement of objects. The animals were required to choose between two objects under five different conditions which utilised different cues to guide the animals choice. The differing pattern of impairments revealed under the five conditions, led the authors to conclude that the deficits seen after fornix transection were not just disruptions of place memory, rather the fornix appears to be important for storing not only the identity of visible stimuli, but also their spatial arrangement within the viewed scene ("*snapshot memory*"). In support for this proposal, Gaffan (1992b) reported that monkeys trained to discriminate among many complex naturalistic scenes were severely impaired by fornix transection.

It is not in the scope of this report to provided a detailed analysis of the precise spatial deficit produced following fornix transection in monkey and man, it will instead focus upon the nature of the fornical deficit produced in rats on two automated tasks, and on various T-maze, and 8-arm radial maze tasks.

1.5 THE DIENCEPHALIC SYSTEM AND MEMORY.

The diencephalic structures that are of primary concern to the evidence presented, and the experiments described in this thesis are the thalamus (particularly the medial dorsal nucleus (MD), and the various anterior nuclei), and the mammillary bodies.

Although in the past it has been exceedingly difficult to assess the role of individual thalamic nuclei, with the development of more precise imaging techniques in human patients, and with the use of selective neurotoxins in experimental animals, it is becoming possible to tease out the possible contributions of at least some individual nuclei.

1.5.1 The Medial Dorsal Nucleus of the thalamus (MD).

The medial dorsal (MD) nucleus is the largest subdivision of the medial thalamus, and is most developed in primates, especially in humans; an increase in phylogenetic evolution that parallels that of prefrontal and cingulate cortices (Bentivoglio et al., 1993). According to Walker (1940), MD lies medial to the internal medullary lamina (IML) in the middle third of the thalamus and has been clearly defined in all mammalian species so far. In primates, MD is most commonly subdivided into three sections: the magnocellular, the parvocellular lateral, and the paralamellar regions; but in rodents, the existence of a distinct parvocellular portion is generally denied (Leonard 1969). Despite this apparent simplification of the structure of MD in rodents, it has been generally accepted that a distinct compartmentalisation into central, medial and lateral subfields is an accurate representation.

Moreover, results from a recent study by Kuroda et al., (1992) indicated that cells and their major dendrites are confined to single segments of MD. As a consequence, these portions of MD may therefore be considered to be relatively independent subnuclei. With regard to rats, Krettek & Price (1977) have subdivided MD into three sections:

1. Central segment: Most prominent in the middle part of the rostrocaudal extent of the nucleus and is readily identified using silver preparations by its very dense fibre plexus.

2. Lateral segment: This extends along the inner face of the internal medullary lamina (IML) and occupies the lateral portion and part of the ventral section of MD.

3. Medial segment: This extends the full length of the nucleus and can be subdivided into anterior and posterior parts. It contains few fibres of passage and consists mainly of small, loosely distributed neurons.

Using the autoradiographic method of demonstrating axonal connections, Krettek & Price (1977) reported that the different subfields of MD apparently have different efferent connections to distinct areas within prefrontal cortex. The central segment projects to the ventral agranular insular area in the dorsal part of the rhinal sulcus (rhinal cortex). The lateral segment projects principally to the anterior cingulate region (area 24) and the medial precentral area. The medial segment projects to the prelimbic region (area 32) and also to the rhinal sulcus.

Groenewegen (1988) has outlined the principal afferent connections of MD in the rat, with a particular emphasis on the relation of MD with the prefrontal cortex. Injections of horseradish peroxidase were placed in the different segments of MD and revealed the organisation of reciprocal MD / prefrontal connections. A number of subcortical structures were found to send fibres to all segments of MD, these consisted primarily of the reticular thalamic nucleus and several regions of the brainstem including the mesencephalic raphe nuclei, the locus coeruleus, and the reticular formation. Excluding these general inputs, the different parts of MD receive a specific input and have different relationships with particular areas of the prefrontal cortex.

Groenewegen (1988) divided the medial segment of the rat MD into three sections and analysed each separately. The rostral part has reciprocal connections with prelimbic and anterior cingulate cortices, and to a lesser degree, with medial orbital and ventral agranular insular regions.

The posterior / ventral portion has its principal afferent connection with the dorsal agranular insular area. The most medial part of the nucleus receives fibres from the infralimbic region, but does not seem to reciprocate them. It also receives input from a large number of limbic structures, principally the amygdala, entorhinal cortex, diagonal band of Broca, lateral preoptic area and supramammillary nuclei; and also from structures related to the basal ganglion region. In addition to this wide range of inputs, the medial portion also receives fibres from the nucleus accumbens, nucleus of the stria terminalis, dorsomedial hypothalamus, subiculum, and finally the parabrachial nuclei.

The central segment of MD receives its most specific inputs from olfactory related structures such as the prepiriform cortex and the olfactory tubercle. It also receives afferents from the nucleus of the diagonal band of Broca, the lateral pre-optic area and the lateral hypothalamus.

The lateral segment of MD receives a small number of fibres from the globus pallidus and the lateral hypothalamus, but its principal afferents arise from structures in the brainstem, notably the substantia nigra, pars reticulata and the dorsolateral tegmental nucleus. See figure 1 over:

Earlier studies had concluded that the MD nucleus projected solely to the prefrontal cortex, which in turn, was the sole projection target of MD. The study by Groenewegen outlined above, and other reports of MD/prefrontal connections in primates (Goldman-Rakic & Porrino, 1985), showed that MD does in fact project to cortical targets outside of prefrontal cortex, notably to anterior cingulate, and premotor cortices.

Groenewegen (1988) concluded that in the rat at least, different parts of the prefrontal cortex could be influenced (via distinct segments of MD) by a wide range of structures that are principally involved in the circuitry of the limbic system. The importance of these reciprocal connections with a wide range of limbic structures cannot be underestimated. The key component of the mnemonic processing capacity of the limbic system is undoubtedly the hippocampus which, although not having a direct reciprocal connection with MD, certainly has indirect ones via the entorhinal and perirhinal cortices and the anterior cingulate area.

Aggleton (1991) pointed out that the hippocampus does indeed provide extensive thalamic inputs, in particular, dense projections arise from the subicular and entorhinal cortices to pass, via the fornix, to the anterior part of the thalamus - particularly the anterior medial (AM) and anterior ventral (AV) sections. Aggleton (1991) argued that these direct connections between temporal and diencephalic regions which are both implicated in the amnesic syndrome would seem to indicate that temporal lobe and diencephalic amnesia's are different facets of the same underlying syndrome.

The major interconnections of MD.

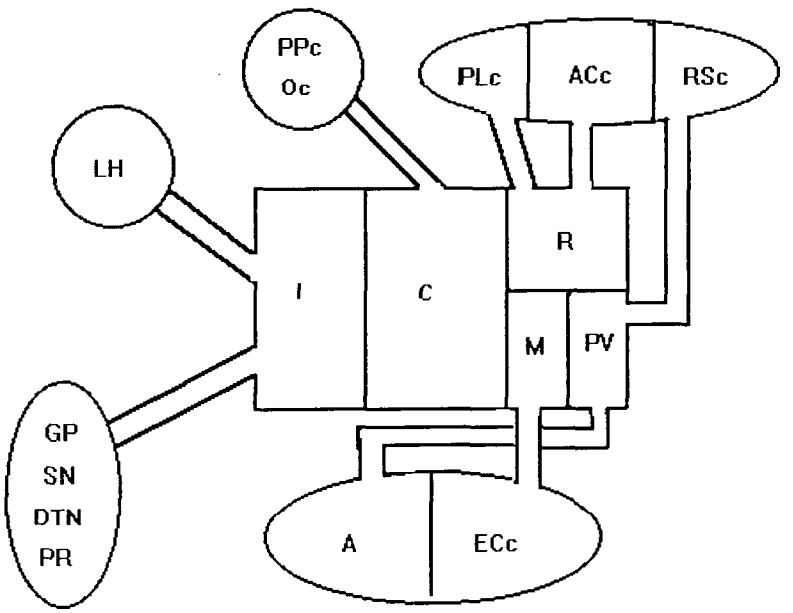


Figure 1. Diagrammatic representation of the major interconnections of MD. L = Lateral portion of MD, C = central portion, R = rostral part of medial portion; PV = parvocellular part of medial portion; M = medial part of media portion. ACc = anterior cingulate cortex; PLc = prelimbic cortex; RSc = retrosplenial cortex; A = amygdala; ECc = entorhinal cortex; LH = lateral hypothalamus; PPc = prepiriform cortex; Oc = olfactory cortex; GP = globus pallidus; SN = substantia nigra; PR = pars reticula; DTN = dorsal tegmental nucleus.

If, (as strongly suggested by such as Squire, 1992), the hippocampus is directly involved in the processing and possible storage of mnemonic information; then the various thalamic nuclei are indirectly implicated in these procedures as well.

Several reviews of thalamic amnesia have emphasised the importance of MD (e.g. Waxman, 1988). To assess the possible role of MD in the memory process, it will be necessary to present evidence from both human and animal studies concerning the effects of damage to this, and related cortical /subcortical areas.

1.5.2 Diencephalic amnesia.

Evidence for a distinct form of amnesia which appears to involve the thalamic nuclei and/or their various interconnections has come from several sources.

1. The '*Wernicke - Korsakoff Syndrome*': The first detailed description of this syndrome came from the writings of Wernicke and Korsakoff in the 1880's. Both described a neurological condition that included a gait disorder, abnormal eye movements, a state of psychological confusion, and more importantly, symptoms of amnesia. This condition was brought about by symmetrical brain lesions along the third and fourth ventricles along with cortical atrophy and cerebral damage as a result of chronic alcoholism. Clinical studies have demonstrated that if a patient in the Wernicke state (i.e. confusional stage) of the syndrome is not treated with large doses of thiamine, then the syndrome could be fatal. If, however, treatment is obtained, then the patient was described as having passed the '*acute Wernicke phase*' and entered the '*chronic Korsakoff stage*' characterised by a severe, permanent amnesic condition (Heindel et al., 1991).

Korsakoff patients may show a normal IQ as measured by the Wechsler Adult Intelligence Scale (WAIS), in spite of demonstrating a severely impaired memory quotient as measured by the Wechsler Memory Scale (WMS) , often up to a thirty point difference (Butters and Cermak, 1980). The majority of neuropathological studies concerning this syndrome point to damage involving several diencephalic structures surrounding the third ventricle of the brain. Of these, damage to the mammillary bodies has been described in all cases of the Wernicke - Korsakoff syndrome (Victor et al., 1971), this structure typically appearing shrunken with neuronal loss being generally found in the medial part of the nucleus. Damage to this structure has also been described in reports of colloid cysts and tumours located in the floor and walls of the third ventricle, areas adjacent to the mammillary bodies (Ignelzi & Squire, 1976).

As a consequence, the role of the mammillary bodies in the human amnesic syndrome seemed secure until doubt was cast by the extensive study of Victor et al., (1971). In that report, the authors looked at 43 Korsakoff patients and noted that in each case, the mammillary bodies were necrotic, but that in 38 out of the 43 cases additional damage was also found to MD.

It was subsequently found that only in the cases where damage to MD was also present that any obvious memory impairments were demonstrated. The possible involvement of MD in Korsakoff's Syndrome has however remained contentious.

Two thoroughly studied cases by Mair et al., (1979) revealed bilateral subcortical damage limited to the mammillary bodies and the paratenial nucleus of the thalamus, but not to MD. A more recent report by Mayes et al., (1988) also focused upon two Korsakoff patients who showed severe neuronal loss in the medial mammillary bodies. Once again there was damage to the paratenial nucleus, but this damage did not apparently extend into MD, although both patients showed small unilateral infarctions in the magnocellular part of this nucleus. Such data indicates that a severe anterograde amnesia can occur in Korsakoff patients without direct damage to MD, the close proximity of the paratenial nucleus to MD suggests that some clinical studies may have failed to distinguish between these two nuclei (Aggleton, 1991).

The relative involvement of MD has also been the cause of much debate. Markowitsch (1982) for example, has argued that the critical site is the lateral parvocellular portion of the nucleus, while Victor et al., (1971), defined the critical portion as the more medial magnocellular portion. A third hypothesis has been proposed by Mishkin (1978; 1982). He argued that a severe amnesia only occurred if there was damage to both of two limbic-diencephalic pathways. The first of these pathways projects via the fornix from the hippocampus to the mammillary bodies, the anterior thalamic nuclei, cingulate cortex, and back again to the hippocampus. The second of these pathways projects from the amygdala, to MD, and then possibly to orbitofrontal cortex, and back again to the amygdala.

Recent advances in neuro-imaging techniques such as Magnetic Resonance Imaging (MRI) has further fuelled the controversy. For example, Jernigan et al., (1991) reported that Korsakoff patients exhibited widespread reductions in grey matter in the anterior diencephalic structures, orbito-frontal cortex and the medial temporal lobe. Other authors have also raised the possibility of hippocampal damage in Korsakoff's syndrome (Mayes et al., 1988), a position strengthened by the Hata et al., (1987) finding of reduced blood flow in the temporal limbic structures of Korsakoff patients.

One of the key problems in evaluating the deficits produced by Korsakoff syndrome is that of the greatly varied neuropathology demonstrated in patients. Mayes et al., (1988) pointed out that frontal and parietal lobe, hippocampal and thalamic pathologies are variable features of the syndrome, and even that damage to the cholinergic basal forebrain may be crucial.

2. Paramedian thalamic infarcts: Thalamic haemorrhage is relatively frequent, and there have been numerous reports of amnesia following such an event, (e.g. Choi et al., 1983; Guberman & Stuss, 1983). The occurrence of amnesic symptoms following thalamic infarction appears to be dependant upon the location of the infarction. Bogousslavsky et al., (1988) described four principal arterial regions in the thalamus, with an amnesic syndrome only being associated with infarction of the paramedian territory. Interestingly, such lesions were often unilateral with the left side being the most affected; in these instances, verbal memory showed the greater deficit (Graff-Radford et al., 1990).

Von Cramon et al., (1985) pointed out that the paramedian arteries supply both the posterior part of the internal medullary lamina (IML), and part of MD, but do not serve the anterior nuclei. The authors then presented 6 cases, 4 of whom suffered chronic amnesia, whilst 2 did not. Interestingly, the 2 patients who showed no obvious mnemonic impairments had suffered greater damage to MD than the 4 cases who were impaired.

Von Cramon et al., (1985) concluded that the critical sites were the mammilothalamic tract (MMT) and the IML, which had both been damaged in the 4 cases showing mnemonic impairments. It is perhaps important to note that a lesion of the IML would disrupt the connection between MD and its neocortical projection sites - principally the anterior cingulate gyrus, whose own particular role in learning and memory will be considered in a later chapter.

1.5.3 Further evidence for the role of MD in diencephalic amnesia.

The case for the involvement of MD in human diencephalic amnesia is still under review, for, as far as human case studies are concerned, a convincing example of a memory impairment in a person with confirmed damage restricted to MD has yet to be reported.

It can be also argued that other clinical examples are unclear. It was, for example, proposed that the well described patient N.A. provided an example of diencephalic amnesia in which there was thalamic, but not mammillary bodies damage (Squire & Moore, 1979). However, more recent research using the MRI method has shown that N.A. may have a lesion involving both rostral and caudal parts of the internal medullary lamina (IML) extending to ventral areas of MD, he also appears to have damage to the mammillary bodies, mammillothalamic tract, and the hypothalamus (Squire et al., 1989a). It must be pointed out though, that the degree of accuracy of current imaging techniques are not yet precise enough to pinpoint the exact limitations of lesions, nor indeed of the structures involved.

In his extensive review of the possible involvement of MD in both animals and man, Markowitsch (1982) presented a large number of clinical reports which yet again implicated MD but which provided no convincing proof. There are many studies which have reported a correlation between lesions in MD and the occurrence of amnesic deficits, but in the majority of cases, MD damage occurred in conjunction with other related diencephalic and/or cortical damage.

For example, circumscribed lesions of MD which included the surrounding IML have been performed as a surgical treatment for the relief of pain (Spiegel & Wyciss, 1953), and in the control of aggressive behaviour (Poblete et al., 1970). Memory related changes were seldom reported and several studies emphasised the lack of any enduring memory disturbance at all. Spiegel & Wyciss, (1953) noted that patients suffered disorientation in time (chronotarxis) and considered this syndrome to be similar in some respects to that seen in Korsakoff patients. What differentiated the chronotarxis seen in MD lesioned patients' from those suffering from Korsakoff's syndrome was that in the MD patients, the disorientation typically only lasted a few days, likewise if any other disorders in memory were noted, they disappeared in a short space of time.

Reports which have described cases in which damage was confined solely to MD either suffered from the problem of indirect (and hence potentially inaccurate) detailing of the lesions (Squire & Moore 1979), or revealed no impairment when damage was limited to MD (Kritchevsky et al., 1987). In the Kritchevsky et al., (1987) study, two patients with non - haemorrhagic infarcts of the thalamus were comprehensively assessed on a wide range of standardised neuropsychological tests. Neither patient showed any discernible memory impairment, both for verbal and non-verbal material.

Unfortunately, even this report remains inconclusive as MRI scans revealed that one patient had a right - sided lesion involving about 15% of MD, and the second patient had bilateral lesions affecting about 15% of the left part of MD, but only 5% of the right portion. Kritchevsky et al., (1987) concluded that two possible reasons could account for the lack of any amnesic deficit. Firstly, the amount of MD damage could have been less than required to cause amnesia; and secondly, amnesia related to thalamic lesions may well require damage to a second structure, possibly the mammillothalamic tract or to the anterior thalamic nuclei.

These points could be of crucial importance in assessing the role of MD in humans, with both being equally important. As Markowitsch (1982) concluded, the majority of reports involving human clinical cases with lesions involving MD do suggest some role for this structure in the mnemonic process. However, as yet, there has not been reported a single case in which damage has been confined solely to MD, and which has resulted in a measurable impairment of learning and memory.

Due to the rather confusing and somewhat conflicting data just reported, it is necessary to turn to animal studies of diencephalic amnesia; principally because it is with the use of experimental animals that circumscribed lesions can be produced, and furthermore, the results can be assessed using a variety of sensitive tasks.

1.5.4 Experimental animal studies involving damage to MD.

Initial research involving experimental animals did not provide much evidence for the possible role of MD. Markowitsch (1982) outlined and discussed several areas of research which can be summarised :

1. Stimulation studies: In one of the first experiments in which the thalamus was electrically stimulated Sachs (1909) found that such stimulation resulted in head, neck, and eye movements. In other studies, emotional changes and arousal reactions resulted from stimulation of MD. In particular, types of attack behaviour were noted (Siegal et al., 1972), and fear reactions were elicited (Wester, 1972). Studies relating electrical stimulation of MD to the mnemonic process were few, but Briese & Olds (1964) noted that stimulating MD during the retention intervals in a delayed response task yielded higher error scores.

When interpreting findings from stimulation studies, it must be borne in mind that such stimulation would not be limited to the structure focused upon, but will also affect surrounding regions thereby making it difficult to rule out the possible involvement of related structures.

2. Electrophysiological studies: Studies relevant to this report have focused upon the attempt to relate firing changes of single units in MD during tasks that have utilised learning and memory. For example, Fuster (1973) reported that the most prominent feature of unit activity in MD neurons was an increase in firing rate during the early part of the delay in a delayed response task. This finding need not of course reflect the activity of MD in the short term memory processing during the delay, but may just represent an arousal process enduring beyond the end of the delay period.

3. Metabolic Activity studies: The so-called '*2-Deoxyglucose (2-DG) technique*' as developed by Sokoloff et al., (1977) allows the uptake of this compound during certain tasks to be measured in various cortical/subcortical regions at a later date, and has offered the opportunity to examine functional mnemonic activity in different brain regions in normal animals. Using this method Friedman et al., (1990) recorded the uptake of 2-DG in five diencephalic regions in monkeys who performed either one of three tasks that engaged working memory. Mean local glucose uptake was significantly higher in the parvocellular and magnocellular components of MD, and interestingly, also in the anteroventral (AV) and anteromedial (AM) thalamic nuclei; but the mammillary bodies showed no such increase. The authors argued that both MD and anterior thalamic nuclei represented diencephalic components of a neural circuit mediating working memory.

1.5.5 Lesion studies involving non-human primates.

Initial studies using monkeys focused on discrete lesions of MD and revealed that such damage did not lead to an impairment in tasks such as delayed response (Peters et al., 1956). More recent studies have however, shown that more extensive aspiration or radiofrequency lesions of MD can impair performance on both delayed response and delayed alternation tasks (Isseroff et al., 1982), a delayed nonmatching-to-sample (DNMS) task (Aggleton & Mishkin 1983a; Zola-Morgan & Squire, 1985), and 2-choice visual discriminations (Gaffan & Murray, 1990). The impairments described however were often not severe and in all cases there was additional slight damage to the fornix which made interpretation of the results difficult.

These problems were recently addressed by Gaffan & Watkins (1991) in which six monkeys were trained to associate visual stimuli with the delivery of various amounts of food reward. MD lesions produced a severe impairment both in memory for the quantity of reward, and also in the qualitative absence or presence of reward. Such deficits would seem to indicate that lesions of MD can impair both long and short term memory processing. The authors concluded that the effects of MD lesions were not limited to short-term memory tasks, but could also produce a severe impairment in a long-term memory task.

The authors also argued that function of MD may be related to the effects of reward on the animals learning, (this notion that MD may well be involved with different kinds of mnemonic processing will be assessed more fully in the discussion of experiment 1). However, Gaffan & Watkins (1991) admitted that their results did not differentiate between the effects of lesions in the medial part of MD and the effects of lesions in the other midline structures which were included in the ablations. Thus, in common with other studies (for example Zola-Morgan & Squire, 1985), these results cannot with certainty be ascribed to the inclusion of MD in the lesion.

1.5.6 Lesion studies involving rats.

Considerably more research has been carried out on the effects of MD damage on spatial learning in rats, however, like the primate studies, the results have been inconsistent. For example, significant impairments have been reported for acquisition of the radial arm maze (Stokes & Best, 1988), spatial DNMS (Mair et al., 1992), and the acquisition and subsequent performance of a non-recurring items DNMS task (Mumby et al., 1993). Deficits have also been reported at the learning and subsequent reversal of brightness and tactile discriminations (Tigner, 1974), reinforced spatial alternation (Vicedomini et al., 1982), and go / no - go alternation (Winocur, 1985). In contrast, other studies have provided no support for the involvement of MD. It was found, for example, that MD lesions had no apparent effect on spatial alternation (Tigner, 1974), the Morris water maze, and the radial arm maze (Kolb et al., 1982).

Two recent studies have further raised a set of issues concerning the possible role of MD which must be addressed if some sort of reasonable hypothesis is to be formulated. Stokes & Best (1990a) evaluated the ability of rats with MD lesions to perform a task differentiating between reference and working memory.

In this task, only 4 of the arms of an 8 - arm radial maze were baited, and the animals had to restrict their entries to arms which were baited, and had to avoid never baited arms. Despite postoperative acquisition trials, the MD animals failed to acquire the task to a level comparable to that of controls. The lesioned animals made both reference memory error (entering never baited arms), and working memory errors (re-entering baited arms). Stokes & Best (1990a) interpreted these results as a non-specific, non-memory dysfunction induced by MD damage; a dysfunction that could interfere with either attention or motor processes. This hypothesis of a non-mnemonic role for MD raises some intriguing possibilities for current research on MD (see General Discussion). It is possible however that other midline, non - specific thalamic nuclei included in the lesion, may have contributed to the behavioural deficits obtained.

Another study, that of Hunt & Aggleton (1991), raised the possibility that in conventional surgical techniques, fibres of passage are damaged along with neurons located in the nucleus of MD. This is of particular importance regarding the debate concerning MD as it not only contains fibres of passage, but is also bounded by a major pathway - the IML. Small differences in lesion location could, theoretically, produce a marked difference in behavioural performance. Using either a neurotoxin (which spared fibres of passage) or the less restrictive radiofrequency lesions, the authors found that while cellular damage to MD disrupted learning on the DNMS task, some impairments on a spatial alternation task may have reflected damage to the adjacent anterior thalamic nuclei. Interestingly, Hunt & Aggleton (1991) found evidence that the extent of MD damage correlated with the size of the deficit, and in particular, damage to the medial portion of MD (which is connected to a wide range of limbic structures and related cortical regions), appeared particularly important in producing the deficit. This assumption that the more of MD that suffers damage will lead to a greater impairment ties in with the human case study reported by Kritchevsky et al., (1987).

1.5.7 The effects of unilateral lesions of MD in both humans and animals.

Various studies have assessed the effects of unilateral thalamic damage in humans with the general finding that such lesions lead to moderate, though not severe neuropsychological deficits (Wallesch et al., 1983). With regard to unilateral damage to MD in humans and monkeys several reports have been of interest.

For example, Sandson et al., (1991) reported a the case of a patient who developed a dramatic change of personality and behaviour following a discrete left-sided thalamic lesion involving MD. Neuropsychological testing revealed three types of impairment, firstly, a deficit in complex executive behaviours most often associated with frontal lobe function; secondly, a naming deficit; and thirdly, a retrieval deficit for verbal material. It is particularly interesting to note that the patient suffered an impairment in executive functions, usually indicative of frontal lobe pathology. With the close links between MD and prefrontal cortex (Groenwegen, 1988), such a deficit should perhaps be expected.

In a study using monkeys, Gaffan & Murray (1990) looked at the effects of a unilateral lesion in MD crossed with a unilateral lesion in the amygdala, and compared such "*disconnection lesions*" with bilateral ablations of amygdala, ventromedial prefrontal cortex, or MD alone. While the bilateral lesions of the amygdala, or MD had severe effects on performance of a 2-choice visual discrimination task, unilateral lesions resulted in milder impairments. The authors argued that the three structures were functionally related to each other but were not serial stages in a single functional pathway.

1.5.8 The role of other thalamic nuclei in diencephalic amnesia.

The anterior thalamic nuclei.

The nuclei of the anterior thalamus have long been considered as important components of a neural circuit linking the hippocampal formation with medial frontal cortex. Rose and Woolsey (1948) were the first to correlate so-called "*limbic cortex*" (areas 24, 25 and 29) with specific thalamic nuclei. These regions of medial frontal cortex received projections from the anterior dorsal (AD), anterior ventral (AV), and anterior medial (AM) nuclei, each of which were viewed as being dependant upon a cytoarchitecturally defined limbic cortical field. Although it is now known that the input to each cortical region does not derive exclusively from a single thalamic nucleus, but rather, may originate from neurons located in more than one nucleus; the term '*limbic thalamus*' is thus still used to designate the anterior group of nuclei (Bentivoglio et al., 1993).

The anterior group of nuclei are situated between fibres of the IML that splits in the rostral and most dorsal pole of the thalamus. The three nuclei of this group (AM, AV, and AD) display some cytoarchitectonic distinctions but share common connections and functions (Bentivoglio et al., 1993).

In the rat, the key connections of the anterior thalamic nuclei are with the mammillary bodies, via the mammillothalamic tract, and with the hippocampus, passing exclusively through the fornix, this connection being reciprocal (Aggleton et al., 1986). Regarding cortical association areas, the anterior thalamic nuclei possess strong reciprocal connections with the cingulate cortex, particularly with the retrosplenial region, with the AM and AV nuclei having the densest links. Conversely, the anterior thalamic nuclei are the principal source of thalamic projections to cingulate cortex, with all three nuclei sending dense afferents to retrosplenial cortex, and lighter projections to anterior cingulate cortex (Shibata 1993a; 1993b). Greater detail of cingulate-anterior thalamic connections will be presented in chapter 3.

So, it can be argued that the anterior thalamic nuclei are in a strategic anatomical position, receiving dense projections both directly from hippocampus and mammillary bodies, but also forming indirect links with the hippocampus via cingulate/retrosplenial cortices. Judging by these links, it would perhaps be surprising if the anterior thalamic nuclei were shown to provide no contribution to at least some aspects of learning and memory, see figure 2 over:

Aggleton & Sahgal (1993) have provided a review of both clinical and direct experimental evidence that the anterior thalamic nuclei may be involved in mnemonic processing. The authors argued that while there is only limited clinical evidence of a direct role for the anterior thalamic nuclei in diencephalic amnesia, careful analysis of clinical cases reveals that connections to and from these nuclei are disrupted in many cases. For example, in Korsakoff's syndrome, while the anterior nuclei are not always directly affected, the mammillary bodies (a source of the major hippocampal input to the anterior nuclei) are almost invariably damaged.

The major interconnections between hippocampal system, anterior thalamus and frontal cortices.

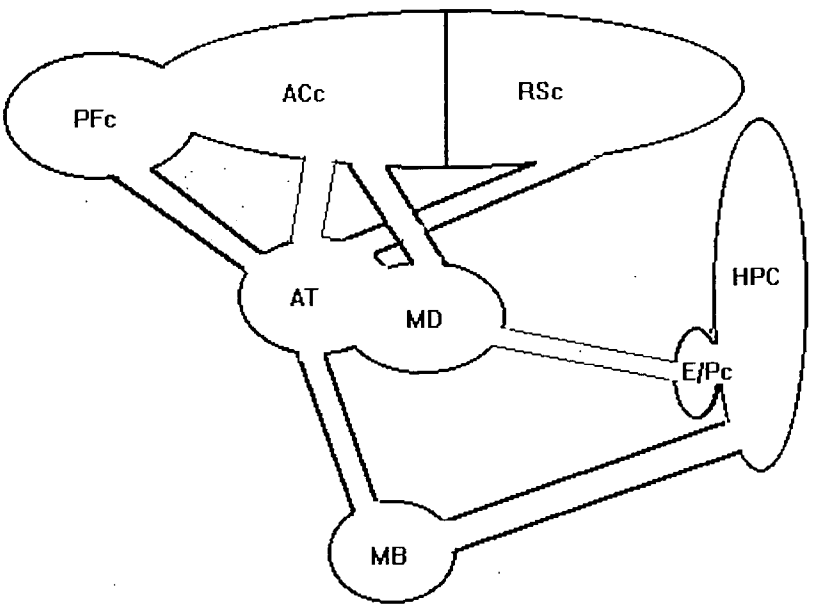


Figure 2. Diagrammatical representation of the major interconnections between the hippocampal system, the thalamic nuclei, and frontal cortices, which may form a mnemonic circuit. HPC = hippocampus; E/Pc = entorhinal / perirhinal cortex;, MB = mammillary bodies; AT = anterior thalamic nuclei; MD = medial dorsal thalamic nucleus; ACc = anterior cingulate cortex; RSc = Retrosplenial cortex; PFc = prefrontal cortex.

Mark et al., (1970) however reported a case study of a patient in whom lesions of the anterior thalamic nuclei were made during treatment for chronic depression. Following surgery, the patient demonstrated both spatial and temporal confusion, and showed impairments in recent memory. Following the patient's death, it was confirmed that the surgery had bilaterally destroyed the anterior thalamic nuclei, but importantly, MD and the mammillary bodies had not been affected.

1.5.9 Experimental evidence concerning the anterior thalamic nuclei.

The main difficulty encountered in attempting to create animal models of diencephalic amnesia has been the problem of assessing the contribution of individual nuclei. For example, monkey studies have revealed that lesions of the anterior thalamic nuclei can produce severe impairments in recognition tasks (Aggleton & Mishkin, 1983a); however, in such studies the MD and midline nuclei typically also suffer damage, thus making a proper interpretation difficult.

It is important to be able to carry out surgical lesioning which is limited as far as possible to individual structures. The use of neurotoxins is of considerable help in this matter as their effect is often contained within the boundaries of the targeted nuclei, and they do not affect fibres of passage. Aggleton et al., (1991a) compared the effects of selective neurotoxic damage to the mammillary bodies, and anterior thalamic nuclei (efferent targets of the hippocampus), on an automated spatial DNMP task. The performance of these two groups was compared with that of animals with hippocampal system damage (radiofrequency lesions of the fornix). The authors found that both fornix and anterior thalamic lesions led to a comparable severe spatial memory impairment. However, mammillary body damage had no effect, which indicated that while the contribution of the anterior thalamus to spatial working memory depends upon direct hippocampal afferents, it need not rely on the indirect hippocampal connections via the mammillary bodies. Of importance to this debate may be the proposal that the connections between the anterior thalamic nuclei and posterior cingulate cortex, may form part of a mnemonic system (Gabriel et al., 1980).

1.5.10 Summary.

The previous sections have presented strong evidence that both medial temporal, and diencephalic damage in both human patients and experimental animals, lead to an anterograde amnesic syndrome, various questions are raised by such evidence which need to be answered before further material can be presented.

1.6 TWO FORMS OF ANTEROGRADE AMNESIA ?

It has been argued with fairly clear conviction that neurological injury to fairly circumscribed regions of the brain can lead to severe, yet selective memory impairments, classically defined as an '*amnesic syndrome*'.

Such a syndrome can result from a variety of causes, having their principal effects on two key regions - the medial temporal, or the diencephalic system. In recent years a key concern has been whether the supposed two forms of the syndrome result in qualitatively different kinds of memory impairment.

1.6.1 Medial temporal versus diencephalic amnesia.

As Parkin (1984) has suggested, the possibility that the amnesic syndrome has two different forms has several important implications. Principally, if the two syndromes do differ in a significant manner, then much of the evidence concerning their effects would be negated, as mixed aetiology groups could not be compared as they have in the past; this would be the case for both human and animal studies. The existence of two separable syndromes would also create much confusion as current anatomical considerations regarding their functions have focused on the connectivity of a variety of cortical/subcortical structures. In this way, one is given the impression of an interconnected memory network linking both medial temporal and diencephalic structures with neocortical association areas in prefrontal cortex. For example, von Cramon et al., (1989) argued that learning and memory depended upon associative information stores in different neocortical areas which are closely linked with, and heavily dependant upon, the interdependent limbic/diencephalic networks. An integrated memory network clearly requires that these two systems function in co-operation, and also that the interaction between them and the neocortical association areas, be guaranteed.

A qualitative dissociation between the two networks would therefore indicate that different memory systems exist in parallel, and yet share a great many features and functions. Such neurological redundancy is difficult to comprehend and so the most parsimonious solution is to assume that the system as a whole is composed of several components, each performing a slightly different operation, which is integrated to perform a single task.

If Parkin (1984) was correct, it would have important implications for future research on learning and memory. Parkin proposed that there were qualitative differences between medial temporal, and diencephalic amnesia's, namely in confabulation, retrograde amnesia, rapid forgetting, frontal lobe symptoms, and the effects of interference. It was, for example, thought that medial temporal amnesics (i.e. H.M.), showed rapid forgetting compared to diencephalic amnesics (i.e. Korsakoff patients), who tended to exhibit normal forgetting rates.

Several experimental studies have supported this theory. Parkin et al., (1990) compared human patients with diencephalic damage (Korsakoff's) against ones with medial temporal lobe damage (Herpes simplex encephalopathy) on a recency judgement task. All patients were presented with four pictures and, after one minute of distraction, both groups could identify the targets with little difficulty. However, on subsequent trials, items that had been used as distracters were used as targets, and vice versa, and the subjects were asked to select, in a recognition test, the items that they had been asked to remember most recently. The diencephalic group performed close to chance, while the medial temporal group continued to perform well. Parkin et al., (1990) ascribed this difference to a particular inability of the diencephalic group to retain contextual information regarding the targets used on a particular trial. The ability of the temporal lobe patients to perform well on this task was interpreted as suggesting a different form of deficit, one of consolidating memory *per se.*

In a recent study, Hunkin et al., (1994) compared the performance of two groups, one with diencephalic damage, and the other with medial temporal damage, on a temporal list discrimination task. Measures of recognition and temporal discrimination were collected, and while both groups were similarly impaired on the recognition measures, only the diencephalic group showed an impairment on the temporal discrimination measure. Hunkin et al., (1994) argued that the diencephalic patients demonstrated an impairment in a specific aspect of memory performance (memory for temporal context) and represented a qualitative difference between medial temporal and diencephalic pathology.

Other reports have, however, focused upon the degree of similarity between the two groups of patients. Squire et al., (1989b) provided evidence that, at least as far as supposed differences in retrograde amnesia were concerned, patients with medial temporal or diencephalic pathologies showed very similar rates of retrograde amnesia. Even more recently, Mckee & Squire (1992) have re-evaluated previous evidence concerning supposed differences between the two groups and have argued that past reports have not tested the two groups in a comparable manner. For example, they discovered that Huppert & Piercy (1982) had assessed the forgetting rate of patient H.M. (who was found to display abnormally rapid forgetting compared with a group of Korsakoff patients); but the methodology of the memory assessment could well have found in favour of the Korsakoff patients due to the fact that H.M's initial learning level was not matched to that of the other groups.

In subsequent studies, both Freed et al., (1987), and Freed & Corkin (1988), did match H.M's initial learning level to that of the other groups and no abnormal rate of forgetting was reported. Mckee & Squire (1992) also presented evidence showing that both types of amnesics performed in a similar manner at both retention and recognition tasks, provided that they were carefully matched in initial performance.

To summarise the question concerning the apparent differences between medial and diencephalic amnesia's, although the neuropathology associated with the two types can be readily differentiated, even among living patients (Squire et al., 1990), it has not been fully established that the two forms result in qualitatively different patterns of impairment. Though it is reasonable to assume that medial temporal and diencephalic structures may make slightly different contributions to mnemonic processing, each region can be regarded as an essential component in a larger functional system, such that a similar amnesic state can result from damage to any portion of the network. This strongly suggests that the critical areas are both functionally and anatomically connected.

1.7 NEUROANATOMICAL CIRCUITS GOVERNING ANTEROGRADE AMNESIA.

Of crucial importance to this thesis and the experimental work presented in it, is the discussion and evaluation of the proposed neuroanatomical circuitry that governs memory (of particular concern is that system which presumably deals with spatial working memory). Several such circuits have been proposed, the majority sharing the common theme of the connections between hippocampus and frontal cortex being the crucial route, with possible other links via a variety of subcortical nuclei. Perhaps the first such circuit was described by Broca (1878) as the *"grand lobe limbique"* which referred to the ring like structures that form the hillus of the cerebral hemisphere and includes the hippocampal formation and pericallosal cingulate cortex.

Later, Papez (1937) explicitly connected this circuit with learning and emotion and further defined the nature of the circuit as in figure 3 over:

Papez Circuit.

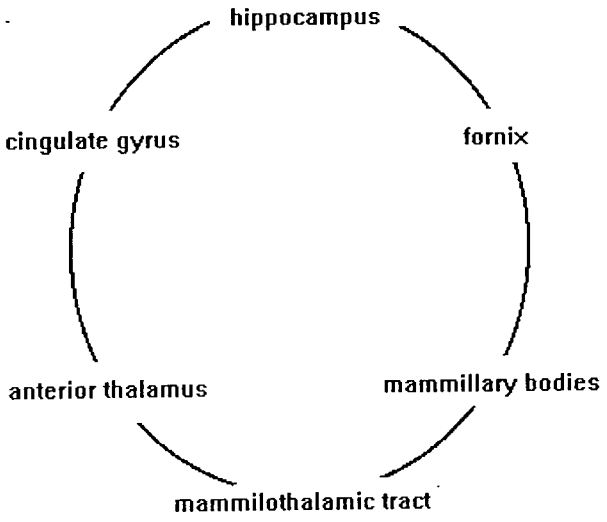


Figure 3. A diagrammatical representation of Papez (1937) proposed circuit underlying emotion.

The importance of the various thalamic nuclei was however rather neglected until Rose and Woolsey (1948) correlated what they termed "*limbic cortex*" with specific thalamic nuclei. They argued that the so-called *limbic cortex* included both anterior and posterior cingulate regions, plus infralimbic cortex which received projections from the various anterior thalamic nuclei, as well as from laterodorsal, and mediodorsal nuclei.

In 1969 Delay and Brion put forward the simpler theory that the hippocampus, fornix, and the mammillary bodies constituted a single functional system for memory, and that the same functional consequence (namely amnesia) resulted from the interruption of this circuit at any of the structures or pathways along it. Mishkin (1982) proposed the concept of two parallel memory circuits that could partially compensate for one another if damage occurred, one of which was the same as that described by Delay and Brion (1969), while the second emphasised the amygdala / MD connection.

Selemon & Goldman-Rakic (1988) have outlined an elaborate anatomical circuit consisting of a large number of cortical and subcortical areas which receive input from both prefrontal and parietal cortices. Again, the principal structure of this circuit is the hippocampal system, with the various thalamic nuclei and cingulate cortex, also featuring prominently. The authors argued that the common efferent pathways of this system could mediate many aspects of spatial function, a function that could encompass attention and memory.

So, a variety of hypothetical neuroanatomical circuits governing learning and memory have been proposed, with the current state of knowledge focusing broadly upon the circuit envisaged by Papez (1937), though with some amendments. The origin of the circuit is the hippocampal formation which, through its principal efferent the fornix, is connected directly to both the mammillary bodies and the anterior thalamus. While the role of the mammillary bodies may yet prove to be of significance, the swing in emphasis onto the role of the various thalamic nuclei has increased.

Either or both hippocampal / medial frontal connections (one passing from hippocampus via the fornix to the anterior thalamic nuclei and thence to prefrontal cortex, or the other emphasising the indirect hippocampal connection with MD to prefrontal cortex, and back to hippocampus) may prove to be vital components of the mnemonic circuit. It is more likely however that each serves some different purpose, one perhaps governing some aspect of spatial working memory, while the other may govern some non-mnemonic, non-spatial mnemonic , or emotional functions.

1.8 MEASURING ANTEROGRADE AMNESIA IN EXPERIMENTAL ANIMALS.

Milner's description of the global amnesia suffered by patient H.M in the 1950's created a flurry of experimental interest regarding the localisation of memory functions. Up to this point, experimental animals had been used by such as Lashley, but with not a great deal of success, from the mid 1950's onward the use of animals (particularly non - human primates and rats) became much more common, the types of surgery used to mimic the kinds of damage suffered by human patients, and the tests used to assess any deficit resulting from lesion damage, became more refined.

1.8.1 The development of animal tests of learning and memory.

Gaffan (1972) pointed out that previous tests of memory in animals with medial temporal damage may not have been comparable to those kinds of tests which revealed a memory deficit in human amnesic patients. Furthermore, an impairment was not always found when severely amnesic human patients were tested using tasks that did demonstrate an impairment in animals. Gaffan realised that the vital question of whether animals showed an anterograde amnesia in the same way as humans could not be demonstrated by conventional tasks, but only with specifically designed tests analogous to the tests that clearly revealed impairments in humans.

It is not in the scope of this thesis to describe and evaluate every type of test that has been used to assess learning and memory in animals, rather it will focus upon several tasks that are regarded as benchmark tests of spatial working memory, with relation to hippocampal and thalamic dysfunction, both in human patients, and experimental animals.

1.8.2 Tests assessing working memory processing.

There are a variety of tests that have been developed to measure spatial aspects of working memory. Delayed response tasks have been used for a long time (e.g. Jacobsen 1935). The task typically involved the provision of information to the animal (e.g. the location of hidden food) which it could then use subsequently to gain a reward (i.e. the selection of the appropriate location to retrieve the food). Such tasks required the animal to store this initial piece of information in memory until they were required to make a response. As pointed out by Dunnett (1989), if the animal could succeed in this when the delay between presentation and response was short, then it had clearly learned the task contingency. The rate of forgetting could be represented by the slope of the delay-performance function, and an increase in the slope of the forgetting function thus suggested a deficit in short-term retention, providing that the groups had been matched at short delays and ceiling effects had been controlled for, see figure 4 over:

Hypothetical Memory Performance as a Function of Delay.

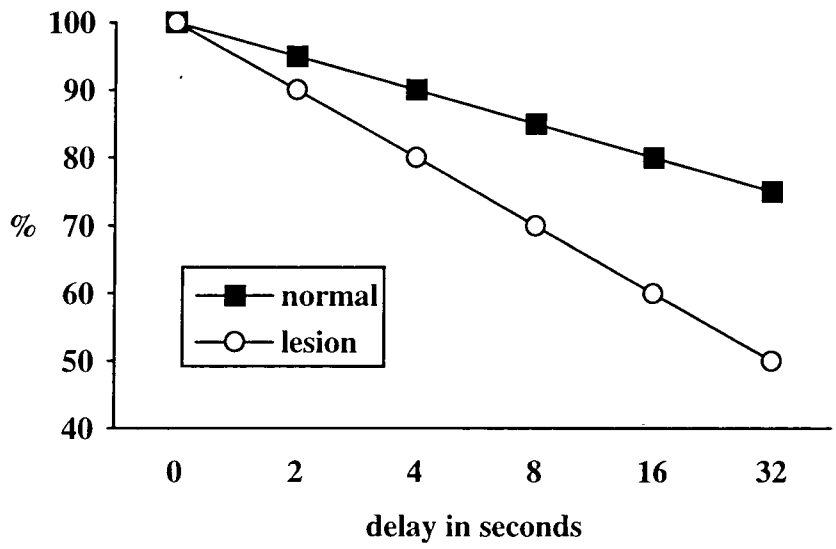


Figure 4. A simplified illustration of a hypothetical curve of memory performance as a function of delay for normal and lesioned subjects on a typical delayed response task. As the delay increases, the performance of the lesioned group worsens relative to that of the normal animals.

Initial findings from such tests did not bode well, for example Squire & Zola - Morgan (1983) reported that monkeys with damage to the hippocampus, amygdala, or conjoint lesions of both, performed normally on the test. In contrast, such lesions generally led to a severe impairment in the non - spatial (DNMS) version of the test (Mishkin, 1978). It was however realised that the length of the delay between sample and choice was crucial, the study of Squire and Zola - Morgan only used delays of up to 5 seconds; when longer delays were employed deficits were indeed found (Zola - Morgan et al., 1989b).

With the rapid development of tests of associative and recognition memory both taxing the spatial and non - spatial components of memory, human and primate data could be compared more accurately. This was, initially, not the case for rodent studies; the difficulty in developing an analogous set of tests for the rat being rather more difficult.

Traditional tests of testing recognition memory in rodents have differed from primate tests in a number of ways. The principal difference lies in the fact that rats (the main animal used in rodent studies) are much more reliant on spatial cues, and so recognition tasks have tended to exploit the ease at which these animals can remember different locations. This had led to a constraint on rodent tasks of recognition memory, namely that only a limited number of spatial locations to be remembered can be used; for example the 2 arms of a T-maze etc. This problem was alleviated somewhat by the introduction of the radial-arm maze test.

1.8.3 The radial-arm maze.

Olton & Samuelsen (1976) introduced a new rodent spatial test of memory designed to specifically investigate the characteristics of working memory. The apparatus was an 8-arm maze with the arms radiating away from a central platform like spokes on a wheel. The basic radial maze procedure was to bait each arm and then allow the animal to enter the arms at will, until all of the arms had been visited. The optimal strategy for the animal was to choose each arm once, and not to repeat choices of any arm.

Olton & Samuelsen (1976) reported that normal rats performed well, and did not rely on response chains, general algorithms, or odour markings to choose correctly. The authors argued that the animals formed (in working memory), a list of places which had been chosen, and, as other arms were visited, this list was developed.

Olton et al., (1977) described further modifications to this general procedure, including using a maze with 17 arms to tax the capacity of working memory more, and adding a guillotine door to each arm, serving to confine the animal to the centre, thus reducing the formation of response patterns. Using these modifications, the authors reported that the animals still performed well, and there was interference among choices, so that the probability of a correct response decreased as the number of choices increased.

According to Olton (1978), this testing procedure demonstrated several important characteristics:

1. It tested memory for a list of items.

2. The task was learnt quickly and was performed well by normal animals.

3. The task was flexible and with small modifications, could assess different types of memory, i.e. working or reference memory processing.

4. It evoked behaviour that appeared to be relatively natural, being similar to food-searching behaviour in semi-natural environments.

Olton (1978) described a series of experiments looking at the performance of animals with damage to the fornix. These animals showed severe impairments on this task, exhibiting a variety of behavioural changes which established this test as a benchmark task of hippocampal dysfunction. Using further modifications to the general testing procedure, Olton & Papas (1979) further demonstrated that lesions of the fornix resulted in severe deficits in working memory processing, whilst leaving reference memory processing unaffected.

1.8.4 The automated delayed nonmatching-to-position (DNMP) task.

Another key development in rodent studies has been the introduction of an automated matching / nonmatching-to-position (DMP/DNMP) task which was designed to be closely analogous to primate delayed response, and delayed alternation tasks. By using spatial information to be remembered on a trial by trial basis, it is also closely analogous to the paired-trial T-maze delayed-alternation task devised by Rawlins & Olton (1982). According to Dunnett (1993), the essential features of this task were as follows:

1. Two response levers, placed one either side of a central food hopper which were independently retractable, and under computer control.

2. Food pellet rewards were delivered to a central food hopper, covered by a hinged perspex panel. A mechanical switch detected when the panel was opened.

3. A house light in the centre of the ceiling illuminated the chamber, and a panel light illuminated the food hopper. Three additional stimulus lights were available, located above each lever, and above the food hopper respectively.

In such a task, as described by Dunnett (1989), the animal was housed in a conventional operant chamber and was faced with two retractable levers.

One of the two levers provided the sample to which the rat must respond; then, after a variable delay, both levers were presented simultaneously, and the animal must respond to the same (matching) or the alternate (non - matching) lever to obtain a reward. The additional task of having to respond to the central flap throughout the delay period provided a supplementary distracter task which helped prevent the animal from adopting a positional strategy to solve the task.

As this test taxed the ability of the animal to remember which of two spatially discrete samples had most recently been selected, it closely resembled the delayed response task and as such, could be seen to provide a powerful means of assessing cognitive capacity. The measuring of performance using a number of accuracy and bias indices derived from '*Signal Detection Theory*' (Fray & Colliver 1973; Sahgal 1987), has strengthened the role of this test. Such measures can provide a wealth of detail concerning an individual animals' daily performance, covering not only percent correct at consecutive delay intervals, but also mean latencies to respond to the lever presentations, an important clue to the motivational state of the animal. The left and right sample trials provide independent sets of trials for analysis, enabling the researcher to distinguish between changes in the discriminability of the memory trace, and response bias, which underlie the delay-dependant deficit typically noted.

1.9 A RAPPROCHEMENT BETWEEN HUMAN AND ANIMAL STUDIES ?

A crucial point is how far one can compare results from human and animal measures ? In order to develop an accurate model of human and animal amnesia one must be sure that the tests involved are of an equivalent nature else comparisons would be futile. For example, if a human amnesic patient displayed an impairment on a delayed response task while a monkey with qualitatively similar experimental lesions did not, then this could indicate either that the tests were not analogous, or that there were some differences in functional anatomy between the two species.

As reported in an earlier section, initial studies comparing the performance of humans and animals on similar tests found many differences; with continued refinement however, such large differences have subsequently been rarely reported. Many studies have compared the performance of humans, monkeys and rodents on similar tasks, with broadly similar results being demonstrated.

1.9.1 Monkey studies.

Squire et al., (1988) assessed the ability of amnesic patients to perform 5 different tasks that have figured prominently in the development of monkey models of amnesia. Four of these tasks - DNMS, object-reward association, concurrent discrimination, and object discrimination, were failed by monkeys with medial temporal or diencephalic damage (Aggleton & Mishkin 1983b; Mahut et al., 1982; Moss et al., 1981; Zola-Morgan & Squire 1985). The last task, that of 24 hour concurrent discrimination could be successfully learnt by monkeys with medial temporal damage, and has been considered to provide a measure of the kind of learning that is spared in amnesia (Malamut et al., 1984). For the first four of these tasks, the human amnesics were duly impaired (just as were the monkeys), but were also impaired on the 24 hour discrimination task.

Squire et al., (1988) argued that such tasks did appear to be sensitive to the memory functions affected in human amnesia, and could also provide valid comparisons with monkeys. With regards the 24 hour discrimination task, the authors concluded that such a task may be learnt differently by monkeys and humans; in that monkeys may have approached the task incrementally in a skill like manner, whilst humans appeared to have approached the task as if it were a test of declarative memory, i.e. in which an explicit attempt was made to memorise the correct stimuli.

Other studies have demonstrated a similar rapprochement between human and monkey amnesics, i.e. in both DNMS and concurrent learning (Aggleton et al., 1988; Bowden et al., 1992); object discrimination (Kessler et al., 1986); and in delayed response (Oscar-Berman & Zola-Morgan, 1982).

1.9.2 Rat studies.

The vast amount of research on learning and memory in rats has focused on much the same areas of interest as the work performed with monkeys and humans. Until fairly recently, it was not easy to reconcile the three sets of findings as the tasks used were not altogether compatible. However, work by such as Aggleton (1985), and Dunnett (1990), has shown that rats can perform memory tasks analogous (though not precisely so), to those used with monkeys and humans. The value of such studies is threefold:

Firstly, the testing procedures are comparable to those used with humans and monkeys thus aiding cross-species comparison. Secondly, rats are able to learn procedures such as DNMS rapidly, and perform at a high level of accuracy. Finally, such findings add weight to the argument that rats are capable of learning a variety of complex rule governed behaviours which can be described in a similar manner to such behaviours as shown by monkeys and humans. Experiments utilising rats instead of monkeys have revealed very similar findings to primate studies, namely that the tests are taxing essentially the same kinds of declarative memory processes, and that damage to corresponding areas of the rodent brain have quantitatively similar effects as the same lesions in monkeys.

For example, in humans, a transient cerebral ischemia can produce irreversible neuronal damage and hence permanent learning and memory deficits (Zola-Morgan et al., 1986), the same kinds of impairments are found in non-human primates (Zola-Morgan et al., 1992). A study by Wood et al., (1993) attempted to recreate the same types of ischemic episode in rats and then tested the effects using a novel non-spatial DNMS recognition task developed by Mumby et al., (1990) thought to be analogous to that used with human patients and monkeys. Following surgery, ischemic rats were significantly impaired in both learning and performing the DNMS task, and extensive pre-surgical training did not reduce the impairment (Wood et al., 1993). Such findings support the validity of a direct comparison of ischemia-induced memory deficits between rats and primates.

1.10 AIMS AND RATIONALE.

The preceding sections have provided an overview of the evidence concerning the existence of an anterograde amnesic syndrome which appears to have similar characteristics in both humans, and in experimental animals. Though the causes of such a syndrome can vary, the severity of the amnesia seems to depend upon damage to one, or several, neuroanatomical circuits interlinking both medial temporal and diencephalic regions with other areas of association cortex.

A complex series of medial temporal/diencephalic connections has been revealed so that damage to such a circuit at any point may have a variable effect upon mnemonic processing. The magnitude and nature of the effect may depend on the relative significance of the structure or pathway affected.

Much of the evidence concerning anterograde amnesia has focused upon the role of the hippocampal formation. While the integrity of the hippocampal system seems to be of vital necessity for an intact mnemonic system, the functional outcome of damage to the extended hippocampal formation (notably the thalamic nuclei, mammillary bodies, and cingulate cortex) remains contentious. The relative involvement of each of these structures in the types of mnemonic processing typically impaired in the anterograde amnesic syndrome, has been the source of much disagreement.

Over the years, a wealth of evidence attesting to the importance of each part of the extended hippocampal system has been put forward, much of it being contested at a later date. What is required is a systematic investigation of the functional mnemonic consequences of relatively circumscribed damage to each of these extended structures, compared to the effect of similar damage to the hippocampal system. If damage to one, or all, of these regions, produces the same functional consequence (namely anterograde amnesia), as damage to the hippocampal system, then it can be assumed that they are part of the same neuroanatomical circuit governing learning and memory.

The rationale behind this thesis is as follows:

1.10.1 Comparable Anatomy.

The basic anatomy of the hippocampal formation and its extended system is very similar in humans, primates and rodents, especially in terms of its subcortical connections. Though the mnemonic capacities of each species is likely to be different, the functional consequences of hippocampal system damage to learning and memory could be very similar.

1.10.2 The fornix model of hippocampal dysfunction.

Jarrard (1993) pointed out that the interruption of the fimbria/fornix is often used as a model of hippocampal dysfunction, but argued that such a procedure suffers from several problems.

Firstly, this lesion spares the major output projections from the hippocampus that course through the subiculum and entorhinal cortex. Secondly, disruptions to the hippocampus can lead to seizures that can have widespread effects on other brain regions, while leaving its cells intact.

Several studies have examined this problem. For example Olton (1978) investigated the functional effects of a variety of lesions which served to disconnect the important elements of the septohippocampal system. Bilateral destruction of the fimbria/fornix had a comparable effect with the same lesion in entorhinal cortex, and contralateral lesions in both areas also had the same result. Olton (1978) concluded that lesions in the septal pole and the temporal pole of the hippocampus had an equivalent behavioural effect.

More recently, a study by Aggleton et al., (1992) found support for Olton's (1978) findings by comparing the effects of radiofrequency lesions of the fimbria/fornix, with extensive aspiration lesions of the hippocampal region, on the performance of rats on an automated test of spatial working memory (delayed non-matching-to position). Both lesions were found to produce equivalent performance deficits on this task, the pattern of which led the authors to conclude as being consistent with a mnemonic deficit.

These findings indicated that the fornix model of hippocampal dysfunction relating to spatial working memory at least, was valid, and it could thus be argued that disrupting the fornix can tell us much about the functions of the hippocampus. Throughout the experimental work described in this thesis, the destruction of the fornix by radiofrequency lesions will be used, and assumed to accurately reflect hippocampal dysfunction.

1.10.3 Several pathways subserving spatial working memory ?

Evidence from human patients and experimental animals, presented in previous sections has strongly indicated that there may be several pathways mediating mnemonic processing, these pathways are likely to consist of the following structures and connections:

Pathway 1: **Rhinal cortex ---------- MD ---------- Ant' Cing' cortex ---------- HPc**

Pathway 2: **HPc ---------- Ant' Thal' ---------- Retrosplenial cortex ---------- HPc**

\ |

Mammillary Bodies

Pathway 3: **HPc --------- Ant' Thal' ---------- Prefrontal cortex**

It is hypothesised that damage to any of these circuits may result in some kind of mnemonic deficit, but that deficit may well critically depend upon which part has been damaged. Damage to one or several of the structures contained in each circuit have been implicated in learning and memory deficits in both humans and animals but some structures are likely to perform different roles in mnemonic processing. It is likely that severe damage to each circuit as a whole, or to the key structure in both (the hippocampus), will result in the most severe mnemonic impairment. Relatively circumscribed damage to one or several parts of the circuit may not have such a severe effect, depending on their relative involvement to mnemonic processing. Though damage to one part may produce a severe mnemonic deficit, it is more likely that damage to several sites in tandem, will produce a deficit equivalent of hippocampal system damage.

This of course depends on whether each circuit is engaged in parallel or serial processing. There may well be several serial circuits which are each responsible for one aspect of working memory, or one large parallel circuit which subserves all of working memory, its various elements governing some individual aspect of it.

It is the principal aim of this thesis to investigate the functional consequences of circumscribed damage to key structures of the three proposed circuits, and to determine the possible roles of each circuit (if any) in the processing of spatial working memory in the rat. Thus, the experiments described in this thesis will focus upon the consequences of producing selective damage to several parts of the extended hippocampal system (structures and regions that are most likely to form the key components in the suggested mnemonic circuits) and comparing such effects to those found after damage to the hippocampal system (the fornix) on certain tests of spatial working memory.

1.10.4 The tests chosen.

To measure the mnemonic functioning of the hippocampus and its extended system, a range of tasks have been chosen that have, in previous reports, demonstrated their sensitivity to hippocampal dysfunction in the rat. Such tests typically focus upon spatial working memory (the kind of memory which appears to be particularly sensitive to hippocampal dysfunction in rodents). The tests chosen will consist of two automated operant tasks, and two non-automated maze type tasks.

The automated tasks (DNMP, and spatial discrimination and reversal), enable the experimenter to control the relevant cues with ease, and provide an in-depth and sophisticated array of performance measures and indices, through which any behavioural or mnemonic impairments can be recorded. In addition, the spatial discrimination task can be regarded as largely a test of reference memory, i.e. memory based upon rules or procedures, while the DNMP and maze tasks are principally tests of working memory, i.e. memory for specific locations visited or actions carried out during the performance of a task.

Most importantly, all of these tests have been shown to be very sensitive to hippocampal system damage (Aggleton et al., 1986; Aggleton et al., 1992; Markowska et al., 1989; Olton & Papas 1979; Rawlins & Olton, 1982). These tests are regarded as benchmark tasks of hippocampal function and so the performance of animals with damage to the extended hippocampal system on these tasks should reveal much about the relative importance of these regions to spatial working memory. Damage to the anterior thalamic nuclei is known, for example, to impair performance on the DNMP task, while mammillary body damage does not (Aggleton et al., 1991a). Though the effects of damage to MD, and of various regions of medial frontal cortex have been reported on some T-maze, and radial maze tasks, the functional effects of neurotoxic damage to these same regions on such tasks, and specifically, on the automated tasks, remain as yet unknown.

These tasks can also differentiate between allocentric and egocentric spatial processing. This differentiation is important as it has been shown that different structures may mediate these two types of spatial memory (Kesner et al., 1989).

1.11 SUMMARY.

The aims of this thesis are thus twofold:

1. To determine the effects of lesions to several structures and areas of the extended hippocampal system on various tasks of spatial working memory that have been shown to be sensitive to hippocampal system damage, and reflect egocentric and allocentric spatial processing.

2. To apply any findings to each of the three hypothetical circuits presumed to subserve spatial working memory in the rat.

Experiment 1 will consider the effects of neurotoxic lesions to the thalamic mediodorsal (MD), nucleus on the acquisition and performance of two automated tests of spatial working memory (DNMP) and reference memory (lever discrimination and reversal).

Experiment 2 will focus upon the effects of neurotoxic lesions to two regions of cingulate cortex, and radiofrequency lesions of the fornix on the same two tasks, and a further task (forced-alternation in a T-maze).

Experiment 3 will consider the effects of neurotoxic lesions to the entire cingulate cortex, and radiofrequency lesions of the fornix, and cingulum bundle, on the performance of the same three tasks.

Experiment 4 will analyse the effects of lesions at different sites along the cingulum bundle, and of lesions of the fornix, on the acquisition of the DNMP and T-maze tasks.

Experiment 5 will consider the effects of damage to the cingulum bundle, and lesions of the fornix, on several tests which clearly differentiate between egocentric and allocentric memory (T-maze, Cross-maze, and 8-arm radial maze).

CHAPTER 2. EXPERIMENT 1. THE EFFECT OF DORSOMEDIAL THALAMIC LESIONS ON AUTOMATED TESTS OF SPATIAL WORKING MEMORY.

2.1 INTRODUCTION.

To briefly summarise some key points from the preceding chapter, it can be argued that the role of MD in learning and memory is contentious. Neuropathological studies in humans have linked damage to MD with diencephalic amnesia's of varied aetiology (Markowitsch, 1982; Speedie & Heilman, 1982; Victor et al., 1971). Unfortunately, the lack of cases with damage confined to MD, coupled with the close proximity of MD to other nuclei or fibre tracts also implicated in amnesia (von Cramon et al., 1985; Markowitsch 1988), has left uncertain the contribution of this nucleus to human memory processing.

A similar situation is found with regard to the evidence from experimental animals, with some studies reporting severe deficits on memory tasks following MD damage, and others not. For example, MD lesioning in rats has been reported not to impair performance in the T-maze (Brito et al, 1982), the radial maze (Kolb et al., 1982), and the Morris water task (Kolb et al., 1982). However, severe impairments following MD damage have been reported on the radial maze (Stokes & Best, 1988; 1990a; 1990b), spatial DNMS (Mair et al., 1992), reinforced spatial alternation (Vicedomini et al., (1982), and the acquisition and performance of a non-recurring items DNMS (Mumby et al., 1993). A further complication is the recent proposal by Hunt & Aggleton (1991) that these inconsistent effects may be due to the production of inadvertent damage to the adjacent anterior thalamic nuclei in such studies.

The aim of this experiment was to examine the acquisition and performance of rats with intrathalamic injections of N-methyl D aspartic acid (NMDA) on an automated test of working memory, delayed nonmatching-to-position (DNMP). The rationale for this experiment arose from the opportunity to produce selective MD lesions with a neurotoxin that causes little, if any, damage to fibres of passage (Olney, 1984).

The particular memory task, (DNMP), was selected for several reasons. Firstly, it has recently been confirmed that automated tests of spatial working memory are sensitive to diencephalic amnesia in humans (Joyce & Robbins, 1991). Secondly, the demands of the DNMP task share many features with delayed response and delayed alternation tasks which are regarded as benchmark tests of prefrontal cortex function in animals. With the strong connections between MD and prefrontal cortex (Groenewegen, 1988; Krettek & Price, 1977), it would be surprising if damage to MD did not result in similar impairments in the analogous DNMP task. Thirdly, a number of previous studies have revealed that this task is sensitive to the effects of hippocampal system damage and to prefrontal cortex damage in the rat (Aggleton et al., 1991a; Dunnett, 1990) although the effects of MD damage are unknown.

