Contribution of the Laterodorsal, the Anteroventral, and the Anterior Thalamic nucleus to tasks of Spatial and Non-Spatial working Memory

Submitted in fulfillment of the requirements for the Degree of Master of Arts by thesis alone in the University of Canterbury by

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2001

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Abstract

The role of the anterior thalamus (AT) in spatial working memory is well established. The involvement of the AT nuclei in tasks of object recognition is less clear. Two tasks of working memory were used to assess the extent to which NMDA lesions to the AT of rats, or a constituent structure, the anteroventral nucleus (AV), impair spatial and nonspatial working memory. An additional group consisted of lesion to the laterodorsal (LD) thalamic nucleus. Impairment was found for the AT group on an operant, spatial working memory task, the delayed non-matching to position task (DNMP). Neither the AV nor LD groups revealed any deficits on this task. Variations of intertrial interval (ITI) revealed that ITI of ten seconds produces proactive interference on the DNMP as compared with ITI of thirty seconds. All groups performed significantly better in the latter condition. The object recognition task revealed that all rats were able to recognize a familiar object after delays of up to thirty minutes, but no group differences were found. No evidence was found for the inclusion of the LD as part of the AT complex.

ACKNOWLEDGEMENTS

Firstly I would like to thank John Dalrymple-Alford, my supervisor, for the guidance and support given in producing and finishing this project, particularly during the last few weeks when imminent deadlines loomed.

I would also like to thank Anthony McLean, my co-supervisor, for many hours spent developing computer files and programmes to help analyse the data produced.

Trish Meatchem was vitally helpful in caring for my animals and in giving advice on the best ways to work with them.

Michael Christie was extremely helpful in the early days of neurological surgery giving good, clear, and helpful advice in this regard. Michael's help was also invaluable in dealing with small technical problems occurring with the operant boxes and Med PC programmes throughout the duration of the project.

Glenn Lewis and Howard Patterson both provided a lot of support and help with the experimental equipment.

Finally I would like to thank my partner, Maggy, and my brother and mother for their support in finishing this thesis.

CONTRIBUTION OF THE ANTEROVENTRAL, THE LATERODORSAL, AND THE ANTERIOR THALAMIC NUCLEI TO TASKS OF SPATIAL AND NONSPATIAL WORKING MEMORY.

General Introduction

The role of the various diencephalic structures in declarative working memory processes is far from clear. Many neural substrates are thought to be crucial to these processes, in particular the hippocampal formation (HF), the anterior thalamic nuclei (AT), and the mammillary bodies (MB). An additional nucleus, the lateral dorsal thalamic nucleus (LD), may also play an important role in these processes given the dense interconnections it shares with all these structures. The LD in fact has been considered by many to be part of the AT which traditionally consist of the anteroventral (AV), the anterodorsal (AD), and the anteromedial (AM) thalamic nuclei (Warburton et al, 1997; Bentivoglio, Kultas-Ilinsky, & Ilinsky 1993).

The current research aims to help clarify a number of pertinent questions. Given the lack of work into the role of the LD, this study examined the extent to which this nucleus shares functions with the AT and thus whether it should be considered part of this system. Given the similarities of LD neural connections to those of the conventional AT, this study assessed the influence of LD lesions on an automated working memory task, the delayed non matching to position task (DNMP). The DNMP task is known to be sensitive to AT damage. Positive findings here would be supportive of the view that the LD should be considered a part of the AT. However, it is important to note that many authors consider that full AT lesion effects are only produced when the entire AT is destroyed (see Aggleton et al, 1996). Thus the extent of the deficit produced by an AV lesion group was also assessed to discover the extent of the deficit produced by small lesions to particular AT nuclei on the DNMP task and, secondly, to use this as a comparison against any deficit produced by LD lesions. The role of particular AT nuclei on this task, such as the AV, and possibly the LD, will help clarify the extent to which discrete AT lesions can impair memory.

An object recognition task was also used to examine whether these AT nuclei, and the

LD, have an influence on non-spatial item recognition memory. Recently Aggleton & Brown (1999) suggested that two separate neural systems are involved in non-spatial and spatial working memory, and that these two processes are governed by separate and relatively independent neurological systems. The AT is believed to be part of the spatial system, along with the HF and the MB (a view which can be traced back to Delay & Brion, 1969). Given that the conventional AT lie outside Aggleton's object recognition (non-spatial) memory system one would expect no involvement of these nuclei in this type of memory process. The role of the LD here is less clear given that it has dense connections with one of the potential components of the nonspatial system, namely the entorhinal cortex (note that it is the perirhinal cortex of the rhinal cortices that is considered most important on this task. See later section on Aggleton and Brown's (1999) memory model).

Human Amnesia and the Anterior Thalamus

Damage to diencephalic structures in the brain has long been implicated in anterograde amnesia. In humans, this damage occurs through a variety of means including stroke, as part of degenerative processes such as Wernicke-Korsakoff's (WKS) or Alzheimer's disease (AD), and after traumatic head injury (e.g. Graff-Radford, Tranel, Van Hoesen & Brandt, 1990; Haut, Young & Cutlip, 1995; Mayes, Mindal, Mann & Pickering 1988; Parkin, Rees, Hunkin & Rose, 1995). However, the anatomical complexity of the anterior thalamic region means that it is rare to find relatively discrete injury in humans. Thalamic infarcts shed light on this matter as they frequently lead to memory impairment. Early interest in diencephalic structures involved in memory loss tended to focus on the mediodorsal thalamic nucleus (MD). This was due to the observation that this nucleus is frequently damaged in Korsakoff's disease (Victor, Adams, and Collins, 1971) and other types of diencephalic amnesia (e.g. Markowitsch, 1982). However, the role of the MD in human amnesia was uncertain given the fact that damage was rarely confined to this nucleus. Also its proximity to other potentially important nuclei or fiber tracts implicated in human amnesia made it difficult to verify the specific significance of MD damage. The consequences of thalamic infarcts often involve many structures but the weight of the evidence now favours the view that damage anterior to the DM is likely to be equally, if

not more critical in producing memory loss. Obviously this clearly implicates the AT which lie immediately anterior to the DM (Graff-Radford et al. 1990; Parkin, Rees, Hunkin & Rose, 1994). In fact these authors conclude that lesions in the general area of the thalamus can be small as long as they are strategically situated so as to disrupt key limbic system structures. Third ventricular tumors also indirectly implicate the AT by causing damage to the MB (Mckentee et al. 1976). Removal of third ventricular cysts and following the repair of aneurysms, particularly those of the anterior communicating artery, implicate the AT by causing damage to the fornix, a structure densely connected to the AT (see Gaffan, Gaffan, and Hodges, 1991).

The AT is not always damaged in Korsakoff's syndrome cases, but the MB, which provides a major source of AT afferents, is always effected in those cases which reveal memory deficits (Victor et al, 1989). This interconnection between the AT and the MB indirectly points to the potential importance of the AT in the memory loss associated with this disorder. In their study, Victor and colleagues even found that 35% of their cases actually revealed damage which extended to the AT. Given the size and position of the thalamus it is frequently difficult to accurately assess how much damage has occurred there. Cravioto, Korein and Silberman (1961) found that in 22 cases of Wernicke's encephalopathy, all had sustained at least some damage to the AT.

