An examination of the effects of thalamic lesions on learning and memory in the rat

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An Examination of the Effects of Thalamic Lesions on Learning and Memory in the Rat

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Abstract

The study examined the effects of lesions of the thalamic nucleus medialis dorsalis (MD) made by neurotoxin in three cohorts of rats to help understand the contribution of this nucleus to learning and memory. The lesions typically provided comprehensive damage to MD, while the use of an excitotoxin helped to minimise damage to fibres of passage or adjacent fibre tracts. This excluded one confounding influence that may have been present in some previous studies. Some MD lesions also affected the anterior thalamic nuclei, and this additional damage led to spatial memory impairments, helping to confirm the value of results from rats with lesions confined to MD. Whilst the groups with MD lesions were largely unimpaired on non-spatial tests of visual recognition and discrimination, they were impaired on a configural discrimination task. The MD lesions did not impair spatial non-matching to sample in a T-maze, nor the acquisition or performance over delay conditions of the standard radial maze task. There were impairments, however, when the radial maze was rotated during the delay, requiring a strategy shift. Similar impairment was found when a matching, rather than non-matching, strategy was required on the T-maze task and also when only some arms were rewarded on the radial arm maze task for reference memory measurement. No impairment was seen when the T-maze matching task was reversed to the non-matching variant, emphasising the lesion rats' preference for pre-existing rules. In addition, some evidence was found that MD lesions brought about increased activity, but had no effect on conditioned place preference. The study concludes that MD damage in rats does not directly cause memory deficits. The influence that MD damage has on memory is, however, similar to that associated with damage to prefrontal cortex causing deficits in rule-switching ability, a higher order frontal lobe function.

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Introduction

1.1 - There have been many attempts to define memory. Often, they describe memory as though it were a simple, mechanical procedure, imparting no sense of the depth and complexity of memory functioning; for example "the ability to receive a sensory impression, to retain it, and to recall it at the appropriate moment" (Brierley, 1977, p199). This process of memorising is, in fact, exceedingly complex and seems at times to be impossible to isolate as a defined system, being bound up with a confusion of other contributive and dependent processes from perception to action. Nevertheless, certain brain structures are clearly of especial importance in memory, and deserve the attention of careful research. The role that science can play here is to establish painstakingly and empirically the links between brain structures whose evolution can be delineated with their particular contribution to the functioning of memory.

The thalamic nucleus medialis dorsalis (MD) has at some time been associated with a wide range of functions of the brain, including motor programming (Vanderwolf, 1971), sleeping behaviour (Marini, Gritti, and Mancia, 1988), speech production (Bogousslavsky, Ferrazzini, Regli, Assal, Tanabe, and Delaloye-Bischof, 1988), pain sensation (Hoff, Pateisky, and Wanko, 1953), olfactory assessment (Mair, Capra, McEntee, and Engen, 1980), multimodal sensory discrimination (Mair, Doty, Kelly, and Wilson, 1986), and time programming (Spiegel, Wycis, Orchinik, and Freed (1956). Despite this, MD ranks amongst a number of relatively well-defined structures that are pre-eminently implicated in normal mnemonic functioning, making it a target for especial interest in memory research. This study will attempt to isolate and illuminate some aspects of the role it plays in mnemonic functioning.

1.2 - Anatomical Properties and Connections of MD

The thalamus forms the dorsal part of the diencephalon; the other parts being the hypothalamus and epithalamus. The brains of all mammalian species include a thalamus, or more correctly two thalami; one in each hemisphere, connected through the third ventricle by the massa intermedia. The medial thalamus, bounded by the thalamic midline and the lateral border of the internal medullary lamina, is generally accepted to be the part of the thalamus that, in humans, is most involved in memory processes such as the recognition and recall of people, places, facts and events; evidence for this coming from both clinical and experimental studies (Bentivoglio, Aggleton, and Mishkin, 1997). Structures comprising the medial thalamus are the anterior nuclei, midline nuclei, intralaminar nuclei, the medial portion of the ventral nuclei, the medial pulvinar, and MD. When the lateral dorsal nucleus is added to this group, it is often termed the *limbic thalamus*. MD is the largest structure in the medial thalamus, and is most frequently implicated in studies of memory.

The characteristically strong connections of MD with prefrontal cortex have generally been used to define pre-frontal cortex by delineating it from (pre)motor cortical areas in the frontal lobe, although this definition is now regarded as overly simplistic (Groenewegen, Wright, and Uylings, 1997). Further, the view of thalamic nuclei as simple relays to the cortex is now largely seen as outdated, current opinion ascribing much more interesting aspects to their function (Sharman and Guillery, 1996).

In primates MD can be readily divided into three regions on cytoarchitectonic grounds. These are the medial magnocellular portion (MDmc), the lateral parvocellular portion (MDpc), and the far lateral multiformis portion (MDmf) (Olszewski, 1952; Akert, 1964; Tobias 1975; Goldman-Rakic & Porrino, 1985). Olszewski (1952) also referred to a small

densocellular portion in the most caudal part of the nucleus. Although MD in the rat is much more homogeneous in its cytoarchitectonic structure, fibre preparations have helped to distinguish a medial, a central, and a lateral region (Cornwall & Phillipson, 1988; Krettek & Price, 1979; Leonard, 1969), and Groenewegen (1988) added a paralamellar area in the extreme lateral region.

In order to establish an analogy between MD in rats and primates (including humans), it is necessary to compare these divisions on the basis of their projections. Thus, in the far lateral part of MD, the rat's paralamellar segment would appear to correspond with the pars multiformis in primates; both having substantial reciprocal connections with the frontal eye fields (Groenewegen, 1988; Olszewski, 1952). This then leaves the apparent anomaly of the rat having three remaining segments (medial, central, and lateral) to compare with only two in primates (magnocellular and parvocellular). This anomaly may be resolved by the discovery that the central portion of MD in the rat has heavy inputs from olfactory regions such as the ventral orbital cortex, prepiriform cortex, and olfactory tubercle (Churchill, Zahm, and Kalivas, 1996; Groenewegen, 1988; Reep, Corwin and King, 1996). Similar rhinencephalic afferents in the primate terminate in MDmc (Ray and Price, 1993; Russchen, Ameral, & Price, 1987) (Figure 1). Given that the rat has evolved to rely heavily on olfaction, it seems reasonable to regard this central portion as a relatively specialised part of what would be MDmc. Other evidence indicating that the combined central and medial portions of MD in the rat correspond to MDmc in the monkey comes from the finding that in both it is these regions of MD that are connected with the ventral and orbital portions of the prefrontal cortex and receive limbic inputs from structures such as the amygdala and entorhinal cortex (Cornwall & Phillipson, 1988; Groenewegen, 1988; Nauta, 1961; Reep, Corwin, and King, 1996; Russchen et al, 1987). Similarly, both the lateral MD in the rat and MDpc in the monkey are connected with the dorsal and lateral prefrontal cortex (Figure 1). Thus it can be seen that the divisions of MD

are comparable in rats and primates; the central and medial portions in rats being analogous with MDmc, and likewise the lateral portion being analogous with MDpc.

The major anatomical connections of MD in the rat, which are illustrated in Figures 1 and 2, can be subdivided into four groups. First there are limbic system structures such as the amygdala and lateral hypothalamus. These connect largely with the medial and central portions of MD. Second, there are basal forebrain structures such as the ventral pallidum and olfactory tubercle, which also connect with the medial and central portions. Third, there are areas of the frontal cortex such as the prelimbic and medial precentral which connect with the medial, central, and lateral portions. The frontal eye fields are an exception to this, having reciprocal connections with the paralamellar portion of MD. The remaining major connections that do not fit into these three groups include such structures as the substantia nigra and thalamic reticular nucleus, and these connect diffusely throughout MD. It should be noted also that MD's cholinergic innervation in the rat has been shown to derive from the pedunculopontine tegmental nucleus rather than from the basal forebrain (Hallinger, Levey, Lee, Rye, and Wainer, 1987).

An examination of the connections of MD in the monkey (Figures 1 & 2) reveals a very similar picture to that in the rat with, once again, the major connections being with limbic, basal forebrain, and frontal cortical regions. Connections between the thalamus and the cortex are largely ipsilateral both in rats (Pirot, Jay, Glowinsky, and Thierry, 1994) and in monkeys (Dermon and Barbas, 1994). However, contralateral connections between MD and the cortex have been noted both in monkeys (Dermon and Barbas, 1994) and rats (Negyessy, Hamori, and Bentivoglio, 1998).

In summary, it can be seen that the rat MD bears many connectional similarities with the primate MD, and as a consequence may prove to have similar functions. This may lead to

an experimental situation in which suitably analogous memory tasks can be applied to rats, monkeys, and humans with the ultimate aim of being able to use the rat as a model of human diencephalic amnesia.

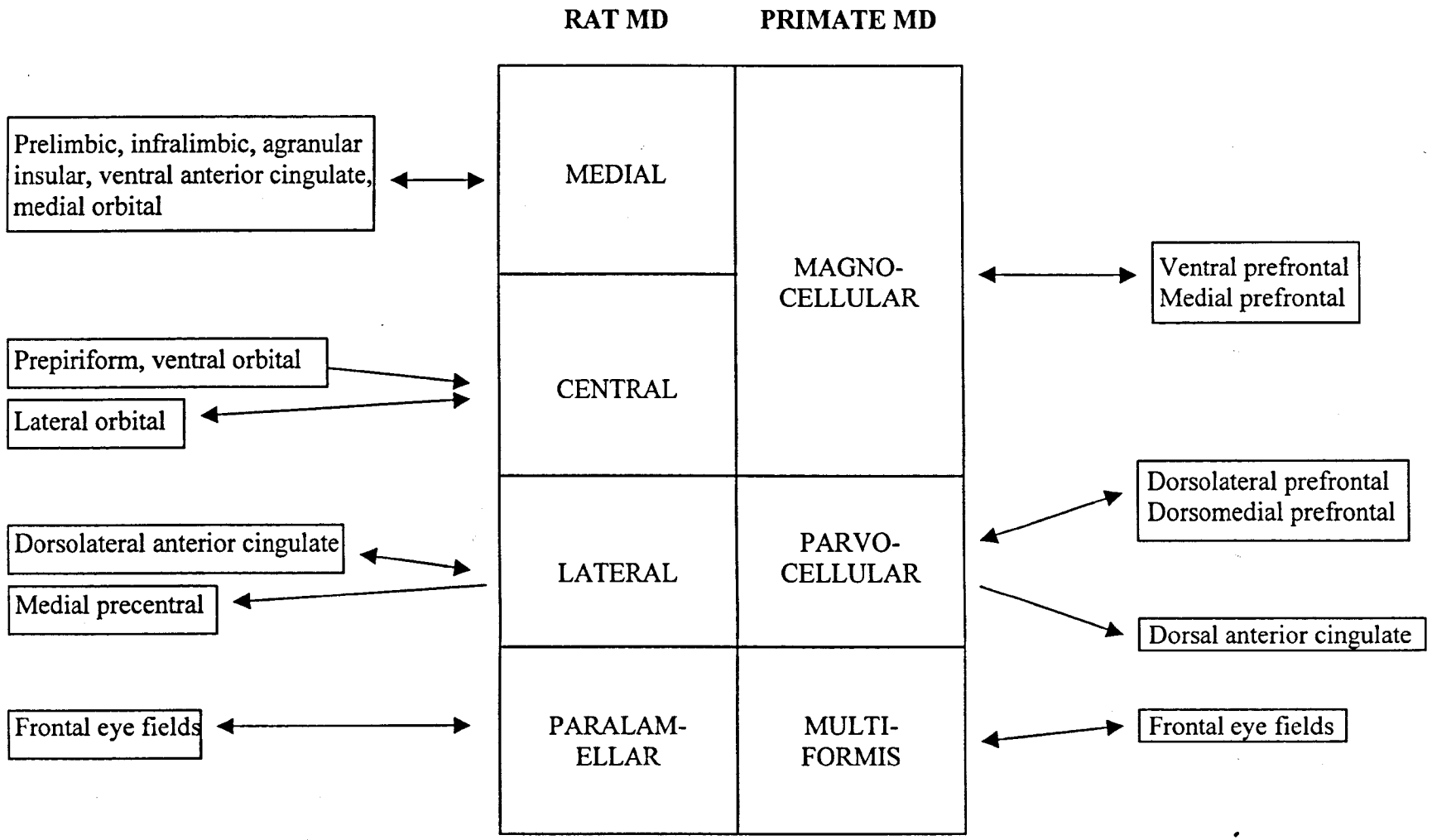

Fig. 1 - Frontal cortical connections of MD

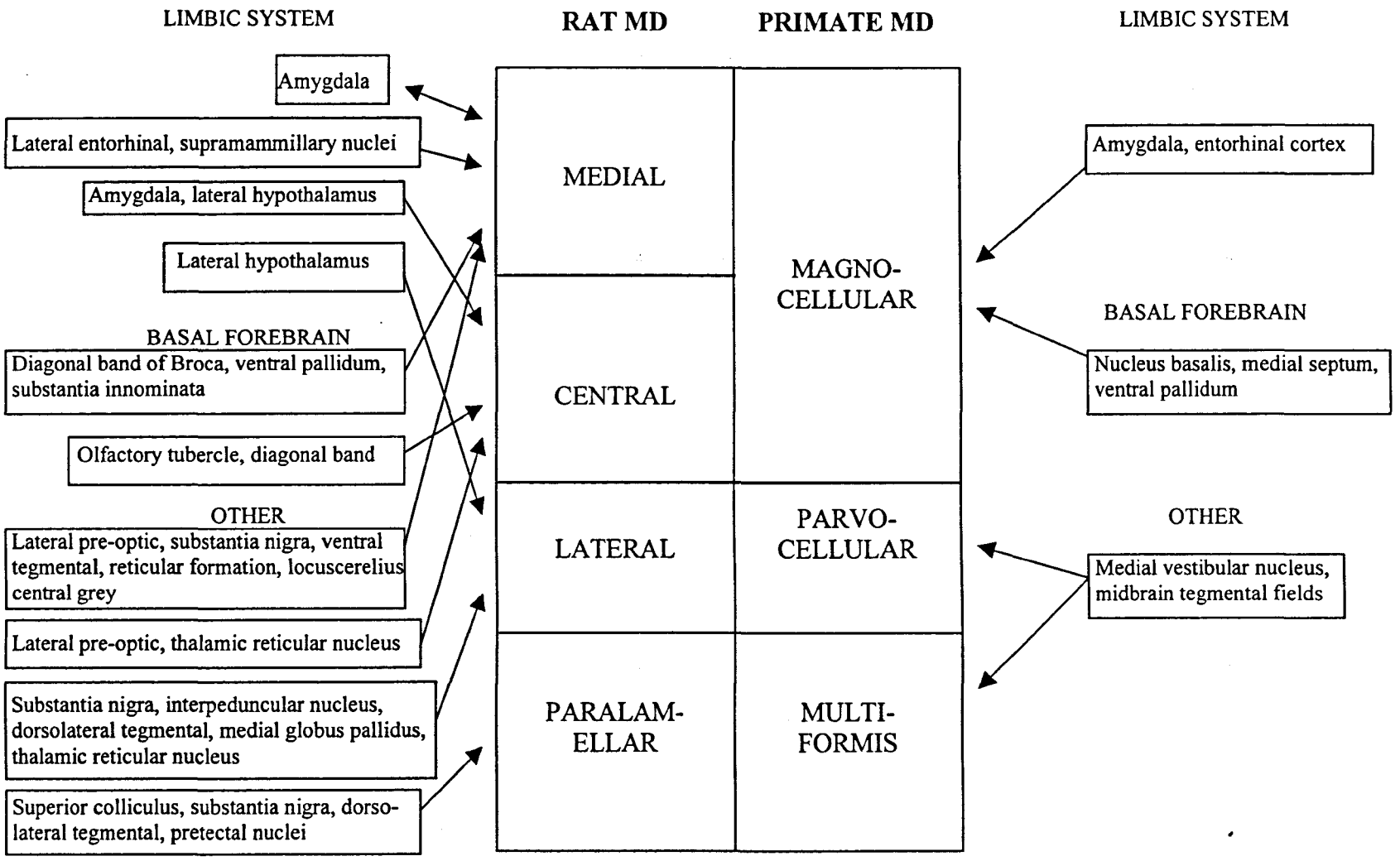

Fig. 2 - Subcortical connections of MD

1.3 - Classifications of Memory Processes

Most workers in the field of memory have found it possible to recognise three types of memory in terms of duration, i.e. the brief sensory store *iconic* or *echoic memory, short-term memory,* and *long-term memory.* Although the actual lengths of time ascribed to these terms vary widely, they cannot be regarded as merely relative terms, as the physiological mechanisms behind them can be differentiated.

Furthermore, long-term memory can be seen to have a structure comprising *procedural* memory (knowing *how)* and *declarative* memory (knowing *that)* (Tulving, 1985). Procedural memory is then subdivided into classical conditioning, motor skills, and priming; declarative memory into *episodic* and *semantic* memory (Parkin, 1987; Tulving, 1985). Episodic memory is memory for autobiographical events (facts about the world gained from unique personal experiences) as distinct from semantic memory which is memory for language, facts, general concepts, and rules about the world. Although episodic memory would seem necessarily to be a uniquely human facility, recent work with birds has shown at least an episodic-like memory function in non-human animals (Griffiths, Dickinson, and Clayton, 1999).

Another important differentiation that has often been made, both in animal and human memory research, is that of *working memory* and *reference memory* (Honig, 1978; Olton, Becker, & Handelman, 1979). This distinction in animal, i.e. non-human, research is made on the premise that the process of learning a general rule (reference memory) differs from the ability to learn a piece of information necessary to complete a single subsequent task (working memory). Therefore, in an experimental context like this study, working memory would provide the information needed to perform a single trial correctly, and reference memory would provide the rule as to how to perform all such trials. Whether

individual brain structures can be closely related to such particular types of memory is not clear. Some studies, however, have claimed to make this relationship in attempting to understand the neuronal mechanisms of memory (Funahashi and Kubota, 1994).

1.4 - Diencephalic Amnesia and the Study of Memory

In order to attempt to relate memory functions to specific brain loci, much use has been made over the years of cases of memory loss. This can be found in amnesic syndromes in human clinical cases, or deliberately created by producing brain lesions at sensitive sites in animals. In clinical amnesic syndromes in humans, the cause is usually brain damage of some kind cased by disease, trauma, or surgical intervention.

Amnesia is often divided into anterograde amnesia, with memory loss acting on material encountered after the onset of brain injury or degeneration, and retrograde amnesia, pertaining to material acquired before the onset. Although both types of amnesia can be present in the same subject, anterograde amnesia has been of particular interest as the learning deficit can occur in the face of normal or near-normal performance in a wide range of other cognitive tasks. This points to the fact that certain brain structures can be regarded as being pre-eminently concerned with the encoding, storage, or retrieval of new memories.

Anterograde amnesia is usually divided into two classes, reflecting the two anatomical regions within the brain with which amnesia is most often associated. These regions are; the temporal lobe, which encompasses structures such as the hippocampus, fornix, and amygdala; and the diencephalon, which includes, amongst other structures, the mammillary bodies and several thalamic nuclei including MD. The basal forebrain and the

frontal lobes, which have strong anatomical links with these two regions also have amnesic syndromes associated with them. Therefore, as this series of animal experiments is based on MD, evidence from human diencephalic amnesia has been examined as a background to the work. As can be seen from the studies described below, however, there are considerable difficulties in obtaining accurate data from human cases, and this highlights the need for precise examination of the mnemonic effects of diencephalic lesions in animals.

Human cases of surgically induced diencephalic amnesia are rare, and little knowledge has been gained from this source, compared with temporal lobe amnesia. It is, in any case, an unwanted side-effect of neurosurgery whose replication is likely to be avoided avidly. Accidental brain damage has supplied some data, but cases of circumscribed trauma lesions causing amnesia are rare. More information has been gained from lesions caused by disease processes that have led to amnesia, such as tumours and infarction. However, the greatest cause of diencephalic amnesia and also the best studied is Korsakoff's syndrome. The amnesia caused by Korsakoff's syndrome has been useful to the study of memory because it is relatively selective in some cases, leaving the intellect virtually intact. This is, however, a feature that is merely relative to other neurological disorders, and it must be noted that in the vast majority of cases the syndrome is associated with a reduction in I.Q.

1.5 - Evidence from Cases of Human Diencephalic Amnesia

1.5.1 - Korsakoff's Syndrome

Korsakoff's syndrome is a symptom-complex related to vitamin deficiency causing widespread degeneration of the brain. It is, however, notable for being associated with relatively small neuropathological lesions that give rise to a severe amnesic syndrome that is disproportionate to any other impairments in cognitive functioning (Kopelman, 1995). These small lesions have classsically been described in the medial thalamus and mammillary bodies.

In 1887, Korsakoff, a nineteenth century Russian physician, first described this syndrome in cases of uterine infection, puerperal septicaemia, typhus, tuberculosis, diabetes mellitus and cases of poisoning by arsenic, lead, carbon monoxide, and ergot. However, during his studies of the syndrome most of his evidence came from cases of chronic alcoholism, which is still the case today.

Gudden, in 1896, linked Korsakoff's findings with the neuropathology of two chronic alcoholics and one case of sulphuric acid poisoning described by Wernicke in 1881. The term *Wernicke-Korsakoff syndrome* is sometimes used because of this association, but generally Korsakoff's syndrome or psychosis is distinguished as being the chronically amnesic form of the syndrome. Korsakoff described patients with "a derangement of memory and of the association of ideas" (Victor & Yakovlev, 1955, p.396), and is recognised currently as an abnormal mental state in an otherwise alert and responsive patient, characterised by a severely affected memory and learning (Kopelman, 1995). The Wernicke symptoms are less cognitive, applying more to motor dysfunctions, for example

nystagmus, dysarthria, gaze palsies, dysphagia, and gait disturbance as well as a global confusional state. Victor, Adams, and Collins (1989) distinguish Korsakoff's psychosis as affecting memory disproportionately, whilst leaving other areas of brain-functioning relatively clear.

Korsakoff's syndrome has long been linked with thiamine (vitamin B_1) deficiency which can cause a long-term inability to metabolise glucose, resulting in a distinctive, widespread brain tissue degeneration. Although a whole range of backgrounds to this kind of nutritional deficiency is possible, in recent times the sort of prolonged and severe thiamine deficiency required to produce this state is almost exclusively found in chronic alcoholics, whose general malnutrition combines with the reduced ability of the gut to absorb thiamine in the presence of alcohol. The relationship between chronic alcohol intake, thiamine deficiency, and learning impairments has been widely demonstrated in rats (Homewood, Bond, and MacKenzie, 1997; Homewood, Bond, and Mc Gregor, 1991; Irle and Markovitsch, 1983b; Langlais and Savage, 1995; Mair, Knoth, Rabchenuk, and Langlais, 1991; Zimitat, Kril, Harper, and Nixon, 1990). Similarly, Korsakoff-like anterograde memory deficits and associated neuroanatomical changes were induced in thiamine-deprived monkeys (Witt and Goldman-Rakic, 1983a & b) and in mice (Tako, Beracochea, Lescaudron, and Jaffard, 1991). The reversibility of this Korsakoff model in rats when treated with thiamine has been also been demonstrated (Zhang, Weilersbacher, Henderson, Corso, Olney, and Langlais, 1995).

That the syndrome is relatively common can be seen by a reported prevailance of 1.7 per cent in Perth, Australia (Harper, 1979) and a diagnosis in 0.8 per cent of all autopsies in Oslo, Norway (Torvik, Lindboe, and Rogde, 1982). Enlarged ventricles are found on pathological investigations in fifty to one hundred per cent of alcoholics (Parsons & Prigatano, 1977).

Since the beginning of this century four *cardinal elements* of Korsakoff's syndrome have been recognised in relation to memory deficits. These are, in order of their relative frequency of occurrence; anterograde amnesia, retrograde amnesia, disorientation in space and time, and confabulation. The anterograde amnesia element is highly characteristic of the condition, and was well described by Korsakoff. Anterograde amnesia is amenable to study in animals and thus is especially interesting for the purposes of this study. However, retrograde amnesia does occur in Korsakoff subjects, and Korsakoff himself described patients in whom the impairment involved memories from up to 30 years earlier. (Kopelman, 1995). More recent neuropsychological studies confirm this (Cohen and Squire, 1981; Parkin, Montaldi, and Leng, 1990; Squire, Haiste, and Shimamura, 1989), and retrograde amnesia covering 25 years of a Korsakoff patient's life is well decribed by Sacks (1985). Retrograde amnesia can also be seen to span episodic and semantic memories (Kopelman, 1989). Disorientation in space and time is a memory dysfunction sometimes noted in the syndrome and usually takes the form of an upsetting of the chronological order of events. Confabulation, the tendency to invent or improvise events and to substitute them for gaps in memory, is also a feature sometimes found in Korsakoff's syndrome. However, a more typical pattern is for Korsakoff patients to be non-fluent in accessing memories in conversation rather than showing florid confabulation (Kopelmann, 1995). The confabulation that is present is likely to be due to the frontal lobe dysfunction characteristic of Korsakoff's syndrome (Baddeley and Wilson, 1988; Kapur and Coughlan, 1980; Kopelman, Ng, Van den Brouke, 1997; Luria, 1976).

Studies of Korsakoff subjects using tests analogous to animal studies of memory have shown impairments in both spatial (Oscar-Berman & Zola-Morgan, 1980; Oscar-Berman, Zola-Morgan, Oberg, & Bonner, 1982) and non-spatial tasks (Aggleton, Nicol, Huston, &

Fairbairn, 1987; Kessler, Irle, and Markowitsch, 1986; Oscar-Berman & Bonner, 1985 & 1987).

Precisely which brain structures are most critically affected in Korsakoff's syndrome is important to the study of memory and has been much researched. Damage to the mammillary bodies and the mediodorsal thalamic nuclei was noted in Korsakoff patients as long ago as 1896 by Gudden, who described shrinkage, loss of cells, and gliosis at these locations. The relative importance of these structures, though much disputed over the years, remains unconcluded.

It was once widely assumed that mammillary body damage was the pre-eminently important factor in the syndrome, based on a number of studies that stressed their importance (Benedek & Juba, 1944; Delay, Brion, & Elissalde, 1958a; Gruner, 1956; Malamud and Skillicorn, 1956; Orthner, 1957, Remy, 1942). However, these studies can be criticised in that they tended to base their observations of mammillary body damage on gross pathological changes visible to the naked eye, which could be misleading. This is well illustrated by Delay et al's (1958a) description of mammillary bodies appearing atrophic and yellow, yet on microscopic analysis they found loss of neurones to be only slight. Cravioto, Korein, and Silberman (1961) stress the importance of the mammillary bodies in their study of 28 cases, all of which showed bilateral mammillary body changes, and half of which were gross lesions. However, of the twenty two brains which were examined microscopically, consistent thalamic damage was found in all of them, particularly in the anterior nuclear group.

The large-scale and authoritative study of Victor et al (1989) also found little evidence of gross pathological lesions in the thalamus, but mammillary body lesions were apparent to the naked eye in 74 per cent of cases. However, despite these gross pathological findings,

their study provides the most widely accepted evidence for the predominant involvement of the thalamus and particularly the mediodorsal nucleus. Over 80 per cent of the Korsakoff patients examined post-mortem showed changes in the thalamus when this was examined microscopically. Of these cases, 88.4 percent had lesions of the mediodorsal nucleus, principally in the magnocellular portion, and 85 per cent lesions of the medial pulvinar nucleus. The authors noted the ambiguous boundary between these two nuclei, but implied that the lesions of the latter constituted an extension of those of the former. In fact, MD was affected in every case of thalamic involvement, and in 20 per cent of these cases it was the only thalamic nucleus found to be involved.

Although the authors did not claim to decide with certainty which structure was pre-eminently critical in memory function between the mammillary bodies, the mediodorsal nucleus, and the medial pulvinar nucleus, they did provide strong evidence in favour of the mediodorsal nucleus as follows:

Of the 43 brains examined post-mortem in the study, only 5 were found with no change in the mediodorsal nuclei. These were also the only five cases which in life had shown no memory deficit, and the mammillary bodies were affected in all five cases. The authors conclude that the mammillary bodies may be significantly affected in cases where there is no memory deficit. They sum up by stating their belief that the amnesic defect is related to lesions in the diencephalon, specifically in the medial dorsal nuclei and possibly in the medial pulvinar. Victor (1988) draws on what he terms "unambiguous evidence" from both human and animal studies to argue the irrelevance of mammillary bodies in the causation of Korsakoff's syndrome, declaring that medial thalamic lesions alone are quite sufficent to produce a severe and enduring amnesic state.

In contrast to this view of the lack of importance of the mammillary bodies, Torvik (1987) describes a study of 45 cases of Wernicke's encephalopathy. In this study, the majority of cases had lesions of both the mammillary bodies and the thalamus. However, eleven of 21 chronic cases had lesions restricted to the mammillary bodies, and three of these cases showed memory loss. One interpretation of the more usually observed lack of amnesic effect with mammillary body lesions alone is that a conjoint mammillary body and medial thalamic lesion is necessary (Mayes, Meudell, Mann, and Pickering, 1988). This view is supported by lesion studies in monkeys that show a lack of effect on recognition memory with lesions of mammillary bodies alone (Aggleton and Mishkin, 1985; Zola-Morgan,1989), although Gaffan and Watkins (1991) found a deficit with MD lesions alone that, as with perirhinal lesions, was manifest with decreased set size. Mair, Warrington, and Weiskrantz (1979) described a severe anterograde and retrograde amnesia in two human patients with Korsakoff's syndrome who had only a thin band of gliosis running through the parataenial nucleus between the third ventricle and the magnocellular portion of MD.

Evidence that neither mammillary body nor MD damage is sufficient in itself to cause amnesic Korsakoff's psychosis is given in a recent large-scale post-mortem study (Harding, Halliday, Caine, and Kril, 1999 in press). This shows that the loss of neurones in both structures is equally substantial in both amnesic and non-amnesic alcoholic patients. Loss of neurones in the anterior thalamic nuclei, however, is confined to the Korsakoff patients, indicating the possibility that these nuclei hold the critical role in the amnesic syndrome.