An impairment on this DNMP task would indicate that a rhinal cortex - MD - anterior cingulate cortex circuit, may be important for spatial working memory. Staubli et al., (1987) have proposed that MD plays an important role in the establishment of memory at the time of learning, and Gaffan & Watkins (1991) have argued that MD damage can impair both long- and short-term memory. In the light of these proposals concerning the contribution of MD to learning and memory the present study examined the effects of MD lesions both on the acquisition of the DNMP task (MDpre) and on the performance of rats that had received surgery after DNMP acquisition (MDpost). Each group of animals was finally tested on a spatial discrimination and reversal task in the same operant chambers. Rats with damage to MD have been reported to be impaired on tasks of visual discriminations (Tigner, 1974).

2.2 MATERIALS AND METHODS.

2.2.1 Subjects.

The study involved 37 naive, male rats of the pigmented DA strain (Bantin and Kingman, Hull). Throughout the period of the experiment the animals were housed individually under diurnal conditions (14h light/10h dark), all testing occurring at a regular time during the light period. The animals were fed approximately 15g of laboratory diet (Labsure ERM) daily so that they did not drop below 85% of normal body weight. At the start of testing the animals were aged approximately 4 months and weighed between 200 - 300g. All animals had free access to water.

2.2.2 Apparatus.

All testing was carried out in 7 operant chambers (Campden Instruments Ltd., Loughborough) under the on-line control of 2 Spider microprocessors (Paul Fray Ltd., Cambridge). Each chamber was fitted with two retractable levers situated 7.5cm either side of a central food tray. This food dispenser, which delivered 45mg pellets (Campden Instruments), had a hinged perspex lid at which nose pokes could be recorded. A light was located inside the food dispenser and a house light was located in the centre of the roof. A further light was situated above each lever.

2.2.3 Procedure for Experiment 1a: DNMP.

10 animals received MD surgery. After being allowed to recover for two weeks, this group (MDpre, n=10), and the remaining normal control animals (CON, n=27) were given the same, standardised training protocol.

1. Magazine training: The animals were allowed to eat ten reinforcement pellets that had been placed in the food tray with all of the house lights on. Training stopped when the animal ate all pellets during a period of 10 minutes on three consecutive daily sessions.

2. Autoshaping: A randomly selected lever was inserted into the chamber and the light above it lit. The lever remained '*out*' until the animal responded by pressing, at which point the lever was retracted, the food tray light came on and a single reward was delivered (Sahgal, 1983). If no response was made after 10s the lever was retracted. Autoshaping finished when the animal had responded to at least 50 out of 60 lever presentations over three consecutive sessions. A formal correction procedure was also applied (Sahgal, 1983).

3. Delayed non matching-to-position (DNMP): During the first stage of testing (0's delay) a trial would consist of the following sequence of events. After a 10s inter-trial interval, either the left or the right hand lever emerged and the corresponding lever light illuminated. The rat then had to respond to the lever within 10s, upon which the lever would retract, a pellet would be dispensed and the magazine tray illuminated. The animal then had to approach the tray and, within 10s, operate the magazine flap.

Both levers then emerged, and the stimulus lights above them were illuminated. The rat now had to respond, for a food pellet, to the lever which had not appeared as the sample; these choice responses also had to be made within 10s. Incorrect responses, or a failure to respond, resulted in a '*time-out*' of 10s, during which all lights in the chamber were switched off, and both levers retracted; these were the only occasions when the chamber was unlit, (see figure 5).

A session consisted of 60 trials. A formal correction procedure was used to encourage an animal to respond on the opposite lever if it had developed a pronounced side bias (Sahgal, 1983).

Delays between the sample and choice lever presentations were introduced once the animals had reached a criterion of 90% correct on three consecutive sessions. For all subsequent trials magazine responses following the sample presentation were ineffective (but were recorded) until the appropriate delay interval had lapsed; these nose-pokes at the central magazine flap provide a distracter task which served to keep the animal centralised between the two levers, and thereby helped to prevent the animal from adopting a mediating position strategy to solve the task (Dunnett, 1989).

The first response after these nose-pokes resulted in the choice levers being presented, providing this response occurred within 10s of the end of the delay (see figure 5). The animals were first given a retention delay of 2s with the same performance criterion as 0s. Following this the number of trials was increased to 96 and each session contained an equal mixture of 0, 2 and 4s (four sessions), 0, 2, 4 and 8s (four sessions, and 0, 2, 4, 8, 16, and 32s (six sessions) delay intervals.

The DNMP Procedure.

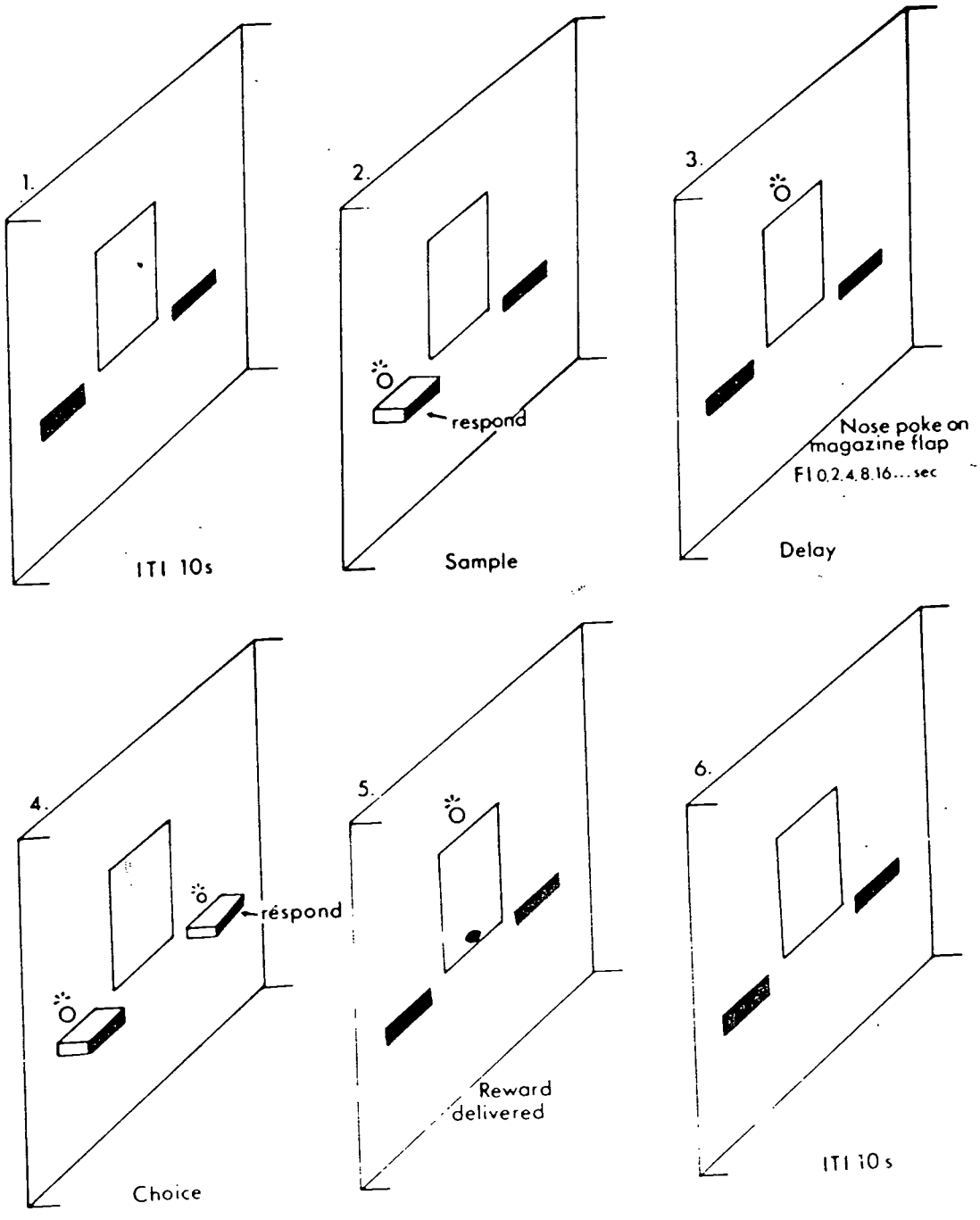


Figure 5. Schematic diagram showing the delayed nonmatching-to-position (DNMP) procedure, taken with kind permission from Aggleton & Sahgal (1993). The figure which shows the sequence during a correct DNMP trial, depicts the inside of the operant chamber with retractable levers, food magazine flap, centre light, lever lights, and food reward.

4. Post-operative performance: Following completion of this protocol, 15 of the CON group received surgeries (7 MDpost, 8 Controls). Approximately one month after surgery, these 15 rats, and the rats operated prior to acquisition (the MDpre group), were retested on the DNMP task. Due to testing constraints, the remaining 12 normal control animals were removed from the experiment. All animals received 15 sessions with a balanced, mixture of 0 - 32s (as in the final stage of acquisition).

5. Performance measures and analyses: The data from the 0 - 32s delay condition on the DNMP task were analysed to provide three indices of accuracy and three of response bias. These indices (barring percent correct) were derived from nonparametric signal detection models (Fray & Colliver, 1973). Briefly, the theory of signal detection (TSD) proposes that performance is governed by two independent processes, the accuracy of information processing, and the motivational state, or bias, of the subject. Any alteration in performance could be due to one or both of these processes and it is important to distinguish between them. The indices described in this, and the succeeding experiments, consist of three measures of accuracy - percent correct, and a further two derived from TSD, consisting of A' and SI (sensitivity indices); and three motivational indices - B" (perceptual bias), RI (response bias), and Iy (accuracy between the two levers).

It should be noted that only the absolute values of B" and RI were analysed, since only the magnitude of the bias is of interest, rather than position or direction (for example, left versus right position bias). As described by Aggleton et al. (1991a), the indices can be calculated from the hit (*h*) and false positive (*f*) probabilities. In this DNMP task in which two choices are possible, *h* = the number of correct left responses divided by the total number of correct left plus incorrect responses; *f* = the number of incorrect left responses divided by the total number of incorrect left plus correct right responses. Thus,

$$A' = 0.5 + [(h - f) + (h - f)^2] / [4*h*(1 - f)]$$

$$SI = [h - f] / [2*(h + f) - (h + f)^2]$$

$$B'' = [(h - h^2) - (f - f^2)] / [(h - h^2) + (f - f^2)]$$

$$RI = [h + f1] / [1 - (h - f)^2]$$

The final index (Iy) contrasts accuracy between the two levers, and provides a simple index of the subject's tendency to select one or other side of the operant chamber for responding; in some cases, this index is regarded as being superior to B" or RI (Sahgal 1987). Thus,

Iy = (absolute value of left minus right lever corrects) (total number of corrects).

These various measures are of interest as they may help to distinguish between different types of impairments.

Finally, estimates of general responsivity were also recorded. These included (a) latency to respond to the sample lever - pooled across delays, since delay was unpredictable at the sampling stage, (b) latency to make the first magazine response ('*nose-poke*'), (c) rate of responding to the magazine flap, or nose-pokes/s, excluding the 0 delay condition, (d) choice (and average choice) latencies and (e) the number of missed trials. The justification and calculation of these signal detection measures and bias indices has been described in detail elsewhere (Aggleton et al., 1991a; Sahgal 1987).

In the light of the recent controversy concerning data analysis in monkey studies (Alvarez-Royo et al., 1992; Ringo, 1991; 1993) it must be emphasised that the data was transformed as appropriate (arcsin: all accuracy and bias indices; logarithmic: latencies; square-root: misses) and analysed by parametric analysis of variance (ANOVA). This included 1-factor (lesion group) independent measure, and 2-factor (lesion group, delay) mixed measure analysis, using a modified ANOVA program for microcomputers (Lane, 1981). When the F-ratio was significant ($P<0.05$), the means were compared using the Newman-Keuls procedure (Winer, 1971). For the sake of brevity, analyses failing to reach at least a $P< 0.1$ level are not presented in detail.

2.2.4 Procedure for Experiment 1b: Spatial discrimination and reversal.

One day after completing the DNMP task, the animals were trained on a spatial discrimination task in the same operant chamber. For each session the animal was presented with both levers, one of which had been arbitrarily designated as S+, the other as S-.

The pair of levers was extended until one was pressed (there were no 'miss' trials) at which time both were retracted. Responding on the correct (S+) lever was followed by the delivery of one reward pellet. Responding on the incorrect lever (S-) was recorded as an error and no reward given. The intertrial interval (ITI) was set at 7s. Each session consisted of 30 trials, the correct lever always being on the same side.

The animals received 16 daily sessions, the position of the correct lever on each session following a balanced, pseudorandom sequence, as such, a session could either be 'consistent' with the previous session (i.e. the S+ lever was in the same position as in the previous session), or it could be a reversal (i.e. the S+ lever was now the lever that had been the S- lever in the previous session), see figure 6.

2.2.5 Surgical and histological procedures.

For the MD surgeries the animals were anaesthetised by intraperitoneal injection (4 ml/kg) of a solution containing 42 mg/ml of chloral hydrate and 9.7 mg/ml pentobarbitone sodium (Equithesin). Each animal was then placed in a stereotaxic frame (David Kopf Instruments, Tujunga). The scalp was cut and reflected to expose the skull. Following craniotomy, a single injection of 0.36µl of 0.12M NMDA (Sigma Chemical Company Ltd., Poole) dissolved in phosphate buffer (pH 7.2) was made through a 1 µl Hamilton Syringe in each hemisphere. The stereotaxic co-ordinates relative to ear-bar zero, with the incisor-bar set at +5.0 relative to the horizontal plane, were as follows: AP +3.7, LAT ±0.7, the depth at both injection sites was -6.2mm below the top of the cortex. Each injection was made gradually over a five minute period and the needle allowed to remain in situ for a further five minutes before being withdrawn. After completion of the lesion sulphanilamide powder was applied and the skin sutured. The animals acting as non surgical controls (Control group) were just injected with the same dose of Equithesin and allowed to recover.

The Lever Discrimination and Reversal Procedure.

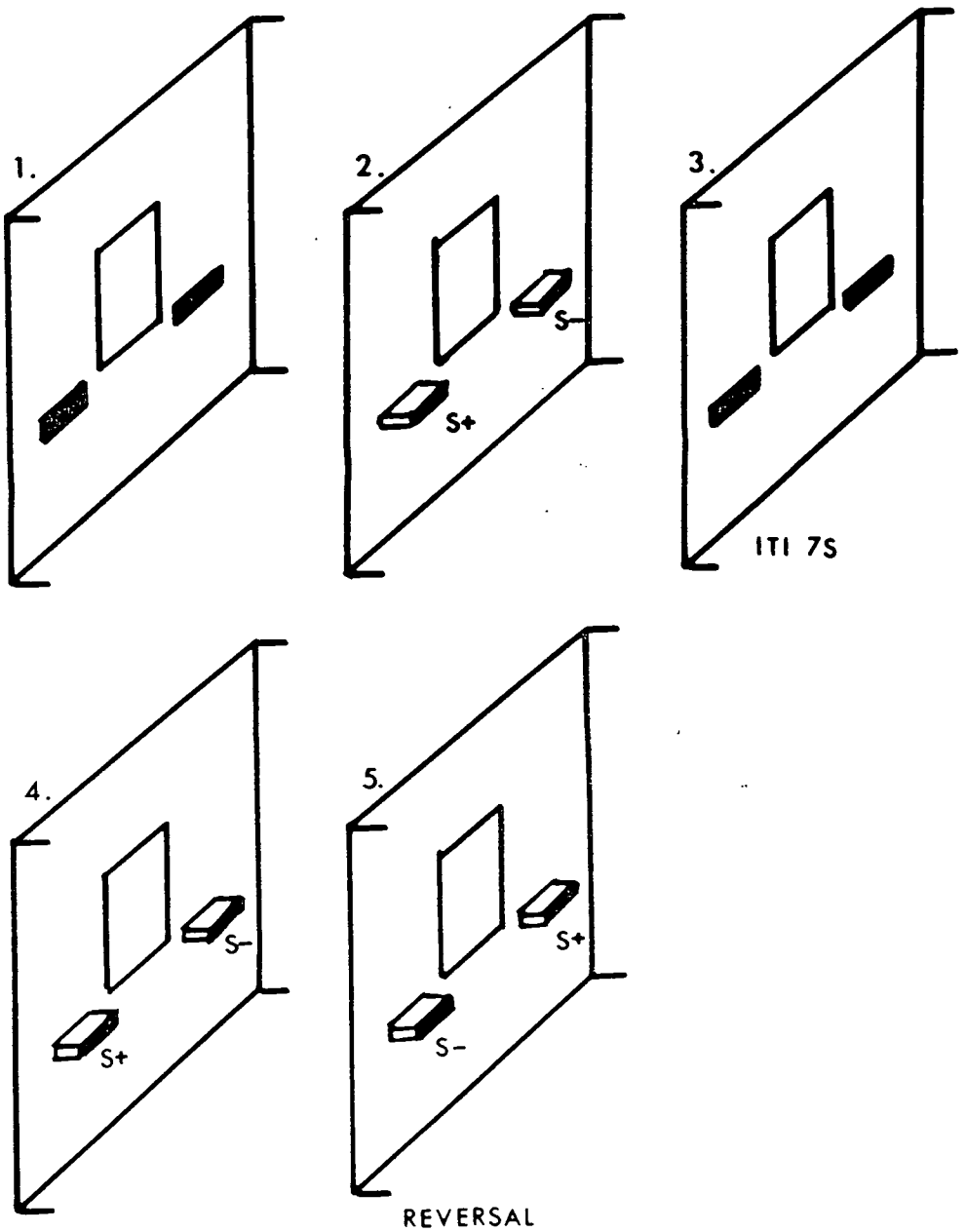


Figure 6. Schematic diagram showing the lever discrimination and reversal task. The figure shows the sequence during the initial lever discrimination and the subsequent reversal.

On completion of the experiment, each rat was killed and was perfused intracardially with 5% formol saline. The brain was then rapidly removed and placed in 5% formol saline. Subsequently, the brains were blocked, embedded in wax (Paraplast), and cut in 10μm coronal sections. Every tenth section was mounted and stained with cresyl violet, a Nissl stain.

2.3 RESULTS.

2.3.1 Histological analysis.

Every animal receiving an MD surgery displayed extensive cell loss within the nucleus and no animals were excluded on histological grounds. As there was no discernible difference between the location and extent of the MDpre and MDpost surgeries these two groups are described together. Figures 7 and 8 over, show the largest and smallest of the MDpre and MDpost lesions, and a photomicrograph of a typical MD lesion, respectively.

All 17 animals showed extensive damage throughout nucleus medialis dorsalis, the NMDA causing a complete loss of cells in the affected areas (figure 8). In all cases there was shrinkage of tissue associated with the lesion. Estimates of the extent of the lesions, based on planimetric measurements of four standard sections, indicated that they varied from 72% to 100% (median 88%) of the total area of MD. The only other regions to show consistent cell loss were the midline paraventricular nucleus, those parts of the paratenial nucleus lying immediately in front of MD, and the medial edges of nucleus lateralis dorsalis (Figure 8). In nine cases the lesion extended dorsally to involve the lateral habenula. Bilateral cell loss was seen in the caudal half of the anterior dorsal nucleus in six cases while unilateral damage was observed in a further seven cases. Slight, unilateral cell loss was found in the most caudal part of the anterior ventral nucleus in five cases. The anterior medial nucleus was always unaffected. In four cases there was a very restricted region of cell loss in the dentate gyrus associated with the injection tract.

Reconstruction of MD surgeries.

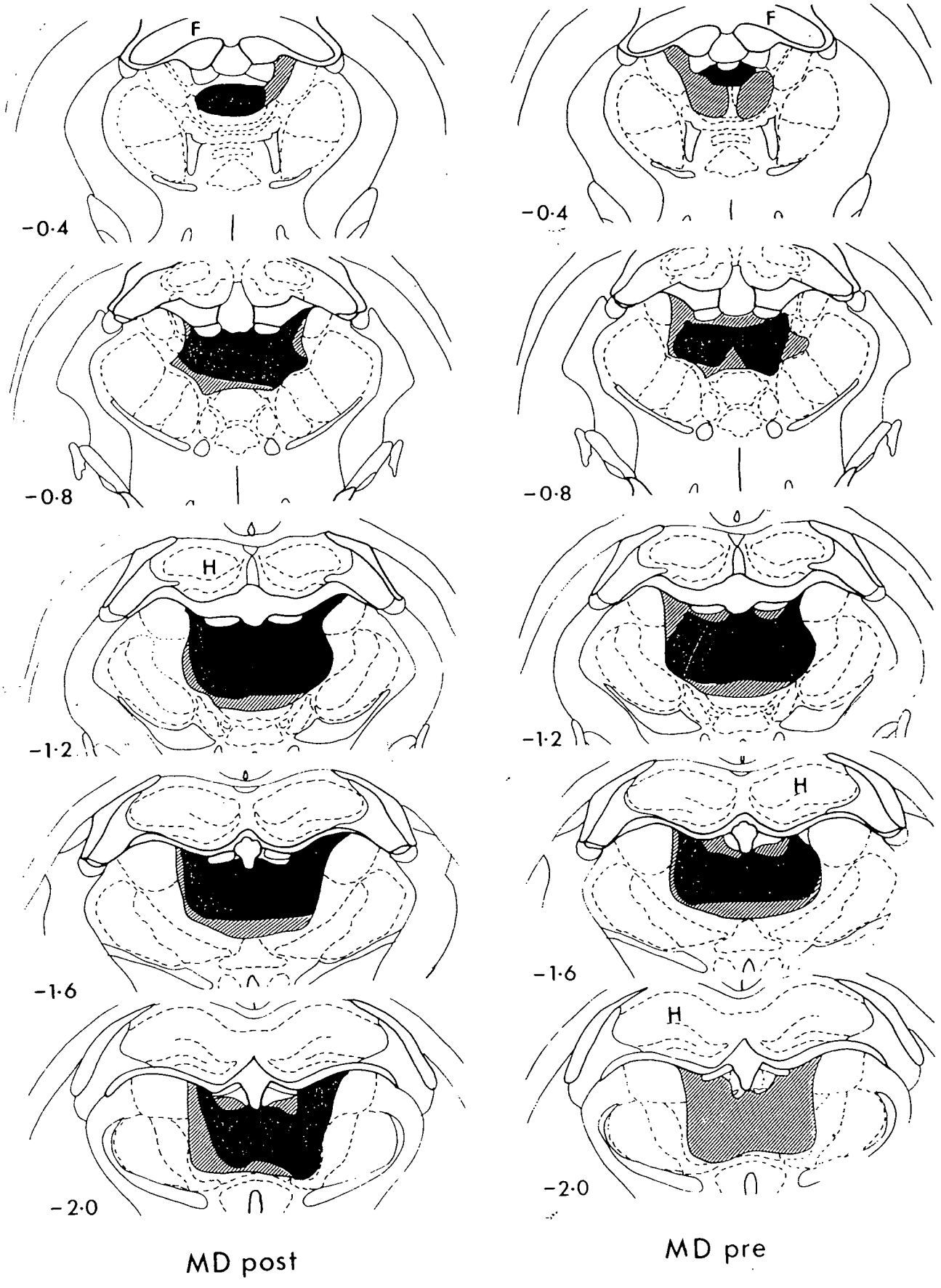


Figure 7. Coronal sections showing the MDpre and MDpost cases with the largest (diagonal lines) and smallest (black) lesions. The numbers refer to the approximate corresponding levels in the atlas of Pellegrino and Cushman (1967). F = fornix; H = hippocampus.

Photomicrograph of the Appearance of MD Following the Injection of a Neurotoxin.

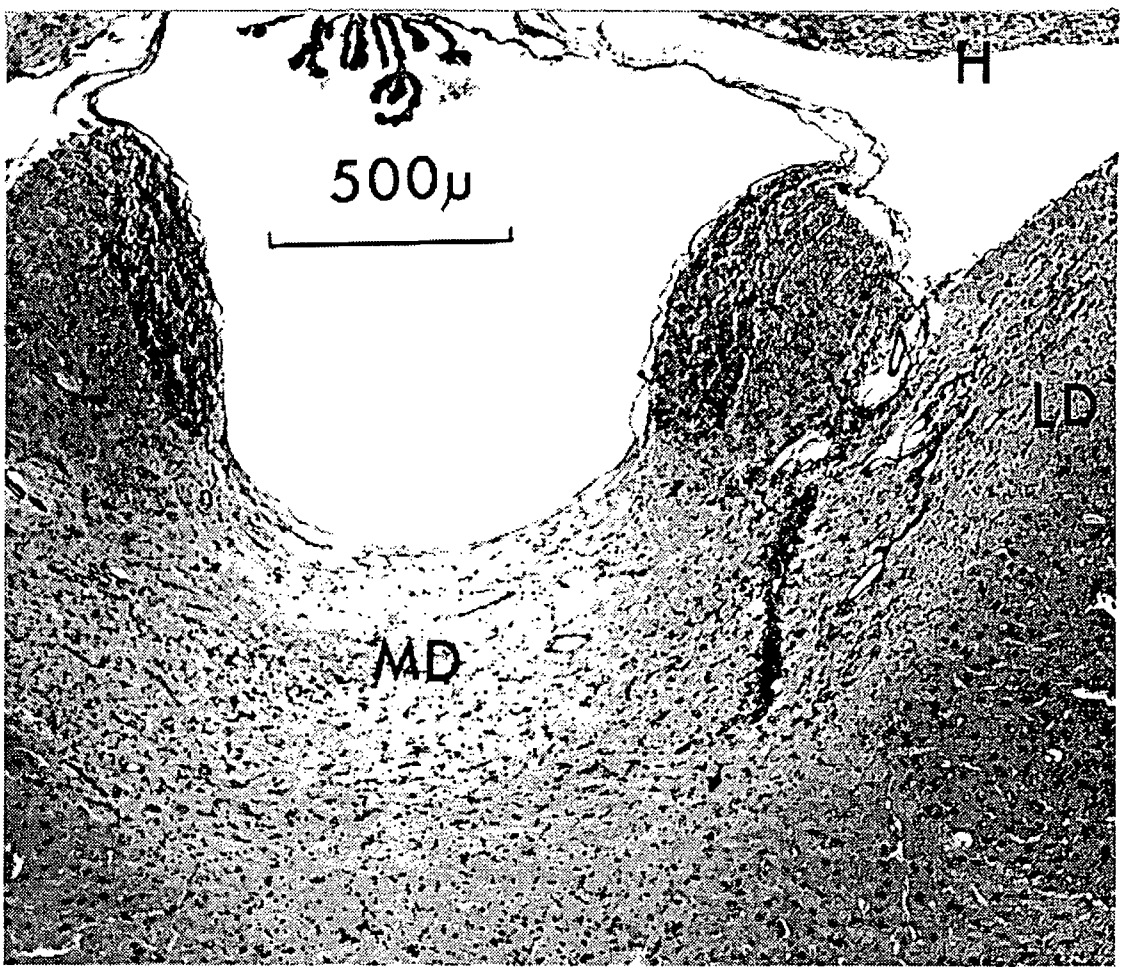


Figure 8. Photomicrograph of coronal section (Nissl stain) showing typical appearance of MD lesion following injection of NMDA. H = hippocampus; LD = nucleus lateralis dorsalis; MD = nucleus medialis dorsalis.

2.3.2 Experiment 1a: DNMP.

1. The effects of MD lesions prior to acquisition (MDpre versus CON).

a) Magazine Training: The mean number of sessions taken to reach the magazine training criterion with the relevant standard deviations in parentheses, were as follows: CON = 7.3 (2.7), and MDpre = 5.2 (1.6). An unpaired t-test (two-tailed) revealed a significant group difference ($t, 35 = 2.23$, $P<0.05$) which reflected the greater number of trials taken to reach criterion by the CON group.

b) Autoshaping: During this procedure, one animal from the CON group, failed to learn the autoshaping procedure and so was excluded from further testing, as a consequence, the group sizes were MDpre, n=10, and CON n=26. The corresponding scores for the autoshaping procedure were as follows: CON = 9.3 (3.0), and MDpre = 9.3 (5.0). As there was a significant difference between the variance of the two groups ($F\ 1,34 = 2.71$, $P<0.05$) a Mann-Whitney test was used to compare the two groups. This test revealed no significant group difference ($U = 111$, $P>0.05$, two-tailed).

c) DNMP at short delays: During this procedure, one of the MDpre animals became ill and was removed from the experiment, leaving the MDpre group with 9 animals. The mean number of sessions taken by each group to reach criteria at the 0s and 2s delay conditions were as follows: 0s: CON = 13.6 (5.4), and MDpre = 16.2 (3.4); 2s: CON = 6.8 (2.7), and MDpre = 6.7 (0.8). There was no significant group difference on either of these conditions ($t = 1.32$, $P>0.05$, and $t = 0.02$, $P>0.05$ respectively).

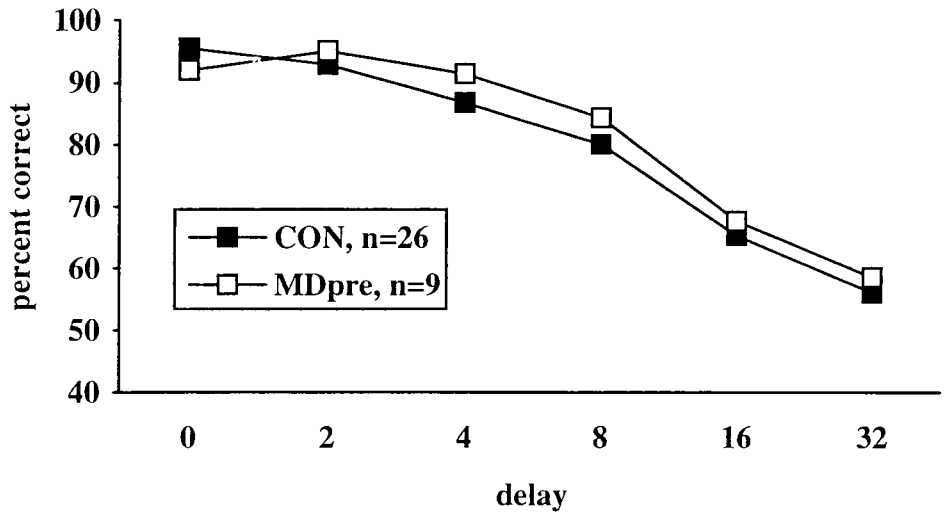
When each group had achieved criteria at the shortest delays, they were given eight more sessions four when the delay intervals ranged from 0-4s, and four when the delay intervals rangéd from 0-8s, in each case over 96 trials per session. The mean overall percent correct scores of each group at this condition were as follows: 0-4s: CON = 94.0 (2.7), and MDpre = 96.2 (1.8); 0-8s: CON = 95.2 (2.5), and MDpre = 94.5 (3.2). On the 0-4s condition a t-test revealed that the MDpre group had in fact performed significantly better than the CON group ($t = 2.25$, $p<0.05$). However, on the 0-8s condition the same test revealed no such group difference ($t = 0.61$, $P>0.05$).

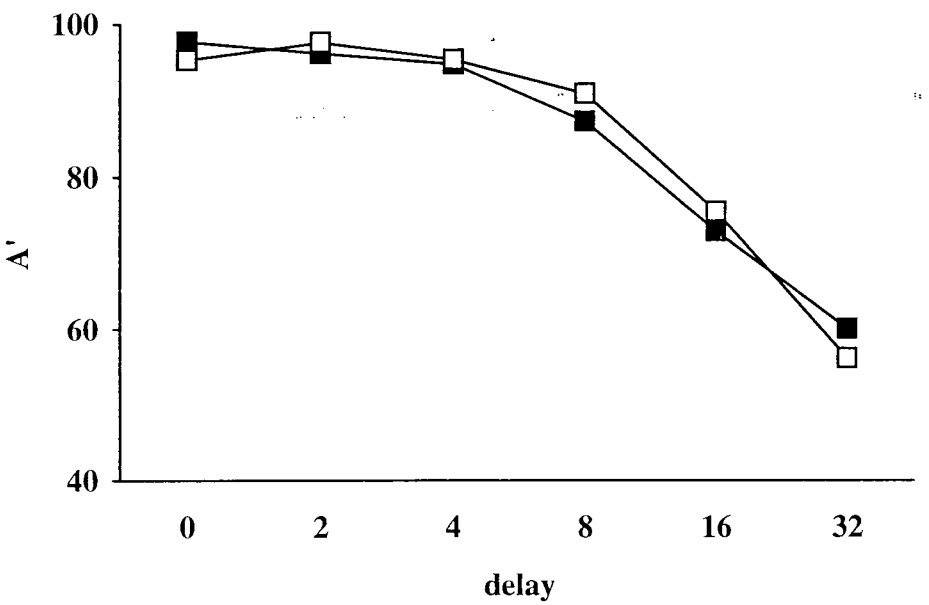
d) DNMP Performance at the longest delays: Both groups were then compared on their performance over six sessions with retention delays of 0 - 32s (figure 9). The mean percent correct scores of each group on this final six sessions were as follows: CON = 80.6 (4.2), and MDpre = 82.2 (0.8). As the difference between the standard deviations of the two groups was significant ($F_{1,33} = 3.3$, $P<0.05$) the groups were compared using a Mann-Whitney test. This test revealed no significant group difference between the mean percent correct scores of each group ($U = 90.0$, $P>0.05$, two-tailed).

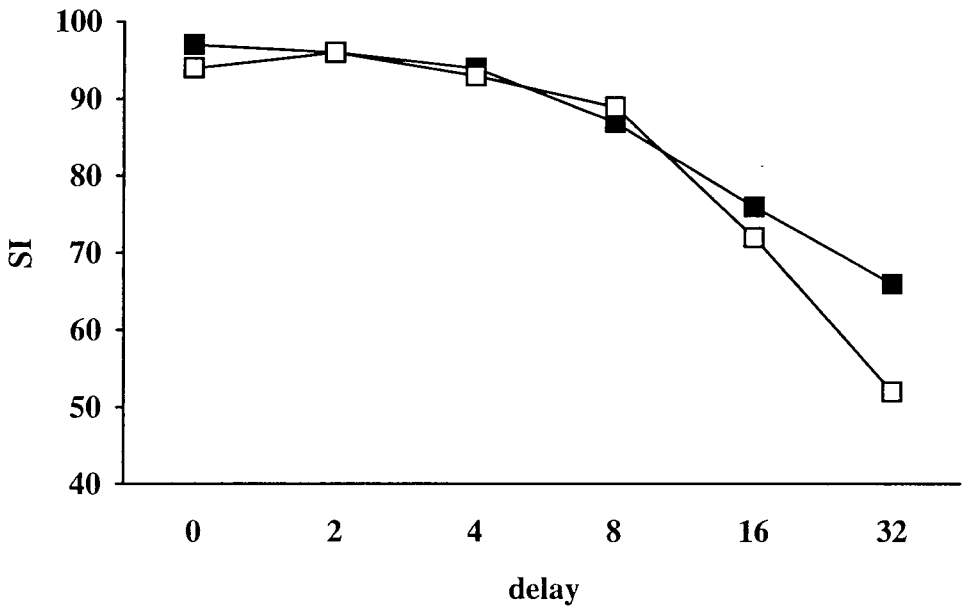
e) Accuracy measures: The percent correct measure provided the expected effect of delay ($F_{5,165} = 244.66$, $P<0.001$), which reflected the poorer performance of both groups as the delays increased. There was however, no evidence of a group effect ($F<1$) or of an effect of session ($F<1$), and no other comparisons were significant at the $P<0.05$ level. Similar results were found for the two TSD measures, A' and SI. In both cases there was a highly significant effect of delay ($P<0.001$ in both cases), but no evidence of a group effect (the maximum $F_{1,33} = 1.57$, $P>0.05$) or of a group X delay interaction (maximum $F_{5,165} = 1.90$, $P>0.05$), see figure 9.

f) Bias indices: None of the three bias indices (Iy, B", RI) provided evidence of a group effect at the $P<0.05$ level (Figure 9). There were, however the expected delay effects for all three indices (all $P<0.001$). The only significant interaction concerned the group X delay effect for index RI ($F_{5, 165} = 2.43$; $P<0.05$). This indicated a greater increase in response bias among the CON animals with delays of over 4s, see figure 9.

DNMP Bias Indices (CON Versus MDpre).



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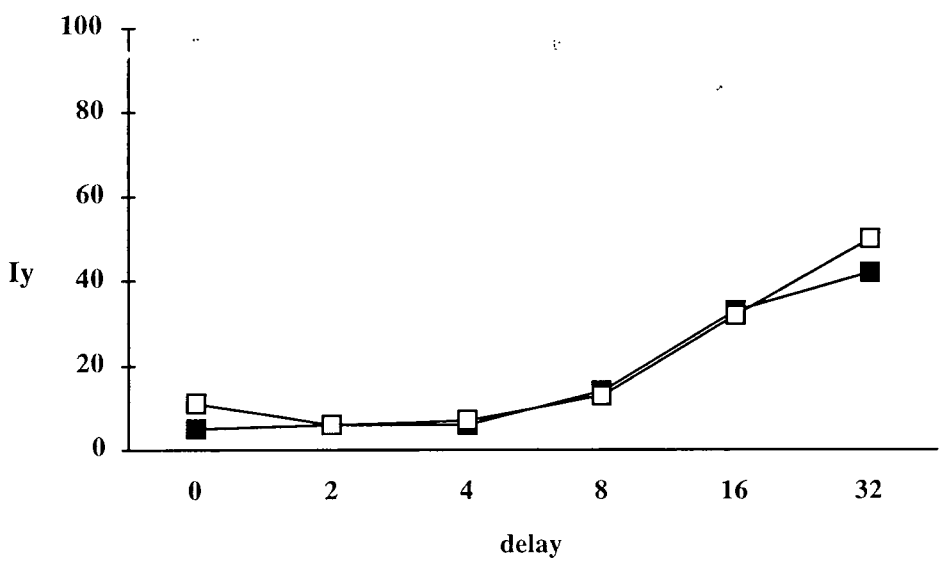
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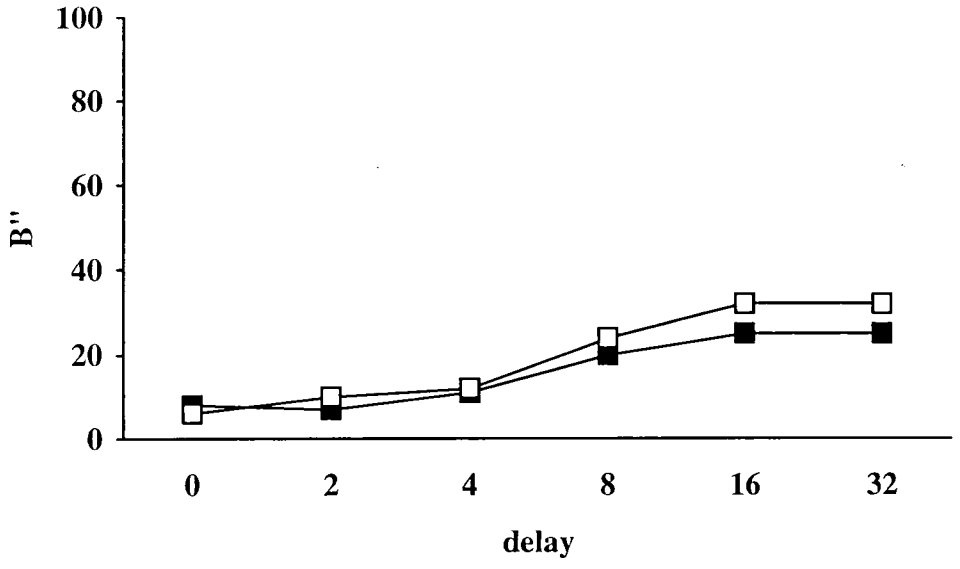


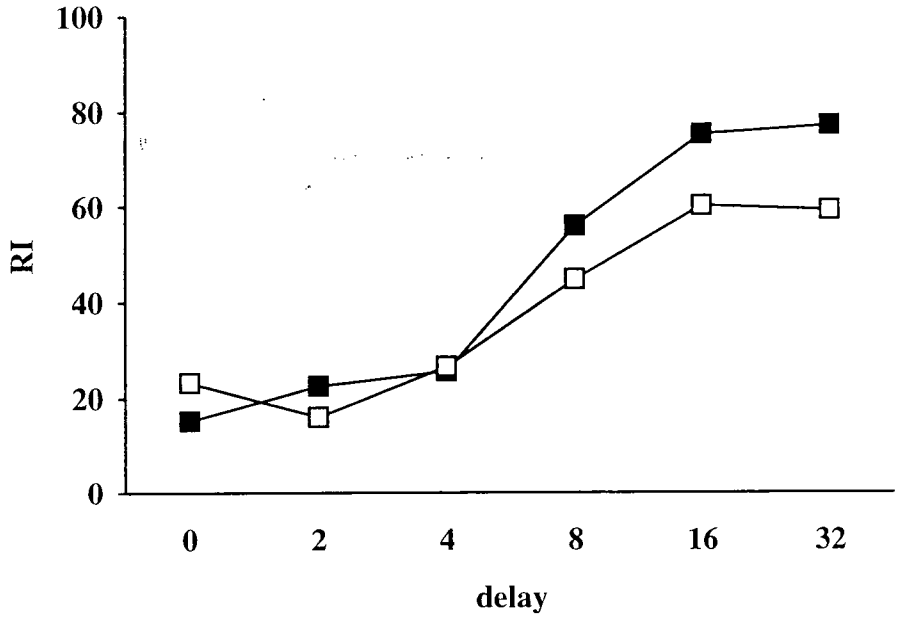


Figure 9. Accuracy (first three graphs) and bias (final three graphs) indices as a function of lesion and delay. See text for explanation of indices. All scores have been converted to read from 0 to 100. In the case of the bias scores, 100 represents a complete bias and 0 neutral, or no bias.

g) General responsivity: The various measures of latency all indicated that the MDpre group were slightly slower to respond than the CON group. This difference was, however, only significant for the latency to first magazine response ($F_{1,33} = 11.53$, $P<0.005$) as the other two measures (latency to sample, and choice latency) failed to reach significance (both $P>0.05$). Although the MDpre group made relatively more '*misses*' with the longer delays than the CON group, as reflected in the group X delay interaction ($F_{5,165} = 2.52$, $P<0.05$), there was no clear overall group difference ($P>0.05$). Similarly, there was no evidence that the two groups differed in their overall magazine response rate ($F<1$).

2. The effects of MD lesions after acquisition (MDpost Versus CON Versus MDpre).

Following completion of this protocol, 15 of the CON animals received surgeries, 7 having the same MD lesions as the MDpre group, and the remainder acting as controls. Approximately 1 month after surgery the three groups (MDpre, n=9, MDpost, n=7, and CON, n=8) were re-tested on the DNMP task. After a single practice session using 0s delays, all animals received 15 sessions comprising 96 trials using retention delays of 0 - 32s.

a) Percent correct scores over all 15 sessions: The mean percent correct score for each of the 15 postoperative sessions is shown in figure 10 over. An analysis of variance using this data indicated that while there was a marked effect of session ($F_{14, 294} = 6.17$, $P<0.001$), there was no clear group effect ($F_{2,21} = 3.30$, $P>0.5$), and no group X session interaction ($F<1$). As can be seen from figure 10, the suggestion of a group effect came from the high performance of the MDpre group and not from any impairment associated with the MDpost group. The session effect reflected the gradual improvement in post-operative performance among the test animals.

DNMP Percent Correct Scores (CON Versus MDpre and MD post).

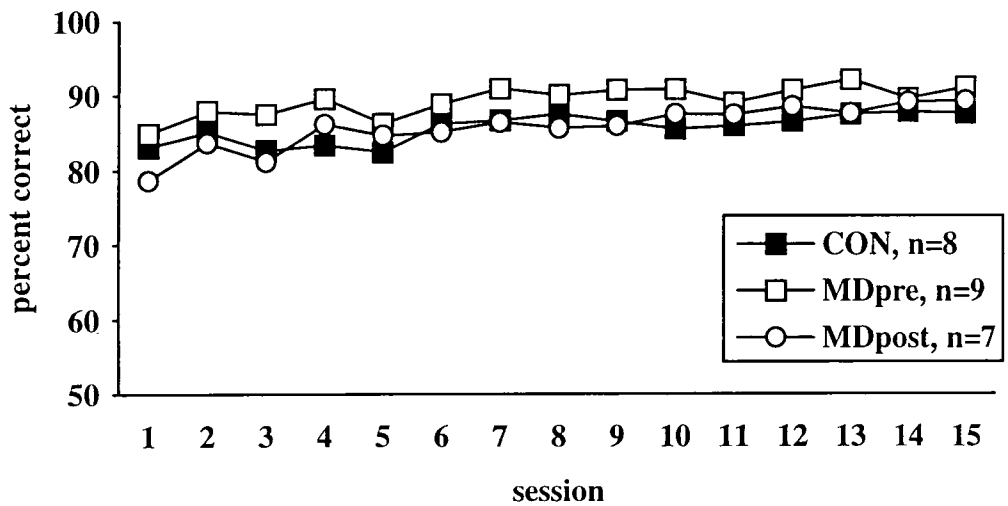


Figure 10. Mean percent correct scores of the three groups over each of the 15 postoperative sessions using all 6 delays.

b) Analysis of performance over the final 5 sessions: More detailed comparisons using the full range of response measures focused on the final five sessions where the animals maintained a stable level of performance (Figure 11). Initial comparisons between just the MDpre and MDpost groups for these five sessions found no evidence of a group effect for the three accuracy measures of percent correct, A', and SI, ($F_{1,14}$ = 1.38, F<1, F<1, respectively). In view of this lack of difference between the MDpre and MDpost animals, the two groups were combined to form one MD group (n=16) which was then compared with the CON group. This provided a large group of animals with MD lesions and, hence, a more powerful test of the effects of this surgery.

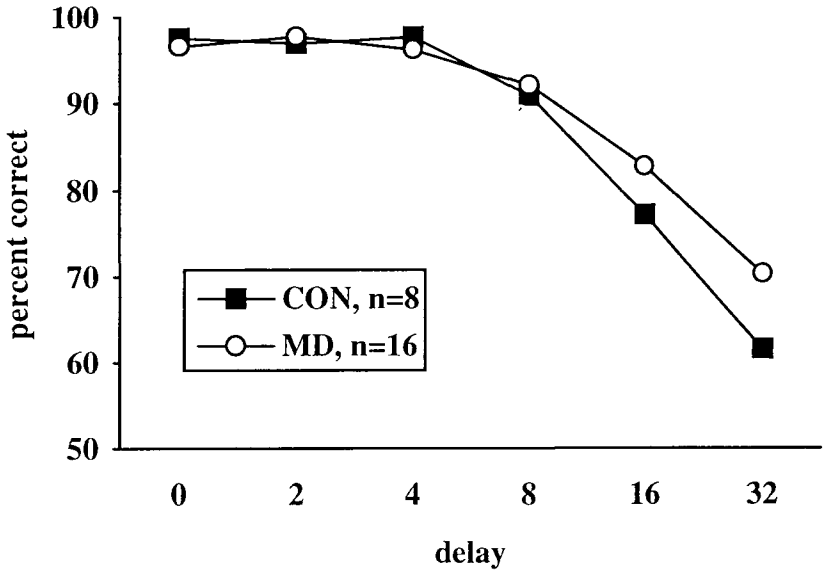
c) Accuracy measures: An analysis of percent correct performance by the two groups revealed the expected significant effect of delay ($F_{5,110}$ = 106.8, P<0.001), but no group effect ($F_{1,22}$ = 0.97 P>0.05) or group X delay interaction ($F_{5,110}$ = 1.51) was noted. The results for the two TSD measures (A' and SI) were very similar to each other.

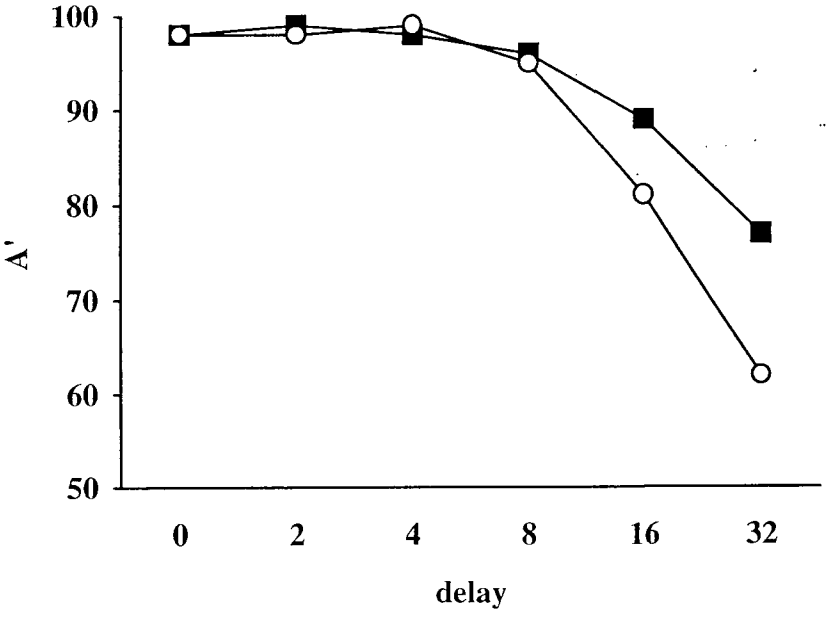
As expected, both revealed a highly significant effect of delay ($F_{5,110} = 24.2$, $P<0.0001$, and $F_{5,110} = 53.7$, $P<0.0001$ respectively), but there was also an indication of a possible group effect (both $0.1>P>0.05$). In addition, there was a significant group X delay interaction for both measures ($F_{5,110} = 2.96$, $P<0.05$, and $F_{5,110} = 3.40$, $P<0.01$ respectively). It can, however, be seen that for both indices (A ' and SI) these interactions reflected poorer performance by the CON animals at the longest delay intervals (Figure 11).

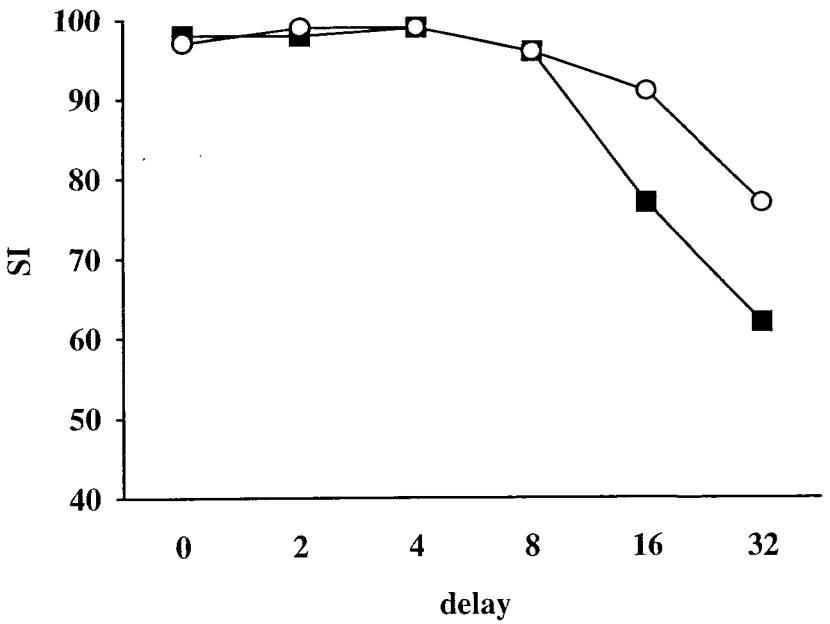
d) Bias indices: None of the three bias indices (Iy, B", RI) provided evidence of a group effect ($F_{1,22} = 0.5$, 0.9, and 0.6, respectively, with $P>0.05$ in each case), neither were there any significant interactions between group and delay for any of these three measures ($F_{5,110} = 2.21$, 0.19, and 0.21 respectively with $P<0.05$ in each case. There were, however, delay effects for all three indices ($F_{5,110} = 53.9$, 40.1, and 50.9 respectively, with $P<0.0001$ in each case). See figure 11 over.

e) General responsivity: Analysis of the general responsivity measures (latency to respond to the sample lever, latency to make the first nose-poke, rate of responding to the magazine flap, average choice latencies, and number of trials missed) revealed no significant group differences, or any significant group X delay interactions ($F_{1,22} = <1$, and $F_{1,110} = <1$ in each case).

DNMP Bias Indices (CON Versus MD).



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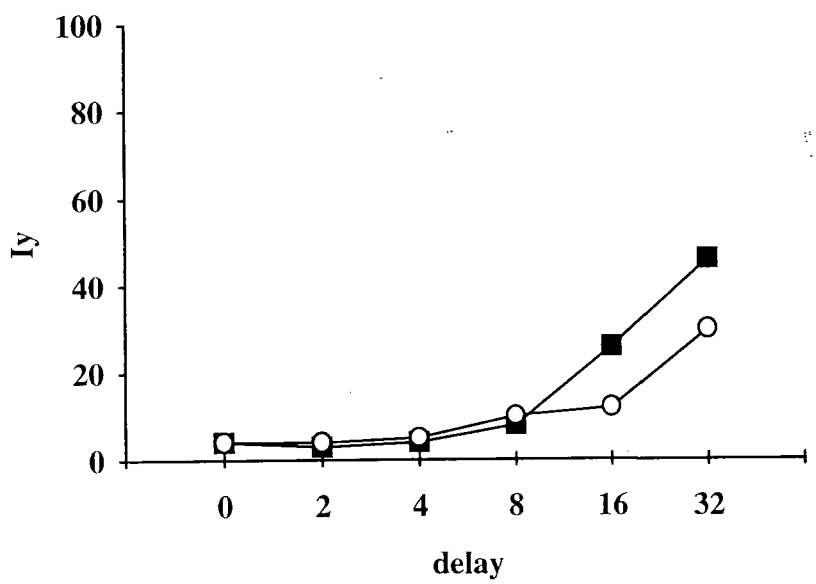
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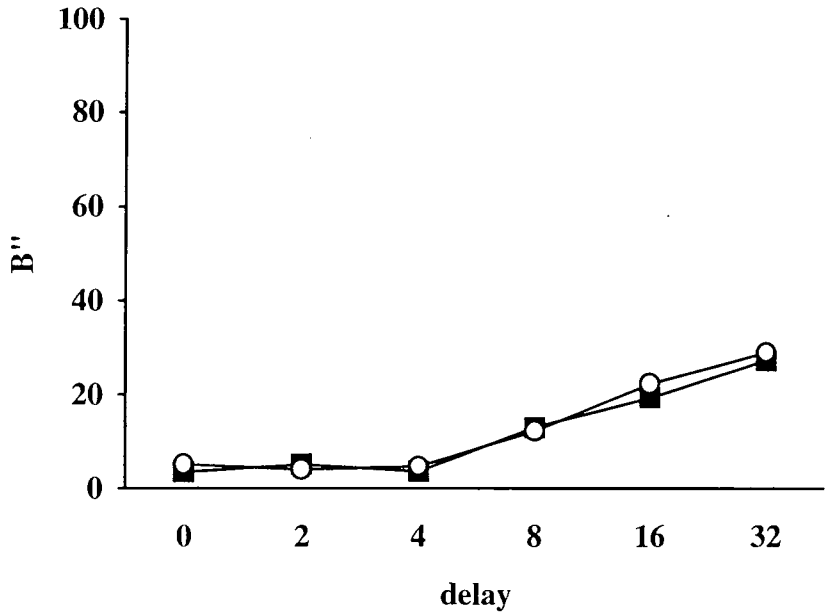


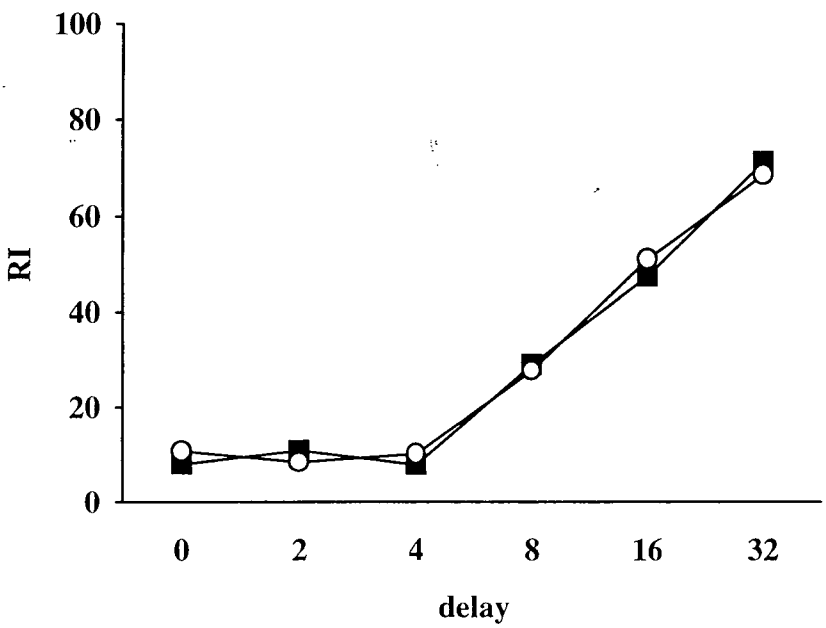


Figure 11. Accuracy (first three graphs) and bias (final three graphs) indices as a function of lesion (Controls vs. MDpre and MDpost combined) for all of the scores from the last five sessions taken together. See text for explanation of indices. All scores have been converted to read from 0 to 100. In the case of the bias scores, 100 represents a complete bias and 0 neutral, or no bias.

2.3.3 Experiment 1b: Spatial discrimination and reversal.

The last part of the study used a spatial (lever) discrimination task with reversals between specified sessions. During the running of this task one of the MDpost animals became ill and its results were excluded from the final analyses.

a) Comparison of the two MD groups: The mean percent correct scores of the two MD groups over all of the 15 sessions were as follows: MDpre = 69.9 (7.7), and MDpost = 62.9 (7.8). The scores of each group were then compared using a t-test (two-tailed) which revealed no significant difference between the two groups ($t,13 = 1.71$, $P>0.05$). They were thus combined prior to comparison with the CON group.

b) Performance over the initial acquisition session: The scores of each group on the first session which reflected the initial acquisition of the task were as follows: CON = 57.8 (11.3) and MD = 55.4 (18.4). A subsequent t-test (two-tailed) revealed no significant difference between the acquisition scores of the two groups ($t,21 = 0.33$, $P>0.05$).

Spatial Discrimination and Reversals.

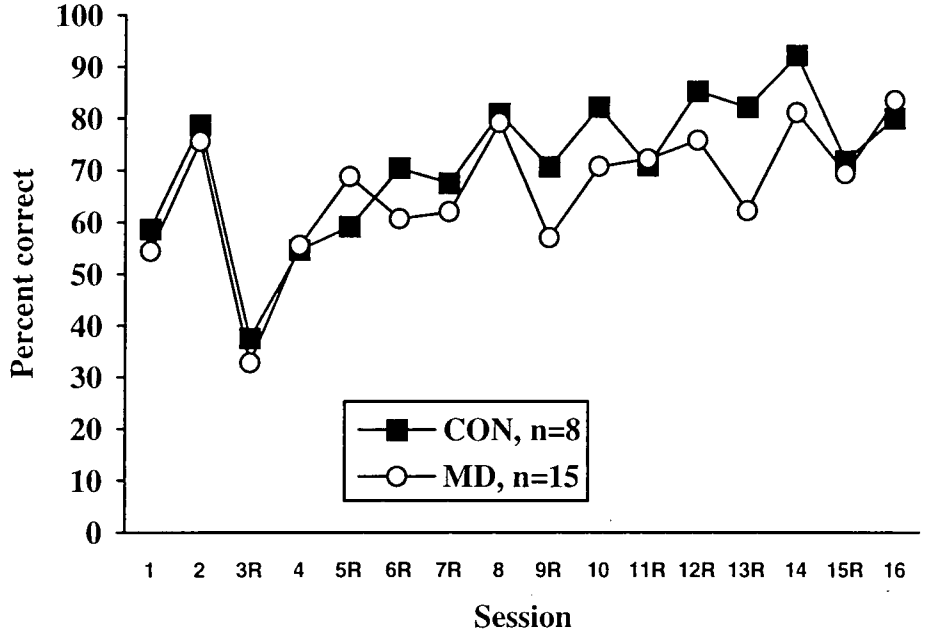


Figure 12. Mean percent correct scores for the two groups on the lever discrimination and reversal task. Sessions where the correct lever changed from the preceding session are labelled as 'R'.

c) Performance on the 'consistent' sessions: Excluding the first session, the scores of each animal could be divided between those six sessions where the correct lever was in the same '*consistent*' position as in the previous session, and between those nine sessions when the lever was in the opposite position i.e. a 'reversal' session. The mean scores for each group on the 'consistent' sessions were as follows: CON = 78.5 (4.9) and MD = 74.5 (7.9).

The scores of each group were then compared using a t-test (two-tailed) which revealed no significant difference between the two groups ($t,22 = 1.3$, $P>0.05$).

d) Performance on the 'reversal' sessions: The mean scores for each group on the 'reversal' sessions were as follows: CON = 67.9 (6.6), and MD = 63.0 (9.3). The scores of each group were then compared using a t-test (two-tailed) which revealed no significant group difference ($t,22 = 1.33$, $P>0.05$).

e) Performance on 'consistent' versus 'reversal' sessions: Subsequent comparisons using a two-way ANOVA on the scores of both groups on the 'consistent' versus 'reversal' sessions revealed no significant group difference ($F\ 1,21 = 2.25$, $P>0.05$) see figure 13. There was a strong effect of the reversal as both groups performed significantly worse on those sessions when the correct arm was 'reversed' ($F\ 1,21 = 57.1$, $P<0.0001$). There was however no evidence of an interaction between lesion and type of session ($F\ 1,21 = 0.04$, $P>0.05$).

Spatial Discrimination and Reversals, 'Consistent' And 'Reversal' Sessions.

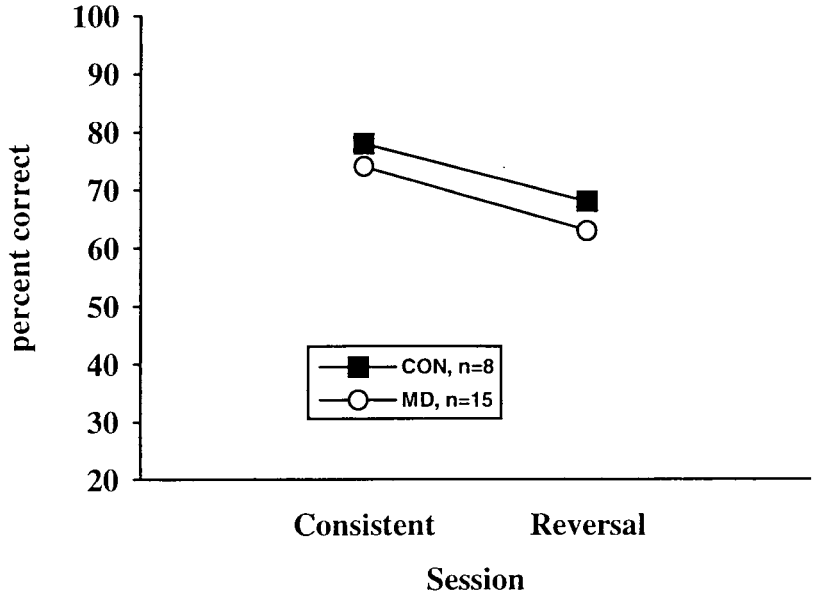


Figure 13. Mean percent correct scores on the lever reversal task grouped according to those days on which the correct lever was 'consistent' with the previous session and those days on which it was a 'reversal' from the previous session.

2.4 DISCUSSION.

Extensive neurotoxic lesions of the medial dorsal (MD) nucleus of the thalamus did not impair either the acquisition, nor the subsequent performance of an automated DNMP task; indeed, following a detailed analysis of performance, the MD group showed slightly superior performances during initial acquisition, and for indices A' and SI over longer delays. Somewhat slower response latencies shown by the MD group during the initial acquisition phase were moderate and not significant. This clear lack of effect of MD lesions on the DNMP task were found whether the lesions were made before or after acquisition of the task.