While it is comparatively rare to find relatively discrete AT injury in humans, Parkin et al (1993) found a case, JR, who had sustained a relatively discrete left thalamic infarct. MRI confirmed that JR had sustained damage to various structures of the left thalamus including the hippocampal pathway, the amygdalar pathway, and the pathway from the perirhinal cortex to the dorsomedial thalamic nucleus. Psychometric testing revealed verbal memory deficits comparable to Wernicke Korsakoff's syndrome despite near normal performance on nonverbal tests of memory. The localisation of the lesion is consistent with the view that lesions to the AT can produce anterograde amnesia, particularly when the hippocampal pathway plus either the amygdalar pathway or the perirhinal pathway to the dorsomedial thalamic nucleus are damaged (see Graff-Radford et al 1990).

Aggleton and Saunders (1997) proposed that amnesics can be divided into two major types. Those with disfunctions due solely to selective lesions within an extended hippocampal system (comprising the HF, the AT, the fornix, the MB and the cingulum bundle), and those with additional cortical and subcortical damage which extends the types of memory loss (Aggleton and Shaw, 1996). While the norm is for widespread damage, it is thought that the core deficits observed in anterograde amnesia are due to damage to any part of this system. To support this conclusion Aggleton and Saunders conducted an analysis of the findings of 112 amnesics who had undertaken the Warrington recognition memory test (RMT), and placed them into 11 distinct pathological groupings. Seven subjects were of particular interest as they presented with relatively specific damage to the HF, MB, or the fornix, and thus damage confined to the proposed extended hippocampal system. Analysis of the RMT scores of these subjects revealed that their amnesia did not include a recognition memory deficit as compared with their age matched norms. On the strength of these findings the authors suggested that damage limited to any part of this extended HF system (including the AT) can largely spare item recognition. Assessment of these seven subjects revealed that they had a significant and severe deficit in delayed recall, a valid test for amnesic severity. They concluded that the extent of the amnesic state in these subjects, as reflected by the delayed recall deficit, was comparable to that of the other subjects despite displaying relatively intact item recognition memory.

In a similar vein, Baxendale (1997) found that out of 104 epileptic patients tested on the RMT, those with combined cortical and HF damage performed significantly worse than those with selective HF damage, suggesting that cortical damage extends the type of memory loss found after HF insult, as assessed by the RMT, a recognition memory test. In addition it was found that word recognition performance was normal for those with selective left HF damage, and face recognition normal for those with selective right HF damage, suggesting that unilateral HF damage has no consistent effect on recognition memory. Clearly if HF injury impairs recognition then left HF damage should impair word recognition (a left hemisphere process), and right HF damage should impair face recognition (a right hemisphere process).

More recent evidence obtained from 12 patients who had received surgery for colloidal cyst in the third ventricle also provided evidence consistent with Aggleton and Shaw's (1996) proposal (Aggleton et al. 2000). Unlike the other 9 patients, three patients experienced bilateral interruption of the fornix and showed pronounced impairments in learning and recall, but sparing and only mild impairments in recognition memory tests. A great deal of clinical data is therefore consistent with the proposal that damage to the AT or to the structures or pathways that link the AT with the HF impairs memory for new events (see also McMackie, Cockburn, Anslow, and Gaffan, 1995) but has a comparatively lesser effect on item recognition.

Animal Models of Diencephalic Amnesia

A growing number of investigations on the effects of experimental thalamic lesions in animals (see Aggleton & Brown, 1999) have surfaced over recent years. The use of animals is particularly helpful as key neurological sites can be targeted relatively specifically rather than relying on clinical evidence which tends to involve widespread damage and possible hidden pathology. With this type of study the role of particular nuclei or systems can be specifically examined. Much of the current research focus is directed on the proposal that the AT constitute a critical site in the extended hippocampal system already mentioned (Aggleton and Sahgal, 1993, Aggleton & Brown, 1999), because of their direct and indirect reciprocal connections with the HF (Shibata, 1992, 1993a; 1993b; 1996). There are now several reports that various spatial memory tasks are particularly sensitive to the effects of large AT lesions. These include delayed non-matching to position (operant DNMTP; Aggleton, Keith, and Sahgal 1991), forced T-maze alternation (e.g. Aggleton, Neave, Nagle and Hunt, 1995), and performance in a radial maze and Morris water maze (Aggleton, et al, 1996; Sziklas & Petrides, 1999; Sutherland & Rodriguez, 1989).

The Anterior Thalamic Complex; Neuroanatomical Considerations

The extensive connections that the AT share with the HF, both directly and indirectly, suggest an important role for this nucleus in memory. Indirectly, the AT receive a

massive input from the MB via the mammillothalamic tract which is not reciprocated (Veazy, Amoral, & Cohen, 1982). They are also densely innervated directly from the HF via the fornix (Aggleton, Desimone, and Mishkin, 1986) and project back to the HF reciprocally via the presubiculum (Swanson, Kohler, and, Bjorkland, 1987). Additional indirect reciprocal connections exist between the AT and the HF via the cingulate region (van Groen and Wise, 1992). The AT also project to the entorhinal cortex via the subicular region.

Conventionally the AT consist of the anterodorsal (AD), anteromedial (AM) and anteroventral (AV) nuclei, but the nature and extent of the consequences of damage to these individual nuclei remains an open issue (Dalrymple-Alford, Gifkins & Christie, 1999). The AD is a group of cells in the rostral thalamus which lie along the border of the stria medullaris. Lateral to the AD is the AV; the AM lies ventral and medial to the AV. The AM projects to the presubiculum and the projections of both the AV and AD terminate in the post, pre, and subiculum proper (Finch 1993). The AV receives HF afferents from the presubiculum and parasubiculum, the AM from the subiculum. All these connections pass through the fornix (Aggleton, Desimone, and Mishkin, 1986). The inclusion of the laterodorsal (LD) nucleus as part of this thalamic group has also been suggested, primarily because it has similar neural connections to those of the conventional AT (Warburton et al, 1997; Bentivoglio, Kultas-Ilinsky, & Ilinsky 1993). Clearly the AT are thus likely to play a pivotal role in the limbic system. Also there is evidence for a functional integration of these diencephalic and temporal limbic systems suggesting a crucial role of the AT in normal working memory (see Aggleton and Brown, 1999).

Aggleton and Brown's (1999) Episodic Memory and Item Recognition Systems

Recently, Aggleton and Brown (1999) proposed a model involving two separate and independent memory systems that may underlie the range of deficits shown by human amnesics. Their paper extended the previous consideration by Aggleton and Shaw (1996) on the anatomy underlying anterograde amnesia. As indicated earlier, an extended hippocampal system was proposed consisting of the AT, HF, fornix, and the MB, damage

to which is a common feature of anterograde amnesia. They suggested that the distinction between temporal lobe and diencephalic amnesia is of limited value and placed critical importance on HF efferents via the fornix to the diencephalon. This system is believed to support the encoding of spatial information in the rat and context/event-dependent information in humans, and thus is necessary for effective recall of episodic memory. In addition to this HF-AT axis, further projections exist back from the AT complex to the HF and temporal cortex, which also help support episodic memory. A second and independent system consisting of the rhinal cortices (the perirhinal cortex in particular), the MD and the association cortex is believed to encode familiarity based, non-spatial processing, that facilitates item recognition. In cases of amnesia with widespread damage to both these systems, severe deficits in both item recognition and episodic memory are commonplace (Aggleton and Shaw, 1996).