Evidence from imaging techniques on Korsakoff's patients is equivocal and, as yet, largely unenlightening. Computer tomography (CT) scans on Korsakoff patients, usually compared with normal and alcoholic control groups, are limited in their spatial definition

and cannot show small sub-cortical structures such as mammillary bodies with any useful precision (Fazio, Perani, Gilardi, Colombo, Cappa, Vallar, Bettinardi, Paulesu, Alberoni, Bressi, Franceschi, and Leenzi, 1992), although magnetic resonance images (MRI) have shown small mammillary bodies in Korsakoff cases (Squire, Amaral, and Press, 1990). Jacobson (1987) and Jacobson and Lishman (1990) used CT techniques in Korsakoff patients to show thalamic hypodensity. Positron emission tomography (PET) scans, however, have demonstrated decline in cortical glucose metabolism in Korsakoff patients (Paller, Archaya, Richardson, Plaisant, Shimamura, Reed, and Jagust, 1997), but have not shown evidence of sulcal or ventricular enlargement (Carlen, Wilkinson, and Wortzman, 1981).

It seems reasonable then to conclude that, within the considerable experimental constraints associated with human subjects, anatomical and functional studies of Korsakoff patients do indicate some possible links between memory function and the thalamus. The case for MD's pre-eminence in this involvement, however, remains unproven.

1.5.2 - Other Thalamic Damage

1.5.2.1 - Tumours

The disturbances of memory often described as Korsakoff-like symptoms associated with tumourous growths in the diencephalon have been used to provide evidence of the link between memory and diencephalic structures. According to Markowitsch and Pritzel (1985) tumours of the dorsal midline structures of the thalamus are frequently found to be the cause of memory disturbances; their removal sometimes resulting in complete recovery of memory function.

One advantage in studying tumours involving MD is that their extent and consequent mnemonic effects may not include effects on other structures implicated in memory function, especially the mammillary bodies and anterior thalamic nuclei. Such studies are, however, quite rare. A bilateral thalamic tumour with no mammillary body involvement was reported by Sprofkin and Sciarra (1952) to be associated with amnesic symptoms. Unfortunately no specific description of the thalamic structures involved was given in this study. McEntee, Biber, Perl, & Benson, (1976) described another such case in which amnesia was associated with bilateral thalamic tumour, described as invading the medial and posterior thalamus and including the mediodorsal nucleus, without involvement of the mammillary bodies or anterior thalamus. The authors also recognised, however, that tumours can cause damage distal from their site due to the surrounding oedema they create.

Tumours on the floor and/or walls of the third ventricle close to MD have sometimes been implicated in memory disturbance syndromes, but, similarly, may be exerting an influence on structures elsewhere. Weisenburg, in 1911, reviewed a number of cases of memory disorders and concluded that the third ventricle tumour was causing increased pressure on the cerebral cortex rather than the damage being done directly to diencephalic structures (Brierley, 1977). Williams and Pennybacker (1954) found that third ventricle tumours had resulted in amnesia due to increased pressure on diencephalic structures. Although it was assumed that the structures most affected by this pressure were the mammillary bodies, the nature of ventricular pressure cannot lend itself to much selectivity, and other structures bordering on the ventricle may be similarly implicated, especially the thalamus.

The surgical removal of tumours of the diencephalon in cases showing amnesic symptoms has produced effects on subsequent memory performance varying from complete

restitution to a worsening of memory functions (Foerster & Gagel, 1934; Geffen, Walsh, Simpson, and Jeeves, 1980). Williams and Pennybacker (1954) found that aspiration of fluid from the third ventricle as well as tumour removal resulted in improvement in memory defects, but the report of this is poorly documented.

1.5.2.2 - Circulatory Disturbances

After damage caused by chronic alcoholism, the most frequent cause of diencephalic damage is disturbances in blood circulation (Markowitsch & Pritzel, 1985). Occlusion or rupture of the major arteries can disturb or prevent normal metabolism in the pertinent brain area, often causing widespread and enduring amnesic states. In the case of thalamic damage, the relevant vessels are the paramedian thalamic arteries. Such damage is usually accompanied by mnemonic disturbances, and one case, a unilateral infarction of the thalamus, resulted in a deficit restricted to verbal memory (Michel, Laurent, Foyatier, Blanc, and Portafaix, 1982).

Computer tomography (CT) scans (e.g. Speedie and Heilman, 1982; von Cramen and Eilert, 1979; Winocur, Oxbury, Roberts, Agnetti, and Davis, 1984) and magnetic resonance imaging (MRI) techniques (Bogousslavsky et al, 1988) have allowed damage to the thalamic nuclei to be identified more accurately. These studies described cases of restricted medial thalamic damage, both unilateral and bilateral, of ischemic origin associated with memory disturbances. Kritchevsky, Graf-Radford, and Damasio (1987), however, found no memory impairments in a group of patients with MRI-identified MD thalamic lesions in which the mammillo-thalamic tract was seen to be intact. The MD damage in all of these cases (Kritchevsky et al, 1987) was, however, small (around 30 per cent). Further, von Cramen, Hebel, and Schuri (1985) carefully examined CT data from

their own six patients with medial thalamic damage and related it to performance on tests of memory function. The two patients without significant memory dysfunction had MD damage that spared the mammillothalamic tract and ventral part of the lamina medullaris interna.

Amnesia caused during cardiac arrest has frequently been attributed solely to damage to the medial temporal lobe (O'Rourke, Saykin, Gilhool, Harley, O'Connor, and Sperling, 1993; Rempel-Clower, Zola-Morgan, Squire, and Amaral, 1996; Sass, Lencz, Westerfeld, Novelly, Spencer, and Kim, 1991; Zola-Morgan, Squire, and Amaral, 1986). Consequently, patients who have suffered anoxic-ischemic brain damage are largely regarded as models of medial temporal lobe amnesia. Markowitsch, Weber-Luxemburger, Ewald, Kessler, and Heiss (1997), however, have demonstrated medial thalamic damage in one such amnesic patient using positron emission tomography (PET) that was not shown by MRI. Since memory disorders caused by medial diencephalic damage resemble those caused by medial temporal lobe damage (Bentivoglio, Aggleton, and Mishkin, 1997), the possibility arises that some amnesic effects reported in previous MRI studies could be at least partly attributable to medial thalamic damage.

In a review of the mnemonic effects of thalamic infarction, Aggleton and Brown (1999) conclude that infarction in the anterior thalamus is associated with anterograde amnesia, and that mammillo-thalamic tract damage plays a critical role in this. Infarction in the mediodorsal part of the thalamus, however, seems more associated with executive control, whilst damage caused to the internal medullary lamina by infarction may accentuate both of these effects (Aggleton and Brown, 1999).

1.5.2.3 - Trauma

Thalamic damage sustained in accidents can have an effect on memory; memory disturbances being the most frequently reported behavioural consequence of traumatic brain lesions. Retrograde amnesia is more common in trauma than damage caused by tumours or circulatory disturbances, and a number of cases have been described in which head injuries have caused symptoms paralleling those of Korsakoff patients (Markowitsch and Pritzel, 1985). However, the nature of most head injuries makes the correlation of memory functions with a relatively circumscribed lesion difficult (Brierley, 1977). A well-known exception to this is case N.A.

Case N.A., a 22 year old United States Air Force technician, sustained a brain injury as a result of a mishap with a miniature fencing foil paper knife in 1960. The resulting, apparently very localised, diencephalic damage and amnesia have been intensively studied over the subsequent years. The location of the lesion was identified in 1978 by computer tomography scan to be in the mediodorsal region of the left thalamus (Squire and Moore, 1979). The resulting anterograde amnesia is markedly worse for verbal than for non-verbal material, but his intellect remains high with an I.Q. of 124. In fact, he has achieved higher scores than control subjects in many perceptual and cognitive tasks (Squire and Zola-Morgan, 1983). His amnesia differs from that of Korsakoff patients in that there is almost no retrograde amnesia, no impairment in tasks involving the temporal order of recent events, and he is able to exhibit release from proactive interference (Markowitsch and Pritzel, 1985).

A subsequent study on Case N.A. (Squire, Amaral, Zola-Morgan, Kritchevsky, and Press, 1987) used magnetic resonance imaging to reveal evidence of damage to structures other than MD, notably the mammillo-thalamic tract. This finding highlights the difficulty of

obtaining reliable and detailed anatomical evidence from human subjects. Further, a similar penetrating injury to the mammillary bodies has been associated with a deficit for largely verbal material, but with recognition remaining almost normal (Dusoir, Kapur, Bymes, McKinstry, and Hoare, 1990). A later imaging study has, however, revealed some abnormality in left hippocampal structure and function (Kapur, Scholey, Moore, Barker, Mayes, Brice, and Fleming, 1994).

1.5.2.4 - Surgically induced lesions

Although there has been a significant number of studies of surgical thalamic lesions performed on human subjects for clinical reasons, information on the mnemonic effects of these interventions is rare. There are several reasons for creating such lesions (Markowitsch and Pritzel, 1985), e.g. pain relief, control of aggressive behaviour, and control of epilepsy. As these are attempts to modify the emotional behaviour of individuals, the post-operative effects on memory have not necessarily been central to the descriptions of post-operative behaviour changes. Furthermore, the fact must be noted that such surgery is confined to patients whose abnormal behaviour has been attributed to abnormal brain activity. This makes interpretation of any data thus gained difficult to apply to any general theory of thalamic memory function.

Of those studies which have noted amnesic effects after lesions of MD, Spiegel, Wycis, Orchinik, and Freed (1955, 1956) provide a little evidence of a similarity to Korsakoff's syndrome. They describe a temporal disorientation (chronotaraxis) similar in some respects to Korsakoff's syndrome, but differing significantly in its duration. The symptom lasted generally for a few days or weeks with only one case lasting for as long as six months. The location of the lesions in these cases also remains unconfirmed.

There is also some evidence for the involvement of the anterior thalamic nuclear group from human surgical lesion cases. Although Brierley (1977) asserts the opinion that no such lesions, surgical or tumourous, have been shown to correlate with a true amnesia, Mark, Barry, McLardy, and Ervin, 1970 describe memory loss in a patient who received bilateral radio-frequency lesions to the anterior nuclei. Markowitsch and Pritzel (1985) suggest, however, that lasting impairments are found only when lesions are made in patients with other existing damage to the central nervous system, e.g. Parkinson's disease.

1.6 - Animal Evidence

Animals, especially other mammals mammals, have been used in the attempt to develop models for human amnesic syndromes because of the difficulties and limitations of using purely human data for the study of memory and learning systems. As human studies of memory have made great use of memory losses and deficits to infer the existence and morphology of memory and learning mechanisms, so animal studies have been needed to establish the necessary and sufficient conditions under which the clinical syndromes occur. In this way, the memory and learning systems and inter-relationships of anatomical structures apparent in man may be paralleled, tested, and perhaps confirmed.

The main limitation of using human amnesic patients in the study of learning and memory is that, for obvious ethical reasons, the therapeutic benefit to the individual always has to take precedence over the gaining of useful scientific data, even though this may assist in the understanding of the basis of the problem and therefore ultimately be useful in future therapy (Olton, 1985). Animals are therefore widely used to gain precise knowledge

relatively rapidly. Other difficulties encountered in human studies include the size of sample groups available to the scientist, the necessarily long delay in obtaining pathological data (although increased availability of scanning technology is decreasing this problem), the relatively uncircumscribed nature of uncontrolled lesion induction, and the interference of other extraneous factors in clinical subjects.

Although invertebrates are often used to study neural mechanisms at the cellular level, and to build up phylogenetic trends in order to infer the human situation (Markowitsch and Pritzel, 1985), non-human mammals, principally monkeys and rats, have almost exclusively been used to produce models of human learning and memory. Despite the long history of memory studies using animals, it is only relatively recently that reliable and appropriate methods of memory testing have been developed that enable animal models to yield the kind of valuable knowledge that they now provide (Squire and Zola-Morgan, 1985).

Addressing the controversy over the relative importance of thalamic nuclei versus mammillary bodies, Victor et al (1989) reviewed some of the animal experimental literature. After pointing out the difficulty of directly equating animal behaviour in Korsakoff's syndrome, they cautiously suggest that animal studies do bear out their conclusions that in humans the thalamic nuclei, and especially the mediodorsal, are of primary importance. They go on to point out that the mammillary body lesions produce very variable behavioural results depending on the type of memory task used, and that lesions of the thalamic nuclei must be bilateral and virtually complete in animals. This latter point is also made by Squire and Zola-Morgan (1983) and Stokes and Best (1990c). However, the difficulty of producing such virtually complete lesions that are also confined to individual nuclei must not be underestimated (Hunt and Aggleton, 1991). The

anatomical descriptions in those studies that report virtually complete lesions must consequently be scrutinised very carefully for evidence of damage to adjacent structures.

Before dealing with the classical lesion and behaviour studies of MD in monkeys and rats, it may be useful briefly to examine the link between chronic alcohol intake, thiamine deficiency, and learning impairments in animal studies. Using rats, a number of studies have succeeded in demonstrating that there is a causal relationship between these elements (Homewood, Bond, and MacKenzie, 1997; Homewood, Bond, and Mc Gregor, 1991; Irle and Markovitsch, 1983b; Langlais and Savage, 1995; Mair, Knoth, Rabchenuk, and Langlais, 1991; Zimitat, Kril, Harper, and Nixon, 1990). Similarly, Korsakoff-like anterograde memory deficits and associated neuroanatomical changes were induced in thiamine-deprived monkeys (Witt and Goldman-Rakic, 1983a & b) and in mice (Tako, Beracochea, Lescaudron, and Jaffard, 1991). The reversibility of this Korsakoff model in rats when treated with thiamine has been also been demonstrated (Zhang, Weilersbacher, Henderson, Corso, Olney, and Langlais, 1995).

1.6.1 - Experimental lesion studies in monkeys

Electrophysiological studies in which evoked potentials and single unit activity in MD in monkeys were correlated with learned behaviour have been reported (Alexander and Fuster, 1973; Kubota, Niki, and Goto, 1972). These studies, however, provide no strong evidence for a memory-related role for MD (Markowitsch, 1982). Similarly, the proximity to the medial thalamus of several major fibre tracts make stimulation studies in monkey MD (Briese and Olds, 1964; Olds, 1966) of little value when attempting to attribute brain functions to specific nuclei (Markowitsch, 1982).

Those lesion studies in monkeys that use behavioural testing of learning and memory are particularly of interest in the current study. However, other changes following MD lesions are worth noting as they may have secondary effects on more pertinent behavioural test results. Motor disturbances, principally of the extremities, have been observed (Showers, 1958; Brierley and Beck, 1958), as have changes in emotionality (Brierley and Beck, 1958; Butter and Snyder, 1972). All these studies used methods of lesion-making (aspiration and coagulation) which may have disrupted fibres of passage to bring about the observed changes.

There is little evidence of impairment on sensory discrimination tasks (Aggleton and Mishkin, 1983a; Chow, 1954; Schulman, 1964; Thompson and Myers, 1971). Studies using delayed response-type tasks give conflicting results, with some studies reporting no impairments (Chow, 1954; Peters, Rosvold, and Mirsky, 1956; Walker, 1940), whilst others (Isserhoff, Rosvold, Galkin, and Goldman-Rakic, 1982; Schulman, 1964) found severe and long-lasting impairments on this class of task.

Loss of recognition memory is often regarded as a core deficit in anterograde amnesia. Since recognition memory is readily testable in animals, usually by testing recognition of an object after a variable delay, the use of such tests in assessing animal models of anterograde amnesia has been widespread. The methodology was developed for use in monkeys with large medial temporal lesions (Mishkin, 1978; Zola-Morgan et al, 1982) and was subsequently used with large (Aggleton and Mishkin, 1983a) and more restricted (Aggleton and Mishkin, 1983b; Zola-Morgan and Squire, 1985) medial diencephalic lesions. All these studies used aspiration lesions and reported severe recognition memory deficits, the size of the deficit seeming to correspond with the extent of medial thalamic damage. Using similar lesion techniques, Gaffan and Murray (1990), Gaffan and Watkins (1992), and Parker, Eacott, and Gaffan (1997) found recognition deficits in monkeys with

lesions aimed at medial magnocellular MD tested on a computer-based visual discrimination task. The severity of the deficits was seen to be as great as that associated with perirhinal lesions (Parker et al., 1997).

From studies that have used monkeys, then, it is possible to say that the clearest effect of MD lesions on learning and memory is shown in tasks that address recognition memory. However, it must not be forgotten that since these studies all make use of aspiration techniques to ablate the nucleus, it is not possible to account for the damage to fibres of passage which may be affecting the behavioural outcome of the lesions.

1.6.2 - Experimental lesion studies in rats

The rat has been claimed to be an excellent model of human memory (Kesner, 1990) in that it displays serial position, serial anticipation learning, temporal coding, and repetition lag functions as well as utilisation of retrospective and prospective codes, nearly equivalent to that of humans. The clear practical advantages of using rats, combined with the above qualities, have led to a much larger number of studies using rats to measure the mnemonic effects of lesions in MD compared with monkeys.

As in monkeys, lesions to rat MD have been used to investigate more general behavioural functions outside learning and memory (Table 1). Although these studies by no means represent a comprehensive or systematic basis for understanding the non-mnemonic behavioural effects of MD lesions in rats, some inferences may be drawn from the pattern of results shown here. The most frequently observed measure of behaviour is activity, either in the rats' home cage (Beracochea et al, 1989; Vanderwolf, 1971) or in test apparatus (Kolb, 1977; Waring and Means, 1976), and the majority of studies suggests

that activity is unaffected by MD lesions. The level of activity displayed by animals is an important factor in behavioural testing, since changes in activity levels could lead to differences in performance that could be misinterpreted as deficits in learning and memory. Similarly, the way that rats deal with food and water, either in time spent eating and drinking (Beracochea et al, 1989), food hoarding (Kolb, 1977), or foraging behaviour (Schacter et al, 1991), could also be important, especially where behavioural testing involves food reward. In this case it appears that behaviour toward food and water is altered in rats with MD lesions (Table 1). Aside from being a factor in the interpretation of experimental results, there is also the possibility that this alteration in normal behaviour may indicate something about the way the lesion affects normal cognitive functions in the animal, particularly the role of executive functioning. This may be especially pertinent in the case of lesions to MD, since this structure has such direct and extensive anatomical links with the prefrontal cortex, the brain area known to be largely directed towards executive function.

Tables 2, 3, and 4 list experiments that more directly test the cognitive, and principally learning and memory, effects of MD lesions. Clearly, all three tables show the lack of consistency in the results from such tests. Table 2, dealing with non-spatial discrimination or recognition tasks, shows studies that cover a range of discrimination modalities, including vision, touch, audition, olfaction, and combinations of these. None of these types of task or modalities appears to form any consistent pattern of deficit. Other non-spatial learning and memory tasks that do not involve recognition or discrimination are shown in Table 3. Despite a near consensus of deficits being apparent after MD lesions in this class of task, it is difficult to draw any useful inferences since the nature of the tasks varies widely and the number of studies is in any case low. Table 4, deals with tests of spatial learning and memory, and, in contrast with Table 3, represents the largest and most comprehensive class of task that has been applied to rats with MD lesions. Again, despite

this relative thoroughness of investigation, there is still a confusingly wide array of deficits, and those not consistently reported.

One possible approach in the interpretation of these conflicting results is to recognise that conventional lesion-making techniques (electrolytic and radio frequency) destroy fibres of passage as well as cells located within the target area. Thus, the behavioural consequences of the lesion may not be confined to the function of the target structure. This problem is of particular concern for a sub-cortical nucleus like MD that not only contains fibres of passage, but is also bounded by a major fibre pathway, the internal medullary lamina. It is therefore possible that differences in lesion location and size could have marked behavioural consequences. This view is strongly suggested in a review article (Markowitsch, 1988) that largely uses examples from cases of human diencephalic amnesia. Accordingly, the outcome of the few animal studies that have used neurotoxins that help spare fibres of passage (Schwarcz, Hokfelt, Fuxe, Jonsson, Goldstein, and Terenius, 1979) may be of particular interest. Looking at the results of these studies in isolation then, it is certainly true to say that they are less likely to bring about significant deficits and changes in behaviour than are radio frequency or electrolytic lesions. This, at least, bears out the supposition that the destruction of fibres of passage may bring about behavioural changes that are not directly accountable to the structure being studied. Indeed, the one study that directly compared the effects of lesions made by neurotoxin and by radio frequency (Hunt and Aggleton, 1991) found a deficit with the latter and normal performance with the former lesion in groups of rats tested on the same task in the same experiment.

This apparent effect of lesion method, however, does not in itself explain the confusing pattern of results. The neurotoxic lesion studies, taken in isolation, still show a broad array of effects, with evidence of impaired spatial memory in a range of RAM tasks, acquisition

of delayed non-match to sample object recognition, temporal alternation, and place preference acquisition. The fact that negative findings for most of these classes of tasks have been more widely reported can provide no real basis to show that learning and memory depend on the normal functioning of MD in the way that, for example, the hippocampus (Olton and Papas, 1979) or anterior thalamic (Aggleton and Brown, 1999) lesions can be seen to function. It might be, however, that this apparently unhelpful pattern could, by its nature, imply something important about MD's function. Since MD has broad and important connections with many cortical sites, it might be expected that it would have an influence on a wide array of classes of information being processed. Further, if these MD-cortical connections form part of a series of parallel cortical connection systems, then disruption of MD's functioning would rarely be crucial in itself, but might provide the kind of results seen in Tables 1 to 4 above. The functions of MD, then, may be seen as providing a variety of influences on information being processed in learning and memory.

TASK	AUTHORS	LESION	RESULT
Emotionality	Waring & Means, 1976	electrolytic	+
Exploration	Weiss & Means 1980	electrolytic	-
Exploration	Mair, Robinson, Koger, Fox, & Zhang, 1992	NMDA	-
Running wheel activity	Kolb, 1977	electrolytic	-
Open field activity	Waring & Means, 1976	electrolytic	-
Activity	Vanderwolf, 1971	electrolytic	+
Activity	Beracochea, Jaffard, and Jarrard, 1989	ibotenic acid	-
Food hoarding	Kolb, 1977	electrolytic	+
Eating/drinking	Beracochea, Jaffard, and Jarrard, 1989	ibotenic acid	+ at night
Central place foraging	Schacter, Phelps, Brodbeck, Mogenson, & Roberts, 1991	electrolytic	+

Table 1 - Findings of studies making general behavioural observations on rats with MD lesions
+ = changed behaviour; - = unchanged behaviour

TASK	AUTHORS	LESION	RESULT
Tactile discrimination	Weiss & Means, 1980	electrolytic	- anterograde, + retrograde
Visual-tactile discrimination	Waring&Means, 1976	electrolytic	+
Roughness discrimination	Tigner, 1973	radio frequency	+ acquisition, + reversal
Visual discrimination	Slotnic & Kaneko, 1981	electrolytic	-
Tone/light discrimination	Means, Hershey, Waterhouse, & Lane, 1975	electrolytic	+
Brightness discrimination	Tigner, 1973	radio frequency	+ acquisition, + reversal
Odour discrim. & DNMS	Staubli, Schottler, & Nejat-Bina, 1987	electrolytic	+
Odour discrim. & DNMS	Zhang, Burk, Glode, & Mair, 1998	NMDA	-
Odour discrim. & reversal	Slotnik & Kaneko, 1981	electrolytic	-post-op. retention, + reversal
Odour discrim. & detection	Eichenbaum, Shedlack, & Eckman, 1980	radio frequency	- detection, + discrimination
DNMS object recognition	Hunt and Aggleton, 1991	ibotenic, RF	+ acquisition, - performance
DNMS object recognition	Mumby, Pinel, & Dastur, 1993	electrolytic	+ acquisition, + performance
DNMS	Mair , Robinson, Koger, Fox & Zhang, 1992	NMDA	- acquisition, - performance
Place discrimination	Tigner, 1973	radio frequency	-

Table 2 - Findings of studies using non-spatial discrimination or recognition tasks on rats with MD lesions
+ = deficit; - = no deficit

TASK	AUTHORS	LESION	RESULT
Learned food preference memory	Winocur, 1990	electrolytic	- anterograde, + retrograde
Active avoidance	Vanderwolf, 1966	electrolytic	+
Delayed alternation (Skinner box)	Peinado-Manzano & Pozo-Garcia, 1991	electrolytic	+ acquisition, + performance
Place preference acquisition	McAlonan, Robbins, & Everitt, 1993	ibotenic acid	+
Temporal altn. in a straight alley	Beracochea, Jaffard, & Jarrard, 1989	ibotenic acid	+

Table 3 - Findings of studies using non-spatial tasks other than discriminations or recognition on rats with MD lesions
+ = deficit; - = no deficit

TASK	AUTHORS	LESION	RESULT
T-maze spontaneous alternation	Weiss & Means, 1980	electrolytic	+
T-maze spontaneous alternation	Tigner, 1973	radio frequency	-
T-maze spontaneous alternation	Greene & Naranjo, 1986	electrolytic	-
T-maze spontaneous alternation	Vicedomini, Corwin, & Nonneman, 1982	electrolytic	+
T-maze spontaneous alternation	Hunt & Aggleton, 1991	ibotenic acid, RF	- ibo, + RF
T-maze delayed alternation	Kessler & Markowitsch 1981	kainic acid	+ (*HPC)
T-maze post-operative retention	Brito, Thomas, Davis, & Gingold, 1982	electrolytic	+
T-maze reversal	Means, Hershey, Waterhouse, & Lane, 1975	electrolytic	+
Radial arm maze (RAM), no visual cues	Stokes & Best 1988	electrolytic	+
RAM working memory, visual cues	Stokes & Best 1990a	electrolytic	+
RAM working memory & reference memory, visual cues	Stokes & Best 1990b	electrolytic, ibotenic acid	+
RAM arm selection accuracy	Stokes & Best 1990c	ibotenic acid	+
RAM working memory	Kolb, Pittman, Sutherland & Whishaw, 1982	electrolytic	-
RAM delay	Kessler, Markowitsh, & Otto, 1982	ibotenic acid	+
RAM working memory	Beracochea, Jaffard, & Jarrard, 1989	ibotenic acid	-
Skinner box delay non-match to position	Neave, Sahgal, & Aggleton, 1993	NMDA	-acquis. -perfor.
Skinner box discrimination reversal	Beracochea, Jaffard, & Jarrard, 1989	ibotenic acid	-
Skinner box discrimination reversal	Neave, Sahgal, & Aggleton, 1993	NMDA	-
Water maze	Kolb, Pittman, Sutherland & Whishaw, 1982	electrolytic	-

Table 4 - Findings of studies using spatial memory tasks on rats with MD lesions
+ = deficit; - = no deficit; *HPC = substantial damage to hippocampus reported

1.7 - General Aims of the Present Study

This series of experiments builds upon a previous study of the role of MD in memory (Hunt and Aggleton, 1991) which primarily examined working memory in rats with circumscribed medial dorsal thalamic damage. This study found that rats with both neurotoxin (ibotenic acid) and radio-frequency lesions made in MD were subsequently impaired on the acquisition of a non-matching to sample test of object recognition. These lesion groups were, however, found to be unimpaired on performing the task following acquisition with the imposition of intra-trial retention intervals of up to 60 seconds. A second experiment tested the same groups of rats on a spatial task, delayed forced alternation, in which the rats were tested with delays similar to those in the recognition experiment in both spaced and massed trials. Damage to MD had no effect on acquisition or on spaced trials, but a slight deficit was found in the animals with radio frequency lesions under massed trial conditions. Much clearer deficits were, however, present in those animals in which the lesion was found to extend appreciably into the anterior thalamic nuclei. This effect highlights both the important role that the anterior thalamic nuclei play in spatial memory and the danger of falsely attributing deficits to target brain structures without making adequate histological verification of the extent of lesions.

Some findings in this previous study (Hunt and Aggleton, 1991) were equivocal, reflecting the pattern of results in this area in general (Tables 1-4). It may therefore be useful to be re-explore some of these findings as a starting point, using different and extended experimental approaches. The limited scope of the previous study necessarily demanded a very small range of tasks that could be applied to the rats, and consequently the types of memory functioning that could be examined. The present series of experiments sought to extend this to investigate other ways in which MD may be exerting an influence on learning and memory, expressed within a similar framework of interpreting behavioural

deficits in rats with MD lesions. For example, where the previous study used a task that looked at the ability of rats with MD lesions to choose unfamiliar objects presented to them in a Y-maze (non-matching to sample), the present study used several tasks to ask how these rats recognise or make discriminations of both objects and places. Similarly, where the previous study used a single T-maze task to look at spatial working memory, this study will use a number of variants of both the T-maze and radial arm maze tasks to look for deficits of both working and reference memory in a spatial context.

There is considerable advantage in using lesion-making techniques that spare fibres of passage. This study will use the neurotoxic compound N-methyl-D aspartic acid (NMDA) that destroys cell bodies within rats' MD but spares fibre tracts such as the internal medullary lamina and the mammillo-thalamic tract. The resulting behavioural observations ought therefore to be a purer representation of the function of the nucleus, and may thus make a contribution to the small but growing number of studies that have questioned MD's mnemonic functions in this way. A disadvantage of using neurotoxins is that their perfusion through brain tissue can be unpredictable and their effect may spread to structures outside the target nucleus. The previous study (Hunt and Aggleton, 1991) found that the ibotenic acid used to make the lesions in MD had a propensity to extend its effect into the anterior thalamic nuclei, leaving the spatial memory deficits thus caused open to misinterpretation as effects of damage to MD itself. Only very careful examination and reporting of the histological preparations can avoid such misinterpretation of behavioural findings. It is clear from an examination of the literature that a number of the potentially relevant studies in this area have failed to do this adequately, thus depleting the already small stock of useful experimental findings in this area. The present study will be mindful of this possibility and will describe fully the extent of the lesions, making appropriate changes to the interpretation of behavioural analyses.

One inference that can be made from the previous study and from the pattern of results from other studies (Tables 1 - 4) is that brain damage, in this case circumscribed experimental lesions to MD, could effect behavioural changes that are not directly a function of mnemonic ability, but which may have an effect on the performance of tasks aimed at measuring learning and memory. Because rats' memory can be expressed and measured only in behavioural terms, the possibility that what is being measured is an aspect of behaviour and not memory has to be seriously considered. Changes in such factors as motor activity, attention, emotion, arousal, the perception of reward, and the tendency to perseverate, which may be quite subtle in their expression, could all be misinterpreted as pure memory deficit. This possibility could account for the range of seemingly inconsistent results to be found in the literature of previous studies. Although the use of a neurotoxin to make the experimental lesions goes some way to reducing this potentially confounding factor by minimising the disruption to the functioning of other structures in the brain, MD's own contribution to such indirect changes in the mediation of memory will be investigated.