On a second spatial learning task involving spatial discriminations and their subsequent reversals, a similar lack of effect was noted. These findings are consistent with a number of other studies that have reported little or no evidence that MD lesions in rats produce any impairments on spatial tasks, particularly with regards to spatial alternation (Greene & Naranjo, 1986; Hunt & Aggleton, 1991), spatial reversal (Tigner, 1974), the Morris water task, and the radial arm maze (Béracochéa et al., 1989; Kolb et al., 1982; Olton, 1978).

The inconsistency of the evidence for the involvement of MD in spatial memory is however, marked. For example, a number of studies have reported severe impairments following MD lesions, both for spontaneous and reinforced alternation (Vicedomini et al., 1982; Weiss & Means, 1980), delayed alternation (Winocur, 1985), and for performance on the radial arm maze (Stokes & Best, 1988; 1990a - c). Other reports have revealed less clear cut impairments, for example, in tests of spatial alternation (Brito et al. 1982), spatial reversal (Means et al., 1975) and in a modified version of the radial arm maze (Kessler et al., 1982).

There are a number of possible explanations for the inconsistencies in the literature concerning the effect of MD damage on spatial memory.

2.4.1 Damage to fibre tracts.

The MD lesions described in this experiment were produced by means of a neurotoxin, which, although causing extensive damage to MD, did not cause any noticeable damage to fibres of passage.

A large majority of previous reports did not use neurotoxins and there is a strong possibility that the deficits reported following MD lesioning in such studies, could have reflected additional damage to fibres of passage. Damage to the region of the medial diencephalon is strongly implicated in the memory impairment associated with the amnesic syndrome in both human clinical studies, and in work using experimental animals. The point under contention is that of the relative involvement of the individual diencephalic structures, for example, there being only circumstantial evidence for the proposed roles of MD and the mammillary bodies.

As Markowitsch (1988) has argued: "*...identifying only one or two loci as crucial must face counter evidence in the form either of damage to these loci without severe amnesia, or of severe amnesia with medial diencephalic damage, but excluding these particular loci*".

Markowitsch (1988) further pointed out that no brain structure can act on its own but is dependent upon its afferent and efferent connections with other structures. This interdependency between structures must be regarded as crucially important to this debate, as must the importance of the fibre connections between the structures.

As McEntee et al., (1976) have reported, small lesions in a number of different sites in the diencephalon (the anatomical relationships between which remain unclear), can produce remarkably similar and profound memory impairments. They concluded that it is the interaction between structures which regulates the flow, and the representation of, mnemonic information. This conclusion was echoed by Markowitsch (1988) who stated that a disruption of the diencephalic fibre system may actually have a greater effect than damage to the structures that they connect (the key fibre pathways of the diencephalic region are the mammillothalamic tract (MMT), and the internal medullary lamina (IML))

Experimental evidence for this proposal was provided by Graff-Radford et al., (1990). Their in-depth analysis of four amnesic patients, combined with autoradiographic studies in monkeys, supported the idea that a severe amnesic condition resulted when there was damage to the MMT and also to the ventroamygdalofugal pathway (VAFP). These authors specifically noted that the hippocampal and amygdaloid pathways lay adjacent to each other, anteriorly in the thalamus of the monkey, and in human patients the lesions causing the severest amnesia were located anteriorly, and involved the region where these pathways lay adjacent.

von Cramon et al., (1985) have reported much the same, in an analysis of CAT scans of a total of eleven patients, all having selective vascular lesions of the thalamus, these authors noted that anteriorly located lesions caused the severest amnesia's, whereas posterior lesions (not affecting fibre pathways), had far less severe effects. von Cramon et al., (1985) concluded that: "*The mammillothalamic tract and the ventral portion of the lamina medullaris interna are the most likely candidates in the mediation of the memory process, and a combined lesion of these structures may be responsible for thalamic lesions in man.*"

Similar evidence was presented by Dusoir et al., (1990). Their study of amnesic patient B.J. revealed damage to the mammillary bodies and to the MMT, but importantly, no abnormality was discovered in the body of the thalamus. Finally, MRI studies of patient N.A. have shown a lesion involving both rostral and caudal groups of IML nuclei (Squire et al., 1989a).

Conversely, in a longitudinal study of three patients with both bilateral and unilateral thalamic infarcts, Stuss et al., (1988) reported that the structures suffering the most damage were MD, ventral anterior thalamus, and the MMT. All the patients were severely impaired on a variety of neuropsychological memory tests, and interestingly, the two patients with unilateral infarcts (one left and one right), showed hemispheric asymmetry of memory function. The patient R.M. with a predominant right thalamic lesion was relatively impaired with nonverbal material, while patient I.G. with left thalamic damage had more difficulty with verbal information. Another case study of unilateral damage to MD in which various mnemonic and behavioural impairments were recorded also involved damage to both the IML and the MMT (Sandson et al., 1991).

Animal evidence for the role of diencephalic fibre pathways in amnesia is as yet limited but promising. Mair et al., (1992) for example, have reported that limited lesions of the lateral internal medullary lamina (L-IML) had little effect on the ability of rats to perform a spatial DNMS task, but complete lesions of the L-IML had a severe effect. A similar result was also reported by Mair & Lacourse (1992).

A cautionary note must be made concerning damage to fibres of passage and the possible effects of such damage.

Hunt & Aggleton (1991) compared two groups of animals, one group received conventional radiofrequency lesions, (which typically damage fibres of passage), and the other group received lesions using ibotenic acid (this neurotoxin spares fibres of passage). Both were compared on a spatial (delayed forced-alternation), and a nonspatial (DNMS) test of working memory.

If damage to fibres of passage was crucial then one would expect that the radiofrequency group would have been more impaired than the neurotoxin group. Only one difference between the two groups (on the T-maze massed/delay condition) was reported, the authors concluding that although cellular damage to MD may disrupt learning, some of the impairments on spatial tests may be due to damage to the adjacent anterior thalamic nuclei.

2.4.2 The extent of damage to MD.

It had been reported by Victor et al., (1971) that the amnesic symptoms of Korsakoff's syndrome were consistently associated with lesions encompassing the medial portion of MD that were found to extend beyond the probable site of the boundary with the medial pulvinar. They also noted a relatively high incidence of pathology extending into other adjacent structures including paratenial, medial ventral, and lateral dorsal thalamic nuclei.

Similarly, in monkeys, Isseroff et al., (1982) reported that the effects of medial thalamic lesions on delayed response and delayed alternation tasks depended on the extent of posterior damage to MD, a correlation they related to the failure of small MD lesions to disrupt delayed response performance.

Aggleton and Mishkin (1983a; 1983b) showed that lesions of either the anterior or posterior areas of the medial thalamus alone produced slight impairments, which became greater when the lesions encompassed the entire nucleus. In their first report, the lesions were large, and included all of the midline nuclei in addition to MD, a correspondingly marked behavioural deficit was recorded. In addition, the anterior nucleus of the thalamus was consistently damaged, and all animals sustained damage to the MMT which in turn affected the mammillary bodies. In the second report, the lesions of MD were smaller, and the behavioural deficit was reported to be less severe.

The lesions centred on the magnocellular portion of MD, but the extent of damage to MD was never greater than 50%, and it was always limited to the anterior half of the nucleus.

Rodent studies have yielded a similar set of results. Hunt & Aggleton (1991) reviewed the effects of MD lesions in rats on spatial working memory. Evidence was found of a link between anterior thalamic damage and the presence of an impairment in the MD group. In that report, the lesions of MD were fairly circumscribed, although damage was present in medial portions of the lateral dorsal nuclei (LD), the lesions also consistently damaged the anterior medial nucleus (AM), anterior ventral nucleus (AV), and some of the anterior dorsal (AD) nucleus.

2.4:3 The relationship between MD and prefrontal cortex (PFc).

The failure of MD lesions to affect either the acquisition or subsequent performance of the DNMP task must be considered in the light of the association between MD and the prefrontal cortex. As has been pointed out, the DNMP task shares many demands with tests of delayed alternation and delayed response, tests which have been shown to be highly sensitive to prefrontal cortex damage (Larsen & Divac, 1978; Kolb et al., 1974). Some studies using non-human primates have reported an impairment in both delayed response, and delayed alternation following MD damage (Schulman, 1964; Isseroff et al., 1982), which attest to the close relationship between MD and prefrontal cortex. It must be pointed out however, that such results are not always observed (Aggleton & Mishkin, 1983b; Peters et al., 1956).

In rodent studies using delayed alternation and delayed response, it has often been noted that rats with medial prefrontal damage are poor at such tasks. Of particular relevance to this experiment, is the finding of Dunnett (1990), who reported that rats with aspiration lesions of the medial prefrontal cortex were impaired on an automated DMP task which is almost identical to the DNMP task described in this experiment. Interestingly, the Dunnett study noted two types of deficit, one group of animals showed severe delay dependent deficits, while another group were severely impaired across all delays, neither being found in this experiment.

The finding that damage to the prelimbic region was closely linked with the DMP deficit may however explain why MD damage does not mirror the effects of prefrontal cortex damage on such tasks. The prelimbic cortex receives heavy projections from the hippocampus, as well as from MD (Jay & Witter, 1991; Krettek & Price 1977); and given that the hippocampus is assumed to be vital for normal DNMP and DMP performance (Aggleton et al., 1991a; 1992; von Cramon et al., 1985), it is possible that Dunnett's effects mirror hippocampal, but not MD involvement.

Though an intact prefrontal cortex also appears to be important for the normal performance on this task, precisely which regions are important for spatial working memory, is, as yet unknown. The following chapter will outline in more detail, the potential role of the various regions of prefrontal cortex to learning and memory, and will be followed by direct experimental evidence concerning the role of cingulate and retrosplenial cortices to this, and other tasks.

2.4.4 Comparison of MD lesions with limbic system lesions.

On a final note, the finding that lesions of MD had no effect on performance on the DNMP task can be directly compared with the effects of other limbic lesions on this task. It has repeatedly been reported that lesions of the hippocampus, or the fornix produce severe performance deficits on this spatial task (e.g. Aggleton et al., 1992). The opposite dissociation has, however, been reported for the equivalent non-spatial DNMS task, i.e. while lesions of the hippocampus have little effect (Aggleton et al., 1986), rats with ibotenate lesions of MD were found to be impaired (Hunt & Aggleton, 1991). Such a double dissociation indicates that the contributions of these structures to the mnemonic process are likely to be very different.

CHAPTER 3. THE INVOLVEMENT OF THE FRONTAL CORTICES IN LEARNING AND MEMORY.

3.1 THE FRONTAL LOBES.

Over the years there have been many claims regarding the possible role of the frontal lobes, particularly with regards intellectual functioning. Until quite recently however, there was little evidence that the frontal lobes were involved in memory processing. The fact that patients with frontal lobe damage performed normally on a wide range of memory tasks fostered the idea that they had little or no disorders of memory (Iversen 1983). However, a list of frontal impairments has gradually emerged; typically, such patients show impairments in maze learning (Milner, 1965); poor memory for the temporal ordering of events (Corsi, 1972); failure to show release from proactive interference (Moscovitch & Milner, 1982), and an increase in errors of intrusion and omission (Kolb & Milner, 1981).

While it seems clear that lesions of the frontal lobes do not (as yet currently demonstrated), produce deficits in many standard tests of memory performance; such lesions do produce disturbances in certain memory functions, disturbances which are not incidentally observed in medial temporal patients, but are seen in certain types of amnesic syndrome. Such a dissociation suggests that frontal lobe damage may contribute to amnesic syndromes (Kolb & Whishaw, 1990). It has been further suggested by Warrington & Weiskrantz (1982) that the disconnection of temporal lobe structures from those in the frontal lobes (due to interruption of the fornix - mammillary body pathway) may be responsible for amnesia.

According to von Cramon et al., (1989), learning and memory (in man at least), depends upon the interaction between the associative information areas of the neocortex, and the phylogenetically older, subcortical limbic brain structures. In the rat such links between limbic and diencephalic structures, and a variety of frontal cortical areas have also been demonstrated (Leonard, 1969; Krettek & Price, 1977; Swanson, 1981), these connections being both direct and indirect. Any analysis of the role played by structures such as the hippocampus, or the thalamic nuclei must take into account the inter-connections between these structures and the frontal cortices, as it is becoming clearer that any explanation of learning and memory in both humans and animals is dependent upon the associations between a variety of different subcortical and neocortical regions.

The importance of the frontal lobes in the governing of cognitive and social behaviour has been long recognised, but an integrated view of frontal lobe organisation and function has proved difficult to assess (Goldman-Rakic, 1984a). In humans, the frontal lobe occupies a large proportion of the cortex and is generally described as that portion of the cerebrum lying anterior to the central sulcus. Traditionally, three broad divisions of this region of cortex have been proposed: the primary motor cortex, premotor cortex, and the prefrontal cortex. A full description of the complex inter-connections of the frontal cortex is out of the scope of this thesis which will only focus upon those areas of medial frontal cortex that have been implicated in learning and memory processing.

3.2 PREFRONTAL CORTEX (PFc).

If one uses Rose and Woolsey's (1948) definition of prefrontal cortex as that part of the cortex that receives afferents from the medial dorsal nucleus of the thalamus (MD), then it is possible to identify a region of prefrontal cortex in all mammalian brains. Furthermore, in rodents and primates there is a topographical organisation to these projections such that different cortical subfields can be identified. Although the thalamic projections are not quite as discrete as once supposed, a comparison of the cortico-cortical connections in rodents and primates reveals a close parallel in the general pattern of connections indicating that quantitative comparisons are valid between humans, monkeys and rodents (Kolb, 1990a; 1990b; Uylings & van Eden, 1990), though some authors do not quite agree (for example Preuss, 1995).

A large portion of frontal cortex receives both direct and indirect connections from the limbic system, particularly from the amygdala and hippocampus, via the various thalamic nuclei. Some of these connections include pathways such as the cingulum bundle, fimbria-fornix, stria terminalis, ventral amygdalofugal pathway and the mammillothalamic tract. Structures in the limbic and diencephalic systems are therefore in a unique position to influence the frontal association areas, especially prefrontal cortex, possibly with regards mnemonic processing.

3.2.1 PFc and cognitive functioning.

It has long been known that damage to prefrontal cortex can interfere with the performance of everyday life activities bar the most routine, in particular, prefrontal cortex damage tends to affect decision making, planning, and the application of strategies (Joyce & Robbins, 1991; Shallice & Burgess, 1991).

For example, Eslinger and Damasio (1985) reported the case of a patient, who, following the removal of a large meningioma from orbitofrontal cortex, was severely impaired on all but the most routine organisational skills, while performance on a range of IQ. and neuropsychological tests was above average.

Similar findings have been reported more recently. For example, Owen et al., (1990) assessed 26 patients with frontal lobe damage on a battery of tests of spatial working memory and planning. While they showed no short term memory capacity deficit, they were markedly impaired on a computerised version of the '*Tower of London*' test, a task which requires higher level planning ability. The authors concluded that the frontal lobe patients were impaired on such tasks because of their tendency to use inappropriate organisational strategies.

Similarly, Della Malva et al., (1993) investigated sequencing tasks (tasks which required overriding the selection of familiar, routine associations, when these were inappropriate within the general context of the task) and found that patients with focal frontal lobe lesions encountered difficulties when they were required to efficiently complete a shift to a new and correct alternative response. Finally, Saver and Damasio (1991) reported that damage to the ventromedial frontal cortex produced abnormalities of decision making that were especially marked in social conduct, their patient displayed severe impairments in general decision making, such as deciding which clothes to wear, or in which restaurant to eat.

3.2.2 PFc and memory.

Prefrontal cortex is characterised by granular cells, and is organised into modular units with distinct inputs and outputs which form extensive links between the medial dorsal nucleus of the thalamus, and with the hippocampus and adjacent cortex (Goldman-Rakic, 1984b). It has been hypothesised by a number of authors that such links may be part of the circuitry used for spatial short-term memory (e.g. Goldman-Rakic, 1984b). Experiments on primates that have monitored electrical activity in single neurons in the prefrontal cortex have provided evidence for such a theory.

Goldman-Rakic (1990; 1992) described several such experiments, with the predominant finding being that such recordings taken while the animal performed tasks that depended on specific delayed-response skills, have revealed a range of responses among prefrontal neurons.

Some neurons showed heightened electrical activity when information was presented, others became active during the delay period, when the animal was remembering, and another set activated when the animal began a motor response.

In another task, each monkey was trained to fix its gaze on a small spot in the centre of a screen upon which a visual stimulus appeared briefly in one of several locations. Following a variable delay, the animal had to return its gaze from a central fixation spot to the area where the stimulus initially appeared. Using this task it has been demonstrated that certain prefrontal neurons possess what was termed "*memory fields*" i.e., when a target disappeared from sight, an individual neuron switched into an active state, and remained activated until the end of the delay period, when the animal made a response. A given neuron always appeared to code the same visual location. The neurons capable of retaining the visual and spatial co-ordinates of a stimulus are organised together within a specific area of prefrontal cortex, and, according to Goldman-Rakic (1992), form the basis of a spatial working memory system. Fuster (1990) has also argued that PFc is responsible for bridging the temporal gaps, in what he termed the "*perception-action cycle*" in other words, for mediating cross-temporal contingencies of behaviour

Another important feature of prefrontal cortex is its selective dopamine input, dopamine axons are concentrated in the frontal, anterior temporal, and cingulate cortices of rodents, monkeys, and man. It has been shown for example that dopamine antagonists injected into the prefrontal cortex of rhesus monkeys induced errors and increased latency in performance on an occulomotor task requiring memory-guided saccades (Sawaguchi & Goldman-Rakic, 1991), though such a deficit may reflect some non-specific mnemonic deficit (Dunnett, 1990).

A recent study by Kapur et al., (1994) has provided strong evidence for the involvement of the prefrontal cortex in memory processing. In this experiment, normal human subjects performed different cognitive tasks involving the encoding of verbal stimuli whilst undergoing a PET scan. When the subjects carried out deeper processing operations on the stimuli, increased neural activity (as measured by regional cerebral blood flow) was seen in the left inferior prefrontal cortex. The authors argued that although this region was unlikely to be the storage site for episodic memories, this area was probably part of a complex cortical and subcortical network subserving memory functions.

3.2.3 The effects of PFc damage on learning and memory.

The first evidence that prefrontal lesions might interfere with memory came from Jacobsen's (1936) report of delayed response deficits in chimpanzees with frontal lesions. There is corresponding evidence from both monkeys and rats that prefrontal damage impairs the performance of delayed nonmatching-to-sample (DNMS) tests at successively longer delays (Kolb et al., 1989), the source of the deficit on such tasks has remained controversial. Kolb (1990a) pointed out that a successful performance on such tasks requires three operations. Firstly, sensory information must be received and appropriately processed. Secondly, this information must be held "*on-line*" for a temporal interval until a behaviour is produced or a decision is reached; and thirdly, the appropriate response needs to be made. It is the second operation that has been hypothesised to be dependent upon prefrontal cortex (Goldman-Rakic, 1987).

Prefrontal cortex is made up of several subregions (these will be discussed in more detail in later sections), that are likely to be functionally distinct. Very little work has investigated the effects of discrete lesions in each of these regions, and then compared the subsequent behavioural changes. Nevertheless, it is becoming clearer that some areas of prefrontal cortex are concerned with mediating certain aspects of learning and memory - particularly with regards to spatial memory, and the organising and sequencing of behaviour (Kolb, 1990b). Schacter (1987) reviewed the evidence for frontal lobe lesions and their effects on learning and memory and concluded that the frontal lobes were involved in the spatiotemporal contextual processing of mnemonic information, which is processed in a relatively automatic fashion. This argument has been challenged by Lewis (1989), who questioned the role of the frontal lobes in the spatial context of memory, insisting that the hippocampus was the critical structure.

The possible dissociation between prefrontal cortex and hippocampal functions in rats has been noted in several studies using complex maze learning, and conditional discrimination learning (Winocur & Moscovitch, 1990; Winocur 1991). Using a delayed matching-to-sample (DMS) task, Winocur (1992) found that while rats with damage to prefrontal cortex were impaired at the basic task when there was a minimal delay, those with hippocampal damage were unaffected. The introduction of successively longer delays had no effect on the prefrontal group, but led to a severe impairment in the group with hippocampal damage.

This result, and those from the other studies listed above, were interpreted in terms of a functional dissociation, whereby the hippocampus was regarded as being critically important for episodic memory functions, while the prefrontal cortex was seen as being involved in acquiring skill, and rule-based behaviours.

A similar dissociation has been proposed by Kesner (1993). In a previous experiment Kesner & Holbrook (1987) tested rats with prefrontal lesions for item and order memory for a list of items (spatial locations in a maze). These animals could not remember the sequential presentation of specific spatial locations in the radial maze, indicating an inability to organise temporal information - a deficit noted in human frontal lobe patients (Petrides & Milner, 1982). A later experiment (Kesner, 1993) showed that animals with hippocampal lesions did not show a temporal order deficit, indicating that they could still utilise a temporal order strategy.

3.2.4 The role of the PFc ?

There have been many attempts to develop a theory of the general role of the prefrontal cortex in the control of behaviour in general, and although there are many differences between the theories, they often present a common theme in that many consider that prefrontal cortex is involved in the temporal organisation of behaviour (e.g. Goldman-Rakic, 1987). If this is the case, it seems likely that prefrontal cortex will consist of several systems providing temporal organisation of behaviours related to different types of sensory input, as lesions in different regions of prefrontal cortex can produce deficits in different delay tasks which may require different types of information (Passingham, 1985). One key to understanding prefrontal organisation may be found in the study of cortico-cortical inputs to the various subregions of prefrontal cortex.

A considerable problem with assessing the results of experiments describing the effects of prefrontal cortex lesions is that authors are often vague as to precisely which regions of prefrontal cortex have been damaged. Another problem concerns the type of surgical method used, typically, radiofrequency or aspiration techniques are performed which cause widespread and indiscriminate damage, often to adjacent regions, and/or underlying fibres of passage. As stated earlier, the region of prefrontal cortex consists of several interdependent, and yet independent regions, each of which may contribute to behaviour in different, but as yet, unspecified ways. It is not in the scope of this thesis to focus on prefrontal cortex as a whole, but rather to assess the possible contributions of various subregions, and/or relevant fibre tracts.

3.3 THE ROLE OF MEDIAL FRONTAL CORTEX (MFc) IN THE RAT.

The previous section has outlined the possible role of prefrontal cortex in learning and memory and also outlined potential problems in the analysis of supposed prefrontal deficits. This section will focus upon that part of rodent prefrontal cortex - medial frontal cortex, whose subregions have been directly implicated in some aspects of learning and memory.

Though the area known as medial frontal cortex (MFc) had been defined in primates and carnivores, it was not assumed until the 1960's that an analogous region in rats could be discerned (Kolb, 1990b). The key problem with the designation of frontal regions was that non-primate species have a rather more modest, or even total lack of the frontal granular area. The solution to this problem was to define frontal cortex not by its cytoarchitecture, but by its thalamic afferents.

This followed from Rose & Woolsey's (1948) suggestion that all mammals appear to have some cortical area near the frontal pole that received projections from the medial dorsal nucleus of the thalamus. So, despite several differences of opinion as to whether the various regions of MFc do receive connections from MD (e.g. Krettek & Price, 1977 versus Sarter & Markowitsch, 1983), MFc is typically described as the projection area of MD.

3.3.1 Cytoarchitecture of rodent MFc.

Krettek & Price (1977) have identified several distinct medial frontal cortical regions in the rat:

a). Precentral area: This region encompasses the areas 4 and 6 as designated by Brodmann. The zone is divided into a medial precentral area, which probably corresponds to the frontal eye field of primates; and a lateral precentral area which has been defined as the rats' primary motor cortex (Hall & Lindholm, 1974).

b). Prelimbic rostral area: Three regions can be recognised on the medial cortical surface to the precentral area; the infralimbic area, the prelimbic area, and the anterior cingulate area which correspond to Brodmann's areas 25, 32, and 24 respectively.

c). Orbital area: The cortex forming the ventral aspect of the frontal lobe, divided into 4 sub regions on the basis of their thalamic connectivity; these include the medial orbital, ventral orbital, ventral lateral orbital, and lateral orbital zones.

d). Agranular insular area: This region of rodent cortex includes 3 sub-regions, only two of which are regarded as equivalent to frontal cortical areas of primates. The first is the ventral agranular insular area which forms the dorsal bank of the rhinal fissure caudal to the lateral orbital area; the second is the dorsal agranular insular area lying dorsal to the rhinal sulcus; and the third is the posterior agranular insular area which is the region that is probably not equivalent to any prefrontal area in primates.

3.3.2 Cortical/subcortical connections of rodent MFc.

1. Afferents: Until Leonard (1969) demonstrated that MD projected to the anterior cingulate and prelimbic regions, little was known of projections to the frontal cortex. Since Leonard's study, there have been a variety of reports which have outlined major afferents from the thalamus, basal forebrain, and brainstem (e.g. Divac, 1979). With regards the thalamus, there appears to be a topographical organisation of the projections from MD to MFc that seems to respect the different cortical sub fields, (Divac et al., 1978).

Furthermore, the different subregions of MD have distinctive inputs which presumably influence prefrontal function. Other thalamic inputs to the MFc include the anteromedial nucleus (AM) which projects to anterior cingulate, prelimbic, and retrosplenial, regions (Shibata, 1993a; 1993b; Shibata & Kano, 1993); the anterodorsal (AD) and anteroventral (AV) nuclei which project to the anterior cingulate and retrosplenial regions, and the laterodorsal nucleus (LD) which projects to the retrosplenial region (Horikawa et al., 1988; Sripanidkulchai & Wyss, 1986). These will be discussed in more detail in a later section.

MFc also receives extensive connections from other subcortical areas, thus different regions of the MFc share reciprocal pathways with the substantia nigra, amygdala, hypothalamus, and hippocampus (Krettek & Price, 1977; Divac et al., 1978; Swanson, 1981). In terms of cortico/cortical projections, MFc receives two main projections: the first from sensory regions (predominantly from the visual area); and the second from the posterior parietal region. The connections between MFc and the hippocampal formation are reciprocal, and as yet, their precise nature and functions remain unknown.

Finch (1993) had proposed that they may simply route information into the hippocampus for processing and then back out for use by other structures; or they could represent dynamic loops essential to the mechanisms of information processing. Physiological data has increased our knowledge of the nature of these reciprocal connections in that stimulation of either hippocampus or MFc evokes synaptic responses and long-term potentiation (LTP) in the corresponding region (Finch, 1993; Laroche et al., 1990).

2. Efferents: Knowledge of the efferent connections of rodent frontal cortex is limited, but several studies have revealed that the anterior cingulate, prelimbic and agranular insular cortices all project to posterior cingulate, retrosplenial, entorhinal and presubicular cortical regions; while the orbital and insular areas project to the pyriform cortex (Domesick, 1969). The projections to the entorhinal cortex are particularly important, as they provide a direct and reciprocal link with the hippocampus.

3.3.3. Behavioural effects of MFc lesions in the rat.

Medial frontal damage has been reported to lead to a wide variety of behavioural changes, virtually every species-typical behaviour is disrupted, as is performance on a wide variety of learning tasks. Both sexual and social behaviour are affected, the effects being apparently dependent on whether the damage has affected orbitofrontal or medial frontal regions (Kolb, 1984). Emotional behaviours, as measured by classical conditioning techniques are also affected (Morgan et al., 1993). One possible reason for such effects is the possibility that MFc lesions impair behavioural flexibility, a prime example being that of the poor performance of animals in tests of reversal learning; for example, Divac (1971) reported that MFc lesioned animals learned the correct response as rapidly as controls, but when they had to shift their response, they tended to perseverate on the initially correct solution. Moreover, these animals were often slower to adopt a correct strategy to solve a variety of tasks, but, once adopted, they appeared to learn as rapidly as normal animals.

Shallice (1982) made the important point that MFc damage reliably produces an impairment on "*non-routine*" tasks where information must be constantly monitored for appropriate decision making. Deficits are less likely on "*routine*" tasks where a response strategy can be formed on the basis of predictable relationships. Consistent with this hypothesis are reports showing large effects of MFc damage on tests of conditional learning, and on tests that require behavioural shifts.

Winocur & Moscovitch (1990) compared the effects of MFc lesions with hippocampal lesions, on the ability of rats to learn complex mazes. Both groups were found to be impaired but there were subtle differences in their respective deficits; the hippocampal group showed a sparing of memory for the general skills of maze learning , but were impaired on the maze they had been trained in; the MFc animals showed no impairment of the familiar maze, but failed to transfer this knowledge to running in a similar maze.

With regards to working memory, rats with MFc lesions have been found to be impaired at a variety of delay-type tasks which are sensitive to medial temporal damage; in particular, MFc lesioned animals show deficits on delayed response (Kolb et al., 1974), delayed alternation (Larsen & Divac, 1978; Brito et al., 1982), and DNMS (Kolb et al., 1989). Interestingly, human patients with MFC damage have also demonstrated an impairment in delayed alternation and delayed response tasks, and an increase in perseverative responses (Freedman and Oscar-Berman, 1986).

Rats with MFc lesions are also impaired on the acquisition of the majority of spatial tasks including the radial arm maze (Becker et al., 1980; Kesner, 1989), and the Morris water task (Kolb et al., 1983). In this latter example, the authors compared animals with lesions of either MFc or MD on the acquisition of the Morris task and the 8-arm radial maze. The MFc group failed to learn to swim from different locations to the hidden platform in the Morris task, and were also impaired at learning the location of the rewards in the radial maze.

It is tempting to compare such results to the similar failure of hippocampectomized animals in such tasks (O'Keefe & Nadel, 1978). However, animals with MFc lesions, although demonstrating acquisition impairments, do not show corresponding deficits in the retention of such tasks. For example, Sutherland (1985) reported that rats trained on the Morris task pre-operatively were indistinguishable from normal controls during post-operative testing, thus implying that MFc may be crucial to the initial learning process, but not so vital once learning is complete.

3.4 CINGULATE CORTEX.

Cingulate cortex forms a ring or cingulum around much of the corpus callosum, and its defining anatomical features are a prominent deep layer of pyramidal neurons and input from the anterior nuclei of the thalamus.

The cingulate gyrus is regarded as a major part of the '*anatomical limbic system*' as it is a principal component of Broca's "*grand lobe limbique*", and holds a prominent position in Papez's description of the circuitry involved in emotion and affect. The cingulate gyrus is not supplied by an independent arterial system, rather the anterior cerebral artery supplies most of cingulate cortex, as well as adjacent frontal and parietal cortices. A consequence of this, is that infarcts of a single branch of this system can cause extensive damage beyond cingulate cortex and produce complex neurological deficits (Vogt, 1993). Classically, cingulate cortex has been considered to be involved primarily in emotion but recent findings have focused on this regions' role in sensory, motor, and cognitive processes.

Anatomical studies have revealed that the major inputs to the cingulate gyrus are from the anterior and midline nuclei of the thalamus (Shibata, 1993a, 1993b), the basal forebrain, particularly the septum and diagonal band of Broca (Gaykema et al., 1990, Swanson & Cowan, 1979; Saper, 1984) and the cerebral cortex (Vogt et al., 1979); the cingulate gyrus is believed to send efferent projections back to the anterior and dorsal thalamic nuclei, as well as to specific regions of the limbic cortex, particularly the subiculum, perirhinal and entorhinal cortices (Pandya et al., 1981; Shibata, 1993a; 1993b).

These results suggest that the cingulate gyrus is not only a key element in limbic system circuitry, but also that it may mediate widespread limbic system influences on the cerebral cortex (Sutherland et al., 1988). From results showing that each subdivision of the anterior thalamus projects to a distinct region of retrohippocampal cortex via cingulate cortex, Shibata (1993b) has proposed that each of these projections and interconnections may have a modulatory effect upon the activity of those retrohippocampal neurons concerned with learning and memory.

Cingulate cortex in the rat can nominally be divided into two areas, both of which have rather different inputs, and project to different target structures. Vogt (1993) and Vogt et al., (1981) described these two regions as anterior cingulate (occupying area 24), and posterior cingulate (occupying area 29). According to these reports, the essential cytoarchitectural differences between anterior and posterior divisions of cingulate cortex are that the anterior areas lack a layer IV and are characterised as agranular, whereas the posterior region has the granular layers II - IV, Moreover, both regions receive different thalamic and cortical afferents.

With regard to monkeys, Vogt et al., (1979) argued that anterior and posterior cingulate cortices are functionally distinct regions of the limbic system, and noted that cingulate cortex is cytoarchitecturally heterogeneous, in that anterior and posterior parts have different thalamic and cortical connections. This finding in monkeys appears to be echoed in rats (Zeng & Stuesse, 1991).

3.4.1 Area 25: Infralimbic cortex (ILc).

The rodent infralimbic cortex (ILc) is the most ventral portion of the MFc, and its position along the medial bank of the cerebral hemisphere opposite the visceral sensory cortex has led some authors to propose that this region may function as a "*visceral motor cortex*" (Hurley-Guis & Neafsy, 1986). Some authors have, however, been divided as to whether ILc should be included under the term MFc. Krettek & Price (1977) argued that this region does not receive projections from MD, while Sarter & Markowitsch (1984) presented evidence that it did receive MD projections.

A recent study by Freedman & Cassell (1991) has cleared up much of the confusion. These authors defined the thalamic afferents of this area using a retrograde fluorescent labelling technique. They found that injections into the ILc resulted in labelling in the mediodorsal, intralaminar, and midline thalamic nuclei, which, if correct, indicated strongly that ILc should be classified under the heading MFc. Indeed, the evidence for a reciprocal connection from ILc to MD has been fairly consistent, for example, Groenewegen (1988) noted that injections of horseradish peroxidase in MD produced retrograde labelling in ILc. (Recent reviews of cingulate cortical function e.g. Vogt et al., (1992) have included ILc as part of anterior cingulate cortex).

More recent studies (Takagishi & Chiba, 1991; Hurley et al., 1991), have focused upon the efferent connections of ILc using anterograde tracing methods. They found that axons exit ILc in one of three efferent pathways. The dorsal pathway innervates prelimbic and anterior cingulate cortices, the lateral pathway courses through the nucleus accumbens to innervate the insular, perirhinal and piriform cortices; and the ventral pathway innervates the thalamus (particularly paraventricular and medial dorsal nuclei), hypothalamus, and amygdala. In addition to receiving reciprocal projections from MD, ILc also has other properties that characterise both rodent and primate MFc, namely that it receives strong projections from the basolateral amygdala (Krettek & Price, 1977), and secondly, the concentration of dopaminergic fibres in ILc is indistinguishable from that in the more dorsally situated prelimbic cortex (van Eden et al., 1987).

3.4.2 Area 24: Anterior cingulate cortex (ACc).

The anterior cingulate cortex (ACc) has several anatomical definitions, Brodmann referred to it as '*area 24*'; Krettek and Price (1977) identified dorsal and ventral areas which a modified Brodmann's scheme referred to as '*areas 24a and 24b*'; whereas Zeng and Stuesse (1993) referred to these dorsal and ventral aspects as '*CG1-CG3*". (Throughout this thesis, the ACc will be referred to as area 24.) This region in both monkeys and rats, receives thalamic afferents mainly from midline and intralaminar nuclei (Vogt et al., 1979, Horikawa et al., 1988) and has been implicated in numerous functions some of which include emotion, pain, maternal behaviour, motor control, and attention (Vogt et al., 1992; Neafsey et al., 1993). Some evidence concerning the proposed functions of ACc are outlined below

a). Emotion: With regards human studies, infarction of the anterior cerebral artery is usually associated with large lesions of ACc and adjacent frontal cortex. Laplane et al., (1981) reported a case in which no motor deficits were recorded, but the patient displayed a confabulatory amnesic syndrome, indifference, docility, and a severe lack of attention. These symptoms followed an infarction of both anterior cerebral arteries, which destroyed the rostral part of the anterior cingulate gyrus, small areas of the adjacent medial frontal cortex, and underlying white matter. Importantly though, there was corresponding bilateral damage to the fornices, and it is possible that this damage had the greater impact in terms of the learning deficit. In another case study, Angelini et al., (1980) noted that a tumour in the right anterior cingulate cortex resulted in the patient displaying serious behavioural abnormalities including lack of social restraint, heightened sexuality, eating disorders, and aggressiveness. No changes in intelligence or memory were noted.

Specific localised damage to ACc is rare, but surgical procedures involving such lesioning were carried out as a measure against obsessional states, severe aggression, intractable pain, and epilepsy in the 1950's and 1960's. Whitty & Lewin (1960) described several such cases and revealed that anterior cingulectomies (involving removal of much of area 24 and the underlying white matter), sometimes appeared to produce a marked improvement in obsessive behaviours, aggression, and anxiety.

Interestingly, such surgeries also often resulted in transient memory impairments, similar to those seen in Korsakoff patients. With the fairly limited surgical techniques of the time, however, the authors admit that there was a strong likelihood of damage to other regions of the frontal lobes.

Ballantine et al., (1987) reviewed 198 psychiatrically disabled patients who were treated with small ablations of the cingulum bundle and found that patients with the major affective disorders were greatly helped by the procedure. A recent report (Benes, 1993) has indicated that the pathophysiology of schizophrenia may involved discrete alterations of intrinsic circuits within MFc, she noted that post-mortem studies of the anterior cingulate cortices of schizophrenic patients were characterised by a decrease in neuronal density, and a decrease in the size of neurons, particularly in layer II.

Electrical stimulation studies in humans have been carried out in epileptic patients and by far the most consistent result of stimulation was a "*reaction of wakefulness*", consisting of a body posture and facial expression suggestive of heightened attention, but in the absence of a stimulus to focus upon. In addition, many subjects appeared to experience affective changes and behaved in a way that suggested anguish, or even fear; more often though, the subjects appeared jovial and reported pleasant reactions (Talairach et al., 1973). In experimental animals, several studies have reported that both lesioning, and electrical stimulation of ACc can affect emotional states. For example, Ward (1948) reported that ACc lesions in monkeys reduced aggression and fear, and also produced inappropriate social behaviours. Such changes in social behaviours may however, be only transient (Mirsky et al., 1957). The cingulate gyrus was first stimulated in monkeys by Smith (1945) who reported a variety of emotional and autonomic behaviours being produced as a result of such stimulation.

b). Pain: There is a nociceptive region in ACc, which may be involved in affective responses to noxious stimuli. Ablations of the rostral cingulum bundle reduce responsivity to noxious stimuli, and abolish pain in chronically ill patients; patients with such lesions of the cingulum bundle frequently report that they can still localise the source of a noxious stimulus but no longer mind it (Ballantine et al., 1975). In animals, the contribution of AC to avoidance learning is well established, it is assumed that ACc is involved in the animals' ability to predict and/or avoid noxious stimuli, since the unconditional stimulus is aversive foot shock (Gabriel et al., 1991).

c). Attention: Studies that have utilised positron emission tomography (PET) have demonstrated that ACc exhibits differentially high metabolic activity in subjects performing cognitively demanding stimulus-response tasks. For example, when normal human subjects respond to a noun by producing an associated verb, as opposed to simply repeating of the noun (Petersen et al., 1988), between the congruent and incongruent versions of the Stroop colour / word task (Pardo et al., 1990), or whilst performing subspan / supraspan auditory-verbal memory tasks (Grasby et al., 1993).

The contribution of ACc to attentional processing is further enhanced by reports of neglect following anterior cingulate damage (Watson et al., 1973).

d). Visceromotor functions: Emotional activity can be generally associated with autonomic outflow; alterations in autonomic state serve a social function in communicating emotion and in preparing the organism for '*fight*', '*flight*', sexual, or eating behaviours. Neafsey et al., (1993) reviewed the roles of areas 24 and 25 in visceromotor activity, and conclude that area 25 with its direct projections to the parasympathetic nucleus of the solitary tract, and dorsal motor nucleus of the vagus, is the key region, but both are involved in, for example, autonomic adjustments associated with classical conditioning (Gibbs & Powell, 1991).

e). Skeletomotor functions: Electrical stimulation of ACc evokes skeletomotor responses, and lesions of ACc disrupt motor function, particularly with regards vocalisation, for example, vocalisation in monkeys is usually evoked by stimulation around the rostrum of the corpus callosum (Smith, 1945). The relevance of these vocalisations to emotional expression is emphasised by reports revealing that the separation cry in infant monkeys is impaired by lesions of rostral ACc (MacLean & Newman, 1988).

A recent report by Paus et al., (1993) focused on the role of the anterior cingulate region in the control of occulomotor, manual and speech responses. PET scans of normal human subjects performing a variety of tasks, revealed specific changes in regional cerebral blood flow in certain subregions of ACc, depending on the type of response used. They noted that ACc became particularly activated when the subject was forced to choose from a set of competing responses rather than relying on well-established stimulus-response associations.

With regards emotion, the ACc must be seen in the light of its connections with the amygdala. According to Vogt et al., (1992), the amygdala plays a role in the evaluation of the motivational significance of sensory stimuli, in particular, it contributes to conditioned fear. Rostral parts of ACc receive inputs from the basal and laterobasal nuclei of the amygdala (Vogt & Pandya, 1987), while posterior cingulate cortex does not. It is likely that the amygdala and ACc operate in tandem to produce affective behaviours, i.e. signals arising in the amygdala may contribute to the motivational drive underlying processes carried out by the ACc.

Vogt et al., (1992) have considered these properties in the light of effector, or executive functions and propose that ACc is primarily concerned with controlling output, whether to Visceromotor, endocrine, or skeletomotor systems. Emotional states are of course closely related to effector processes as each emotion achieves expression through autonomic, endocrine, and skeletomotor output; and also in that most emotions involve a tendency to act.

3.4.3 Area 32: Prelimbic cortex (PLc).

Due to its close proximity, this region is often described as being part of anterior cingulate cortex, yet the two regions (although connected) have been shown to be anatomically different. Zeng & Stuesse (1991; 1993) compared the connections within these two regions of cingulate cortex and found that cortical connections were more widespread for PLc (they referred to it as Cg3). They concluded that the ACc (consisting of anterior cingulate and infralimbic cortices and referred to as Cg1/Cg2) was more closely allied with somatic motor control, whilst PLc projected more to visceral motor area, and to parts of the limbic system. In short, cingulate cortex, although closely connected and having a few common projections, was nevertheless anatomically heterogeneous.

Other neuroanatomical and electrophysiological data have indicated that prelimbic cortex (PLc) has extensive direct connections with the hippocampal formation, for example, Ferino et al., (1987), showed that following microinjection of horseradish peroxide into MFc, labelled cells were observed in the pyramidal layer of the CA1 sub field of Ammon's Horn. Jay et al., (1989) have shown that such projections, which originate in the temporal part of the CA1 sub field and in the parasubiculum, are restricted to the PLc region. Furthermore, PLc has dense projections to the medial region of the lateral septum, which in turn connects via the medial septum, to the hippocampus (Swanson, 1981).

The PLc does not only share connections with the hippocampus, it also shares reciprocal connections with the thalamus - notably with MD (Krettek & Price, 1977). Condé et al., (1990) focused upon the thalamic afferents of this region and found that the PLc could be distinguished from the medial precentral (PrCm) and dorsal anterior cingulate (ACd) regions because it received afferents from a large number of neurons from both medial and lateral MD, whereas only a few neurons from lateral MD projected to the other regions.

Moreover, the paratenial and paraventricular thalamic nuclei project exclusively to PLc, while the central midline nucleus projects mostly to PLc. From this anatomical evidence PLc must surely be regarded as being a functional part of the rodent medial frontal cortex, and for the purpose of this study, it will be defined as such.

Due to the close anatomical relationship between PLc and the septohippocampal system, it would be expected that lesions in these structures would have similar functional consequences. For example, lesions of the posterior dorsal septal area impairs the performance on T-maze alternation in rats (Thomas, 1978), as does lesions of the prelimbic sector of MFc (Brito et al., 1982; Thomas & Brito, 1980). Both sets of authors have argued that PLc is the critical region of MFc that is involved in T-maze alternation. An interesting finding emerging from such studies is the lack of correspondence between lesions in PLc and MD. As stated earlier, PLc has extensive anatomical connections with MD which indicates that the two structures might share similar functional attributes. Brito et al., (1982) showed that MD was not critical for the retention of delayed alternation, but argued that PLc was. Such findings do not support the hypothesis put foreword by Kolb (1977) who suggested that MD and PFc constituted an interdependent functional system in the organisation of behaviour.

In a large study utilising a battery of 14 neuropsychological tests, Brito & Brito (1990) compared the performance of rats with either lesions of the septohippocampal system, or with lesions of PLc. A variety of deficits were recorded for both lesion sites, for example, septal lesioned animals performed worse at T-maze alternation and DNMS than did the PLc lesioned group. The PLc lesioned animals however, were severely impaired at contingently reinforced alternation at long inter-trial intervals, DNMS at long inter-run intervals, and successive tactile discriminations; and transiently impaired at T-maze alternation at short inter-trial intervals. The authors argued that such data were consistent with the hypothesis that PLc is involved in working, but not reference memory processes. The results further suggested that there is a hierarchy such that the septohippocampal system plays a larger role in working memory processes than does the PLc.

3.4.4 Area 23,29,30 and 31: Posterior cingulate cortex (PCc).

A great many studies have failed to implicate posterior cingulate cortex (PCc) in either emotional, visceromotor, or other function commonly ascribed to the ACc. Instead, much of the evidence concerning the possible functions of PCc suggests that this region is involved in assessing the environment and in spatial memory (Vogt et al., 1992).

It must be pointed out however that there are likely to be cross-species differences regarding possible roles of PCc; for instance, rodents lack an area 23 and instead possess an area 29 which is much larger than in monkey or human and which essentially covers PCc. This report will first present a general assessment of PCc across the species, and will then focus more closely on the retrosplenial cortex (RSc) of the rodent, with particular reference to spatial memory.

3.4.5 Possible functions of PCc.

a). Eye movements: In several species, the electrical activity of PCc is modulated during eye movements, neurons in the visuospatial area undergoing shifts of firing frequency at or after the time of the corresponding eye movement, thus suggesting that the PCc is involved in monitoring, rather than controlling movement (Olson et al., 1993).

b). Vision: PCc neurons which are responsive to visual stimuli have been encountered in all species that have so far been tested. In the monkey, it has been reported that the physical characteristics of the stimulus are a key determinant of response level, for example, small spots presented onto a screen in front of the animal do not elicit neuronal responses, whereas large, textured stimuli do (Olson et al., 1993). This is interpreted by Vogt et al., (1992) in the light of PCc neurons responding to visual stimuli to the degree to which they are visually salient.

c). Somatic function: One part of PCc (area 23c), contains neurons that project to the spinal cord and appear to be activated in conjunction with hand movements (Shima et al., 1991), Vogt et al., (1992) argued that this region is involved in assessing the state of the skeletomotor system.

d). Spatial orientation and memory: A variety of studies have indicated that PCc is involved in the orientation of the animal in its environment, and in spatial working memory. For example, Chen et al., (1991) have reported that single neurons in rodent PCc are sensitive to the angle of the animals' body in relation to the environment, and to bodily movements. Other findings relating to the possible spatial learning role of the medial frontal cortex have been reported by Sutherland et al., (1988). They examined spatial learning in rats with either anterior cingulate, posterior cingulate, or total cingulate lesions. Using the Morris water task, they found that the animals with total cingulate, or posterior cingulate lesions alone, failed to learn the task, or show any significant improvement.

In contrast, the animals with only anterior cingulate damage showed a less severe impairment, and with only slightly more training than control animals, acquired near normal place - navigation accuracy. These results implied that the posterior cingulate cortex may play a role in the use of topographical information, while the role of the cingulate cortex remained rather unclear.

3.4.6 Area 29: Rodent retrosplenial cortex (RSc).

The rodent retrosplenial cortex (RSc) is adjacent to the splenium of the corpus callosum, and includes one division of dysgranular, and three divisions of granular cortex, referred to as areas 29a-29c (Vogt 1993). These regions receive heavy projections predominantly from anteroventral, and anterodorsal thalamic nuclei, and lighter connections from anteromedial, lateroposterior and laterodorsal thalamic nuclei (Horikawa et al., 1988; Sripanidkulchai & Wyss, 1986; Tengelsen et al., 1992). These regions also receive cholinergic projections from the basal forebrain, principally from the septum, nucleus basalis magnocellularis (NMB) and the diagonal band of Broca (Bigl et al., 1982; Gaykema et al., 1990; Saper, 1984). Finally, they receive extensive cortical inputs from the visual cortex, particularly from 18b, and with the motor cortex - area 8 (Vogt and Miller, 1983). Finally, RSc shares reciprocal connections with the stria terminalis, parasubiculum, presubiculum, and with anterior cingulate cortex (Zilles & Wree, 1985; Meibach & Siegel, 1977).

More recent reports have further revealed the extent of the reciprocal connections between RSc and structures in the medial temporal lobe. Wyss & van Groen (1992) found that both retrosplenial granular, and agranular (Rgb / Rga) cortex receive dense projections from the hippocampal formation which are topographically organised. They suggested that these elaborate connections implied a close link between the two regions, especially with regards to memory processing. A further finding (van Groen & Wyss, 1992), revealed that retrosplenial dysgranular cortex in particular shared reciprocal connection with the thalamus, the hippocampus and neocortex.

The fibres connecting RSc with hippocampus and thalamus are carried in the cingulum bundle, damage to which would severely interrupt the alternative pathway from hippocampus to anterior thalamus and also would interrupt information carried from anterior and lateral dorsal thalamic nuclei to RSc and the medial temporal lobe. The suggestion that RSc serves to integrate information from a variety of cortical and sub cortical structures, each associated in some way with the possible processing and / or storage of memories seems a plausible one. Hence, the potential role of retrosplenial cortex in at least some aspect of mnemonic processing must be regarded as likely.

3.4.7 RSc and memory in human case studies.

Human data regarding the possible role of retrosplenial cortex in an amnesic syndrome is scarce. One case study was reported by Valenstein et al., (1987). Their patient, T.R., developed both retrograde and anterograde amnesia comparable to that seen in medial temporal and diencephalic cases, following a haemorrhage situated near the splenium of the corpus callosum. Neuroradiological studies revealed no damage to the hippocampus, thalamus, basal ganglia, or frontal cortex, but the cingulum bundle and retrosplenial cortex were affected. The authors argued that this damage had served to disconnect the hippocampus from the thalamus; specifically, they proposed that such damage would severely disrupt the posterior cingulum which serves to connect the hippocampus, and parts of the thalamus, with the frontal lobes.

More recently, Bowers et al., (1988) re-examined patient T.R. using three memory tasks assessing his ability to judge when a previously learned event had occurred. T.R. was severely impaired in acquiring temporal information about new stimuli, and his defect could not be attributed to a failure of recognition memory, or due to frontal lobe dysfunction. The authors suggested that the pattern of T.R.'s impairment revealed a specific deficit in "*time-tagging*" new incoming information.

Some recent experiments using PET scanning have focused upon this region in normal subjects whilst they performed memory related tasks. In one such study by Grasby et al., (1993) the authors measured regional cerebral blood flow while the subjects performed two auditory - verbal memory tasks. Increases in blood flow were recorded in retrosplenial cortex, as well as in both prefrontal and anterior cingulate cortices.

3.4.8 RSc and memory in experimental animal studies.

In non-human primates, physiological studies have indicated that retrosplenial cortex is involved in the circuitry underlying the memory process. For example, Matsunami et al., (1989) investigated the activity of retrosplenial cortex using radioactive 2-deoxyglucose (2-DG) while the monkey was performing a visual tracking task utilising a delay component. A large increase in 2 - DG uptake in retrosplenial cortex was recorded, as was a corresponding increase in structures such as the hippocampus and MD. This finding was echoed by Sawaguchi & Goldman-Rakic, (1991), they reported that injections of a dopaminergic agonist into the prefrontal cortex during an occulomotor task requiring memory guided saccades, induced errors and increased performance latency.

A variety of studies have investigated the possible role of RSc in learning and memory, using monkeys and rodents, with the results often being contradictory. For example, Murray et al., (1989) compared the performance of monkeys with hippocampal system damage and with complete lesions of cingulate cortex on a specially designed T-maze which shared the same features of the T-maze commonly used with rodents. In the second experiment, spatial position was used as a conditional cue to indicate which of two objects the animal should displace. In the spatial working-memory task (T-maze), monkeys with fornix damage were severely impaired, but the group with complete cingulate cortex lesions were only moderately impaired. In the conditional tasks, neither fornix, nor cingulate groups were impaired.

In a parallel experiment, Markowska et al., (1989) compared the relative roles of fornix and cingulate cortex in rats using the same tasks as in the alternate experiment using monkeys. In rodents, the equivalent lesions and tests led to severe impairments in the T-maze task, but again, no impairment in the conditional spatial discriminations. Though the cingulate monkey group were not severely impaired on the T-maze task, they were impaired to some extent, such differences between rats and monkeys may reflect the degree of cingulate cortical damage. In any case, the dissociation between performance on T-maze and spatial conditional discriminations suggests that the hippocampal system and its connections via the fornix are involved to some degree in spatial memory, and that conditional discriminations do not require this type of memory.

A recent review of posterior cingulate cortex by Sutherland & Hoesing (1993) has focused primarily on the role of area 29 with particular regards to spatial memory in rats. The authors described their own series of experiments mainly comparing hippocampectomized rats with animals having bilateral lesions of RSc on the Morris water task. Typically, both groups differed significantly from controls in escape latency, and all other measures of performance, but were strikingly similar to one another. Similar results were obtained for acquisition of the task, and interestingly, the performance of rats with damage to ACc was superior to that of rats with combined damage, or damage to RSc alone. Even if rats received extensive pre-training prior to RSc lesioning, they still showed a clear navigational deficit. The authors proposed that RSc (in the rodent at least), was part of the essential circuitry interfacing a spatial mapping system with perceptual systems and movement.

3.5 SUMMARY.

The evidence outlined in the sections above strongly implies that ACc is involved in orientation within, and interpretation of, the environment, whilst the functions of posterior cingulate cortex (particularly the retrosplenial region) cover the monitoring of visual events, spatial orientation and memory. Both regions are anatomically heterogeneous, yet share many connections with other cortical and thalamic sites, as well as with the hippocampal system. They are also closely inter-linked together.

Several researchers have argued that the functions of these regions are likely to be integrated and co-ordinated. For example, Vogt et al., (1992), have referred to the ACc as being the "*executive region*", and the PCc as being the "*evaluative region*", concluding that output functions of the ACc executive region may be directed by activity in the evaluative area of PCc.

The subtle differences in connectivity between the two regions points to a similar conclusion, in a simplified sense, one can note that the ACc shares its principle connections with the amygdala (crucial for motivation and emotion), whilst PCc/RSc are connected principally with the hippocampal formation (crucial for spatial orientation and memory). Both regions have extensive thalamic links, though, once more, there are subtle differences, with ACc receiving its principal thalamic connection from the anteromedial nucleus (AM) with lighter connections from the anterodorsal (AD) and anteroventral (AV) nuclei; whilst RSc is principally connected with the AD, AV and laterodorsal nuclei (LD) nuclei. The differing contributions of the thalamic nuclei to learning and memory is not yet clear, yet will presumably reveal certain differences.

3.5.1 Damage to fibres of passage ?

The possibility must be considered that the deficit found following lesioning of RSc may not be due to damage to that site alone, but is the result of damage to fibres of passage - i.e. to the cingulum bundle. Previous studies (e.g. Meunier & Destrade, 1988), had discovered that deficits following RSc damage in mice, did not occur when fibres of passage were spared following a cytotoxic lesion. Sutherland & Hoesing (1993) further addressed this point in an experiment in which RSc was completely aspirated in one hemisphere, while the other hemisphere either suffered no damage, or the lesioning was carried out using a cytotoxin. The authors argued that if damage to fibres of passage was the cause of the deficits previously reported, then receiving cytotoxic lesions should be similar to that of the animals with unilateral aspiration lesions.

If, on the other hand, the effects were attributable to damage to RSc neurons alone, then the performance of rats with cytotoxic lesions should be similar to that of rats with bilateral aspiration lesions. Unilateral RSc damage caused little disruption in the acquisition of the Morris task, and the cytotoxin lesion group performed in a comparable manner to the bilateral aspiration group, indicating that RSc neurons and not fibres of passage made a significant contribution to the learning of the task.

3.5.2 The cingulum bundle (CB).

The key fibre pathway of the cingulate gyrus is referred to as the '*cingulum bundle*' which has generally been described as the major association pathway of the cingulate cortex (Domesick, 1970). Both short fibres which serve to connect adjacent areas of cingulate cortex, and longer fibres connecting cingulate cortex to the hippocampal region, have been described (Cajal, 1955; White, 1959). Domesick (1970) examined the degeneration in this tract following variously placed lesions in the anterior thalamus to discover precisely which parts of the bundle enclose thalamocortical fibres; the study revealed that the vast majority of the bundle's fibres were of thalamic origin. More recently, several authors have outlined in detail the organisation, course and composition of the cingulum bundle in the rhesus monkey (Mufson & Pandya, 1984), and the rat (Wouterloud et al., 1990; Kristt, 1991; Shibata, 1993a; 1993b). The important findings from these reports can be summarised as below, showing basically that the bundle consists of three major fibre components :

1. Thalamic: The major fibre connection with the cingulum bundle is from the thalamus with fibres from the anterior nuclei (AM, AIM, AV), and lateral dorsal (LD) nuclei extending to frontal, and retrosplenial cortices, and to the parahippocampal gyrus and presubiculum. Furthermore, fibres carried by the cingulum bundle travelling from the reuniens nucleus of the thalamus (NRT) exclusively innervate field CA1 of the hippocampus, the dorsal part of the subiculum, and both pre- and parasubiculum.

2. Cingulate gyrus: Fibres from the anterior cingulate region are directed to both premotor and prefrontal regions as well as to posterior cingulate and retrosplenial cortices. Fibres from posterior cingulate cortex extend to the PFc and also to the presubiculum, and parahippocampal gyrus.

3. Association cortex: An association component arises from PFc and the posterior parietal region; cingulum bundle fibres from PFc extend to the retrosplenial cortex, while those from the posterior parietal region extend caudally to the parahippocampal gyrus and presubiculum, and also to the PFc.

Although these three fibre systems intermingle in the cingulum bundle, they each exhibit a distinct topography and presumably are independent. Such findings attest to the complexity of the cingulum bundle, and also emphasise that any procedure disrupting the bundle must necessarily lead to extensive disconnection's among a variety of cortical, subcortical, and limbic structures. The results of Saper (1984) showing that axons from the nucleus basalis magnocellularis (NBM) and septal region also pass into cingulate and retrosplenial cortices via the cingulum bundle, adds a further complication, as cholinergic mechanisms have been implicated in certain forms of learning and memory (Dunnett, 1990; Dunnett et al., 1988; 1990b; Marston et al., 1993). With regards to this thesis, it can be hypothesised that damage to the cingulum bundle may lead to a disruption of mnemonic processing, with particular reference to the cingulate, retrosplenial and limbic connections.

3.5.3 Behavioural effects of CB damage in humans.

It could be assumed that disruption of the cingulum bundle would disrupt not only direct thalamic connections with the hippocampus, but would also disrupt thalamic and hippocampal links with cingulate cortex, and links between anterior and retrosplenial cortices. Despite the potentially large effects on learning and memory of direct cingulum bundle damage, evidence concerning specific damage to the cingulum bundle and its resulting effects are indirect, though promising.

Bilateral lesions of the cingulum bundle have been termed '*cingulumotomies*" and have been reported to be effective in the treatment of various neuropsychiatric disorders (Foltz and White, 1962; Santo et al., 1990). Further evidence has come from reports of '*cingulotomies*' in which part of ACc and the cingulum bundle are removed. Neuropsychiatric disorders such as obsessive-compulsive psychosis, personality and aggressive disorders, depressive syndromes and self destructive behaviours, have been '*treated*' by such surgeries with variable results (Devinsky & Luciano, 1993; Robertson et al., 1990). In a review of such studies, González et al., (1980) however, concluded that cingulotomies and cingulumotomies had little therapeutic value and often appeared to cause affective disorders, whilst having only a transient effect on pain relief.

The evidence for any effect of such surgeries on learning and memory is promising but is heavily obscured by other anatomical damage and is often contradictory. For example, Fedio & Ommaya (1970) reported that large bilateral lesions of the cingulum bundle did not result in any obvious memory deficit.

However, in a later report, Ommaya & Fedio (1972) studied immediate memory for verbal material in neurosurgical patients undergoing electrical stimulation of left and right cingulum, and the hippocampus. A severe memory disorder for verbal memoranda accompanied left, but not right, cingulum stimulation; the converse was true when non-verbal recognition was tested.

In another study, Angelini et al., (1980) reported the case of a boy with a focal lesion of the right cingulum subjected to cingulectomy for the removal of a tumour. After the operation, no evidence was seen that the procedure had affected higher cognitive functions, particularly memory. Again, the anatomical descriptions of the case are rather vague, and there is a possibility that the lesion opposite the genu of the corpus callosum, may not have affected the cingulum bundle. The unilateral nature of the cingulum lesion may also have a bearing on the resulting absence of lack of mnemonic deficit.

More encouraging evidence (albeit indirect) has been revealed in several studies. In the first, Bowers et al., (1988) discussed in more detail the case of patient T.R., whose amnesic deficits were originally described by Valenstein et al., (1987). This patient developed both retrograde and anterograde amnesia following a haemorrhage which damaged the splenium of the corpus callosum, and also affected part of retrosplenial cortex. Bowers et al., (1988) argued that T.R., was impaired because the cingulum bundle was damaged, and thus the hippocampus and thalamus were "*disconnected*" from frontal association areas. However, the possibility of fornical damage in this patient cannot be ruled out.

Another case was described by von Cramon et al., (1989); the patient was a woman with a small haemorrhage from a ruptured cryptic angioma in the left retrosplenial area. Early CAT scans revealed blood in the left lateral ventricle, indicating a disruption of the left portion of the splenium; further M.R.I scans illustrated a circumscribed lesion which had destroyed the left posterior cingulum bundle. Initially, her verbal learning and memory functions were considerably impaired, but six months later her test performance had achieved normality. Von Cramon et al., (1989) concluded that a retrosplenial disruption of the posterior cingulum bundle produced a memory and learning dysfunction but had no long - lasting effect.

A final example was reported by Rudge and Warrington (1991) who described the neuropsychological abnormalities found in 9 patients with tumours involving the splenium of the corpus callosum, the outstanding feature of which was a profound memory deficit. The authors argued that the impairment was due to damage of the fornix which is certainly possible, but admitted that damage to other regions (they indicated retrosplenial cortex) could not be ruled out. It is possible in the case of these patients that resultant damage to the cingulum bundle could also have played a key role in the mnemonic deficits seen.

In all of the human studies described above there is considerable lack of detail concerning the precise regions surrounding the cingulum bundle that were stimulated or damaged, and so exact interpretation of the results is difficult. What is obviously required is an animal model utilising circumscribed and controlled damage to the fibre tract, and the effects (or lack of) that such damage has upon a range of tasks which reliably assess learning and memory. Such a study has not yet been reported.

3.6 OVERALL SUMMARY.

Previous sections have presented evidence for the role of medial frontal cortex in mnemonic processing. With regards rodents, that area of frontal cortex known as cingulate cortex appears to be the key region relating to mnemonic processing. Within cingulate cortex, damage (in both humans and experimental animals) to the two principal divisions - anterior cingulate and retrosplenial cortices, have most strongly indicated that these regions are involved in learning and memory, with the role of the retrosplenial region being perhaps the most crucial.

The anatomical connectivity of these areas also attests to their possible mnemonic role, the anterior cingulate region shares its principal connections with MD, whilst the retrosplenial region shares its principal connections with the anterior thalamic nuclei. Furthermore, both regions are linked by the cingulum bundle which serves to connect these areas with the hippocampus.

The next chapter will present experimental evidence concerning the functional effects of neurotoxic lesions on both cingulate regions, on tasks of spatial working memory in the rat.

CHAPTER 4. EXPERIMENT 2: THE CONTRIBUTION OF ANTERIOR CINGULATE AND RETROSPLENIAL CORTICES ON TESTS OF SPATIAL WORKING MEMORY.

4.1 INTRODUCTION.

As seen in chapter 3, evidence from the effects of brain injuries in human patients' and in experimental animals, indicates that the cingulate cortex contributes to learning and memory (Valenstein et al., 1987; Murray et al., 1989; Matsunami et al., 1989; Sutherland et al., 1988; Winocur, 1992). As the cingulate cortex contains several distinct cytoarchitectonic areas it is likely that these different regions will contribute to the mnemonic process in different ways. This view is reinforced by the finding that the various regions within cingulate cortex have quite different patterns of connectivity with other limbic regions thought to be involved in learning and memory.