The basic idea of this extended HF-AT system can be traced back to Delay & Brion (1969). They suggested the importance of a single and independent functional system consisting of the HF, the fornix, and the MB in human memory. Although the AT were not specifically mentioned within this formulation, the dense interconnections of the AT with all these nuclei suggests an important role of these nuclei within such a system. Damage to any part of this system was expected to produce an impairment in the recall of episodic information and thus was thought critical to the core deficits of anterograde amnesia. More recently Gaffan (1991) also placed importance on the link from the HF to the MB and AT via the fornix on normal episodic memory.

Aggleton and Brown's (1999) model has a number of central features. HF efferents via the diencephalon are effectively seen as functional extensions of the HF and are considered as vital to normal HF function. The principal thalamic targets of these extensions are the AT nuclei (including the LD). Diencephalic structures can in turn influence temporal processing through return connections that are mainly via the cingulum bundle. The principal axis of this system is that between the AT and HF, and the extensions beyond this point are relatively diffuse. As a consequence damage to structures beyond the AT are expected to have a comparatively lesser effect on episodic memory. That is, the AT are considered a critical nodal point in the extended

hippocampal system (Aggleton & Sahgal, 1993), and the extensions beyond this axis are considered less important in episodic memory.

This model leads to a number of predictions. To begin with, given that this system is believed crucial to the effective encoding and thus the normal recall of episodic information, damage here can result in severe anterograde amnesia. Damage to any part of this extended system is expected to produce similar types of deficits in the recall of episodic memory. It is also expected that damage to this system need not necessarily produce deficits in recognition. Part of the rationale of this view is that recognition is believed to compose of two independent processes (Mandler, 1980) only one of which is hippocampally dependent (e.g. Aggleton and Brown, 1999). One of these processes is the recollection of a stimulus, the act of remembering, which is a hippocampal process. However an additional process that can guide recognition, as opposed to recall, is one based on judging the familiarity of a stimulus. The latter process does not require intact hippocampal function but rather the functioning of the other perirhinal dependent recognition memory system already mentioned. Thus recognition memory may be unaffected despite extensive hippocampal damage and loss of episodic memory.
The previous section on human populations reveals a dissociation between these two processes in that some human amnesics appear to have a marked deficit in recall but can still perform recognition tasks such as face and word recognition. The specificity of these deficits appears to relate to the extent to which the areas of damage are specific to this extended hippocampal system. However in the majority of cases damage is much more widespread and marked deficits in both recall and recognition are observed (see Aggleton and Saunders, 1997).

Early Experimental Work on Anterior Thalamic Damage In rats and Mice

Some early reports suggested that AT lesions, especially selective AM and AV lesions, were without effect on learning and memory. Greene and Naranjo (1986) found that lesions to either the AV or the AM had no effect on a task of delayed alternation and performed similarly to MD lesioned rats, whereas an MB lesion group revealed marked deficits. These workers suggested a potential redundancy of function in the AT and the

likelihood that to obtain a noticeable effect a large lesion targeting the entire AT would be necessary (see Warburton et al, 1997). An earlier study (Greene, Stauff, and Walters, 1972) also produced negative results when output fibers of the AT nuclei were sectioned. Beracochea, Jaffard, and Jarrard (1989) produced ibotenic acid lesions to the AT or MD in rats. Subsequent testing on an eight arm radial maze revealed no performance differences between either of the lesion groups and controls. On a temporal alternation task however they found that while the MD rats required more time to learn the task, an AT lesioned group showed greater deficits at longer delays. They concluded that the MD is mostly involved in the registering of new info and the AT in its maintenance over time. A later study, with mice, did reveal that AT lesions produced a delay dependant deficit on a T-maze alternation task. However, this effect was only evident over a very long delay of six hours while no effect was observed at the shorter delay of five minutes. The fact that the AT lesioned mice could perform the task adequately at short delays suggested that these effects were a true delay dependant deficit and not due to a loss of procedural knowledge (Beracochea and Jaffard, 1994).

Such evidence supported the view that the general disinterest of the AT in the literature was due both to a lack of effects after small lesions to these nuclei, and of only moderate effects after larger AT lesions (see Aggleton & Brown, 1999; Warburton et al, 1997). It was felt that other hippocampal and fornical outputs might support spatial memory thereby minimising the likely importance of the AT per se. Alternatively it was posited that given the shape and placement of the AT, it was a difficult task to produce complete lesions of these nuclei and thus difficult to assess a true AT lesion effect (Aggleton et al, 1996; Beracochea & Jaffard, 1994).

Experimental Work on the Mediodorsal Thalamic Nucleus and Rat Models of Korsakoff's Syndrome.

Early interest into the role of thalamic structures in the diencephalon focused on the MD. This interest arose due to the observation that this nucleus is frequently damaged in Korsakoff's disease (Victor, Adams, and Collins, 1971) and other diencephalic amnesias (e.g. Markowitsch, 1982). However, the role of the MD in human amnesia was uncertain

given the fact that damage was rarely confined to this nucleus and its proximity to other potentially important nuclei or fiber tracts implicated in human amnesia. Animal studies of spatial memory after MD lesions produced inconsistent results, possibly in part due to the fact that lesions to this nucleus tended to extend to fibers of passage or adjacent regions and thus implied nuclei adjacent to the MD (see Hunt and Aggleton, 1991). Hunt and Aggleton (1991) helped to clarify the confusion. Employing a T-maze alternation task they found that MD lesions only produced deficits on massed trials, suggesting that perhaps these rats were sensitive to proactive interference (interference produced by a previous trial on a subsequent trial). A "real" spatial deficit not obviously linked to proactive interference only occurred when additional damage had occurred to the AT. The authors thus suggested a link between coincidental AT damage and an "MD impairment". Their analysis of previous studies into the MD's role in spatial memory found four that revealed no lesion effect (see Hunt and Aggleton, 1991 for a review). Of the six studies showing a MD deficit it was discovered that in at least half of these studies additional damage had extended to the AT. The remaining "positive" studies revealed extra damage in many of the rats, particularly to the mammillothalamic tract or the dorsal hippocampus. This analysis suggested that at least some of the spatial deficits observed after MD damage could be due to lesions extending to the AT and hinted at an important role of the AT on tasks of spatial working memory. Pertinent here is a follow up study by Neave, Sahgal, and Hunt (1993) showing that selective MD lesions, if anything, improved performance of an operant-delayed-non-matching-to-position (DNMP) task. No deficits were evident either in the acquisition or postoperative performance of this task. Their selective MD lesions produced substantial damage to the MD region but had only minimal impact on the AT region. They indicated that their MD lesions affected the medial portions of the LD unilaterally and sometimes caudal aspects of the AV unilaterally. Subsequent work has confirmed that MD lesions have an impact on spatial working memory when the lesions include damage to the AT region but also suggest that some mild spatial memory impairments occur after restricted MD lesions when the task demands encourage perseverative behaviour (Hunt & Aggleton, 1998a & 1998b).