1.8 - Methodology

The type of experimental strategy used in this study is well founded. It uses groups of laboratory animals as a model to test and specific, circumscribed hypotheses; the results of which can be used to address larger questions of more general interest. Essentially, the experimenter takes a group of genetically similar animals and causes, in some of those animals, controlled, circumscribed damage to a site in the brain that is of interest. The animals are otherwise treated in exactly the same way throughout their lives, with great care being taken to expose the control animals to the same environment and experience as the experimental animals, including the performance of sham surgeries on the control

animals analogous to the lesion-making techniques in the experimental groups. Thus, in each experiment in this study, the animals are divided into *sham* and a *lesion* groups, and both are exposed post-operatively to laboratory situations that address the relevant brain structure, and behavioural observations are recorded. The experimenter can thus assume that any behavioural differences between these two groups are dependent upon the lesion and, by implication, that the target structure is likely to be involved in that particular activity. It is, however, important to note that what is being studied is, in fact, a reflection of how the brain functions without the brain structure in question, and not directly the target structure's function.

In creating damage to a defined structure in the brain, it is necessary not only to restrict damage to that structure, but also to destroy as large a proportion of it as is possible. This is, in itself, difficult to achieve and the task is further complicated by the presence of fibres of passage that may be present in such structures which, if destroyed, may have effects in other, possibly unrelated, brain areas. The present study uses micro-injections of neurotoxic substances for lesion-making in order to minimise such inadvertent damage to fibres of passage. Careful examination of appropriate histological preparations of brain tissue is also carried out at the end of the experiments in order that accurate assessment of the extent of damage caused can be made, thus reducing the possibility of making false attributions of observed behavioural differences.

The study used groups of animals that were just large enough to give a statistically unambiguous answer. Since the strength of any effects found was unknown, the study aimed to use between eight and ten animals for each lesion group, and to have a similar size control group for each lesion group. An inbred strain of rats was chosen that is relatively small in size and may be assumed to have relatively good vision, not being albino or albino-derived. The rats used were thus suited to the type of behavioural tasks to

be applied. Since inbred strains, by definition, show only very tiny genetic differences between individuals, their use presents less opportunity for genetic variables to confound the behavioural test results. Further, variations in brain morphology between individuals are likely to be minimised, avoiding inaccuracies in targeting brain structures during stereotaxic surgical procedures.

1.9 – Summary of Aims

1.9.1 – To investigate whether deficits in spatial and non-spatial learning and memory are produced by MD lesions in rats.

1.9.2 – To look for behavioural changes in rats following MD lesions that may contribute to deficits in learning and memory, such as activity, exploration, and the association of places with food reward.

1.9.3 – To examine alternative interpretations of the effects of MD lesions on learning and memory in rats.

1.9.4 - To apply careful methods of investigation to differentiate effects directly attributable to MD from those that may result from damage to adjacent structures or fibres of passage and may previously have been reported as MD deficits.

CHAPTER TWO

Cohort 1 - tests of non-spatial reference memory and spatial working memory.

2.1 - Introduction

Although evidence from both clinical and animal studies has indicated that the thalamic nucleus medialis dorsalis has an important role in some aspects of learning and memory (Markowitsch, 1982, Victor et al, 1989, Aggleton and Mishkin, 1983a, Aggleton and Mishkin, 1983b, Zola-Morgan and Squire, 1985), its involvement remains poorly defined. This first series of experiments was intended to build directly on the findings of a previous study (Hunt and Aggleton, 1991), extending and refining the range of types of memory task with which rats with MD lesions were challenged. The previous work had examined the role of MD in rats performing a limited range of spatial and non-spatial working memory tasks. These next three experiments, using the Grice box, radial arm maze, and T-maze, aimed to apply a similar approach using tests of non-spatial and spatial working and reference memory.

Although the experiments in a previous study (Hunt and Aggleton, 1991) were designed primarily to examine working memory, the contribution of reference memory elements cannot be eliminated, since rule-learning was necessary to the performance of the tasks. For example, on the Y-maze delayed non-matching to sample task, designed to tax non-spatial working memory, the non-matching rule must first be acquired before delay conditions can be introduced. In fact, it was the examination of data from this acquisition stage of the experiment which revealed a learning impairment in the MD lesion group. The first in this new series of experiments therefore set out specifically to address the non-spatial rule-learning abilities of rats with MD lesions principally through a concurrent object discrimination task, but also tested discriminations serially, including a configural object discrimination.

A number of recent studies have used concurrent object discriminations to assess the effects of selective limbic lesions in rats (Aggleton, Kentridge, & Sembi, 1991; Rothblat, Vnek, Gleason, & Kromer, 1993; Wible, Shiber, & Olton, 1992). In such tasks the animals are required to learn a number of discriminations at the same time. This differs from "serial discriminations", the more standard design in which a new discrimination is not given until the previous one has been learnt. It has been reported that lesions of the fornix (Aggleton et al, 1991; Wible et al, 1992), the hippocampus (Wible et al, 1992), and the parahippocampal region (Rothblat et al, 1993) all impair the acquisition of concurrent object discriminations by rats. In contrast, lesions of the amygdala have no apparent effect on their own (Aggleton et al, 1991), nor do they enhance the effects of hippocampal system damage (Aggleton et al, 1991, Wible et al, 1992).

Interest in the performance of animals on such tasks stems from the fact that human amnesic subjects are impaired on learning concurrent visual discriminations. Deficits have been found among amnesic subjects irrespective of the type of discriminative stimuli (Aggleton, Nicol, Huston, & Fairbairn, 1988; Gaffan, Aggleton, Gaffan, & Shaw, 1990; Squire, Zola-Morgan, & Chen, 1988) or the type of feedback used to indicate correct responses (Gaffan et al, 1990; Squire et al, 1988). Furthermore, impairments have been found in amnesics with a variety of aetiologies and, hence, a variety of pathologies (Aggleton et al 1988; Aggleton, Shaw, & Gaffan, 1992; Kessler, Irle, & Markowitsch, 1986; Oscar-Berman & Zola-Morgan, 1980; Squire et al, 1988). It is therefore supposed that concurrent discrimination tasks provide a sensitive assay for both temporal lobe and diencephalic amnesia.

Studies using monkeys have indicated that mammillary body lesions have no effect on this task (Zola-Morgan, Squire, & Amaral, 1989), indicating a possible contribution from MD, and while monkeys with MD lesions can still learn single discriminations (Aggleton & Mishkin, 1983; Zola-Morgan & Squire, 1985), they are impaired on the acquisition of multiple discriminations presented serially (Gaffan & Murray, 1990). These lesions can also impair discriminations that require the subject to remember the amount of reward associated with a particular stimulus (Gaffan & Watkins, 1991).

Studies using rats have also indicated that damage to medialis dorsalis can disrupt a wide range of discrimination tasks (Staubli, Schottler, & Nejat-Bina, 1987; Tigner, 1974; Waring & Means, 1976, Weiss & Means, 1980) and can abolish conditioned place preference learning (McAlonan, Robbins, & Everitt, 1993). As a consequence it has been suggested that medialis dorsalis is involved in reward-related processes (McAlonan et al, 1993), such as the way in which rewards help the learning of performance rules (Gaffan & Murray, 1990; Gaffan & Watkins, 1991; Staubli et al, 1987). If this is the case it might be predicted that damage to MD would disrupt the learning of concurrent discrimination tasks when sufficient practice has led to the development of efficient learning strategies by normal subjects.

Experiment 1, besides examining such concurrent learning, included a condition in which the stimuli to be discriminated are presented once per session. The rationale for this comes from the seemingly paradoxical finding that combined removal of the amygdala, hippocampus, and rhinal cortex in monkeys has *no* effect on the learning of concurrent discriminations when each trial for a given pair of stimuli is separated by 24 hours (Malamut, Mishkin, & Saunders, 1984; Phillips, Malamut, Bechevalier, & Mishkin, 1988).

One of the standard tasks used to assess spatial working memory in rats is the radial arm maze (Olton and Samuelson, 1976). In some studies, medialis dorsalis lesions whether made by neurotoxic (Beracochea, Jaffard, and Jarrard, 1989) or electrolytic (Kolb, Pitman, Sutherland, and Whishaw, 1982; Olton and Samuelson, 1976) means have had no disruptive effect on acquisition or performance. One of these studies (Kolb et al, 1982) also included an explicit reference memory component in which a number of arms were never baited. The thalamic lesions did not affect the ability of the rats to learn to avoid these arms, and so spared both working and reference memory. In contrast, a series of studies by Stokes and Best (1988; 1990a,b,c) described deficits in both the working and reference memory components of the radial arm task in groups of rats with nucleus medialis dorsalis lesions. These deficits were found whether the lesions were made by electrolytic or neurotoxic methods. Using a modified radial arm maze with additional interlinking arms, evidence of a mild acquisition deficit was again found following neurotoxic lesions of the nucleus medialis dorsalis (Kessler and Markowitsch, 1982). The same study also found evidence of a mild deficit when a delay of one hour was interposed between trials.

In view of the disruptive effects of even small amounts of anterior thalamic damage, it is possible that many of the reported spatial memory deficits associated with medial dorsal thalamic damage are a consequence of anterior thalamic involvement (Hunt and Aggleton,1991), or of damage to the intralaminar nuclei (Burk and Mair, 1998). In the case of conventional lesions, this involvement may include damage to tracts that are linked with these nuclei, most notably the mammillothalamic tract (Thomas and Gash, 1985). With these possibilities in mind, the current study re-investigated the effects of neurotoxic lesions confined to the region of nucleus medialis dorsalis on tests of spatial working memory. The tests included variants of the radial arm maze task and T-maze alternation.

The radial arm maze tests included conditions designed to determine whether any of the subjects had acquired an alternative strategy (i.e. one that did not make demands on working memory). For this reason, Experiment 2 examined whether rats had learnt to solve the task by constantly turning in one direction or by using odour trails to avoid repeat visits to the same arm (Buresova and Bures, 1981).

Experiment 3, the final test given to this cohort of animals, was a simple spatial learning task in a T-maze, forced-choice alternation. A previous study (Hunt and Aggleton, 1991) comparing the effects of radio-frequency and neurotoxic (ibotenic acid) MD lesions on this task, had found no consistent evidence that MD damage alters either acquisition or subsequent performance of the task. The inclusion of this task helps to confirm or further test the lack of acquisition deficit found in that study (Hunt and Aggleton, 1991) using massed trials, and acts as a further assay for the effects of damage to the anterior thalamic nuclei.

2.2 - General Methods

The following description of methods applies to all experiments performed throughout the study.

2.2.1 - Subjects

The subjects were all naive male rats of the pigmented Dark Agouti (DA) strain (B&K Universal Ltd, Hull), approximately 12 weeks old and 210-250g in weight at the start of the experiments. They were housed in individual cages in a holding room with a photo

period of 14:10 hours light:dark, and each was randomly assigned to one of two surgical groups: MD (lesions of the mediodorsal nucleus of the thalamus) or SHAM (surgical controls). Following recovery from surgery and throughout the subsequent testing period they were maintained on approximately 15g of laboratory diet (RM1(E) - Special Diets Services, Witham, Essex) per day, and their body weights were monitored so that they remained at no less than 85% of normal.

2.2.2 - Surgical Procedure

Each animal was deeply anaesthetised by intraperitoneal injections of pentobarbitone sodium ("Sagatal", Rhone Merieux, Harlow) at a dose rate of 6mg/100g. The animal was then placed in a stereotaxic headholder (David Kopf Instruments, Tujunga, U.S.A.), the scalp retracted, and a small craniotomy made to expose the dura above the target region. In the MD lesion groups a single injection of 0.36µl of a 0.2 molar solution of *N*-methyl-D-aspartic acid (Sigma Chemical Co. Ltd., Poole), dissolved in phosphate buffer (pH 7.2), was made through a 1µl micro-syringe (Hamilton Instruments, Bonaduz, Switzerland) in each hemisphere. Each injection was made over a period of five minutes and the needle was left in position for a further five minutes before being retracted. The injection co-ordinates relative to ear bar zero with the incisor bar set at +5.0 were: AP = 3.7, Ht = 4.6, LAT = $\pm$ 0.7. Following removal of the needle from the second hemisphere the skin was sutured and wound powder (Acramide, Dales Pharmaceuticals, Skipton) applied to the area. The SHAM groups received identical treatment with the exception that the micro-syringe needle was introduced into the brain to a position just above the target nucleus, and no injection was made.

A heated pad was kept under the rats at all times during surgery to maintain near-normal body temperature, and a 6ml subcutaneous injection of isotonic saline (Animalcare Ltd, York) was made at the beginning of each surgery to prevent dehydration. The eyes were protected from both dehydration and excessive light by the application of ophthalmic ointment (Chloromycetin, Parke-Davis, Pontypool) after mounting the rats in the stereotaxic frame. Immediately following surgery a further saline injection was made along with etamphylline (Millophyline, Arnold's, Romford; 35mg/kg, s.c.), a respiratory stimulant.

2.2.3 - Histological Procedure

At the end of the study each rat was perfused intracardially with 0.9% saline followed by 5% formol saline. The brains were subsequently blocked, embedded in wax (Paraplast), and cut in 10 micron coronal sections. Every tenth section was mounted and stained with a Nissl stain (Cresyl violet).

2.2.4 - Statistical Analysis

Where appropriate, parametric tests were used to compare the groups' scores. Evidence of heterogeneity of variance was found in some instances and modifications of analyses were carried out accordingly. Student's *t*-tests were modified where appropriate by Levene's test (SPSS, Chicago, IL). Logarithmic transformations were carried out where indicated prior to analysis of variance. All *t*-tests were one-tailed unless otherwise stated. Analyses of simple effects following analysis of variance were based on just the level of the within-subject variable at which the effect was being tested (Keppel, 1973). The error bars shown

on figures represent standard error of the means. For those behavioural tests sensitive to anterior thalamic damage, a second series of analyses was conducted. These analyses excluded those MD rats with bilateral damage in any anterior thalamic nucleus.

2.3 - EXPERIMENT 1 - Object Discrimination.

2.3.1 - Method

2.3.1.1 – Subjects - The subjects were 17 rats as described in General Methods. Prior to surgery each rat was randomly allocated to one of two surgical groups. Nine rats were assigned to the group which was to receive neurotoxic lesions to the nucleus medialis dorsalis (MD1) and the remaining 8 formed the surgical control group (SHAM1).

2.3.1.2 - Surgical, Histological, and Statistical Procedures - Procedures were as described in General Methods.

2.3.1.3 - Test Apparatus - All tasks were carried out in a Grice box, composed of aluminium walls and door and a laminate plastic floor (Figure3). The small, rectangular start box (13x18cm) was separated from the funnel-shaped test area by a guillotine door. The far wall of the test area was 43cm wide and 43cm from the guillotine door. Two side-walls of equal length (46cm) joined the ends of this far wall to the entrance of the start box. All walls of the apparatus and the guillotine door were 24cm high. The floor contained two food circular food wells, 2.5cm in diameter by 0.5cm deep, each located 35cm from the start box. An aluminium partition protruded 16cm from the middle of the far wall and ensured that the rats could not run directly between the two food wells.

The floor of the food wells was made of perforated zinc sheet, and directly underneath each was a tray containing the same type of reward pellets used in testing. This

arrangement, which was to prevent the animals from using olfactory cues in performing the discriminations, meant that the rats could not see the hidden pellets but could presumably smell them. Thus, in the event that the presence of food could be detected even when covered by an object, both the positive and negative food wells would smell similar.

Illumination was by fluorescent room light suspended 132cm above the apparatus giving a luminance level of 100 lux at the position of the food wells.

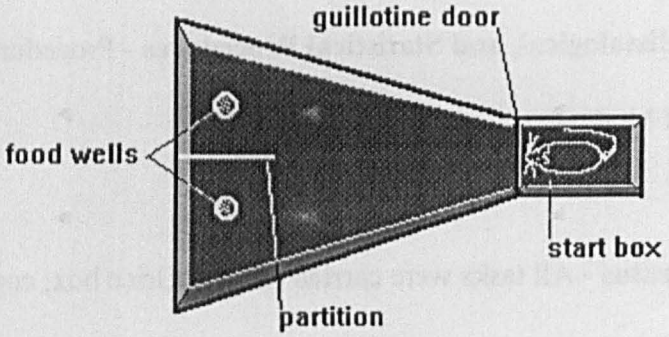

Figure 3 - The Grice box used in all object discrimination tasks in Experiment 1

The objects used for the various discriminations differed in their size, shape, and colour (usually patterns of black and white). Multiple copies were made of all stimuli and they were sealed with coats of clear varnish to help eliminate olfactory cues. All of the objects had sufficiently large bases to cover the food wells completely.

Object discrimination 1 - This used two differently shaped wooden objects (Figure 4), one painted black, the other white (maximum height 41mm).

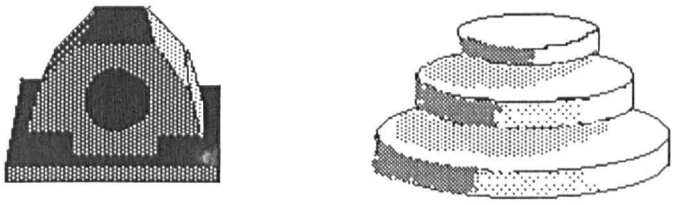

Figure 4 - The black and white discrimination stimuli used in Object Discrimination 1.

Concurrent discrimination - The 10 pairs of stimuli used for the concurrent discriminations were made either of wood or metal (S+ and S- pairs were always of the same material). All were covered with paint or clear varnish, and care was taken to make the individual stimuli as distinctive as possible both from one another and from those used in other conditions. The tallest of the stimuli was 92mm high and the shortest 11mm.

Object discrimination 2 - This used two different wooden objects painted in distinctive patterns, similar in construction to those used in the concurrent discriminations.

Configural discrimination - Each stimulus consisted of a 5cm square base on which were set four equal lengths of varnished wooden dowel rods (40mm long, 13mm diameter). Some of these rods were glued lengthways on the base, others upright, and one piece was glued across the top end of the vertical rod(s). The two stimuli differed in the number and position of the vertical and horizontal rods (Figure 5).

2.3.1.4 - Testing Procedure - Pre-training began a minimum of 14 days after surgery. During pre-training the animals were trained to run from the start box to find food pellets

(45mg, Campden Instruments Ltd., Loughborough) in either food well by pushing aside a flat wooden disc.

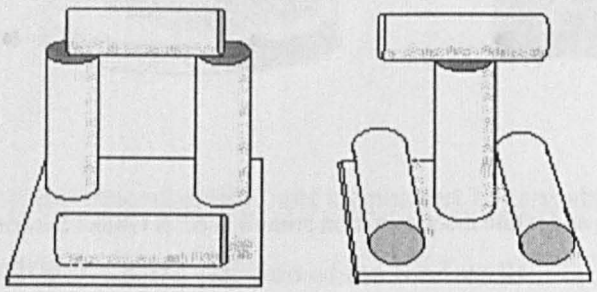

Figure 5 - Discrimination objects, which differed in the number and position of the vertical and horizontal rods, used in c*onfigural discrimination.*

For all of the following discrimination tasks the animals received 3 food pellets following a correct choice. A choice was defined as moving a stimulus object with front paws or snout. After making a choice the animal was picked up and returned to the start box. Correction trials were not run. The left-right positions of the stimuli varied according to a random schedule. The animals received one session per day, 5 days per week. Care was taken when baiting the food wells not to provide the animals with additional cues. Throughout testing the experimenter was unaware to which group the rats belonged.

Object discrimination 1. - Each animal received 20 trials per session. Half of the animals in each group were allocated the black object as the positive stimulus (S+), while the S+ for the other half was the white object. Training continued until the rat reached a criterion of at least 38 correct trials over two consecutive days. After an interval of 4 days they were tested on the concurrent task.

Concurrent discrimination. - The procedure was the same as in the previous task except that each session now consisted of 26 trials in which three different pairs of discriminations were interspersed. Two object pairs (A^1 vs A^2, B^1 vs B^2) were tested for just one trial every session (Single Trial condition). A further two object pairs (C^1 vs. C^2, D^1 vs. D^2) were tested for four trials on each session (Four Trial condition). Finally, two more pairs of objects (E^1 vs. E^2, F^1 vs. F^2) were used for 8 trials on every session (Eight Trial condition). All animals received 32 sessions. The discriminative stimuli for the Four and Eight Trials conditions were replaced by new pairs of stimuli for sessions 17-32, i.e. the second half of the total sessions. Whilst the order of the stimulus pairs was kept constant across all sessions, the various conditions were interspersed and the left-right positions of S+ randomised in order to prevent the development of side preferences. It was arranged that the choices made by the rats in Session 1 determined the actual S+ and S- stimuli. Thus, for each of the three conditions one of the S+ stimuli corresponded to that object selected by the rat on its very first exposure (discriminations A, C, and E, i.e. on trial 1 bait both objects), while the other S+ was the object not selected (discriminations B, D, and F, i.e. on trial 1 bait neither object). Four days after completion of the concurrent test the rats were trained on two discriminations presented serially.

Object discrimination 2 and configural discrimination. - For both discriminations the rats received 20 trials per session, and were tested to a criterion of 19 correct trials in one session. For half of the animals in each group the S+ corresponded to the object selected on Trial 1 (both baited). For the other half the object selected on Trial 1 became S- (neither baited). Immediately after completing object discrimination 2, which used two distinctive painted wooden objects, the rats were tested on the configural discrimination, which was run in the same way.

2.3.2 - Results

2.3.2.1 - Histological Analysis

Damage within nucleus medialis dorsalis - All animals in the MD1 group had extensive bilateral lesions within nucleus medialis dorsalis. The largest and the smallest of the MD1 lesions are depicted in Figure 7. In most cases the lesion affected at least 80% of the nucleus; the only sparing occurring at the most lateral and ventral limits of the nucleus. The region composing nucleus medialis dorsalis was always shrunken and within the extent of the lesion there was almost a complete loss of neurons, but no evident gliosis (Figure 6). In two cases there were small infarcts confined to nucleus medialis dorsalis, while a more extensive infarct, also confined to the nucleus, was found in another case.

Damage to other structures - most cases showed a loss of cells in the medial portion of nucleus lateralis dorsalis (Figure 7). One case showed unilateral damage to the intralaminar nuclei. In three cases there was also extensive bilateral damage to the anterior dorsal nucleus, resulting in total or near-total loss of the nucleus. The other anterior thalamic nuclei were almost completely spared in all cases (although in three cases the lesion extended rostrally to encroach into the most caudal margins of the anteroventral thalamic nuclei). In all MD1 cases there was damage to the mid-line nucleus paraventricularis, and to that part of nucleus parataenialis at the rostral level of medialis dorsalis. In most cases there was a restricted zone of damage where the tract passed through the dentate gyrus. The habenula did not appear to suffer neurotoxin damage. The needle tract could be seen entering the hippocampus, dorsal to medialis dorsalis in all SHAM1 cases.

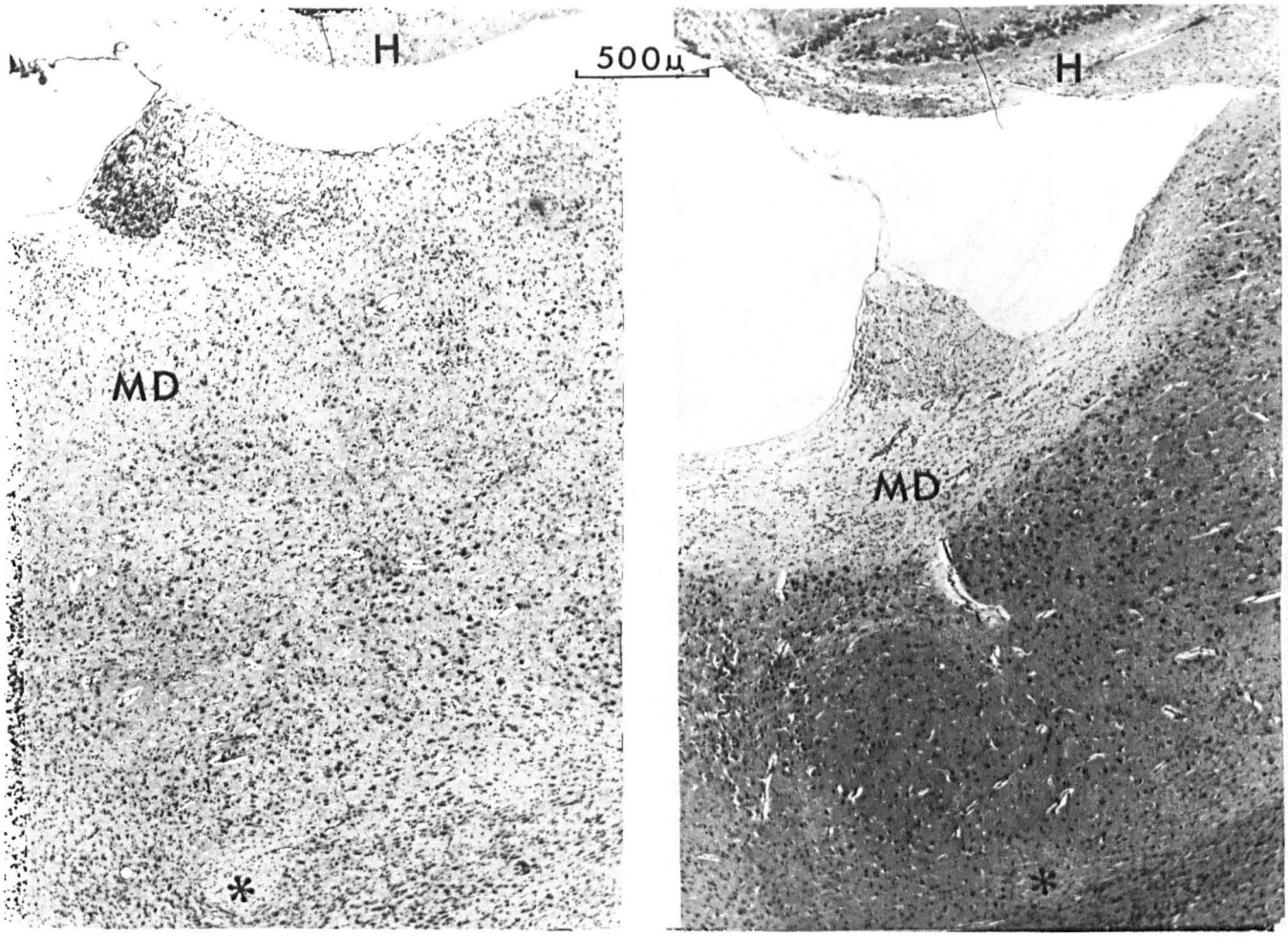

Figure 6 - Photomicrograph of coronal sections (Nissl stain) showing the appearance of nucleus medialis dorsalis in a normal animal (left) and in the MD1 animal with the median sized lesion (right). The photomicrograph shows not only the loss of neurons within nucleus medialis dorsalis, but also the resultant contraction of the region. For purposes of comparison the mammillothalamic tract is marked with an asterisk. H = hippocampus; MD = nucleus medialis dorsalis.

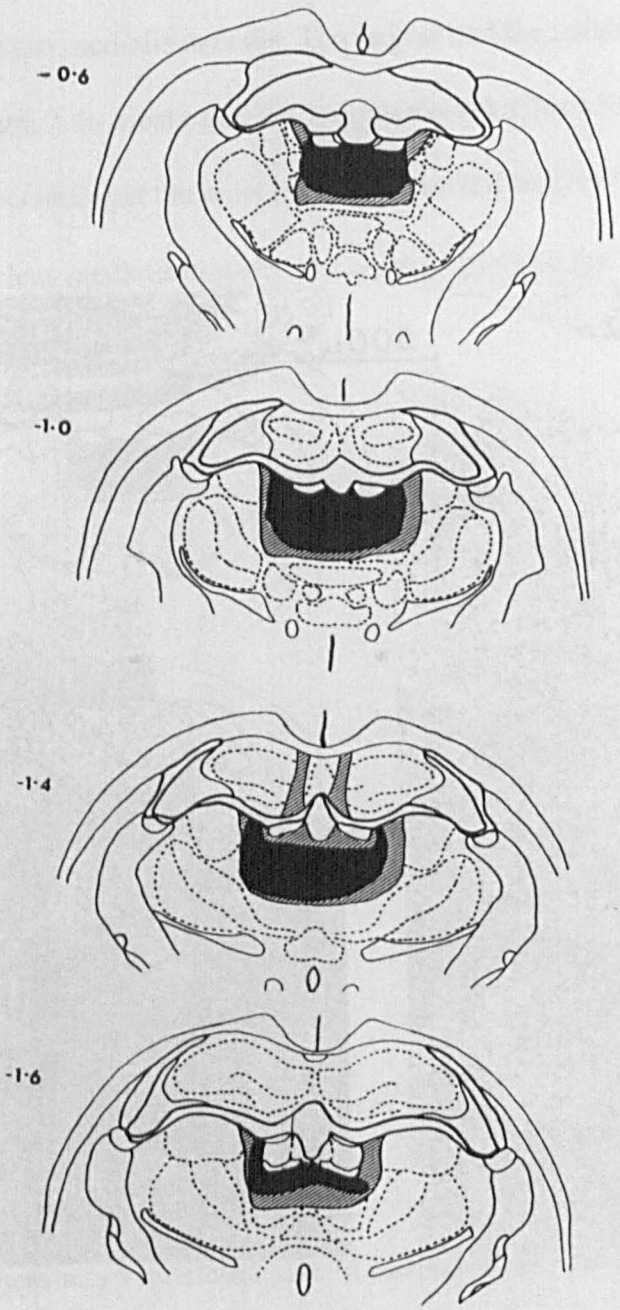

Figure 7 - Diagrammatic reconstruction of the lesions of nucleus medialis dorsalis. The coronal sections depict the smallest (black) and largest (diagonal lines) extent of cellular loss. The numbers refer to the approximate corresponding AP levels from the stereotaxic atlas of Pellegrino and Cushman (1967).

2.3.2.2 - Behavioural Analysis

2.3.2.2.1 - Single Discriminations

Object Discrimination 1 - The acquisition performance of the two groups (Figure 8) was assessed by comparing the total number of errors in reaching the learning criterion. Levine's test for equality of variance showed that the data variance of the two groups was unequal, and a t-test which was therefore adjusted accordingly showed no significant difference between the groups ($t = 0.38$, $df = 13.58$, $p = 0.35$).

Object Discrimination 2 - Comparisons of the performance of the two groups on this task produced no evidence of a lesion effect (Figure 8). A t-test on the number of errors to criterion confirmed this ($t = 0.03$, $df = 15$, $p = 0.49$).