The purpose of this experiment was to compare the behavioural contributions of anterior and posterior cingulate cortex to spatial memory. The experiment thus compared the behavioural effects of neurotoxic lesions in the anterior and posterior (retrosplenial) cingulate cortices on various tasks of spatial memory. These consisted of the automated DNMP and automated spatial discrimination tasks described in experiment 1, and a forced - alternation task performed in a T-maze.

The automated DNMP task shares many similarities with delayed alternation and delayed response tasks, both of which have been reported to be sensitive to prefrontal damage in rats (Larsen & Divac, 1978; Kolb et al., 1974). Furthermore, the automated DNMP task described in this report has been shown to be sensitive to prefrontal damage (Dunnett, 1990). The T-maze, is a task known to be sensitive to both hippocampal, and medial frontal damage (Aggleton et al., 1986; Brito and Brito, 1990). Both tasks are also sensitive to anterior thalamic nuclei damage (Aggleton et al., 1991a; Hunt & Aggleton, 1991), which is relevant given the connections between these nuclei and cingulate cortex.

This experiment will also compare cingulate cortical lesions with those of the fornix; the rationale for including a fornix group is that comparisons can be drawn between damage to sites in MFc and lesions of the hippocampal system; damage to the fornix having been reported to produce impairments in a variety of spatial memory tasks in both rats and monkeys (Aggleton et al., 1991a; 1992; Sutherland & Rodriguez, 1989; Gaffan & Harrison, 1989; Rawlins & Olton, 1982).

Of particular importance in this experiment is the use of neurotoxic lesions, as several studies which have reported severe impairments following medial frontal damage, have tended to use either radiofrequency lesions (e.g. Sutherland et al., 1988), or aspiration lesions (e.g. Dunnett, 1990). Both methods cause damage to underlying fibres of passage, the particular fibre tract at risk in such lesions is the cingulum bundle, which links the hippocampal formation and anterior thalamus with cingulate cortex (Mufson & Pandya, 1984). As a consequence, conventional lesions may be measuring the effect of disrupting thalamic / hippocampal connections rather than the loss of cingulate cortex.

In the light of the results from the previous experiment, it can be hypothesised that anterior cingulate cortex damage will have little, or no effect, upon the DNMP task, given that MD damage caused no impairments on this task (recall that MD shares connections with this region of cingulate cortex). Given also that retrosplenial cortex has dense connections with the anterior thalamic nuclei, damage to this region should produce some form of impairment.

4.2 MATERIALS AND METHODS.

4.2.1 Subjects.

The study involved 73 (two cohorts of 49 and 24 animals) naive male rats of the pigmented DA strain (Bantin and Kingman, Hull). Throughout the period of the experiment the animals were housed individually under diurnal conditions (14hr light/10hr dark), all testing occurring at a regular time during the light period. The animals were tested for five days a week, and prior to test days fed approximately 15g of RMI laboratory diet (Special Diet Services, Witham, Essex) daily so that they did not drop below 80% of normal body weight. At the start of testing the animals were aged 4 months and weighed between 208-280g. All animals had free access to water.

4.2.2 Apparatus for experiments 2a and 2b: DNMP and spatial discriminations.

All testing was carried out in 7 operant chambers (Campden Instruments Ltd., Loughborough) under the control of 2 Spider microprocessors (Paul Fray Ltd., Cambridge). Each chamber was fitted with two retractable levers situated 7.5cm either side of a central food tray.

This food dispenser which delivered 45mg pellets (Campden Instruments), had a hinged perspex lid at which nose pokes could be recorded. A light was located inside the food tray and a house light was located in the centre of the roof, and a further light was positioned above each lever.

4.2.3 Apparatus for experiment 2c: T-maze alternation.

The floors of the T maze were 10cm wide and made of aluminium. The stem of the maze was 80cm long with a guillotine door located 33cm from the beginning. The cross piece was 136cm long and at each end there was a food well 4cm in diameter and 0.75 cm deep. The walls of the maze were 17cm high and made of clear Perspex. The maze was supported on two stands 93cm high. Lighting was provided by fluorescent lights suspended 92cm above the apparatus, the luminance light levels at the choice point and food wells being 320 and 280 lux respectively.

4.2.4 Procedure for experiment 2a: DNMP.

a) Training: The full training procedure consisting of magazine training, autoshaping and initial training on the DNMP task has been fully described in experiment 1a, see figure 5. Once the animals reached the criteria of three successive daily sessions performing at 85% + at delays of 0 - 2 seconds, they each performed a further 14 sessions containing an equal mixture of 0, 2 and 4 s (four sessions), 0, 2, 4 and 8s (four sessions), and 0, 2, 4, 8, 16, and 32 s (six sessions).

b) Postoperative performance: Following completion of this protocol, all animals in both cohorts received either sham, anterior cingulate, retrosplenial, or fornix surgeries. In the first cohort, postoperative complications reduced the number to 20 animals, and the second cohort were reduced to 21 animals giving a total of 41, separated into shams (n=14), anterior cingulate (n=9), retrosplenial (n=10), fornix (n=8). Approximately six weeks after surgery, all animals were retested on the DNMP task. They firstly received seven daily sessions consisting of 60 trials of 0s delays, followed by a further two sessions of 0, 2 and 4s, over 60 trials, and finally fifteen sessions of 96 trials, each comprising a balanced mixture of 0, 2, 4, 8, 16 and 32s. Data analyses were carried out on this final series of fifteen sessions.

c) Performance measures and analyses: The data from the 0-32s delay condition were analysed to provide the various indices of accuracy, responsivity and bias that have been fully described in experiment 1a. Once again, the data in this report was transformed as appropriate (arcsin: all accuracy and bias indices, logarithmic: latencies, square-root: misses), and analysed by parametric analysis of variance (ANOVA), including 1-factor (lesion group) independent measure, and 2-factor (lesion group, delay) mixed measure analysis. As in experiment 1a, when the F-ratios were significant, the means were compared using the Newman-Keuls procedure (Winer, 1971).

4.2.5 Procedure for experiment 2b: Spatial discrimination and reversal.

One week after completing the DNMP task, the same animals were trained on a spatial discrimination task which has been fully described in experiment 1b. A slight modification was made to the programme so that a record could be made of the first ten trials of each session. This was done because the effects of the previous session on present performance might be expected to be most apparent. Again, each daily session consisted of 40 trials, the correct lever always being on the same side throughout the session. All animals received 20 sessions, the designation of which lever was to be '*correct*' on the day followed a balanced, pseudorandom sequence.

4.2.6 Procedure for experiment 2c: T-maze alternation.

Testing began approximately 8 weeks after completion of experiment 1b. Each animal was given one or two days of pretraining in order to run reliably down the stem of the maze to find food pellets in both of the food wells. At the start of each trial, which consisted of two stages, three food pellets (45mg Campden Instruments, Loughborough), were placed in each food well and a wooden block was placed at the neck of the '*T*' to close off one arm.

On this '*information run*' the animal was thus forced to enter the open arm and was allowed to eat the pellets there. The animal was then picked up and confined in the start box for a delay, consisting of 15s before the wooden block was removed. The door to the start box was then opened and the animal allowed a free choice between the two arms of the maze.

On this '*choice run*' the animal was deemed to have chosen when it had placed a back foot in one of the two arms, no retracing was permitted.

If the rat had alternated, i.e. had entered the arm not previously visited on the information run', it was allowed to eat the pellets, and was then returned to its cage. If the other arm was chosen, i.e. the same arm as visited on the information run, the rat was confined to that arm for approximately 10s, and then returned to its cage (see Figure 14, over).

The rats were tested in groups of 4 with each rat having one trial in turn, so that the intertrial interval ranged between 1 and 5min, depending upon the delay. The animals received 6 trials per session and a total of six daily sessions. This was followed by a further ten sessions in which the six trials were divided equally between those with retention delays (i.e. the time spent in the start box prior to the 'choice run') of either 10s, 30s, or 60s, making a total of 20 trials at each delay.

4.2.7 Surgical and histological procedures.

Animals in the first cohort were anaesthetised by intraperitoneal injection (4ml/kg) of a solution containing 42mg/kg of chloral hydrate and 9.7mg/kg of pentobarbitone sodium (Equithesin), while those in the second cohort received an intraperitoneal injection (60mg/kg) of pentobarbitone sodium. Each animal was then placed in a stereotaxic headholder (David Kopf Instruments, Tujunga), the scalp retracted to expose the skull, and a small craniotomy made above the target structure.

For the anterior cingulate (ACc) lesions, injections of 0.28 µl of 0.09 M N-methyl-D-aspartic acid (NMDA) (Sigma Chemical Co. Ltd., Poole) dissolved in phosphate buffer (pH 7.2) were made through a 1 µl Hamilton syringe into two sites in each hemisphere. The stereotaxic co-ordinates relative to ear-bar zero, were: AP +7.6, LAT ±0.7 and AP +6.3, LAT ±0.7. The depth at both sites was 2.2mm below the top of the cortex. Each injection was made gradually over a four minute period and the needle was left in situ for a further 4 min before being withdrawn. After completion of the injection, sulphanilamide powder was applied, and the skin sutured.

T-maze alternation procedure.

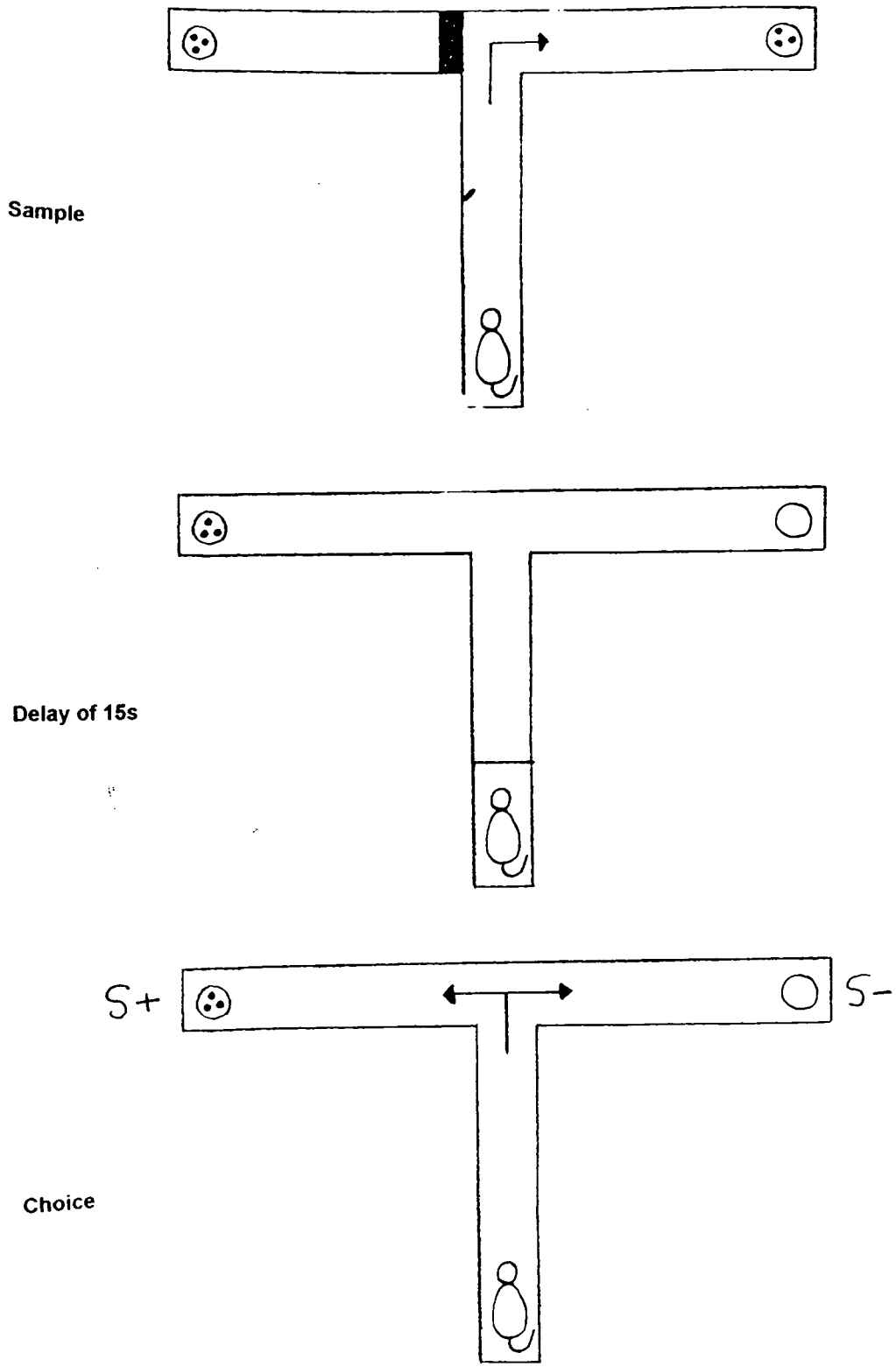


Figure 14. Forced-alternation in the T-maze. Top: Sample phase. Middle: Delay phase. Lower: Choice phase.

The procedure for the retrosplenial (RSc) surgeries was virtually the same, the only difference being that the RSc group received three injections of 0.25 μl of 0.09 NMDA per hemisphere. The co-ordinates , relative to ear bar zero were: AP +3.7, LAT ±0.7; AP +2.8, LAT ±0.7; and AP +0.9, LAT ±0.7. The two rostral injections were placed 1.9mm below the cortex while the most caudal injection was placed 2.2mm below the cortex.

The surgical control animals (SHAM) received a craniotomy and the dura was cut in the appropriate place, the animal then received sulphanilamide powder and the skin was sutured. For half of the SHAM animals, the placement matched that of the ACc group, while for the other half, it matched the RSc lesion.

For those animals receiving fornix lesions (Fx) the initial stages were the same as those described above, but the actual lesion was made by radiofrequency. A Radionics TCZ (Radionics Inc., Burlington) electrode (0.3mm tip length, 0.25mm diameter) was lowered vertically into the fornix and the tip temperature raised to 75ºC for 60s using an RFG4-A Lesion Maker (Radionics Inc., Burlington). Two lesions were made in each hemisphere the stereotaxic co-ordinates of the lesions relative to ear-bar zero were: AP + 5.3, LAT ±0.9 and AP +5.3, LAT ±1.7. The depth at both sites was 4.5mm and 3.7mm below the top of the cortex respectively.

On completion of the experiment the animals were killed and perfused intracardially with 5% formol-saline. The brains were then rapidly removed and placed in 5% formol-saline. Subsequently, the brains were blocked, embedded in wax (Paraplast), and cut into 10- μm coronal sections. Every tenth section was mounted and stained with Cresyl violet, a Nissl stain.

4.3 RESULTS.

4.3.1 Histological Analysis.

Following histological analysis, one animal in the sham group was excluded owing to vascular damage to the cortex. One animal in the ACc group was also excluded as the lesion was unusually small.

Figure 15 shows the extent of the largest and smallest of the ACc lesions. These surgeries resulted in a localised region of cell loss that was always restricted to area 24 (Vogt & Peters, 1981) Within the extent of the lesion there was a complete loss of cells. In contrast, there was no evidence of direct damage to the corpus callosum, the cingulum bundle, or the fornix. While the lesions involved much of area 24a, they sometimes spared the most dorsal parts of area 24b. In addition, the pregenual portions of the cingulate cortex and the posterior transitional region were often spared. There was no evidence of any thalamic degeneration.

Figures 15 and 16a shows the extent of the retrosplenial lesions which produced considerable damage to area 29. All of the surgeries resulted in extensive cell loss throughout the rostral and middle portions of the RSc, usually involving all of areas 29b, 29c, and 29d. The lesions extended caudally behind the splenium both ventrally and laterally to reach the border of the subicular complex (areas 29a and 29b). While all of the subfields of area 29 (a-d) were involved in the lesions, the only parts of RSc to be spared were the rostral transitional zone with area 24 and the most caudal regions behind the splenium.

Diffusion of the injectate consistently resulted in a localised patch of cellular damage in that part of the CA1 field of the hippocampus closest to the RSc. In addition, all cases had a clear region of cellular shrinkage and loss restricted to the anterior ventral nucleus of the thalamus. The extent of this degeneration matched very closely the borders of the nucleus, although in some cases the region of AV closest to the anterior dorsal thalamic nuclei appeared normal (see figure 16b). In spite of the extensive cell loss in area 29, the cingulum bundle appeared to be spared.

Figures 15 and 17 show the extent of the fornix lesions which produced very extensive damage to the fibre tract itself and in most cases also extended into the most rostral head of the hippocampus. In six of the eight animals the tract was completely severed bilaterally while in the remainder only the most lateral tips of the fimbria were spared. In nearly all cases, the lesion, in addition to damaging a small part of the corpus callosum involved the very most dorsal limit of the anterior ventral, and anterior dorsal thalamic nuclei. The cingulum bundle was always spared and there was no direct damage to the cingulate cortex. Following histological analysis, the groups contained 13 SHAM, 8 ACc, 10 RSc, and 8 Fx animals, see figures 15 - 17 over:

Reconstruction of anterior cingulate, retrosplenial, and fornix surgeries.

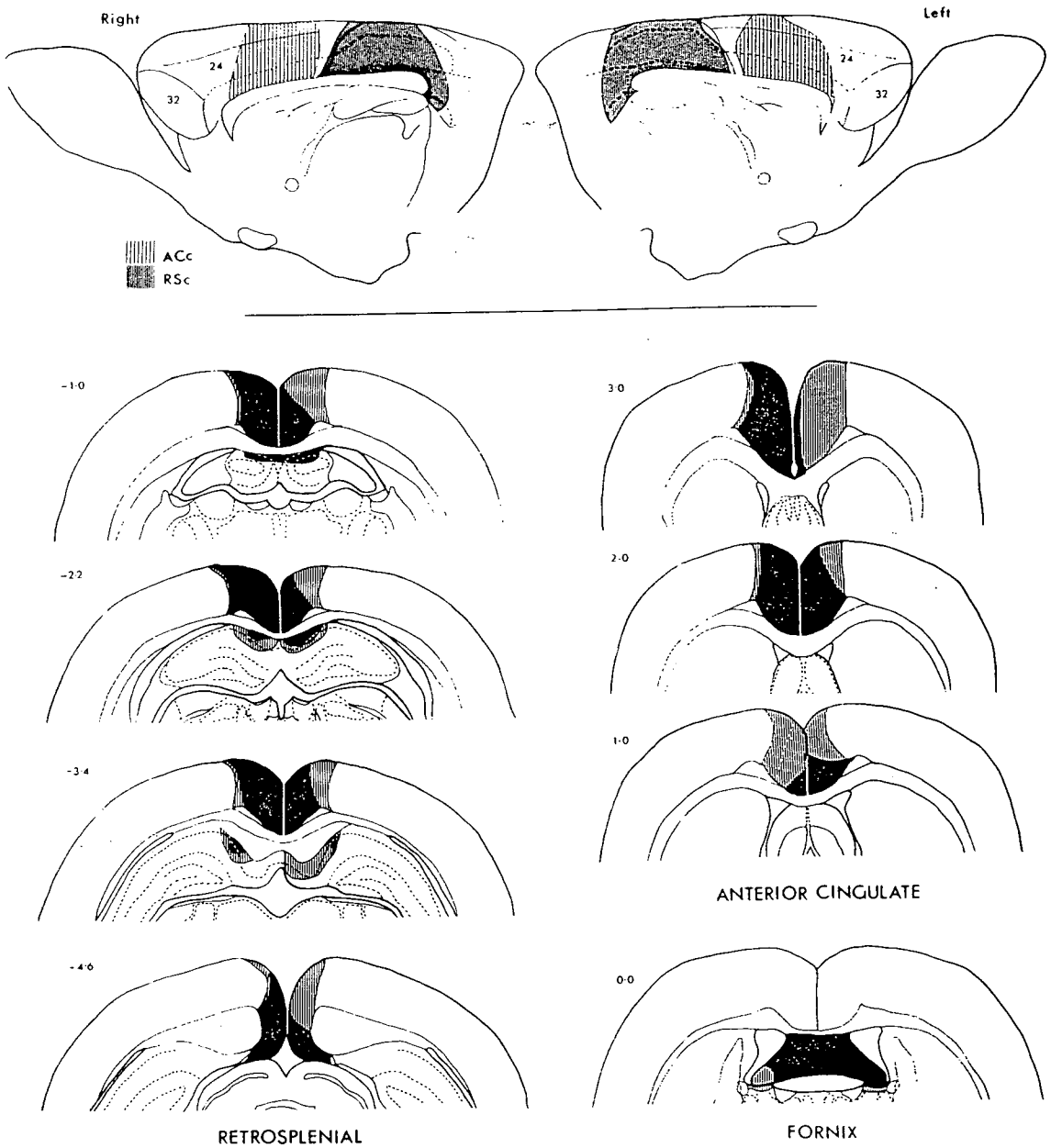


Figure 15 Diagrammatic reconstruction showing the extent of the retrosplenial (RSc), anterior cingulate (ACc) and fornix (Fx) lesions. Upper: View of medial surface of right and left hemispheres showing extent of lesions in cases with mid-sized ACc (vertical lines) and RSc (black) surgeries. The numbers refer to the cortical areas described in Vogt & Peters (1981). Lower: Series of coronal sections showing the extent of the largest (vertical lines) and smallest (black) lesions in each of the three groups. The numbers correspond to the approximate position from Bregma (Pellegrino & Cushman, 1967).

Photomicrograph of appearance of retrosplenial cortex following injection of a neurotoxin.

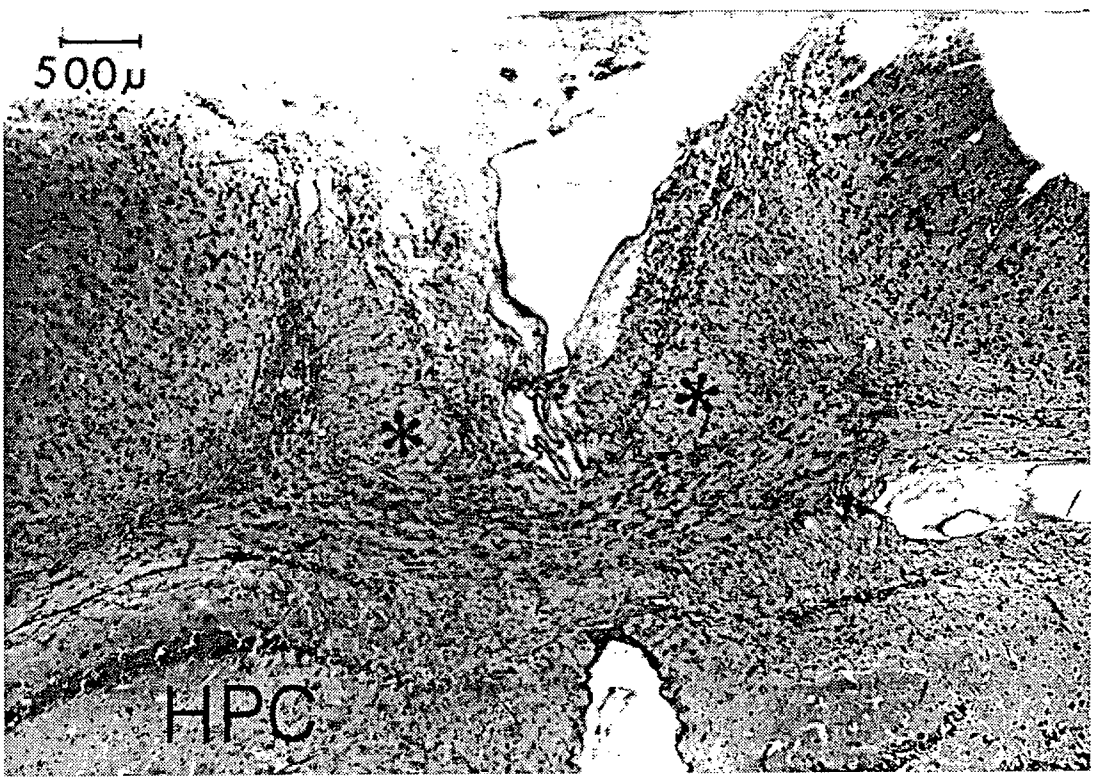


Figure 16a. Photomicrograph of a coronal section (Nissl stain) showing the appearance of retrosplenial cortex following an injection of NMDA. Note the sparing of the cingulum bundle (asterisk) and the small zone of cell loss in the hippocampus. The anterior cingulate lesions were very similar and are thus not represented. HPC = hippocampus.

Photomicrograph of appearance of the anterior ventral (AV) thalamic nuclei after a neurotoxic lesion of retrosplenial cortex.

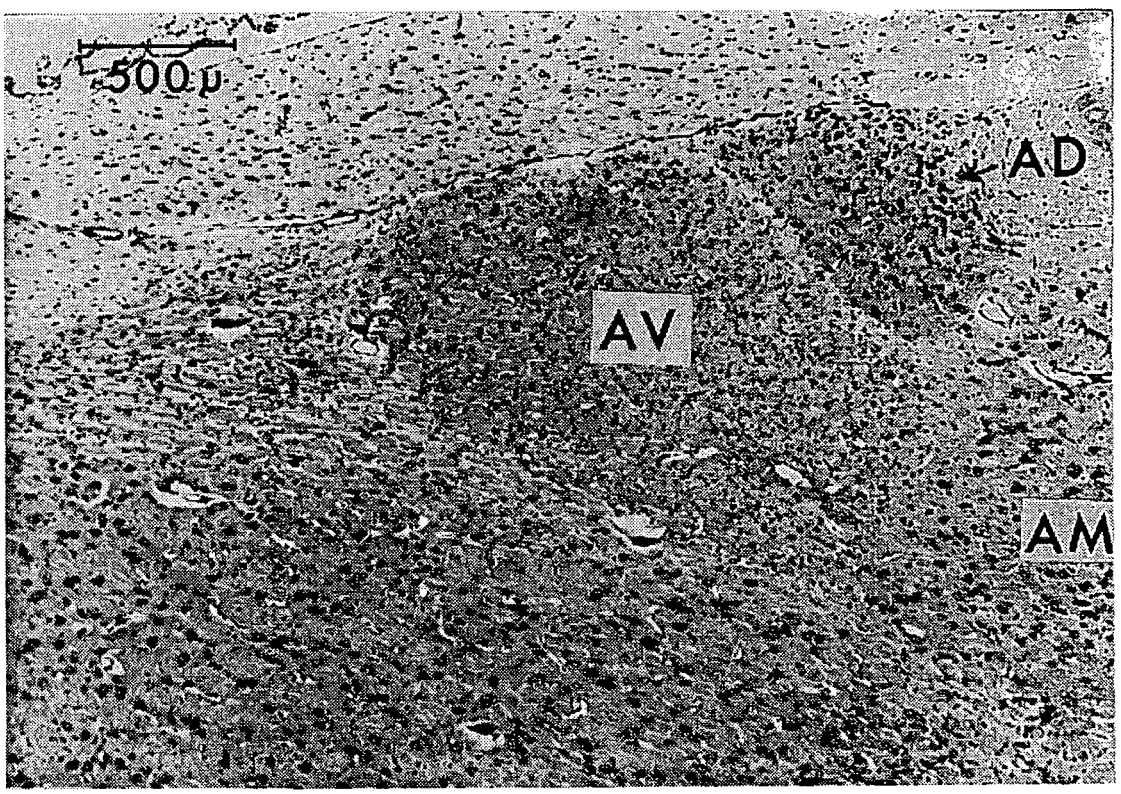


Figure 16b Photomicrograph of a coronal section (Nissl stain) showing the appearance of the area of cell loss and shrinkage in the anterior ventral nucleus (AV) in an RSc case, note the normal appearance of the adjacent anterior medial (AM) and anterior dorsal (AD) nuclei.

Photomicrograph of the fornix following a radiofrequency lesion.

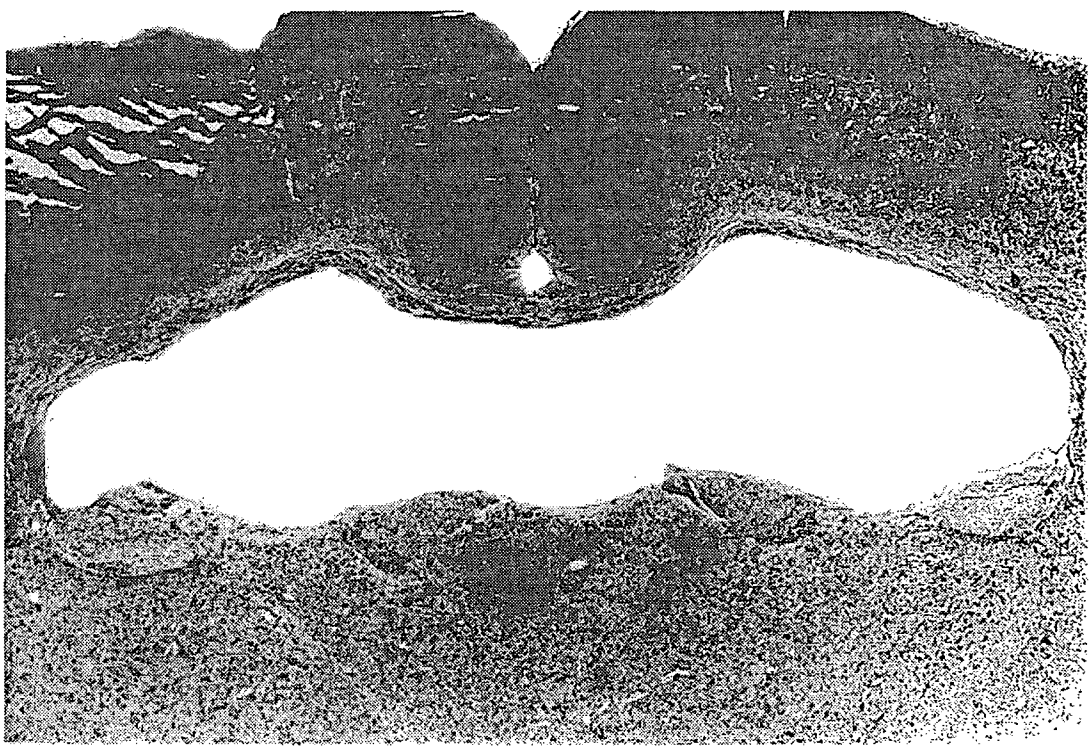


Figure 17. Photomicrograph of coronal sections (Nissl stain) showing the appearance of the fornix following a radiofrequency lesion.

4.3.2 Experiment 2a: DNMP.

Following surgery, all animals were retested on the DNMP task. After 5 practice sessions on the 0 delay condition, and 2 sessions on the 0-4 delay condition, all animals received 15 daily sessions on the final condition (variable retention intervals of 0-32s).

1. Comparison of different cohorts: There were no systematic differences between the rats in the two cohorts; the mean percent correct for the two cohorts over the final six sessions prior to surgery were found to be comparable (83.0% and 83.9% respectively).

2. Comparison of sham groups: The retrosplenial sham, and anterior cingulate sham groups, were compared across all 15 post-operative sessions utilising the 6 delays. A t-test (two-tailed) revealed no significant difference between the two sham groups ($t, 28 = 0.82$, $P>0.05$). Both sham groups were thus combined to form one group (SHAM, $n = 13$).

3. Accuracy measures: The mean percent correct scores for each group over all 15 daily post operative sessions are shown in figures 18 and 19. An analysis of variance using this data revealed a significant group effect ($F\ 3,35 = 5.46$, $P<0.01$), and a non-significant trend for a group X delay interaction ($F\ 15,175 = 1.71$, $P=0.053$). There was also a marked effect of session ($F\ 14,490 = 7.05$, $P<0.01$), a clear delay effect ($F\ 5,175 = 538.84$, $P<0.01$), and a session x delay interaction ($F\ 70,2450 = 1.33$, $P< 0.05$). A subsequent Newman-Keuls test showed that the significant group effects reflected the poorer performances of the Fx animals. Neither ACc nor RSc groups differed from the SHAM animals, but all three groups differed from the Fx group ($P<0.05$). The session effect and the session X delay interaction reflected the gradual improvement in post-operative performance among all test animals.

DNMP Percent Correct Scores .

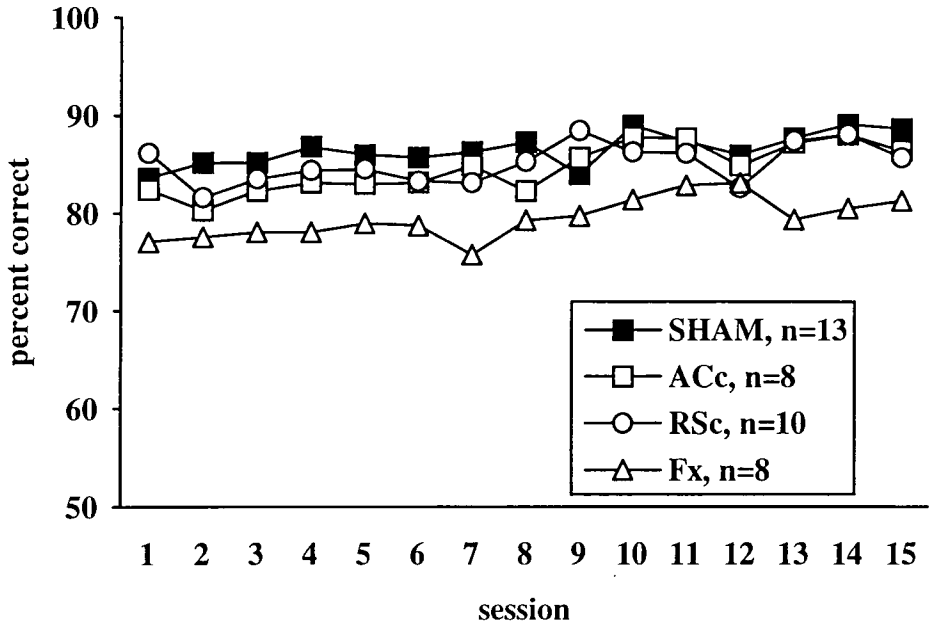


Figure 18. Mean percent correct scores on the DNMP task over each of the fifteen postoperative sessions. Delay data have been pooled.

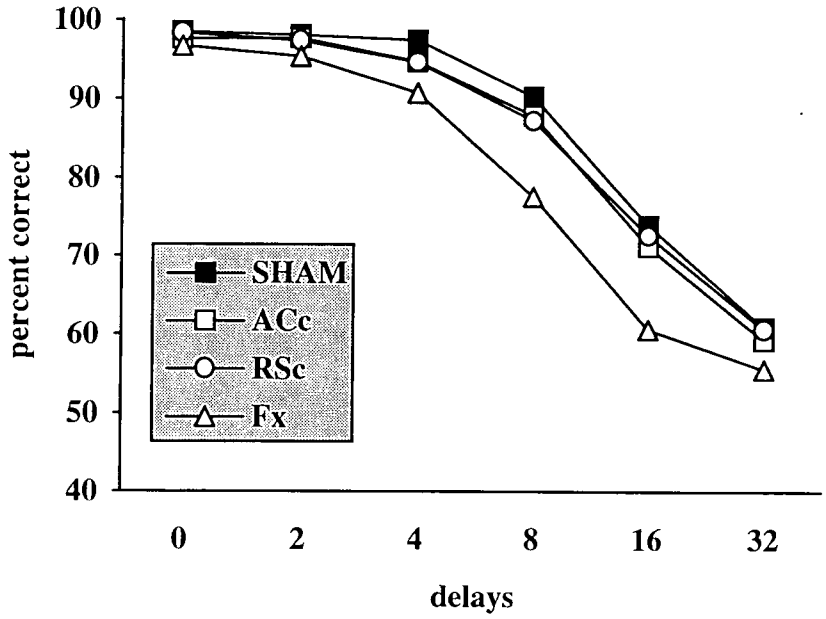
The same pattern of results was found with the two TSD measures of sensitivity, A' and SI (Figure 19). Both revealed highly significant effects of session and delay, ($P<0.01$), but also revealed a group effect in both cases (A', $F_{3,35} = 4.26$, $P<0.05$; SI, $F_{3,35} = 4.85$, $P<0.01$). As before, the group effects reflected the abnormal behaviour of the Fx group which differed from the other three groups ($P<0.05$). There were no other group differences.

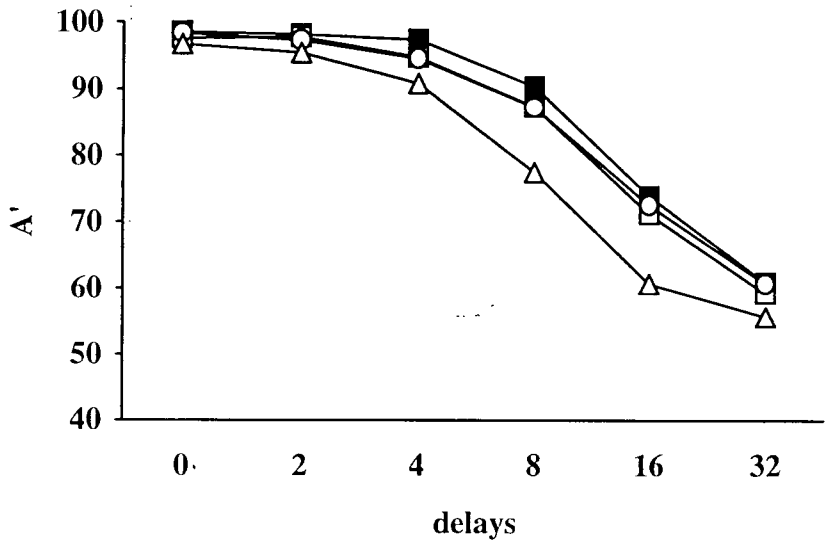
4. Bias indices: All three bias indices (Iy, B", and RI) demonstrated both session and delay effects as expected ($P<0.01$ in all cases). Furthermore, each of the bias indices revealed a highly significant group effect (Iy, $F_{3,35} = 3.05$, $P<0.05$, B", $F_{3,35} = 3.41$, $P<0.05$, RI, $F_{3,35} = 5.12$, $P<0.01$, and for one of the indices there was also a highly significant group x delay interaction (B", $F_{15,175} = 1.88$, $P<0.05$), see Figure 19.

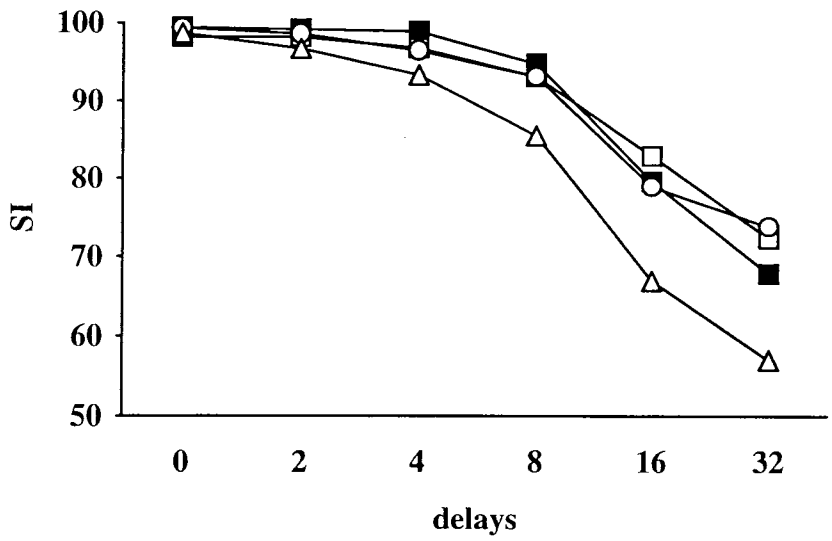
For all three measures of bias, the significant group effects could be attributed to the Fx group which consistently showed higher levels of bias than the SHAM animals. In addition, for B" only, both the ACc and RSc groups differed from the SHAM animals (both $P<0.05$) showing higher levels of this measure of perceptual bias. For RI, all three groups differed from the Fx animals, but did not differ from one another. Finally, for Iy, only the Fx group were significantly different from the SHAM group, see figure 19.

5. General responsivity: There were no group effects for a number of general measures of responsivity; these consisted of: mean latency to sample ($F_{3,35} = 2.03$, $P>0.05$), total number of misses ($F_{3,35} = 1.89$, $P>0.1$), and magazine response (nose-poke) rate ($F_{3,35} = 2.18$, $P>0.05$). Group differences were found however, for mean latency of first magazine response ($F_{3,35} = 3.84$, $P<0.05$, and for the mean choice latency ($F_{3,35} = 2.97$, $P = 0.05$. In both cases, the Fx group responded with a significantly shorter latency than the SHAM animals. In addition, both ACc and RSc animals had a significantly shorter mean latency to make the first magazine response than the SHAM's ($P<0.05$).

DNMP Bias Indices.



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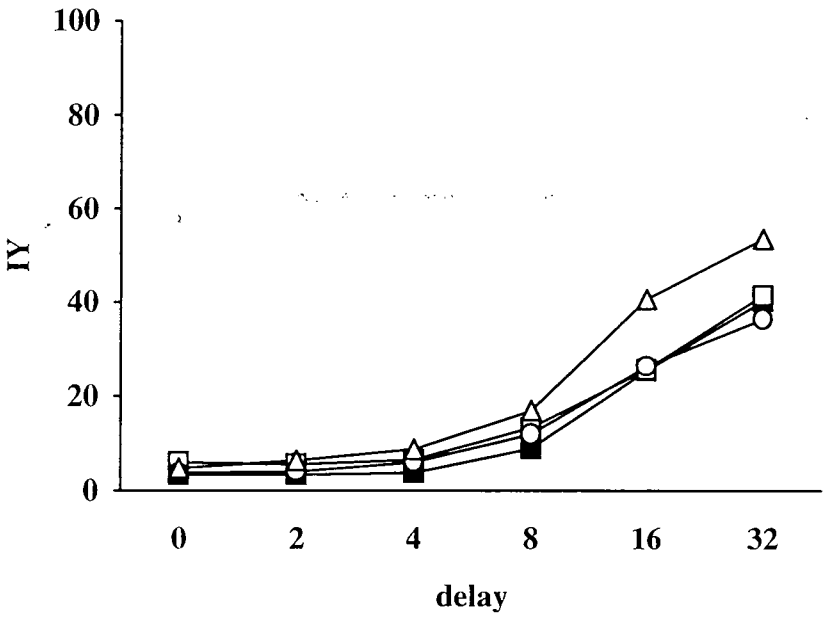
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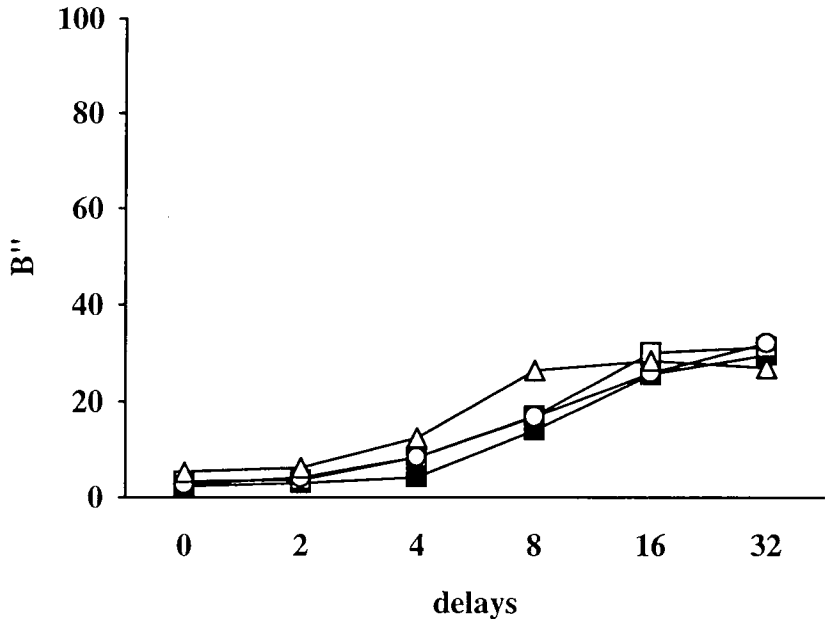


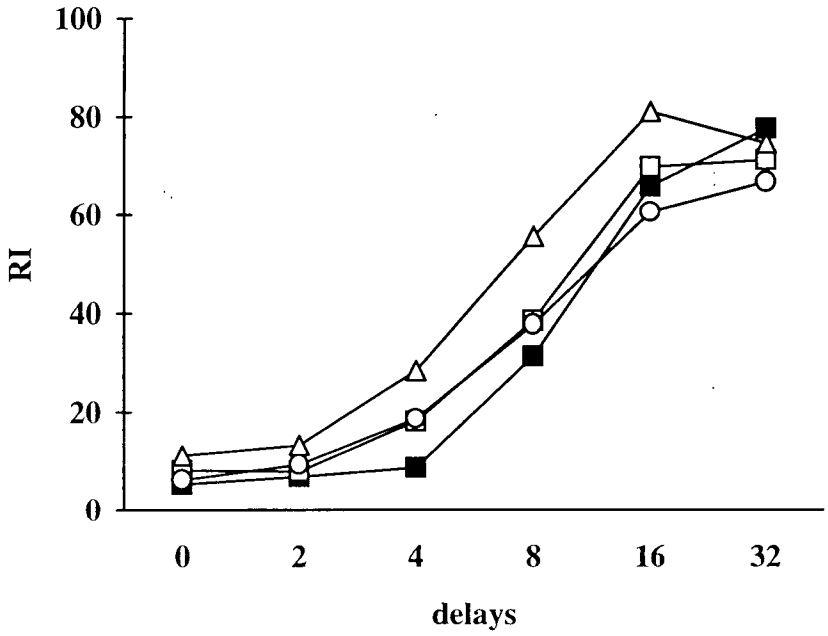


Fig 19. Accuracy (first three graphs) and bias (final three graphs) indices as a function of lesion (See text for explanation of indices). All scores have been converted to read from 0 to 100, and absolute values are shown (note the different scales on the vertical axis). In the case of the bias scores, 100 represents a complete bias and 0 neutral, or no bias.

4.3.3 Experiment 2b: Spatial discrimination and reversal.

The second experiment consisted of a spatial (lever) discrimination task (sessions 1 and 2) followed by a series of reversals between specified sessions.

a) Initial discrimination: The mean percent correct scores for each group over the initial two discrimination sessions were as follows: SHAM = 73.8, ACc = 77.4, RSc = 69.3, and Fx = 82.2. An ANOVA carried out on these mean scores revealed no overall group difference $F_{3,35} = 1.57$, $P>0.05$.

b) Remaining 18 sessions: The mean percent correct responses for the four groups over the remaining 18 sessions were as follows: SHAM = 78.2, ACc = 78.7, RSc = 79.0, and Fx = 72.4, (figure 20). An ANOVA revealed a significant group difference ($F_{3,35} = 3.85$, $P<0.05$), which a subsequent Newman-Keuls analysis showed that this group difference reflected the poorer performance of the Fx group, who differed significantly from the ACc and RSc groups ($P<0.05$), and also from the SHAM group ($P<0.01$).

By excluding the first session, the scores could be divided between those nine sessions where the correct lever was in the same position as in the previous session, and those ten sessions where the correct lever reversed sides between sessions (see figure 21).

c) 'Consistent' sessions: The mean percent correct scores for each group on the 'consistent' sessions, with the standard deviation in parenthesis, were as follows: SHAM = 85.2, (5.5), ACc = 85.3 (3.7), RSc = 86.4 (3.4), and Fx = 84.3 (5.3). An ANOVA carried out on these scores revealed no significant group difference ($F_{3,35} = 0.28$, $P>0.05$).

d) The 'Reversal' sessions: The mean percent correct scores for each group on the 'reversal' sessions, with the standard deviation in parenthesis, were as follows: SHAM = 72.4 (5.1), ACc = 74.2 (9.5), RSc = 71.8 (6.8), and Fx = 63.3 (5.4). An ANOVA revealed an extremely significant group difference ($F_{3,35} = 7.9$. $P<0.001$) which a subsequent Newman-Keuls test showed to be due to the poorer performance of the Fx animals. This group differed from the SHAM ($P<0.001$), ACc ($P<0.01$) and RSc groups ($P<0.05$). There were no other group differences.

Spatial Discrimination and Reversal.

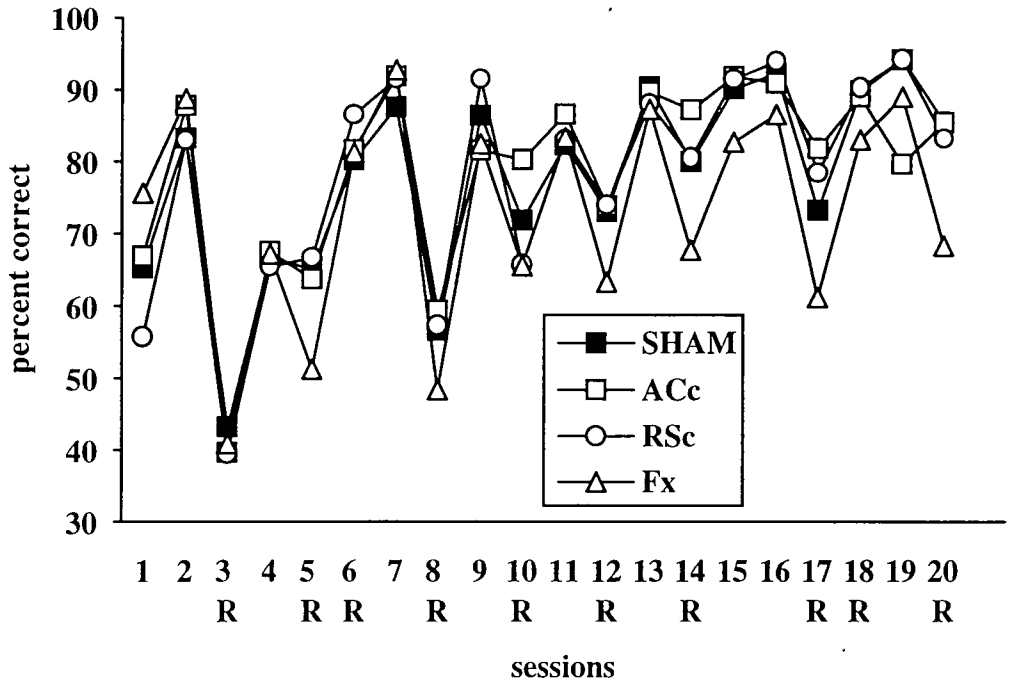


Figure 20. Mean percent correct scores on the lever discrimination and reversal task over the 20 sessions. Sessions where the correct lever changed from the preceding session ('reversal') are labelled as R.

e) 'Consistent' versus 'reversal' sessions: Subsequent analysis of these two sets of scores using an ANOVA, just revealed an overall group difference ($F_{3,35} = 2.9$, $P=0.05$) which reflected the poorer performance of the Fx group. The analysis also showed a strong effect of the type of session indicating that all of the groups had performed considerably worse on the reversal session ($F_{1,35} = 207.0$, $P<0.001$). Finally, a group X type of session interaction was revealed, indicating that the Fx group had been particularly badly affected by the 'reversal' sessions ($F_{3, 35} = 4.45$, $P<0.01$), see figure 21.

f) The first ten trials of each session: A final set of analyses considered only the first ten trials of each session. These were selected for two reasons, firstly, because the effects of the previous session (either 'consistent' or 'reversal') on present performance might be expected to be most apparent; and secondly because performance on the remaining 30 trials of the session would be expected to reach ceiling. Figure 21 shows the mean percent correct scores of the first ten trials on the 'consistent' and 'reversal' sessions.

As expected, there was a highly significant effect of the type of session ($F_{1,35} = 327.1$, $P<0.0001$), showing how the animals performed better when the correct lever was in the same position as in the previous session. There was also a significant group effect ($F_{3,35} = 3.69$, $P<0.05$) which reflected the poorer performance of the Fx group. This lower level of performance was only observed, however, on the reversal sessions. Consistent with this was the highly significant group X type of session interaction ($F_{3,35} = 7.16$, $P<0.01$). There was no evidence that either of the cingulate cortex lesioned groups performed abnormally on this task.

Spatial Discrimination and Reversals, 'Consistent' and 'Reversal' Sessions.

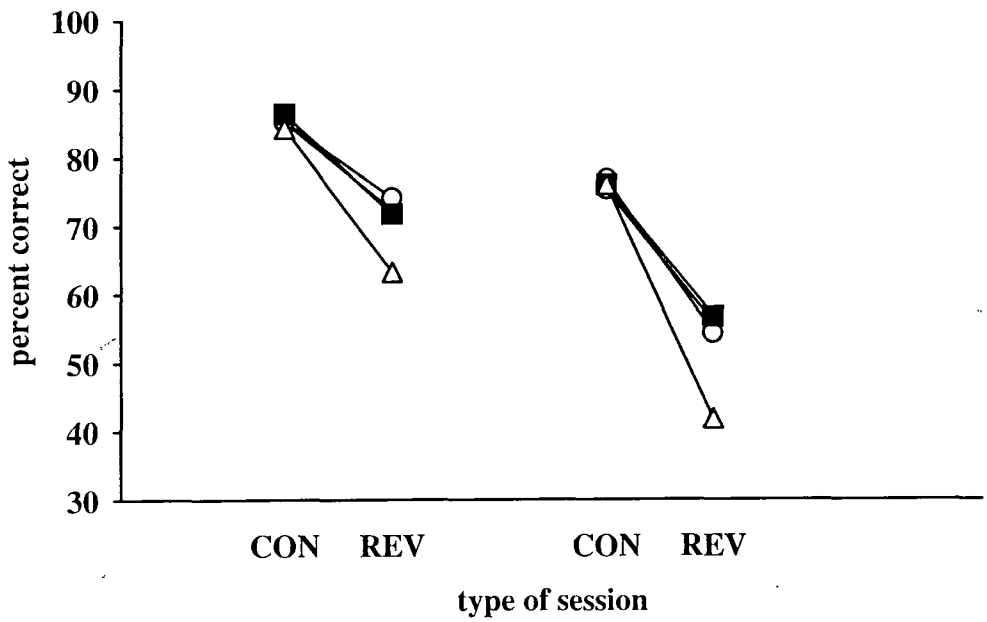


Figure 21. Mean percent correct scores on the lever reversal task grouped according to those days on which the correct lever was 'consistent' with the previous session and those days on which it was a 'reversal' from the previous session. Left: using the scores from each entire session. Right: using the scores from the first 10 trials of each session.

4.3.4 Experiment 2c: T-maze alternation.

a) Acquisition: During the initial six acquisition sessions, each animal performed a total of 36 trials with a retention delay of 15s (Figure 22). Scores were analysed using an ANOVA which revealed a highly significant group effect ($F_{3,35} = 34.6$, $P<0.001$). Subsequent Newman-Keuls tests showed that this reflected the poor performance of the Fx group, who were significantly worse than any of the other three groups ($P<0.01$). While the RSc group produced the second lowest score during the initial acquisition (Figure 22), this group did not differ significantly from the SHAM animals.

b) Delays: All of the animals then performed a total of 20 trials at each of three delays (10s, 30s, and 60s). The mean percent correct scores four the four groups are shown in Figure 22 over. An ANOVA revealed a highly significant group effect ($F_{3,35} = 51.01$, $P<0.001$), a delay effect ($F_{2,70} = 7.18$, $P=0.01$), but no clear group X delay interaction ($F_{6,70} = 1.67$, $P>0.1$). Subsequent Newman-Keuls tests confirmed that the group effect reflected the exceptionally poor performance of the Fx group, who differed significantly from all the other groups ($P<0.01$). Although the RSc group showed the next lowest level of performance, their scores did not differ from those of the SHAM animals ($P>0.05$).

c) Acquisition / delays combined: In the light of the slightly lower RSc scores a final analysis compared the total scores of all animals taken from both acquisition and delay conditions. While there was a large group effect ($F_{3,35} = 66.03$, $P<0.001$), reflecting the Fx deficit, but there were no other group differences.

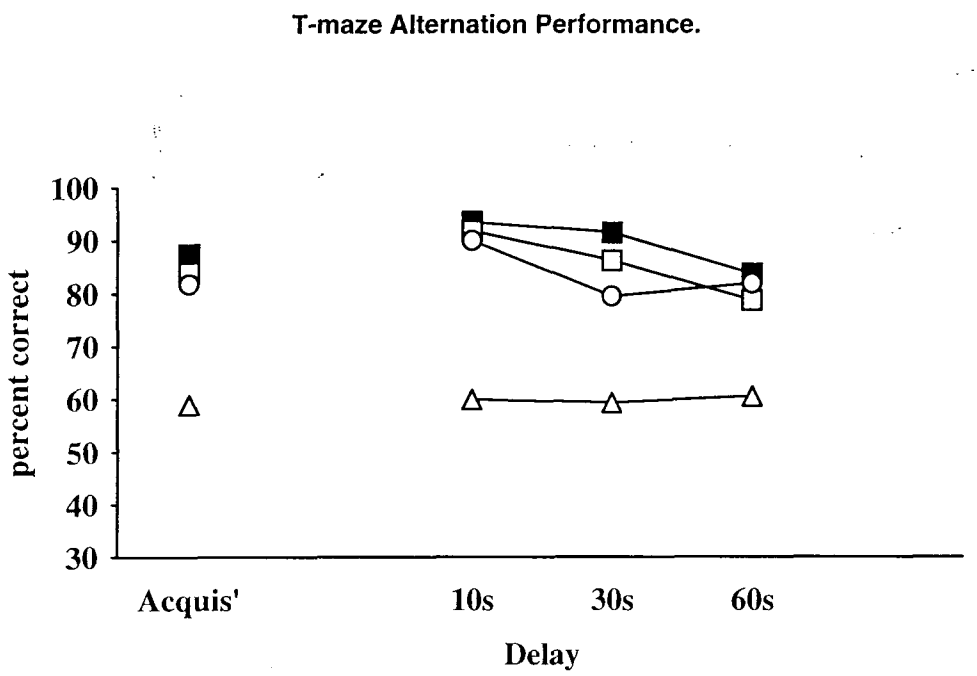


Figure 22. Spatial forced-alternation in the T-maze. The graph shows the mean scores of the four groups over the six acquisition sessions, and the mean scores of the same groups when tested for 20 trials over each of the three retention intervals.

4.4 DISCUSSION.

While fornix lesions produced clear deficits on all three tasks of spatial memory, selective cingulate cortex lesions had no marked effect. Thus, neurotoxic lesions of either ACc, or RSc produced no impairment on an automated DNMP task, a position discrimination and reversal test, and a forced-alternation task. These findings indicate that spatial performance can remain largely unaffected by cingulate cortical damage, a finding which is somewhat surprising given the evidence that RSc in particular, may perform an important role in the processing of spatial information (Sutherland & Hoesing, 1993).

4.4.1 Experiment 2a: DNMP.

This is a test of spatial working memory which can be solved by a number of spatial strategies. These include the use of egocentric memory (response related cues) and the memory for specific local cues, i.e. '*perspex door is on the correct side*'. The task was selected as it was not only sensitive to hippocampal system damage (Aggleton et al., 1992; Dunnett, 1985; 1990) and to anterior thalamic nuclei damage (Aggleton et al., 1991a; Aggleton & Sahgal, 1993) but also because it allows the measurement of a large number of factors related to performance. In view of the dense interconnections between anterior thalamus, hippocampus and RSc, it might be predicted that the RSc group would be impaired on this task.

In fact, there was no evidence that the RSc group were impaired on the DNMP task. The finding that the RSc lesions also consistently produced retrograde degeneration throughout the anterior ventral nucleus of the thalamus indicates that damage to this thalamic structure is not sufficient to produce the performance deficit associated with anterior thalamic damage on this task (Aggleton et al., 1991a). Indeed, the only apparent change in RSc and ACc groups was a slight increase in perceptual bias, as measured by B".

A recent report by Paus et al., (1993) concerning the role of human ACc in the control of various occulomotor, speech, and manual response tasks may provide some explanation for the failure of ACc lesions to impair performance on DNMP. In this report, normal subjects performed oculomotor, manual or speech tasks while regional cerebral blood flow changes in ACc were measured using PET. Task-specific changes in blood flow were found in different subregions of ACc, depending on the output system employed.

The authors proposed that ACc participates in motor control by facilitating the execution of appropriate responses and/or suppressing the execution of inappropriate ones. In this case, human ACc was activated mainly when the subject was forced to choose from a set of competing responses, rather than relying on a set of well-established stimulus-response associations. It could be argued that the DNMP task does indeed rely upon well-established responses, and, providing that ACc performs a similar function in both humans and rodents, it is perhaps not so surprising that ACc lesions had little effect on this task.

In contrast, lesions of the hippocampal system - the fornix, produced both a significant loss of accuracy and an increase in bias. This loss of accuracy was consistent with the findings of a number of previous studies looking at the effects of damage to the fornix, or to the hippocampus itself on the DNMP task (Aggleton et al., 1991a; 1992; Dunnett, 1985). A minor difference between this experiment and two of those studies was the extent to which the Fx group showed high levels of bias (Iy, RI, and B"). Aggleton et al., (1991a) reported that fornix lesions increased Iy (the measure of relative accuracy between the two levers), and Aggleton et al., (1992) found that fornix lesions increased B" (the measure of perceptual bias).

In this experiment, all three bias measures were affected, and there were also decreases in the latencies of some of the response measures. A possible explanation for this difference with previous DNMP studies is that the data analysis in this experiment used the data from every post-operative session whereas the other studies used only a subset of post-operative sessions. The consequence of such selective data usage may be to obscure significant trends which were highlighted in this experiment.

While the changes in bias reported in this experiment are consistent with other reports of increases in perseveration and activity following hippocampal system damage (Gray & McNaughton, 1983; Means & Douglas, 1970), the various types of TSD analysis carried out also indicated that such bias effects were not the sole cause of the severe Fx group deficit on the DNMP task.

4.4.2 Experiment 2b: Spatial discrimination and reversal.

Neither the RSc, nor the ACc groups showed any evidence of an impairment on either the initial discrimination, or the subsequent reversals. This lack of impairment following cingulate damage is consistent with previous studies which have also reported that animals with cingulate cortex damage can perform accurately on tests of spatial memory that can be solved using egocentric, or specific local cues (Markowska et al., 1989; Meunier & Destrade, 1988; Murray et al., 1989; Sutherland & Hoesing, 1993).

A recent report (Marston et al., 1993) used a very similar lever discrimination task to the one used in this experiment and demonstrated similar findings. In their study, rats with lesions of the septal vertical limb nuclei of the diagonal band of Broca (VDB), which produces significant disruptions of cholinergic activity in cingulate cortex and the hippocampus, were unimpaired in the initial discrimination, and subsequent reversals.

In this current experiment it was found that the Fx group could also learn the initial lever discrimination (sessions 1 and 2) at a normal rate. This finding is in agreement with other studies of position discriminations (Gray & McNaughton, 1983; Means & Douglas, 1970; O'Keefe & Nadel, 1978; Rasmussen et al., 1989), although it has been reported that hippocampal damage can disrupt position discriminations when they are counter to the animals' preference (Gray & McNaughton, 1983; Means & Douglas, 1970). In the light of this, it is therefore not surprising that the Fx group were impaired on the reversal sessions and this impairment is consistent both with previous studies of reversal learning (Gaffan, 1972; Gray & McNaughton, 1983) and with the high levels of bias recorded by the Fx group in the DNMP test.

Of particular interest was the finding that the Fx deficit was only associated with those sessions in which the animals had to switch levers. Thus, the Fx group could perform as accurately as the controls over the initial trials in which the lever was on a side consistent with the previous session. This shows that the Fx animals were still able to remember the previous correct lever over a period of 24 hours. This indicates that the fornix deficit may not reflect a mnemonic impairment, but rather a behavioural one - probably the result of perseveration; though it is also possible that the Fx group relied to an unusual degree on response related cues, leading to severe difficulties on the reversal sessions, but enabling the animals to perform normally on the consistent sessions.

This normal performance on the consistent sessions may indicate that fornical connections need not be crucial for the efficient use of local or egocentric cues. This possibility is supported by a number of other studies (Gaffan & Harrison, 1989; Markowska et al., 1989; Meunier & Destrade, 1988; Rasmussen et al., 1989).

The apparent lack of impairment of the Fx group on this part of the lever reversal task appears initially to be at odds with the pattern of the Fx deficit in the DNMP procedure, as both tasks use the same apparatus and share many similarities. There are two possible reasons for this difference. First, in the discrimination task, the animals do not have to nose-poke throughout the session and so may have been able to adopt a positional strategy, i.e. sitting next to the correct lever. Second, as pointed out by Dunnett et al., (1990a) the automated DMP/DNMP tasks contain much higher levels of proactive interference which increase task difficulty and decrease the likelihood of the animals adopting positional strategies.