Rat models of Wernicke Korsakoff's (WK) disease have also implicated a role of the AT in memory loss. The pyrithiamine deficiency model is used to mimic a WK state in

rats given that thiamine deficiency is widely considered to be a major contributing factor to WK (see Langlais, Mandel and Mair, 1992). Early studies concluded that the crucial damage in these rats was to the lateral internal medullary lamina (IML). When the lateral IML is lesioned directly, deficits are produced in operant delayed matching tasks that are not evident in rats with MD lesions (Burk & Mair, 1998). However, other findings suggest that PTD treatment also frequently damages the AT region. Even those PTD rats that sustain no damage to the IML show AT damage, including 40-50% cell loss in the AV. All rats, whether IML lesioned or not, reveal deficits on an operant DNMP task. This clearly suggests that it is not only IML damage which is crucial to the memory loss after PTD treatment, but point to at least an important role of the AT here (Langlais and Savage, 1995).

Recent Experimental Work Using Large Anterior Thalamic Lesions

Despite the early studies, much recent work has emerged targeting the AT nuclei specifically. Typically, damage is produced in all three of the conventional AT nuclei. Often the lesions are targeted at the AV and the AM and often extend to the adjacent AD. Early research using electrolytic lesions of the AT revealed post-operative acquisition deficits on the Morris water maze (Sutherland and Rodriguez, 1989), and impairments on an operant delayed alternation task (Peinado-Manzano & Pozo-Garcia,1991). More recently Aggleton et al (1995) showed a significant and profound deficit in AT lesioned rats when performing a T-maze alternation task. On initial acquisition, the AT rats were more impaired than MB rats, and equally so to fornix rats. On a subsequent continuous alternation task, the MB rats were indistinguishable from the AT rats while the fornix rats revealed a greater deficit. All three lesion groups were impaired relative to controls on these tasks. A possible reason for the more marked MB effect in the continuous alternation condition is that they may be more sensitive to proactive interference (see Beracochea & Jaffard, 1987, 1994). The AT rats reveal no such interference on this task (see Beracochea & Jaffard, 1987; Beracochea & Jaffard, 1990).

Warburton and Aggleton (1998) found that rats with AT lesions revealed reference memory deficits on the Morris water maze which were greater than fornix lesioned rats

on a number of performance measures. They concluded that the deficits observed in spatial memory tasks in AT lesioned rats could not be totally accounted for by disconnection of the fornix input to the AT. Since then a follow up study by Warburton et al. (1999) reported that large AT lesions extending into much of the DM produced greater deficits than those observed after more restricted AT lesions as assessed by the Morris water maze and T-maze forced alternation. Close analysis of the histological results suggest the possibility that some additional damage to the LD had occurred in the group with large AT lesions. The authors concluded that this extended damage was the likely cause of the greater deficits observed in this latter group rather than any inherent of the DM. Evidence was also found for a loss of procedural information in this latter group.

The effects of large AT lesions on allocentric and egocentric spatial memory tasks have been recently assessed by another group (Sziklas and Petrides, 1999). A task that required the turning of the body to the left or right depending on which of two large objects were presented was tested on rats with AT lesions. It was found that on this task AT rats performed comparably to controls. This suggests that these rats were able to associate egocentric cues with different visual cues. The AT group, however, did reveal a deficit in a task where rats need to associate spatial cues with particular objects, demonstrating a deficit in allocentric spatial associative conditioning. On an eight arm radial maze the AT rats revealed an allocentric spatial memory deficit consistent with other similar studies (e.g. Aggleton et al 1996, Warburton et al 1997). Taken together these results reveal that lesions to the AT, like those to the HF and MB, produce profound deficits in the normal working of allocentric spatial learning and memory. Note that these results suggest that AT lesioned rats are able to distinguish between two different visual items and thus "recognise" them.

Warburton et al (2000) used a disconnection procedure to test the notion that HF projections via the fornix to the AT form functional components of a spatial working memory system. In this study unilateral lesions were produced either ipsilaterally or contralaterally to both the AT and the fornix of rats. An additional group received bilateral lesions of the fornix. Compared to SHAM surgery control group, all rats revealed a T-maze alternation deficit. However, the unilateral lesions produced only a minor deficit. In the second part of the study an additional group was included which

received crossed unilateral lesions to the fornix and AT contralaterally with an additional hippocampal transection to prevent cross-hemisphere interactions. Assessment of the working memory function in this final group revealed profound deficits in the Morris water maze, forced alternation in a T-maze, and eight arm radial maze performance. These effects were significantly greater than those of a contralateral group that had not received hippocampal transection. These results suggest that while the disconnection of the AT from its HF inputs produces impairments on a range of memory tasks, there also appear to be a wide range of routes that can subserve this function.

Other relevant studies dealing with the effects of AT and LD lesions on the DNMP task or object recognition will be addressed later.

Recent Work on Partial Anterior Thalamic Damage

While most of the experimental evidence for a role of the AT in amnesia comes from lesions that inflict widespread damage to this nuclear complex, comparable injury in humans is likely to be less complete, or less specific. Research into the effects of discrete lesions of individual nuclei in the AT region, especially the AV nucleus, has found that these can lead to marked impairments in spatial memory. Byatt and Dalrymple-Alford (1996) found that small lesions to either the AM or the AV produced deficits in working and reference memory compared to control rats on a twelve arm radial maze. Despite a lack of effect in the first five blocks of testing, the trials that followed revealed that both lesion groups made significantly more revisits to baited arms than the controls. The same pattern of results was found for a reference memory (never baited arms) component.

Using a rewarded forced alternation task, Aggleton et al (1996) found that AM and AV/AD lesions in rats produced a deficit only in the initial acquisition of the task, whereas AT rats displayed a more persistent deficit. Subsequent testing revealed that all groups could use allocentric cues except the AT group, the AV/AD and AM groups now performing comparably to the SHAM group. Thus on this task it was demonstrated that selective lesions to particular AT nuclei produce spatial working memory deficits but that more complete AT lesions produce greater and more persistent memory loss. Further analysis revealed that all lesion groups were impaired on an eight-arm radial-maze

relative to controls. A marked deficit was found in the AT group, an intermediate deficit in the AV/AD group, and a lesser but evident deficit in the AM group.

These two studies are important because they reinforce the view that even partial damage to the AT, in particular the AV, may exacerbate the severity of memory loss caused by an insult elsewhere in the extended HF system (Dalrymple-Alford et al 1999; Beracochea & Jaffard, 1991). For example, such minor damage may constitute a significant part of the neurodegenerative and behavioural complex induced by alcohol or thiamine deficiency (e.g. Langlais & Savage, 1995; Robinson & Mair, 1992). In addition, understanding the behavioural profile of components of the AT, and their potentially negative (secondary) effects on other connected regions, may also provide insights for future recovery or ameliorative programmes.

The Laterodorsal Thalamic Nucleus

A comparative examination of the effects of LD lesions would help clarify the role this nucleus plays in memory, and whether it should be considered as an integral part of the AT complex. Both the conventional AT and the LD share dense reciprocal interconnections with the HF (Shibata 1996) and the cingulate cortices. These similarities thus raise expectations that their functions would be comparable.

Previous work has found that temporary anaesthetisation of the LD leads to impairment on an 8-arm radial maze (Mizumori, Miya & Ward 1994). Fifteen trials were scheduled daily on this task, but every day between trials five and six the LD was reversibly inactivated. A 2% tetracaine solution was used which was believed to lead to partial inactivation of the LD that lasted 15-20 minutes. The first five and last two trials of every day were used as a within subject baseline against which performance was compared. On the two trials following LD inactivation, trials 6 and 7, accuracy within the maze was impaired as demonstrated by a significant increase in the mean number of errors made. The authors concluded that the LD provides important spatial information to the HF playing an important mnemonic function in the neural system that mediates accurate spatial navigation.