Configural Discrimination - Evidence was found on this final discrimination that the two groups differed in their ability to learn the task (Figure 8). A t-test on the number of errors made in reaching the set learning criterion revealed that the MD1 group was significantly impaired ($t = 2.46$, $df = 9.55$, $p = 0.02$). Again the t-test has been adjusted to take account of the unequal nature of the variances.

2.3.2.2.2 - Concurrent Discriminations

Performance scores on this task were taken from the number of correct trials in each of the three conditions over successive blocks of 4 sessions. In the case of the Four and Eight Trial conditions the animals received a new set of stimuli on session 17 (the beginning of

block 5). As a consequence, sessions 17-32 (blocks 5-8) were primarily considered as completely new sets of discrimination data. Comparisons were made using an analysis of variance (using SPSSx), with the factors *group* and *block.*

One Trial condition: both groups showed evidence of learning the two discriminations (Figure 9), even though they received just one trial per day on each of them. This is reflected in the highly significant block effect [$F(7,105) = 6.04$, $p<0.001$]. There was, however, no group effect [$F(1,15) = 1.65$, $p = 0.22$], nor was there a group x block interaction [$F(7,105) = 1.46$, $p = 0.19$].

Four Trial condition: comparisons of the two groups on the first part of this condition (sessions 1-16) and on the second part (sessions 17-32) showed no evidence of any difference in performance. Again, there was a highly significant block effect as the animals improved with practice on each set of discrimination data [blocks 1-4, $F(3,45) = 45.45$, $p<0.001$; blocks 5-8, $F(3,45) = 15.81$, $p<0.001$]. Both groups' performance fell on Block 5 (Figure 10) when the now-familiar discrimination stimuli were replaced by a new set.

Eight Trial condition: The animals' performance was similar in pattern to that seen in the four trial condition, with a highly significant block effect on both sets of discrimination data [blocks 1-4, $F(3,45) = 102.7$, $p<0.001$; blocks 5-8, $F(3,45) = 44.65$, $p<0.001$]. Again, there was a marked falling of performance on block 5 after the stimuli had been replaced (Figure 9). There was, however, a significant difference between the groups on the first set (sessions 1-16), reflecting an initially poor performance of the task by the MD1 group [$F(1,15) = 5.14$, $p = 0.04$]. The MD1 group did subsequently make up this deficit over subsequent blocks, showing a steeper learning curve than the SHAM1 group (Figure 11),

and from blocks 3 to 8 the performance of the two groups appears virtually indistinguishable.

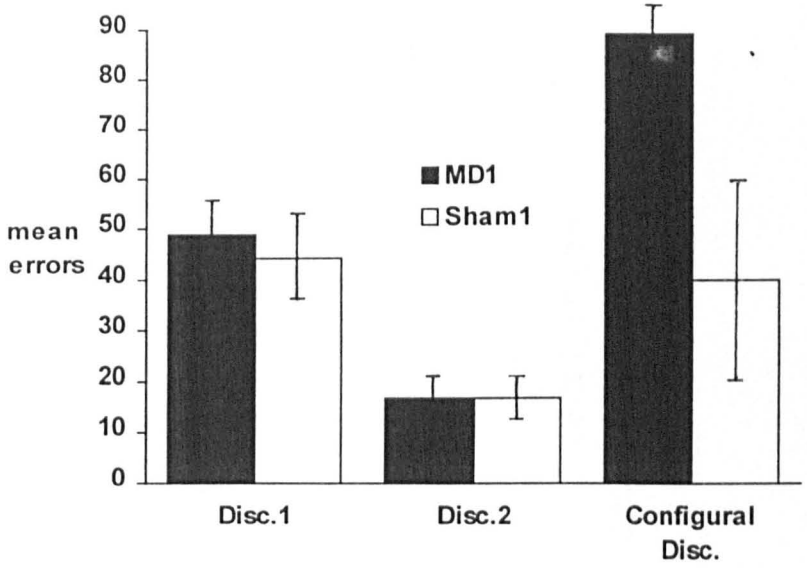

Figure 8 - Mean errors to criterion of the MD1 and Sham1 groups on Discrimination 1 (black vs white), Discrimination 2, and Configural Discrimination. Discriminations 1 and 2 were separated by the concurrent task. Vertical lines indicate standard error of the mean (and in all subsequent figures in Chapter 2).

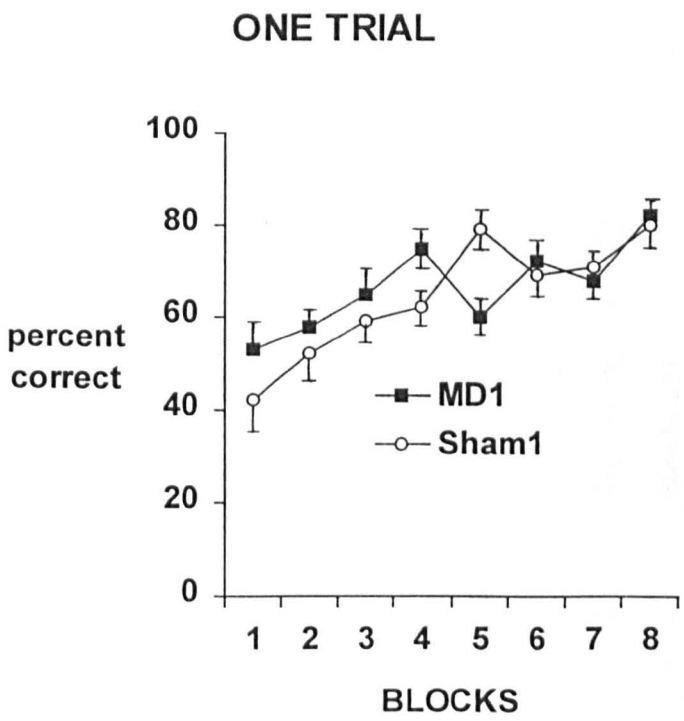

Figure 9 - Concurrent discrimination: One trial condition. Each block corresponds to four sessions.

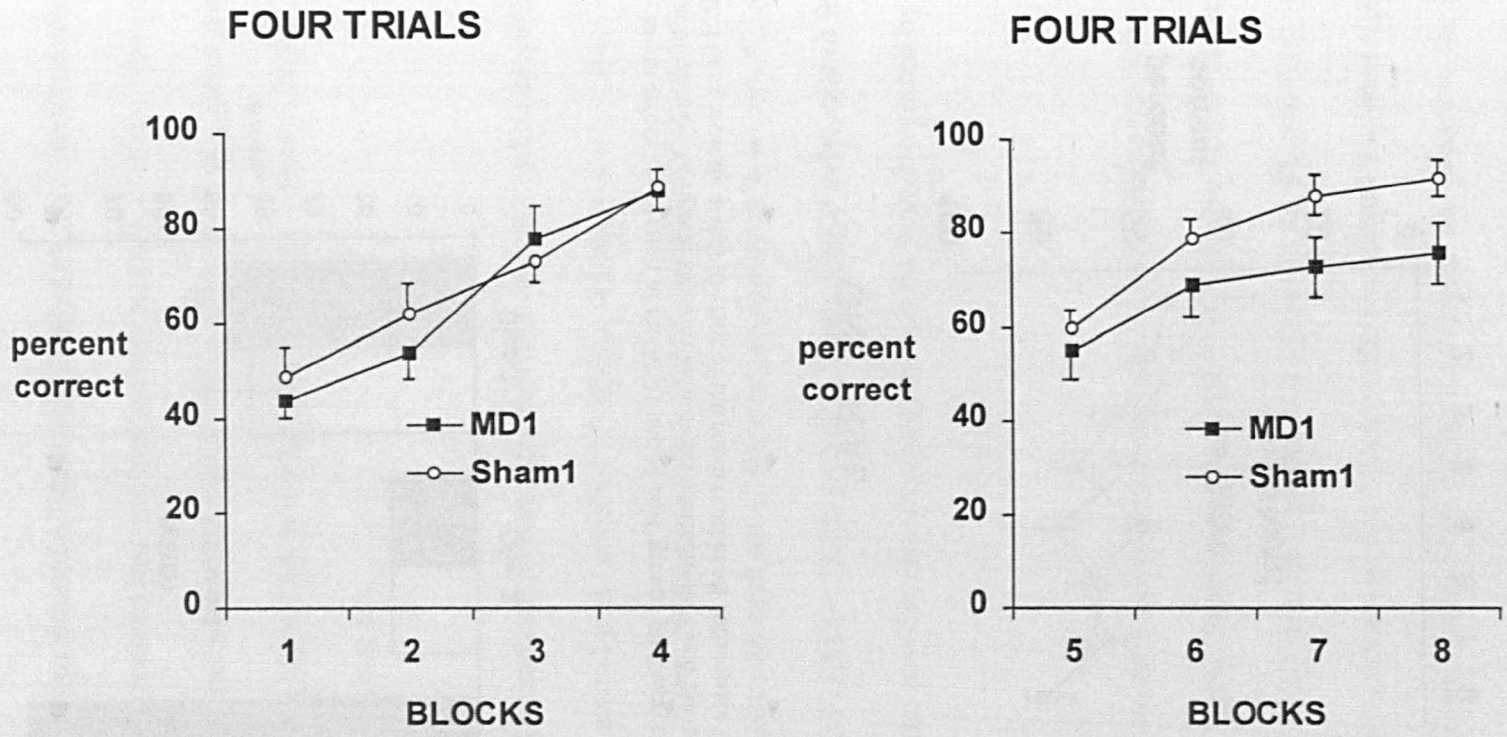

Figure 10 - Concurrent discrimination: Four Trial condition. Each block corresponds to four sessions (32 trials). New pairs of stimuli were presented after completion of Block 4.

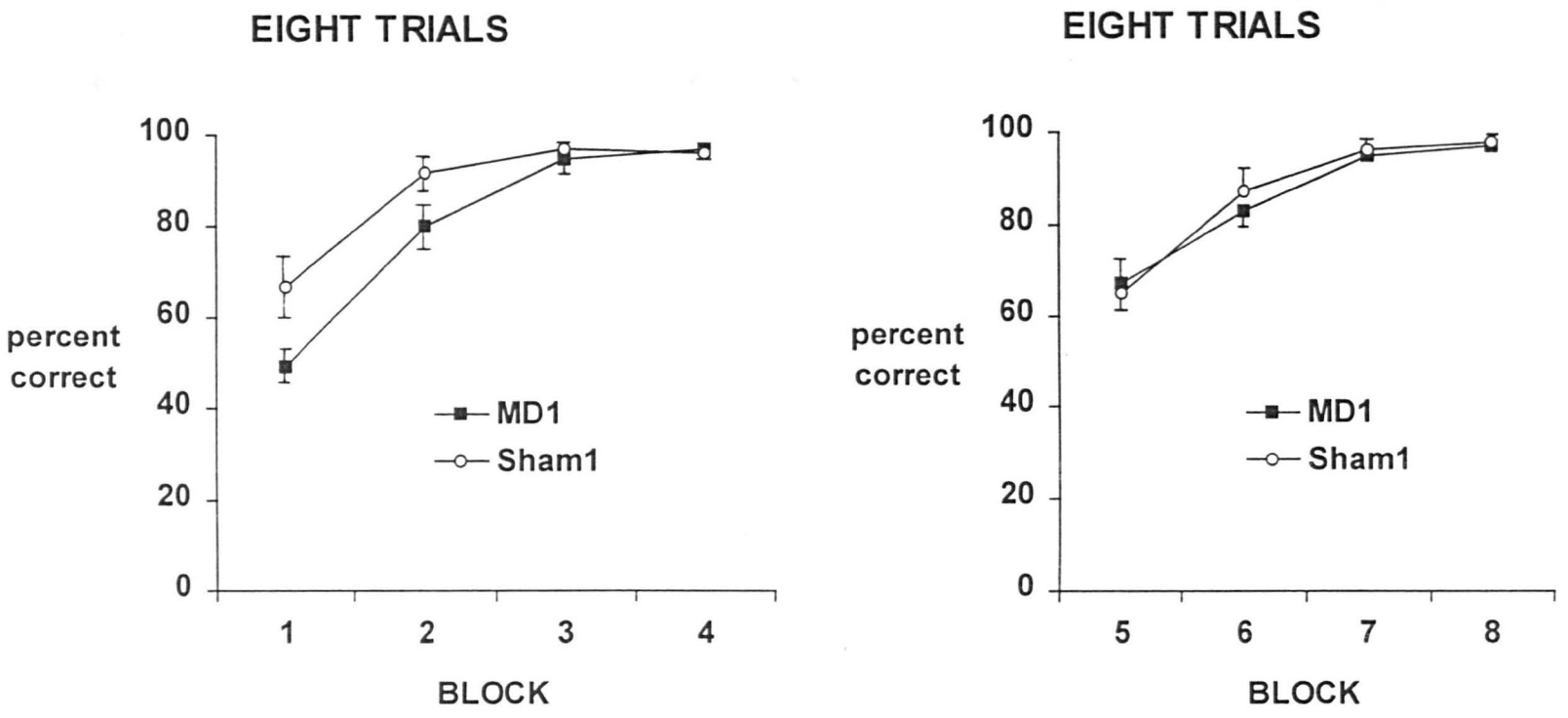

Figure 11 - Concurrent discrimination: Eight trial condition. Each block corresponds to four sessions. New pairs of stimuli were presented after completion of Block 4.

2.4 - EXPERIMENT 2 - Radial Arm Maze

2.4.1 - Method

2.4.1.1 – Subjects - The subjects were the same animals as in Experiment 1 with the exception of one animal from the MD1 group which was excluded because of illness. The groups were therefore: MD1 n = 8, and SHAM1 n = 8.

2.4.1.2 - Test Apparatus - The apparatus was a radial arm maze comprising a central octagonal atrium with eight arms radiating from it. The atrium was 34cm in diameter and constructed of a varnished plywood floor with transparent acrylic sheet walls 24cm in height. The eight arms were each 86cm in length and 10cm in width and, like the atrium, were constructed of a plywood floor and clear acrylic walls. A food well in which reward pellets could be placed, 2cm in diameter and 0.5cm deep, was located 2cm from the end of each arm. A clear acrylic guillotine door was located at the junction of each arm to the atrium, and these could be raised and lowered either together or independently by a system of overhead cords. The whole maze sat on a circular plywood turntable which could be rotated through 360 degrees by the experimenter. Each arm was identified to the experimenter by a printed number, and positions were marked on the floor beneath the maze so that its position on rotation could be replicated exactly. The testing was carried out in a room in which there were salient visual cues such as a door, a sink, a table and chair, and several wall posters, and lighting was provided by three fluorescent lights 140cm above the apparatus.

2.4.1.4 - Testing Procedure - Pre-testing exposure to the maze was begun one week after all the animals had finished Experiment 1, and about 12 weeks after surgery. These

pre-training habituation sessions continued daily for five days and involved free access to the apparatus which had reward pellets (Campden Instruments, Loughborough) placed in and around the food wells. Formal training then followed, each rat receiving one session per day.

Each session began with the baiting of all maze arms with three reward pellets placed in each food well. The rat was placed in the central area and all the doors were raised, allowing the rat access to all rms. The rat was deemed to have entered an arm when all four paws were in the arm, the doors were then lowered and the rat allowed to eat the reward pellets. The door to that arm only was then raised, allowing the rat back into the hub, and immediately closed again when the rat had passed through. This procedure was repeated until all eight arms had been visited. In this way the inter-trial delay was minimal and determined by the animal. The session was aborted if the rat had not completed the task after 10 minutes, or if it made no response at all for 2 minutes, and the data for that session was discounted. Criterion was deemed to have been reached when all eight arms had been visited in 10 or fewer attempts on each of 5 consecutive days. Throughout all testing in this experiment the experimenter was unaware of the group designation of each rat.

On the session following acquisition, each rat was further tested by increasing the retention delay and by assessing the effect of turning the maze mid-session. On "turned" days (session 2 and each alternate session subsequently), the rat was initially run in the maze using the same method as in the acquisition phase, but after the rat had selected four different arms, it was taken out of the central area and placed in its own cage for 60 seconds. During this time the maze was rotated on its turntable 45 degrees clockwise. As a consequence, each arm was now in the former position of its adjacent arm, and the

remaining reward pellets in the food wells were moved accordingly anticlockwise. Arm number 1 thus occupied the position formerly occupied by arm number 2 and so on. This meant that all of the distal spatial cues remained unaltered, but local intra-maze cues had changed position. At the end of the 60 second delay the rat was replaced in the centre of the maze and allowed to continue until it had visited all eight arms.

On the interleaved, alternate sessions ("control delay"), testing procedure was exactly the same as the "turned delay" condition, except that a "sham" maze-turn was carried out in which the experimenter turned the maze 45 degrees clockwise, and immediately returned to its original position. This was done in order to avoid any association being made between the procedure of turning the maze and the changed conditions on "turned" sessions. To this same end, the experimenter also made the motions of apparently moving the reward pellets, but in fact leaving them in place. The rat was replaced in the centre of the maze at the end of the 60 seconds delay and continued the task until all eight arms had been visited. Each animal was given a total of five "turned delay" and five "control delay" sessions.

2.4.2 - Results

2.4.2.1 - Behavioural analysis - The behavioural analyses initially considered all of the MD1 animals with substantial bilateral cell loss in nucleus medialis dorsalis. Subsequent analyses examined whether encroachment into the anterior thalamic nuclei had contributed to any of the lesion effects. This set of analyses was in response to the debate over whether some of the effects ascribed to medialis dorsalis lesions might be a result of damage to adjacent nuclei.

The initial analyses compared the total number of errors required to complete the acquisition criterion (Figure 12). This score did not include errors made on sessions that were void, i.e. when the choice behaviour of the rat was too slow. Although the MD1 group made more errors than the SHAM1 group in reaching the acquisition criterion (mean MD1 = 11.13; mean SHAM1 = 6.5), this difference narrowly failed to reach the significance level [t (14) = 1.54, p = 0.073]. Similarly, the MD1 group required more test sessions (including void sessions) to complete the acquisition criterion (mean sessions, MD1 = 8.13; SHAM1 = 6.13) (Figure 12). This group difference was significant [t(14) = 2.44, p = 0.015].

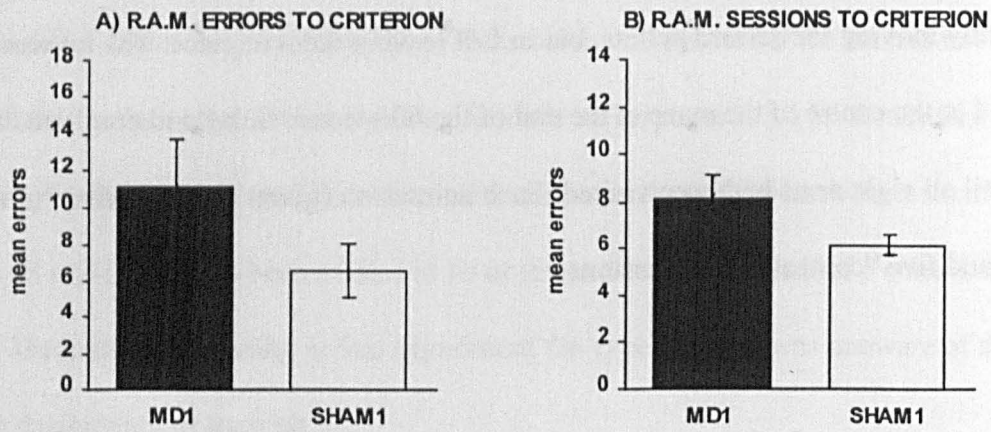

Figure 12 - Acquisition of the Radial Arm Maze task depicted by A) mean errors to criterion and B) mean sessions to criterion.

Inspection of the arm selection by individual animals failed to indicate whether the MD1 rats had learnt to adopt an egocentric strategy (i.e. choose an immediately adjacent arm and always turn in one direction). This was formally examined by calculating the number of sequential choice responses. To measure this, the first choice of an animal was ignored, but the second to eighth choice each animal made was given a score of +1

(clockwise) or -1 (anticlockwise) if the arm selected was immediately adjacent to the arm that the animal had come from. Any other choice was given a score of 0. In this way an absolute score of 7 would reflect a perfect sequential strategy while a score near zero would reflect a failure to use this strategy. Inspection of the absolute scores of the two groups for the last five acquisition sessions failed to provide evidence for the use of such a strategy (mean scores: MD1 = 1.75, s.e.m. = 0.92; SHAM1 = 0.25, s.e.m. = 0.75), nor was there evidence of a group difference [t (14) = 1.26, p = 0.23, two-tailed].

Following acquisition, the effects of increasing the retention delay and of turning the maze were assessed. These analyses just used the errors made after the delay (i.e. after the first four correct choices had been made) and, in fact, none of the rats made an error during the first four choices of either condition. An analysis of variance was then used to compare performance over the five sessions with the 'turned delay' condition, the five sessions of 'control delay', and the last five acquisition sessions (Figure 13). This analysis showed a significant effect of condition [$F(2,28) = 28.47$, $p < 0.001$], a near-effect of group [$F(1,14) = 4.10$, $p = 0.06$], and a highly significant group x condition interaction [$F(2,14) = 5.91$, $p = 0.007$]. Analysis of the simple effects showed that there were no differences between the groups [$F < 1$] on the acquisition and control delay conditions, but that a highly significant difference emerged at the turned delay condition [$F(1,14) = 10.9$, $p = 0.005$]. This reflected the poorer performance of the lesion group at this one condition (Figure 13).

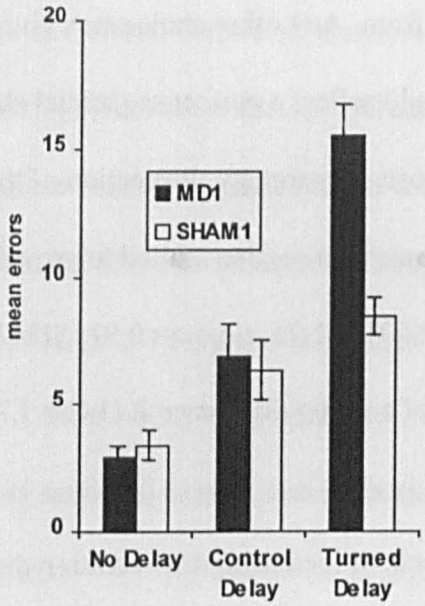

Figure 13 - Radial arm maze: performance over delay conditions. In 'turned delay' sessions the maze was rotated by 45° during the 60s delay period. 'Control delay' sessions included the 60s delay but the maze remained in the same position throughout the session. In both conditions the delay was imposed after four arms had been entered.

The possibility was also examined that the MD1 group were using scent trails as cues as well as the extra-maze cues, and hence were liable to make more errors when the maze was turned. Thus on the first trial after the maze was turned it was noted whether the actual arm chosen by the rat had already been visited on that session (because of the arm rotation). It was predicted that rats more reliant on odour cues would show a higher preference for arms that had not been entered that session. A comparison of the number of odour "free" choices over the five sessions indicated that the MD1 group was indeed more likely to select such arms than the SHAM1 animals [Mann-Whitney U (8,8) = 15, p=0.042].

Finally, attention was given to the possible contribution of any damage to the anterior thalamic nuclei. Three of the MD1 cases displayed marked or complete bilateral cell loss in the anterior dorsal nucleus although the other anterior nuclei were spared. Two of

these three cases made the greatest number of errors to criterion, and when these three cases were removed from the analysis the borderline acquisition deficit completely disappeared [revised mean errors to acquisition MD1 = 7.2, s.e.m. = 0.1.39, t(11)= 0.35]. A similar result was found for sessions to criterion [mean sessions = 7.4, s.e.m. = 0.93, t(11)=1.25].

Similar comparisons on the 'turned delay', 'control delay', and 'no delay' conditions in which the same three MD1 cases were removed (Figure 14) showed that there was still a significant effect of condition [$F(2,22) = 17.21$, $p<0.001$] but there was now no evidence of a group effect [$F(1,11) = 1.47$, $p = 0.25$] or of a group x condition interaction [$F(2,22) = 2.42$, $p = 0.11$]. In spite of the lack of main effects, analysis of the simple effects showed that that this subset of MD1 animals still performed poorly on the 'turned delay' condition [$F(1, 11) = 4.75$, $p = 0.052$].

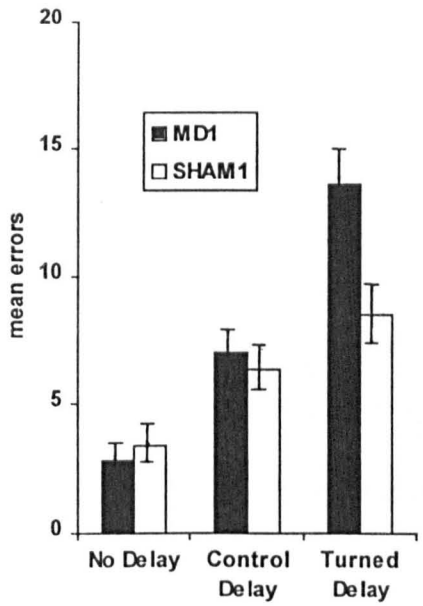

Figure 14 - Radial arm maze: performance over delay conditions and excluding those subjects in the MD1 group with substantial anterior thalamic lesions.

2.5 - EXPERIMENT 3 - T-Maze Non-Matching

2.5.1 - Method

2.5.1.1 – Subjects - The subjects were the same as in Experiment 2 (MD1 n = 8, SHAM1 n = 8).

2.5.1.2 - Test Apparatus - The T-maze used in this task was identical to the one used in a previous study (Hunt and Aggleton, 1991). It had an aluminium floor 10cm wide and clear acrylic sheet walls 17cm high. The stem of the maze was 80cm long with an aluminium guillotine door 33cm from the beginning. The cross arm was 136 cm long with a food well 4cm in diameter and 0.75 cm deep located in the floor 2cm from each end. The maze was supported on stands 93cm high and was illuminated by fluorescent room-lights suspended 92cm above the apparatus. At the choice point and food wells the luminance levels were 320 and 280 lux respectively. Testing was carried out in a different room from either Experiments 1 or 2, but, as in the room used for Experiment 2, it contained salient visual cues.

2.5.1.3 - Testing Procedure - Pre-test exposure to the maze began two weeks after all the animals had completed the R.A.M. tasks. Only one session of pre-training was required since the T-maze apparatus was similar in appearance and behavioural requirements to the R.A.M.. Each trial in this experiment consisted of two stages; an "information" run and a "test" run. At the beginning of each trial the experimenter placed three food reward pellets in each food well and closed off one arm of the maze with a wooden block adjacent to the choice point. The rat was then placed at the start

point and the guillotine door was raised, allowing the rat to run through towards the choice point. On this "information" run the rat was forced by the presence of the wooden block to enter the opposite arm, where it was allowed to eat all the pellets in the food well at the end of that arm before being picked up and returned to the start box. No retracing back into the stem of the maze was permitted once the rat had entered the arm. Once back in the start box the experimenter removed the wooden block and raised the guillotine door to allow the animal to run up the stem towards the choice point for a second time (the "test" run). The delay between the end of the "information" run and the beginning of the "test" run was approximately 10 seconds. This time the animal was faced with two open arms at the choice point, and was deemed to have made a choice when all four of its paws were in one arm. At this point the wooden block was placed behind it to prevent retracing into the stem or entry into the unchosen arm. If a correct choice was made, i.e. the arm entered was the opposite one to the one entered on the information run, the animal was allowed to eat the reward pellets before being returned to the start box for trial 2. If an incorrect choice was made the rat was confined to the arm without food reward for 10 seconds before likewise being returned to the start box. One daily session consisted of 6 of such trials, and 5 sessions were performed by each rat (total 30 trials). The trials were "massed", that is the intertrial interval was restricted to 10 seconds as a previous study had indicated that that this condition could possibly be sensitive to dorsomedial thalamic damage (Hunt and Aggleton, 1991).

2.5.2 -Results

2.5.2.1 - Behavioural Analysis - Both groups learned to perform this task at a high level almost immediately (Figure 15), and no difference emerged between the two groups (correct score means: MD1 = 24.8, SHAM1 = 24.5, maximum score = 30).

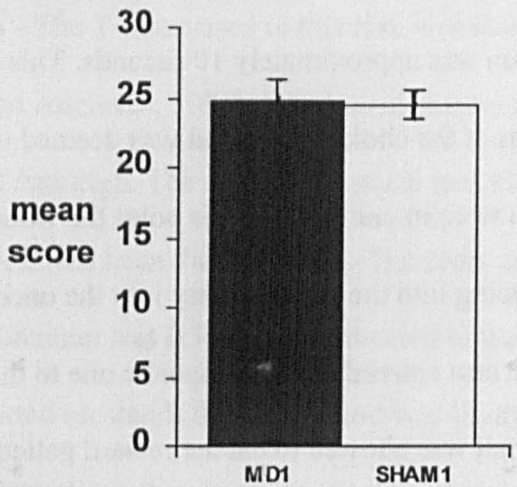

Figure 15 - Performance of the two groups on the T-maze non-matching task.

2.6 - Discussion

Experiment 1 was based on proposals that MD is important for the acquisition of reward-based performance rules, and is implicated in anterograde amnesic syndromes. The first stage of the experiment examined whether these lesions disrupted the learning of simple object discriminations. The results from two such tasks (Object Discriminations 1 and 2), in which just a single pair of objects was tested over successive sessions, showed that extensive lesions to MD did not impair discrimination

learning. This is consistent with the findings of some studies (Aggleton and Mishkin, 1983; Slotnick and Kaneko, 1981; Zola-Morgan and Squire, 1985) but contrary to others (Tigner, 1974; Waring and Means, 1976; Weiss and Means, 1980).

The concurrent learning task was divided into three distinct, simultaneous conditions. The first of these involved the trials given only once every 24 hours. It was found that rats were able to learn discriminations based on just one trial per day, and that extensive lesions to MD failed to disrupt this learning. This resembles the lack of effect observed after extensive limbic lesions in monkeys. However, it remains to be discovered if such a lack of impairment follows MD lesions in monkeys. The second concurrent condition involved giving the animals four trials per day on a particular discrimination. Consequently it resembled the rate of stimulus presentation used in more typical concurrent discrimination studies. No evidence of any impairment was found using either of the two sets of discrimination objects. The third concurrent condition using eight trials per day on each discrimination comprised 60% of all the concurrent trials and was learnt very rapidly over sessions. It did reveal relatively slower learning by the MD rats on the first block of four trial sessions, no significant difference between the groups on the second block, and virtually identical performance thereafter. This finding at first sight resembles those from studies of olfactory discrimination learning, where MD lesions lead to a learning deficit that could be overcome with extensive training (Staubli, Schottler, and Nejat-Bina, 1987). However, the initial deficit is quickly overcome and it would be difficult to argue that the deficit is one of *learning,* but more likely derives from some other, unknown difficulty in discrimination in the MD1 group which can be rapidly compensated for by *efficient* learning. It would appear therefore that MD lesions can sometimes disrupt initial performance in the early stages of a learning procedure, but that other systems ensure that the animal is still able to learn the

task, albeit less efficiently overall. A more specific proposal is that MD contributes to the encoding aspects of a task (Hunt and Aggleton, 1991; Staubli et al, 1987), and hence lesions to this region can alter responsiveness to reward (Gaffan and Murray, 1990; Gaffan and Watkins, 1991). While this latter function may depend, in part, on inputs from the amygdala (Gaffan and Murray, 1990), it appears that other inputs are involved (Aggleton et al, 1991; McAlonan et al, 1993). An alternative proposal is that MD lesions produce an animal that is more inflexible, such that a prior bias towards the incorrect response may result in the appearance of an initial acquisition deficit.