4.4.3 Experiment 2c: T-maze alternation.

This task was more a test of allocentric memory i.e. spatial memory based on the relative placement of distal cues. Once again, the Fx group was severely impaired and their scores did not differ greatly from chance. This reflects a much more severe deficit than those seen in the DNMP and lever discrimination and reversal tasks, and serves to highlight the sensitivity of the hippocampal system damage to tests of allocentric memory. Other studies have also reported the severe effects of hippocampal damage in such tasks (Morris et al., 1982; O'Keefe & Nadel, 1978). While the results from the T-maze procedure differ from those studies in that the relevant spatial stimuli was not learnt prior to surgery, evidence from other studies indicates that this is not sufficient to account for the difference in magnitude of the Fx deficit (Markowska et al., 1989; Sutherland & Rodriguez, 1989).

The normal performance of the two cingulate lesion groups on the T-maze task is in contrast to other reports indicating that such lesions can produce marked deficits on tests of allocentric memory. For example Markowska et al., (1989) have reported that aspiration lesions involving most of the combined region damaged by the ACc and RSc surgeries produced a severe deficit on a test of T-maze alternation, a deficit that was comparable to that produced by lesions of the fornix.

Similarly, Sutherland et al., (1988) compared the effects of retrosplenial, anterior cingulate, and prelimbic cortical lesions on the Morris swim task. In that study, the retrosplenial lesions produced a severe impairment, while the anterior cingulate and prelimbic lesions also impaired performance, but to not as great extent as the retrosplenial damage.

A range of other studies have shown that medial prefrontal lesions in general can markedly impair allocentric memory performance (Kolb, 1984; Kolb et al., 1982; Thomas & Brito, 1980). Such previously reported deficits associated with cingulate damage are in striking contrast to the results from this experiment; there are a number of important factors which may have contributed to this outcome.

4.4.4 The extent of the lesions.

The extent of the lesioning is of key importance as it has been often reported that large lesions of MFc lead to a greater impairment than do smaller ones. This has been found in a variety of tasks, including the Morris task (Sutherland et al., 1988), T-maze (Silva et al., 1986; Shaw & Aggleton, 1993), and in the radial maze (Silva et al., 1986). A critical factor arising out of several reports is the inclusion/exclusion of prelimbic cortex (area 32), an area which receives direct inputs from the hippocampus (Jay & Witter, 1991), and according to several studies is critical for performance in spatial learning tasks (Brito et al., 1982; Thomas & Brito, 1990; Brito & Brito, 1990).

Of particular relevance to this report is the finding by Dunnett (1990) that aspiration lesions of the medial prefrontal cortex can severely disrupt DMP when tested in a comparable manner to that used in this experiment. The prefrontal lesions in Dunnett's report began close to the level of the genu of the corpus callosum and continued rostrally to the frontal pole (thus including prelimbic cortex) the consequence of which is that Dunnett's experiment and experiment 2a of this thesis examined the effects of damage to different parts of the anterior cingulate region; the difference in results is perhaps then, not so surprising. Consequently, it is important to keep distinct any damage to the prelimbic and anterior cingulate cortices, as lesions not solely confined to the respective areas of cingulate cortex will be difficult to interpret.

Although the lesions performed in this experiment did not affect either prelimbic cortex or other regions of the medial frontal cortex (there was some slight hippocampal damage in the retrosplenial group), the anterior cingulate lesions were not complete, i.e. they did not totally destroy all of area 24. It is possible that it may be necessary to produce much more extensive lesions of this region in order to fully determine its possible contribution to spatial memory. A similar problem was encountered with the lesions of retrosplenial cortex, although these lesions were larger and covered almost all of area 29, they were still not total as the difficulty in destroying all of the retrosplenial cortex without leaving sparing is acute. It could be the case that critical components of retrosplenial cortex were left relatively undamaged.

This leaves open the possibility that it may be necessary to remove both areas 24 and 29 in tandem to appreciate the full contribution of cingulate cortex to these tasks. Further evidence for this proposition is provided by those reports which have indicated that the anterior thalamic nuclei project to both regions, although the projection to anterior cingulate cortex is lighter (Horikawa et al., 1988; Shibata, 1993a; 1993b).

4.4.5 Damage to fibres of passage.

A related issue concerns the possibility of damage to the cingulum bundle, the important fibre pathway which forms an interconnection between septum and anterior thalamus to the medial temporal lobe via cingulate cortex (Domesick, 1970; Mufson & Pandya, 1984; Saper, 1984). Presumably, bilateral damage to this fibre tract at any position along its course could severely disrupt communication between the anterior thalamic nuclei, medial frontal cortex and the hippocampus, though few results have been published about the direct behavioural effects of deliberate and selective lesioning of this tract. Typically, those studies which have used electrolytic or aspiration lesioning techniques of cingulate cortex have presumably also damaged the cingulum bundle.

This issue was considered by Meunier & Destrade (1988). In their study, they compared the effects of electrolytic lesions in ACc versus RSc in mice, on the acquisition and performance of the Hebb-Williams mazes. They found that the RSc lesions (which damaged both cingulum bundle and callosal fibres) had a severe effect on performance, whilst the corresponding ACc lesions (which did not damage the cingulum bundle) did not.

Meunier & Destrade (1988) hypothesised that cingulum bundle damage was the cause of the impairment and tested this by including a group of animals with ibotenic acid lesions of the same regions (ibotenic acid does not damage fibres of passage). Such lesions had no effect upon the acquisition of the mazes.

4.4.6 Retrograde thalamic degeneration.

A further complicating factor was highlighted by Sutherland et al., (1988) who reported that cingulate lesions may result in retrograde degeneration in the anterior thalamus, which may be further exacerbated by damage to the cingulum bundle, thus, thalamic and not cingulum bundle damage may be responsible for some of the observed deficits. In this experiment, degeneration in the anterior ventral nucleus of the thalamus was noted, and, due to the lack of impairment seen in the retrosplenial group, it is likely that damage to this nucleus is not sufficient to produce the spatial learning impairments associated with more extensive anterior thalamic damage (Aggleton et al., 1991a; Aggleton & Sahgal, 1993). The finding of a lack of impairment in the animals with cingulate cortex damage who also demonstrated degeneration in the AV nucleus supports the findings of a recent report by Aggleton et al., (manuscript submitted). The authors compared the effects of neurotoxic lesions in AV, AM, and AV + AM in rats, on a range of maze-type tasks. The consistent finding was that while animals with AV and AM damage alone were only mildly impaired only those with combined AM + AV damage were severely impaired.

To summarise the key points of this experiment it appears that anterior cingulate cortex does not appear to contribute greatly to spatial working memory as measured by the tests used in experiments 2a - 2c. This is not surprising as the previous experiment showed that total neurotoxic damage to MD (which shares connections with ACc) also had no effect on the performance of the DNMP task. Secondly retrosplenial cortex, also does not appear to contribute to spatial memory to the degree that has been emphasised in previous reports (e.g. Sutherland & Hoesing, 1993; Vogt et al., 1992). This is somewhat surprising given the important links between hippocampus and anterior thalamus with this region. This is not to say that this region of cortex provides no contribution to learning and memory, but that its degree of involvement is likely to be more complicated than previously thought.

Of critical importance is the extent of the damage within this region, and in the type of lesioning performed. Thus, the involvement of other regions of medial frontal cortex (notably prelimbic cortex), the anterior thalamic nuclei, and the cingulum bundle, may prove to be of critical importance. The following experiments will address these issues in greater detail, and will concentrate upon an analysis of the effects of damage to cingulate cortex in its entirety, and to the cingulum bundle.

CHAPTER 5. EXPERIMENT 3: THE CONTRIBUTION OF TOTAL CINGULATE CORTEX, AND THE CINGULUM BUNDLE ON TESTS OF SPATIAL WORKING MEMORY.

5.1 INTRODUCTION.

The discussion of the previous experiment has highlighted the fact that other regions of medial frontal cortex, and the fibre tracts underlying them, may be of key importance in assessing the contribution to spatial memory of this region. It is not within the scope of this thesis to include a full analysis of prelimbic cortex, or prefrontal cortex, but instead will focus upon the possible role of the cingulum bundle, and of the cingulate cortex as a whole. To summarise the discussion from the previous experiment, three key reasons for the lack of effect of anterior cingulate and retrosplenial cortices on the tests of spatial working memory were proposed.

Firstly, in some studies using similar methods to the experiments previously described in this thesis (e.g. Dunnett, 1990), anterior cingulate lesions have included other regions of prefrontal cortex, most notably the prelimbic cortex (area 32), a region which receives direct hippocampal inputs (Jay & Whitter, 1991), and which appears to contribute to spatial learning (Brito et al., 1982; Brito & Brito, 1990).

Secondly, the lesions described in experiment 2 did not fully encompass anterior cingulate cortex or retrosplenial cortex, the obvious solution being to produce much more extensive lesions in order to fully evaluate the relative contributions of these regions. There is also the possibility that it may be necessary to remove both ACc and RSc in tandem to assess the contribution of the cingulate region to these tasks. Evidence that the anterior thalamic nuclei project to both regions (Horikawa et al., 1988; Shibata, 1993a; 1993b) adds further weight to the idea of total cingulate removal.

Thirdly, several previous reports describing severe effects of cingulate cortical damage, have tended to use either radiofrequency, or aspiration techniques which can destroy fibre pathways (for example Dunnett, 1990).

The key fibre pathway under question here is the cingulum bundle, damage to which would not only disrupt fibres connecting different parts of cingulate cortex, but would also disrupt connections from septum and anterior thalamus to the hippocampal formation (Domesick, 1970; Mufson & Pandya, 1984; Saper, 1984; Wouterlood et al., 1990).

The purpose of this experiment was to compare the behavioural effects of lesions encompassing total cingulate cortex (ACc plus RSc), and the cingulum bundle, with those arising from damage to the hippocampal system (the fornix), on various tasks of spatial memory. These behavioural tasks consisted of the automated DNMP and automated spatial discrimination tasks described in experiments 1 & 2, and a forced-choice alternation task performed in a T-maze as described in experiment 2. This experiment includes a fornix lesion in order to make direct comparisons with the effect of hippocampal system damage. Many previous studies have shown that damage to the fornix produces impairments in a variety of spatial memory tasks in both rats and monkeys (Aggleton et al., 1991a; 1992; Sutherland & Rodriguez, 1989; Gaffan & Harrison, 1989).

Neurotoxic lesions were used to produce the cortical damage, whilst radiofrequency lesions were used to produce both fornical, and cingulum bundle damage. While the latter produced some damage to cingulate cortex this was considered less than that in the total cingulate neurotoxic group, and the co-ordinates were selected to minimise damage outside the region of the cingulum bundle.

5.2 MATERIALS AND METHODS.

5.2.1 Subjects.

The study involved 39 naive male rats of the pigmented DA strain (Bantin and Kingman, Hull). Throughout the period of the experiment the animals were housed individually under diurnal conditions (14hr light/10hr dark), all testing occurring at a regular time during the light period. The animals were tested for five days a week, the day before testing each receiving approximately 15g of RMI laboratory diet (Special Diet Services, Witham, Essex) daily so that they did not drop below 80% of normal body weight. At the start of testing the animals were aged 4 months and weighed between 215-245g. All animals had free access to water.

5.2.2 Apparatus for experiments 3a and 3b: DNMP and spatial discriminations.

All testing was carried out in 7 operant chambers whose characteristics have been fully described in the two previous experiments.

5.2.3 Apparatus for experiment 3c: T-maze alternation.

All testing was carried out in a T-maze, the characteristics of which have been fully described in the previous experiment.

5.2.4 Procedure for experiment 3a: DNMP.

1. Training: The full training procedure consisting of magazine training, autoshaping and initial training on the DNMP task has been fully described in experiments 1 and 2a. In this current experiment, the training procedure was modified slightly so as to reduce the amount of pretraining and also to introduce a longer delay of 64s which, in terms of length of delay, would make the DNMP task more analogous to the T-maze task. Once the animals reached the criteria of two successive daily sessions at 85% + at delays of 0 - 2 seconds, they each performed a further 8 daily sessions containing an equal mixture of 0, 2 and 4s (four sessions), 0, 2, 4, 8, 16, and 32 s (two sessions), and 0, 4, 8, 16, 32, and 64s (two sessions).

2. Post-operative performance: Following this, all rats received the appropriate surgery, approximately six weeks after which, all animals were retested on the DNMP task; firstly for 3 daily sessions of 0 - 8s delays, over 60 trials, a further ten sessions comprising a balanced mixture of 0, 2, 4, 8, 16, and 32s, over 96 trials, and finally, five sessions of 96 trials, each comprising a balanced mixture of 0, 4, 8, 16, 32, and 64s. Data analysis was carried out on the final fifteen daily sessions.

3. Performance measures and analyses: The data from the 0-32s and the 0-64s delay conditions were analysed separately to provide the various indices of accuracy, responsivity and bias that have been fully described in the previous experiments.

Once again, the data in this experiment was transformed as appropriate (arcsin: all accuracy and bias indices, logarithmic: latencies, square-root: misses), and analysed by parametric analysis of variance (ANOVA). As in the previous experiments , when the F-ratios were significant, the means were compared using the Newman-Keuls procedure (Winer, 1971).

5.2.5 Procedure for experiment 3b: Spatial discrimination and reversal.

One week after completing the DNMP task, all animals were trained on a spatial discrimination task which has been fully described in experiments 1b and 2b. All animals received 20 daily sessions, the position of the '*correct*' lever for each session following a balanced, pseudorandom sequence.

5.2.6 Procedure for experiment 3c: T-maze alternation.

Testing began approximately 12 weeks after completion of the lever discrimination testing. A full description of the T-maze procedure has been presented in experiment 2c. Briefly, all animals received six acquisition trials per daily session for a total of six days, with a 15s delay. This was immediately followed by a further 10 sessions in which the 6 trials were divided equally between those with retention delays of either 10s, 30s, or 60s, making a total of 20 trials at each delay.

5.2.7 Surgical and histological procedures.

A total of 39 rats received surgeries. Five animals died following surgery leaving a total of 34 animals divided into four groups; surgical controls (SHAM n=12), total cingulate cortex (TCc n=8), cingulum bundle (CB n=8), and fornix (Fx, n=6).

Each animal was anaesthetised by intraperitoneal injection (6ml/kg) of Sagatal. The animal was then placed in a stereotaxic headholder (David Kopf Instruments, Tujunga), and the scalp retracted to expose the skull. A craniotomy was then made above the sagittal sinus and the dura cut to expose the cortex above the appropriate region.

For the total cingulate cortex (TCc) lesions injections of 0.3 μl of 0.09M N-methyl-D-aspartic acid (NMDA) (Sigma Chemical Company Ltd., Poole) dissolved in phosphate buffer (pH 7.2) were made through a 1 μl Hamilton Syringe into 5 sites in each hemisphere. The stereotaxic co-ordinates relative to ear-bar zero, with the incisor-bar set at + 5.0 relative to the horizontal plane for the surgical groups were as follows: for the first site AP +7.6, LAT ±0.7, then for all the rest AP +6.0, +4.3, + 2.6, +0.9, with the LAT being ±0.8 in all cases. The depth at each of the five sites was 2.0mm, 2.0mm, 1.7mm, 1.7mm and 2.2mm from the top of cortex respectively. Each injection was made gradually over a 4-min period and the needle allowed to remain in situ for a further 4 minutes before being withdrawn. After completion of the surgical procedure sulphanilamide powder was applied, and the skin sutured followed by an intraperitoneal injection of 6ml of saline plus 0.3ml of milophyline.

The initial stages of the cingulum bundle lesions (CB) were the same as those described above for the TCc group but the actual lesion was made by radiofrequency. A Radionics TCZ (Radionics Inc., Burlington) electrode (0.3mm tip length and 0.25mm diameter) was lowered vertically into 3 sites per hemisphere and the tip temperature raised to 75°C for 60s using an RFG4-A Lesion Maker (Radionics Inc., Burlington), at one depth, and then the tip was raised by 0.3mm, and another lesion was made (for the same duration, and using the same temperature). The co-ordinates of these lesions, relative to ear-bar zero were as follows: AP +7.9, LAT ±1.1, AP +3.7, LAT ±1.0, and AP +1.4, LAT ±0.9. The depth at all three sites was 2.1mm (-0.3mm), 1.7mm (-0.3mm), and 1.9mm (-0.3mm), below the top of cortex respectively.

The animals in the fornix (Fx) group underwent the same procedure as the CB group with only 2 radiofrequency lesions being made in each hemisphere. The stereotaxic co-ordinates of the lesions relative to ear-bar zero were AP +5.3, LAT ±0.7 and AP +5.3, LAT ±1.7. The depth at both sites was 4.5mm and 3.7mm below the top of cortex respectively.

The animals acting as surgical controls (SHAM's) received the same treatment as the TCc group, but no injection of NMDA was made.

On completion of the experiment the animals were killed and perfused intracardially with 5% formol-saline. The brains were then rapidly removed and placed in 5% formol-saline. Subsequently, the brains were blocked, embedded in wax (Paraplast), and cut into 10- μm coronal sections. Every tenth section was mounted and stained with Cresyl violet, a Nissl stain.

5.3 RESULTS.

5.3.1 Histological Analysis.

The lesions in the TCc group consistently involved the entire extent of the cingulate (anterior cingulate and retrosplenial) cortices that lie dorsal to the corpus callosum. The cell loss in the anterior cingulate cortex began at the genu and continued caudally to the retrosplenial cortex. The lesions extended dorsally to include ACAv, ACAd, and parts of the adjacent secondary motor area (Swanson 1992), see figure 23. The retrosplenial damage was continuous and extended caudally up to and beyond the splenium. Throughout the level of the corpus callosum both the ventral and the dorsal parts of retrosplenial cortex showed an almost complete loss of cells. Caudal to the splenium however there was noted a sparing of retrosplenial cortex, but the involvement of the ventral retrosplenial cortex often continued to near its caudal limit (figure 24). Some cellular loss was observed in those parts of the hippocampal field CA1 in closest proximity to the ventral retrosplenial cortex (figure 24). In all cases there was evidence of cellular degeneration in the anterior ventral (AV) thalamic nucleus, although the cingulum bundle appeared to be unaffected.

The cingulum bundle (CB) surgeries involved three bilateral radiofrequency lesions placed at different AP levels. In all cases there was very considerable, bilateral damage to the bundle, at more than one level (figure 23). Consistent with this was very marked cellular loss in the anterior ventral (AV) thalamic nucleus, which sometimes extended into the most lateral part of the anterior medial (AM) nucleus. The lesions did not involve the fornix, but in four of the eight animals there was very minor damage to the dorsal limit of the hippocampus (principally to the alveus) in a very restricted anterior - posterior portion below the mid AP lesion. It should be noted that in three of these four cases this hippocampal damage was only unilateral.

The extent of the damage to those parts of the anterior cingulate and retrosplenial cortices adjacent to the cingulum bundle sites was variable, but in the majority of cases the lesions were quite selective, with limited direct cortical damage (figure 24). Even in those cases with the greatest cingulate cortex damage (figure 23) the extent of the lesion at the level of the radiofrequency probe was less than that of the NMDA cases and there were always areas of intact cortex between the three radiofrequency lesion sites.

The fornix (Fx) lesions were extensive with three out of the six animals receiving such surgeries demonstrating a completely severed tract. In two of the remaining animals the fimbria/fornix was completely cut in one hemisphere, and only the most lateral tips of the fimbria were spared. In the animal with the smallest lesion the most lateral tips were of the fimbria were spared bilaterally (figure 23). In four of the Fx animals there was very slight involvement of the most dorsal limbs of the anterior ventral (AV) and anterior dorsal (AD) thalamic nuclei.

Following histological analysis none of the animals were discarded from their respective surgical groups and so the groups consisted of 12 SHAM, 8 TCc, 8 CB, and 6 Fx animals.

Reconstructions of total cingulate cortex, cingulum bundle, and fornix surgeries.

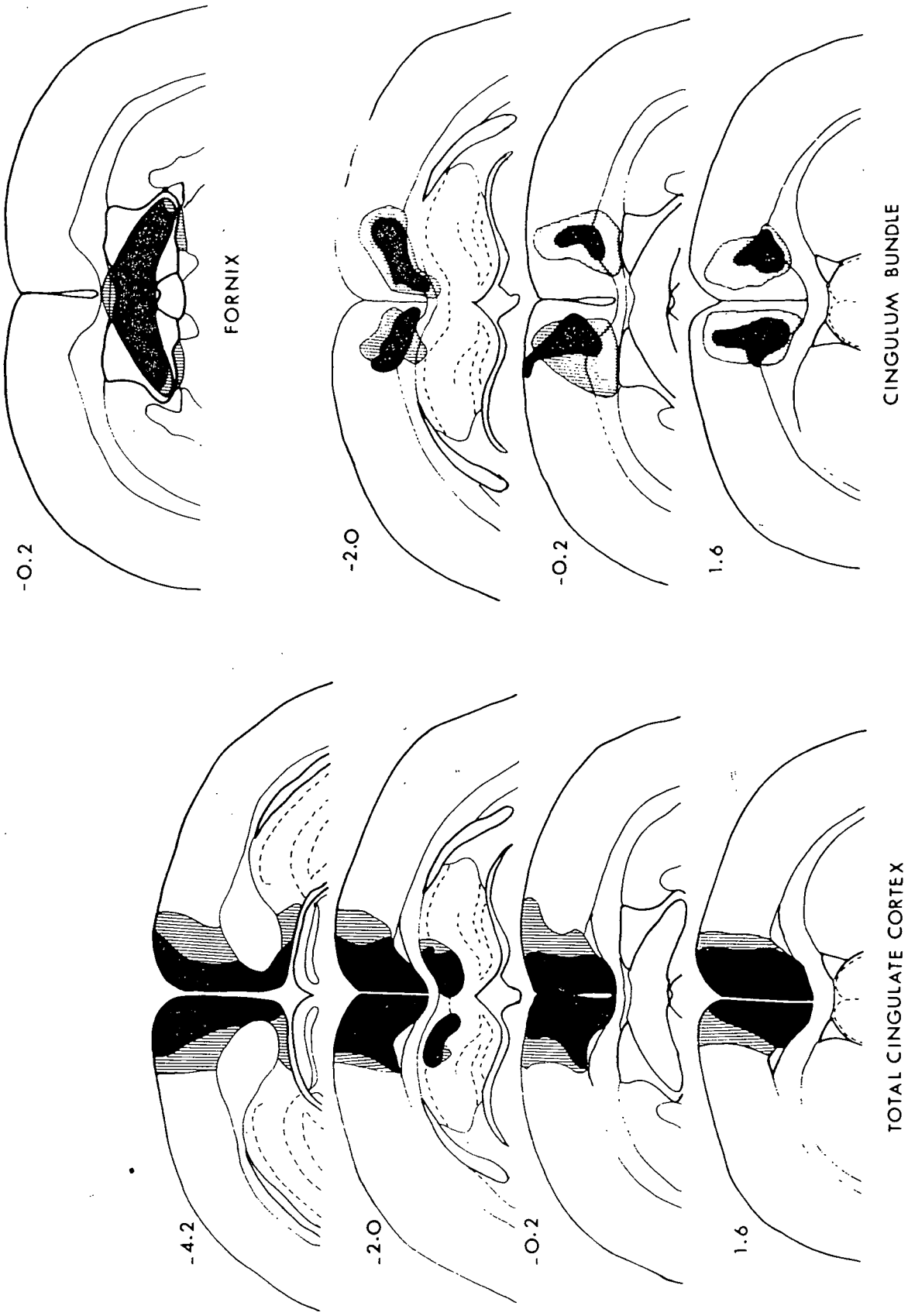


Figure 23. Diagrammatic reconstruction's showing the extent of the total cingulate (TCc), cingulum bundle (CB) and fornix (Fx) lesions.

Photomicrographs showing the appearance of the cingulate cortex following injection of a neurotoxin, and the cingulum bundle following a radiofrequency lesion.

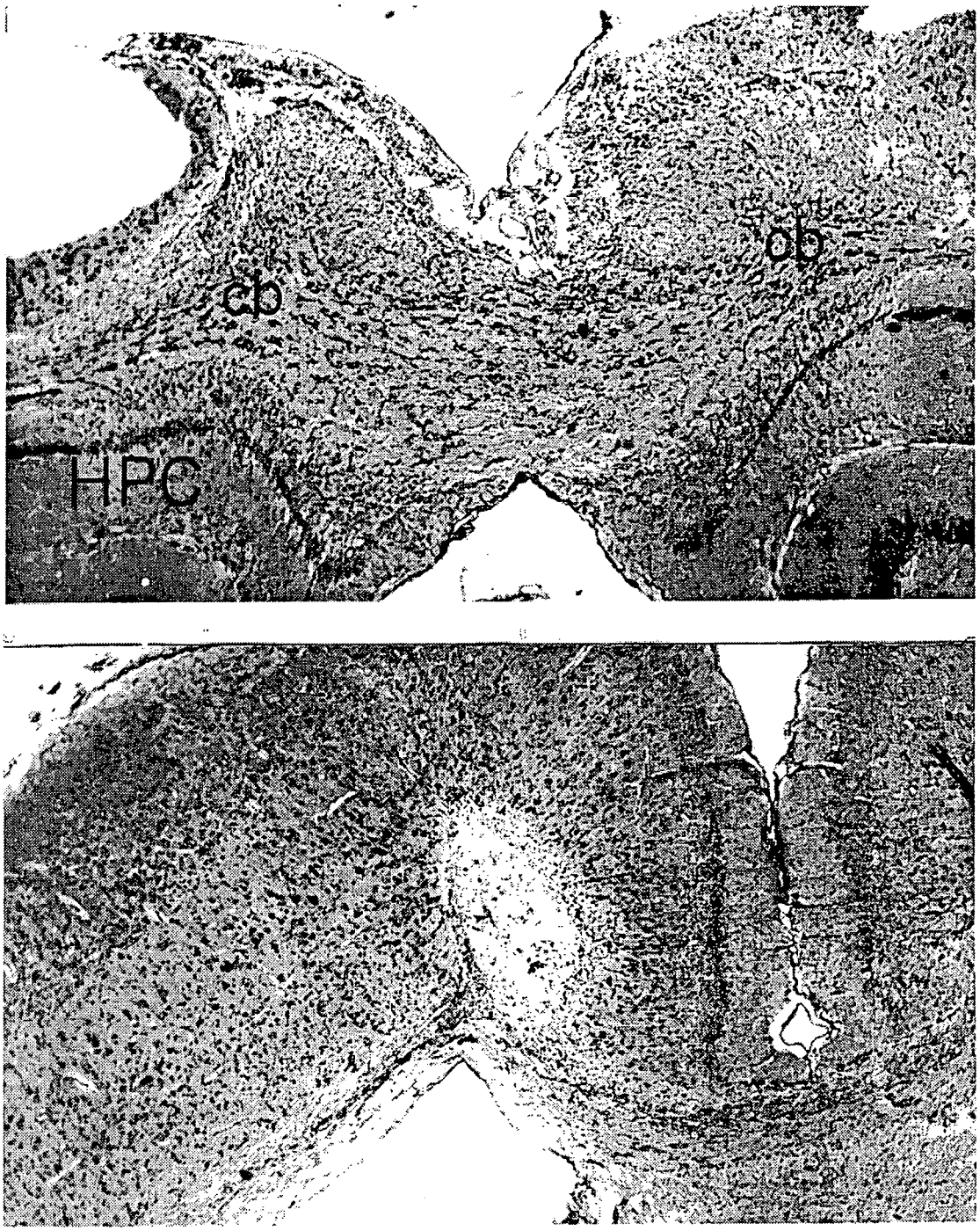


Figure 24. Photomicrograph of upper: coronal section (Nissl stain) showing the appearance of cingulate cortex following injection of NMDA, and lower: coronal section (Nissl stain) showing the appearance of the cingulum bundle following a radiofrequency lesion. HPC = hippocampus; cb = cingulum bundle.

Photomicrograph showing the appearance of the thalamic AV nucleus following neurotoxic lesions of cingulate cortex.

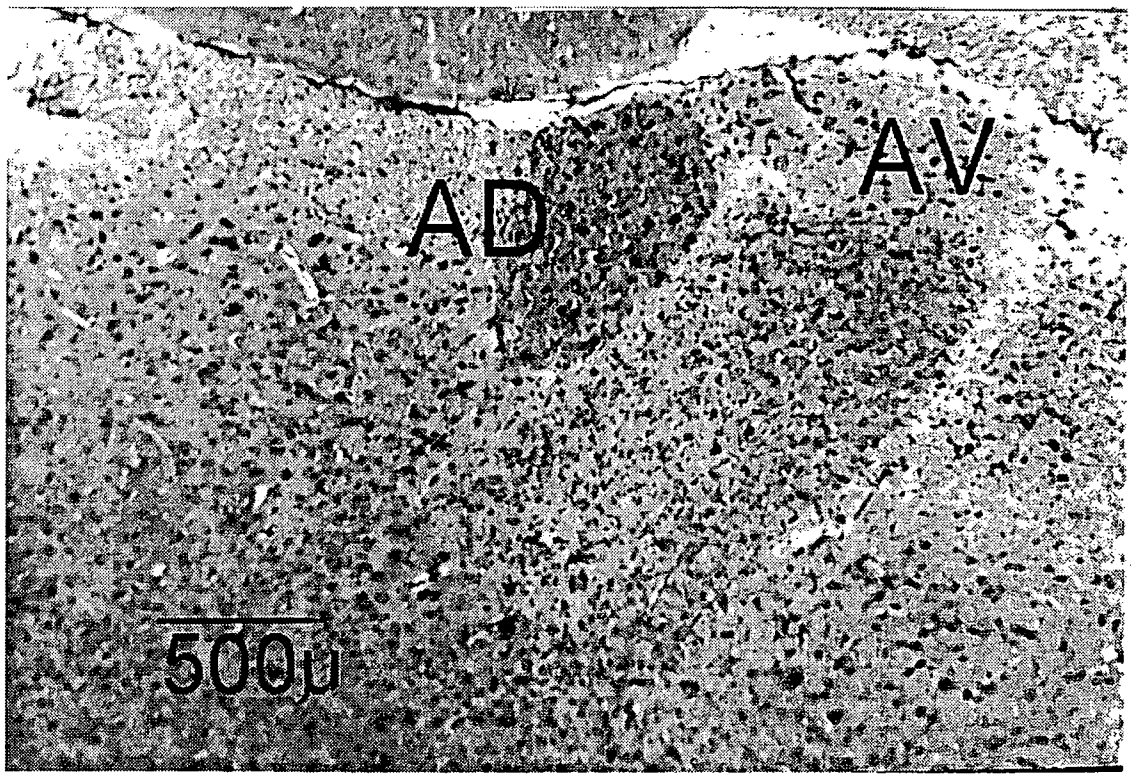


Figure 25. Photomicrograph of coronal section (Nissl stain) showing the appearance of retrograde degeneration of AV following radiofrequency lesioning of the cingulum bundle. AV = anterior ventral nucleus; AD = anterior dorsal nucleus.

Note that the fornix damage was comparable to that reported in experiment 2 and presented in figure 17 thus no photomicrograph is presented here.

5.3.2 Experiment 3a: DNMP.

1. Accuracy measures (32s): An analysis of variance using the percent correct scores over the 10 daily sessions comprising 0-32s delays revealed a significant group effect ($F_{3,31} = 4.93$ $P<0.01$), and a group X delay interaction ($F_{15, 155} = 1.78$ $P<0.05$); there was also an effect of delay ($F_{5,155} = 408.37$ $P<0.001$). A subsequent Newman-Keuls test revealed that the group effects reflected the significantly poorer performance of the Fx group. Neither the TCc or CB groups differed from the SHAM group, but all three differed from the Fx animals ($P<0.05$), see figure 26.

DNMP Percent Correct Scores.

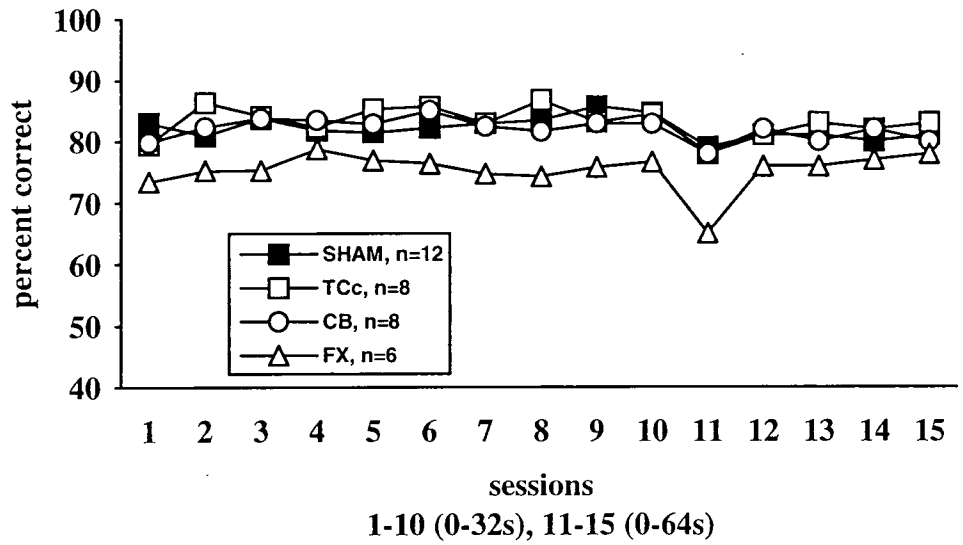


Figure 26. Mean percent correct scores on the DNMP task over each of the 15 postoperative sessions using pooled delay data from the two delay conditions, 0-32s (sessions 1-10), and 0-64s (sessions 11-15).

A similar pattern of results was found for the two TSD measures of sensitivity, A' and SI. Both of these measures not only revealed significant effects of delay ($P<0.001$), but also indicated a significant group effect (A', $F_{3,31} = 4.30$, $P<0.05$; SI, $F_{3,31} = 3.09$, $P<0.05$). In addition, index A' demonstrated a significant group X delay interaction ($F_{15\ 155} = 1.84$, $P<0.05$).

In the case of A' the group effects once more reflected the abnormal behaviour of the Fx group which differed from all the other groups ($P<0.05$), but for index SI, the Fx animals only differed from the CB group ($P<0.05$), see figure 28.

2. Accuracy measures (0-64s): The average percent correct scores over the final five daily sessions are shown in figure 26, and the percent correct performances of each group as a function of delay are shown in figure 27. Over the final 5 sessions utilising the longer delay, all three accuracy measures revealed the expected effects of delay ($P<0.001$ in all cases), and index SI demonstrated a day X delay interaction ($P<0.01$). Furthermore, the percent correct measure revealed both a day effect ($P<0.001$), and a group X delay interaction ($P<0.05$), this interaction reflecting the poor performance of the Fx group (see figure 26).

None of the accuracy measures however demonstrated a significant group effect over all five sessions combined ($F_{3,30} = 2.46$, $P>0.05$), though there was a significant group difference on the very first session. This reflected the severe effect of this sudden increase in delays of the Fx group ($F_{3,33} = 6.98$, $P<0.01$), see figure 26. The most likely reason for the lack of overall group effect is that the longer delays produced a floor effect (see figure 27). As a consequence, these conditions reduced the number of informative delays (those not affected by ceiling or floor effects) and hence reduced any apparent group difference. Also, the scores of the TCB and CB groups (which are essentially the same as those of the SHAM group) served to add 'noise' to the analysis.

When the scores from these groups are ignored, and the SHAM and Fx groups compared using a t-test (two-tailed with 8 degrees of freedom), a significant group effect becomes clear ($t= 2.85$, $P<0.05$). In the 0-32s sessions it was not necessary to remove these groups from the analysis to see the group effect, as in those sessions, the ceiling and floor effects were not as pronounced.

DNMP, Percent Correct Scores As A Function Of Delay

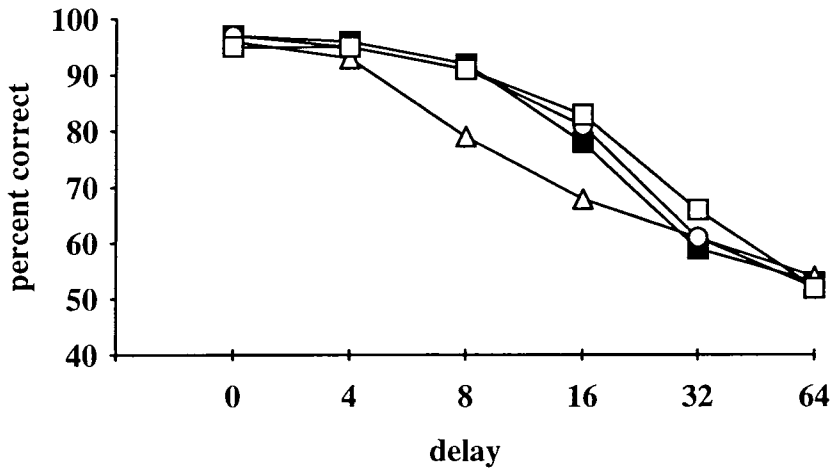
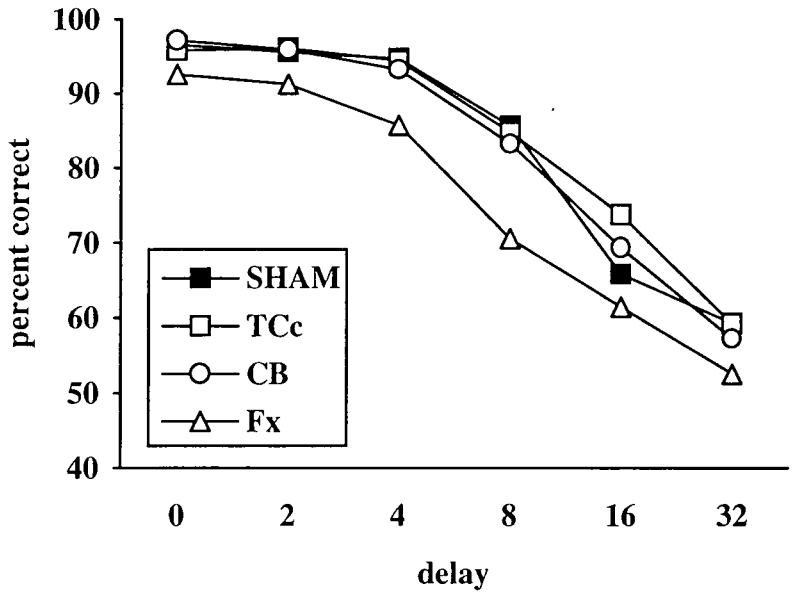


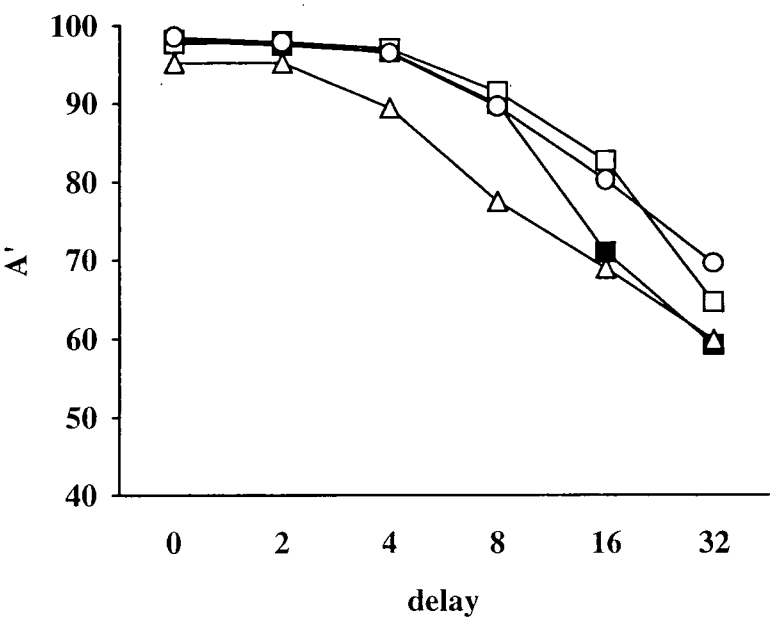
Figure 27. Percent correct performance on the DNMP task as a function of delay over the final five sessions combined.

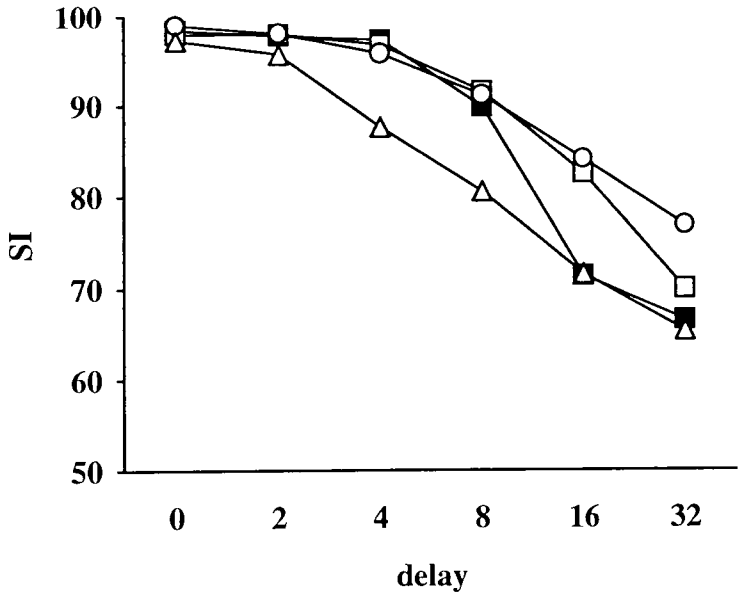
3. Bias indices (0-32s): As expected from the previous experiments, the three bias indices (Iy, B" and RI) demonstrated delay effects (P<0.001) in all cases, whereas only index B" revealed a significant effect of session (F 9,279 = 1.99, P<0.05). In addition, each of the measurements of bias revealed a significant group effect (Iy, F 3,31 = 4.69, P<0.01; B", F 3,31 = 5.51, P<0.01; and RI, F 3,31 = 4.89, P<0.01), and for one of the indices there was also a significant group X session interaction (Iy, F 27,279 = 1.74, P<0.05).

For all three measures of bias the significant group effects could be attributed to the Fx group which consistently showed higher levels of bias than the other three groups. For index RI, all three groups differed from the Fx group but did not differ from one another (P<0.05), but for indices Iy and B" the Fx group differed from both SHAM and CB groups but not from the TCc group. Finally, on index Iy, the SHAM and CB groups differed (P<0.05). See figure 28.

DNMP, Bias Indices.



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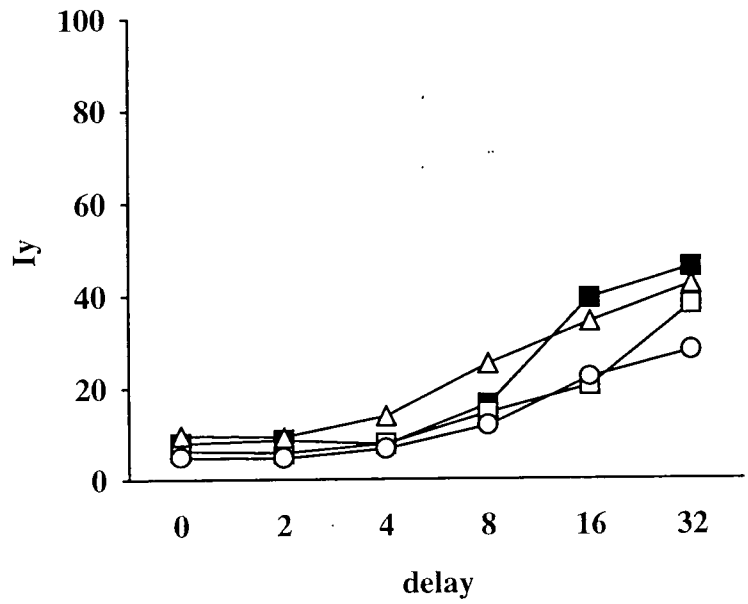
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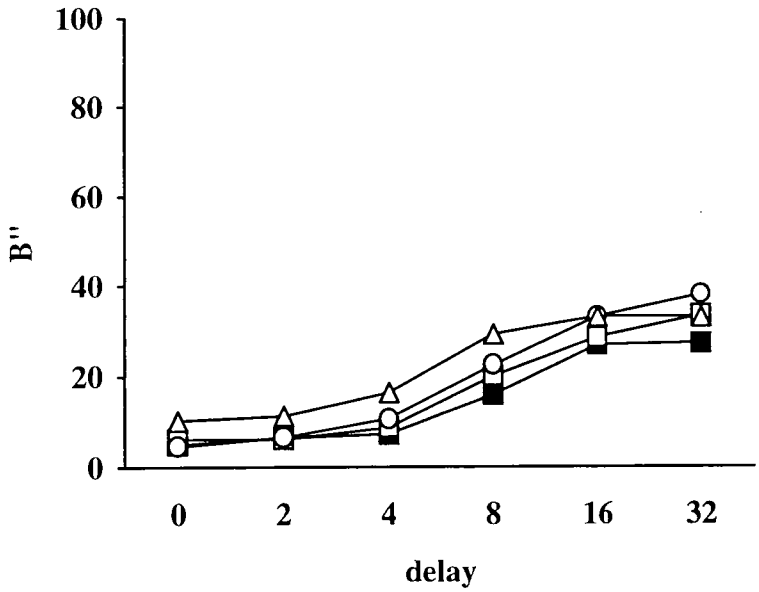


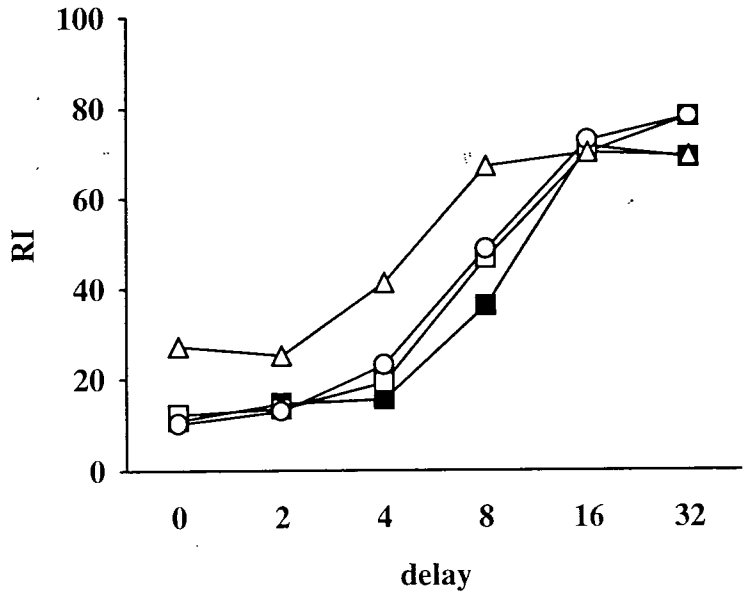


Figure 28. Accuracy (first three) and bias (final three) indices as a function of lesion for the ten 0-32s delay sessions. See text for explanation of indices. All scores have been converted to read from 0 to 100. In the case of the bias scores, 100 represents a complete bias, and 0 neutral or no bias.

4. Bias indices (0-64s): All three bias indices showed the expected effect of delay ($P<0.001$) in all cases, and both B" and RI showed a significant effect of session ($P<0.001$) in both cases. Lastly, only index RI revealed a significant group X delay interaction ($P<0.05$), which was due to an increase in bias shown by the Fx group. Once again, no significant group effects were revealed.

5. General responsivity (0-32s): There were no group effects for several of the general measures of responsivity. These were total number of misses recorded ($P>0.05$), and magazine response (nose-poke) rate ($P>0.05$). Group differences were found, however, for mean latency of first magazine response ($F_{3,31} = 6.85$, $P<0.01$), mean latency to sample ($F_{3,31} = 12.21$, $P<0.001$), and finally for the average choice latency ($F_{3,31} = 3.15$, $P<0.05$). In the case of the mean latency to sample, although both the SHAM and Fx groups differed from each other, they were also both significantly quicker to respond than the TCc and CB groups. In the case of mean latency of first magazine response, the group difference here reflected the significantly slower response time of the CB group which differed from all three other groups ($P<0.05$), there were no other group differences.

6. General responsivity (0-64s): Responsivity data from the 0-64s delay sessions (unlike that gained from the 0-32s) revealed significant group effects for both the total number of misses recorded ($F_{3,30} = 3.12$, $P<0.05$) and for the magazine response (nose-poke) rate ($F_{3,30} = 4.25$, $P<0.05$). Subsequent Newman-Keuls tests revealed that the SHAM group made significantly more misses than the other groups ($P<0.05$ in each case) and both the fornix and SHAM animals made significantly fewer nose-pokes than the TCc or CB groups ($P<0.05$ in each case). No other group differences were revealed.

5.3.3 Experiment 3b: Spatial discrimination and reversal.

Experiment 3b consisted of a spatial (lever) discrimination task (sessions 1 and 2) followed by a series of reversals between specified sessions. Several comparisons were made:

a) The initial 2 sessions (discrimination): The mean correct responses with their standard deviations in parenthesis for the four groups over the first two discrimination sessions were as follows: SHAM = 69.8 (12.6), Fx = 49.2 (26.8), TCc = 72.9 (12.5), and CB = 65.9 (11.8). An ANOVA carried out on these scores revealed a significant group effect ($F_{3,33} = 3.05$, $P<0.05$) which reflected the significantly poorer performance of the Fx group relative to the TCc and SHAM groups, see figure 29.

b) The remaining 18 sessions: The mean percent correct responses for the four groups over the following 18 sessions were as follows: SHAM = 77.2 (3.8), Fx = 75.1 (4.4), TCc = 79.7 (3.7), and CB = 76.9 (4.7). An analysis of these sessions revealed no significant group effect ($F_{3,30} = 1.53$, $P>0.05$) see figure 29.

Spatial Discrimination and Reversal, Percent Correct Scores.

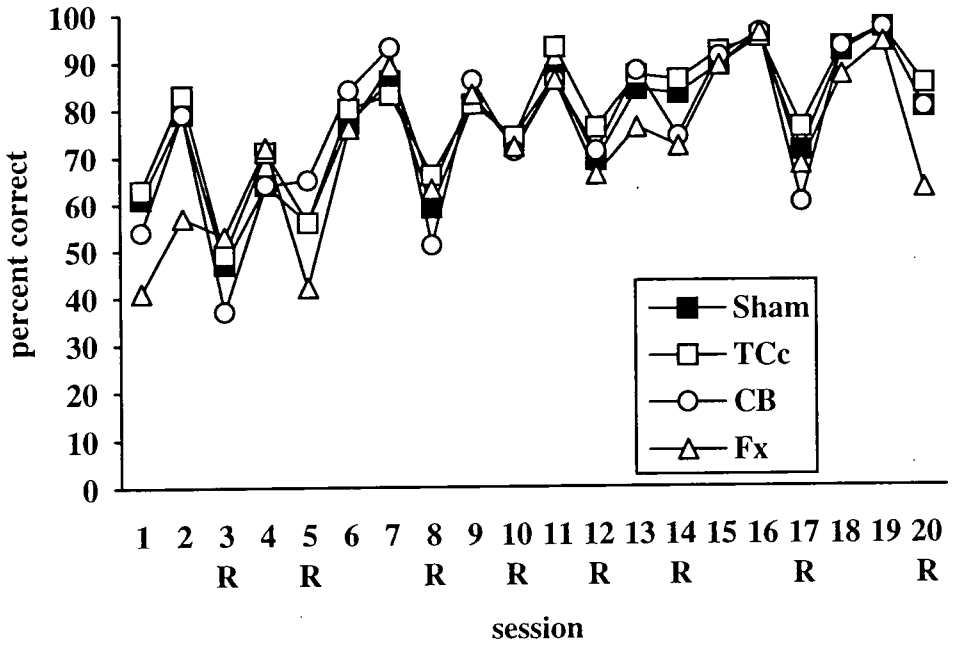


Figure 29. Mean percent correct scores on the lever discrimination and reversal task over 20 sessions. Sessions where the correct lever changed from the preceding session ('reversal') are labelled as 'R'.

By excluding the first session, the scores could be divided between those nine sessions where the correct lever was in the same position as in the previous session, and those ten sessions where the correct lever reversed sides between sessions (Figure 30).

a) 'Consistent' versus 'reversal' sessions: The mean percent scores of each group over both 'consistent' and 'reversal' sessions is shown in figure 30 (right side). While the mean scores on the '*consistent*' sessions were all similar, a clear difference arose for the '*reversal*' sessions. Subsequent analysis of these two sets of scores confirmed that all groups performed considerable worse on the reversal sessions ($F_{1,30} = 164.9$, $P<0.001$). The analysis also revealed a significant group effect ($F_{3,30} = 3.42$, $P<0.05$); a Newman-Keuls test showed that this reflected the poorer performance of the Fx group on the reversal sessions relative to the TCc group ($P<0.05$). There were no other group differences.

b) The first ten trials of each session: A final set of analyses considered only the first ten trials of each session, when the effects of the previous session might be expected to be most apparent. The mean percent correct scores for the first ten trials over both sessions are shown in figure 30 (left side). An ANOVA was performed on the 'consistent' and the 'reversal' trials and found the expected effect of session, ($F_{1,30} = 100.82$, $P<0.001$), as all groups performed better on the 'consistent' but there was no overall group effect ($F<1$), see figure 30 (left).

Spatial Discrimination and Reversal, 'Consistent' and 'Reversal' Sessions.

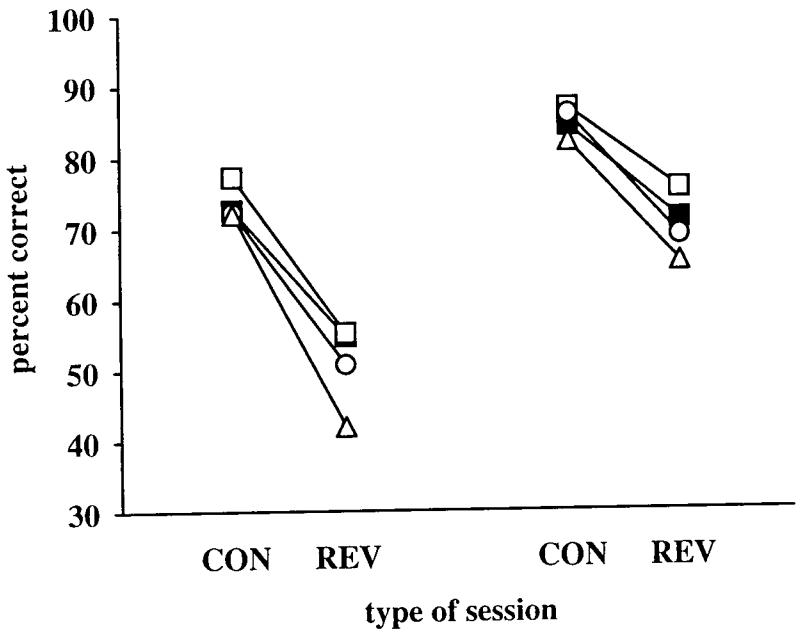


Figure 30. Mean percent correct scores on the lever reversal task grouped according to those days on which the correct lever was 'consistent' with the previous session and those days on which it was a 'reversal' from the previous session. Left: using the scores from the first ten trials of each session. Right: Using the scores from each complete session.

5.3.4 Experiment 3c: T-maze alternation.

a) Acquisition: During the six acquisition sessions each animal performed a total of 36 trials with a retention delay of 15s, the mean scores for each group are shown in figure 31. Analysis of these scores using an ANOVA revealed a highly significant group effect ($F_{3,30} = 25.1$, $P<0.001$), which a subsequent Newman-Keuls test showed to be due to the poorer performance of both the Fx and CB groups, who differed from both SHAM and TCc groups ($P<0.01$ in each case), but did not differ from one another.

b) Delays: All groups then performed a total of twenty trials at each of three delays (10s, 30s, and 60s), the mean percent correct scores of each group are shown in figure 31. As expected, these scores revealed a highly significant effect of delay ($F_{2,56} = 6.52$, $P<0.01$), and also a very significant group effect ($F_{3,28} = 12.65$, $P<0.001$). As with the acquisition trials, a Newman-Keuls test revealed that this group effect reflected the poorer performances of both Fx and CB groups both of whom differed from SHAM and TCc groups ($P<0.01$ in both cases), but did not differ from one another ($P>0.05$).

c) Acquisition and delay scores combined: A final analysis which compared the total scores of each group taken over both acquisition and delay sessions showed the expected Fx and CB group effects ($F_{3,30} = 24.3$, $P<0.001$). A subsequent Newman-Keuls test showed that both Fx and CB groups differed from the SHAM and TCc groups ($P<0.001$ in both cases), but did not differ from one another.

T-Maze Alternation, Percent Correct Scores.

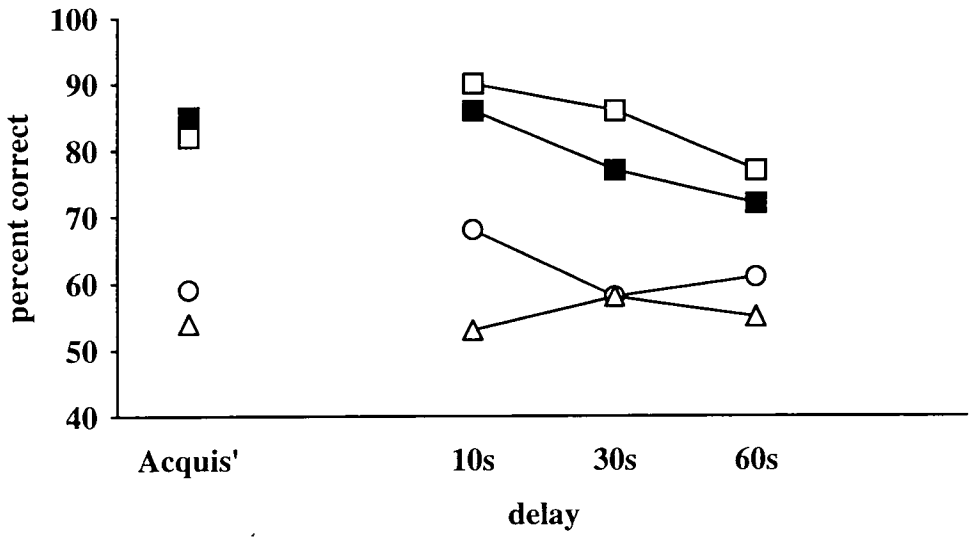


Figure 31. Spatial forced-alternation in the T-maze. The graph shows the mean scores of the four groups over the initial six acquisition sessions, and the mean scores of the same groups when tested for 20 trials over each of three retention intervals.

5.4 DISCUSSION.

Experiments 2a-2c revealed that lesions to either ACc or RSc had no discernible impairment upon an operant DNMP task, a lever discrimination and reversal task, and a test of forced-alternation in the T-maze, findings in contrast to those reported by such as Sutherland & Hoesing (1993). Two reasons were proposed for this lack of impairment, firstly that it may be necessary to damage the entire cingulate cortex to reveal a deficit; or secondly, that the contribution of fibres of passage - the cingulum bundle in particular, may be crucial.

To assess these conjectures, the experiments described above (3a-3c) investigated the effects of damage to total cingulate cortex (TCc), and to the cingulum bundle (CB). As in the previous experiment (2a-2c) a fornix lesioned group (Fx), was included.

While the animals with Fx damage showed severe impairments on the DNMP and T-maze, and a moderate impairment on the lever-discrimination task (much as in the previous experiment), the TCc animals showed no impairment on all three tasks. Interestingly, the animals with CB damage showed a dissociation in that they performed normally on the DNMP and lever reversal tasks, but were severely impaired (to a level which mirrored the performance of the Fx group) on the T-maze forced-alternation.

5.4.1 Experiment 3a: DNMP (0-32s condition).

As in the previous experiment (2a), radiofrequency lesions of the Fx produced a severe impairment on this test of spatial working memory, the performance measures consistently revealed a loss of accuracy, and an increase in bias. These results are consistent with those reported in other studies focusing on this task (Aggleton et al., 1991a; 1992; Dunnett, 1985). The results of experiment 2a had indicated that the Fx animals also tended to respond with shorter latencies to some of the responsivity measures, i.e. the mean latency to make the first magazine response; these findings were repeated in this experiment with the Fx animals again responding rapidly to the levers, and to the magazine flap, but this time not being significantly quicker than the SHAM group. Only in the mean latency to respond to the sample lever, were the FX and SHAM groups significantly quicker than the TCc and CB groups.

The TCc group showed no deficit in accuracy as measured by the percent correct scores, or the two measures derived from the theory of signal detection (A' and SI). Their performance matched that of the SHAM group at the shorter delays, and even exceeded it at the longer delays (see figures 26, 27, and 28). In the previous experiment (2a) both ACc and RSc groups along with the Fx animals, had shown an increase in perceptual bias as measured by index B".

In this experiment, the TCc group showed only a small increase compared to the SHAM group on this measure, but this difference was not significant. Indeed, on another bias measure (Iy), they showed much less bias than the SHAM group at the two longest delays, though did not differ overall (see figures 26, 27, and 28). Of the various measures of responsivity, the TCc group did show one significant difference from the other groups. On the measure of the latency to respond to the sample lever, the TCc group demonstrated significantly slower response times compared to both the SHAM and Fx groups.

The effects of selective lesions of the cingulum bundle on this task are as yet unknown, but clearly this group showed no performance deficit on the DNMP task, and performed at a level consistent with that of the SHAM and TCc groups (figure 26). Indeed, like the TCc group, the CB animals performed at a greater level than the SHAM group at the two longer delays (16s and 32s) as measured by the A' and SI accuracy measures (figure 27). Only one measure - the bias index Iy revealed a difference between SHAM and CB animals, with the CB group in fact performing with significantly less bias at the longest delay (32s).

5.4.2 Experiment 3a: DNMP (0-64s).

The final five sessions used a maximum delay of 64s, as opposed to 32s. Initially no significant group effects were found for both accuracy or bias performances. This result is surprising as a previous report (Aggleton et al., 1991a) using similar delay intervals, had found a severe deficit for both fornix and anterior thalamic nuclei lesions. That study was similar to this in that the animals first performed four sessions comprising 0-32s delays. and then a further four sessions of 0-64s delays; the subsequent data was pooled and revealed a severe fornix, and anterior thalamic impairment.

It can be seen from figure 26 that the performance of the Fx group remained fairly constant at around 75% during the 0-32s delay sessions, but suffered a severe drop in accuracy on the first session using 0-64s delays. Subsequently the performance of the Fx group improved on these longer delays, reaching, and maintaining the 75% level again on the second session. The performance of the other groups dropped slightly to a level almost comparable to that of the fornix animals.

However, the apparent lack of a clear group difference is obscured by the amount of variance added to the data by the TCc, and CB groups which performed comparably to the SHAM group and added little to the interpretation of the data. When the data from these two groups are removed, a significant group effect between SHAM and Fx groups becomes apparent. It is further evident from figure 27 that the inclusion of the 64s condition served to provide little additional information as each group performed at a comparable 'floor' level at this delay. Thus the inclusion of this data point also served to obscure the interpretation.

5.4.3 Experiment 3b: Spatial discrimination and reversal.

The lever discrimination and reversal task is a spatial test which can be solved using mainly 'egocentric' cues, though it remains unknown as to whether the animals do use such cues to solve the task. Neither the TCc, nor the CB groups showed any impairment on either the initial discrimination, or the subsequent reversals on this task. This lack of impairment following total cingulate cortex removal is consistent with the findings from experiment 2b which reported no impairment on this task following lesions of either ACc or RSc, and is also consistent with studies reporting no effect of cingulate cortex damage on egocentric spatial tests, in both rats (Markowska et al., 1989; Sutherland & Hoesing, 1993), and monkeys (Murray et al., 1989).

Like the DNMP task, the effect of selective cingulum bundle lesions on such discrimination and reversal tasks are as yet unknown, but the results from this experiment clearly show no discernible impairment. With the large amounts of similarity of this task to the DNMP task, perhaps such a result is not surprising. Experiment 2b had highlighted the lack of difficulty for the Fx animals to learn the initial discrimination, though they were impaired on the subsequent reversals. In this replication, however, it was found that the Fx group were impaired at the initial discrimination (the first two sessions).

Inspection of the individual scores for the Fx animals indicate that the poor performance of this group as a whole was heavily weighted by the abnormal performance of one animal who only averaged 2.5% over the first two sessions. This animal's performance improved over the subsequent sessions and became no different to the other animals in the group.