The only other study to examine the role of the LD was undertaken by Warburton and colleagues (1997). In this study lesions were produced in the AT, and the fornix of rats. A third group received lesions to both the AT and LD (an AT + LD group). All groups revealed an equally severe and long lasting deficit in the acquisition of a T-maze alternation task. Later analysis of the ability of the rats to use allocentric cues revealed that while all groups had a profound deficit, the AT+LD group performed significantly worse than the fornix group. However, the AT+LD group was not obviously more impaired than the AT alone group. The graphed data for the T-maze alternation task revealed some evidence that the AT +LD group performed worse in two of the delay conditions than the AT alone group. However, this effect was not statistically significant. The authors noted that a lack of statistical significance may in part have been due to near chance performance postoperatively, and thus floor effects. The role of the LD per se was not verified as a lesion group of just the LD was not included. Clearly, in order to verify the effects of an LD lesion on memory tasks an LD alone group is required. The above results could have occurred for a number of reasons other than the addition of the LD lesion to an AT lesion.

Lastly, it is useful to recall the Warburton et al. (1999) study which reported that extensive lesions to the AT which spread into adjacent nuclei produce significantly inferior performance on the Morris water maze and T-maze forced alternation tasks, including an apparent loss of procedural knowledge. The authors concluded in this study that the likely cause of the increased deficit found in these rats was probably due to damage that had spread to the LD, although DM damage also occurred.

Operant Delayed-Non-Matching-to-Position and the Anterior Thalamic Nuclei

The operant delayed non matching to position task developed by Dunnett (1985) is a useful tool for examining the effects that lesions and other manipulations have on the working memory of rats. In this paradigm it is possible for the mnemonic consequences of experimental manipulations to be distinguished from other nonspecific effects. The traditional view is that if a rat performs at high and near normal levels at very short delays it shows us that the rat is attending to the task and has learnt the discrimination

rule. However, effects that occur as a consequence of increasing delay suggest a specific mnemonic deficit, that is deficits in the animals working memory capacity, and not nonspecific effects on motivation, attention, sensory or motor capacities. Alternatively, deficits occurring at zero or very short delays are believed to reflect a loss of procedural information, or possibly a decrease in motivation or attention. The DNMP task is frequently used as it has been confirmed as a task sensitive to diencephalic amnesia in humans (Joyce & Robbins, 1991).

A concern relevant to all tasks of spatial working memory is the extent to which subjects are able to employ strategies to solve the task. Chudasama and Muir (1997), using a video recorder, found that on the DNMP task some rats employ strategies involving orientation of the body towards or away from the non-matching sample during the delay stage prior to the choice phase. When a rat uses such a strategy the task is no longer taxing working memory per se, but rather the ability of the rat to learn and use a strategy to solve the task. Chudasama and Muir (1997), in an attempt to minimize the use of such strategies, and to replicate the design of many previous DNMP studies (e.g. Aggleton and Hunt 1991, Aggleton and Sahgal, 1993), employed a strategy where the rat had to press a magazine flap on the front wall of the chamber during the delay period that intervened between the sample and choice phase of the trial. They found that inclusion of the rats discovered to be using such strategies influenced their results significantly.

Aggleton, Keith, and Sahgal (1991) preoperatively trained rats to perform the DNMP task. Substantial postoperative deficits were reveaed in rats with AT lesions after reintroduction to the DNMP task. In this case the effects of lesions to the AT were virtually indistinguishable from those in a fornix lesion group. By contrast MB lesions showed no postoperative effect as compared with SHAM controls. Analysis of the data showed that the lesion effect only became evident at delays greater than zero. Activity level measures of the AT were also comparable to the control group suggesting that differences between the groups were due to a true mnemonic deficit, and not to nonspecific impairments. The only difference between the AT and fornix rats was that the fornix rats produced a more pronounced side bias with increasing delays. A related study by Aggleton and Sahgal (1993) assessed the DNMP performance of hippocampal and fornix lesioned rats. The results of that study suggest that AT lesions produce virtually

equivalent deficits to hippocampus and fornix lesions on this task. Limited evidence emerged suggesting slightly faster forgetting in the HF group. This effect was attributed as likely to be due to a secondary consequence of the main lesion effect. Taken together, these results suggest that AT, fornix, and HF lesions all produce similar deficits on a task of spatial working memory, DNMP, and are virtually indistinguishable from one another.

Proactive Interference on Operant Matching Tasks

A concern pertaining to all tests of working memory is to what extent the nature of the trials themselves assess mnemonic capacity, and to what extent errors on the task reveal true limits in working memory. Proactive interference refers to the extent to which the memory load of a previous trial interferes with the ability of an organism to perform the next trial. Errors produced by proactive interference do not reflect a true limitation of working memory per se, but rather the extent to which working memory can be "reset" from one trial to the next. Thus, where proactive interference is occurring the extent to which an error reflects limitations of working memory cannot be accurately estimated. One influence on proactive interference is the duration of the intertrial interval (ITI), the amount of time that elapses between two trials. Where this interval is too short the organism is unable to reset working memory from the previous trial in order to successfully carry out the next trial. A certain period is believed to be necessary to "reset" working memory so that information from the previous trial does not "interfere" with the next trial (Roitblat, 1993).

It has been shown that short ITI's can impair a rat's ability to perform memory tasks such as the operant DNMP task. Dunnett and Martel (1990) showed that responses made on a previous trial can interfere with the choice accuracy of rats during the subsequent trial on the operant DNMP task. Specifically, as the delay duration increases the likelihood of a correct choice decreases if the previous trials correct response was on the opposite side as that of the current trial (indicating proactive interference). This effect of proactive interference was abolished with increases of ITI from 5 seconds to 15 or 45 seconds.

Subsequent research has confirmed Dunnett and Martel's (1990) findings. Savage and Parsons (1997) use an operant delayed-matching-to-position task carrying out manipulations of ITI. In the training period a long ITI of 30 seconds was used. Manipulations of ITI followed involving sessions of either ITI 30 seconds or of ITI 7 seconds. The procedure involved an ABAB design where one ITI 30 trial was followed by one ITI 7 trial, followed by another ITI 30 trial, and ending with a final ITI 7 trial. Comparing the ITI conditions revealed that normal rats performed significantly worse on the short ITI procedure. This finding suggests that the rats suffered a greater degree of proactive interference during the short ITI trials and reveal that rats are susceptible to this kind of proactive interference on operant matching tasks. Similarly Harper, McLean, and Dalrymple-Alford (1994) reported that normal rats perform significantly worse under short ITI conditions on a similar task, with the increased proactive interference reflected by increased rates of forgetting. Taken together these studies suggest that rats are prone to interference from previous trials and that short ITI's encourage poor performance due to the effects of proactive interference.

In the Aggleton et al. (1991) AT lesion study it was concluded that no build-up of proactive interference occurred as performance in the first 12 trials in a session was not significantly better than that in the last 12 trials. In fact performance was actually better over the last 12 trials of a session. The idea was that if proactive interference was occurring it should build up through a session, leading to gradually worsening performance on the last trials of a session. However, evidence from both Dunnett and Martel (1990) and Harper et al (1994) suggests that the critical factor in proactive interference is prior trial information, rather than gradual build up over a session. Of relevance here is the study by Beracochea and Jaffard (1994) where no evidence was found for a particular sensitivity of AT lesioned mice to proactive interference buildup on a sequential spontaneous alternation task using a 30 second ITI. Thus there is still no clear information on the effects of proactive interference on AT or LD lesioned rats. Previously comparatively short ITI's of 10 seconds have been used (e.g. Aggleton et al, 1991) so the extent to which previous results have reflected proactive interference effects is not known.