The final test in Experiment 1 was a single, "configural" discrimination. The group of animals with MD lesions were clearly impaired in this task in contrast to all the other object discriminations in this experiment. The question then arises as to why the MD1 group should be differentially affected by a discrimination in which the objects are composed of the same elements. A possible explanation is that the raised levels of interference in this type of task are affecting the MD1 rats to a greater extent than the controls. Perirhinal cortical lesions have been seen to upset configural discriminations in monkeys (Buckley and Gaffan, 1998) and, given the anatomical links between these two areas, this may be of importance. MD lesion rats were unimpaired on the Y-maze delayed non-match to sample task in the previous study (Hunt and Aggleton, 1991). The difference here could also lie in MD rats' lessened ability to reject information which is present in both objects in a trial but which is positive in only one. Hence, in attempting to discriminate between two objects which have a high level of common features, as in this final task in Experiment 1, the MD lesion animals may be more likely to make mistakes by being unable to withhold a positive response to a stimulus which may only superficially appear to be positive. Again, this would suggest a certain inflexibility of responding to test stimuli in the MD1 group, and prompts further investigation of the

way in which animals with MD lesions make decisions on such tasks and how they behave in detail under test conditions.

Experiment 2 suggested that neurotoxic lesions of nucleus medialis dorsalis can induce a borderline deficit on the acquisition of the standard radial-arm maze task, but careful analysis revealed that the deficit was more likely to be a consequence of bilateral damage in one of the anterior thalamic nuclei. This finding is consistent with the discovery that highly selective lesions in the anterior thalamic nuclei are sufficient to produce mild deficits in tests of spatial working memory (Aggleton, Hunt, Nagle, and Neave, 1996; Byatt and Dalrymple-Alford, 1996). Subsequent testing, in which a delay of 60 seconds was interposed between choices ("control delay"), again indicated that neurotoxic lesions of the medial dorsal nucleus are not sufficient to produce a more rapid loss of spatial working memory.

The effect of moving the arms of the radial maze, but not the actual positions of those yet to be visited ('turned delay') did appear to reveal an effect of damage to nucleus medialis dorsalis. Although this effect was somewhat more marked in those cases with additional anterior thalamic damage, there was still evidence of a significant group difference after excluding those animals with bilateral anterior thalamic damage. Thus these results indicate that the medial thalamic lesions had disrupted the animals' response strategy in a way that presumably relates to the differential use of intra-maze and extra-maze cues. Normal rats performing radial-arm maze and T-maze tasks typically rely on distal allocentric cues (Olton, 1978; Olton and Papas, 1979), and will show overshadowing of intra-maze cues by extramaze (allocentric) cues (Diez-Chamizo, Serio, and Macintosh, 1985). This overshadowing is relevant to the performance of the SHAM1 animals on the radial-arm maze task as it explains why

their scores were not affected by the rotation of the actual arms ('control delay' versus 'turned delay'). Furthermore, inspection of their scores showed no evidence of an egocentric strategy. Thus, the SHAM1 animals appeared to rely on distal allocentric cues and were able to ignore potentially misleading intra-maze cues. In contrast, the MD1 animals were more disrupted, indicating a greater attention to intra-maze cues when there is a conflict between the two sets of information. One likely source of intra-maze cues comes from odour trails, and there is evidence that this information is available to rats in the radial arm maze (Buresova and Bures, 1981). Support for this interpretation comes from the evidence of a bias by the MD1 rats to select arms that had not been previously entered. This was observed after the delay in the 'turned delay' condition, where this strategy conflicted with the use of allocentric information and so led to errors. While this evidence is consistent with a reliance on intra-maze cues, it should be noted that an increased selection of unentered arms could occur for a variety of other reasons.

Finally, Experiment 3 showed no evidence that the MD1 animals were impaired on a 'massed' T-maze forced alternation task which again tests spatial working memory. This confirms that this lesion group did not differ grossly from MD lesioned rats in a previous study (Hunt and Aggleton, 1991) in their ability to acquire a spatial working memory task. This contrasts with the deficit in performance of the MD1 group in the "turned maze" condition of Experiment 2, in which this group's response strategy was disrupted on a spatial task. With this evidence, further examination of the ways that medial dorsal thalamic lesions affect the response strategies and exploratory activity of rats under maze-testing conditions would seem to be indicated.

CHAPTER THREE

Cohort 2 - tests of spatial working memory and spatial reference memory, spontaneous object recognition, and levels of exploration

3.1 - Introduction

The radial arm maze is one of the standard tasks used to assess spatial working memory in rats is (Olton and Samuelson, 1976). One study using radial arm maze testing to assess the effects of thalamic lesions (Kolb et al., 1982) also included an explicit reference memory component in which a number of arms were never baited. The thalamic lesions did not affect the ability of the rats to learn to avoid these arms, and so spared both working and reference memory. In contrast, the same method was used in a study by Stokes and Best (1990a), who described resulting deficits in both the working and reference memory components of the radial arm task in groups of rats with ibotenic acid lesions of nucleus medialis dorsalis. Experiment 2 on the previous cohort of rats found marginal deficits with MD lesions on the rate of acquiring the basic (all arms baited) radial arm maze task. The first aim of Experiment 4 was therefore to re-examine this task in the light of the previous equivocal acquisition result. Subsequently, Experiment 4 sought to examine further whether the medial thalamic deficits that can emerge on tests of 'working memory' actually reflect the working memory component or whether they might arise from some other aspect of task performance. As a consequence, rats with neurotoxic lesions of nucleus medialis dorsalis were tested on a variant of the radial-arm maze task in which there is an explicit reference memory, as well as a working memory component. This involved training rats when half of the arms are never baited from session to session, while the other half are always baited at the start of each session (Olton and Papas, 1979).

Following this, Experiment 5 addresses the question of whether MD is involved in the recognition of objects. The results from Experiment 1, which used the Grice Box method to test visual discrimination, had proved somewhat equivocal, finding little substantial effect of MD lesions overall, but with some deficits on two of the nine analyses carried

out. A previous study (Hunt and Aggleton, 1991) had tested recognition (non-spatial delayed non-matching to sample) in a Y-maze, and had found that rats with MD lesions were impaired on acquisition of the task but unimpaired on its subsequent performance over delays of up to 60 seconds. This followed reports of deficits on similar tasks after aspiration or radio-frequency lesions in Monkeys (Aggleton and Mishkin, 1983a, Zola-Morgan and Squire, 1985), which were interpreted as consistent with the supposed contribution of MD damage to human diencephalic amnesia. Experiment 5 applies a method of testing non-spatial memory which does not involve rule-learning, first described by Ennaceur and Delacour (1988). The test is based on the differential exploration of familiar and novel objects by rats and is entirely based on observations of their spontaneous behaviour. Although the results of this test could conceivably be affected by changes in activity or neophobic differences, it may still be described as a relatively pure non-spatial working memory test, free of reference memory components.

One possible explanation for the somewhat vague pattern of often weak deficits emerging from rats performing memory tasks following MD lesions is that the deficits are associated not with any deficiency in mnemonic ability, but rather with some other incidental influence on the rats' performance of the tasks linked with lesions to MD. An obvious candidate for this would be increased activity levels and/or decreased inhibition of exploratory behaviour. Since Experiment 5 is based upon exploration of objects, it is possible to use overall exploration time data from this experiment as an effective, if somewhat crude, means of determining whether rats with MD lesions are indeed more exploratory. Experiment 6, a test of emergence time, addresses the possibility that rats with MD lesions might experience decreased levels of inhibition when emerging from a relatively "safe" environment into one which is likely to be perceived as more "threatening". Experiment 7, the exploration of an open arena, puts animals in a visually

exposed environment in which exploration levels in normal rats are likely to be inhibited; exploration being movement around the arena, especially toward the more exposed centre, and time spent in these central areas.

3.2 - EXPERIMENT 4 - Radial Arm Maze

3.2.1 - Method

3.2.1.1 – Subjects - The new cohort of subjects were 17 experimentally naive rats as described in General Methods in Chapter 2. Prior to surgery they were randomly assigned to one of two surgical groups. Ten rats were assigned to the MD2 group and seven to the SHAM2 group.

3.2.1.2 - Surgical and Histological Procedure - Procedures were as described in General Methods in Chapter 2.

3.2.1.3 - Test Apparatus - The apparatus was the same eight-arm radial maze which was described in Chapter 2 (Experiment 2), and testing was carried out in the same room as that experiment.

3.2.1.4 - Testing Procedure - Pre-testing exposure to the maze was begun two to three weeks after surgery and continued daily for five days during which the animals were free to explore the maze and eat reward pellets. Following this the acquisition phase was conducted using the same procedure and acquisition criterion as described for Experiment 2.

On the test day following each rat's acquisition of the task testing continued with the next phase of the experiment designed to differentiate reference and working memory errors. One rat from the MD2 group failed to reach the acquisition criterion until the 50 testing sessions and had to be excluded from this subsequent testing. Each session began as in the acquisition phase but with only four of the eight arms baited. The pattern of arm baiting was determined before any rats began this phase of the experiment, and was different for each member of a surgical group. However, the baiting was matched between the two groups and was constant for each subject throughout the remaining test period. No more than two adjacent arms were baited and patterns which could be simply solved, for example by constant 90 degree turns, were avoided. The test session ended when all four baited arms had been visited, and each subject underwent 25 daily testing sessions. The experimenter scored each error as either *reference memory,* in which the subject entered arms which were never baited, or *working memory,* in which it re-entered arms which had already been visited. Re-entries to never-baited arms were therefore scored as *reference memory* errors only.

3.2.2 - Results

3.2.2.1 - Histological analysis

Damage within MD: all seven rats in the MD2 group had extensive lesions within nucleus medialis dorsalis. The largest and smallest of these lesions are depicted in Figure 16. The pattern and character of damage within the nucleus in six of these cases was similar to that reported for the MD1 group in Chapter 2, with very small areas of sparing occurring only

at the most rostral margins of the nucleus. The seventh case had a somewhat larger area of rostral sparing in the right hemisphere. There was one MD2 case with bilateral infarction restricted within the mediodorsal nucleus.

Damage to other structures: Damage to nucleus lateralis dorsalis was observed in five of the seven cases, but it was always limited to the medial portion of the nucleus. One case showed unilateral damage to the intralaminar nuclei. There was bilateral damage to the anterior dorsal nucleus in two cases, while four cases showed unilateral damage in the same nucleus. Very limited unilateral damage to the anterior ventral nucleus was also noted in four cases, and more widespread unilateral damage was apparent in one case. The pattern of damage, and sparing, of the mid-line thalamic nuclei, the habenula and the dentate gyrus matched that reported for the MD1 group.

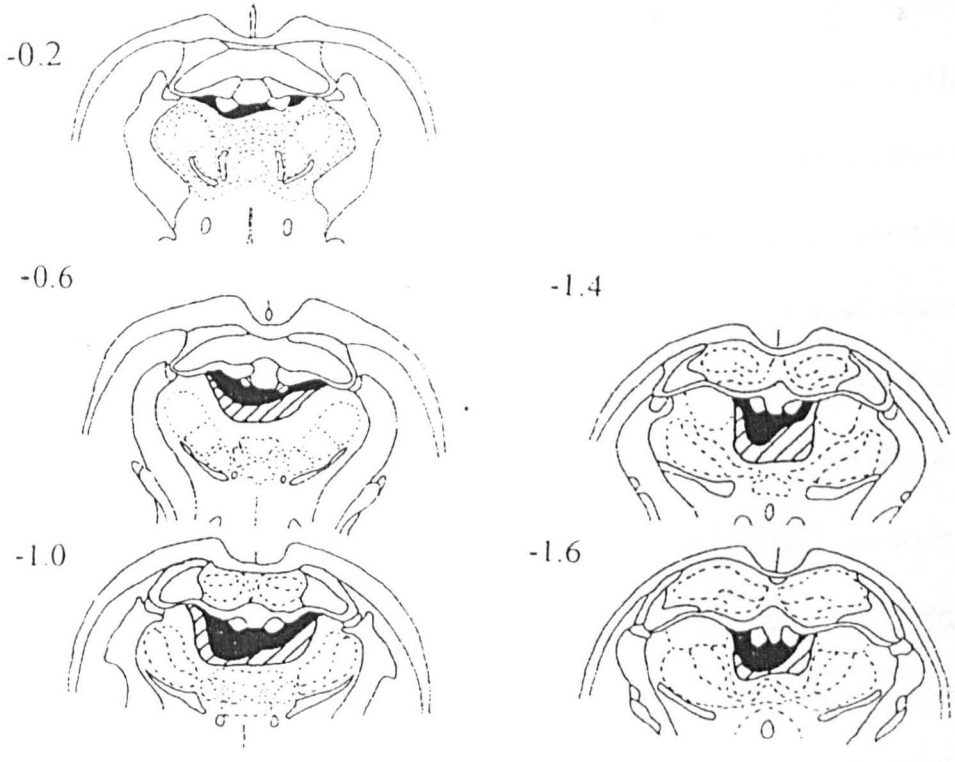

Figure 16 - Diagrammatic reconstruction of the lesions of nucleus medialis dorsalis. The coronal sections depict the smallest (black) and largest (diagonal lines) extent of cellular loss. The numbers refer to the approximate corresponding AP levels from the stereotaxic atlas of Pellegrino and Cushman (1967).

3.2.2.2 - Behavioural analysis

Acquisition - Student's t-tests, adjusted for inequality of variance using Levene's test, revealed that the MD2 group made significantly more errors in completing the acquisition criterion [mean errors SHAM2 = 5.2, s.e.m.= 0.71; MD2 = 64.1, s.e.m = 24.8; t (6.01)= 2.38, $p<0.03$]. The MD2 animals also required significantly more sessions to criterion [mean SHAM2 = 7.3, s.e.m = 0.84; MD2 = 22.7, s.e.m = 5.66; t(6.27) = 2.70, p = 0.02]. One rat in the MD2 group performed exceptionally poorly and did not reach the acquisition criterion until the fifty-first test session (the next poorest animal required 32 sessions).

Working/reference memory - Figure 17 shows that the MD2 group produced more errors on both the working memory and reference memory conditions than did the SHAM2 group (overall means: reference memory errors: MD2 = 77.5; SHAM2 = 61.1; working memory errors: MD2 = 37.3; SHAM2 = 10.1). Both conditions were analysed jointly in a two-way ANOVA with a between-subjects factor of group and two within-subjects factors of condition, i.e. reference memory or working memory error, and block (the data was blocked into five blocks each of five sessions: Figure 18). This revealed a significant effect of condition [$F(1,14) = 328.1$, $p<0.001$], as many more "reference" memory errors were made overall, but no effect of block [$F(4,56) = 1.65$, $p = 1.74$], indicating a lack of improvement over sessions. There was a group effect [$F(1,14) = 13.13$, $p = 0.003$], but no condition x group interaction [$F(1,14) = 2.27$, $p = 0.15$], even though the MD2 group tended to make disproportionately more working memory errors. There was, however, a significant group x block interaction, reflecting the MD2 group's increase in reference memory errors and decrease in working memory errors on block 4. The remaining interactions were non-significant, i.e. condition x block [$F(4,56) = 0.82$, $p = 0.52$] and

condition x group x block [F(4,56) = 0.45, p = 0.78]. Analysis of the simple effects showed that the MD2 group made significantly more errors on both measures [working memory errors: F(1,14)= 12.3, p=0.004; reference memory errors [F(1, 14) = 7.50, p=0.016].

Again, the effect of damage extending outside the nucleus medialis dorsalis on tests of spatial performance was considered. Two rats in the MD2 group had bilateral lesions of the anterior dorsal thalamic nuclei, while another had bilateral damage in the anterior ventral nucleus. This rat required 51 sessions to reach the acquisition criterion. The preceding analyses were repeated, again adjusting for inequality of variance using Levene's test, with the data from these three rats excluded. Even after exclusion of these rats there was evidence of acquisition impairment in the MD2 group. Thus, the mean errors to criterion for the revised MD2 group was 38.3 [t(3.02) = 2.84, p = 0.03], and the mean sessions to criterion were 16.3 [t (3.34) = 2.44, p = 0.042].

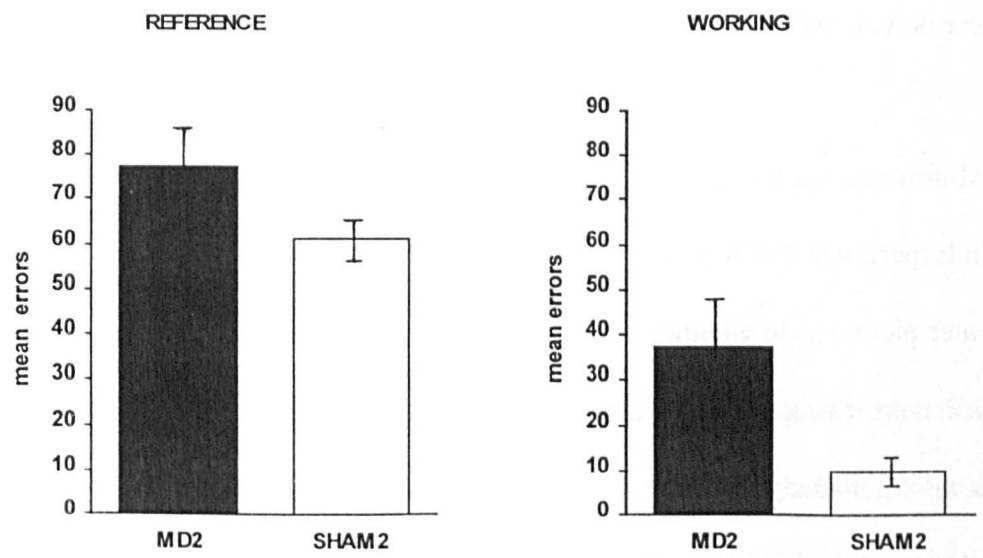

Figure 17 - Performance of the radial arm maze task. The graph on the left shows the mean number of reference memory errors for the two groups, while that on the right shows the mean number of working memory errors. Vertical lines indicate standard error of the mean (and in all subsequent figures in Chapter 3).

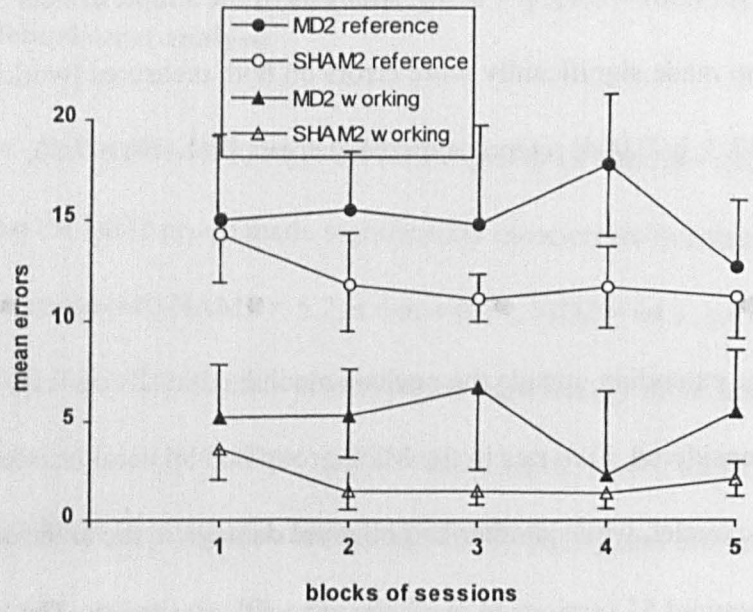

Figure 18 – Performance of the radial arm maze task measured over five blocks of five sessions, showing mean errors on both reference memory and working memory components of the task.

The performance of the same revised MD2 group was examined on the working and reference memory versions of the task. These analyses showed that there was still a significant effect of condition [$F(1,12) = 317.1$, $p < 0.001$] and of group [$F(1,12) = 18.92$, $p = 0.001$] but no effect of block [$F(4,48) = 1.67$, $p = 0.36$]. All other components of the analyses were non-significant.

As the radial-arm maze acquisition procedure in this experiment was identical to that employed in Experiment 2 (Chapter 2), it was feasible to combine the results, and so derive a clearer picture as to whether damage to medialis dorsalis is sufficient to impair task acquisition. As it was first necessary to determine comparability of the two control groups' data, an initial analysis was carried out. This showed that the error and session scores to criterion of the SHAM1 and SHAM 2 groups were comparable [errors, $t(16) = 0.93$; sessions, $t(16) = 1.02$, $p=0.32$, two tailed]. This combined SHAM group (n=18) was then used for comparisons with the MD rats with no bilateral anterior thalamic damage

(MD n = 9) and those with bilateral anterior thalamic damage (MD+ANT, n = 6) in an analysis of variance.

Comparison of the error scores to complete acquisition revealed a highly significant group effect (means: MD+ANT = 58.17, MD = 21.00, SHAM = 5.78), [$F(2, 30) = 6.16$, $p=0.006$]. Subsequent Newman-Keuls tests showed that the MD+ANT group differed from the SHAM rats ($p<0.01$) and from the MD animals ($p<0.05$), but the MD group did not differ significantly from the SHAM rats. Analysis of the session to criterion data produced exactly the same pattern of results (means: MD+ANT = 20.33, MD = 11.33, SHAM = 6.78). There was once again a large group effect [$F(2,30) = 6.57$, $p=0.004$], and Newman-Keuls tests showed that the MD+ANT group differed from both the SHAM ($p<0.01$) and MD ($p<0.05$) groups, but the MD group did not differ from the SHAM group.

3.3 - EXPERIMENT 5 - Spontaneous Object Recognition

3.3.1 - Method

3.3.1.1 - Subjects and surgical and histological procedure- were as Experiment 4. However, during the course of this experiment one animal displayed symptoms of illness and was therefore excluded from this and all subsequent testing.

3.3.1.2 - Test apparatus - The apparatus consisted of an open box (100cm x 100cm x 50cm high) made of aluminium with the inside painted grey. The floor was covered with grade 10 wood flake animal bedding (Datesand, Manchester, UK) of the same type used in the animals' home cages. The objects to be discriminated were in triplicate and made of glass, plastic or metal. The weight of the objects ensured that they could not be moved by the rats. A fluorescent light was positioned 2.1 metres above the floor of the testing box and video camera was mounted 1.75 metres above the test box on a tripod so that the test sessions could be relayed to the experimenter who was seated 3 metres from the test box observing the video monitor screen. The sessions were also recorded on video tape in case the need arose to re-examine the sessions later.

3.3.1.3 – Testing procedure - All rats received five habituation sessions in which they were allowed 3 minutes to explore the apparatus. Testing began forty-eight hours later and consisted of two daily sessions per delay condition, in order to counterbalance the side on which novel and familiar objects appeared. These sessions were a minimum of 24h apart. Rats were first exposed to a matching pair of novel stimuli (A1 and A2) for three minutes in the test box (sample phase). After a delay of either 15 min or 60 min they were returned to the apparatus. The box now contained a third, identical version of object A (A3) and a novel object (B). The time spent exploring each of the two objects in the sample phase and the test phase was recorded. Exploration of an object was defined as directing the nose to the object at a distance <2cm and/or touching the object with the nose. Behaviours such as turning around or sitting on the object were not considered exploratory. New sets of objects were used for each of the four sessions, and the use of any particular object as a sample or test stimulus was counterbalanced within a session. Throughout this test and the remaining tests of this cohort, the rats were kept on ad. lib. feeding.

3.3.2 - Results

3.3.2.1 - Behavioural analysis - The first analyses considered the total times spent exploring the two identical objects in the sample phase (e1). These measures are potentially valuable as group differences at this stage could potentially confound the recognition test. The exploration times (e1) for each of the two delay conditions were analysed separately using Student's t-tests (two-tailed). The only evidence of difference concerned the 60 minute delay condition with the MD2 group displaying slightly elevated duration of exploration. [$t(14) = 1.87$, $p = 0.08$].

Comparisons of object preference were then performed for each of the two delay conditions using (1) d1, the discrimination index, which is the difference in time spent exploring the two objects over the entire choice phase (e.g. B-A3) and (2) d2, the discrimination ratio, which is the discrimination index divided by the total time spent exploring the two objects in the choice phase e.g. B-A3/B+A3 i.e. (d1/e2).

Analysis of variance using the factors delay and group was applied to the d1 scores of the two groups over the two delay conditions. This analysis revealed a significant effect of delay [$F(1,14) = 11.99$, $p = 0.004$] but no effect of group [$F(1,14) = 0.01$, $p = 0.94$], nor a delay x group interaction [$F(1,14) = 0.02$, $p = 0.90$]. In view of the possibility that the two groups might differ in their respective levels of total exploration (Table 5), the more useful measure of object recognition is likely to be d2, the discrimination ratio, which takes into account individual differences in total exploration during the choice phase. The same analysis was applied to the d2 scores of the two groups, this time revealing no significant effect of delay [$F(1,14) = 0.38$, $p = 0.55$], nor of group [$F(1,14) = 1.43$, $p = 0.25$], nor a delay x group interaction [$F(1,14) = 0.24$, $p = 0.63$].

15 minutes delay				
	e1	e2	d1	d2
MD2	87.33	124.33	35.67	0.30
SHAM2	72.90	91.40	34.80	0.38

60 minutes delay				
	e1	e2	d1	d2
MD2	60.00	65.00	21.17	0.30
SHAM2	46.70	62.20	21.30	0.34

Table 5 - Mean observed measures of the two groups on the spontaneous object recognition test (mean seconds) over two delay conditions; e1 = time spent exploring both objects in sample phase, e2 = time spent exploring both objects in choice phase, d1 = discrimination index, d2 = discrimination ratio.

In order to establish that recognition of novel objects was occurring during the choice phase (e2), Student's t-tests (one-tailed) were used to compare the time spent exploring novel and familiar objects on the two conditions by each of the separate groups. Student's t-tests were used to compare the time spent exploring novel and familiar objects on the two conditions by each of the separate groups. This was done in order to establish that recognition of novel objects was occurring during the choice phase (e2). Both groups spent significantly longer in exploring the novel objects on the 15 minute delay condition [MD2: $t(5) = 6.72$, $p<0.001$; SHAM2: $t(9) = 9.17$, $p<0.001$]. At the 60 minute delay condition the SHAM2 group showed a significant difference [$t(9) = 4.85$, $p<0.001$], but the MD2 group narrowly failed to reach the 0.05 significance level [$t(5)= 1.97$, $p = 0.053$]. These within-group comparisons are, however, influenced by the differences in group sizes, so that the comparisons of MD2 rats have less power.

3.4 - EXPERIMENT 6 - Emergence

3.4.1 - Method

3.4.1.1 - Subjects - were as Experiment 5.

3.4.1.2 – Test apparatus - The apparatus consisted of a 30cm length of PVC pipe 12cm in diameter and closed at one end. This was stabilised by PVC feet to prevent rolling and the open end could be closed off by a heavy aluminium-faced block. The apparatus was placed at the centre of a table measuring 2m by 0.8m which was covered in white polythene sheeting and illuminated by a 250W floodlight suspended 75cm above the surface of the table.

3.4.1.3 – Testing procedure - Testing took place ten days after the end of the object recognition test and comprised a single session. Each rat was placed inside the apparatus and the opening was closed off for a period of 2 minutes. At the end of the 2 minutes the floodlight was switched on and the aluminium-faced block removed so that the rat could emerge. The experimenter, who was unaware of the rats' group at the time of testing, sat level with the open end of the apparatus and 60cm from it. Three timings were recorded for each animal: nose emergence, forepaws emergence, and hind paws emergence. The test was terminated when the rat's hind paws emerged or, if this did not happen, after 10 minutes.

3.4.2 - Results

3.4.2.1 - Behavioural analysis - Mean values for the nose and forepaw emergence measures were: nose, MD2 = 8.0 sec; SHAM2 = 15.4 sec; forepaws, MD2 = 31.2 sec; SHAM = 86.5 sec (Figure 19). Student's t-tests (two-tail, log transform) showed that the difference between the two groups in the emergence of the nose [$t(14) = 1.39$, $p = 0.09$) or the forepaws [$t(14) = 1.67$, $p = 0.06$] narrowly failed to reach significance. Hind paws emergence data were not compared due to a ceiling effect resulting from the failure of seven of the SHAM2 group and four of the MD2 group to complete the test within the 10 minute period.

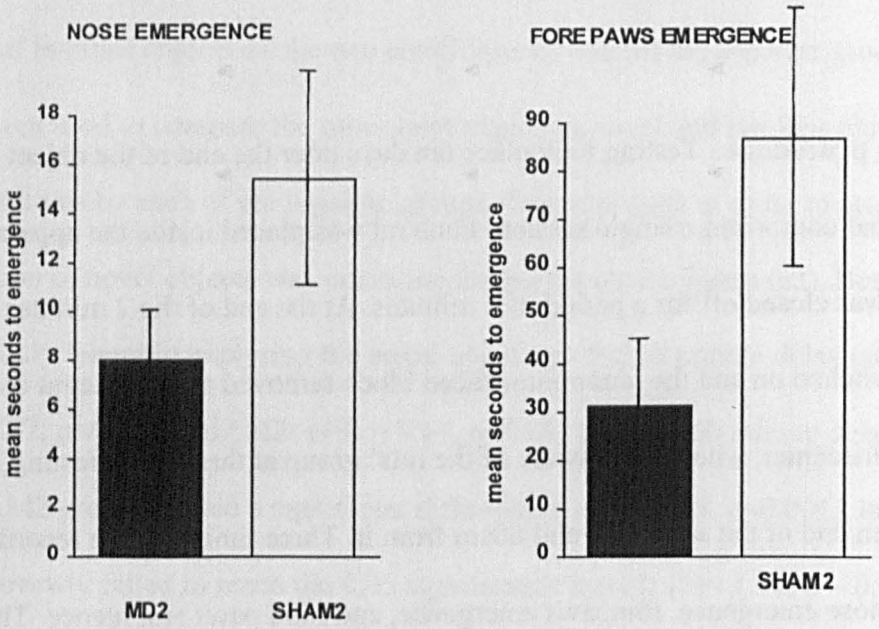

Figure 19 - Mean times in seconds for the emergence of the nose (left) and fore paws (right) of the two groups of rats.