The reason for this bad start is probably due to perseverative effects continued from the previous DNMP test. When this animal's score was ignored from the analysis of the initial discrimination, the Fx group average rose accordingly and the overall group effect disappeared, (P>0.05). While the difference between this and the previous experimental result concerning the initial discrimination could be reconciled, the finding of no overall group differences on the subsequent 18 trials when the correct lever reversed sides is slightly harder to explain.

Experiment 2b had shown that the Fx group could learn the initial discrimination, but failed to rapidly shift their response when the correct lever reversed position, and so performed badly with respect to the other groups. In this experiment, the Fx group were unimpaired overall on the subsequent 18 sessions containing reversals. Importantly however, as in experiment 2b, there was still a significant group difference when the sessions were split between '*consistent*' and '*reversal*' sessions, the Fx animals again demonstrated a severe impairment when the lever reversed sides. This demonstrates that this task is indeed sensitive to hippocampal system damage.

5.4.4 Experiment 3c: T-maze alternation.

Experiment 2c had demonstrated that lesions of ACc or RSc had no significant effect on the ability of animals to perform a forced-choice alternation task in the T-maze, both over a set of acquisition trials, and subsequent sessions using a range of delays. In this experiment the performance of animals with lesions of total cingulate cortex was found to be unimpaired, both over acquisition and delay sessions, thus confirming that cingulate cortex is not involved in some aspects of spatial memory based upon the use of allocentric cues. Such a finding is in direct contrast to that of Markowska et al., (1989) who reported that aspiration lesions of cingulate cortex (ACc plus RSc) produced a severe deficit. This deficit (found on a DNMS T-maze discrimination task), was comparable to that of Fx lesioned animals, and indicated that cingulate cortex may be vital for certain types of allocentric spatial memory, e.g. the temporal order of allocentric information.

When one considers the performance of the CB animals on this T-maze task, it is not difficult to reconcile the above discrepancy. All of the studies which have reported severe deficits following cingulate cortex (particularly retrosplenial) damage, have used either aspiration, electrolytic, or radiofrequency methods which may have consistently damaged the cingulum bundle. In experiment 3c, selective damage to the cingulum bundle resulted in a severe impairment on the T-maze task, an impairment seen both in the initial acquisition sessions, and in the subsequent delay sessions; a deficit which was strikingly similar to that produced in the Fx group, and which was not seen in animals with either total cingulate, or retrosplenial cortex damage. This finding is in accordance with the report of Meunier and Destrade (1988) showing that cingulum bundle damage was the cause of the impairment of maze learning following electrolytic RSc lesioning in mice.

However, in contrast, Sutherland & Hoesing (1993) compared the effects of RSc lesions carried out using different methods, on the performance of rats on the Morris task. They completely aspirated area 29 in one hemisphere and either left the other hemisphere undamaged, or used a neurotoxin; rats were also included with bilateral aspiration lesions of area 29. They argued that if fibres of passage were crucial to spatial memory, then the performance of the neurotoxin group should be similar to that of rats with only unilateral aspirations; conversely, if the neurons of retrosplenial cortex are crucial, then the performance of the neurotoxin group should be similar to that of the bilaterally aspirated group. Unilateral aspiration lesions caused little disruption of place navigation, whereas bilateral aspiration lesions led to severe deficits; interestingly, the neurotoxin group did not differ from the bilateral aspiration animals.

Sutherland & Hoesing (1993) concluded that retrosplenial cortex made a significant contribution to spatial memory, while the contribution from fibres of passage was negligible. However, their conclusions are somewhat weakened by the fact that they failed to include a group with bilateral neurotoxic lesions. If such a group had been impaired, then direct evidence for the importance of retrosplenial cortex, and not the cingulum bundle, would have been provided. It should lastly be argued that the present findings do not imply that the retrosplenial cortex is not important for some spatial processing, just that it does not appear necessary for those spatial abilities tested in these three spatial tasks.

5.4.5 An egocentric / allocentric dissociation.

The dissociation of effect between lesions of the cingulum bundle on DNMP performance and the acquisition and performance on the T-maze, hints at the possibility that the severity of such lesions may depend upon the type of spatial task that is used to test them. As stated earlier, the DNMP task is probably primarily a test of egocentric memory, while the T-maze probably taxes essentially allocentric processing. From this it is difficult to avoid the conclusion that the cingulum bundle may well mediate allocentric but not egocentric spatial memory.

Such a dissociation has been described before regarding the differing effects of parietal versus medial prefrontal lesions on such tasks (Kesner et al., 1989). In that report, animals with aspiration lesions of medial frontal cortex (including anterior cingulate and prelimbic cortices), were impaired on an egocentric but not on an allocentric maze task, a dissociation which has been replicated by Aggleton et al., (in press). Unfortunately, Kesner et al., (1989) failed to mention if the cingulum bundle had been damaged.

Of closer interest to this experiment is the study reported by King & Corwin (1992) who examined the role of cingulate and posterior parietal cortex in the performance of both egocentric (adjacent arm test in the radial maze) and allocentric (cheeseboard maze) tasks. In their study, animals with aspiration lesions of anterior cingulate cortex showed severe impairments in the egocentric, but not the allocentric task, with the opposite finding being true for the parietal group. This dissociation between egocentric and allocentric tests is opposite to that reported in experiments 3a-c.

It is not a straightforward task to account for this contrary finding, as the differences in surgical techniques and testing procedures, make valid comparisons difficult. In the King & Corwin (1992) study the aspiration lesions of cingulate cortex removed all of dorsal ACc sparing those parts of ACc that were lesioned in experiments 3a-c. The lesions described in the King and Corwin report also caused damage to some of rostral RSc, and possibly damaged the prelimbic, and infralimbic regions. If damage was caused to the prelimbic and infralimbic regions then the egocentric deficit displayed by this group would not be surprising as damage to these regions causes an impairment on egocentric tasks (for example Dunnett, 1990).

The cingulum bundle was only affected in its rostral aspect and therefore may not have been damaged enough to affect the anterior thalamic / cingulum bundle fibres, and hence performance in the allocentric task.

To summarise the discussion of this experiment: Firstly damage to both anterior cingulate and retrosplenial cortices in tandem is not sufficient to affect the performance of rats on either the DNMP, lever discrimination, and delayed alternation tasks. This is in accordance with the findings of experiments 2a - 2c, and indicates strongly that these regions of medial frontal cortex are not as important for spatial working memory processing (as measured on three tasks) as has been suggested.

Secondly if cingulate cortex is not as important as previously thought, then the role of the underlying fibre tract - the cingulum bundle assumes greater prominence. This experiment has shown that radiofrequency damage to the cingulum bundle can have a dissociable effect on performance depending on whether the task assesses egocentric or allocentric processing. It remains to be seen however how different types of lesions of the cingulum bundle (i.e. ones confined to the anterior or posterior ends, or of crossed lesions) affect the performance on such tasks. It may be necessary to damage the bundle along its entire length, or selective, limited lesions may only be required to produce a deficit. The dissociation between types of tasks is also worthy of further consideration, as such a dissociation implies the existence of separable neural circuits governing the processing underlying the successful performance on such tasks.

Finally, the deficits associated with damage to the hippocampal system (destruction of the fornix) described in experiments 2a-2c have been replicated in this series of experiments.

CHAPTER 6. EXPERIMENT 4: THE EFFECTS OF DISCRETE CINGULUM BUNDLE LESIONS ON THE ACQUISITION AND PERFORMANCE ON SEVERAL TESTS OF SPATIAL WORKING MEMORY.

6.1 INTRODUCTION.

The two previous experiments had indicated that the spatial working memory deficit (as measured on the DNMP and delayed-alternation tasks) commonly described following lesions of the retrosplenial region of cingulate cortex, may be due to damage to the fibre tract underlying this area of cortex. Neurotoxic lesions of anterior cingulate cortex, retrosplenial cortex, and anterior cingulate + retrosplenial cortex had no effect on the ability of rats to perform three tasks of spatial working memory. Conversely, radiofrequency lesions of the cingulum bundle, whilst having no effect on the performance of an automated DNMP, and the acquisition of a lever discrimination task, had a severe effect (comparable to animals with hippocampal system damage) on the acquisition and performance of a forced-choice alternation task in the T-maze. The intriguing dissociation described in experiments 3a-3c was assumed to lie in the nature of the tasks chosen, i.e. between egocentric and allocentric spatial processing, with lesions of the cingulum bundle apparently causing a deficit in allocentric, but not egocentric tasks.

One problem with this proposed dissociation is that it might reflect a quite different aspect of learning and memory, namely a dissociation between acquisition and performance. This is because all of the DNMP experiments carried out so far, have involved the extensive training of naive animals prior to surgery, while the T-maze apparatus and testing procedures were only used postoperatively. This is not strictly true however for the lever discrimination task, but it involved exactly the same apparatus and many of the same elements of the already well-rehearsed DNMP task, so that the process of acquisition was presumably rapid and straightforward. It is therefore possible that the T-maze deficit seen following lesions of the cingulum bundle may not have reflected a dissociation between allocentric and egocentric processing, but rather between acquisition and performance. The cingulum bundle may thus be of crucial importance while a task is being acquired (as with the T-maze) but may be less so if the task has been learned before surgery and is thus well practised (as with the DNMP, and to some extent, the lever discriminations).

Thus the main aim of this experiment was to evaluate the role of the cingulum bundle in the acquisition of the DNMP task, any deficit found will indicate that this fibre tract is somehow important for the acquisition of egocentric working memory processes (the DNMP is however not a pure test of egocentric processing since how animals do perform this task is as yet unconfirmed). A lack of impairment on the DNMP acquisition, but an impairment on the T-maze task will reconfirm the findings from the previous experiment, and indicate that the cingulum bundle may well mediate certain aspects of allocentric memory processing.

The second aim of this set of experiments is to evaluate the effects of lesions at different sites along the cingulum bundle. Although the bundle is a continuous fibre tract, it consists of several major fibre components originating principally from thalamic and neocortical areas, each of which enter and leave the tract at various points along its route (see section 3.5.2). The last experiment reported the effects of extensive cingulum bundle damage, which consisted of 3 bilateral radiofrequency lesions, each made at two depths along its length. The effects of restricted or crossed lesions of the cingulum bundle on the DNMP and T-maze tasks have not been reported.

This current experiment will thus compare the effects of bilateral radiofrequency damage to the bundle made at several anterior / posterior (A/P) points (one group with two symmetrical bilateral lesions, anterior and posterior; and one group with a single symmetrical bilateral lesion made only at the posterior end, adjacent to retrosplenial cortex). A further group will receive crossed-lesions (two asymmetric controlateral lesions, one anterior, and one posterior). The use of this asymmetric 'crossed-lesion' group was to alleviate the possibility of causing bilateral symmetrical cingulate cortex damage, yet should still cut the tract bilaterally. Although destroying the bundle bilaterally at several sites can produce a mnemonic impairment (as has been shown in the previous experiment), it is unclear whether this impairment simply reflects the disconnection of the anterior thalamic connections to association cortex or the temporal lobe, or whether associated damage to the fibres coursing through the cingulum bundle from prefrontal and cingulate cortices contribute to the impairment. If the former is true then we would expect that a bilateral lesion anywhere along the fibre's length, after the anterior thalamic fibres join, would produce an impairment functionally equivalent to that seen in the previous experiment. If the latter is true, then the posterior and crossed lesions may have little functional effect.

A further complicating factor is that the lesions reported in experiment 3 were very extensive and caused additional cortical damage. To reduce any extraneous damage to cingulate cortex, the surgical co-ordinates were modified slightly, and the lesions were only made at one depth. This procedure will help to rule out the possibility that the T-maze deficit of the cingulum bundle group in the previous experiment was due to damage to both cingulum bundle and cingulate cortex.

6.2 MATERIALS AND METHODS.

6.2.1 Subjects.

The study involved 41 naive male rats of the pigmented DA strain (Bantin and Kingman, Hull). Throughout the period of the experiment the animals were housed individually under diurnal conditions (14hr light/10hr dark), all testing occurring at a regular time during the light period. The animals were tested for five days a week, the day before testing each receiving approximately 15g of RM1 laboratory diet (Special Diet Services, Witham, Essex) daily so that they did not drop below 80% of normal body weight. At the start of testing the animals were aged 4 to 5 months and weighed between 203-254g. All animals had free access to water.

6.2.2 Apparatus for experiment 4a: DNMP.

All testing was carried out in 7 operant chambers whose characteristics have been fully described in the preceding experiments.

6.2.3 Apparatus for experiment 4b: T-maze alternation.

All testing was carried out in a T-maze, the characteristics of which have been fully described in the preceding experiments.

6.2.4 Procedure for experiment 4a: DNMP.

Approximately 2 weeks after surgery all animals were given the same standardised training protocol which followed closely (though was not identical) to that reported in experiment 1a.

1. Acquisition and performance: The magazine training and autoshaping procedures remained exactly the same, but there were several small differences for the DNMP procedure. In this experiment the initial training consisted solely of 0s delays, the animal reaching criteria at 85% + correct, out of 60 trials for three consecutive days. This was followed by further sessions involving equal numbers of 0, 2, and 4 s delays, criteria being reached when the animal responded at 85%+ out of 60 trials for three consecutive days. To provide an indication of the speed of acquisition, the number of sessions taken to reach criteria in all of the training stages was recorded, as well as the number of correction trials needed in both the autoshaping procedure and the DNMP trials in the 0, and 0 - 8s delay training sessions.

When all the smaller delay criteria had been met, the number of daily trials was increased to 96 and each session contained an equal mixture of 0, 2, 4, and 8s delays presented in a pseudorandom order. Following 2 daily sessions using these delays, a further 4 sessions were carried out using a balanced mixture of longer delays consisting of 0, 2, 4, 8, 16, and 32s delays.

2. Performance measures and analyses: The data from the 4 sessions consisting of 0-32s were analysed separately to provide the various indices of accuracy, responsivity and bias that have been fully described in the previous experiments. Once again, the data in this experiment was transformed as appropriate (arcsin: all accuracy and bias indices, logarithmic: latencies, square-root: misses), and analysed by parametric analysis of variance (ANOVA). As in the previous experiments, when the F-ratios were significant, the means were compared using the Newman-Keuls procedure (Winer, 1971).

6.2.5 Procedure for experiment 4b: T-Maze alternation.

Testing began approximately 2 weeks after completion of experiment 4a. A full description of the T-maze procedure has been presented in the two preceding experiments. Briefly, all animals received six acquisition trials a day for six days, using a 15s delay. This was immediately followed by a further 10 sessions in which the 6 trials were divided equally between those with retention delays of either 10s, 30s, or 60s, making a total of 20 trials at each delay.

6.2.6 Surgical and histological procedures.

A total of 41 rats received surgeries. These were divided into four groups; total cingulum bundle, crossed cingulum bundle, posterior cingulum bundle, and surgical shams. Following surgery, 3 animals in the SHAM group, 1 in the CCB group, and 1 in the TCB group died leaving 9 TCB, 8 CCB, 7 PCB, and 12 SHAM animals. Each animal was anaesthetised by intraperitoneal injection (6ml/kg) of Sagatal, and then placed in a stereotaxic headholder (David Kopf Instruments, Tujunga). The scalp was retracted to expose the skull. A small craniotomy made above the sagital sinus and the dura cut to expose the cortex above the target region. In all cases the incisor bar was set at +5.0.

For all lesions involving the cingulum bundle a Radionics TCZ (Radionics Inc., Burlington) electrode (0.3mm tip length, 0.25mm diameter) was lowered vertically into the appropriate site and the tip temperature raised to 75C for 60s using an RFG4-A Lesion Maker (Radionics Inc., Burlington).

For the total cingulum bundle (TCB) group, two lesions were made in each hemisphere. The stereotaxic co-ordinates of the lesions relative to ear-bar zero were: AP + 5.3, LAT ± 1.1, and AP + 1.4, LAT ± 0.9. The two rostral lesions were placed 2.1mm below the cortex, while the two caudal lesions were placed 1.9mm below the top of the cortex (see figure 32). For the crossed cingulum bundle group (CCB), one lesion was made in either hemisphere using the same co-ordinates as the TCB group. This resulted in four animals with left rostral and right caudal lesions, and four animals with right rostral, and left caudal cingulum bundle lesions (see figure 32). For the posterior cingulum bundle group (PCB), one lesion was made in either hemisphere, the co-ordinates were as follows: AP + 1.4, LAT ±0.9, with the depth being 1.9mm below the top of the cortex (see figure 32). The surgical controls (SHAM) received a craniotomy, and the dura was cut in the appropriate places.

On completion of surgery, animals in all groups received sulphanilamide powder and the skin was sutured. This was followed by an intraperitoneal injection of 6ml sodium chloride + 0.3ml of milophyline. On completion of the experiment the animals were killed and perfused intracardially with 5% formol-saline. The brains were then rapidly removed and placed in 5% formol-saline. Subsequently, the brains were blocked, embedded in wax (Paraplast), and cut into 10- mm coronal sections. Every tenth section was mounted and stained with Cresyl violet, a Nissl stain.

Representation of the cingulum bundle lesions.

TCB.

```
          ==========X==========X==========
rostral   ____________________________________   caudal
          ==========X==========X==========
```

CCB.

```
          ==========X====================
          ____________________________________
          ====================X==========
```

PCB.

```
          ====================X==========
          ____________________________________
          ====================X==========
```

Figure 32. Diagrammatical representation of lesion placement in the cingulum bundle groups. Each diagram represents an overhead view of the cingulum bundle tracts. The solid black line represents the sinus midline, while the dotted lines represent the bundle. An ***X*** *represents the site of a radiofrequency lesion. In the CCB group a right rostral / left caudal lesion is represented.*

6.3 RESULTS.

6.3.1 Histological Analysis.

Figures 33a and 33b shows the extent of the largest and smallest of the cingulum bundle surgeries in each of the three surgical groups. These surgeries involved bilateral radiofrequency lesions placed at different AP levels and each resulted in both localised damage to the bundle itself, and more generalised cortical damage surrounding the lesion. The more anterior cingulum bundle lesions produced moderate damage to anterior cingulate cortex, while the more posterior cingulum bundle lesions produced additional damage to retrosplenial cortex. The extent of this cortical damage was variable, but in the majority of cases the lesions were quite selective, with limited direct cortical damage. In most cases there was also additional minor damage to the corpus callosum at the lesion sites, though in all cases bar one, the hippocampus and the fornix suffered no obvious direct damage. The one animal in the CCB group that received direct hippocampal damage was discarded.

While the cingulum bundle lesions typically resulted in moderate to severe tract damage the most medial aspects of the bundle were consistently spared. The degree of the severity of the bundle damage was reflected in associated cellular degeneration in the anterior ventral nucleus of the thalamus (AV). Those animals receiving total cingulum bundle damage had a correspondingly greater degree of AV degeneration, while animals with only moderate cingulum bundle damage displayed little or no AV degeneration. No other thalamic nuclei were affected.

Following histological analysis six animals with only very minor damage to the cingulum bundle were rejected (three CCB, two PCB, and one TCB). The final groups thus consisted of 12 SHAM, 8 TCB, 5 CCB, and 5 PCB animals.

Note that the retrograde degeneration seen in the anterior ventral (AV) nucleus of the thalamus appeared identical to that recorded in experiment 3 (see figure 25, chapter 4), and so again no photomicrograph is reproduced.

Reconstructions showing the total cingulum bundle, and crossed cingulum bundle surgeries.

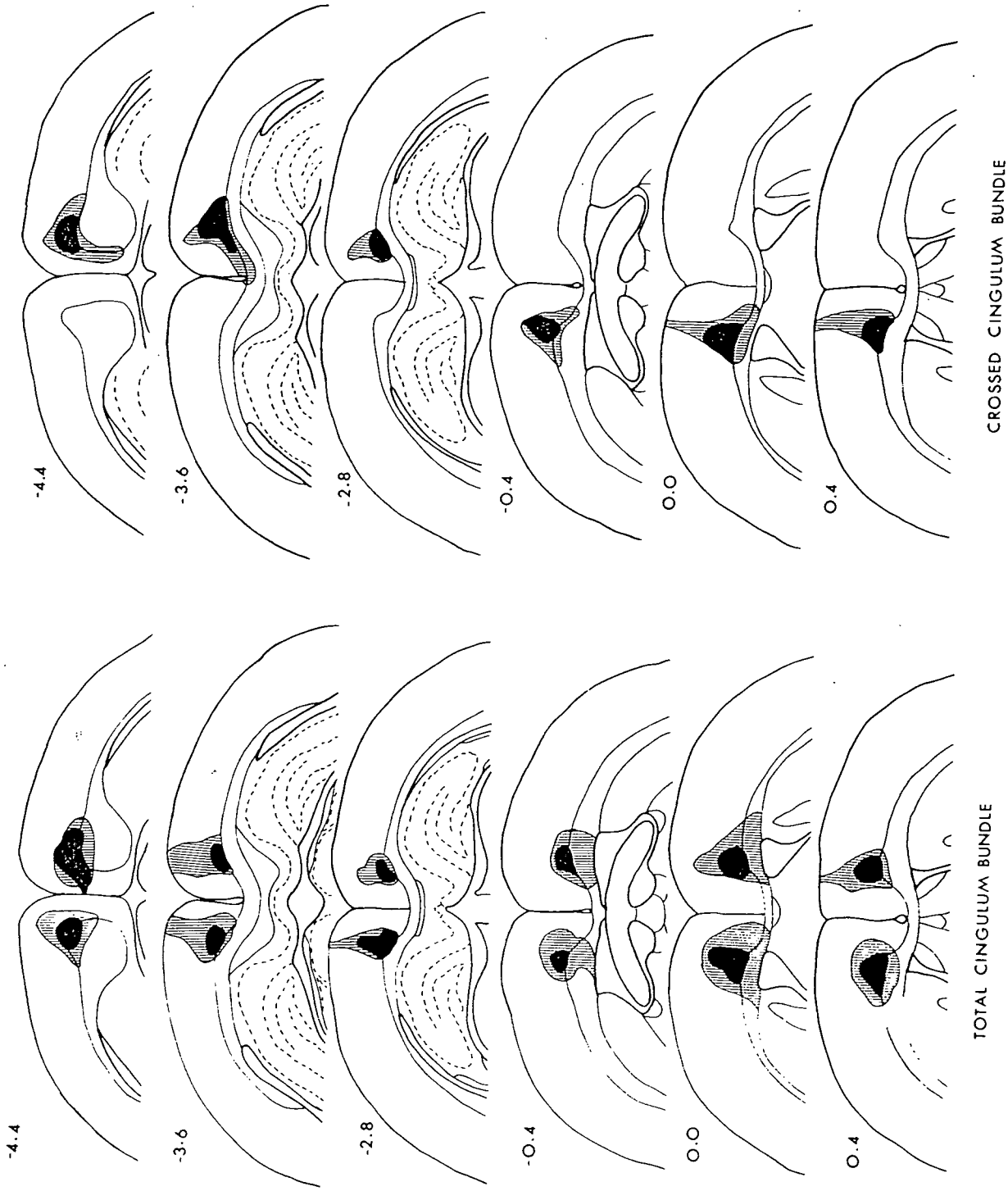


Figure 33a. Diagrammatic reconstruction's showing the total cingulum and crossed cingulum bundle lesions.

Reconstruction showing the posterior cingulum bundle surgeries.

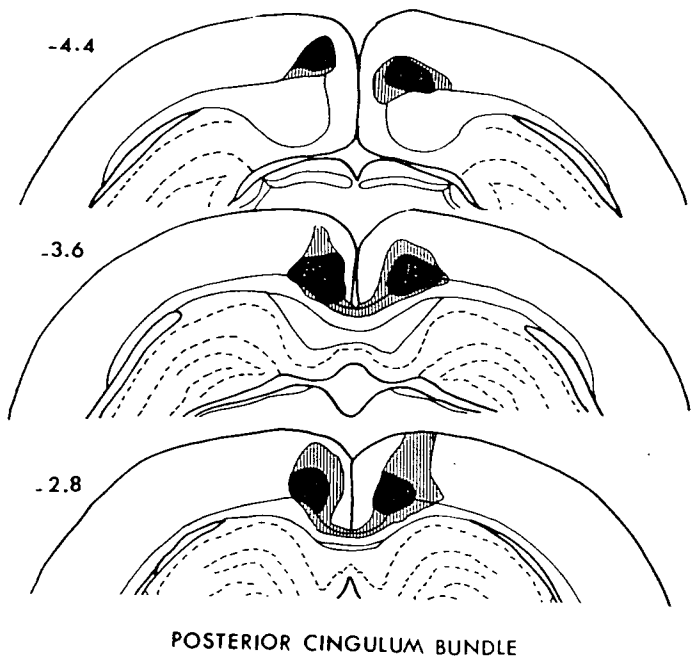


Figure 33b. Diagrammatic reconstruction showing the posterior cingulum bundle lesions.

Photomicrographs showing the appearance of the cingulum bundle following radiofrequency lesioning.

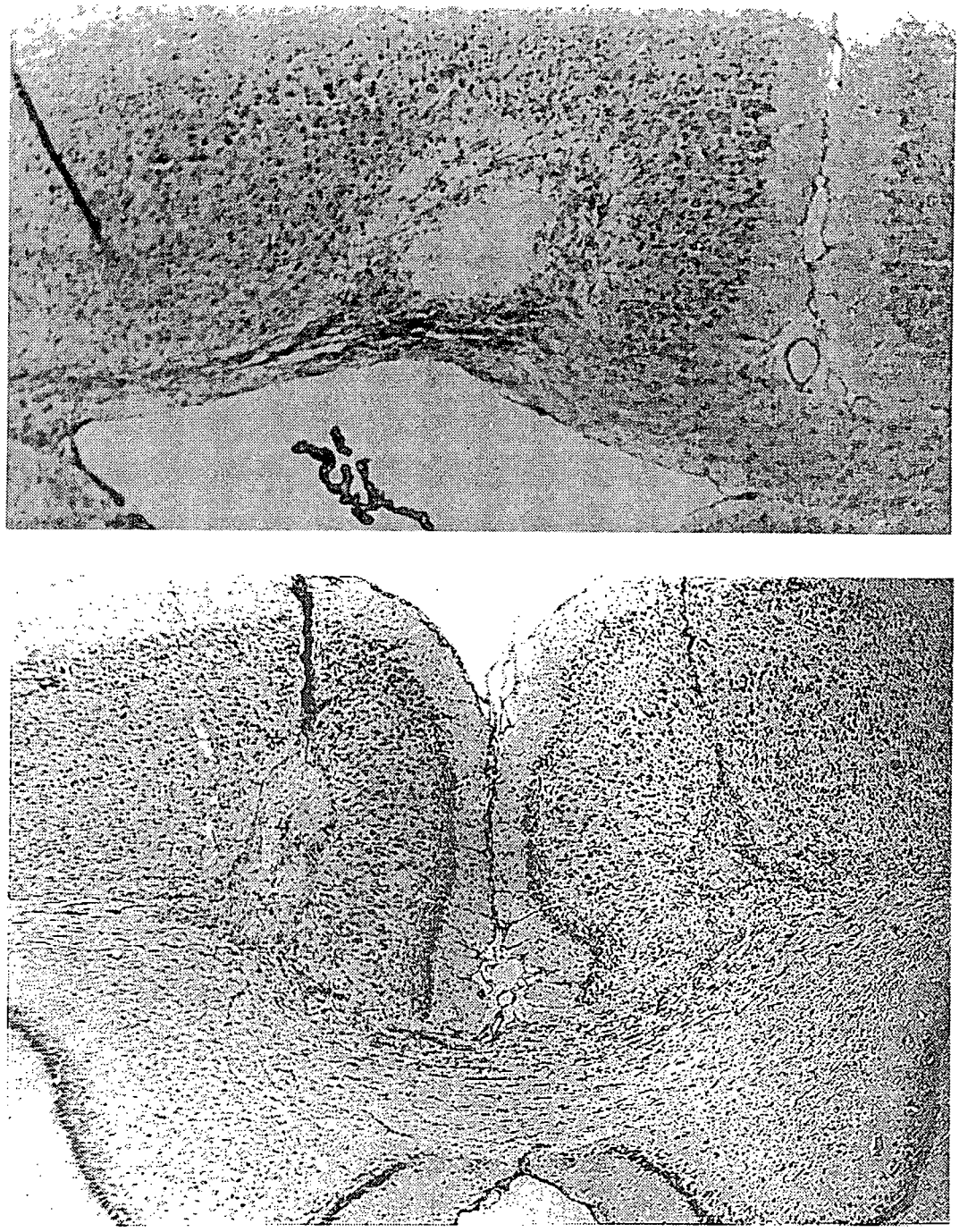


Figure 34. Photomicrographs of coronal sections (Nissl stain) showing the typical appearance of the cingulum bundle following radiofrequency lesioning. Upper: A rostral lesion. Lower: A caudal lesion. Note that the lesions are not taken from any particular group.

6.3.2 Comparison between the cingulum bundle groups.

One of the principal aims of this experiment was to compare the effects of lesions at different sites along the cingulum bundle. All three groups had bilateral damage to the cingulum bundle, so, unless other routes coursing through cingulate cortex are critical, all three groups should show similar performance levels. Thus, each of the cingulum bundle groups were firstly compared with each other on the acquisition and performance of the DNMP task, and the acquisition and performance of the T-maze forced-alternation task.

a). DNMP acquisition: The mean scores for each of the CB groups on each element of the DNMP acquisition (number of trials to criteria) were as follows (standard deviations in parentheses): magazine training: TCB = 5.3 (2.8), CCB = 4.4 (1.7), and PCB = 3.65 (0.5); autoshaping: TCB = 8.8 (3.9), CCB = 7.8 (3.2), and PCB = 8.8 (3.6); 0s : TCB = 12.9 (4.6), CCB = 11.6 (2.1), and PCB = 12.2 (3.3); 0-4s: TCB = 8.1 (2.5), CCB = 8.4 (2.5), and PCB = 7.6 (3.7). A one-way ANOVA carried out on each of these sets of scores revealed no significant group differences ($F<1$ in each case).

b). DNMP Performance: The mean percent correct scores of each of the CB groups on the performance of the DNMP over a range of delays were as follows: 0-8s: TCB = 86.6 (5.7), CCB = 83.9 (6.7), and PCB = 85.4 (3.7); 0-32s: TCB = 84.9 (2.9), CCB = 83.0 (2.1), and PCB = 81.9 (2.2). An ANOVA carried out on these sets of scores again revealed no significant difference between the three CB groups ($F<1$ over the 0-8s sessions, and $F_{2,15} = 2.38$, $P>0.05$ over the 0-32s sessions).

c) T-maze alternation: acquisition trials: The mean scores for each group on the initial 6 acquisition sessions of this task were as follows: TCB = 27.5 (2.5), CCB = 27.8 (3.4), and PCB = 28.8 (2.6). An ANOVA again revealed no group difference ($F<1$) and no group X session interaction ($F<1$).

d) T-maze alternation: delay trials: The mean scores for each group on the subsequent ten sessions, which comprised a total of twenty trials, each at 10s, 30s, and 60s, were as follows: 10s: TCB = 18.3 (0.4), CCB = 18.6 (1.5), and PCB = 17.6 (1.9); 30s: TCB = 16.9 (1.5), CCB = 17.6 (1.8), and PCB = 17.2 (1.9); 60s: TCB = 15.3 (1.3), CCB = 16.8 (0.8), and PCB = 16.2 (2.1). Again an ANOVA revealed no group difference ($F<1$ over the 10s and 30s trials, $F_{2,15} = 1.89$, $P>0.05$ over the 60s trials).

As no group differences were found between the cingulum bundle groups on any element of the acquisition or performance of the two tasks, all three groups were combined to form one cingulum bundle (CB, n=18) group.

6.3.3 Experiment 4a: DNMP acquisition and performance.

Both SHAM and CB groups were compared on the mean number of sessions required to reach criteria on each element of the acquisition of the DNMP task, see figure 35.

1. Magazine training: A t-test revealed no significant difference ($t, 28 = 0.064$, $P>0.05$).

2. Autoshaping: A t-test revealed no significant difference ($t,28 = 1.76$, $P>0.05$).

3. DNMP at short delays: A t-test revealed no significant difference over the 0s, or 0-4s delay sessions ($T, 28 = 1.25$, and $t,28 = 0.80$, respectively).

4. DNMP performance at longer delays.

i) 0-8s performance: The mean percent correct scores for each group over the two sessions comprising 0-8s are shown in figure 36. An ANOVA revealed that while there was no significant group difference ($F<1$) there was a significant group X session interaction ($F, 1,29 = 13.29$, $P<0.01$). This was due to the fact that while the SHAM group performed slightly better than the CB group on the first session, the CB group performed much better than the SHAM group on the second session.

ii) 0-32s: The mean percent correct scores for each group over the 4 sessions comprising 0-32s are shown in figure 36. The data from these final 4 sessions were analysed using the accuracy and bias indices described in an earlier section.

DNMP Acquisition Performance.

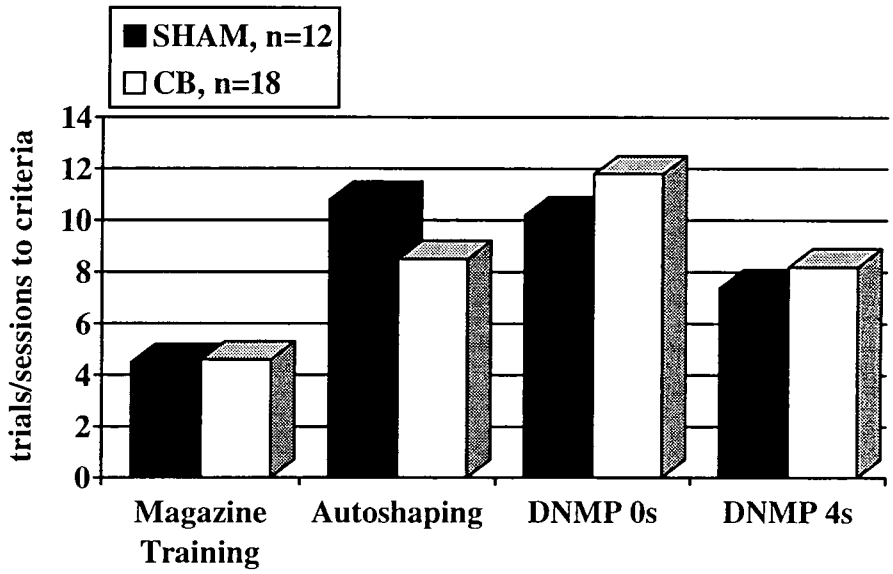


Figure 35. The average number of trials or sessions to criteria for both groups, on each of the elements of DNMP acquisition.

a) Accuracy measures: An analysis of variance using the percent correct scores over the 4 daily sessions comprising 0-32s delays revealed a significant group effect ($F_{1,28} = 12.10$ $P<0.01$), and the expected delay effect ($F_{5,140} = 214.34$ $P<0.0001$). The significant group effect was due to the relatively poorer performance of the SHAM group, see figures 36 and 37. A similar pattern of results was found for the two TSD measures of sensitivity, A' and SI. Both of these measures not only revealed significant effects of delay ($P<0.001$ in both cases), but also indicated a significant group effect (A', $F_{1,28} = 12.49$, $P<0.01$; SI, $F_{1,28} = 10.09$, $P<0.01$), see figure 37. Once again, these reflected the lower scores of the SHAM group.

DNMP, Percent Correct Performance.

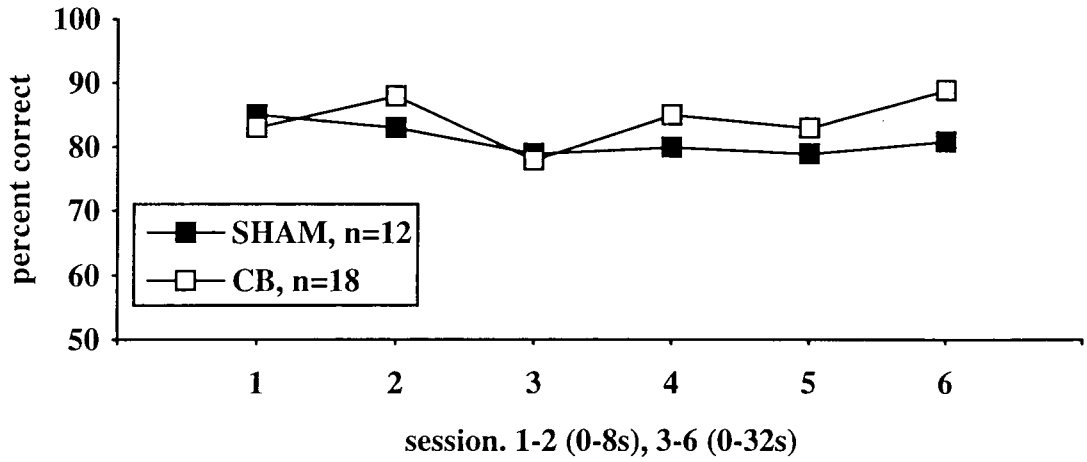
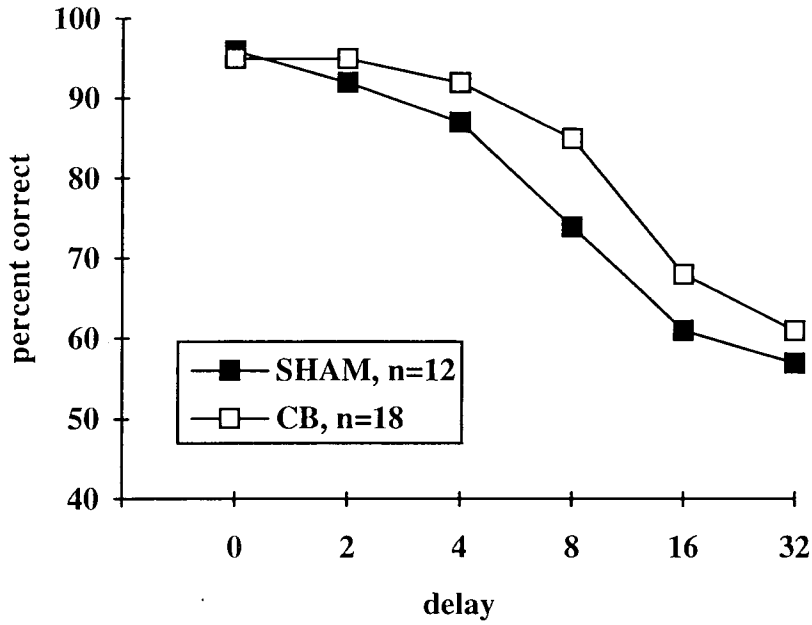


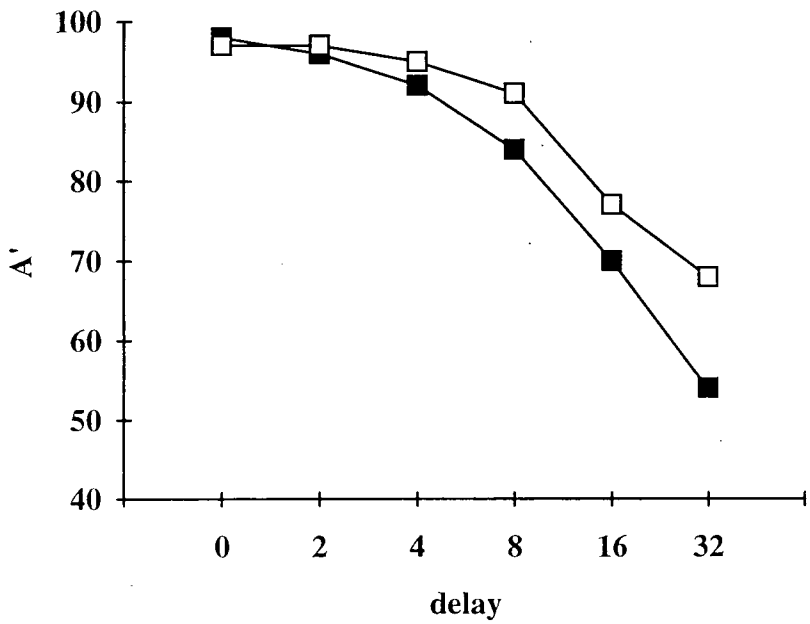
Figure 36. Mean percent correct scores on the DNMP task over each of the 6 postoperative sessions using the results from all delays. Delay data have been pooled. Sessions 1 and two represent the data from the 0-8s delay condition, while sessions 3-6 represent the data from the 0-32s delay condition.

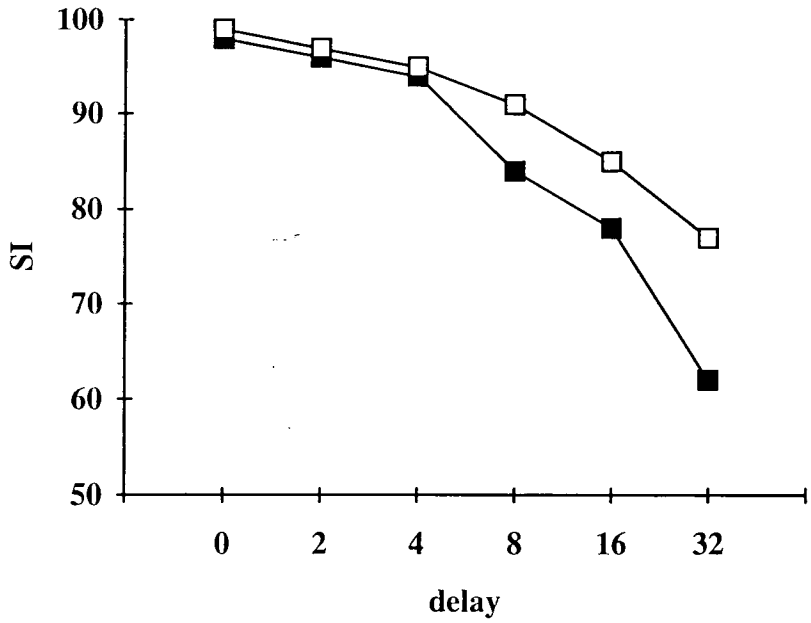
b) Bias indices: As expected from the previous experiments, all three bias indices (Iy, B" and RI) demonstrated the expected delay effects ($P<0.001$) in all three cases. In addition, both Iy and RI each revealed a significant group effect (Iy, $F_{1,28} = 17.64$, $P<0.001$; and RI, $F_{1,28} = 7.36$, $P<0.05$). Finally both Iy and B" both revealed a significant group X delay interaction (Iy, $F_{5,140} = 4.33$, $P<0.01$; B" $F_{5,140} = 5.35$; $P<0.01$). In each case, the significant group effect reflected the poorer performance of the SHAM group, see figure 37.

c) Responsivity measures: For all of the measures of responsivity (total misses, average choice latency, nose-poke rate, mean latency to sample, and mean latency to first nose-poke) no significant group differences were found ($F<1$ in all cases except nose-poke rate when $F = 3.42$).

DNMP, Bias Indices.



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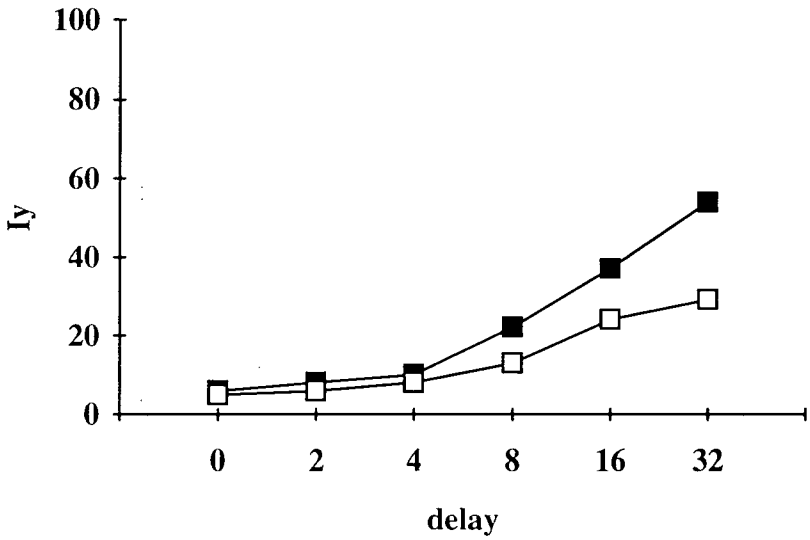
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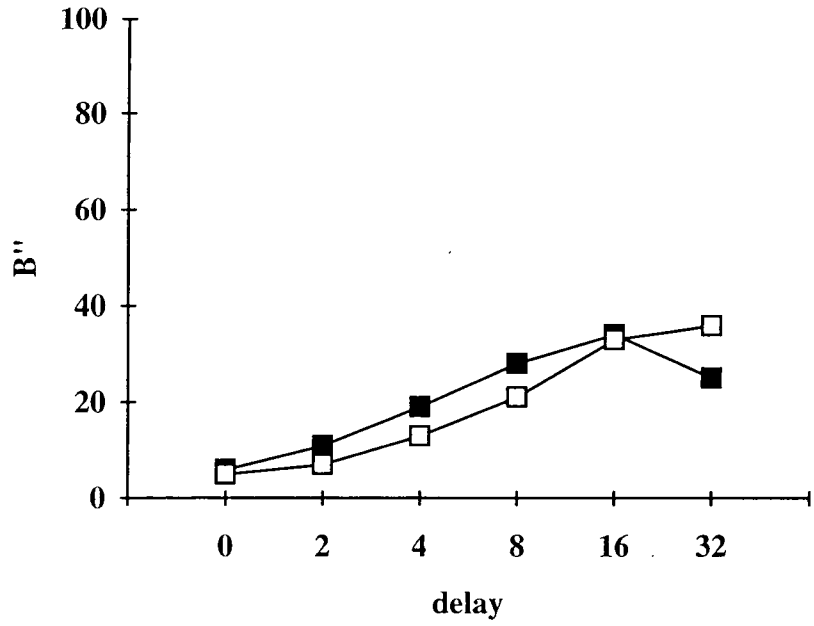

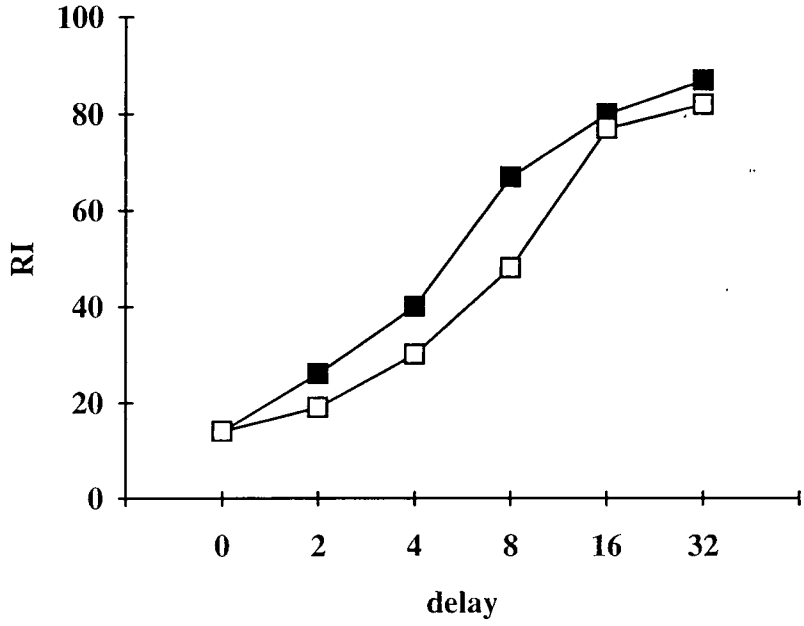

Figure 37. Accuracy (first three graphs) and bias (last three graphs) indices as a function of lesion and delay. See text for explanation of indices. All scores have been converted to read from 0 to 100. In the case of the bias scores, 100 represents a complete bias and 0 neutral, or no bias.

6.3.4 Experiment 4b: T-maze alternation.

During this experiment one of the SHAM animals became ill and so was removed from the experiment leaving the SHAM group with 11 animals.

a) Acquisition performance: During the initial six acquisition sessions each animal performed a total of 36 trials with a retention delay of 15s. The mean percent correct scores of each group are shown in figure 38. An ANOVA carried out on this data revealed a highly significant group difference ($F_{1,27} = 17.6$, $P<0.001$), but there was no group X session interaction ($F<1$). The CB group clearly performed worse than the SHAM group.

b) Delay performance: All of the animals then performed a total of 60 trials comprising 20 trials at each of three delays (10s, 30s, and 60s). The mean scores for each group are shown in figure 38. A comparison between the scores of each group using a 2-way ANOVA revealed the expected effect of delay ($F_{2,54} = 19.12$, $P<0.0001$), as the performance of all animals was affected by the longer delay intervals. Importantly though, while there was no group X delay interaction ($F<1$), there was an overall significant group difference ($F_{1,27} = 5.52$, $P<0.05$), see figure 38.

T-Maze Alternation, Percent Correct Performance.

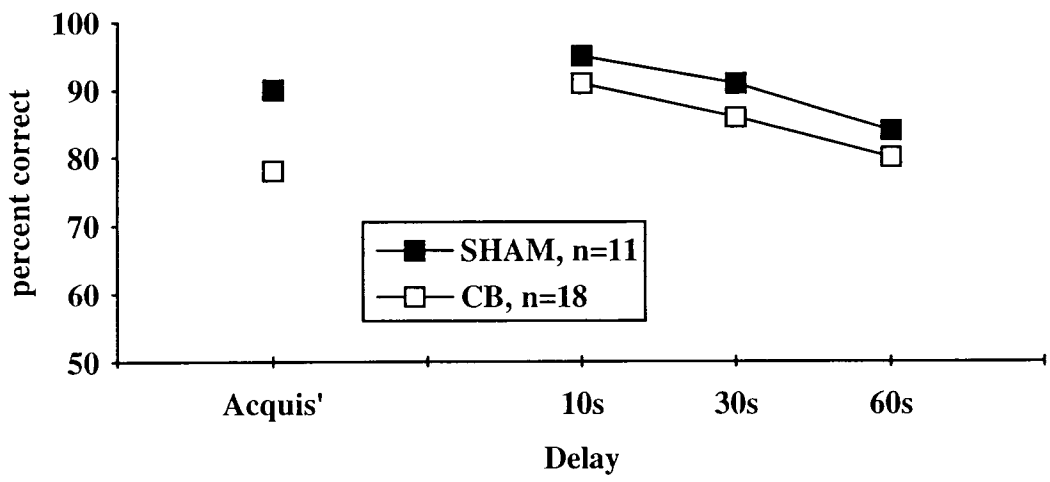

Figure 38. Spatial forced-alternation in a T-maze. The graph shows the mean scores of each group over the initial six acquisition sessions, and the mean scores of the same groups when tested for twenty trials over 3 retention intervals.

6.4 DISCUSSION.

The previous set of experiments (3a-3c) had revealed a dissociation between the performance of animals with total cingulum bundle lesions on several tests of spatial working memory. These animals were unimpaired on the performance of the DNMP task, and on a lever discrimination and reversal test, yet were severely impaired on the acquisition and performance of a forced-alternation task in the T-maze. In this current experiment, a double-dissociation was noted, as the animals with cingulum bundle damage showed a significant facilitation of performance on the DNMP task, but were significantly impaired on the T-maze alternation task.

In the discussion of the previous set of experiments, it was hypothesised that the dissociation reflected the key difference between the DNMP / Lever discrimination tasks and the T-maze task, namely that the former are thought of as taxing essentially egocentric spatial processing, while the latter is thought to tax primarily allocentric processing. The performance deficit of the cingulum bundle animals on the T-maze task (an impairment which was comparable to that shown by animals with hippocampal system damage) was therefore assumed to reflect the specific involvement of the bundle in allocentric spatial processing. The possibility still remained however, that the dissociation could have reflected an impairment in acquisition rather than performance, as the lesioned animals in that set of experiments (3a-3c) had not been tested on the acquisition of the DNMP task.

One aim of this current set of experiments (4a and 4b) focused on the acquisition of the elements of the DNMP, by animals with varying types of lesions of the cingulum bundle. Focusing on the acquisition of the DNMP task may also serve as a more sensitive comparison as the extensive pretraining of the animals on the DNMP task in the previous experiment, may have attenuated any deficit.

6.4.1 Aim 1: The effects of cingulum bundle damage on two tasks.

1. Experiment 4a: DNMP acquisition and performance.

In all the acquisition stages of the DNMP procedure, which included learning to operate the magazine flap, pressing the levers to gain a reward, and in the performance of the DNMP procedure at delay of 0s and 4s, no group differences were recorded. Furthermore, none of the cingulum bundle groups showed a deficit in performance when the delay intervals of the DNMP procedure were increased. In fact, in the final four sessions in which the delay interval ranged from 0s - 32s, the CB group as a whole performed significantly better than the SHAM group. The analyses based on TSD revealed that the SHAM group not only showed a decrease in accuracy, but also showed an increase in bias.

These present results thus confirm the finding from experiment 3a, and further show that animals with lesions restricted to the cingulum bundle, are unimpaired on the acquisition and performance of the DNMP task. The lack of impairment on the acquisition of the various elements of the DNMP task is important as it might be argued that acquisition is likely to be a more sensitive measure of potential impairment than is final performance on a well-learned task.

The fact that the animals with CB lesions performed at a level significantly better than that of the SHAM group on the 0-4s and 0-32s sessions on the DNMP task is a little puzzling, but such a facilitation of performance following lesions of cingulate cortex has been reported (Bussey et al., 1993). Of more relevance to this study is the report by Meunier & Destrade (1988) who examined the effects of electrolytic lesions of cingulate cortex in mice (which also included damage to the cingulum bundle) on the acquisition of the Hebb-Williams maze. These authors noted that the animals with posterior cingulate cortex damage (which affected the cingulum bundle in all cases) showed a transient facilitation of learning on the acquisition sessions immediately following surgery. Neurotoxic lesions of the same area of cortex (leaving the cingulum bundle undamaged) had no such effects on the same procedures, indicating strongly that the damage to the cingulum bundle had caused the facilitation.

It is interesting to note that the human patient T.R. who developed amnesia following damage to retrosplenial cortex (including the cingulum bundle) also showed a paradoxical improvement in performance on a recency judgement task, an improvement that was only transitory but which surpassed that of the control group (Bowers et al., 1988).

Why such facilitation should occur is difficult to explain. It is possible that the cingulum bundle damage interfered with some aspect of normal behaviour that actually causes problems with the learning of certain tasks e.g. the tendency to adopt a side preference. Thus SHAM (and by implication, normal animals), may be at a slight disadvantage on the DNMP task compared to animals with cingulum bundle damage. The data from the DNMP bias indices is consistent with this hypothesis as the SHAM group showed consistently higher levels of bias (figure 37). Furthermore, the bias indices (Iy) specifically contrasts accuracy between the two levers, and provides a simple index of the animals' tendency to select one or the other side of the test chamber (Aggleton et al., 1991a). On this measure the SHAM animals showed higher levels of bias, at 8s and above (figure 37).

Such a facilitation was not recorded in experiment 3a but this is not surprising because in that experiment the animals had already been well trained on the DNMP task before undergoing surgery. In the Meunier & Destrade (1988) report, the facilitation was only observed in the sessions immediately following surgery (19 - 35 days after), and the animals subsequently demonstrated an impairment from 45 days after surgery. It is unfortunately impossible to confirm these findings in this current experiment because immediately after the DNMP sessions, the animals were tested on the T-maze task.

An alternative explanation for the difference between CB and SHAM groups in the DNMP part of this experiment could be that the SHAM group performed slightly worse than has typically been found on the DNMP procedure. All three measures of accuracy (figure 37: first three) revealed that the SHAM group was performing at a lower level of performance than the CB group, particularly at the longer delay intervals. The bias indices (figure 37: last three) also revealed that the SHAM performance was relatively poor.

A comparison between the SHAM and CB groups of this experiment with the SHAM and CB groups of the preceding experiment (3a) does suggest that the performance of the SHAM group in this experiment was poor.

If both SHAM and CB groups are compared over the first four sessions of the DNMP task in each experiment utilising retention delays of 0-32s delays a significant group effect is revealed ($F_{3,15} = 6.32$, $P<0.05$) which was due to the better performance of the CB group in this experiment, compared to the SHAM group in the same experiment. Importantly though, the CB group of this experiment did not differ from the SHAM and CB groups of the preceding experiment. However, despite the compelling nature of this argument, it is not strictly justified to compare groups between experiments in this way. Although each group was trained in a very similar manner and carried out the same task, in a similar order, using the same retention delays, there are nevertheless too many differences between the experiments (particularly regarding the amount of pre-training, and the differences in lesion site), that makes such comparisons untenable, albeit interesting.

2. Experiment 4b: Forced alternation in the T-maze.

Experiment 3c had revealed a striking impairment in the acquisition and subsequent delay performance of cingulum bundle lesioned animals on a forced-alternation procedure tested in a T-maze. The results from this current experiment serves to confirm this previous finding. On the acquisition trials, the CB group were markedly impaired in relation to the SHAM animals and this is in accord with the results from experiment 3c. Over the trials using a range of delays however, the findings were not so clear cut. Although the CB group were impaired relative to the SHAM group they were clearly not as impaired as in the acquisition phase, and not as impaired as the group with bilateral cingulum bundle lesions in experiment 3c (see figure 38).

This lack of a severe impairment of the cingulum bundle group in this present experiment in contrast to the much more severe one demonstrated by the cingulum bundle group in experiment 3c may be explained by the differences in the lesioning in the two experiments. In the previous experiment, the cingulum bundle group received 2 lesions at each of 3 sites per hemisphere, and as a result, the cingulum bundle received extensive bilateral damage. Due to the extensive nature of those lesions which caused some additional cortical damage, the lesions performed in this current experiment were deliberately designed to be less extensive.

In this current experiment, although each cingulum bundle group received a bilateral lesion, each animal only received two lesions per hemisphere, and while extraneous cortical damage was reduced, the bundle may not have been completely destroyed. In fact the histological analysis revealed that the most medial aspects of the bundle had indeed been spared. A paper by Greene & Stauff (1974) described the effects of cingulum bundle lesions in rats on an alternation task performed in a T-maze. Although the intention of the authors was to focus upon the effects of fornix transection, the surgical method used involved some cingulum bundle and cingulate cortex damage, though this was not extensive. The authors reported that fornix lesioned animals (with associated cingulum damage) were impaired on T-maze alternation, moreover a small group with cingulum damage but no fornical damage also showed some mild impairments but these were not significant. Although the histological descriptions are vague, it would seem that the cingulum bundle in the cingulum group was not completely destroyed, and so the mild deficits observed are perhaps not surprising. A similar finding had also been reported by Thomas & Slotnick (1962). In that report, rats received lesions of the cingulum and although they showed a deficit on maze learning, this deficit was not significant. Again, the authors admitted that the cingulum bundle had not been completely severed bilaterally.

So the difference between the severity of the impairment in the CB groups from this, and the previous experiment, is probably due to the differences in cingulum bundle disruption; in experiment 3 it being almost complete, while in this experiment it was severe, but not total. The initial severe impairment on the acquisition of the T-maze task does however indicate that the cingulum bundle is important, in at least the early stages of acquiring this allocentric task.

6.4.2 Aim 2: Comparison between the various cingulum bundle lesions.

Another aim of this current set of experiments was to compare lesions made at different sites along the cingulum bundle. The severe impairment reported in the previous experiment on the allocentric T-maze task appeared to reflect the bilateral destruction of the cingulum bundle alone. The lesions in this current experiment disrupted the cingulum bundle at both rostral and caudal positions, and included asymmetric lesions to assess the additive effect of unintentional cingulate cortex damage. The three cingulum bundle groups were thus compared with each other on both the acquisition and performance on the DNMP, and T-maze forced-alternation tasks.

Regarding the DNMP task, no group differences were noted over the acquisition of any of the elements of the task (magazine training, autoshaping, 0s, and 0-4s performance. Also, no significant difference was found between the cingulum bundle groups over the performance of the DNMP task (0-8s, and 0-32s sessions). With regard the T-maze alternation task, no group differences were again noted over both the initial acquisition sessions and the subsequent delay sessions. The cingulum bundle groups were thus combined to form one CB group which was subsequently compared to the SHAM group.

It was interesting to note that the locations of the lesions along the cingulum bundle had no differential effect on the performance of the relevant cingulum bundle groups. This supports the notion that the critical effect of cingulum bundle damage is the disruption of the anterior thalamic connections with association and/or the temporal lobes. The deficits seen are the results of this disruption and do not simply reflect the disruption of other fibres coursing through cingulate cortex. If fibres coursing through cingulate cortex were important then it would have been expected that the TCB group would show a greater impairment than the PCB group, but this was not the case. However, the deficit found on the T-maze task in this experiment (which was not as severe as that reported in the previous experiment), suggests that a complete transection of the bundle must be made in order to produce a clear-cut and long-term effect.

6.4.3 Degeneration in the anterior thalamus.

A final point of interest concerns the degeneration seen in the anterior thalamic nuclei following the cingulum bundle lesions. As in the previous experiments, retrograde degeneration was noted in the anterior ventral (AV) nucleus in the majority of animals with cingulum bundle damage. In fact, associated degeneration of AV, along with bilateral damage to the cingulum bundle damage was a prerequisite for acceptance into the cingulum bundle groups. Such degeneration seems to consistently reflect the effects of cingulum bundle damage, and may thus prove to be a useful assay of such lesioning. In one animal (in whom the anterior bilateral lesions were further forward than the rest, and were at the level of the septum), it was noted that there was no degeneration in AV at all, even though the bilateral lesions of the CB were extensive. The AV connection to the cingulum bundle would appear to occur after this point. Consistent with this is the finding that all the other lesions which were positioned slightly further back, showed retrograde degeneration in AV.

The cingulum bundle surgeries in the previous experiment were all extensive and produced similar amounts of AV degeneration, the ones carried out in this experiment were more limited and were expected to have a more variable effect on the AV nucleus, which was in fact the case. It is interesting to note that the report of Thomas & Slotnick (1962) also observed retrograde degeneration in AV following cingulum bundle damage, the degree of retrograde degeneration being dependent on the extent of the bilateral damage to the cingulum bundle.

6.4.4 Summary.

1. A double-dissociation was found in that animals with bilateral cingulum bundle damage showed a significant facilitation of performance in the DNMP task, yet were significantly impaired in the T-maze alternation task. These findings thus confirm the results from experiment 3 of this thesis, whilst using more selective lesions that caused minimal damage to the surrounding cingulate cortex. The animals in this current experiment were however not as severely impaired as those described in the previous experiment, and therefore the degree of impairment may depend crucially on how badly affected the bundle is. In this current experiment the bilateral damage to the cingulum bundle was very restricted and there was marked sparing of the most medial aspects of the fibre tract.

2. The effects of bilateral lesions placed at different A/P points along the cingulum bundle was assessed. No differences between the three cingulum bundle groups was observed indicating that bilateral damage to the bundle is sufficient to cause an impairment on an allocentric task, and may indeed cause a transitory facilitation on an egocentric task. This also indicates that the deleterious effects of bilateral cingulum bundle damage are due to the disconnection of anterior thalamus with association cortex or hippocampus, and not due to damage caused to other fibres coursing through cingulate cortex.

The crucial findings from this experiment reflect the double dissociation between allocentric and egocentric tasks following cingulum bundle damage, which highlights the very different demands of the two types of task. The next experiment will focus in more detail on this egocentric / allocentric double dissociation.

CHAPTER 7. EXPERIMENT 5: THE EFFECTS OF CINGULUM BUNDLE LESIONS ON THE ACQUISITION AND PERFORMANCE ON TESTS ASSESSING ALLOCENTRIC AND EGOCENTRIC PROCESSING.

7.1 INTRODUCTION.

The previous experiment had confirmed that bilateral damage to the cingulum bundle did not affect neither acquisition, or performance on the DNMP task. However, the same lesions had a severe effect on the acquisition of a forced-alternation procedure in the T-maze, but this deficit was only transitory. When the same groups performed further trials containing a range of delays, the former severe impairment was greatly reduced.

It was concluded that although bilateral damage to the cingulum bundle did produce an impairment on the allocentric T-maze task, the degree of the deficit was contingent upon the amount of damage to the cingulum bundle. As the cingulum bundle is composed of several layers of fibres it may be necessary to totally destroy the bundle to achieve a long-lasting deficit on such allocentric tasks.

The aims of this current experiment are thus twofold. Firstly the dissociation between performance of animals with cingulum bundle lesions on egocentric and allocentric tasks will be further assessed. As the DNMP task does not appear to be sensitive to cingulum bundle lesions, this current set of experiments will focus upon a range of maze-type tasks which can assess both allocentric and egocentric processing.