Object Recognition

As mentioned earlier, it is thought that two separate and independent processes govern spatial and non-spatial (recognition) working memory (Aggleton and Brown, 1999). To recap, it is proposed that the system subserved by the HF-AT axis is involved in the encoding and thus the effective recall of episodic (spatial) memory. This "spatial" system need not be involved in tasks of recognition that tax familiarity based judgements given the distinct nature of the processes that underly recall and recognition. In addition to actively recalling a stimulus, recognition can also occur by judging the familiarity of that stimulus (Mandler, 1980). Thus recognition can occur without recall. In tasks taxing object recognition, the non-spatial, recognition memory system can be used.

The neural bases of this non-spatial system includes the rhinal cortices (particularly the perirhinal cortex) and the MD of the thalamus. Entorhinal connections are particularly interesting here in that the latter structure has attributes of both of the spatial and non-spatial systems. It not only shares some of the connections of the perirhinal cortex, an integral part of the non-spatial item recognition system, but has also connections with the AT, the LD, and of course the HF (see Aggleton and Sahgal, 1993). The involvement of the AT in object recognition has not received much attention; no research has been conducted on the role of the LD in object recognition.

Support for the existence of the non-spatial system has been found in studies measuring the extent to which perhirhinal cortex lesions impair object recognition memory. In fact Mishkin and Murray (1994) suggest that it in the primate the perirhinal cortex is the temporal lobe structure critical for effective recognition in the first few minutes of presentation of an object. Rat studies reveal that perirhinal cortex lesions impair object recognition on delayed-non-matching-to-sample tasks (Enaceur, Neave, & Aggleton, 1996; Mumby and Pinel, 1994; Meunier et al. 1993; Meunier et al. 1996). The specific importance of the perirhinal cortex seems well established. Comparable lesions to the entorhinal cortex lead to comparatively mild and transient deficits in object recognition (Leonard et al. 1995; Meunier et al. 1993).

In response to previous attempts to formulate a truly one trial test of item recognition memory (Mishkin & Delacour, 1975) Ennaceur and Delacour (1988) formulated the

"spontaneous object recognition" test. The procedure they developed is based on the spontaneous exploratory behaviour of rats and consequently does not require the learning of procedural rules. Initially a rat is given an allotted period of free exploration of two identical objects in a large open field, the "sample phase". Following this, and after a variable delay, the rat is reintroduced into the open field. On this "recognition" trial two new objects are in place of the objects previously explored. One of these is a duplicate of those explored in the "sample" phase, the other a novel object never before seen. The use of three identical samples is essential so as to preclude the possibility of a rat recognising the sample as familiar by the odor it left in the sample phase, as opposed to true visual recognition. Using this procedure, Ennaceur and Delacour (1988) discovered that normal rats have a preference for exploring a novel as opposed to a familiar object that is well above normal variations in exploratory behaviour. The authors concluded that object recognition in rats can be measured by the difference in the exploration times of new and familiar objects. Given a free choice rats will spend more time attending to a new, never before seen object, as opposed to a familiar object. They found that measures of recognition on this task are influenced by both the initial amount of time given to explore the "familiar" object or "sample", and the amount of time that elapses between the sample phase and the subsequent recognition phase. In addition other factors that influence recognition can be used such as pharmacological treatments and neurological lesions.

Enaceur and Delaceur's (1988) paradigm has some significant benefits over many other tasks of recognition. To begin with it does not require the learning of a rule and thus does not have a reference memory component. In addition, it is not based on the usual use of food reinforcers or electric shocks which can make generalisations from animal studies to humans difficult; it is rare to find human amnesics performing memory tasks under conditions of extreme fear or hunger.

A study involving lesions to a number of neural sites including the MB, fornix, and the AT assessed spatial and nonspatial memory (Aggleton et al. 1995). The forced alternation task and the spontaneous object recognition task described above were used as the main behavioural assays. No AT lesion impairment on object recognition was reported but closer analysis of the data indicates that perhaps a weak lesion effect was present. This

effect did not reach a statistically significant level. The authors used two delay conditions, one of one minute and another of fifteen minutes. All groups performed equally in the first condition showing the expected preference for the novel object. In the second condition all groups (mammillary body lesion, fornix lesion and control), save the AT group, gave evidence of improved object discrimination with the extended delay. These results are important in that Aggleton and Brown's (1999) model explicitly predicts that the neither the AT or LD groups would be involved in non-spatial processing (i.e. object recognition). By contrast, other workers suggest that the AT may be involved in a wider range of memory phenomena, such as different aspects of consolidation across varying task demands (Dalrymple-Alford et al, 1999; Gabriel & Smith, 1999). Thus an examination of this issue should be especially informative.

The Current Study

The roles of both the conventional AT and the LD in declarative memory processes are far from clear. The importance of the AT in normal spatial working memory is becoming well accepted. Many studies have shown its importance on a number of different spatial tasks such as the Morris water maze, forced alternation in a T-maze, 8 and 12 arm radial maze, and the DNMP task (DNMP: Aggleton, Keith, and Sahgal 1991, T-maze alternation: Aggleton, Neave, Nagle and Hunt, 1995, radial maze: Aggleton, et al, 1996; Sziklas & Petrides, 1999; Morris water maze: Sutherland & Rodriguez, 1989). The role of the LD in spatial memory is far less clear although two studies reveal a possible importance for this nuclei on a T-maze task and on an 8 arm radial maze.

To assess spatial working memory the current study used a DNMP task. This task has been demonstrated to be sensitive to AT damage and is a task similar to memory tests used to assess the severity of human anterograde amnesia. The AT rats were expected to reveal a profound and persistent deficit. The extent to which the AV group was impaired is of interest because to date no studies of the AV on DNMP have been carried out. The results here are of importance to proponents of the view that in order to produce a full and true AT deficit the entire structure needs to be lesioned. Comparatively transient deficits in AV/AD or AM lesioned rats have been found on a forced alternation task as compared

with the more persistent and profound deficits produced by larger AT lesions (Aggleton et al. 1996). However, in that study a significant role for the AV/AD nuclei in particular was revealed on an eight arm radial maze task. Byatt and Dalrymple-Alford (1996) also revealed that AV rats present with spatial memory deficits, this time using a baited/unbaited 12 arm radial maze task. Thus on the radial maze at least it has been shown that partial AT lesions can lead to marked memory impairments, particularly when lesions involve the AV.

The role of the LD is also of importance given that this nucleus lies outside Aggleton and Brown's (1999) spatial memory system, but shares many neural connections. In addition, the LD shares dense connections with the entorhinal cortex, a possible interface of the recognition memory system. An LD deficit on the DNMP task will support the view that this nucleus should be considered part of the AT. Alternatively an LD object recognition deficit will highlight the importance of this nucleus and its entorhinal connections on recognition memory tasks. Clearly many of the findings of this research relate closely to Aggleton and Brown's (1999) model and to some of the predictions it makes regarding object recognition and spatial working memory.