3.5 - EXPERIMENT 7 - Exploration of an Open Arena ("open field")

3.5 1 – Method

3.5.1.1 - Subjects - were as Experiment 5.

3.5.1.2 – Test apparatus - The apparatus consisted of a circular arena 90cm in diameter with a 45cm high wall. The floor and wall of the arena were painted matt black and the floor was marked with two concentric circles 30cm and 60cm in diameter. Each of the outer two rings thus formed was divided by radial lines, the outer ring being divided into 8 sectors, and the middle ring into 4 sectors. The centre circle was undivided. Illumination of the arena was provided by a fluorescent room light 2m above it. A video camera was supported by a tripod 1.75m above the arena and to one side so that the activity sessions could be recorded without the presence of the experimenter in the room and analysed later.

3.5.1.3 - Testing procedure - Each session consisted of a rat being placed in an outer sector and allowed to wander about the arena freely for 5 minutes during which time the video apparatus was recording. When all animals had completed one such session, activity was analysed from the video recording. All line crossings in which all four paws crossed a line were counted, and of these inward line crossings were noted. Also time spent in the 2 inner rings, i.e. away from the arena wall, was recorded. The experimenter was unaware of the rats' groups during this process.

3.5.2 - Results

3.5.2.1 - Behavioural analysis - All line crossings in which all four paws crossed a line were counted, and of these inward line crossings were noted. Also time spent in the inner ring and centre circle, i.e. away from the arena wall, was recorded (Figure 20). The experimenter was unaware of which experimental group the animals belonged to during this process.

The MD2 group made significantly more line crossings [$t(14) = 3.60$, $p = 0.002$] and inward line crossings [$t(14) = 3.35$, $p = 0.003$] than the SHAM2 group. There was no difference between the groups in time spent in the central parts of the arena ($t(14) = 1.44$, $p = 0.09$). Mean values were: line crossings, MD2 = 96.8; SHAM2 = 75.3; line crossings inward, MD2 = 23.8; SHAM2 = 18.2;time spent in middle areas, MD2 = 91.0 sec; SHAM2 = 70.4 sec. It should be noted that these group differences concerning line crossings and inward line crossings persisted after removal of those animals with bilateral anterior thalamic damage ($p = 0.006$ in both cases).

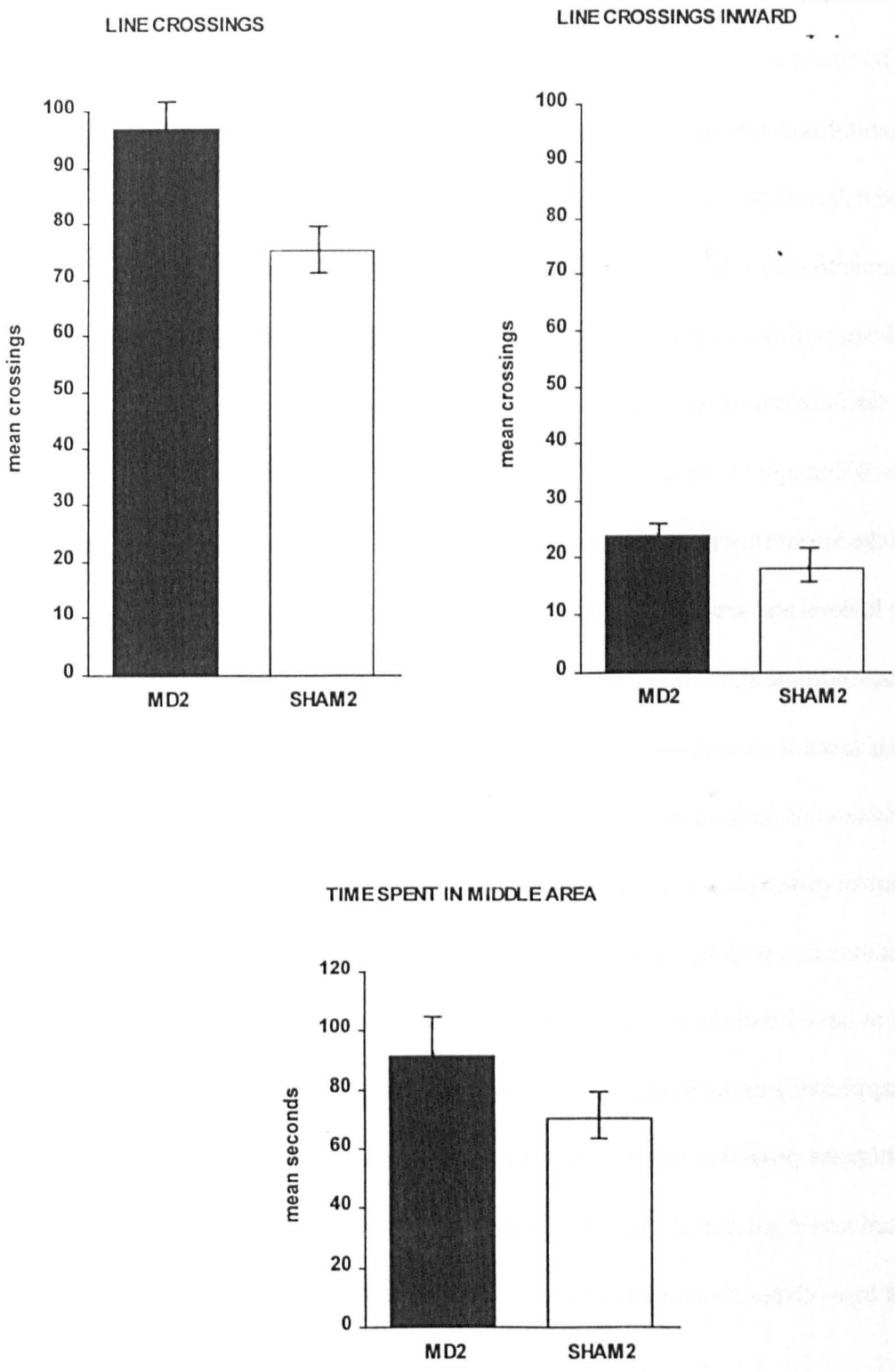

Figure 20 - Open arena exploration: all line crossings (upper left), inward line crossings (upper right), and time spent in the innermost part of the arena (lower).

3.6 - Discussion

Experiment 4 was a radial arm maze task in which reference and working memory components could be differentiated. This followed a repetition of the initial radial arm maze acquisition task in which all arms are baited and visited by the rat. The data from this part of the experiment, as in Experiment 2, showed that lesions of nucleus medialis dorsalis result in a borderline acquisition deficit, which is considerably exacerbated by the presence of damage to the anterior thalamic nuclei. This latter effect probably accounts for much of the variability that is found in the published descriptions concerning dorsomedial thalamic lesions and tests of spatial working memory. This conclusion is supported by two additional findings. First, that discrete lesions within individual anterior thalamic nuclei can impair tests of spatial working memory ((Aggleton, Hunt, Nagle, and Neave,1996). Second, when the radial-arm maze acquisition data were combined, so ensuring a much larger control group (n = 18) and larger thalamic sub-groups, no acquisition deficit was found in those rats with lesions essentially confined to nucleus medialis dorsalis i.e. those that did not have bilateral damage in any of the anterior thalamic nuclei (MD). The MD1 rats also appeared unaffected on the T-maze alternation task even though it used massed trials to increase proactive interference. These results, which are consistent with a previous study (Hunt and Aggleton, 1991), also indicate that damage to this nucleus does not produce a loss of spatial working memory.

In contrast, bilateral medial thalamic damage that included one of the anterior thalamic nuclei resulted in a significant acquisition impairment on the radial-arm maze task when compared not only to the sham controls but also to those animals with more discrete medial thalamic damage i.e. those without bilateral anterior thalamic damage. This additional damage was largely confined to the anterior dorsal nucleus in most cases. This

is of interest as this nucleus contains 'head direction' units (Taube, 1995) as does the lateral dorsal thalamic nucleus (Mizumori and Williams, 1993), both of which are immediately adjacent to medialis dorsalis. Furthermore, a loss of head direction information might be sufficient to disrupt radial-arm maze performance (Mizumori, Miya, and Ward, 1994). These findings lead to the prediction that dorsomedial thalamic lesions will have no effect on the standard radial arm maze task when the lesions are confined to nucleus medialis dorsalis. This view is supported by the failure to observe a radial-arm maze deficit in some other studies of dorsomedial thalamic lesions (Kolb, Pittman, Sutherland, and Whishaw, 1982; Olton, 1978; Beracochea, Jaffard, and Jarrard, 1989). In contrast, a number of studies have described significant impairments after dorsomedial thalamic lesions (Stokes and Best, 1988, 1990a,b; Kessler, Markowitsch, and Otto, 1982), but in at least one of these studies the presence of consistent anterior thalamic involvement is acknowledged (Kessler et al, 1982). In the series of studies by Stokes and Best (1988, 1990a and b), the status of the anterior thalamic nuclei is more difficult to determine. The lesion reconstruction in at least one of these studies (Stokes and Best, 1988) does, however, indicate the consistent involvement of the anterior thalamic nuclei, while photomicrographs show extensive lateral dorsal damage in other studies (Stokes and Best, 1990a and b). In the light of the present study and other recent experiments (Aggleton, Hunt, Nagle, and Neave, 1996), it is clear that it is vital to state precisely the extent of anterior thalamic involvement when attempting the analyse the effects of damage to nucleus medialis dorsalis.

It is not, however, the case that lesions of nucleus medialis dorsalis that spare the anterior thalamic nuclei leave performance of the radial-arm maze task unaffected. As in Experiment 2 where there was evidence that the lesions disrupted performance when the arms were turned, other deficits emerged in Experiment 4 when the radial-arm maze test

was modified in order to distinguish reference and working memory errors. This was achieved by having four arms that were never baited across all sessions, so that entries into these arms were counted as reference errors. The change in the task rules led to clearer deficits in animals with lesions of medialis dorsalis, and these were not dependent on the presence of anterior thalamic damage. Thus, a significant increase was observed in both 'working' memory and 'reference' memory errors, even for those animals lacking bilateral anterior thalamic damage.

Previous studies have also used the combined reference/working memory design to test dorsomedial thalamic lesions, but the results have been inconsistent. While one study reported a lesion related deficit for both reference and working memory errors (Stokes and Best, 1990a), another study found no impairment on either component of the task (Kolb, Pittman, Sutherland, and Whishaw, 1982). The present study is not, however, strictly comparable as in both of these previous studies acquisition began with the modified version of the task i.e. the animals were not first run on the standard radial arm maze task with all arms baited. This difference probably makes the present version more demanding as the animals have to learn to withhold selection of a previously baited arm. Indeed, it is possible that this largely accounts for the deficits in the present study, a view that receives some support from the finding that lesions of the medial frontal cortex, which is closely connected with nucleus medialis dorsalis, produce a disproportionate impairment on the reference memory component (Kolb, Pittman, Sutherland, and Whishaw, 1982).

Experiment 5 was a test of spontaneous object recognition which has been shown to be sensitive to cortical lesions involving the perirhinal cortex (Ennaceur, Neave, and Aggleton, 1996; Aggleton, Keen, Warburton, and Bussey, 1997). This is of interest as the perirhinal cortex projects to nucleus medialis dorsalis, and there is evidence that lesions in

this thalamic region can impair the acquisition (Hunt and Aggleton, 1991) and performance (Mumby, Pinel, and Dastur, 1993) of tasks such as delayed non-matching-to-sample (DNMS). In comparison with the DNMS task, the spontaneous test used in the current study is probably a purer test of recognition (Ennaceur and Delacour, 1988; Ennaceur and Meliani, 1988) as it does not involve acquiring a response rule i.e. a reference memory component. This feature of the task is potentially important as a previous study found evidence that lesions of medialis dorsalis can impair DNMS acquisition (Hunt and Aggleton, 1991), but leave performance over increasing delays intact once the task rule had been mastered. Consistent with that finding, the current study appeared to demonstrate that rats with lesions of medialis dorsalis are able to recognise novel objects. This result will, however, require further examination as the thalamic lesions also affected some aspects of exploration (e.g. open field). As an increase in sample exploration could potentially mask a mild recognition deficit, subsequent experiments will need to use a variant of this task in which sample exploration times are matched between groups (Ennaceur, Neave, and Aggleton, 1997). This is especially important in view of other evidence that medial dorsal thalamic lesions in rats can lead to recognition deficits even after the non-matching rule as been acquired (Mumby, Pinel, and Dastur, 1993).

The possible changes in exploration level in the object recognition task prompted two further tests of activity in the MD2 rats. While the emergence study failed to provide a clear group difference, the MD2 rats typically emerged faster as measured by nose and forepaw latencies. More convincing evidence of a change in exploration was found in the 'open field' task. The rats with medial dorsal thalamic lesions showed significantly higher levels of movement around an open arena, as measured by the number of marked lines that they crossed, and by their movement toward the centre of the open space. As these

changes in exploration were not related to the extent of anterior thalamic damage it is most likely that lesions of nucleus medialis dorsalis were responsible for increased levels of exploration in the object recognition test and the open field study. An increase in levels of activity following neurotoxic lesions of the medial dorsal thalamus has been observed previously (Beracochea, Jaffard, and Jarrard, 1989) and there are obvious parallels with the increase in activity that has been reported after prefrontal damage in rats (Kolb, 1984; Wolf, Dahlin, Hu, Xue, and White, 1995). These changes may also be linked to the deficits in the reference/working memory task if they indicate a release of behaviour and so add to the difficulty of withholding selection of the previously baited arm.

CHAPTER FOUR

Cohort 3 - tests of conditioned cue preference, exploration, spatial delayed matching and non-matching, and activity

4.1 - Introduction

Previous experiments in this study explored the somewhat confusing picture of deficits on learning and memory tasks displayed by rats with lesions to the thalamic nucleus medialis dorsalis. This took two directions; detailed investigations of aspects of spatial and non-spatial learning and memory tasks, and the investigation of aspects of non-mnemonic behaviour in the testing situation which might influence results. Further investigation of non-spatial learning and memory would seem not to be the most clearly indicated direction to follow, since previous experiments showed little indication of such deficits in rats with MD lesions. The next series of experiments, then, aimed to pursue the investigations further along the lines of learning and memory in spatial tasks, and the behaviour of rats with MD lesions in the test situation.

The results of experiments completed so far in this study, far from describing a pattern of clear MD impairment in learning and memory, indicated that MD rats' ill-defined impairments in performance may have more to do with the way that they move around and behave in the test situation. Measures of exploratory behaviour indeed already indicated that rats with MD lesions can be more active in the test environment. This does not, however, explain why their performance in tests of learning and memory is sometimes diminished. In one sense it might be seen as an advantage for rats to be less inhibited when placed in novel, exposed situations when they have, necessarily, to move around the environment in order to achieve reward. It was therefore necessary to examine the relationship of place and reward in the context of learning and memory to establish whether this differs in rats with MD lesions. Experiment 8, therefore, used the

method of "conditioned place preference" to look at the way rats relate reward to environments that are visually distinctive and asked whether rats with MD lesions have a reduced ability to respond to the association of food reward and place. This class of task assesses the ability to associate a reinforcer with a specific cue signal, using a classical conditioning paradigm (Van der Kooy, 1985). As a consequence, it can help to determine whether lesions of MD disrupt reward-related processes. This is of value as such a deficit could affect the acquisition and performance of a wide array of tasks (Sahgal, 1993), which may be relevant to this type of study. Indeed, there is evidence from one study using a cue preference task (McAlonan et al., 1993) that ibotenic acid lesions of nucleus medialis dorsalis can impair conditioning. This study (McAlonan et al., 1993) used the term "conditioned place preference", although it has been pointed out (White and McDonald, 1993) that the term "place" can be misleading in such experimental methods, and should be reserved for locations perceived by relation to distal cues. The present study, which used only local cues, therefore uses the term "cue" as an appropriate term for the rats' immediate environment. However, it should be noted that the task is functionally similar to that used in McAlonan et al's 1993 study, since distal cues were not available to the animals during the conditioning process in either study. In addition to the main aim of this experiment, data from the first session of this experiment, in which rats were placed in an unfamiliar environment and allowed to wander freely, were used to assess further the notion that MD rats show increased levels of exploratory behaviour under such conditions.

Experiment 9 was a repetition of the exploration of an open arena carried out with the previous cohort of animals in Experiment 7, which found that rats with MD lesions

made more exploratory movements in that environment. This positive finding was an endorsement of the notion that an important element in MD rats' behaviour in the test situation may lie in the way they carry out exploratory behaviour. However, the effective size of the lesion group in that experiment had been quite small (n = 6) and since the experiment was quick to run and unlikely to affect subsequent tests it was decided that a repetition to increase the numbers of subjects for analysis would strengthen the validity of the findings.

A previous study (Hunt and Aggleton, 1991) had looked at delayed non-matching to sample in a T-maze, finding that MD lesions had no effect on the acquisition of the task and only affected the group with lesions made by radio frequency means on the most difficult condition of delay. Similarly, Experiment 3 in the present study had established that rats with MD lesions were unimpaired on learning the task (T-maze alternation) and, like the controls and normal rats (Rawlins & Olton 1982) learned the task very readily. Whilst rats find this normal T-maze alternation task easy to learn, they find a matching variant much harder (Rawlins, 1993). Experiment 10, then, examined the possibility that the acquisition of the T-maze matching rule might prove differentially testing for rats with MD lesions, either because removal of the ceiling effect of the readily-learned non-matching rule might reveal the previously hidden deficit, or because adopting the "counter-intuitive" matching rule might discriminate against MD animals. In addition, one possible interpretation of the pattern of deficits encountered in testing on the radial arm maze could be an increase in the propensity of the lesion groups to perseverate in non-matching and thus visiting never-rewarded arms.

While it has been supposed that the prefrontal cortex, definitively connected with nucleus medialis dorsalis, might have a general role in working memory, it is most often thought to be involved in aspects of response or attentional control and their inhibition (Cohen et al., 1996; Robbins, 1996). The T-maze matching to sample task, therefore, would seem ideally suited to tax these latter attributes of memory since, unlike the easily-learnt non-matching T-maze task (Experiment 3), it requires the rats to inhibit the innate foraging response and adopt the opposite response. The behavioural change required does not, however, involve any change in attentional demands since the rats are innately attending to spatial cues throughout. After examining the acquisition of this matching task, the experiment went on to look at the effect of conditions of delay on its performance, and then at reversal to the non-matching rule.

Although a number of experiments in this study have assessed the possibility that rats with MD lesions are more active in an exploratory sense, no attempt has been made so far to examine undirected activity. Experiment 11, the final experiment in this study, used an automated system to address the question whether MD rats would be more active than controls purely in terms of movement rather than exploratory behaviour. The advantage of such automated measures is that they do not involve any possibility of experimenter bias, and provide very large amounts of data measured in a variety of ways. However, in another sense, they provide only very crude, unfiltered information about the rats' activity since, in this system, they are not able to distinguish between such very different activities as ear-washing and running.

4.2 - EXPERIMENT 8 - Conditioned Place Preference

4.2.1 - Method

4.2.1.1 – Subjects - The subjects were 20 experimentally naive rats as described in General Methods in Chapter 2. Prior to surgery they were randomly assigned to one of two surgical groups. Ten rats were assigned to the MD3 group and 10 to the SHAM3 group.

4.2.1.2 - Surgical and histological procedure - Procedures were as described in General Methods in Chapter 2.

4.2.1.2 - Test apparatus - The apparatus was the same eight-arm radial maze which was described in Chapter 2 (Experiment 2), and testing was carried out in the same room as that experiment and Experiment 4. The guillotine doors to six of the maze arms were closed, leaving access from the central area to only two arms. The side and end walls and top of one of these arms was completely enclosed in black polythene sheeting and the other in white polythene sheeting so that the environment for the rats whilst in each arm was visually distinctive. The polythene sheeting could be applied to any of the arms according to the procedure described below.

4.2.1.3 - Testing Procedure - All rats were maintained on a restricted feeding regime for five days prior to testing and throughout the testing period, their body weights were not allowed to fall below 85% of normal. On the first day of testing (session 1), each rat

was put into the centre of the maze and allowed free access for 20 minutes to the two open arms, enclosed in black and white polythene as described above and located opposite each other. No food was present in the maze during this session. Time spent in each of the two arms and the number of entries to each arm were recorded by the experimenter, who sat in the same location, 1.5 metres from the apparatus, throughout all test sessions.

On test days 2 to 9, each rat was randomly assigned two of the maze's eight arms, one black and one white. These arms always had at least two closed arms between them. The pattern of arm assignment for individual rats in the SHAM3 group replicated that for rats in the MD3group. Half of the rats from each group were enclosed by a wooden block in the "baited" arm, and half in the "non-baited" arm for a period of 20 minutes on each test session. Twenty grams of the subjects' normal laboratory rat diet (RM1E, Special Diets Services, Witham, UK) was scattered around the floor of the "baited" arms, while the "non-baited" arms contained no food. The selection of "baited" and "non-baited" was counterbalanced between black and white arms. The confinement to "baited" and "non-baited" arms was alternated for each session so that each rat received equal exposure to "baited" and "non-baited" arms. Prior to each day's testing, the maze was rotated clockwise by one arm, i.e. by 45 degrees, and the polythene covers moved back by one arm into their former position. Each rat thus remained in the same spatial location but in a different arm, in order to prevent the accumulation of olfactory cues in the arms. The procedure for session 1 was repeated on the tenth and final testing day, i.e. the rat was placed for 20 minutes in the apparatus with free access to both arms, neither of which was baited.

4.2.2 - Results

4.2 2.1 - Histological analysis

Damage within MD: all animals in the MD3 group had extensive lesions within nucleus medialis dorsalis. The largest and smallest of the MD3 lesions are depicted in Figure 21. The pattern and character of damage within the nucleus was similar to that reported for the MD1 group in Chapter 2, with very small areas of sparing occurring only at the most lateral, ventral, caudal, and rostral margins of the nucleus. There were, however, no cases of infarction in the MD3 group.

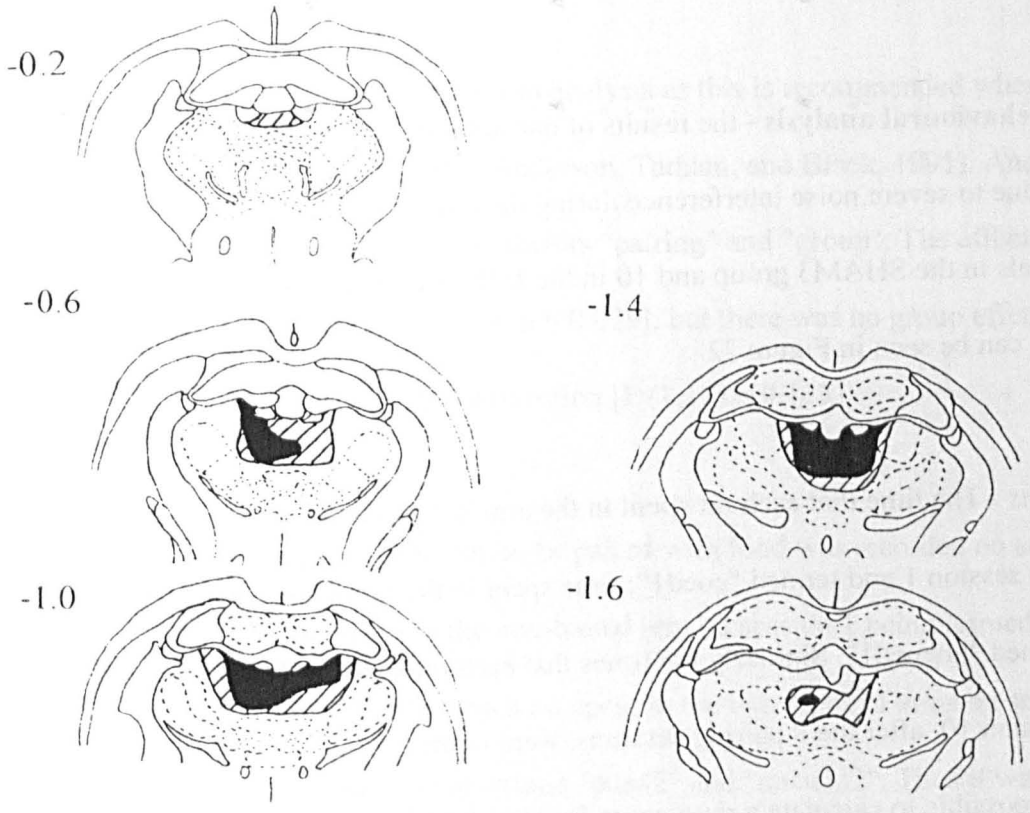

Figure 21 - Diagrammatic reconstruction of the lesions of nucleus medialis dorsalis. The coronal sections depict the smallest (black) and largest (diagonal lines) extent of cellular loss. The numbers refer to the approximate corresponding AP levels from the stereotaxic atlas of Pellegrino and Cushman (1967).

Damage to nuclei of the anterior thalamic group: loss of cells in the medial portion of nucleus lateralis dorsalis occurred in two cases, one unilateral and one bilateral. Damage to the anterior dorsal nucleus was noted in 5 cases bilaterally, and in three cases unilaterally. In all cases the other anterior thalamic nuclei were almost completely spared.

Damage to other structures: The pattern of damage and sparing to mid-line thalamic nuclei, habenula, intralaminar nuclei, and the dentate gyrus was similar to that reported for the MD1 group in Chapter 2. In one MD3 case there was some cortical thinning at the point of needle tract entry with corresponding ventricular enlargement.

4.2.2.2 - Behavioural analysis - the results of one animal from the SHAM3 group were discarded due to severe noise interference during the critical day 10 test session. This left 9 animals in the SHAM3 group and 10 in the MD3 group. The performance of the two groups can be seen in Figure 22.

Time in arms - The time that each rat spent in the arm to be paired with food was recorded on session 1 and termed "cued1"; time spent in the non-baited arm on session 1 being termed "uncued1". Similarly the times that each rat spent in the two types of arms on session 10, after the 8 pairing sessions, were termed "cued2" and "uncued2". Thus it was possible to calculate a ratio score for each rat of cued1 / cued1 + uncued1 and compare this with cued2 / cued2 + uncued2 in order to assess the effect of pairing on preference for each

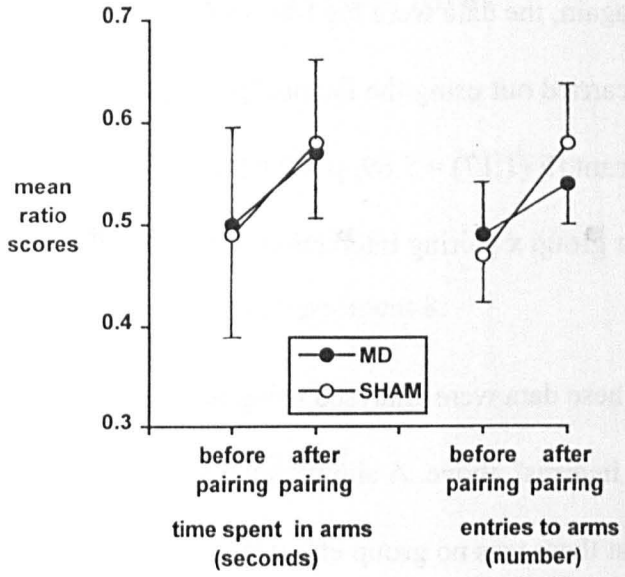

Figure 22 - Conditioned cue preference. The graph on the left shows how both groups of rats spent longer in one distinctive arm of the apparatus after it had been paired with food. The graph on the right depicts the significantly increased number of entries to "paired" arms made by both groups, although there was no significant difference between the groups. Vertical lines indicate standard error of the mean (and in all subsequent figures in Chapter 4).

rat. The data were log transformed prior to analysis as this is recommended when comparing proportional change (Hair, Anderson, Tatham, and Black, 1995). Analysis of variance was then carried out using the factors "pairing" and "group". The effect of pairing was significant [$F(1,17) = 5.69$, $p = 0.029$], but there was no group effect [$F(1,17) = 0.001$], nor group x pairing interaction [$F(1,17) = 0.113$].

The time that each rat spent in the arm to be paired with food was recorded on session 1 and termed "cued1"; time spent in the non-baited arm on session 1 being termed "uncued1". Similarly the times that each rat spent in the two types of arms on session 10, after the 8 pairing sessions, were termed "cued2" and "uncued2". Thus it was possible to calculate a ratio score for each rat of cued1 / cued1 + uncued1 and compare this with cued2 / cued2 + uncued2 in order to assess the effect of pairing on preference

for each rat. Once again, the data were log transformed (Hair et al., 1995). Analysis of variance was then carried out using the factors "pairing" and "group". The effect of pairing was significant [F (1,17) = 5.69, p = 0.029], but there was no group effect [F (1,17) = 0.001], nor group x pairing interaction [F (1,17) = 0.113].

Entries to arms - These data were analysed using the same method of calculating ratio scores as the "time in arms" above. A significant effect of pairing was found [F (1,17) = 7.29, p = 0.015], but there was no group effect [F (1,17) = 0.015] nor group x pairing interaction [F (1,17) = 0.023]. Total entries to arms on session 1 only were also compared between the groups to assess the difference in overall levels of exploratory behaviour (Figure 23). Student's t-test carried out on these data confirmed that the MD3 group made significantly more arm-entries than the SHAM3 group [t (18) = 2.77, p = 0.006).