The first task was the same T-maze alternation procedure as used as in the previous experiments, though in addition a 'double-forced' procedure was included. In this procedure, the animal is first forced to select one arm, then returned to the start arm, and subsequently forced to enter the arm not visited in the first forced-choice. The animal is then given a free choice of both arms and is rewarded for entering the arm not visited on the second forced-choice. This procedure increases the difficulty of the task as proactive interference is increased (making the task more like the automated DNMP task), and should prevent any possible ceiling effects which may mask any mild deficit found during performance of the easier single forced-alternation task (as in the previous experiment).

The second and third tasks used an additional stem added to the T-maze to form a 'cross-maze' and were designed to differentiate between allocentric and egocentric processing. In the second task, the procedure again consisted of two parts with the first part being identical to that performed in the first T-maze alternation task. The second part was also similar in that the animal was rewarded for entering the arm not chosen on the previous information run, but in half of the trials the animal was placed in the start area of the arm on the opposite side to that used on the choice run. The location of the correct choice arm had not altered but the animals' relative position to it had. If the animal was relying upon allocentric room cues to solve the task then it should have little extra difficulty, but an animal relying upon egocentric cues should visit the same arm as on the information run and therefore be incorrect.

The third task again made use of the 'cross-maze' but this procedure was designed to specifically assess egocentric discrimination. The animal was rewarded for always turning in one direction and then (on reaching a criteria) this 'correct' turn was reversed so that the animal was only rewarded for consistently turning in the opposite direction. Using purely allocentric cues has no benefit for the animal in this procedure as the task relies purely on the processing of egocentric information.

The final task consisted of an 8-arm radial maze which is a standard test of spatial memory (Olton, 1978). While this task has been shown to be sensitive to damage to the fornix, the mammillary bodies and the anterior thalamus (Aggleton et al., 1990; Olton & Papas, 1979; Aggleton et al., in preparation) the effect of cingulum bundle lesions is unknown, though as such lesions affected performance in the T-maze alternation task (experiment 4) it is probable that such damage will also affect radial-maze performance.

The second aim of this experiment is to consider the kinds of cingulum bundle lesions necessary to produce a long-lasting impairment. A similar cingulum bundle group to that described in experiment 3a-c who were severely impaired on the allocentric task, (extensive bilateral cingulum bundle damage) will be compared to other groups with only unilateral damage, and with crossed-lesions. This group with extensive bilateral damage (BCB) received two radiofrequency lesions in each hemisphere which were staggered so as to reduce the extent of bilateral damage to the same cortical regions which may confuse the interpretation of the results, (see figure 39).

A second group received crossed lesions of the cingulum bundle (CCB) which consisted of one radiofrequency lesion in each hemisphere, again staggered. The rationale for crossed lesions has been described by Geschwind (1965) and has been utilised in animal models of memory (e.g. Olton, 1978). If the cingulum bundle consists of one complete layer of fibres then a crossed lesion should have the same functional effect as a bilateral lesion. However, if there are several fibres entering and leaving the bundle at various points along its length as proposed by Mufson & Pandya, (1984) then despite the bundle having bilateral damage (as in the BCB group) important fibres which join the bundle along its entire length may remain unaffected. In the former case this group should still be impaired, but in the latter case the group might only show a similar level of impairment (if any) as a group with only unilateral lesions. In the light of the results from the last experiment it is unlikely that the CCB group will demonstrate a severe and long-lasting impairment.

Two further groups were used as controls for the BCB and CCB groups. One cingulum bundle control group received four lesions of the bundle in the same hemisphere, at the same coordinates as the BCB group. As a consequence the extent of cortical damage should be similar in these different groups. This group with extensive unilateral cingulum bundle damage (XUCB) will be interesting as the effects of unilateral damage to the bundle were not assessed in the previous experiment, though the results from experiment 4 would indicate that this group would not be impaired to any large degree. A further group acted as control for the CCB group and received only two lesions along the bundle in one hemisphere at the same coordinates as the CCB group, (see figure 39). This group with limited unilateral lesions of the cingulum bundle (LUCB) provides a comparison with the XUCB group.

7.2 MATERIALS AND METHODS.

7.2.1 Subjects. The study involved 54 naive male rats of the pigmented DA strain (Bantin and Kingman, Hull). Throughout the experimental period all animals where individually housed under diurnal conditions (14hr light/10hr dark), all testing occurring at a regular time during the light period. The animals were tested for five days a week, and prior to test days fed approximately 15g of RM1 laboratory diet (Special Diet Services, Witham, Essex) daily so that they did not drop below 80% of normal body weight. At the start of testing the animals were aged approximately 4 months and weighed between 210-272g. All animals had free access to water.

Cingulum bundle lesions.

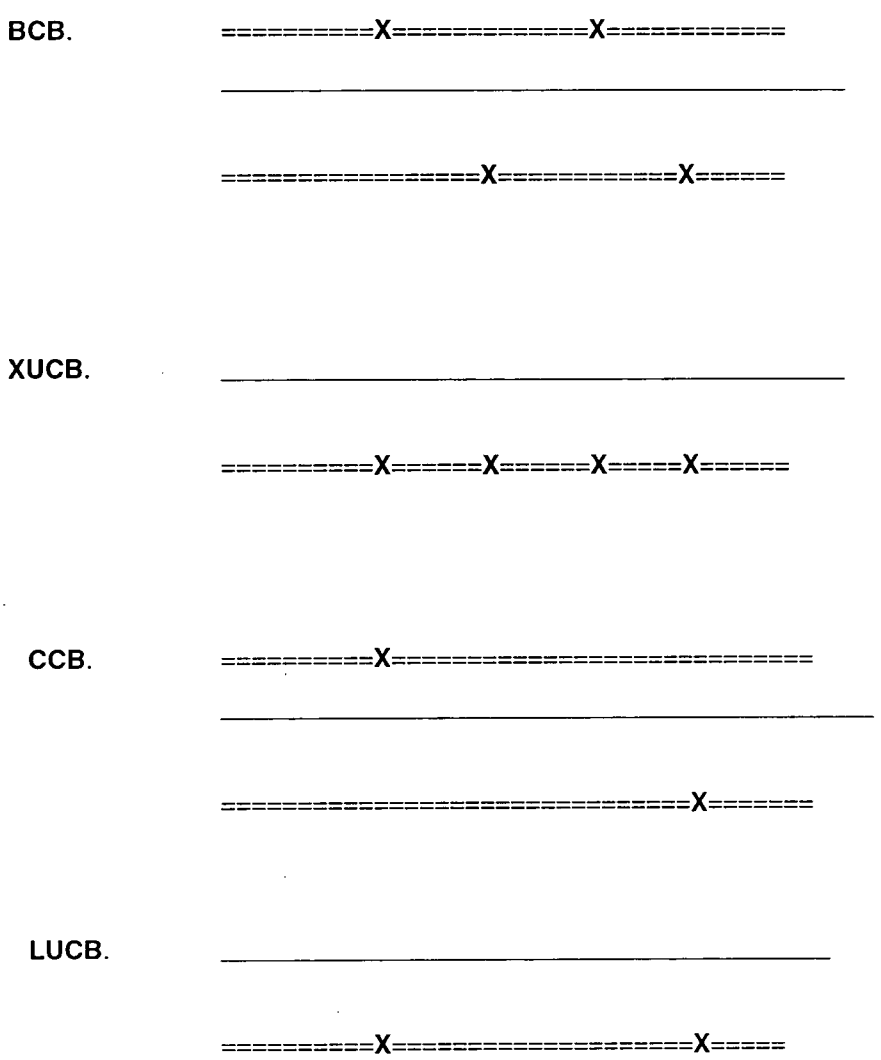

Figure 39. Diagrammatical representation of the various cingulum bundle lesions carried out in this experiment. The diagram represents a top view of the cortex with the unbroken horizontal line representing the sinus midline. The broken lines either side of the midline represent the cingulum bundle. An ***X*** *represents the approximate site of the lesion.*

7.2.2 Apparatus for experiment 5a: T-maze alternation.

All testing was carried out in a T-maze whose characteristics have been described in previous sections.

7.2.3 Apparatus for experiments 5b and 5c: Cross-maze.

All testing was carried out in a 4-arm (cross-shaped) maze. The floors of the maze were 10cm wide and made of aluminium, and the walls were 19cm high and made of clear perspex. The stems were 78cm long, and at the end of each section was a food well 4cm in diameter and 0.75 cm deep. The entire maze was supported by three stands 92cm high. Lighting was provided by fluorescent lights suspended 92cm above the apparatus, the luminance light levels at the choice points and food wells being 320 and 280 lux respectively. A slotted perspex door made it possible to open or close the end of either of the two stem arms.

7.2.4 Apparatus for Experiment 5d: 8-arm Radial maze.

All testing was carried out in an 8-arm radial maze. This apparatus was a standard radial maze but had clear perspex sides added to the arms to prevent the animals from moving directly from arm to arm. Each arm was 87cm long and 10cm wide, with the sides being 24cm high, at the end of which was a food well 2cm in diameter and 0.5cm deep. Each arm led through a clear perspex guillotine door 12cm high to a central circular platform 34cm in diameter. Each of the eight doors were separately connected to a circular board suspended 100cm above the maze enabling the experimenter to open the doors either individually or simultaneously. The maze was mounted upon a turntable which stood 53cm off the ground. This enabled the entire maze to be rotated through 360 degrees, each arm was individually numbered and the floor of the testing room marked so that the position of the maze could be standardised in relation to the room cues. Lighting was provided by 3 fluorescent lights positioned 140cm above the maze. The radial-arm maze was placed in a different room to that used for the other T- and cross-maze procedures.

7.2.5 Procedure for experiment 5a: T-maze alternation.

A full description of the T-maze procedure has been presented in experiment 2c (see figure 14, and accompanying text), though for this series of experiments the acquisition procedure was amended slightly. In the preceding experiments the animals were tested for a total of six trials per day (presented in pseudorandom order giving an equal number of 'left' and 'right') for 6 sessions at 15s delay before moving onto a further series of sessions using longer delays. In this experiment each animal was again presented with six trials per day at 15s delay, but for a total of 15 sessions.

Following these acquisition trials, a further set of six sessions were introduced in which the animal underwent a 'double-forced' procedure. In this, the animals were forced in one direction and then confined to the start-arm, before being forced to the other direction and then confined again before being allowed to select. In this 'double-forced' procedure the animal was therefore rewarded for choosing the arm not visited on the second forced-choice (see figure 40). Following this procedure, each animal performed three final sessions on the 'normal' forced-choice procedure as in the initial training sessions.

7.2.6 Procedure for experiment 5b: Cross-maze (allocentric).

Testing began 4 days after the completion of experiment 5a. The procedure again consisted of two parts, the first part being identical to that described in experiment 5a, i.e. on the 'information' run the animal was forced to run into one randomly selected arm and allowed to eat the reward. All information runs began in the same start area used in experiment 5a. The second part was similar in that the animal was rewarded for entering the arm not previously chosen on the 'information' run, but now on half of the choice trials the animal was placed in the start area of the arm opposite to that used on the information run, see figure 41. In these 'opposite-arm trials' the location of the 'correct' choice arm had not altered but the animals spatial location in relation to it had, i.e. in the previous experiment if the animal had turned left in the 'information' run, it would have been rewarded for turning right on the 'choice' run; in this procedure however, if the animal had turned left on the 'information' run, it would have to turn left again to be correct on the "choice" run.

It must be emphasised that whichever start arm was chosen, the animal was prevented from entering the opposite arm by a wooden block, thus only three out the four arms were in use at any one time. All animals received 6 trials per day for a total of 10 days, giving a total of 60 trials divided equally between choices taken from the 'same-arm' or the 'opposite-arm'. Each session consisted of a mixture of 'same-arm' and 'opposite-arm' trials. The trials were 'spaced' in that each animal received one trial and was then returned to its cage, the ITI being approximately 3 minutes.

7.2.8 Procedure for experiment 5c: Cross-maze (egocentric).

Testing began 7 days after the completion of experiment 5b. At the start of each trial each animal was randomly assigned to one of the four arms and confined at the end by a wooden block. The arm opposite to that start arm had been blocked, so effectively creating a T-maze. The animal was allowed to choose either of the two arms branching out from the "T" i.e. making a body turn to the right or to the left.

On the very first trial of the first session the animal was rewarded for entering either arm, but on all subsequent sessions the animal was only rewarded for turning in the same direction as it had turned on the first trial. For example, if the animal had turned to the left on the initial run, on each subsequent run the animal would only be rewarded for turning left (see figure 42). Each arm was numbered and the rat was assigned to a start arm on a pseudo-random sequence, so that following the first body turn, it could then be placed in any of the three other arms, where it was confined for approximately 10s, before being allowed to make another choice.

Each animal received 12 trials per day, and unlike the previous experiments they were run consecutively i.e. one straight after another. Testing ceased when the animal reached 30/36 correct responses. Upon reaching this criteria, the 'correct' body turn was 'reversed'. i.e. if the animal had been rewarded for always turning left, on subsequent trials it would only be rewarded for turning right (see figure 41). Each animal again received 12 consecutive trials per day up to the same criterion level. If an animal made over 150 errors and still failed to achieve criteria, then it was assumed that the animal had failed to learn the task.

The Double-Forced Procedure in the T-Maze.

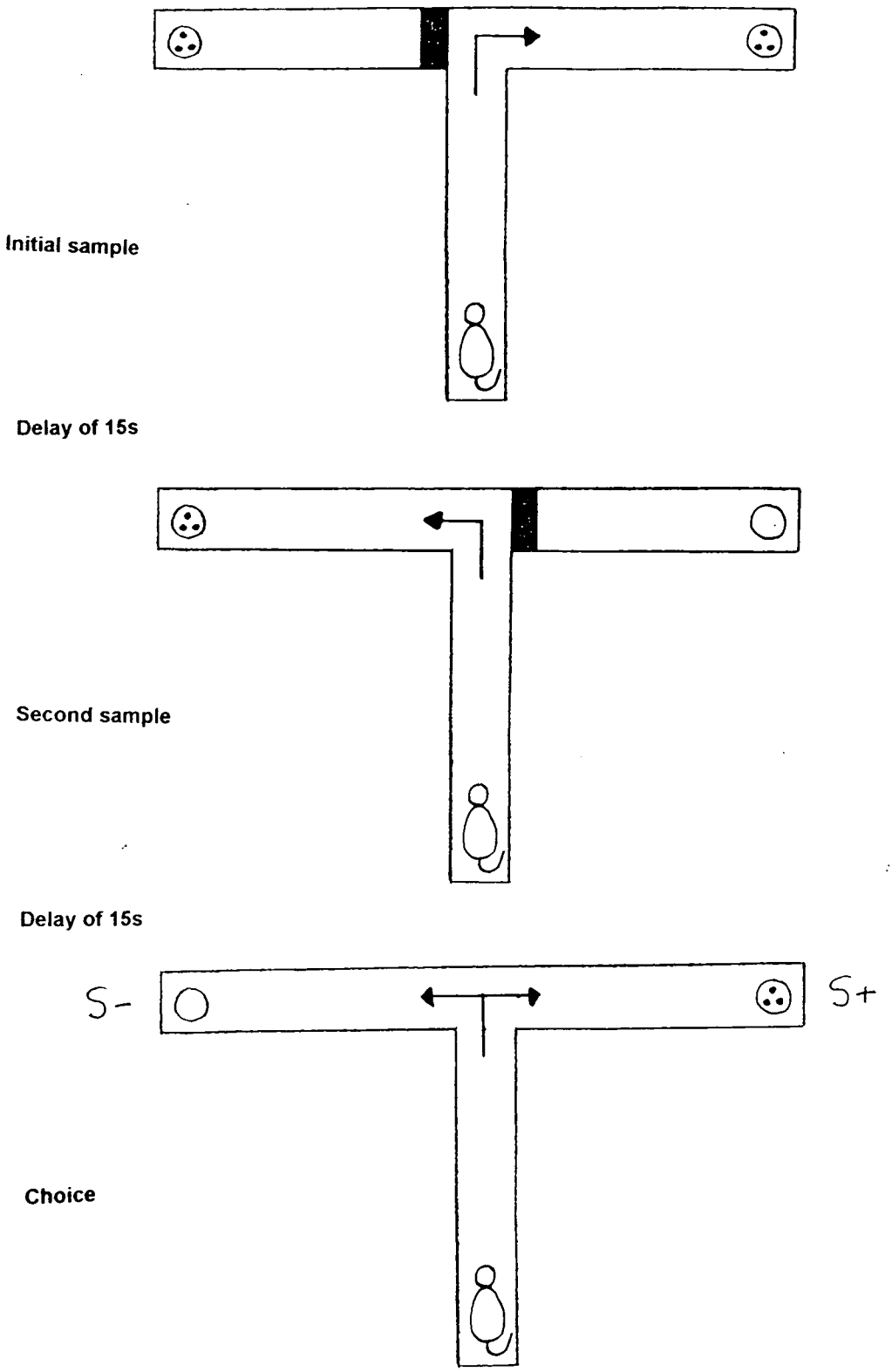

Figure 40. The double-forced alternation procedure used in the T-Maze.

Same-Arm Versus Opposite-Arm Procedure in the Cross-Maze.

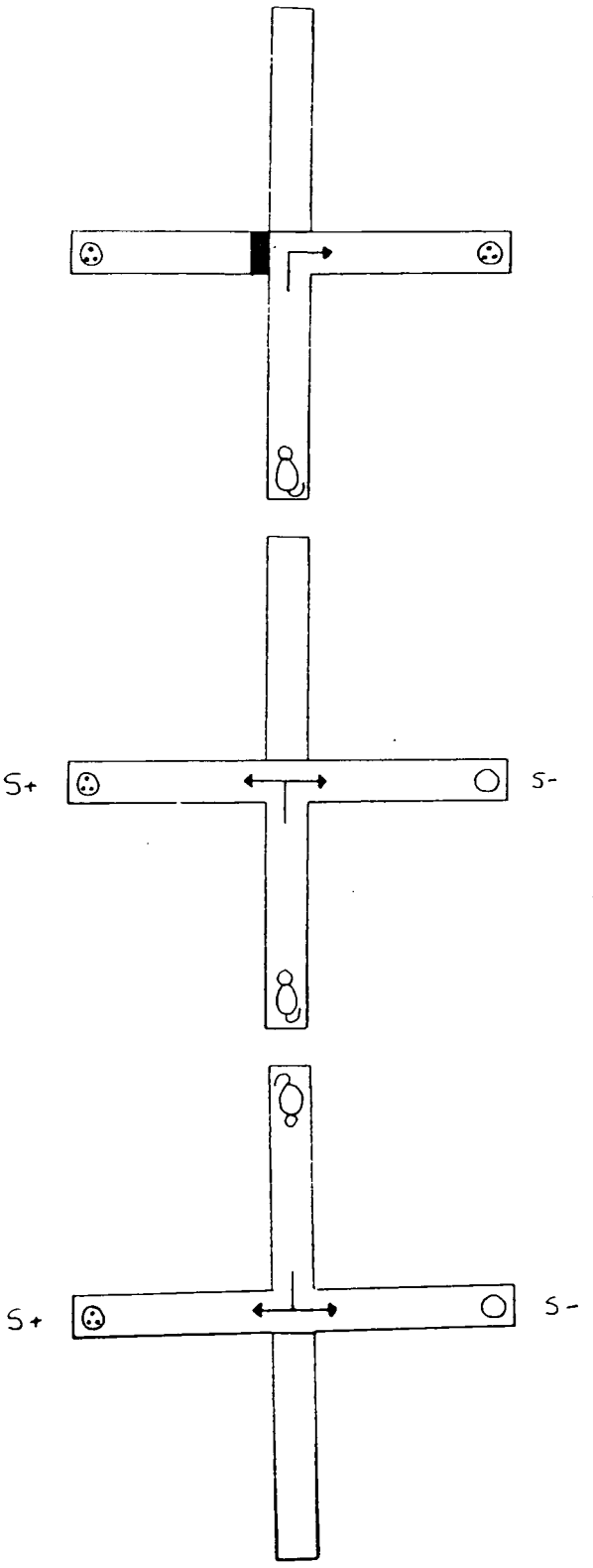

Figure 41. The 'same-arm' versus 'opposite-arm' procedure in the Cross-Maze. Top: initial forced-choice. Middle: subsequent choice in the same-arm as the initial forced-choice. Lower: Subsequent choice in the opposite-arm as in the initial forced-choice.

Egocentric (Body-Turns) Procedure in the Cross-Maze.

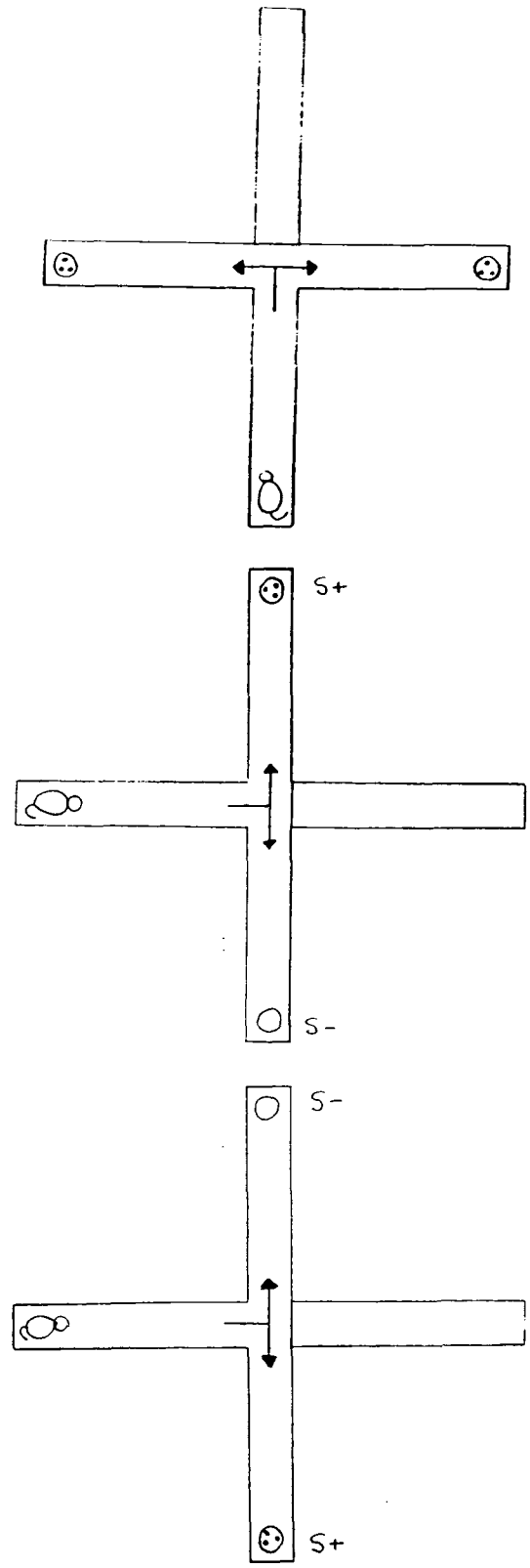

Figure 42. Body-turns procedure in the Cross-Maze.Top: Initial free-choice. Middle: Subsequent free choice up to set criteria. Lower: Reversal of 'correct' turn up to criteria.

7.2.8 Procedure for experiment 5d: 8-arm radial maze.

Testing began approximately 7 days after each animal had finished experiment 5c. Following several habituation sessions in which each animal was confined to the maze for approximately 15 min, with all of the guillotine doors raised and food reward pellets scattered in each arm, the following procedure was carried out.

All eight arms of the maze were baited with reward pellets placed in the food wells. The animal was placed in the central area and all the doors were simultaneously raised. When the animal had made a choice by entering an arm all doors were lowered and the animal was allowed to eat the reward pellets. The door to that arm was then raised allowing the animal to return to the central area, and thence make another choice. This procedure was repeated until all the remaining baited arms had been visited. The session was terminated if the rat had not completed this task within 10min or if it had not made a response for 2 min. Twelve sessions, one per day were performed in total.

7.2.9 Surgical and histological procedures.

A total of 56 animals received surgery with 6 dying post-operatively, this left a total of 50 which were divided into six surgical groups; surgical control (SHAM, n=11), fornix (Fx, n=6), limited unilateral cingulum bundle (LUCB, n=9), extensive unilateral cingulum bundle (XUCB, n=6), crossed cingulum bundle (CCB, n=9), and bilateral cingulum bundle (BCB, n=9). Each animal was anaesthetised by intraperitoneal injection (60mg/kg) of Sagatal, each was then placed in a stereotaxic headholder (David Kopf Instruments, Tujunga), and the scalp retracted to expose the skull. A small craniotomy made above the sagital sinus and the dura cut to expose the cortex above the target region.

For all lesions, a Radionics TCZ (Radionics Inc., Burlington) electrode (0.3mm tip length, 0.25mm diameter) was lowered vertically into the appropriate sites and the tip temperature raised to 75C for 60s using an RFG4-A Lesion Maker (Radionics Inc., Burlington). For the limited unilateral cingulum bundle (LUCB) group, four lesions were made in one hemisphere, at two sites separated by 1.5mm (see figure 39). The stereotaxic coordinates of the lesions relative to ear-bar zero were: AP + 6.5, LAT ±1.1 from central sinus, and AP + 5.0, LAT ±0.8. both sets of lesions were placed 2.2mm and 2.3mm below the cortex respectively.

For the extensive unilateral cingulum bundle (XUCB) group, eight lesions were made in one hemisphere, at four sites along the whole length of the bundle (see figure 39). The stereotaxic coordinates of these lesions relative to ear-bar zero were: AP +7.9, LAT ±1.1, AP +5.3, LAT ±1.1, AP + 3.7, LAT ±1.0, and AP +1.4, LAT ± 0.9. In each case, two lesions were made, firstly at one depth (-2.1mm, -2.1mm, -1.7mm, and -1.9mm from top of cortex respectively), and then the probe was raised by 0.3mm in all cases and another lesion was made.

For the crossed cingulum bundle group (CCB), two lesions was made in either hemisphere using the same co-ordinates as the LUCB group, resulting in four animals of left midline + right caudal, and four animals with right midline + left caudal cingulum bundle lesions (see figure 39). For the bilateral cingulum bundle group (BCB), four lesions were made at two sites in each hemisphere, (see figure 39). The stereotaxic coordinates of these lesions are the same as for the XUCB group, but lesions were made in both hemispheres.

For the animals receiving fornix lesions, two radiofrequency lesions were made in each hemisphere , the coordinates of these lesions relative to ear-bar zero were: AP +5.3, LAT ±0.7, and AP +5.3, LAT ±1.8, the depth from top of cortex was -3.7mm and -3.8mm respectively. Note that both the LAT and depth co-ordinates were altered slightly from the ones used in experiments 4a-4c, this was due to the sparing of the lateral tips of the fimbria which was a consistent feature of using the former co-ordinates (see figure 17a).

The surgical controls (CON) received a craniotomy, and the dura was cut in the appropriate places. Animals in all groups received sulphanilamide powder and the skin was sutured, followed by a subcutaneous injection of 6ml sodium chloride + 0.3ml of milophyline. On completion of the experiment the animals were killed and perfused intracardially with 5% formol-saline. The brains were then rapidly removed and placed in 5% formol-saline. Subsequently, the brains were blocked, embedded in wax (Paraplast), and cut into 10- µm coronal sections. Every tenth section was mounted and stained with Cresyl violet, a Nissl stain.

7.3 RESULTS.

7.3.1 Histological Analysis.

Following histological analysis three animals in the CCB group were rejected as the lesions to the cingulum bundle were inaccurate and the bundle was only partially damaged. One animal in the BCB group was also rejected as it did not receive a full bilateral lesion, the caudal lesion being exceptionally small and causing only minor cingulum bundle damage. The final groups thus consisted of 11 CON, 8 BCB, 6 XUCB, 6 CCB, 9 LUCB, and 6 Fx.

Figures 43a and 43b show the extent of the largest and smallest of the cingulum bundle surgeries in each of the surgical groups. These surgeries involved bilateral or unilateral radiofrequency lesions placed at different AP levels, and each resulted in both localised damage to the bundle itself, and more generalised cortical damage surrounding the lesion. The more anterior cingulum bundle lesions consistently produced additional damage to rostral anterior cingulate cortex (in all but three animals) though caused no obvious damage to the prelimbic region. In one of the BCB animals there was also an indication that these anterior lesions resulted in some minor damage to the most dorsal region of the rostral limit of the lateral septum. The more posterior cingulum bundle lesions consistently produced additional damage to retrosplenial cortex (all animals). The extent of the extraneous damage to anterior cingulate and retrosplenial cortices varied between the groups with the BCB group (as expected) consistently showing a greater degree of cortical damage than the animals with more limited lesions (LUCB and CCB groups).

In both of the groups with bilateral lesions (CCB and BCB) the lesions were extensive, and completely destroyed the cingulum bundle at the appropriate sites. The lesions had been staggered yet in some cases the lesions in the opposite hemisphere overlapped i.e. there was some bilateral damage (6/9 in the BCB group, and 5/8 in the CCB group) though one lesion was always smaller as it was either just beginning or ending. In two of the BCB animals however, two lesions in opposite hemispheres overlapped considerably so that these animals demonstrated extensive bilateral cortical and cingulum bundle damage.

In addition, there was some hippocampal damage limited to the dorsal extent of the CA1 field, in most cases when this hippocampal damage was noted, it was only unilateral and very restricted (5/9 in the BCB group; 1/8 in the CCB group) though two animals in the BCB group received minor bilateral hippocampal damage. The subsequent scores of these two animals with bilateral hippocampal damage was assessed and both fell well within the range of the scores of the other animals in this group who had received either unilateral or no hippocampal damage, so these two animals were not rejected.

In the groups sustaining only unilateral lesions there was not a great difference in the extent of cingulum bundle and cortical damage. Obviously, neither group showed any symmetrical bilateral cortical damage but the XUCB group tended to receive more unilateral hippocampal damage (5/6 in the XUCB group and 3/9 in the LUCB group).

The degree of the severity of the cingulum bundle damage was reflected in associated cellular degeneration in the anterior ventral nucleus of the thalamus (AV). Those animals receiving bilateral cingulum bundle damage had a correspondingly greater degree of bilateral AV degeneration. Thus in the BCB group, 5/9 of the animals showed severe bilateral degeneration, with the remainder showing only moderate bilateral degeneration; while in the CCB group all of the animals showed only mild to moderate bilateral degeneration of AV. In the two groups with unilateral cingulum bundle damage the XUCB group demonstrated severe unilateral degeneration (6/6), while all of the LUCB animals displayed only mild to moderate unilateral AV degeneration. No other thalamic nuclei were affected. In most cases there was also additional damage to the corpus callosum at the lesion sites.

Figure 43b shows the extent of the largest and smallest fornix lesions. All were extensive with all six animals demonstrating a completely severed tract. In all of the Fx animals there was moderate involvement of the most dorsal limits of the anterior ventral (AV) and anterior dorsal (AD) thalamic nuclei. Furthermore the corpus callosum was slightly damaged in each case, and in one animal there was unilateral damage to the overlying cingulum bundle and the surrounding cortex. Note that as all the lesions were produced using the same coordinates, the photomicrographs just represent a typical example of a lesion at a particular site rather than any particular group.

Reconstructions of CCB and UCB surgeries.

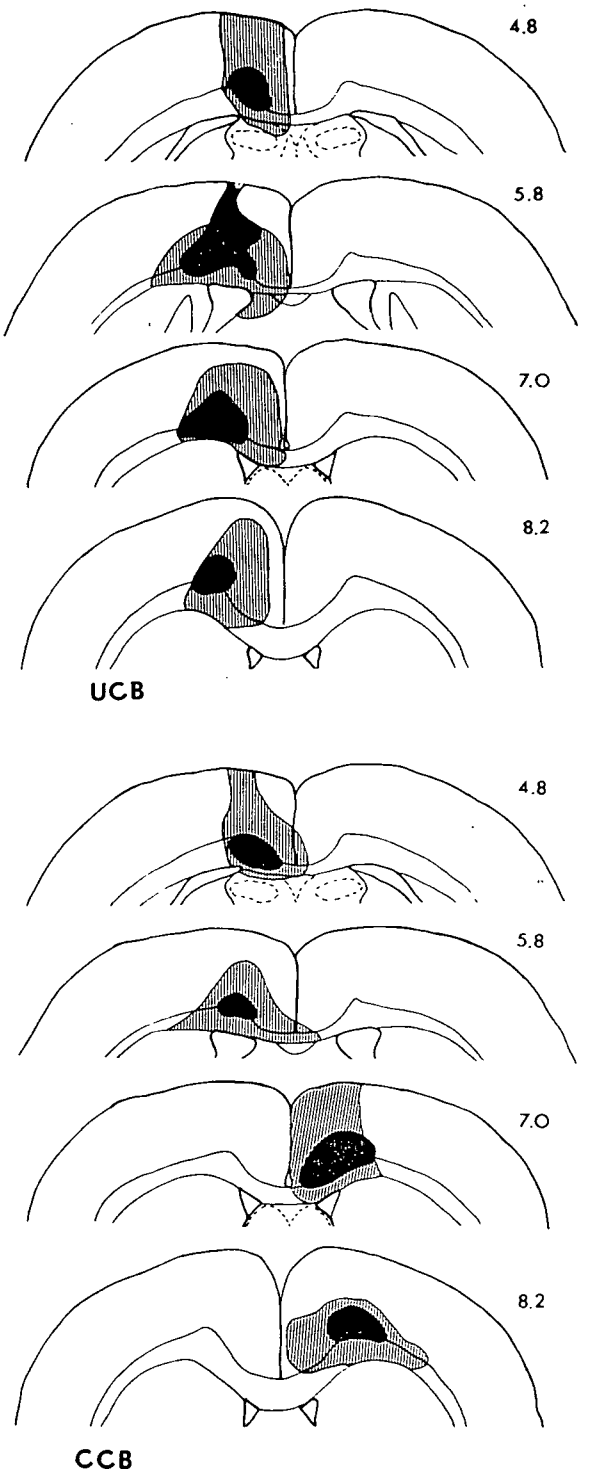

Figure 43a: Diagrammatical reconstruction's showing the extent of the crossed-cingulum bundle (CCB) and unilateral cingulum bundle lesions (UCB).

Reconstruction's of BCB and Fx surgeries.

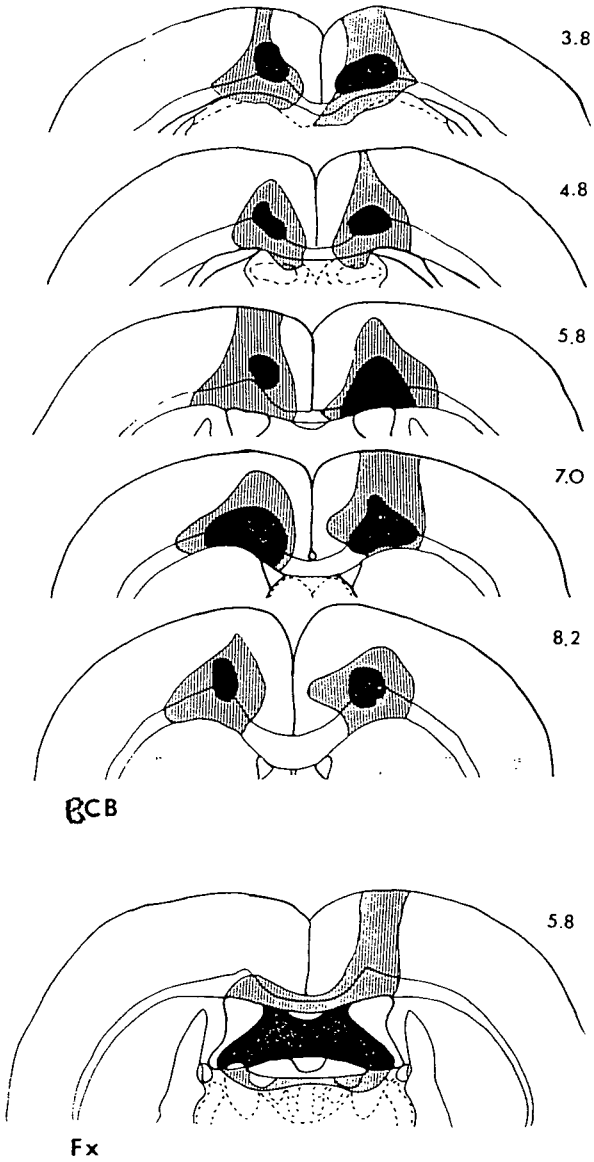

Figure 43b: Diagrammatical reconstruction's showing the extent of the bilateral cingulum bundle (BCB) and fornix (Fx) lesions.

Photomicrographs showing the appearance of the cingulum bundle following radiofrequency lesions.

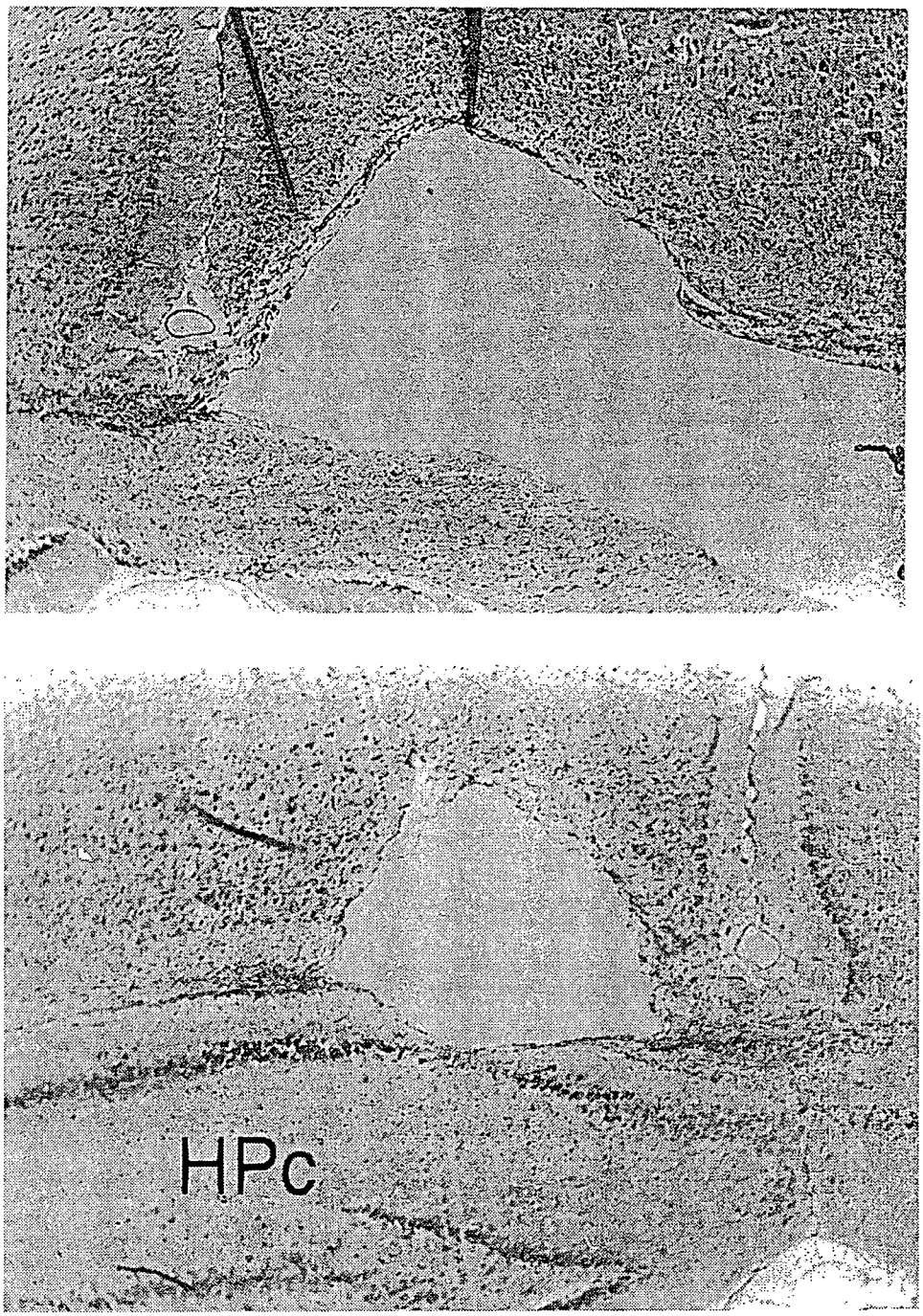

Figure 44. Photomicrographs showing the appearance of the cingulum bundle following radiofrequency lesions. Upper: a rostral lesion. Lower: a more caudal lesion, note the hippocampal damage. HPc = hippocampus

7.3.2. Comparison between XUCB and LUCB groups.

The histological analysis had strongly indicated that there was little difference between the position and extent of the unilateral lesions to the cingulum bundle in the LUCB and XUCB groups. Furthermore both were acting as control groups for the groups with bilateral cingulum bundle damage and as a consequence the performance of the two groups with only unilateral cingulum bundle/gyrus damage were compared across the sequence of behavioural tasks:

1. T-maze acquisition: The performance of the LUCB and XUCB groups were compared over the five blocks of acquisition sessions. An ANOVA revealed no group difference ($F_{1,13} = 3.52$, $P>0.05$), and no group X session interaction ($F_{4,52} = 1.49$, $P>0.05$).

2. Cross-maze (allocentric procedure): A two-way ANOVA was used to compare the two groups on their performance (percent correct) when the start arm was in the 'same' or the 'opposite' position to that used on the initial choice. No overall group difference was revealed ($F_{1,13} = 2.84$, $P>0.05$), and no group X session interaction was noted ($F < 1$).

3. Cross-maze (egocentric): A two-way ANOVA was used to compare the two groups on their performance (number of trials to criteria) on the initial discrimination and the subsequent reversal of body turns in the cross maze. The ANOVA revealed no significant group difference ($F < 1$) but did reveal a significant group X session interaction ($F_{1,13} = 10.48$, $P<0.05$). While the XUCB made on average fewer errors to criteria on the initial discrimination (25.0 versus 39.8), the same group made significantly more errors on the reversal (92.5 versus 58.4).

4. Radial maze: The two groups were finally compared on their performance over the four blocks (12 sessions) on both number correct in the first 8 choices, and total number of arms visited. An ANOVA revealed no significant group difference on either measure ($F_{1,13} = 3.7$, $P<0.05$; and $F < 1$ respectively). There was no group X session interaction in either case.

As there was little difference in the extent of the cingulum bundle damage in both groups with unilateral damage, and as only one significant difference was revealed in the latter stages of testing on the Cross-maze egocentric task, it was considered appropriate to combine these two groups to form a single group (UCB, n=15). As a difference was found on the egocentric (body turns) task, these groups will also be analysed separately on their performance on this task relative to the other groups.

7.3.3 Experiment 5a: T-maze alternation.

Each group performed an initial 15 acquisition sessions comprising 6 trials per day. For analysis these sessions were split into blocks comprising 3 sets of daily sessions (18 trials per block). Following that, each group performed a further two blocks of 6 sessions at the double-forced procedure. Finally each group then performed a final block of 3 sessions on the normal single-forced procedure (see figure 45).

a) Comparison of performance over all 5 blocks combined: The mean scores of each group over all five blocks is represented in figure 45. An ANOVA comparing the performance of each group over the five blocks combined revealed a very strong group effect ($F_{4,40} = 61.37$, $P<0.0001$). A subsequent Newman-Keuls analysis revealed that this group difference was due to the significantly poorer performances of the Fx and BCB groups. Both of these groups differed from all of the other groups ($P<0.05$ in all cases), and also differed from one another, though this is probably due to the increasingly better performance of the BCB group over the final two sessions.

The UCB group were observed to differ from the CON group and figure 44 indicates that this difference is probably due the relatively poorer performance of the UCB group over the first two blocks. An ANOVA compared the scores of the CON and UCB groups over the first two sessions combined, and revealed a significant group difference ($F_{1,24} = 5.31$, $P<0.05$). Another ANOVA comparing the scores of the same two groups over the remaining three blocks showed that this difference had vanished ($F_{1,24} = 1.96$, $P>0.05$).

b) Comparison of performance over the two blocks of double-forced procedure: The mean scores of each group over the 6 sessions using the double-forced procedure are also shown in figure 45.

An ANOVA revealed a strong group effect (F 4,40 = 4.07, P<0.01), which a subsequent Newman-Keuls test showed to be due to the significantly poorer performance of the Fx group. This group differed from the CON, UCB, BCB and CCB (P<0.05 in each case) groups. There were no other group differences.

T-Maze Acquisition, Percent Correct Scores On Normal, And Double-Forced Procedures.

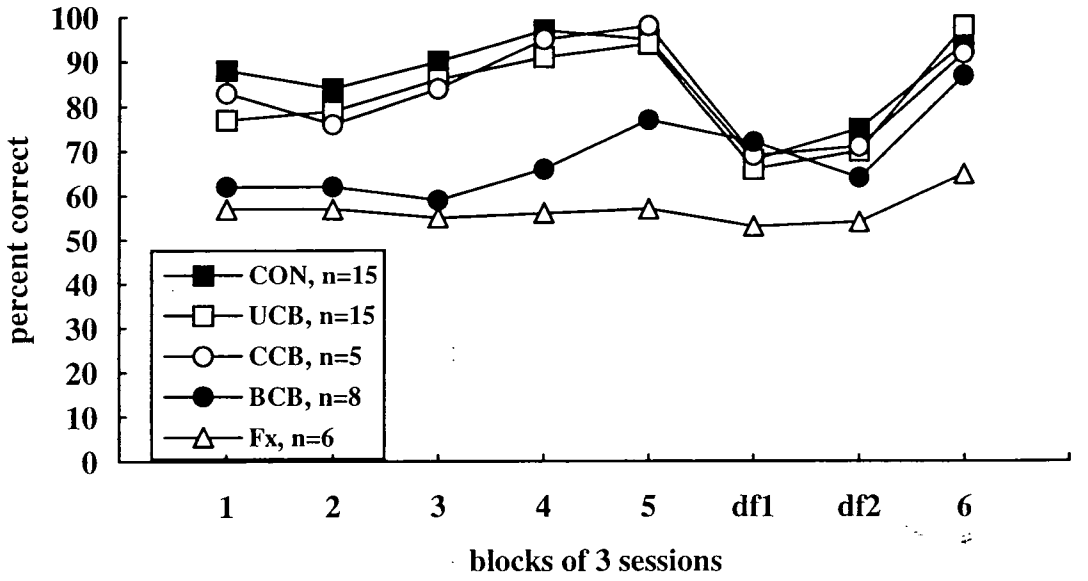

Figure 45. Percent correct performance on the T-maze delayed forced-alternation. Blocks 1 to 5, and 6 consist of the normal forced procedure, while blocks df1 and df2 consist of the double-forced procedure.

c). Comparison of performance over the last block: Following the two blocks of double-forced alternation, each group returned to a block of the 'normal' single-forced procedure, see figure 45. An ANOVA revealed a highly significant group effect (F 4,40 = 13.40, P<0.0001). A subsequent Newman-Keuls test revealed that this strong group difference was due to the significantly poorer performance of the Fx group which differed from all of the other groups (P<0.05 in each case). There were no other group differences.

7.3.4 Experiment 5b: Cross-maze (allocentric).

Each group performed a further 10 sessions comprising of 60 trials, 30 when the arm was the 'same' as that used in the 'information' run, and 30 when the arm was 'opposite' to that used in the 'information' run. The mean correct scores are shown in figure 46.

Same arm versus opposite arm: An ANOVA performed on both sets of scores revealed a significant group difference ($F_{4,40} = 15.92$, $P<0.0001$) reflecting the overall poorer performance of the Fx group. The test also revealed the expected effect of session ($F_{1,40} = 133.78$, $P<0.0001$) as all groups performed worse on the 'opposite-arm' condition than on the 'same-arm' condition. Finally, the test revealed a significant group X session interaction ($F_{4,40} = 5.70$, $P<0.001$), which again reflected the poorer performance of the Fx group.

As the Fx group were largely unaffected by the type of session and performed essentially at 'floor' throughout both conditions, the previous ANOVA which compared both 'same-arm' and 'opposite-arm' conditions was repeated, but without the Fx group. This time, there was no significant group effect ($F_{3,35} = 1.68$, $P>0.05$), nor was there a significant group X session interaction ($F_{3,35} = 1.06$, $P>0.05$), indicating strongly that the significant results gained in the previous test was indeed due to the abnormal performance by the Fx group (see figure 46).

As seen from figure 46, the BCB group performed worse than the other cingulum bundle groups particularly on the 'opposite-arm' sessions. A closer analysis of this was thus carried out. An ANOVA performed on the data from the 'opposite-arm' sessions again without the fornix group however revealed no significant group difference ($F_{3,35} = 1.68$, $P>0.05$).

Same-Arm Versus Opposite-Arm Performance in the Cross-Maze.

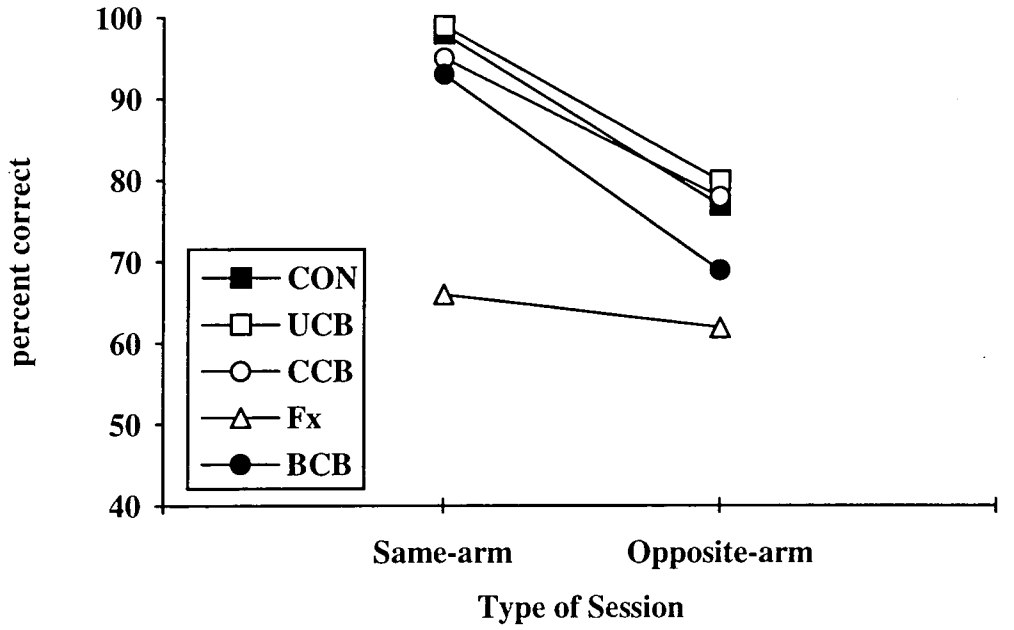

Figure 46. Percent correct performance of each group on the forced-alternation procedure in the T-maze, under two conditions. Firstly, when the choice start- arm was the 'same' as in the sample phase, and secondly, when the choice start-arm was 'opposite' to that used in the sample phase.

7.3.5 Experiment 5c: Cross-maze (egocentric).

Each animal was given an initial choice and then on subsequent trials only rewarded for turning the same way, up to a criteria of 30/36 in 3 consecutive sessions (the first discrimination). When this criteria was reached, the body turn that had been 'correct' was reversed, i.e. if the animal had been rewarded for always turning left, it was now only rewarded for turning right. Again this was performed to a criteria of 30/36 in 3 consecutive sessions. In the first 'choice' phase of this procedure one of the BCB animals failed to reach criteria (scoring over 150 errors) and was thus removed from this part of the experiment. One of the Fx animals also became ill at this point and was removed from the remainder of the experiment.

As the initial comparison between the LUCB and XUCB groups had indicated a significant difference (a group X session interaction on this egocentric task), the UCB groups were split into their respective two groups for this comparison. The mean scores of each group on the first discrimination and the subsequent reversal sessions are presented in figure 47. An ANOVA revealed a significant group effect ($F_{5,37} = 4.69$, $P<0.01$) which a Newman-Keuls test showed to be due to the poorer overall performances of the CCB animals who differed significantly from the other groups ($P<0.05$). There were however no other group differences. The ANOVA also revealed the expected effect of session ($F_{1,37} = 62.61$, $P<0.0001$) as all groups performed worse over the reversal trials. Finally no group X session interaction was revealed ($F<1$), see figure 47.

As seen in figure 47, the BCB group are performing better than all of the other groups in both the initial discrimination and the subsequent reversal so a more detailed analysis was made of these sessions.

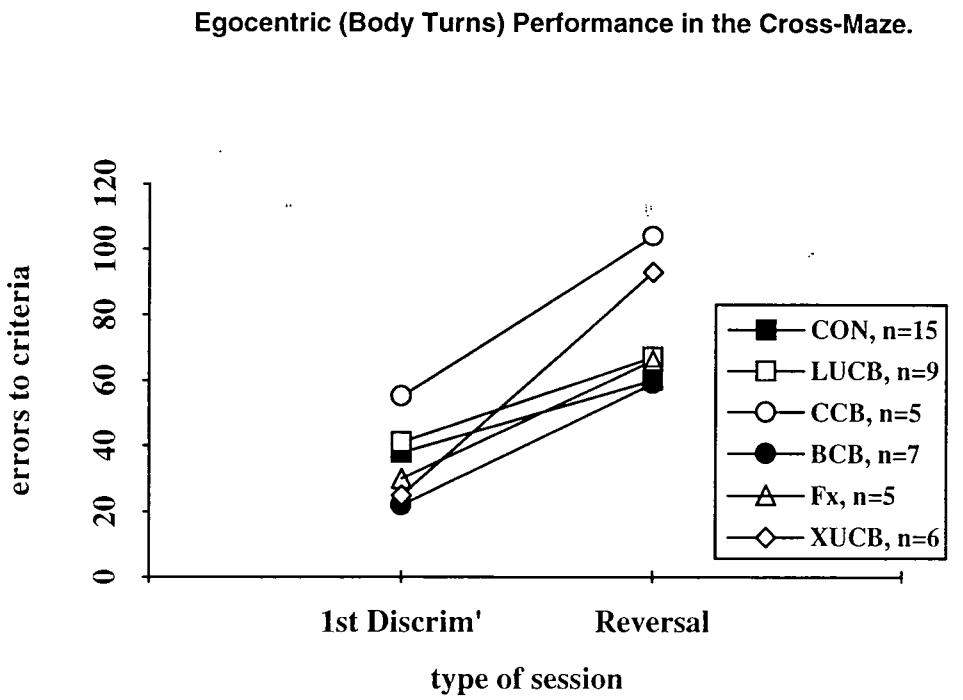

Figure 47. Errors to criteria on the initial choice of body turn, and its subsequent reversal by each group.

Number of errors taken to reach criteria on the first discrimination: An ANOVA revealed the expected significant group difference ($F_{5,37} = 2.78$, $P<0.05$), a subsequent Newman-Keuls test showed that this effect was due to the poorer performance of the CCB group who differed from the BCB and XUCB groups ($P<0.05$ in each case). There were no other group differences.

Number of errors taken to reach criteria on the subsequent reversal: An ANOVA revealed a significant group effect ($F_{5,37} = 3.31$, $P<0.05$) which a Newman-Keuls test showed to be due to the poorer performance of the CCB animals who differed from the CON and BCB groups ($P<0.05$ in each case). There were no other group differences.

One possible reason for the poorer performance of the CCB group was that they could have been experiencing 'carry-over effects' from the previous alternation experiment to a greater degree. To test this, the types of errors made over the first discrimination and the subsequent reversal were compared. Two types of error are possible: either the animal can alternate (i.e. it can firstly turn one way and then subsequently turn the other way, as in the previous experiment), or the animal can select one direction and continually turn in that direction. It would be expected that over the initial discrimination sessions all groups will show a greater percentage of 'alternation' errors, and over the subsequent reversal they would show a greater percentage of 'turning one-way' errors (as they continue to turn in the now incorrect direction). The percentage of 'alternation' errors shown by each group over the two sessions is shown in figure 48.

An ANOVA carried out on the percentage of 'alternation' errors shown by each group on the 'discrimination' and 'reversal' sessions revealed the expected effect of session ($F_{1,37} = 34.58$, $P<0.0001$) as each group produced more alternation errors on the initial discrimination than on the reversal. However, no group effect was noted ($F <1$) and neither was there a group X session interaction ($F_{5,37} = 1.51$, $P>0.05$). This indicates that whatever the reason for the poorer performance by the CCB animals it was not due to carry-over effects from the previous experiment. The poor CCB performance could be attributed to the very bad performances of two out of the five animals, and interestingly the percentage of alternation errors for these two animals was much higher than the other animals.

Percentage of 'Alternation' Errors On The Egocentric Task.

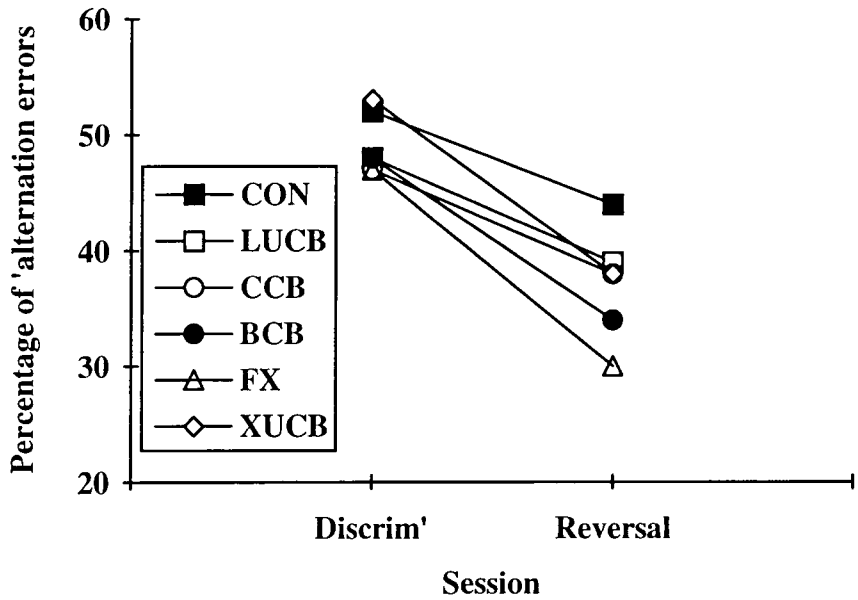

Figure 48. Percentage of 'alternation' errors by each group on the Cross-Maze egocentric task.

The above graph also shows that the CON group made more 'alternation' errors than the other groups on both types of session, this is perhaps not surprising as 'normal' animals show a preference for alternating. The Fx animals (and to a lesser extent the BCB animals) however made much less alternation errors (and thereby many more 'one-way' errors) particularly over the reversal sessions indicating the reluctance of these groups to abandon the previously learned 'correct' turn. The XUCB group, who clearly performed much worse on the reversal sessions than on the discrimination sessions also did not demonstrate 'carry-over' effects (at least on the reversal sessions) as they were in fact remarkably similar to the LUCB animals in terms of 'alternation' errors. Why this group should have performed particularly badly on the reversal sessions is thus difficult to explain as they were not experiencing 'carry-over' effects to any significant degree.

7.3.6 Experiment 5d: 8-arm radial maze.

Each group performed 12 sessions which was split into 4 blocks comprising 3 sessions each. Two types of analysis were carried out on this data, the first relating to the number of correct arms visited by each group out of the first 8 choices, and the second relating to the total number of arms visited in entering all eight arms.

Number of correct choices out of the first 8 over four blocks: The average number of correct choices in the first 8, taken over the twelve sessions as a whole are shown in figure 49. An ANOVA revealed, an extremely significant group difference ($F_{4,39} = 39.44$, $P<0.0001$) but no significant group X session interaction ($F<1$). A Newman-Keuls test showed that this was due to the poorer performances of the BCB and Fx groups, both of whom differed from all of the other groups ($P<0.005$ in each case). They did not however differ from one another ($P>0.05$). There were no other group differences.

Radial Maze: Number of Correct Arms Visited in the First 8 Choices.

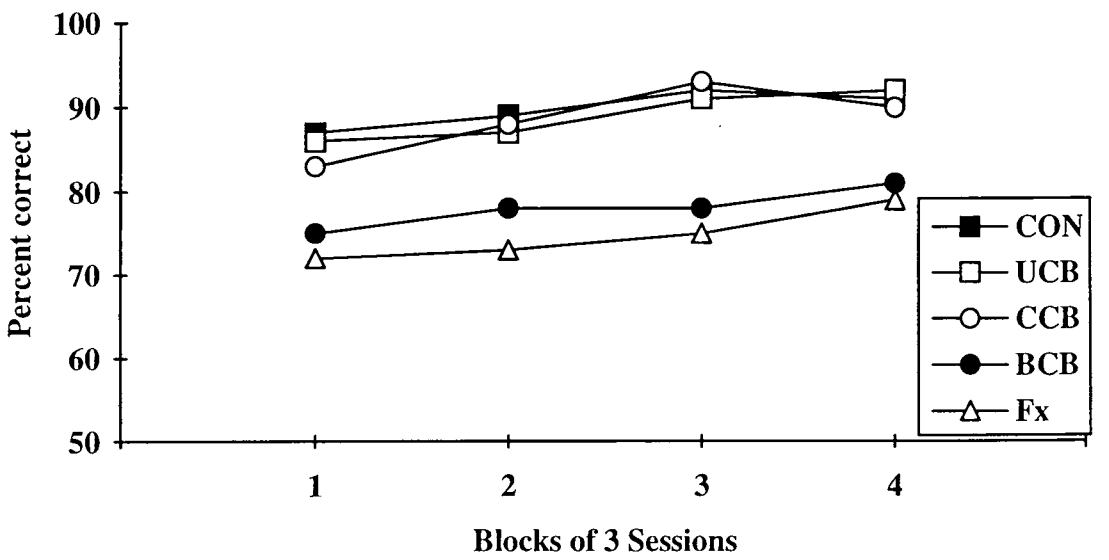

Figure 49. Percent correct scores by each group on the number of correct arms visited in the first eight choices in the radial-arm maze. The data is presented in blocks of three sessions.

c) Total number of arms visited in entering all eight arms: The mean total number of arms visited in entering all eight arms is shown in figure 50. An ANOVA revealed an extremely significant group difference ($F_{4,39} = 49.864$, $P<0.0001$). A Newman-Keuls test showed that this was due to the poorer performance of the BCB, and Fx groups who differed from each of the other groups ($P<0.005$ in each case). These two groups also differed from one another ($P<0.05$) which probably reflected the much poorer performance of the Fx group over the first block. No group X session interaction was revealed ($F_{12, 117} = 1.81$, $P=0.053$) but this interaction only just missed significance.

Radial Maze: Total Arms Visited in Entering All 8 Arms.

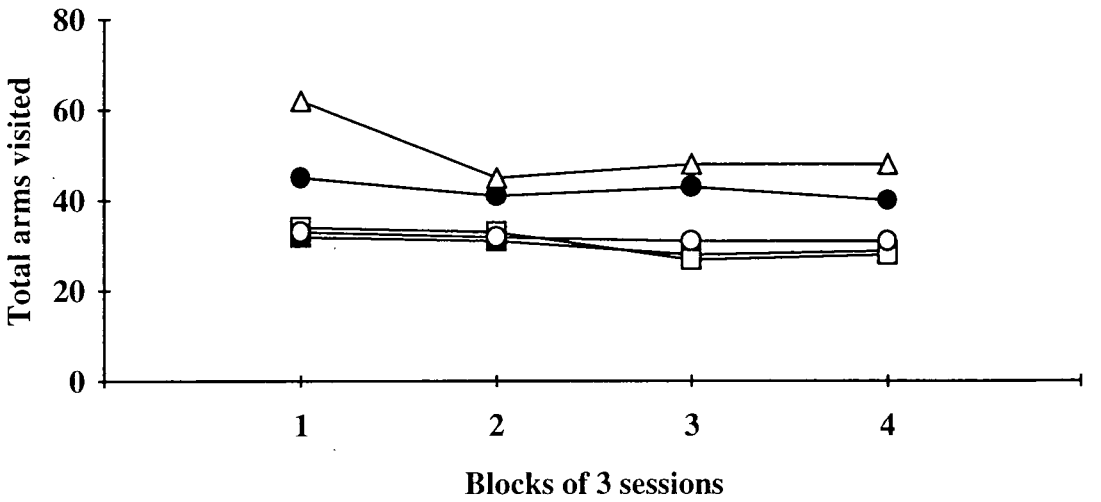

Figure 50. Average number of arms visited by each group in visiting all eight arms of the radial maze. The data are presented in blocks of three sessions.