The extent to which past findings on DNMP in AT lesioned rats have been influenced by proactive interference is not known. Aggleton et al (1991) concluded in their study that no such interference had occurred for their lesion groups as no buildup of interference was observed throughout a session. Dunnett and Martel (1993) and Harper et al (1994) both found that it is previous response that is most important in proactive interference, rather than buildup over many trials. It has also been found that normal rats suffer substantial proactive interference at ITI's shorter than 30 seconds (Savage and Parsons, 1991). The extent to which lesions of the AT, its constituent nuclei, or the LD, sensitize a rat to proactive interference is not known. Lesions to the MB, part of the proposed system, have been shown to sensitize rats to such interference (Beracochea and Jaffard, 1987 & 1994) on a sequential alternation task while AT rats revealed no such interference on that task.

To assess the effects of proactive interference on the DNMP task, and to highlight any interactions that may occur between our lesion groups and this type of interference, the

present study used an ITI manipulation in the post surgical period. ITI's of 30 seconds were mixed with 10 second ITI's in alternating sessions using an ABAB design over a period of 24 days to assess the extent to which the various groups would be influenced by proactive interference.

To ensure that the current study was not influenced by data produced by rats employing mediating strategies this study videoed the behaviour of all rats on the DNMP task. In this way any rats identified as using a mediating strategy of any sort were excluded from the analysis. In addition, a back lever response during the delay period between the choice and sample phases was used in attempt to decrease the likelihood that rats would discover and employ mediating strategies.

A second part of this study employed Ennaceur's (Ennaceur and Delacour, 1988) spontaneous object recognition task. This recognition memory task has been shown to be particularly sensitive to perirhinal cortex lesions (e.g. Ennaceur, Neave, and Aggleton, 1996) but limited work with AT lesioned animals has been done on this task. LD lesions have not been tested on any object recognition paradigms. This task is also of relevance to Aggleton and Brown's (1999) model as it relates to their recognition memory system. In a related study Aggleton et al (1995) concluded that AT lesions produced no impairment in object recognition memory. However, the graphed data suggested that the AT group do not improve discrimination at the longest 15 minute delay, unlike all other groups including the SHAM controls. This trend was not statistically significant but leaves open the question whether a longer delay, or a larger sample size, would reveal recognition deficits in these rats. To examine this issue a spontaneous object recognition task was carried out using three delay conditions the longest of which was 30 minutes. Given the comparatively few studies into the role of the LD in memory, it is not known what effect lesions to this nucleus will have on object recognition. However, given the LD's entorhinal connections, it is possible that this nucleus helps subserve normal object recognition. Also given the similarity in neural connections that the LD shares with the AT nuclei, we can expect a similar role for the LD as the AT on this task. This clearly reflects back on Aggleton and Brown's (1999) formulation in that the LD lie outside both of the proposed networks but share many features of both these systems, especially the

extended HF system.

Summary

The role of the conventional AT, its sub components, and the LD, in declarative memory processes is apparent but requires further investigation. Given the lack of work into the functions of the LD, this study should reveal valuable data concerning the role of this nucleus. Given the similarities of the LD neural connections to those of the conventional AT we expect comparable changes in our DNMP task after lesions to this structure. Findings here will be particularly relevant to proponents of the view that in order to illustrate AT effects one must produce near total destruction to all of the AT. The role of particular AT nuclei on the DNMP task will help clarify some inconsistent findings regarding the extent to which discrete AT lesions can impair memory. Finally, the object recognition component will help clarify the role of the AT and the LD on this behavioural task. Given that both the conventional AT and the LD lie outside Aggleton and Brown's (1999) object recognition memory system, one would expect no involvement of these nuclei here. However, given the entorhinal connections of both the AT and particularly the LD, there might be some influence of these lesions on object/nonspatial recognition.

Method

Subjects

56 naive male hooded rats were trained to perform the DNMP task. The rats were two to four months old at the commencement of training when their free feeding weights varied between 250 and 370 grams. Pre-operatively they were housed 3-4 per cage, and postoperatively 2-3 per cage. Rats were housed under reverse light conditions with lights off between 5:30-17:30. Testing occurred during the dark cycle between the hours 10:00-16:00. Rat's weights were gradually reduced to 85% of free feeding weight and maintained throughout the preoperative training period. They were returned to free food prior to surgery. Nine days after surgery new postoperative 85% target weights were calculated and maintained.

Surgical Procedure

Rats were anaesthetised IP with a pentobarbitone solution (50 mg/ml) twenty minutes after an Atropine injection (0.185 mg/kg). Pentobarbitone was injected at a volume of 1.85 ml/kg of the rat's weight. Prior to the final surgeries a large number of trial surgeries were conducted to locate the coordinate sites for each target nucleus, and to determine the NMDA quantities required to produce lesions of optimal size and location. The incisor bar was set at 5 mm above the inter-aural line. The relative posteriority of the lesions produced appeared to relate to the distance calculated between lambda and bregma in that the longer this distance, the more anterior the location of the lesion. Thus a method was used in which the greater the lambda-bregma distance, the more posterior the lesion coordinate relative to bregma (see Table 1).

Laterality and ventrality measures were as follows: AV (laterality +/- 0.165, ventrality – 0.56), AM (laterality +/- 0.10, ventrality – 0.58), LD 1 (laterality +/- 0.21, ventrality – 0.48), LD 2 (laterality +/- 0.22, ventrality – 0.49). Two sites were used for the LD nucleus given its relative size, the AT involved a lesion to both the AV and the AM, whereas the AV group received lesion to the AV only. All lesions were administered bilaterally using 0.12 M NMDA dissolved in a pH 7.2 solution of phosphate buffer. The

amount of buffer used to produce the 0.12 M NMDA was based on the following equation: wgt/1.765x10^{-5} liters of the phosphate buffer. The volume of the NMDA solution used per site was as follows; AV (only)=0.17 micro-liters; for the AT lesions, AV=0.16mcl and AM=0.14mcl; and for the LD lesions LD 1=0.16mcl and LD 2= 0.14mcl. SHAM rats had all steps of surgery barring the actual injection of the neurotoxin. Of the final 12 SHAM rats there were four for each set of lesion coordinates. Given the very small size of the nuclei being targeted, for SHAM surgeries the needle was lowered to a site 0.15 cm above the appropriate lesion ventralities. This ensured no damage was produced in the relative nuclei of the SHAM group. Twelve of the 56 trained rats died during surgery leaving 44 post-operatively, 12 SHAM, 11AV, 11 LD, and 10 AT rats.

Table 1; Lesion Coordinates for each of the target nuclei relative to bregma based on estimated lambda-bregma distance (cm):

Distance	AV	AM	LD 1	LD 2
0.72-0.73	-0.03	-0.02	-0.13	-0.15
0.70-0.71	-0.02	-0.01	-0.12	-0.14
0.68-0.69	-0.01	-0.00	-0.11	-0.13
0.66-0.67	-0.00	+0.01	-0.10	-0.12

Delayed-Non-Matching-to-Position

Rats were trained in an operant chamber using MED PC automated programmes. To begin with rats were shaped to learn to press the relevant levers for a reward (a diluted condensed milk solution). An automated shaping programme was used to shape lever pressing and to train rats to perform the DNMP task.