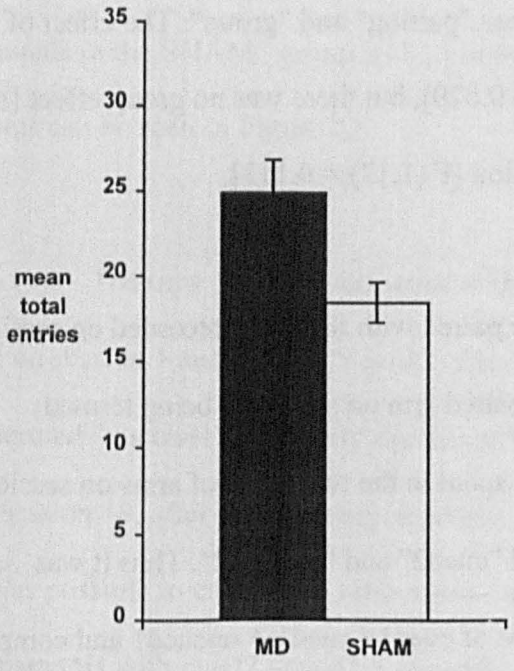

Figure 23 - Conditioned cue preference: exploratory behaviour as measured by entries into arms on the initial test session.

4.3 - EXPERIMENT 9 - Exploration of an Open Arena

4.3.1 - Method

4.3.1.1 - Subjects - were as described in Experiment 8.

4.3.2.2 - Test apparatus - was as Experiment 7.

4.3.1.3 - Testing procedure - was as Experiment 7. Testing began 9 days after the end of Experiment 8.

4.3.2 - Results

4.3.2.1 - Behavioural analysis - Scoring was carried out as in Experiment 7. Despite the higher scoring of the MD3 group on all three measures of arena activity (Figure 24), there was no significant difference between the groups: line crossings ($t = 1.09$, $df = 18$, $p = 0.15$; group means: MD3 = 96.83, SHAM3 = 75.30); inward line crossings ($t = 0.88$, $df = 18$, $p = 0.20$; group means: MD3 = 23.83, SHAM3 = 18.20); time spent in inner segments ($t = 0.20$, $df = 18$, $p = 0.42$; group means: MD3 = 91.00, SHAM3 = 70.40).

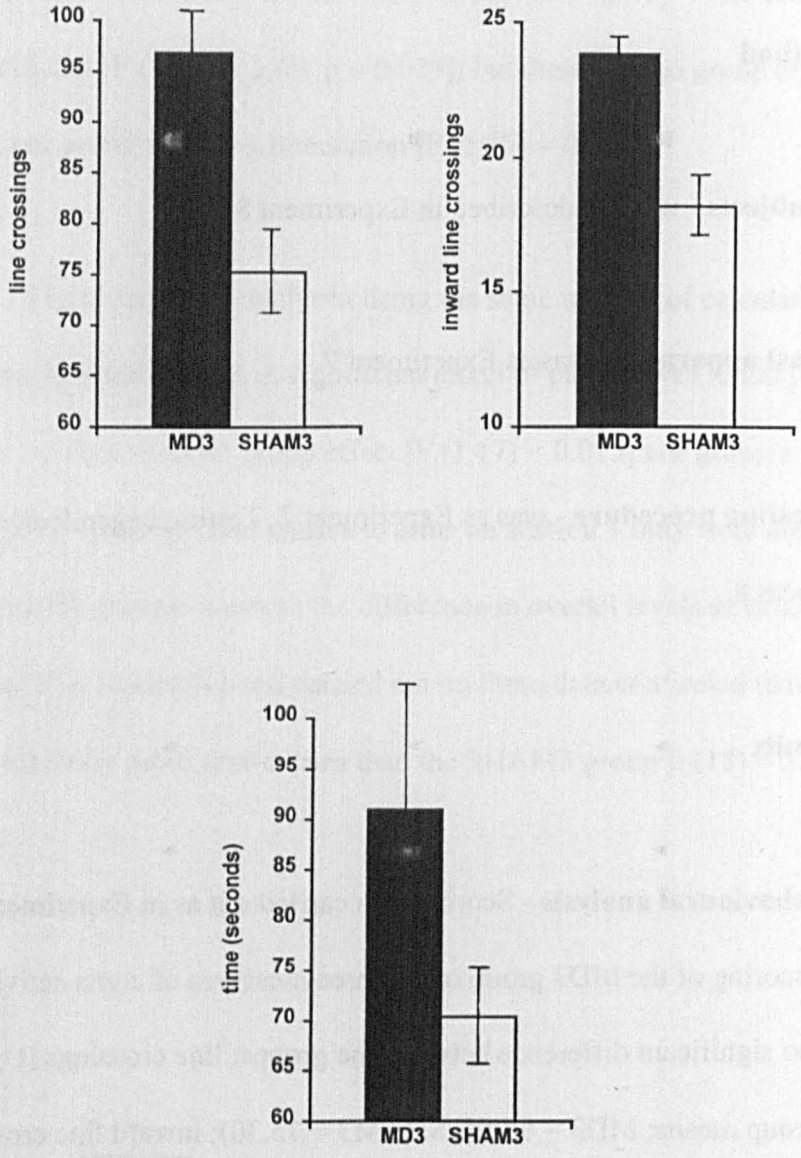

Figure 24 - Three measures of exploration activity in the open arena by the two groups. The upper left chart shows the mean number of times that each group crossed any of the lines marked on the arena floor, whilst the upper right shows the mean number of times that each group crossed lines whilst moving towards the centre of the arena and therefore away from the arena wall. The lower graph depicts the mean time that each group spent in the inner areas of the arena.

4.4 - EXPERIMENT 10 - Spatial Delayed Matching and Non-Matching to Place

4.4.1 - Method

4.4.1.1 - Subjects - were as described in Experiment 8.

4.4.1.2 - Test Apparatus - was as Experiment 3.

4.4.1.3 - Testing Procedure - Three sessions of pre-testing exposure to the maze were given 4 weeks after the end of Experiment 9. This was immediately followed by task acquisition. As in Experiment 3, each trial consisted of two stages; an 'information' run and a 'test' run. At the beginning of each trial, the experimenter placed three reward pellets (45mg) in one food well and closed off the other arm of the maze with a wooden block adjacent to the choice point. The rat was then placed at the start point and the guillotine door raised, so allowing the rat to run to the choice point. On this 'information' run, the rat was forced by the wooden block to enter a predetermined arm, where it was allowed to eat all three pellets. The rat was then picked up and returned to the start box. While the rat was retained in the start box, the experimenter baited the arm just visited by the rat with three reward pellets and also went through the motions of baiting the other arm without actually leaving any reward pellets in the food well. The experimenter then removed the wooden block and raised the guillotine door to allow the rat to run to the choice point for a second time (the 'test' run). The delay between the end of the 'information' run and the beginning of the 'test' run was approximately 10 seconds. On the test run both arms were open and the rat was allowed a free choice. The

rat was deemed to have made a choice when all four of its paws were in one arm. At this point the wooden block was placed behind it to prevent the rat changing its selection. If a correct choice was made, i.e. the rat entered the same arm as on the information run, the rat was allowed to eat the reward pellets before being returned to the start box for trial two. If an incorrect choice was made the rat was confined to the arm without food reward for 10 seconds before being returned to the start box. Thus, the training differed from Experiment 3 in that the rat was required to carry out *matching* the arm on the test run rather than *non-matching*. Each daily session consisted of six trials, and rats were tested in groups of 3 or 4 with each rat having one trial in turn. This spaced method meant that there was an inter-trial interval of 3 to 5 minutes.

When each rat had reached an acquisition criterion of 25 correct trials over five consecutive sessions (30 trials), it moved on to the next stage in which three delay conditions of 10, 20, and 40 seconds were interposed between the information and test runs. Two trials at each condition were given each day in a pseudo-random order, and each rat received 10 such test sessions followed by three sessions (i.e. 18 trials) of undelayed matching to sample.

The next stage began on the next test session and consisted of a reversal to non-matching to sample, i.e. rats were now rewarded for selecting in the choice phase the arm opposite to that entered in the sample phase. All other aspects of this stage of testing were identical to those used in the initial acquisition of the matching task. Testing continued until each rat had achieved the same acquisition criterion as in the matching to place stage (25 out of 30 trials).

4.4.2 – Results

4.4.2.1 - Behavioural analysis

Acquisition of matching to place task - Comparisons using the number of trials to the acquisition criterion (Figure 25) showed that the MD3 group were significantly slower to learn the task [two-tailed Student's t-test: t (18) = 3.25, p = 0.004; group means: MD3 = 151.6, SHAM3 = 109.8]. Errors to criterion (Figure 25) revealed a similar acquisition difference between the two groups [t (18) = 3.68, p = 0.002: group means: MD3 = 75.9, SHAM3 = 54.1].

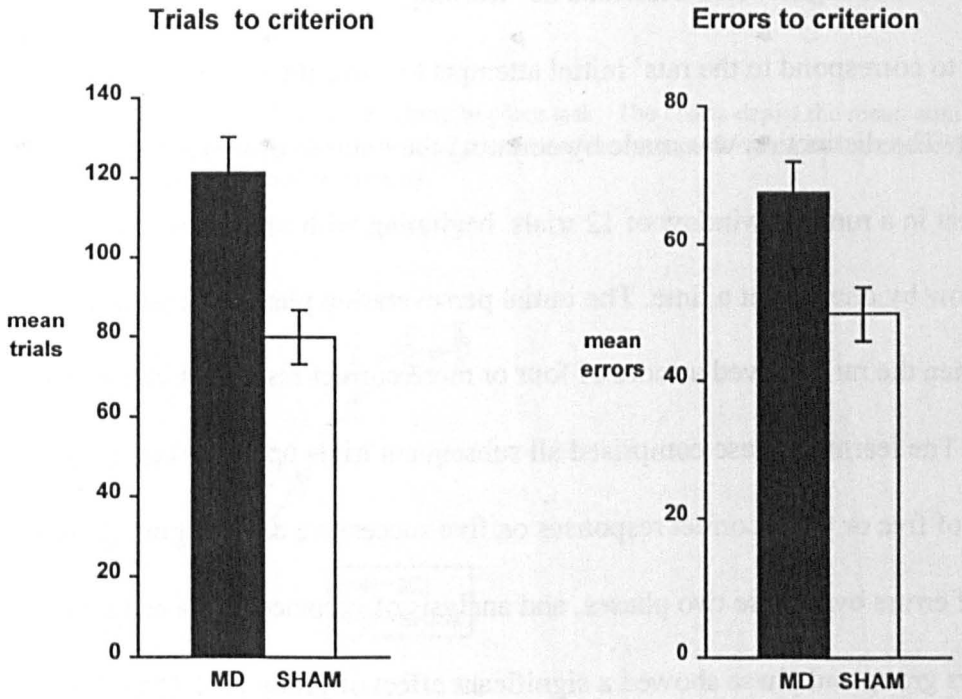

Figure 25. Acquisition of the T-maze matching to place task. Mean numbers of trials to criterion (left) and errors to criterion (right) are shown for both groups.

Both groups began the acquisition phase (Figure 27) by performing well below chance levels (mean group correct trials over the first 30 trials: MD3 = 5.1, SHAM3 = 6.6; chance = 15). Two-tailed t-tests confirmed that both groups' performance at this stage was significantly *below* chance [MD3: $t(9) = 9.71$, $p<0.001$; SHAM3: $t(9) = 13.3$, $p<0.001$]. Furthermore, at this initial stage there was no difference in the performance levels of the two groups [first 30 trials, $t(18)= 0.98$, $p = 0.34$, two-tailed].

To examine more closely the way that the two groups acquired the matching task, the acquisition process was divided into two phases. Very low scores, (3/12 or lower) were described as "perseveration" (probability of scoring 3/12 or lower = 0.073), whilst scores of 4/12 or higher were described as "learning". The "perseveration" scores were assumed to correspond to the rats' initial attempts to solve the matching task by non-matching. The distinction was made by counting the number of correct responses made by each rat in a running window of 12 trials, beginning with trials 1 to 12 and advancing the window by one trial at a time. The initial perseveration phase was deemed to have ended when the rat achieved a score of four or more correct responses in a window of 12 trials. The learning phase comprised all subsequent trials up to the task acquisition criterion of five or more correct responses on five successive days. Figure 26 shows the pattern of errors over these two phases, and analysis of variance of the error data using the factors group and phase showed a significant effect of group [$F(1,18) = 5.74$, $p = 0.028$], but not phase [$F(1,18) = 2.88$, $p = 0.107$] nor group by phase interaction [$F(1,18) = 1.31$, $p = 0.267$]. In spite of the lack of a significant interaction, analysis of the simple effects showed that the two groups differed on the "perseveration" measure ($p<0.05$) but not on the "learning" measure. For purposes of comparison, the acquisition

data are depicted in Figure 27 by blocks of trials only. Unlike the above method of analysis (Figure 26), this more conventional method clearly fails to show the important difference in the way that the two groups of rats learned the task.

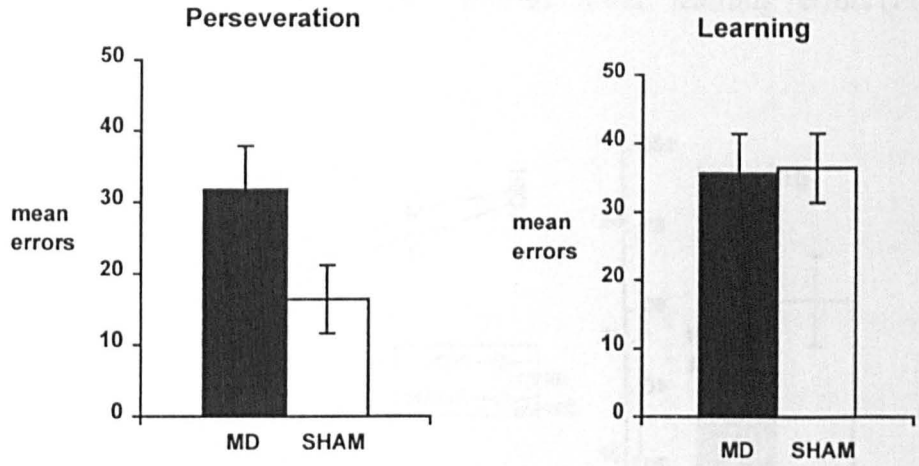

Figure 26 - Acquisition of the T-maze matching to place task. The charts depict the mean number of errors made during the two acquisition phases of *perseveration,* (rats performing below chance) and *learning* (rats performing at or above chance).

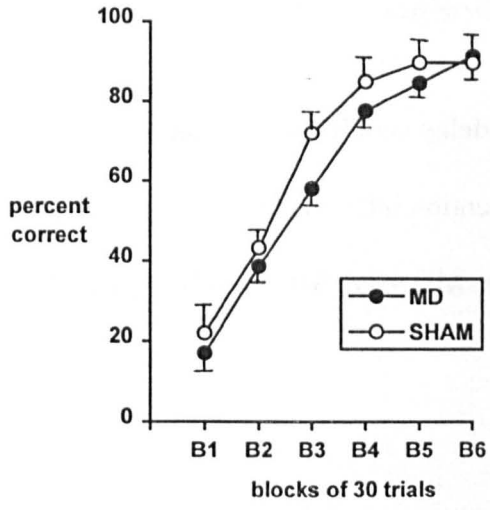

Figure 27 - Acquisition of the T-maze matching to place task. The graph shows the pattern of acquisition over six blocks of 30 trials. Rats that acquired the task before block 6 were assumed to continue performing at the same level as when they reached the acquisition criterion.

Matching to place with delays - The performance of the two groups over the three delay conditions is shown in Figure 28. Analysis of variance was carried out using the factors of group and delay. There was a strong effect of delay [$F(2, 36) = 14.05$, $p < 0.001$], but no effect of group [$F(1, 18) = 0.93$], nor group by delay interaction [$F(2, 36) = 0.66$].

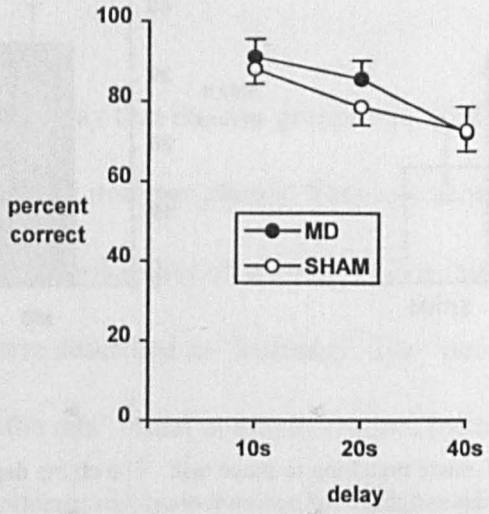

Figure 28 - T-maze matching to place performance. The graph shows the percentage of correct trials (maximum 20) performed by the two groups over three delay conditions following acquisition.

Normal matching to position - Following the delay conditions each rat underwent three sessions of matching to sample with 10 sec retention intervals (18 trials). Both groups performed the task at a high level (mean scores: MD = 16, SHAM = 16.3), and there was no evidence of a group difference.

Reversal to non-matching to place - Overall acquisition of the non-matching task by the two groups barely differed (group means of errors to criterion: MD = 39.7, s.e.m. = 2.45, SHAM = 38.0, s.e.m. = 3.39). However, analysis of the pattern of errors by two phases, carried out in the same way as for the matching to place task, showed that the

two groups differed in the way that they achieved acquisition (Figure 29). Analysis of variance using the factors group and phase showed no effect of group [$F(1,18) = 0.05$, $p = 0.82$], nor of phase [$F(1,18) = 0.01$, $p = 0.915$], but there was a significant group by phase interaction [$F(1,18) = 8.75$, $p = 0.008$]. This interaction arose from the MD animals making more "perseverative" errors and fewer "learning" errors (Figure 29).

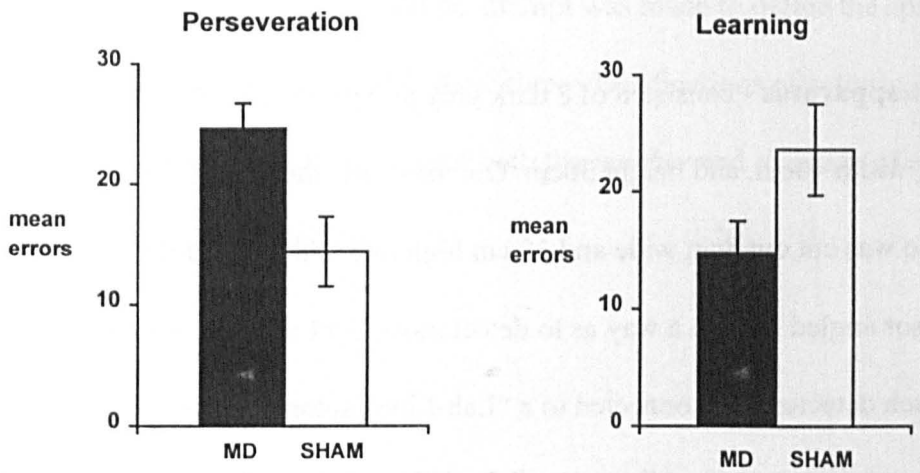

Figure 29 - Acquisition of the reversal to T-maze non-matching to sample task. The mean group error scores for the two groups have been divided into two phases: P = perseveration phase in which rats are performing below chance; L = learning phase in which rats are performing at or above chance.

4.5 - EXPERIMENT 11 - Automated Measures of Activity

4.5.1 - Method

4.5.1.1 - Subjects - were as described in Experiment 8.

4.5.1.2 - Test apparatus - consisted of 8 dark grey polypropylene boxes of dimensions: length 60cm, width 40cm, and height 30cm. On one short side of each box, 3cm from the top, a hole was cut out 8cm wide and 10 cm high into which was fitted a passive infra-red sensor angled in such a way as to detect movement over the whole of the box floor area. Each detector was connected to a "Lab-Linc" automated activity monitoring system (Colbourn Instruments, Allentown, P.A., USA). Galvanised steel mesh sheets were used as lids for the boxes and the floor of each box was covered with grade 14 wood flakes (Datesand Ltd, Manchester, UK) of the same type as that used as bedding in the rats' home cages. The boxes were arranged in a line on the floor of the test room, which was the same room as that used for Experiment 5.

4.5.1.3 - Testing procedure - Testing was begun two weeks after the conclusion of Experiment 10. The 20 rats were tested on one day in two groups of 8 and one group of 4. Each group of rats contained animals of both experimental groups, randomly assigned. Each rat was placed in a test box, the lid was secured, and activity recorded for a period of 72 minutes. At the end of the session the rats were returned to their home

cages and the bedding was replaced with clean wood flakes before the next group was tested. Three days after this first run a second, identical, run was completed.

4.5.1.4 - Data Collection - Activity was classified as either "no movement", "small movements", or "large movements". Small movements were defined as *less* than one second in duration and large movements as *more* than one second in duration. These measurements were of time only, and no attempt was made to define the spatial magnitude of the movements. Within these three classifications of activity, recordings were made over the 72 minute session of both the *number* and *duration* of each type of movement in 36 two minute bins.

4.5.2 - Results

4.5.2.1 - Behavioural analysis - For each rat on each test run there were 6 sets of information recorded (number and duration of no movement events, small movement events, and large movement events) and 36 recordings of each. The 36 recordings were grouped as 6 blocks of 6 and are shown in Figures 30 to 32.

As well as a consistency of performance throughout both the six different measures and two runs, Figures 30 to 32 also show the clear of time effect across the six blocks and apparently a close similarity between the performance of both groups. To confirm this, analyses of variance were carried out using the factors group and block and the results were as follows:

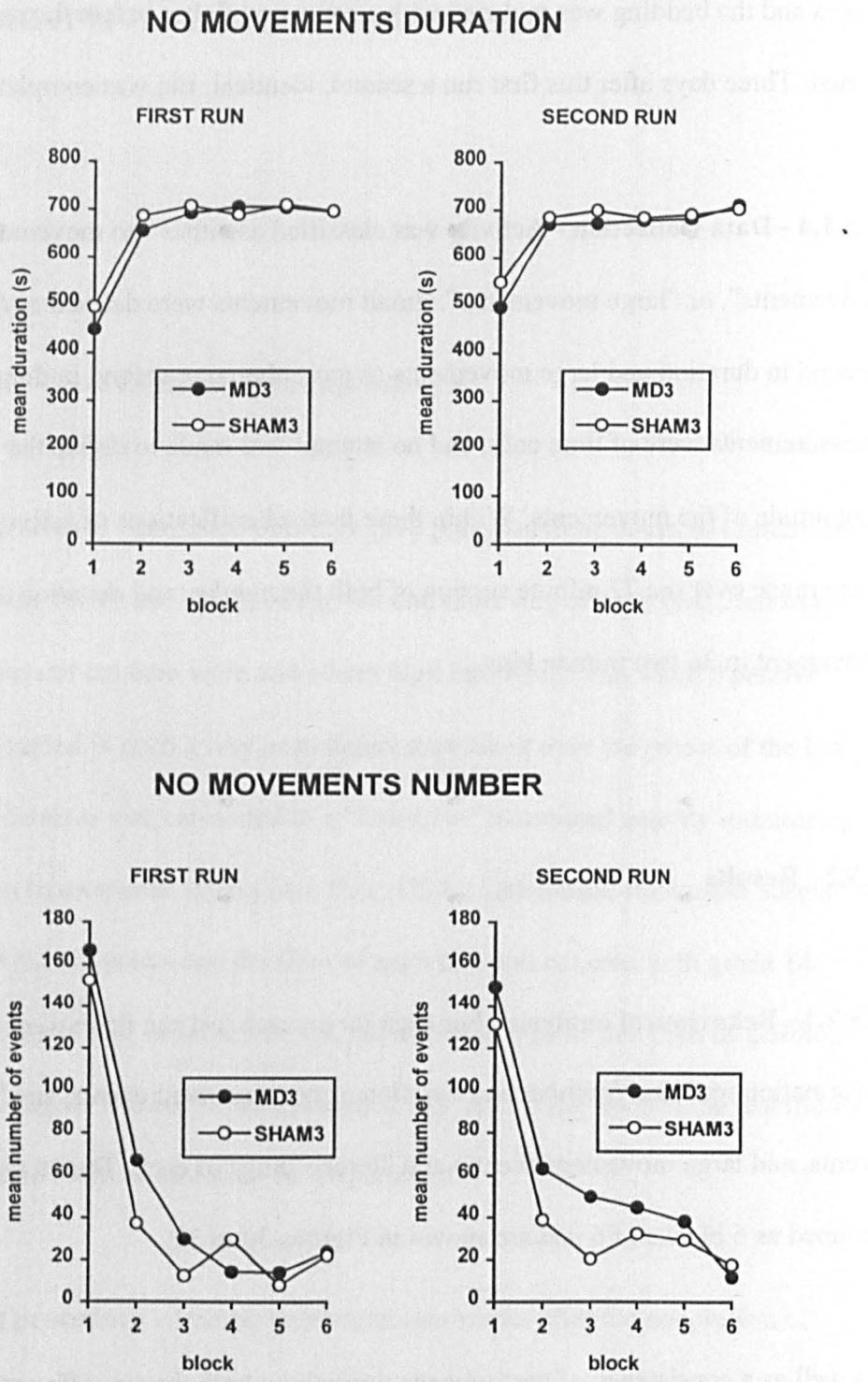

Figure 30 - automated activity monitoring. The upper two graphs show the duration of *no movement* events recorded for both groups over two 72 minute sessions, grouped into 6 blocks of 6 two minute bins. The lower two graphs represent the number of *no movement* events over the same sessions. Error bars have been omitted for the sake of clarity, as in all cases s.e.m. was below 5% of the value.

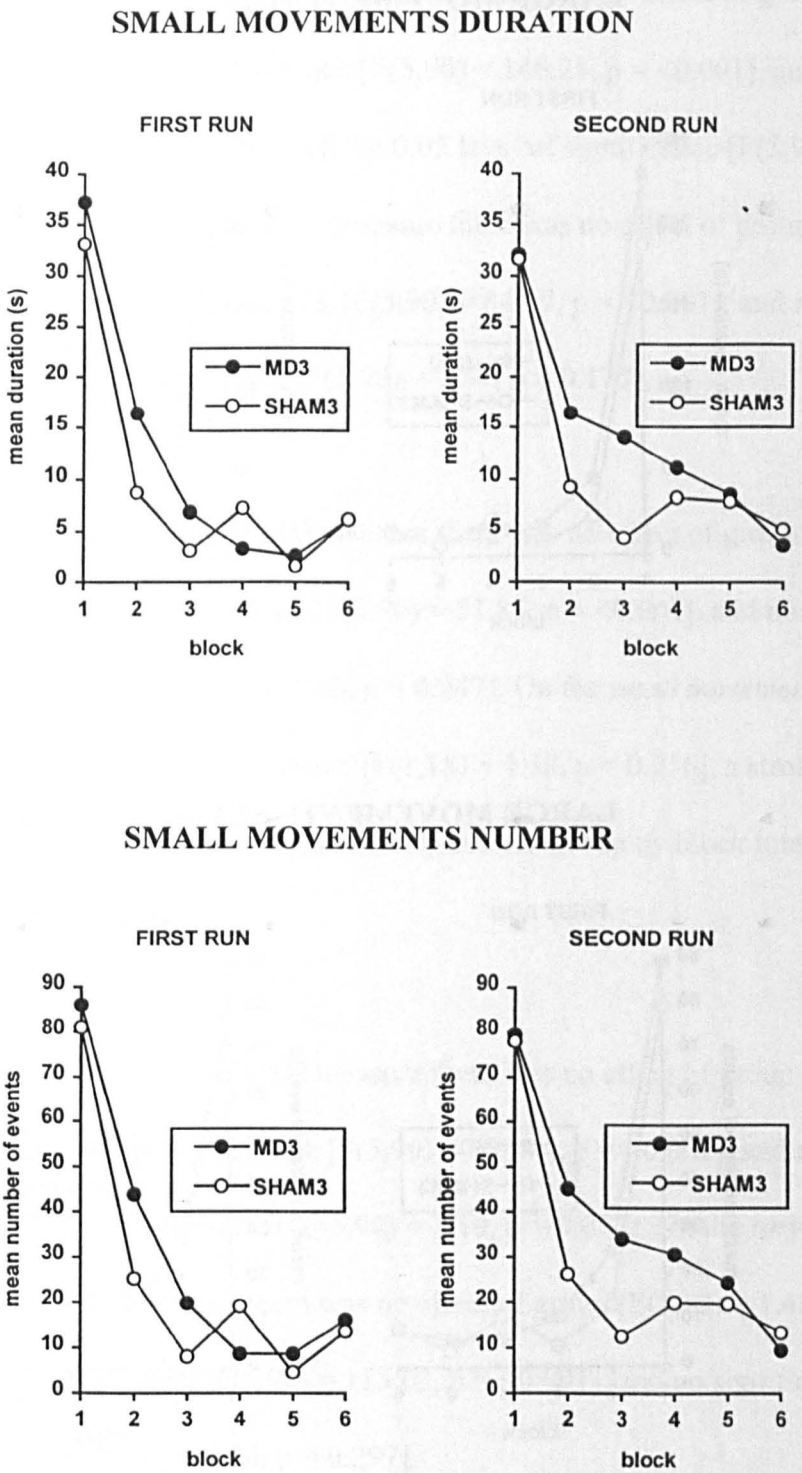

Figure 31 - automated activity monitoring. The upper two graphs show the duration of *small movement* events recorded for both groups over two 72 minute sessions, grouped into 6 blocks of 6 two minute bins. The lower two graphs represent the number of *small movement* events over the same sessions. Error bars have been omitted for the sake of clarity, as in all cases s.e.m. was below 5% of the value.