7.3.7 Degree of Hippocampal damage.

The histology had revealed that a fair proportion of the animals receiving cingulum bundle lesions also received some damage to the hippocampus. Thus, some animals showed unilateral damage (5/8 in the BCB, 1/5 in the CCB, and 8/15 in the UCB groups), while several showed bilateral damage (2/8 in the BCB group). It could be argued that the more severe deficits shown by the BCB group could be explained by the fact that damage was caused to the hippocampus. However, there are two reasons why this is unlikely.

Firstly, the performance of the two animals with bilateral hippocampal damage in the BCB group was not significantly different to that of other animals who received bilateral cingulum bundle lesions, with associated unilateral hippocampal damage ($F<1$ in each comparison). Although the smallness of the group with bilateral hippocampal damage ($n=2$) means that such comparisons must be treated with extreme caution, the lack of a clear difference indicates that the degree of hippocampal damage did not exacerbate their deficits, as animals in the same group with slight or no hippocampal damage were just as impaired on several of the tasks. Secondly, over half of the animals in the UCB group showed some unilateral hippocampal damage, (in some, this damage was moderate to severe) and yet these animals showed no clear deficits in performance on any of the tasks.

7.4 DISCUSSION.

This experiment had two main aims: firstly to judge the effects of different lesions of the cingulum bundle, and secondly to assess the effects of such lesions on tasks assessing both allocentric and egocentric processing.

7.4.1 Aim 1: The effects of different cingulum bundle lesions.

The two groups with only unilateral lesions of the cingulum bundle (LUCB and XUCB) were firstly compared over all of the tasks. In each instance no significant group difference was found, although on the cross-maze egocentric task a significant group X session interaction was noted. This may indicate that unilateral damage to the cingulum bundle might affect the animals' ability to turn a given way.

As no other clear group differences were found it was considered appropriate to combine the two groups and the findings thus indicate that extensive unilateral damage to the cingulum bundle (which affected the surrounding cortex more) does not exacerbate any impairment caused by more limited unilateral damage. As the histology subsequently revealed no great difference in the extent of the lesions in the LUCB and XUCB group (the extraneous damage to surrounding cortex was slightly more in the XUCB group but not dramatically so) such a conclusion is perhaps not surprising.

These two groups were subsequently combined to form one group (UCB) and it was significant that this group only showed a brief transitory impairment on the first two blocks of the T-maze task. This result is similar to that reported in the previous experiment where bilateral damage (but sparing the most medial aspects of the bundle) to the cingulum bundle caused a similar transitory impairment. The lack of a consistent and strong impairment indicates that unilateral or moderate bilateral damage to the cingulum bundle is not sufficient to cause a severe and permanent mnemonic deficit. As both of the UCB groups also showed significant unilateral cortical damage, the finding of only a moderate and transient impairment gives some support to the results from experiment 3 which revealed that extensive cingulate cortex damage did not produce an impairment on the T-maze task.

The group with crossed-lesions of the cingulum bundle (CCB) also showed only a slight performance deficit compared to the control animals over the first two blocks on the T-maze acquisition sessions but this was not significant. The CCB group did show a more severe impairment on the 'choice' phase of the cross-maze 'egocentric procedure' (which will be discussed later). They were unimpaired on the radial maze task. This lack of a severe and long-lasting impairment is not however surprising. Despite the fact that each animal suffered a bilateral lesion of the cingulum bundle the lesions were staggered (so as to reduce adjacent cortical damage) and the histological analysis indicated that the rostral lesion occurred at the most anterior part of the bundle, level with the septum. It is thus quite possible that this anterior lesion occurred before other potentially important fibres have joined the bundle and so this crossed-lesion group received essentially a unilateral lesion (see figure 51).

As this group only showed mild to moderate AV degeneration compared with the moderate to severe degeneration shown in the BCB group this would indicate that the AV fibres joining the bundle had indeed remained relatively unaffected. This further implies that the fibres joining the cingulum bundle from medial frontal cortex are not critical for performance on the allocentric tasks described in this experiment.

The CCB lesion.

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                    {}
                    {}
rostral   ==========X===={}=======================   caudal
          ____________________________________________

          ========================X=============
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*Figure 51. Diagrammatical representation of the crossed-cingulum bundle lesion with **X** representing the position of the lesion in each hemisphere, and {} representing a possible fibre pathway joining the bundle. This figure shows that while the cingulum bundle is damaged bilaterally, other fibres could join after the most rostral lesion, and so we are left with essentially a unilateral lesion, which would not be expected to cause a deficit, as indeed such damage was shown to have no severe or permanent effect.*

The group with bilateral damage to the cingulum bundle (BCB) were impaired on two of the tasks which assessed allocentric processing (T-maze and radial-maze). In the former the deficit was initially severe but was not permanent (figure 45); while in the latter, the group showed no signs of improvement (figures 49 and 50). In the Cross-maze 'egocentric' procedure, the BCB group in fact performed significantly better than the CCB group who were impaired in this phase. In all cases where the BCB group showed an impairment, they performed in a similar manner to the Fx group although the Fx animals often showed a slightly more severe impairment.

This result supports the findings from experiment 3 and indicates that a severe impairment following cingulum bundle damage will only follow if there is bilateral damage to the cingulum bundle at several points along its course so as to completely disrupt connecting fibres. The interesting question of course relates to which fibres are the important ones. Several animals in the CCB group only received bilateral lesions at the most rostral end of the bundle due to an error in the AP coordinates (thereby only presumably disrupting fibres from prefrontal cortex). They demonstrated no AV degeneration and no form of impairment (although the lesions were very small) indicating that prefrontal fibres are not crucial. Animals in the BCB group also received disruption of the prefrontal fibres but also bilateral damage to more caudal portions of the bundle at a level after the fibres from the anterior thalamus had joined (as they all showed severe retrograde degeneration in this nucleus). The more severe impairments showed by this group therefore indicates that it is the efferent connections from the anterior thalamus which are more important. The finding that neurotoxic lesions of the anterior thalamic nuclei produce corresponding impairments on the same tasks as used in this experiment (Aggleton et al., submitted) serves to confirm this speculation.

7.4.2 Aim 2: A dissociation between allocentric and egocentric processing.

A dissociation was found between those tasks that are considered to be taxing primarily allocentric processing and those which are thought to tax essentially egocentric processing, with a clear impairment being found on the former for animals with extensive damage to the cingulum bundle.

1. T-maze alternation.

The BCB group were severely impaired over the initial blocks of the T-maze forced-alternation procedure, but did improve over subsequent blocks, yet they were severely impaired on the acquisition of the radial-maze. The T-maze task would seem to require the use of distal cues for a successful performance, and this form of allocentric processing is clearly disrupted following extensive bilateral cingulum bundle damage. The BCB group did however improve over time and by the final block were performing at a level only slightly lower than the CON group. The reason for this improvement is not clear as the Fx group remained severely impaired throughout, and only showed a small improvement in the very last block (figure 45). The Fx group however may have been relying to a larger extent on egocentric cues.

The cingulum bundle deficit on the T-maze, while often mirroring the performance levels of the Fx lesioned animals, is not the same as its effects are not quite as severe, and not as long-lasting. It may be the case that the cingulum bundle forms only part of a circuit which also contains the fornix (which is crucial for spatial processing), and so animals with cingulum bundle damage may be able to utilise connections with other regions (which also play a role in such processing) which have been left undamaged by the surgery. This could explain the transient nature of the cingulum bundle deficit as opposed to the more long-lasting fornix deficit. However, the need to know more about the precise route of the anterior thalamic fibres through the temporal lobe to the cingulum bundle, is of great importance.

2. Cross-maze (allocentric procedure).

On the cross-maze 'same-arm / opposite-arm' task (which more directly assesses allocentric processing) the Fx animals were markedly impaired over both conditions while the BCB showed a different impairment. On the 'same-arm' trials the BCB animals were unimpaired, and this is to be expected as the task is the same as the T-maze task which this group were performing well in the latter blocks. On the 'opposite-arm' trials however, the performance of the BCB animals dropped markedly and while the Fx animals differed significantly from CON, CCB and UCB groups, they did not differ from the BCB animals. This task difference on the 'opposite-arm' trials was sufficient to cause a drop of performance of the BCB animals larger than that (though not significantly so) of the CON, UCB, and CCB groups.

3. Cross-maze (egocentric procedure).

On the cross-maze 'body-turns' procedure (taxing egocentric processing) it was assumed that the BCB animals would not be impaired and indeed they were not, in fact this group performed slightly better than the other groups, and significantly better than the CCB group. This is not all that surprising because the BCB animals may have been relying more on egocentric (body-turn) cues to perform the allocentric procedure which is why they were impaired. On this task, such egocentric cues were advantageous to them and so they actually performed slightly better than the other groups.

This improvement in performance in an egocentric task is in accordance with the results from the previous experiment which had revealed that the group with bilateral lesions of the cingulum bundle group performed significantly better than the sham animals over the later stages of testing on the DNMP task (thought of as taxing essentially egocentric processing).

The Fx animals performed normally on the initial discrimination phase which is not surprising if the hypothesis concerning their reliance upon egocentric cues is correct. All groups performed worse on the 'reversal' phase but the BCB and Fx groups performed slightly better than the other groups. This was presumably because although these animals may have found it initially difficult to alternate their responses on the 'reversal' phase, once they did, they then found it easier than the other groups and were therefore quicker to achieve criteria.

It was interesting that the CCB group performed significantly poorer than the BCB group on the initial discrimination (figure 47) and this is not easy to explain. The animals did not appear to suffer carry-over effects from the previous experiment as they were no more likely than the other groups (and less so than the CON group) to produce more alternation errors on the initial discrimination sessions (figure 48). One explanation could be that this group had suffered damage to prefrontal cortex at the extreme anterior portion of the cingulum bundle. However, the histological analysis did not reveal any evidence of direct prefrontal cortex damage but there is still a strong possibility that certain prefrontal cortex fibres could have been disrupted. Such damage to this region can lead to deficits on egocentric spatial tasks (Kesner et al., 1989; King & Corwin, 1992).

However, the animals in the BCB group also received similar lesions to the same region of the cingulum bundle (and in fact these lesions were more extensive) and yet they showed no impairment on the egocentric task, in fact performing significantly better than the CCB group on the initial discrimination. If the BCB animals had an allocentric deficit though, this could account for the discrepancy as the CCB animals were presumably relying on allocentric cues (which of course were no use to them in this task) while the BCB group were using egocentric ones which were much more useful. It must be noted that the impairment of the CCB group in this test was due to the very much poorer performance of two out of the five CCB animals who had great problems with this task. There was no obvious reason in the histological analysis which could indicate why these two animals were particularly affected.

4. Radial maze.

Over the four blocks of this allocentric procedure both BCB and Fx animals were significantly impaired over the two measures: number correct in first 8 choices, and total number of arms visited. The BCB group appeared to be not quite as impaired as the Fx animals but maintained an even level of performance throughout (figures 49 and 50) showing no sign of improved performance even on the last block of sessions, unlike their performance in the T-maze experiment (figure 44). In contrast, the Fx group showed a significant reduction in the number of arms visited from the first block, though their level of performance remained impaired over subsequent blocks. This severe and apparently long-lasting impairment shown by the BCB animals (an impairment which is correspondingly similar to that shown by animals with hippocampal system damage) indicates strongly that the cingulum bundle is responsible for processing allocentric information. As the radial-maze task is somewhat harder than the T-maze task (having 8-arms to remember rather than two) it is perhaps not so surprising that the impairment shown was larger and longer-lasting.

7.5 SUMMARY.

1. Bilateral damage to the cingulum bundle at several points along its course leads to an impairment on tasks requiring allocentric processing, and the severity and duration of the impairment may be dependant upon task difficulty. The same lesions produce no deficit (and indeed may produce a slight advantage) on tasks requiring the use of egocentric cues. Although these lesions were planned to be staggered, several animals in this group received lesions that were symmetrical and there was thus significant bilateral cortical damage. Despite this fact, as a high proportion of the CCB group also received symmetrical cortical damage they did not show the same degree of impairments as the BCB group. This indicates strongly that it is not the severity of the cortical damage that is important but the complete disconnection of the cingulum bundle which supports the results from experiment 3 of this thesis.

2. Unilateral or crossed lesions of the cingulum bundle do not lead to a marked impairment on tasks requiring either egocentric or allocentric processing. While such lesions may have a deleterious effect on the initial performance on an allocentric task, this impairment is only transitory. This finding is true whether the unilateral damage occurs at two, or four sites along the course of the bundle. However, as the group with extensive unilateral damage made more errors on the reversal sessions of the Cross-maze egocentric task, the possibility that such extensive damage might have a greater effect on the body-turns procedure cannot as yet be discounted.

3. The precise location of any lesions of the cingulum bundle appears to be crucial and the results are consistent with the view that the key projections (for allocentric tasks) seem to be ones from the anterior thalamic nuclei and not from prefrontal cortex.

4. A cautionary note must be added to the effect that the allocentric tasks all taxed working memory while the egocentric task can be viewed as taxing reference memory. As a consequence this egocentric task is not a true analogue of the allocentric tasks and so the results must be treated with caution. An egocentric task that is also a test of working memory is thus required to address this problem.

CHAPTER 8: GENERAL DISCUSSION.

Recent advances in describing and characterising human memory processes have indicated that the brain supports multiple memory systems. One influential account (Cohen & Squire, 1980) has suggested that these systems can be described as '*declarative*', - concerned with the memory for facts and events, and '*procedural* - concerned with the acquisition of skills and rules. A large body of converging evidence from both human case studies and from experimental animals indicates that these systems are mediated by different neural circuits.

The evidence concerning the differential contribution of these structures and regions in experimental animals is still unclear, but the relative involvement of some areas is less controversial than others. For instance, the evidence for the crucial importance of the hippocampus, and adjacent cortices (entorhinal cortex, perirhinal cortex and the parahippocampal gyrus) in learning and memory in humans and animals is strong (Squire, 1992). Slightly less compelling is the respective evidence for the thalamic nuclei. While they do appear to subserve some aspect of the mnemonic process, the precise role of the various structures is as yet unclear (Aggleton & Sahgal, 1993).

More controversial perhaps are the functions of the medial prefrontal cortices, and in particular the anterior cingulate, retrosplenial, and prelimbic cortices. They have each been implicated in certain forms of mnemonic processing (and a host of other behaviours as well) and share a complicated array of interconnections. The precise nature of these interconnections, and the relative contribution of these regions to various forms of processing, is as yet not fully understood. Due to the wide extent of the connections to and from these areas, their possible roles are likely to be complex as they are in a position to potentially influence many other cortical and subcortical regions.

The aims of this thesis were highlighted in section 1.10 and this general discussion will attempt to address each of the aims:

8.1 Aim 1: The effects of lesions to several structures and regions of the extended hippocampal formation on various tests of spatial working memory.

8.1.1 The effects of MD lesions.

Experiments 1a and 1b compared the effects of medial dorsal (MD) thalamic lesions, with control animals on the acquisition and performance of automated (DNMP and spatial (lever) discrimination) tasks. At no point in the acquisition process did the MD animals show any impairment relative to the control group; furthermore, the MD animals were unimpaired on the performance of this task over a range of delays. Similarly, the MD group showed no deficit in the acquisition and subsequent performance on the lever discrimination and reversal task performed in the same operant apparatus.

The data from experiment 1 is in accord with those studies that have reported no effect of MD damage on the performance of rats on tests of spatial working memory. This is in marked contrast with the reported effect of hippocampal or fornix lesions on the same tasks, as rats with such lesions are typically severely impaired. For example, Aggleton et al., (1992) compared the performance of rats with either radiofrequency lesions of the fimbria/fornix, against animals with aspiration lesions of the hippocampal formation on an automated DNMP task (identical to the task described in this thesis). Both lesions produced equivalent performance deficits on the task, the pattern of deficits being consistent with a mnemonic impairment.

Results from comparable tasks assessing non-spatial memory have revealed the opposite set of impairments, namely, while such as Aggleton et al., (1986) found that hippocampal lesions had no effect on a non-spatial DNMS task, animals with MD lesions have shown acquisition impairments on the same task (Hunt & Aggleton, 1991). These findings have indicated a double dissociation between MD and the hippocampal system and shows that whatever role MD serves, it clearly does not make a significant contribution to spatial working memory as measured using certain tasks.

8.1.2 The role of MD in reward-related processing ?

Despite the controversy surrounding the possible role of MD in the memory process, the possibility that damage to MD may contribute to mnemonic processing cannot yet be ruled out. It remains possible that MD performs some as yet undisclosed mnemonic function which can be qualitatively distinguished from that performed by the hippocampal formation and its related network.

A report by Gaffan & Watkins (1991) focused upon the effects of MD lesions on long-term visual associative memory in the monkey. In that report, the animals were trained to associate visual stimuli with the delivery of various amounts of food reward. They had to choose correctly between pairs of stimuli drawn from a population of 16, 4 of which were associated with no reward pellets, 4 with one, two and four pellets respectively. MD lesions, which were correspondingly similar to those found in human Korsakoff's syndrome, produced a severe impairment, both in memory for the quantity of the reward, and also in the memory for the qualitative absence or presence of reward. The authors argued that this indicated not only that MD damage can impair performance on a long-term memory task, but also that the function of MD could be related to the effects of rewards on the animals' learning, or the retention of a performance rule required by the task, rather than for stimulus-reward associative memory itself.

A more recent report (McAlonan et al., 1993), has provided additional evidence for the role of MD in reward-related processes. The rationale for their experiment lay in a previous finding (Everitt and Robins, 1992), that the basolateral amygdala and ventral striatum were part of a neural system involved in reward-related processes; one of the target structures of this network is MD and so these authors hypothesised that MD may be part of this reward-related circuit.

To test this, McAlonan et al., (1993) examined the effects of neurotoxic lesions of MD on the acquisition of a place preference conditioning task; in this, hungry animals were initially conditioned to drink a sucrose solution from one side of a two-sided partitioned box, and then presented with a choice of sides. MD lesions completely abolished this conditioned place preference, providing that the lesions included the medial-lateral extent of the nucleus (this part receives direct input from the amygdala, and projects to medial frontal cortex). Ingestion of sucrose itself following deprivation was not affected after MD lesioning, and so the result was not secondary to changes in motivation. The authors concluded that reward-related processes may depend on ventral-striatopallidal outflow that engages MD and medial frontal cortex circuitry. This study showed that MD is possibly a site through which emotional information that is processed by the amygdala, may be directly relayed to areas of frontal cortex. The strong connections between MD and the anterior cingulate cortex provides support for this hypothesis as this cortical region has also been implicated in emotional processing in humans and animals (Angelini et al., 1980; Devinsky & Luciano, 1993; Whitty & Lewin, 1960; Talairach et al., 1973; Ward, 1948).

8.1.3 A non-specific role for MD ?

A further theory regarding the possible (non-specific mnemonic) role of MD has been proposed by Stokes & Best (1990a). They argued that previous findings reporting no impairment of MD lesioned animals in which spatial tasks such as the radial maze were used (e.g. Kolb et al., 1982), may not have made the distinction between '*reference*' and '*working*' memory clear. Stokes & Best (1990a) used an 8-arm radial maze, in which only 4 of the arms were baited. Requiring the animal to avoid never-baited arms taxed reference memory, and requiring the animal to avoid re-entering already-baited arms taxed working memory. Animals with MD lesions were impaired on both reference and working memory measures, a finding in contrast to that obtained by such as Kolb et al., (1982).

The authors interpreted their results in terms of a non-specific, non-memory dysfunction, induced by damage to MD. They further argued that such an impairment could either take the form of changes in attention and/or motor processes, i.e. MD damage may increase the susceptibility to interference from previous choices; or that the impairment could result in the animal becoming incapable of distinguishing between the arms of the maze. Finally, the possibility that changes in motor system regulation following MD damage may interfere with task acquisition or performance, i.e. such changes may consist of a decreased ability to inhibit responses, or a tendency to turn in stereotyped directions. Similar points have recently been made by Krazem et al., (1995) regarding the pattern of deficits shown by mice with ibotenic acid lesions of MD on a reversal-set learning task in the T-maze.

Winocur (1985; 1990) has reported some intriguing evidence of the possible nature any impairment associated with MD lesions. In the earlier set of experiments, he compared rats with hippocampal or MD damage on a delayed alternation test. In this task, the hippocampal group performed normally when required to retain specific information over short periods of time, but were severely impaired as the delay interval increased. The MD deficit was more general as the animals were impaired at both short and long intervals, and also showed a mild acquisition impairment. Winocur (1985) interpreted this set of results as implicating the hippocampus in long-term memory, and MD in both short, and long-term memory, and in acquisition, thus raising the possibility of a generalised deficit that affects learning the rule of the task.

The later set of experiments (Winocur, 1990) were concerned with anterograde and retrograde memory for a socially transmitted food preference. In the first experiment, food-preference training was administered postoperatively and memory was tested over a range of delays, both hippocampal and MD groups acquired the preference normally, but the former set of animals displayed a rapid rate of forgetting, indicating anterograde amnesia. In a second experiment the food preference was acquired at different times preoperatively, and retrograde memory tested postoperatively. Both groups showed a memory deficit when training immediately preceded surgery, but the hippocampal group displayed a temporally graded retrograde amnesia. These results confirmed the differential effects of hippocampal and thalamic lesions as reported in the earlier set of experiments. Winocur suggested that the memory loss following MD lesions was related to factors associated with the original learning, while the pattern of hippocampal amnesia reflected disruption at a later stage of processing.

This proposal was supported by the case study of patient B.Y (Winocur et al., 1984) who suffered bilateral thalamic damage and demonstrated consistent impairments in recalling material over short delays. Winocur et al. (1984) argued that the primary deficit showed by B.Y was one of impaired information acquisition, a deficit which would secondarily impair memory.

This does not mean of course that MD performs no role in the acquisition and performance of tasks sensitive to spatial working memory, MD may well contribute to some aspects of spatial mnemonic functioning, but as the evidence presented in the above sections indicates, may well have a more important role in non-spatial, or the emotional aspects of learning and memory.

8.1.4 The effects of cingulate cortex damage on spatial working memory.

The second experiment reported in this thesis compared lesions of anterior cingulate cortex and retrosplenial cortex, with lesions of the hippocampal system (fornix), and control animals on the same automated tasks as on the first experiment, plus a delayed alternation task performed in a T-maze. The third experiment compared the performance of animals with damage to anterior cingulate cortex plus retrosplenial cortex on the same automated and maze tasks. These two experiments showed that selective neurotoxic lesions of anterior cingulate and retrosplenial cortex, or damage to the entire cingulate cortex, did not impair performance on any of the three tasks.

8.1.5 Anterior cingulate cortex damage.

The lack of effect of lesions of anterior cingulate cortex on the spatial tasks reported in this thesis is perhaps not surprising as in both mice and rats this region receives strong connections from MD, but not from the hippocampus (Sif et al., 1989; Vogt et al., 1981). The first experiment had shown that MD damage also had no effect on two automated tasks, and the subsequent experiments revealed that animals with damage to the fornix showed consistent and severe impairments.

The evidence presented in earlier sections had strongly indicated that the anterior cingulate cortex plays a role in a variety of behaviours including attention, pain, and visceromotor control, but particularly this region appears to play a strong role in emotion. Vogt et al., (1992) have described the various functions of anterior cingulate cortex and have emphasised that with its strong reciprocal connections with the amygdala, this region is influential in emotional processing. Evidence from both human and animal studies has tended to confirm this proposal as damage to anterior cingulate cortex produces apathy, inattention, and akinetic mutism in humans (Amyes & Nielson, 1953; Barris & Schuman, 1953), and affects a range of emotional behaviours in animals (Ward, 1948).

Vogt et al., (1992) described the anterior cingulate cortex as an "*executive region*" arguing that it is primarily involved with effector or executive processing; they argued that emotional states are closely related to effector processes in that emotions achieve expression through autonomic, endocrine and skeletomotor outflow. Vogt et al., (1992) further proposed that the amygdala and anterior cingulate region may operate in tandem to produce affective behaviours; as MD and area 24 are also closely linked, it seems likely that these areas form some neural circuit which has the amygdala at its key node. As damage to the amygdala produces impairments on tasks which require an affective component such as avoidance tasks (Cahill & McGaugh, 1990), neophobic responses (Sutherland & McDonald, 1990), and conditioning (Sanares & Campbell, 1989) but does not typically impair performance on tasks assessing spatial working memory (McDonald & White, 1993; Zola-Morgan et al., 1989) then the lack of effect of MD and anterior cingulate cortex damage on the tasks assessing spatial working memory described in this thesis is thus perhaps not surprising.

The results from the second and third experiments of this thesis are thus in agreement with more recent reports which have argued that the anterior cingulate region of medial frontal cortex is not important for certain forms of spatial processing. For example, Poucet (1990) had indicated that while rats with medial frontal damage (including anterior cingulate and prelimbic cortices) suffered a general working memory deficit, their spatial reference memory was not impaired. Also, de Bruin et al., (1994) compared rats with varying degrees of medial frontal damage (anterior cingulate, prelimbic and infralimbic cortices) using the Morris water maze and again concluded that an intact medial prefrontal cortex was not necessary for normal spatial learning and memory processing.

8.1.6 Retrosplenial cortex damage.

The lack of impairment of the animals with neurotoxic damage to retrosplenial cortex is rather more surprising, as several previous reports have shown that this region may be important for some aspects of spatial memory (for example, Sutherland & Hoesing, 1993). Vogt et al., (1992) have described retrosplenial cortex as an "*evaluative region*", and have argued that it is not implicated in emotional processing but rather is concerned with assessing the environment, and spatial processing. In accordance with this idea are the results from several studies which have demonstrated spatial deficits following posterior cingulate cortex lesions in rats (Sutherland et al., 1988) and in monkeys (Murray et al., 1989). Furthermore, single-neuron electrophysiological studies have revealed that neurons in the rats' posterior cingulate cortex are sensitive to the angle of the body relative to the environment, and to bodily displacement (Chen et al., 1991). Similar studies using monkeys have also indicated that neurons in posterior cingulate cortex are involved with visuospatial processing (Olson et al., 1993). Human case studies have also indicated that retrosplenial cortex damage may lead to an impairment in some aspects of memory (Valenstein et al., 1987).

In light of the connections between retrosplenial cortex and the hippocampus and anterior thalamus, the lack of effect of retrosplenial damage in the second and third experiments is doubly surprising. There are dense reciprocal connections between both the hippocampus and anterior thalamic nuclei with retrosplenial cortex in both rats and mice (van Groen & Wyss, 1992; Shibata, 1993; Sif et al., 1989; Wyss & van Groen, 1992).

Experiments 2-4 of this thesis showed that damage to parts of the hippocampal system (fornix) leads to an impairment on the DNMP, lever discrimination, and T-maze tasks while other reports have shown that damage to the anterior thalamic nuclei can also disrupt performance on the same tasks (Aggleton et al., 1991a; Aggleton et al., 1995a, Aggleton et al., in preparation..

One possible explanation for this apparent discrepancy may lie in a closer analysis of the extent of the damage caused to retrosplenial cortex in these other reports. There is a strong possibility that such studies with their use of non-selective lesioning techniques, may have inadvertently damaged other areas of prefrontal cortex or key fibre pathways (such as the cingulum bundle) which themselves may be important parts of a circuit governing spatial working memory. The majority of the experimental animal studies that have reported impairments in spatial memory arising from damage to retrosplenial cortex have used aspiration lesions (for example, Markowska et al., 1989; Sutherland & Hoesing, 1993; Sutherland et al., 1988). The possibility that such techniques may have caused inadvertent damage to the prelimbic cortex, or to the underlying cingulum bundle cannot be ruled out and so make a clear analysis of any results difficult, see sections 8.1.7 and 8.1.8.

In the human case study of patient T.R, who displayed amnesia following damage to the retrosplenial region (Valenstein et al., 1987; Bowers et al., 1988) the authors also indicated that the damage caused to the cingulum bundle may have played a significant role in the mnemonic impairments observed.

8.1.7 The role of prelimbic cortex.

The extent of the damage to cingulate cortex may be of vital importance as it has been often reported that large lesions of MFc lead to a greater impairment on a variety of tasks than do smaller ones (Shaw & Aggleton, 1993; Silva et al., 1986; Sutherland et al., 1988; Wolf et al., 1987). A critical factor arising out of several reports is the inclusion or exclusion of prelimbic cortex (area 32), an area which receives direct inputs from the hippocampus (Jay & Witter, 1991), and according to several studies is critical for performance in spatial learning tasks (Brito et al., 1982; Thomas & Brito, 1990; Brito & Brito, 1990).

Prelimbic cortex receives both strong hippocampal and thalamic connections, and is assumed to play a role in mnemonic processing. A recent study by Aggleton et al., (1995b) focused upon the effect of selective neurotoxic lesions to this region on the performance of the same three tasks reported in experiments 2 and 3 of this thesis. In that report, damage to the medial frontal cortex (which included prelimbic, but largely excluded anterior cingulate cortex) led to a severe impairment on the DNMP task, yet did not affect the acquisition and performance of the same animals on the T-maze forced-alternation task. This result provides strong support for the notion that it is damage to parts of the medial frontal cortex other than anterior cingulate and retrosplenial cortices, that leads to deficits on these spatial tasks.

Another report (Granon et al., 1994) has reported that radiofrequency lesions of prelimbic cortex causes a deficit in working memory in rats which indicates that this region clearly performs some role in mnemonic processing though its exact function remains as yet unclear.

8.1.8 The role of the cingulum bundle.

Experiments 3-5 investigated the effects of damage to the cingulum bundle on a variety of tasks using an operant chamber and more traditional maze-type tasks. These tasks were intended to differentiate between allocentric and egocentric processing. The results from experiment 3 revealed that although lesions of the cingulum bundle did not affect DNMP or lever discrimination and reversal performance, they had a severe effect on the acquisition and performance of the T-maze.

This dissociation is very intriguing, principally because the performance of animals with anterior thalamic damage on the DNMP task is similar to that of fornix animals and is certainly impaired. As the anterior thalamic nuclei project back to the hippocampus via the cingulum bundle, one would have expected cingulum bundle damage to have had a similar behavioural consequence. Two possible reasons for this curious finding were addressed in the discussion of experiment 3; namely, that either cingulum bundle damage only affected the acquisition but not the performance of a spatial task (and so affected the T-maze task to a greater extent), or that the DNMP and T-maze tasks are taxing quite different processes which utilise different neural circuits.

This hypothesis was addressed in experiment 4 which compared a variety of lesions of the cingulum bundle on the acquisition of the DNMP task, and performance on the T-maze task. This experiment revealed that while acquisition of the DNMP was unaffected, performance at longer delays was apparently facilitated by cingulum bundle lesions. Performance in the T-maze alternation task was however affected by cingulum bundle lesions and so this double dissociation may therefore lie in the nature of the processing required to successfully perform the different types of tasks; namely between allocentric or egocentric processing. However, the two tasks also differed markedly on the level of proactive interference with the DNMP task containing far more (Dunnett et al., 1990a). This problem was addressed in the final experiment which described a 'double-forced procedure' used with the T-maze which had the aim of making this alternation task more similar in terms of proactive interference to the DNMP task.

The final experiment focused on different cingulum bundle lesions, and used several maze tests which it can be argued differentiate clearly between allocentric and egocentric processing. Animals with extensive bilateral damage to the cingulum bundle were impaired on those maze tasks that required allocentric processing, (T-maze alternation, Cross-maze 'same-arm versus opposite-arm' procedure, and the standard 8-arm radial-maze procedure). However, the same group were unimpaired on those tasks which required egocentric processing (Cross-maze 'body turns procedure'), and in fact performed slightly better than control animals. Animals with unilateral or crossed lesions of the cingulum bundle showed only a temporary impairment on the first task (T-maze alternation). The crossed group were however impaired on the cross-maze (egocentric) procedure, this was discussed in detail in chapter 7.

Thus, it appears that damage to the cingulum bundle which does not bilaterally sever the bundle, and does not completely disrupt certain connecting fibres (probably those from the anterior thalamus) will not be sufficient to cause a severe impairment on certain spatial tasks. If the damage is bilaterally complete, then it appears that performance on certain tasks (which it can be argued assess allocentric processing) will be impaired but performance on other tasks (which it can be argued assess egocentric processing) will not be affected, and may even show some facilitation. Interestingly, on the double-forced procedure described in experiment 5 (high levels of proactive interference) all groups experienced more difficulty but only the Fx animals were impaired relative to the other groups.

The sensitivity of hippocampal system lesions to increased proactive interference has been documented (Winocur, 1985) but this is often confused with increased difficulty. The 'double-forced' procedure was used in order to make the T-maze task more similar to the DNMP task in terms of levels of interference, and the groups with cingulum bundle damage were unimpaired relative to the control animals. This indicates that whatever the reason for the marked impairment shown by this group on certain tasks it is not the degree of proactive interference; and may therefore lie in the nature of the processing (allocentric versus egocentric).

A major problem with this interpretation however is that the egocentric Cross-maze (body-turns) task is essentially a test of reference memory and is thus not a direct analogue of the allocentric tasks used in the T- and Cross-mazes. Such a conclusion must therefore be treated with caution.

8.1.9 The effects of fornix damage.

In contrast to the cortical and thalamic lesions described above, radiofrequency lesions of the fornix consistently produced a severe and permanent deficit on the acquisition and performance on the majority of the tests described in this thesis. Experiments 2 and 3 revealed a severe fornix impairment on the performance of the DNMP task which consisted of both a significant loss of accuracy and a significant increase in perceptual and response biases. These result are entirely consistent with the results of previous studies which have investigated the effects of fornical damage on the performance of automated operant tasks (Aggleton et al., 1991a; Aggleton et al., 1992; Dunnett, 1990). This impairment was characterised by an increasing loss of performance accuracy as the delay interval increased which is consistent with a mnemonic impairment. Furthermore, the increase in perceptual bias as measured by the indices Index Y, B" and RI is in accord with other reports that have noted increases in perseveration following hippocampal system damage (Gray & McNaughton, 1983; Means & Douglas, 1970).

Finally, the fornix animals displayed differences in their latencies to respond, typically being quicker in reacting to the sample and choice levers, and in operating the magazine flap, in contrast to animals in the other groups. Again, this finding is in agreement with other reports (Gray & McNaughton, 1983; Means & Douglas, 1970, Olton et al., 1978).

The effects of fornix transection on the spatial (lever) discrimination and reversal task (experiments 2 and 3) were almost as clear cut. None of the other surgical groups assessed were impaired on the initial discrimination but all performed at a lower level when the 'correct' lever was reversed. The fornix animals could also learn the initial discrimination but more importantly, were impaired relative to other groups when the 'correct' lever was reversed. In the light of the high levels of bias showed by the fornix animals on the DNMP task it is not surprising that they were impaired on the lever reversal task as they tended to perseverate by continuing to select the now 'incorrect' lever when the initially 'correct' lever was 'reversed'. This impairment in reversal behaviour is consistent with the findings from other studies (Gaffan, 1972; Gray & McNaughton, 1983).

The results from the lever discrimination task are also consistent with a report by Rawlins et al., (1988). In this study the authors compared the ability of rats with fornix damage to follow either a Win-stay / Lose-shift rule, or a Win-shift / Lose-stay rule respectively using a two-lever operant chamber. They found a severe impairment on the Win-shift task but performance on the Win-stay task was unimpaired until the inter-response interval passed 10s. This can be compared to the lever task reported in this thesis as the rats with fornix damage were impaired when the correct lever reversed sides (Win-shift) but were unimpaired when the correct lever remained consistent (Win-stay). Although Rawlins et al., (1988) did find an impairment on the Win-stay version after an interval of 10s while the lever task in this thesis did not, it is not possible to directly compare the two as the ITI used in the lever task reported in this thesis did not exceed 10s.

On the T-maze delayed-alternation task, the animals with fornix lesions were severely impaired on both the initial acquisition sessions, and on the subsequent trials utilising a range of delays (experiments 2, 3, and 5). This impairment was marked, with the animals performing at a level not much above chance. In the final experiment this task was modified somewhat to include a double-forced procedure which increased the level of interference and thereby made the initial task more difficult. The fornix animals continued to perform at a level just above chance (floor effects).

On the cross-maze allocentric task (experiment 5) the fornix group again performed at a consistently low level both when the choice-arm was the same, and when it was opposite to the original start-arm.

On the egocentric version of the task however, the fornix group were unimpaired over both the initial discrimination and the subsequent reversal sessions. This is problematical the previous experiments had clearly shown that the fornix animals tend to perseverate and show higher levels of bias. In this egocentric task, such perseveration would be of assistance on the initial discrimination (which was observed) but would cause great problems on the subsequent reversal (which was not observed). One possible explanation for this result is that the fornix animals were not in fact relying on egocentric processing but rather on allocentric processing to perform the task which would explain their unimpaired performance on the reversal sessions as they did not have to suddenly shift their behavioural response. How rats (both normal and lesioned) do in fact perform this task is however as yet unknown and so this explanation remains speculative.

Another explanation for this result is that as the task was taxing reference and not spatial working memory, the fornix group may have been unimpaired as they were relying to a great extent on reference memory, while the other animals may have been using a mixture of both, or just working memory processing to solve the task. It has been previously reported that lesions of the fornix impair working memory but not reference memory in maze-type tasks (Olton & Papas, 1979) which gives support to this explanation.

On the 8-arm radial maze task (experiment 5) the fornix group were impaired on the two measures of performance (number correct in first 8 arms, and total number of arms visited). They showed impaired accuracy in visiting all eight arms often visiting the arm that they had just visited, and visited many more arms in total before completing the task. This severe deficit on the 8-arm radial maze is consistent with those descriptions of Olton and colleagues (for example, Olton 1978; Olton et al., 1977) who described a very similar set of impairments.

In all of the maze-type tasks the general behaviour of the animals with fornix transections matched the behaviour of those animals with the same damage as described by Olton (1978). This author noticed that rats with such damage exhibited a number of changes in general behaviour on the radial-arm maze. Firstly, they ran faster than normal animals and this led to ballistic tendencies that made sharp turns difficult.

This was also subjectively observed in the maze-tasks performed in the experiments described in this thesis. Although no record was made of the different latencies to respond in the maze-tasks, the fornix animals appeared to run quicker than the other groups. This is borne out by the firm data from the automated DNMP task which did reveal that the fornix animals had significantly shorter response latencies than the other groups. It was also noted that this speed of running made turning difficult, in the T-maze task for example they would approach the T junction so fast that they would lose their footing. The other groups tended to approach, pause, look at both arms, and then make a choice, whereas the fornix animals approached rapidly and entered one arm without pausing to make a choice, the arm entered was often the same one, whether it had been visited on the first forced-choice or not. The need to make formal observations of the latencies to respond in maze-type tasks of animals with differing lesions is therefore necessary, and was unfortunately not considered in the series of maze experiments reported in this thesis.

Olton's (1978) second observation was that fornix animals tended to fall into perseverative patterns by repeating the same sequence of choices. In the experiments described in this thesis this pattern was also noted. In the T-maze and Cross-maze. all of the fornix group would consistently select one arm and consistently visit that arm, while in the radial-maze they tended to adopt a certain pattern of response, entering say, arms 2,4 and 6 in that order several times over. This alteration of behavioural flexibility following fornix transection has been often noted (for example Olton et al., 1977).

The results of fornix transection described in this thesis thus support the general finding that damage to the hippocampal system severely impairs the ability of rats to perform tests assessing spatial working memory, but in particular allocentric processing. Sutherland & Rodriguez (1989) for example have reported that fornix damage severely impairs performance on water-maze procedures which have been argued test allocentric processing. These authors also investigated the effects of damage to a range of structures and regions that receive direct connections from the fornix. They found that damage to the anterior thalamic nuclei, the medial septum, cingulate cortex, nucleus accumbens and the mammillary bodies produced a variable range of deficits with the nucleus accumbens and anterior thalamic group being the worst affected.

All the deficits were however much milder than those seen the fornix group. Sutherland & Rodriguez (1989) concluded that the severe fornix deficit was produced because it destroys many different projections, none of which are individually essential for normal spatial processing.

8.1.10 The fornix model of hippocampal dysfunction.

One of the problems in using the destruction of the fornix as a model of hippocampal dysfunction is that while the severing of the fornix does disrupt hippocampal efferents, a number of target structures are spared which include projections via entorhinal and perirhinal cortices to a variety of cortical association regions. The hippocampus also provides non-fornical connections with the amygdala, retrosplenial cortex, and lateral dorsal thalamic nucleus in particular (Aggleton et al., 1986; Aggleton et al., 1992; Jarrard, 1993). The fornix is thus the major pathway linking the hippocampus with other regions of the brain including both cortical and subcortical sites and it has been assumed that the destruction of the fornix can reveal much about hippocampal system function.

One benefit of using fornix destruction as a model of hippocampal function is that it is much easier to transect the fornix than it is to remove the entire hippocampus. However, while cutting the fornix is likely to produce hippocampal dysfunction, the hippocampal system is not totally isolated and so this leads to the question of whether such a procedure can be regarded as equivalent to hippocampectomy. One recent study (Aggleton et al., 1992) examined this question of whether cutting the fornix could effectively mimic hippocampal system damage by comparing the effects of fornical damage with hippocampal removal. The authors used an automated DNMP task identical to that described in this thesis and reported that the deficit showed by both hippocampal and fornix groups could not be distinguished. The pattern of results showed by animals with equivalent damage to the fornix reported in this thesis, was in accord with the fornix animals of that study. Other reports have also indicated that similar mnemonic deficits can be produced following fornical and hippocampal lesions in rats, whether tested in the radial-arm maze (Olton et al., 1982), or the Morris water maze (Morris et al., 1982), though the results are often affected by 'floor effects'.

It can be concluded from such studies that in the rat at least, fornix transection may be a viable model of hippocampal dysfunction. If that is the case, then it is justified on the basis of the results from this thesis (which revealed a severe fornix impairment on a range of tasks) to argue that the fornix is a vital link between structures in the temporal lobe and the diencephalic region which are responsible for some aspects of mnemonic processing. This is in line with the original theory proposed by Delay & Brion (1969) and further described by Gaffan (1992a).

8.1.11 Gaffan's theories regarding fornix transection.

Gaffan (1992a) has argued that the main feature of anterograde amnesia in humans is an impairment of episodic memory. The author has described several experiments concerned with assessing episodic memory in the monkey following fornix transection (Gaffan, 1992b; 1994). In the former study a discrimination learning task was employed which consisted of pairs of frames from a cinema film. Fornix transection produced a severe impairment in this discrimination task. Gaffan (1992a) argued that memory for complex naturalistic scenes was the basis for episodic memory, this "*whole-scene*" memory allowing individuals to differentiate between memories of similar events (i.e. events with similar component items).

Fornix lesions would impair the discrimination of the spatial organisation of complex scenes and thus make these scenes less distinct from one another in memory. The severe impairments on the various allocentric maze-type tasks shown by the animals with fornix transections in this thesis is consistent with Gaffan's (1992a) theory as in the maze environment the animal is presented with a complex scene in which it has to accurately navigate using distal room cues. The impairment of the fornix animals on the DNMP task is not so easy to reconcile with Gaffan's theory as successful performance in the operant chamber must rely on the accurate assessment of local proximal cues, and not distal cues as these cues are absent.

8.2 Aim 2 : Anatomical circuits governing spatial working memory.

The experiments in this report have attempted to systematically investigate the functional consequences of circumscribed lesions to key parts of several theoretical mnemonic circuits on a set of tasks that are regarded as benchmark tests of spatial working memory.

The experimental evidence described above is in accordance with the notion that there are several interconnected yet dissociable circuits governing the processing of spatial working memory. One circuit can be described as one that links the rhinal cortices with MD, prefrontal cortex, and anterior cingulate cortex, and the second as linking the hippocampal formation and anterior thalamus with areas in medial frontal cortex.

Thus:

Prefrontal cortex

1. **Rhinal / Perirhinal cortex -------- MD -------- Anterior Cingulate cortex.**

8.2.1 An assessment of circuit 1.

The first point on this circuit is entorhinal / perirhinal cortex and while the experimental work reported in this thesis did not directly asses the effects of damage to the rhinal cortex, an experiment recently carried out by colleagues at this laboratory (Ennaceur et al., in press) did so. That experiment compared the effects of neurotoxic lesions of the perirhinal cortex, with radiofrequency lesions of the fornix on the same automated DNMP, lever discrimination and reversal, and T-maze alternation tasks described in this thesis, and a further test of object recognition.

While lesions of the fornix produced a severe impairment on the spatial tests (in accordance with the findings of this thesis) they were unimpaired on the object recognition task; the opposite set of results were found for the animals with rhinal lesions i.e. they were unimpaired on the three spatial tasks but impaired on the object recognition task. Ennaceur et al., argued that this dissociation indicates that the actions of the perirhinal cortex and hippocampus can be dissociated from one another, but as the perirhinal lesions spared the most caudal limits this conclusion remains only tentative.

This dissociation between the hippocampus and entorhinal / perirhinal cortex has been noted by other researchers. For example, Hunt et al., (1994) tested rats with radiofrequency lesions of either entorhinal cortex or the hippocampus alone, or entorhinal cortex plus the hippocampus on both a working and a reference memory task in the 8-arm maze. All of the lesion groups were impaired on the working memory task, but in the second task, only the entorhinal group and the entorhinal plus hippocampal group were impaired.

The group with damage limited to the hippocampus were unaffected. Hunt et al., (1994) argued that these results demonstrated a functional dissociation between entorhinal cortex and the hippocampus, with the hippocampus being important for the encoding of new spatial information in a working memory context, while the entorhinal cortex represents spatial information within a reference memory system.

The second point of the circuit is the medial dorsal nucleus of the thalamus (MD) and the data from the first experiment showed that MD damage had no effect on two tasks of spatial working memory (DNMP and lever discriminations) With regard the T-maze delayed-alternation task, although this first experiment did not assess the effects of MD damage on such a task, previous authors have indicated that MD lesions have no consistent mnemonic effect (for example, Greene & Naranjo, 1986; Hunt & Aggleton, 1991; Tigner, 1974).

The final point of this circuit is the anterior cingulate region of medial frontal cortex (ACc) and experiments 2 and 3 showed clearly that damage to this region had no effect on the performance of the DNMP, lever discrimination, and T-maze alternation tasks. It thus appears that this circuit comprising links between entorhinal/perirhinal cortex, MD and then ACc is not crucial for the processing of spatial working memory as measured in the experiments reported in this thesis. Of course, caution must be exercised as it is not strictly justified to link such null results together.

8.2.2 An assessment of circuit 2.

2. HPc ---------- Anterior thalamus ---------- Retrosplenial cortex

Mammillary Bodies

If the first circuit as described in the previous section is not primarily responsible for the processing of spatial working memory, then the second circuit may well prove to be of greater significance. The first point of this circuit is the hippocampus and experiments 2-4 of this thesis showed that lesions of part of the hippocampal system (the fornix) produced a severe impairment on all of the tasks used.

The second point of this circuit is the anterior thalamic nuclei and the direct contribution of these nuclei were not assessed in these experiments. However, evidence for the contribution of the anterior nuclei of the thalamus (AT), has been presented in a recent review (Aggleton & Sahgal, 1993). These authors assessed the possible role of AT in anterograde memory and pointed out that AT shares dense reciprocal connections with the limbic system - particularly with the hippocampus, and also with the medial frontal cortex - principally with cingulate cortex (Shibata, 1993; Shibata & Kano, 1993). Aggleton & Sahgal (1993) presented evidence from human clinical studies which (although only circumstantial), indicated that damage to the structures and pathways that link AT with the hippocampus lead to an impairment in anterograde memory. Furthermore, they reviewed more convincing experimental evidence from studies using primates and rats (Aggleton & Mishkin, 1983a; Hunt & Aggleton, 1991) which revealed that damage to AT could severely disrupt mnemonic processing.

One experiment which provides direct evidence for the key involvement of the anterior thalamic nuclei was that reported by Aggleton et al., (1991a). In that report the authors compared the performance of rats with either lesions of the fornix, the anterior thalamus or the mammillary bodies on an automated DNMP task which was identical to that reported in experiments 1 - 4 of this thesis. The main findings were that lesions of the fornix or the anterior thalamic nuclei produced a severe impairment on the DNMP task while lesions of the mammillary bodies had no effect. Both of the impaired lesion group performed normally at short delays but were badly affected when the delay between sample and subsequent choice were increased indicating a mnemonic impairment, and the performance of both groups was almost indistinguishable.

With regards performance of animals with anterior thalamic nuclei damage on maze-type tasks, several recent reports have provided some intriguiging information. In one (Aggleton et al., 1995), compared the effects of cytotoxic lesions of the anterior thalamic nuclei with those involving the fornix on a forced-alternation task in the T-maze. Both lesions impaired the acquisition of this task to a comparable degree and both groups performed normally on a subsequent test of object recognition. In another (Aggleton et al., in preparation), rats with either anterior ventral (AV), anterior medial (AM) or combined lesions including both nuclei (Com) were compared on a range of maze tasks almost identical to those described in experiment 5 of this thesis.

On T-maze delayed-alternation, Cross-maze (allocentric), and 8-arm radial-maze, the Com group were severely impaired and the AM or AV animals were moderately impaired. Only on the Cross-maze (egocentric) task did the animals with combined anterior thalamic damage perform better than the control group.

That report thus indicated that hippocampal - anterior thalamic connections are crucial for normal allocentric spatial memory, though other authors have not found such results (for example, Greene & Naranjo, 1986). In the Greene & Naranjo (1986) study however the thalamic lesions were very circumscribed, and Aggleton et al., (submitted) had indicated that it may be necessary to produce combined lesions of several of the anterior thalamic nuclei to obtain a clear effect.

The experiments reported in this thesis have shown that neurotoxic damage to the retrosplenial cortex has no effect on either egocentric or allocentric processing. However, radiofrequency damage to the same region (which destroys the cingulum bundle) can have a dissociable effect, causing impairments on tests assessing allocentric but not egocentric processing. This second circuit can thus be modified as it appears to consist of a link between hippocampus, anterior thalamus and the cingulum bundle (though contributions from retrosplenial cortex cannot be as yet fully discounted).

8.2.3 Posterior Parietal cortex (PPc) contributions.

In humans, damage to the PPc has been associated with a variety of disturbances of 'spatial behaviour' (Butters et al., 1972) but in particular can affect both spatial orientation and memory. Interestingly, human patients with PPc damage display a lack of allocentric spatial awareness and have to fall back on egocentric spatial cues when carrying out spatial tasks (Luria, 1973). Posner et al., (1984) have also reported that the parietal lobes may play a significant role in visuospatial-spatial processing in man, and in shifting attention from one stimulus to another. In monkeys, damage to PPc produces a similar dissociation in that they are impaired at allocentric but not egocentric spatial processing (Pohl, 1973). Friedman & Goldman-Rakic (1994) have recently demonstrated that in monkeys performing a working memory task, there is enhancement of local cerebral glucose in both prefrontal and parietal regions. The authors suggested that these cortical regions represented two important nodes in a neural network mediating spatial working memory.

Parietal lesions in rats also disturb the learning and performance of a variety of spatial tasks, particularly those utilising maze-type and navigational tasks (DiMattia & Kesner, 1988a; Kesner et al., 1989; Kolb et al., 1983; 1989; Kolb & Walkey, 1987), though these deficits are not as severe as those shown by animals with PFc lesions (Kolb et al., 1994). Such lesions also result in impairments in tests assessing spatial working memory (DiMattia & Kesner, 1988b). This parietal damage appears to affect both the initial acquisition and later retention of spatial information (DiMattia & Kesner, 1988a). Recordings of PPc neurons in rats performing a radial-maze task have revealed that this region contains cells that are selective for specific combinations of environmental and spatial features (McNaughton et al., 1989). Davis & McDaniel (1993) have argued that posterior parietal cortex plays an important role in integrating visuospatial stimuli with motor responses.

The PPc region in rats and monkeys receives and sends connections to the prefrontal cortex as well as to different regions of the occipital and somatosensory cortices (Kolb & Walkey, 1987; Pandya & Yeterian 1984), and according to Pandya & Yeterian (1984) the particular region of PPc that appears to most important with regards spatial processing (in monkeys at least) is the caudal inferior parietal lobule - Krieg's area 7, (Krieg, 1946).

A report by Selemon & Goldman-Rakic (1988) using the rhesus monkey, uncovered a large number of cortical and subcortical areas that received input from both PPc and PFc. Some of the key connections were with the cingulate gyrus, parahippocampal gyrus, presubiculum, and the thalamus. Selemon & Goldman-Rakic (1988) argued that these common efferent pathways constituted an elaborate anatomical circuit which could mediate a wide range of spatial processes. These connections from PPc to the regions described in the previous sentence appear to be via the cingulum bundle (Mufson & Pandya, 1984). Kolb & Walkey (1987) examined the posterior association cortex in the rat to see if an analogous region to that of the monkey could be found. These authors carried out several experiments (some anatomical and others behavioural), and concluded that rats did indeed possess an area of posterior parietal cortex that was homologous to that seen in primates. This region of PPc in the rat also receives thalamic inputs, particularly from the lateral posterior and lateral dorsal nuclei (Chandler et al., 1992; Kolb & Walkey, 1987) and also projects to frontal and cingulate cortices (Kolb & Walkey, 1987).

Poucet (1993) has argued (based on initial circuitry proposed by Mishkin et al., 1983) that the neuroanatomical circuit for spatial information consists of two cortical pathways stemming from visual cortex. One of these is directed at the posterior parietal cortex and carries spatial data, whereas the other is directed at inferotemporal cortex and carries data relevant for object recognition. Both circuits then converge upon entorhinal cortex. It thus appears that there exists a very complex set of interconnections uniting PPc and PFc with a range of subcortical targets including the hippocampus and thalamus (some of which are via the cingulum bundle) that seem to be very important for some aspects of spatial mnemonic processing.

8.3 CIRCUITS GOVERNING ALLOCENTRIC AND EGOCENTRIC PROCESSING.

The experiments described in this thesis support the recent proposal by Aggleton et al., (1995b) and indicate that there are two similar circuits governing spatial working memory in the rat, one of which subserves allocentric processing and the other which subserves egocentric processing, see figure 52.

Egocentric circuit : HPc ----------fornix ------- AT ------- PFc.

As damage to the hippocampus, anterior thalamus and prefrontal cortex (especially the prelimbic region) all produce impairments in egocentric processing it is tempting to conclude that these form a circuit which subserves egocentric spatial processing in the rat.

Allocentric circuit : HPc ---------- fornix ------- AT ------- CB ------- RSc/PPc/HPc ?

This circuit is similar to the egocentric circuit as the initial connections are between the hippocampal formation and the anterior nuclei of the thalamus. Instead of then continuing to prefrontal cortex, the circuit appears to involve the cingulum bundle which can then influence cingulate, retrosplenial, and parietal cortices as well as the hippocampal system.

Both of these circuits are closely interconnected and together function to subserve spatial processing, both egocentric and allocentric forms.

Hypothetical Allocentric and Egocentric Circuits.

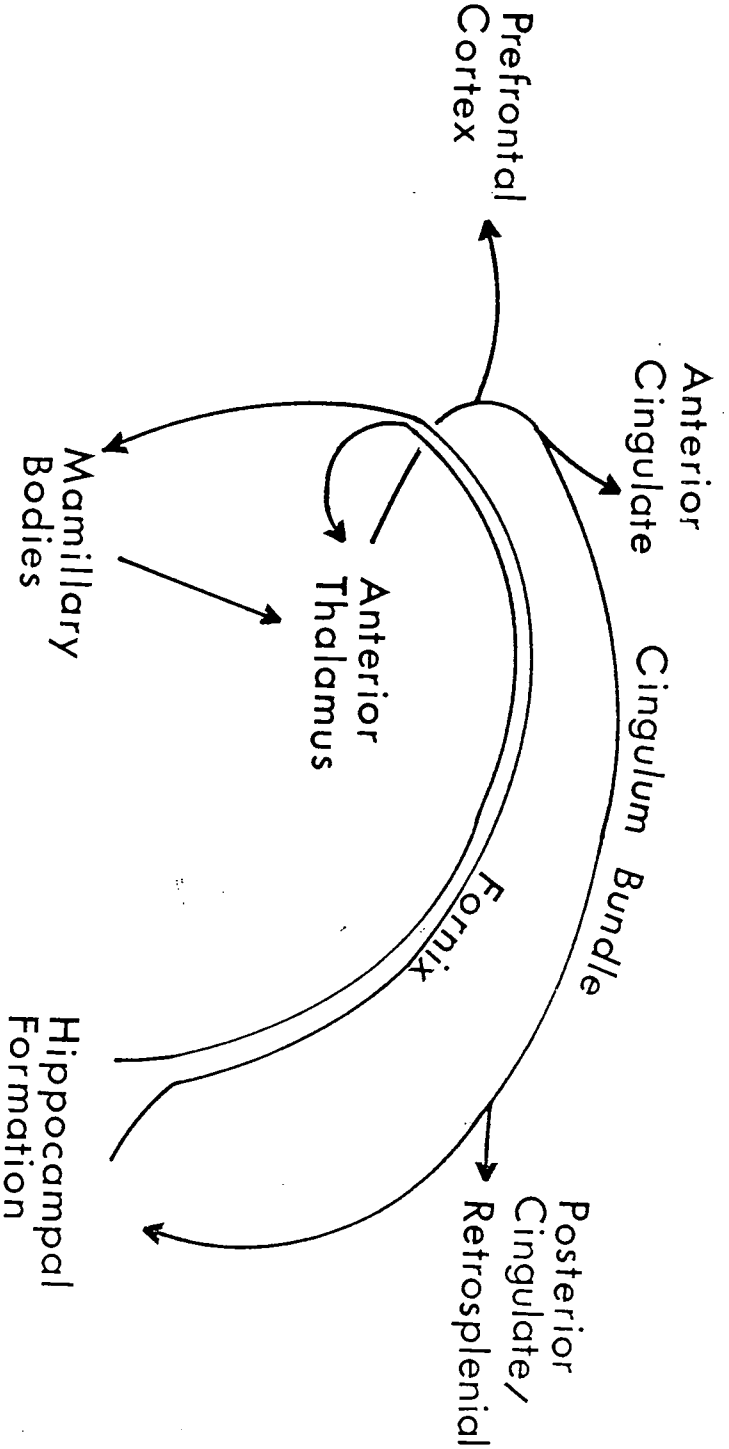

Figure 52. Hypothetical circuits for the processing of allocentric and egocentric spatial memory in the rat.

8.4 A CAUTIONARY NOTE.

Although the evidence reported in this thesis supports the above hypothesis concerning the existence of two neuroanatomical circuits governing different forms of mnemonic processing, it must be admitted that such evidence is based on a small range of tests which are not entirely analogous.

8.4.1 Criticism of the DNMP test.

While the standard maze tasks have consistently found to be sensitive assays of mnemonic processing, and have become benchmark tasks for assessing the effects of lesions, the newer, automated DNMP task can still be regarded as being under evaluation. This task is now widely used in the assessment of rat's working memory and as a sensitive technique for analysing the effects of various drugs on mnemonic performance. However, there are two potentially important problems with this task.

The first is that while it is often assumed that the task requires the successful utilisation of proximal egocentric cues, it is as yet unknown exactly how a rat does perform this task, and in particular which cues it does use to decide which lever to press. Secondly, it has been pointed out by Rawlins & Tsaltas (1983) that the animal can use its body alignment towards the recent lever response as a strategy to help maximise its chance of responding correctly to the choice lever. Although the task as used in the experiments reported in this thesis entailed the animal nose-poking at the central magazine flap throughout the delay this does not guarantee that motor mediation will not be possible. As pointed out by Gutnikov et al., (1994) an animal could respond at the central flap and yet maintain a particular body alignment.

In the Gutnikov et al., (1994) report, the authors described a new operant chamber which contained 5 holes along a curved rear wall, and a food tray on the opposite wall. The five choice holes were crossed by an infrared detector beam and each hole could be separately illuminated. Using just the outside pair of holes, rats could learn both a recency and reward (win-stay/lose-shift) task, though if a random selection of hole-pairs was used performance failed to reach more than 60% correct. In further tests, the authors found that choice accuracy declined as the spatial separation between the holes was decreased and they argued that the simplest explanation for such a pattern of results was that the animals were using a motor-mediated turning strategy.

For this task, when the rat turns from the sample hole to the food tray on the opposite wall it maintains its bodily orientation as it collects the reward and then maintains this bias as it turns again to the choice holes and selects the first illuminated hole it encounters. The authors maintained that this hypothesis could fully account for the observed pattern of results and, more importantly, there was no need to explain the results in terms of mnemonic deficits. Finally the authors argued that while the normal DNMP task may be a useful screening procedure sensitive to a dysfunction in neurochemical or anatomical systems, it is less useful as a test of mnemonic processing as it only clearly shows a disruption of motor activity.

Such a hypothesis is a powerful one as one of the key effects of fornix transection is a disruption of locomotor activity manifesting itself in strong perseverative tendencies, and increased motor-response times. Fornix transection produces a severe impairment on the automated DNMP task which various indices show to be due to increased perceptual and response bias.

While the DNMP task has been compared with the T-maze task in this thesis (as they share many similarities) they differ on one significant point, namely the amount of proactive interference contained in each, and so it can be argued they are not strictly analogous. This was addressed in the final experiment by using a double-forced procedure in the T-maze alternation task (which increased the level of proactive interference). The control group and the cingulum bundle groups were all similarly affected while the fornix group remained at 'floor' so the inclusion of this procedure did not really add any new information.

8.4.2 The maze tasks.

Some problems must be highlighted with regard to the maze tasks used in the experiments described in this thesis. The Cross-maze 'body-turns' task requires the animal to rely on egocentric processing for successful performance and this task was described as being an analogue of the allocentric maze procedures. However, the allocentric maze procedures are a test of working memory while the body-turns task is a reference memory task. This means that a direct comparison between the different types of task is not strictly justified. What was required was an egocentric task which also taxed working memory which will have made such comparisons justified.

An egocentric task which assessed working memory could have been used but it was thought that the degree of similarity between such a task and the preceding Cross-maze task would have been too difficult for the animals to learn, and so an egocentric task assessing reference memory was instead used. An experiment describing the effects of cingulum bundle lesions using such an egocentric working memory task would thus be of great interest. This is currently being planned (Aggleton et al., personal communication).

8.4.3 The lesion method.

While the lesion techniques described in this thesis have produced fairly clear-cut evidence for the existence of dissociable neural circuits governing spatial memory it is accepted that there is a potentially serious flaw in such methods. Any conclusions drawn from a group of animals with such severe neurological damage must be tentative, as it is of course possible that the damage could have had widespread influences upon cortical/subcortical functioning. It is proposed that future research aimed at accurately identifying the neuroanatomical circuits governing spatial memory may have to use techniques that do not involve the destruction and/or disconnection of one or more of the nodes in any proposed circuit.

One such method is the use of radioactively labelled metabolic markers, such as 2 deoxy-glucose, which is based on comparing relative glucose consumption (Sokoloff et al. 1977, Maxwell & Fink 1988). Using this method with monkeys it has been possible to provide direct evidence for the role of certain regions in mnemonic processing. For example, Goldman-Rakic (1984) reported that parts of the prefrontal association cortex are preferentially activated when the animal performs a delayed-response task. Related to this has been the findings that regions such as the hippocampus, the anterior thalamic nuclei, and the mammillary bodies show an increase in 2-DG uptake during the performance of tests of spatial working memory (Friedman & Goldman-Rakic, 1988; Friedman et al., 1990). The performance on a visual tracking task also resulted in an increase in 2-DG in the retrosplenial cortex, with a smaller increase being noted in the anterior thalamus, prefrontal cortex, and hippocampus (Matsunami et al. 1988).

While the 2-DG method has been used primarily with monkeys in an effort to determine the form and function of the neuroanatomical circuits governing learning and memory, it has been little used with rodents. While several papers have reported the pattern of 2-DG labelling in mice during the performance of a variety of learning tasks (Sif et al. 1989, 1991, Bontempi et al. 1990) these studies have not specifically examined spatial working memory. Other researchers have used rats, and have focused upon similar themes (Bryan & Lehman 1988, Maxwell & Fink 1988, Morimoto et al. 1984, Watson et al. 1983). Although some of the studies have used some forms of maze learning, for example Sarter et al. (1989) employed a tunnel maze and compared 2DG uptake in 'experienced' and 'non-experienced' rats, few have directly focused upon differences in 2DG uptake following specific spatial working memory tasks.

The development of a systematic analysis of (i) those regions of the brain that are the strongest candidates for a mnemonic circuit, and (ii) the different types of memory that are being used, is thus long overdue. Such research is currently being planned with normal animals and hopes to confirm (using the 2-DG method outlined above) the major neuroanatomical components of spatial working memory function in the rat, and their interconnections.

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