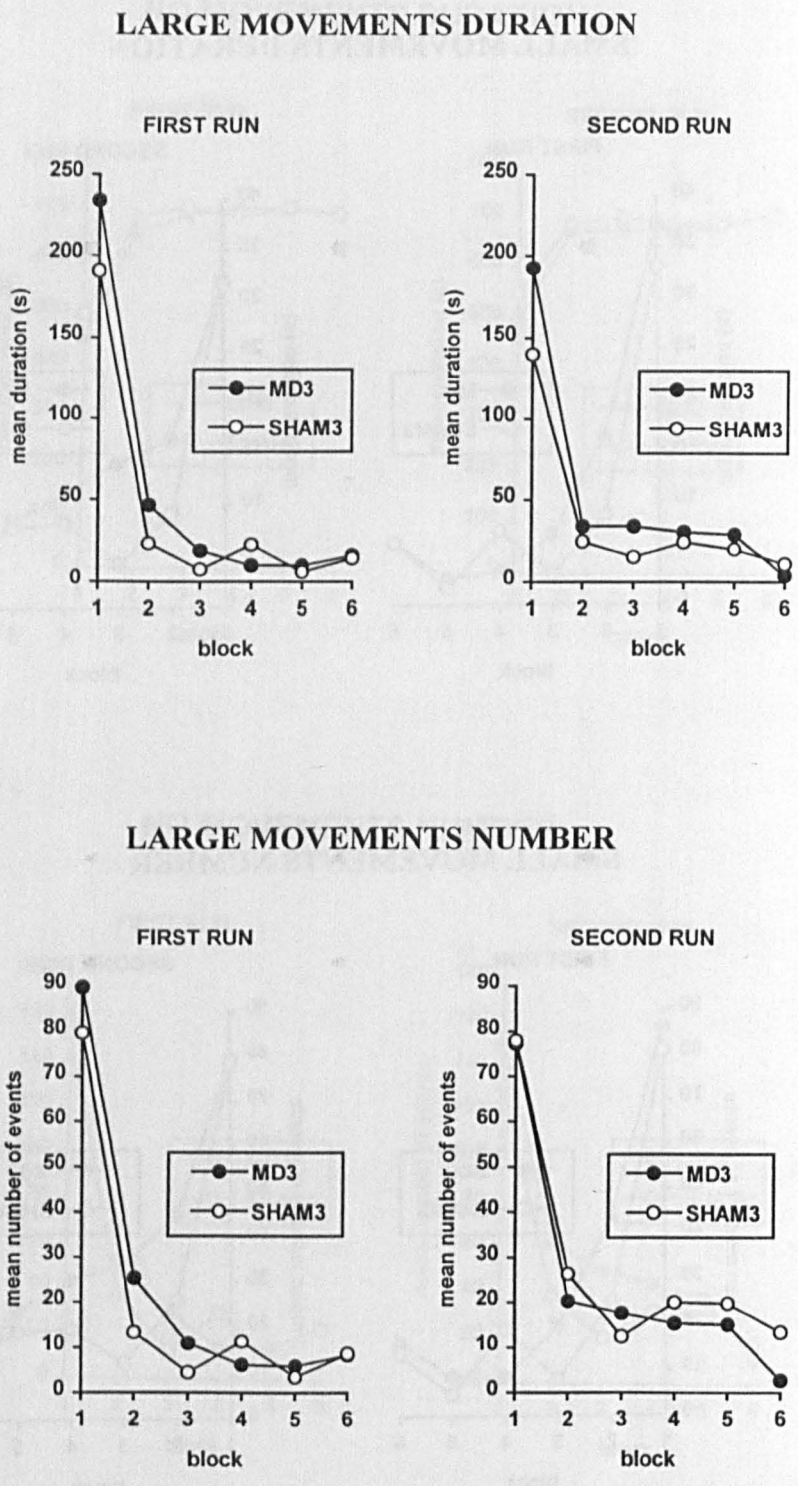

Figure 32 - automated activity monitoring. The upper two graphs show the duration of *large movement* events recorded for both groups over two 72 minute sessions, grouped into 6 blocks of 6 two minute bins. The lower two graphs represent the number of *large movement* events over the same sessions. Error bars have been omitted for the sake of clarity, as in all cases s.e.m. was below 5% of the value.

First Run - On the *no movement duration* measure there was no effect of group [F(1,18) = 2.25, p = 0.151], a strong effect of block [F(5,90) = 146.25, p = <0.001], and the group by block interaction failed to reach the 0.05 level of significance [F(5,90) = 2.22, p = 0.059]. On the *no movement number* measure there was no effect of group [F(1,18) = 1.48, p = 0.24], a strong effect of block [F(5,90) = 84.67, p = <0.001], and no significant group by block interaction [F(5,90) = 1.57, p = 0.175].

On the *small movements duration* (<1s) measure there was no effect of group [F(1,18) = 1.30, p = 0.27], a strong effect of block [F(5,90) = 51.85, p = <0.001], and no significant group by block interaction [F(5,90) = 1.36, p = 0.247]. On the *small movements number* (<1s) measure there was no effect of group [F(1,18) = 1.38, p = 0.256], a strong effect of block [F(5,90) = 52.22, p = <0.001], and no significant group by block interaction [F(5,90) = 1.48, p = 0.204].

On the *large movements duration* (>1s) measure there was no effect of group [F(1,18) = 2.23, p = 0.152], a strong effect of block [F(5,90) = 142.74, p = <0.001], and no significant group by block interaction [F(5,90) = 2.10, p = 0.072]. On the *large movements number* (>1s) measure there was no effect of group [F(1,18) = 1.48, p = 0.239], a strong effect of block [F(5,90) = 115.03, p = <0.001], and no significant group by block interaction [F(5,90) = 1.24, p = 0.297].

Second Run - On the *no movement duration* measure there was no effect of group [F(1,18) = 2.68, p = 0.119], a strong effect of block [F(5,90) = 76.66, p = <0.001], and no significant group by block interaction [F(5,90) = 1.79, p = 0.123]. On the *no*

movement number measure there was no effect of group [F(1,18) = 2.39, p = 0.14], a strong effect of block [F(5,90) = 45.57, p = <0.001], and no significant group by block interaction [F(5,90) = 0.91, p = 0.481].

On the *small movements duration* (<1s) measure there was no effect of group [F(1,18) = 1.33, p = 0.263], a strong effect of block [F(5,90) = 26.63, p = <0.001], and no significant group by block interaction [F(5,90) = 1.32, p = 0.262]. On the *small movements number* (<1s) measure there was no effect of group [F(1,18) = 1.96, p = 0.178], a strong effect of block [F(5,90) = 27.77, p = <0.001], and no significant group by block interaction [F(5,90) = 1.23, p = 0.303].

On the *large movements duration* (>1s) measure there was no effect of group [F(1,18) = 2.82, p = 0.110], a strong effect of block [F(5,90) = 76.98, p = <0.001], and the group by block interaction just failed to reach the 0.05 level of significance [F(5,90) = 2.30, p = 0.052]. On the *large movements number* (>1s) measure there was no effect of group [F(1,18) = 2.95, p = 0.103], a strong effect of block [F(5,90) = 68.92, p = <0.001], and no significant group by block interaction [F(5,90) = 1.66, p = 0.152].

4.6 - Discussion

Experiment 8 examined the way that rats relate reward to place, and asked whether rats with MD lesions behave differently in making this relationship. The experiment made the animals' immediate environments visually distinctive, and measured whether their

preference for place was influenced by previous association with food. The two groups did not differ on either of the two preference measures (time in arms, number of entries to arms), indicating that the difference in the way rats with lesions to the mediodorsal thalamus respond in learning and memory tasks does not lie in the way that they are relating reward and place. This result differs markedly from the findings of McAlonan et al. (1993), who found that ibotenic acid damage to MD in rats completely abolished the acquisition of conditioned place preference. The sizes of the lesions in the two studies appear comparable and the basic task procedures are similar. For this reason the difference in outcome is of interest, but remains unresolved. Despite this, there is no evidence that the present lesion group failed the matching task (Experiment 10) as they were insensitive to the reinforcers or were unable to associate a reinforcer with a specific cue.

Session 1 in Experiment 8, in common with many tests of learning and memory in rats, involved the animals moving around voluntarily in an unfamiliar environment. In this instance data were collected of the number of entries rats made into maze arms, and it was thus possible to compare the total number of entries made by the two groups as a measure of exploratory activity. As might be predicted from previous experiments, the MD3 group made a greater number of entries, further adding evidence to the notion that MD rats are less inhibited in carrying out exploratory activity under test conditions. McAlonan et al (1993) had also carried out several measures of locomotor activity during a similar conditioned place preference study with MD rats and had found "tendencies" and "trends" towards increased activity in the MD rats on the final session which did not, however, reach the $p<0.05$ level of significance. The findings in the

present study, however, are particularly interesting since in this instance the animals were completely experimentally naive; session 1 representing the very first time that they had experienced an environment outside their home cage post-operatively. Hyperactivity was also investigated in Experiment 9 by repeating the arena exploration test of Experiment 7 that had found an increase in exploratory behaviour in the MD2 group, albeit based on somewhat low numbers of animals in the groups. Although there was no evidence that rats in the lesion group were hyperactive in this open field test, other studies have reported an increase in exploration in a variety of test conditions following lesions of the dorsomedial thalamus (Beracochea et al., 1989; Kolb, 1984; Kolb et al., 1982). There is therefore still the possibility that rats with MD lesions may behave with raised levels of activity during maze testing.

The T-maze tests in Experiment 10 revealed a consistent dissociation within the behavioural effects of lesions in nucleus medialis dorsalis. Thus, there was no evidence that the thalamic lesions disrupted the ability of the rats to distinguish which arm had been most recently visited (working memory) but the same rats were impaired at shifting from a preferred response rule. Evidence of their intact spatial working memory comes from the normal performance of the lesion group over retention delays (20s and 40s) that were of sufficient length to preclude possible ceiling effects. Furthermore, the rats with medialis dorsalis lesions persistently performed *below* chance at the outset of matching training, at a level that was comparable to that of the control rats. This unusually poor level of performance is to be expected as normal rats have a very strong, innate bias to alternate in the T-maze (Richman and Dember, 1986), i.e. to turn in the opposite direction to that rewarded in the matching condition. Thus the highly

significant performance *below* chance reflects the comparable ability of both groups of rats to remember the most recently visited arm.

This sparing of spatial working memory is consistent with recent studies that have helped to distinguish the contributions of other nuclei or tracts adjacent to medialis dorsalis. While selective lesions of the dorsomedial thalamus, similar to those in the present task, have little or no effect on non-matching to place tasks (Hunt and Aggleton, 1991; Kessler et al., 1982; Neave et al., 1993), bilateral damage to the anterior thalamic nuclei will produce severe, lasting deficits on the same tasks (Aggleton et al., 1996; Aggleton et al., 1995a). These deficits are still present when the lesions are placed in subfields of the anterior thalamic nuclei (Aggleton et al, 1996; Byatt and Dalrymple - Alford, 1996), highlighting the need to minimise encroachment into these nuclei. Likewise, there is evidence that cutting the mamillothalamic tract is sufficient to impair T-maze alternation (Thomas and Gash, 1985). Thus, although some studies have reported that lesions in the dorsomedial thalamic region can disrupt spatial working memory (Stokes and Best, 1988, 1990a,b,c), careful analysis indicates that in many of these instances the lesions have encroached rostrally to involve the anterior ventral and anterior medial thalamic nuclei.

Despite their intact spatial working memory, the MD animals were impaired at acquiring the matching rule. One possible explanation is that the lesion has a general effect on the ability of rats to learn the reference memory component of a task, in this case, the rule to match. A general failure to learn task rules does not, however, seem likely as the same rats were readily able to learn the non-matching to place task once

they had ceased perseverating on the original rule (Figure 29). Similarly, performance during the "learning phase" of the matching task appeared normal (Fig. 27). Also, previous studies have found that thalamic lesions made in the same manner do not disrupt the ability to acquire the delayed non-matching to position task in an automated chamber (Neave et al., 1993). Similarly, medial dorsal thalamic lesions made by electrolytic means do they affect the ability to learn the position of a platform in the Morris water maze (Kolb et al., 1982). In both instances, medialis dorsalis lesions spared the learning of a response rule in a spatial task.

Having excluded these other possibilities, a logical assumption may be that lesions of nucleus medialis dorsalis lead to a selective deficit in the ability to switch from a preferred strategy to a new strategy. The initial acquisition deficit arose from a failure to switch from an innately preferred strategy (non-matching) to a new strategy (matching), as reflected by a specific increase in "perseverative" errors. Similarly, the abnormal pattern of errors in the subsequent reversal to a non-matching rule also arose from an excess of "perseverative" errors. It is most unlikely that these failures arose from an inability to shift attention to the critical stimulus dimension as in a study of rats with pre-frontal cortical lesions (Dias, Roberts, and Robbins, 1996) since the matching rule uses the same class of stimuli as the preferred, non-matching rule. Consequently, the deficit can be better characterised as a failure to shift response rules. This would also explain why lesions of nucleus medialis dorsalis were seen to have little or no impact on the standard radial arm maze procedure in Chapter 2, as this task accords with natural foraging strategies. In contrast, deficits were found when the procedure was modified in

Chapter 3 so that the selection of some arms is never rewarded as the rat now has to withhold the normal foraging strategy of visiting all arms.

Experiment 11 showed very clearly that the MD3 group were not more active than the SHAM3 group when activity is taken as any sort of movement. The experimental method broke down *measured movement* into a range of *durations* and expressed the data as both number of events and duration of those events. This negative finding is important, since it seems to indicate that the increased levels of activity reported in this and the previous chapter are not merely a result of increased global levels of locomotor activity.

CHAPTER FIVE

Discussion

5.1 - Review of Experimental Findings

The eleven experiments in this study were undertaken using three cohorts of rats at separate times. The type of testing performed on each cohort was deliberately chosen to avoid interference effects between different classes of tasks and behaviour. Especially important in this respect was the use spatial tasks, and for this reason the spatial tasks were dispersed across the three cohorts. It would seem useful, then, to review the findings functionally rather than in chronological order, so that the effects of MD lesions on classes of tasks may be more apparent.

5.1.1 - *Visual recognition and discrimination* – Two types of experiment used visual cues to investigate learning and memory in rats with MD lesions. The spontaneous recognition of objects, which depends upon the normal propensity of rats to attend more to novel objects than to ones they have seen before, was unaffected by MD lesions. This test is regarded as a purer test of working memory than those tests that require the rats to learn a rule via food reward in order to apply working memory. One such test is the Y-maze delay non-match to sample task as used in the previous study (Hunt and Aggleton, 1991) that the present study builds upon. Like the present study, the previous one used a neurotoxin to produce MD lesions, and found that this produced no impairment in working memory, even over incremental delay conditions. The MD group was, however, significantly impaired in acquiring the rule-learning component of the task. This is consistent with the findings of the present study, confirming that MD lesions produced with neurotoxins do not affect non-spatial working memory, either spontaneous or trained to food reward.

MD lesions did not impair the rats' visual discrimination ability when the rat was rewarded with food for selecting the correct, but arbitrarily chosen, object. The MD rats

were, however, impaired in their ability to discriminate objects that differed only in the configuration of their compositional elements. This may be of interest since no one site in the brain has been specifically linked with configural learning, although prefrontal cortex or perirhinal areas would seem to be likely areas for such function. In a further series of object discrimination trials run concurrently, the rats with MD lesions showed impairment only on one (8 trial) condition.

5.1.2 - *Spatial learning and memory* – Experiments were carried out in a radial arm maze and a T-maze to investigate spatial learning and memory using several variants of the basic tasks in each. These spatial test results were carefully examined to include only those rats in which the lesions were confined to MD, as some lesions were seen to extend substantially into the anterior thalamic nuclei. The data from these individuals, all in cohorts 1 and 2 and therefore affecting the radial arm maze tests, were analysed separately, revealing anterior thalamic damage to be the cause of impairments in acquisition and performance of RAM tasks. The implications of this finding are evaluated in section 5.2 below.

The first group of tests exploits the normal ways that rats move around in space in order to find food. In the present study these are the T-maze non-matching task and the radial arm maze (RAM) tasks in which all arms are baited with food. These tasks represent "purer" tests of working memory, since they do not require the rat to learn a new strategy or to suppress a preferred behaviour pattern. MD lesions did not bring about any deficit in T-maze non-matching to sample, confirming the findings of the previous study (Hunt and Aggleton, 1991). In this previous study, rats with MD lesions made with a neurotoxin learned the same non-matching task and performed it under incremental delay conditions without impairment. Acquisition of the basic RAM task and its performance with a delay

condition failed to reveal any deficit in the MD group, although a deficit was seen when the maze was turned during the delay, requiring a shift in strategy from the rats. It should be noted that two cohorts of rats had performed acquisition of the basic RAM task (all arms baited) separately using an identical method, giving two sets of equivocal results that were potentially misleading. The data from the two cohorts was combined to give the acquisition result above.

The second group of tasks specifically required the rats to abandon innate or previously learned strategies and adopt contrary ones in order to obtain the food rewards. The MD rats were significantly impaired in learning such a matching strategy in the T-maze, contrasting with the way they spontaneously perform non-matching in the task. Whilst they appeared subsequently unimpaired in performing a rule reversal to non-matching after having learnt this matching rule, the MD rats differed in the way they learned the task, showing a significant increase in perseverative errors. This difference emphasises their apparent preference for pre-existing rules when responding to a need to change behaviour. Similarly, after acquiring the basic RAM task that utilises only working memory (all arms baited), the MD rats were impaired when the task was changed to allow measurement of reference memory (some arms baited). This task again required the rats to stop using their preferred non-matching rule, since this would lead to the selection of never-rewarded arms.

5.1.3 – *Conditioned place preference* – The rats with MD lesions showed no difference from the operated shams in the way that they associated visually distinctive places with food. This normal pattern of response to reinforcement in a spatial context is an important factor in interpreting the results above.

5.1.4 – *Activity and exploration* – Exploration was explicitly measured in response to informal observations made during tests of learning and memory. These observations had indicated the possibility that exploratory behaviour may have been at a higher level in MD rats during testing. In addition, it was also possible to analyse activity data from some of the learning and memory tests above.

The first test of exploratory behaviour specifically run for the purpose was a test of emergence from a dark, enclosed space into a brightly-lit open area. Rats with MD lesions were always faster to emerge, but analysis showed that the difference in time was marginal and narrowly fell short of significance.

Two cohorts of rats were tested separately on the same measures of arena exploration. Although the rats with MD lesions appeared to be more exploratory throughout both of these sessions, the results were ambiguous. Since the test conditions on both sessions were identical, and since the performance of the two groups of sham operated animals did not differ (t values on all three measures = <1), it is possible to combine the results from the two sessions into a single analysis in order to increase its statistical power. The group numbers, thus combined, become MD2/3 = 16; SHAM2/3 = 20. As can be seen from Figure 32, the MD lesion group scored higher on all three measures of arena activity (group means: line crossings MD2/3 = 89.38, SHAM2/3 = 76.35; inward line crossings MD2/3 = 20.37, SHAM2/3 = 17.25; time spent in inner segments MD2/3 = 79.81s, SHAM2/3 = 70.25). Student's t-tests (one tail) on these data confirm that the combined lesion group differed significantly from the combined surgical sham groups on both of the line-crossing measures, but not on time spent in the inner segments of the arena (all line crossings $t = 2.63$, $df = 24.01$, $p = 0.008$, adjusted for inequality of variance using Levine's test; inward line crossings $t = 1.99$, $df = 34$, $p = 0.03$; time in inner area $t = 0.94$, $df = 34$, p

= 0.18). Thus, the combined analysis reveals greater exploratory activity in the rats with MD lesions.

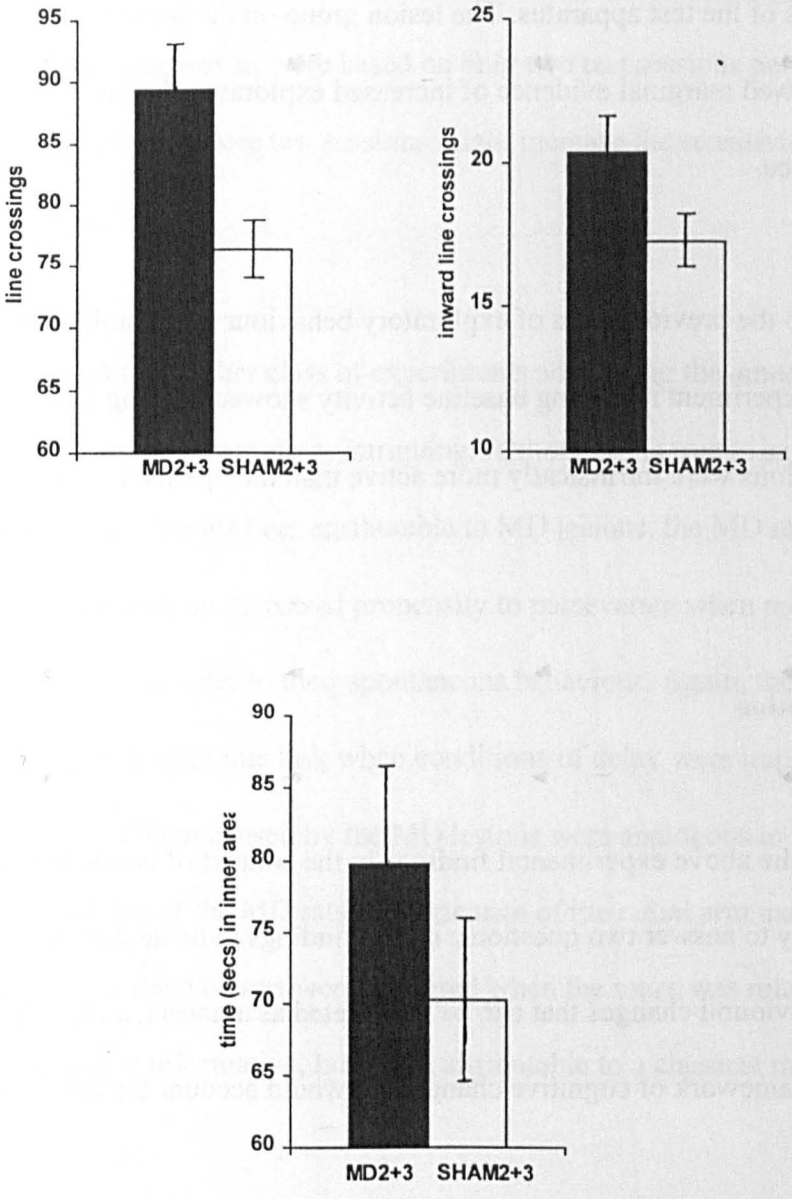

Figure 33 - Three measures of exploration activity in the open arena by the combined groups of cohorts 2 and 3. The upper left chart shows the mean number of times that each group crossed any of the lines marked on the arena floor. The upper right chart shows the mean number of times that each group crossed lines whilst moving towards the centre of the arena and therefore away from the arena wall. The lower graph depicts the mean time that each group spent in the inner areas of the arena. Vertical lines indicate standard error of the mean.

Observations of exploratory behaviour were also made during the conditioned place preference experiment. These showed that the rats with MD lesions displayed more exploratory behaviour than the operated sham rats when first placed in the unfamiliar surroundings of the test apparatus. The lesion group on the spontaneous object recognition test also showed marginal evidence of increased exploratory behaviour, but this fell short of significance.

In contrast to the previous tests of exploratory behaviour in unfamiliar environments, an automated experiment recording baseline activity showed nothing to suggest that the rats with MD lesions were intrinsically more active than the operated sham rats.

5.2 – Evaluation

To evaluate the above experimental findings in the context of issues laid out in Chapter 1, it is necessary to answer two questions: do the findings indicate that the MD lesions were causing behavioural changes that can be interpreted as amnesia, and, if not, is there an alternative framework of cognitive change that would account for these results?

Tests of object discrimination and recognition were one class of experiment that addressed mnemonic ability directly, and no clear amnesic pattern of performance emerged from them. The MD rats were able to acquire a simple object discrimination normally, and performed concurrent object discriminations, shown to be sensitive to memory deficits in rats with damage to the hippocampus and related regions (Aggleton et al, 1991; Rothblat et al, 1993; Wible et al, 1992), with only a mild, initial impairment on the eight trials per day condition. It can be argued that these tasks trained the rats to use a food reward "rule"

to demonstrate the discrimination of objects and that rats' performance on this task owes more to rule-learning than to their discrimination abilities. In the task that looked at *spontaneous* object recognition, described as being a purer assay of working memory (Ennaceur and Delacour, 1988), there was no evidence at all of deficit in the rats with MD lesions. These latter results, however, were based on only two test sessions per delay condition, and it is possible that more test sessions might increase the sensitivity of the test and give different results.

In memory for spatial tasks, the other class of experiments addressing the amnesia question more directly, the results are more intriguing. Although it is true to say that there are no clear mnemonic impairments here attributable to MD lesions, the MD rats did show some learning difficulties, with an increased propensity to perseverate when required to learn a matching task that is counter to their spontaneous behaviour. Again, though, there was no impairment on performing this task when conditions of delay were imposed, as might be expected if the problem caused by the MD lesions were analogous to an amnesic syndrome. This was also true of the MD rats' performance of the radial arm maze task when run with delays. That the MD rats were impaired when the maze was rotated during the delay on this task is very informative, but is not attributable to a classical mnemonic deficit.

An interesting but unintentional effect of the lesion-making procedure was that some rats from the lesion groups in the radial arm maze experiments had damage that encroached substantially into the anterior thalamic nuclei. The clear deficits in task acquisition and performance shown by this sub-group are more consistent with impairments analogous with amnesia, in contrast to the results from the rats with lesions restricted to MD. The finding is important for three reasons. First, it would have been badly misleading for these

deficits to have been attributed to MD damage, and it is quite possible that other studies reporting spatial memory deficits may have done this; the reporting of lesion extent being sometimes far from explicit. Second, it reinforces the view of other studies that the anterior thalamic nuclei play a critical role in spatial learning and memory (Aggleton and Brown, 1999, Aggleton et al, 1995; Byatt and Dalrymple-Alford, 1996). Finally, it emphasises that the testing methods used were sensitive to spatial memory impairments, and thus further helps to confirm that large lesions confined to MD do not produce deficits in spatial working memory. These results highlight the probability that what is being seen in MD rats should not be interpreted as amnesia. One possible interpretation is that this loss of deficit in the remaining purely MD lesion group is the result of lesser, ineffectual lesions of MD. Examination of the histological results, however, confirm that the lesions in the remaining MD group are substantial, affecting almost all of MD's extent, and therefore the behavioural results from this sub-group may be taken as a true reflection of MD function in rats.

Thus, since the behavioural changes in rats with MD lesions cannot be interpreted as amnesic effects, an alternative inference must be made to describe better the cognitive changes that have been observed throughout the series of experiments. One interpretation of the MD rats' pattern of behaviour in many of the tests could be that it is analogous with behaviour more usually associated with damage to the prefrontal cortex (PFC). This proposition would seem unsurprising, given MD's close anatomical links with PFC (Krettek and Price, 1977; Groenewegen, 1988; Ray and Price, 1992). A direct testing of the analogy between the MD impairments and PFC damage in rats is provided by Dias and Aggleton (unpublished results, submitted J. Neuroscience, 1999). The authors report that medial PFC lesions bring about disruption of the ability to shift response rules to match to

place in a T-maze with preserved spatial working memory in a strikingly similar way to that seen in the present study following MD lesions.

A framework that may be used to relate this pattern of MD deficits to PFC functions is provided by Wise, Murray, and Gerfen's (1996) analysis of PFC functions in monkeys. The authors have categorised PFC functions into different levels of behaviour-guiding rules, and propose that the function of PFC in learning and memory centres on the learning of new behaviour-guiding rules and the rejection of old rules. Lower order and higher order rules are differentiated in this model, which also proposes that different subregions of PFC are involved in mediating the two orders of rules. The authors also recognise a third or highest order of rules that deals with temporal ordering. Raggozino, Wilcox, Raso, and Kesner (1999) have tested this proposition in rats and suggest that the rat PFC may be similarly differentiated in function. This study (Raggozino et al., 1999) presents evidence of the prelimbic and infralimbic areas of PFC being involved in the selection of higher order rules, whilst raising the possibility that the lateral orbital area and/or the agranular insular area would be correspondingly involved in lower order rules.

Thus it seems necessary to measure this hierarchy of rule mediation against the pattern of results observed in the present study, if the behaviour of MD rats is to be seen as analogous with PFC dysfunction. The classes of task that in the present study fall into Wise et al's (1996) lower order of abstraction would be object recognition, object discrimination, and place recognition (T-maze non-matching and radial arm maze tasks). The MD lesions failed to produce impairments in any of these classes of task. The higher order of abstraction tasks in the present study would be represented by attention to and memory of stimulus components (configural object discrimination) and the application of abstract rules based on spatial information (T-maze matching). The MD rats were impaired

in both of these tasks in the present study. It therefore appears that the effects of the MD lesions are consistent with impairments in mediating the higher level of cognitive abstraction, but not in the lower level.

This lack of cognitive flexibility in the MD rats is also analogous with dysfunction in primates with frontal damage (Wise et al., 1996, Dias, Robbins, and Roberts, 1996b), and is further seen to extend to humans with frontal damage (Dias, Robbins, and Roberts, 1996a, 1997). The disinhibition in humans with PFC damage results in behaviour being guided by previously acquired responses that are inappropriate to current circumstances, a dysfunction that is very similar to that noted in the MD rats in the present study. The authors (Dias et al., 1996a) also go on to suggest that damage to different areas of PFC is responsible for different types of inhibition loss (attentional selection and affective processing).

The MD lesions in the present study also seemed to bring about increased levels of activity, or rather decreased inhibition of exploratory behaviour in the study. This was noted both in tests specifically designed to test for such behaviour (arena exploration, emergence), and during other tests of learning and memory (spontaneous object recognition, conditioned place preference). This again is analogous with PFC dysfunction in rats (Kolb, 1984), where hyperactivity in some situations has been seen to result from their apparent inability to initiate new response strategies in novel situations. Kolb (1984) also reports impaired performance in rats with PFC lesions on other tasks requiring changes in behaviour, which again is entirely consistent with the impairments described in this study.

5.3 - Conclusions

5.3.1 - Conclusion 1 – MD damage in rats does not cause direct deficits in learning and memory. No evidence was found in the present study to support the proposition that lesions confined to MD bring about direct impairments to learning and memory systems. A number of factors may explain why other studies have ascribed such deficits to MD lesions. The first such explanation is that the physical methods of lesion making (aspiration, radio frequency, electrolysis) that have been widely used in the past have, by their nature, failed to restrict their effects to MD. Damage to fibre tracts such as the internal medullary lamina is usual with this method, as is associated damage to the intralaminar nuclei. Both of these structures may play a more critical role than MD in mediating learning and memory in rats (Burk and Mair, 1998). The second explanation is that lesions, especially neurotoxin lesions, often stray into the adjacent anterior nuclei, as in fact happened in the present study. If the results from such subjects are included in the MD lesion groups, the results are likely to be misleading. Many studies describing deficits following MD lesions fail to describe the extent of the lesions sufficiently adequately for this possibility to be discounted. The present study distinguished those subjects in which lesions extended significantly into the anterior thalamic nuclei, and consequently was able to add to the evidence that the anterior nuclei are critical in spatial learning and memory.

5.3.2 – Conclusion 2 – The indirect effects of MD lesions on learning and memory are akin to those effects usually associated with damage to the prefrontal cortex in that the lesion groups showed deficits in rule switching abilities. This is distinct from inflexibility in attentional switching, being within the same sensory mode and domain of allocentric spatial information, i.e. the rats were able to continue to use their innate preference for spatial cues. Further, the results from the present study, when interpreted in this way,

indicate that MD mediates the "higher" orders of frontal lobe cognition functions and not the lower orders.

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