The DNMP programme that followed used the following procedure. At the beginning of each trial one of two levers (the sample stimulus) was inserted into the chamber as the light above that lever was lit. The rat was required to press the lever within 10 seconds (limited hold), following which the lever retracted and the lights went out. A preset,

variable delay period followed. In order to minimise the likely use of a mediating strategy, e.g. standing in front of the sample lever, the rat was then required to press a lever at the back of the chamber; this lever was lit during the delay period. A video camera was used to record the behaviour of each rat in order to exclude the possibility that any mediating strategies were being used (rats identified as using such strategies postoperatively were subsequently excluded from the analysis). The first press on the back lever after the delay had terminated caused both levers on the front wall to be inserted into the chamber with both the corresponding front lever lights being lit (the choice phase). The rat was now required to press the alternative lever to the sample lever last presented (i.e. non-match). The lever lights served to cue the rat that a new phase in the trial had begun. A time out period with the house lights off was enforced following any error, i.e. pressing the same lever again in the choice phase, or, if the rat did not press the sample lever before the 10 second limited hold timed out. The length of delays was varied up to a maximum of 32 seconds. An intertrial interval (ITI) of 10 seconds was used between all trials during training on the DNMP task. This interval refers to the amount of time that elapsed between any two non-matching trials. Postoperatively, ITI was varied to assess proactive interference. Two ITI conditions were used of 10 and 30 seconds.

Previous studies have trained rats up to a delay of 64 seconds. However, in this study rats could only perform consistently above chance (e.g. at 60%) at delays no greater than 32 seconds. Taking the delays out to 64 seconds revealed that most rats were performing at 50% on the two longest delays, 48 and 64 seconds. It is possible that the added complexity of pressing a back lever on the DNMP task was responsible for the rats not being able to cope with delays longer than 32 seconds. In total, 6 months were spent in training (5 days per week, 96 trials per day, 16 trials per delay type). Five months were spent taking the rats out to delays of 64 seconds. The last month was spent gradually bringing the delays back to a maximum of 32 seconds, arriving at an optimal set of delay types, and gathering pre-operative performance measures. The final delays used were as follows 4, 8, 12, 18, 24, and 32 seconds. Performance measures based on the last twelve days of training were used to allocate rats into groups equal on Log Do and b measures. No sooner than 2 weeks after surgery, all rats were reintroduced to the operant boxes. The

first six days postoperative performance was not used in the analysis as it became clear that all rats performance had dropped markedly postoperatively. After six days on the DNMP task the performance of all rats stabilized. The analysis was performed on the sessions that followed. Sessions 7-18 were the initial postop period and involved an ITI of 10 seconds. On sessions 19-42, twelve sessions each of ITI 10 seconds and ITI 30 seconds were run on alternating days. This design was used to assess the extent to which ITI 10 seconds produced proactive interference compared to an ITI 30 second condition.

Filming of Rats on the Delayed-Non-Matching-to-Position Task

Rats were filmed both pre and post operatively to view their behaviour in order to preclude the possibility that rats were using mediating strategies to solve the task. All rats were observed and videoed for a minimum of three minutes. Rats suspected of using a strategy were videoed for further three minutes. Using this information rats were labeled as "strategists" or "non-strategists".

Preoperatively four rats were found to have obvious and clear strategies that always consisted of the following. The rat would hit the sample lever, turn in a consistent direction and hit the back lever with (often with just one paw). After every hit on the back lever it would twist its body towards the non-matching side. Four "strategists" were discovered out of the 56 trained. One of each of these was assigned to each of the lesion groups.

Spontaneous Object Recognition

Subjects were first familiarised in the open field that would subsequently hold the objects for recognition (a large opaque Perspex box, 100x100cm, walls 50cm high). The floor was covered with sawdust. Five sessions were used in familiarisation, each of which was six minutes long. During this period 3 sessions were analysed (days 1, 3, and 5) to assess the activity levels of the rats. Activity was quantified as the number of line crossings of 20 cm grids superimposed on an image of the open field. The number of such crossings were taken minute by minute throughout the six minutes of each trial. These sessions were all recorded on video.

The spontaneous object recognition task followed. Subjects were placed in the box, facing the wall farthest from the two objects that were now in the box. These two objects were placed in the corners of the opposite wall. The center of each object was 15 cm in from each of the two closest walls. In the sample phase two large identical objects were used. Total time spent exploring the objects during a 3 minute period was recorded. In the second condition (the comparison phase) after a delay of 1, 15, or 30 minutes, the rats were placed again in the box, this time with two different objects. One of these was an identical copy of the objects seen in the first exploration condition. The other was a novel object never before seen. The order in which the rats experienced the delays was systematically varied. All rats had a first trial of 1 minute, followed by a trial of 15 minutes (for half the rats) or 30 minutes (for the other half). The next trial for all rats was another 1 minute trial, which was then followed by a trial of 30 minutes or 15 minutes, based on the delay type each rat had experienced on the previous trial. In this way all rats had 2 trials of 1 minute, one of 15 minutes, and one of 30 minutes. The side on which the novel object was placed (relative to the rat) was also systematically varied such that each rat had two occurrences on the left, and two on the right respectively. The extent to which the new object was recognised as novel was estimated by the difference in time spent exploring the novel as opposed to the familiar object. No pre-training was given as this is a "spontaneous object recognition task" and relies on the rat's natural exploratory behaviour. For greater detail of this task see Aggleton et al (1995) or Ennaceur and Delacour (1988).

During the object task two rats did not explore the sample during the sample phase and were excluded from the object task analysis. One of these was an AV rat and the other an AT rat.

Technical difficulties on the Delayed-Non-Matching-to-Position Task

Intermittent technical difficulties with one of the operant chambers meant that all postoperative DNMP data was lost for four of our subjects. This included one SHAM control rat, one AV rat, and two AT rats.

In addition two rats (one LD and one SHAM) were excluded from the operant task

because they failed to respond on more than 30% of their trials, over two standard deviations more than the average "percent trials missed" by the group.

Statistical Analysis

Power

The usual sample sizes used in this type of research are relatively small i.e. N's of between 4 and 10 subjects per group are commonplace. This reflects the difficult and time-consuming aspects associated with this area. Aggleton et al's (1991) experiment is a useful guide for effect sizes found in this type of research since they used the same task (DNMP) and had the same number of experimental groups (AT, fornix, mammillary and control). The F statistic from that study was used to calculate the effect size, (f) of 0.67 (d value of 1.34). The proposed sample size of N=12 per group for the current work (u=3) has a 96% chance of detecting a significant group effect size of 0.67 at a=0.05. The situation vis a vis pair-wise comparisons is more problematic, because it is difficult to estimate the between-group effect sizes from the data provided in the relevant reports. Mizumori et al (1994) included two conditions namely baseline and temporary lesion, rather than two experimental groups, they found an effect size of f=2.48. Byatt & Dalrymple-Alford (1996) found an effect size of f=1.15 in an experiment that included AV and AM lesions plus a control group. Obviously these effect sizes provide a general guide given that each compares various experimental groups to the control group, but none of the experiments cited uses only the lesion sites suggested for the proposed study, i.e. AT, AV, and LD. One approach for the AV vs control difference is to assume that, in the context of lesion work where only large effect sizes might be considered important, we should want to detect a d of 1.00 and accept a one-tailed a of 0.10 (the lesion should only produce worsened performance). In such a case the power of the study would be 77%. Thus we have every chance statistically significant differences will emerge. In either case, descriptions using relative effect sizes and confidence intervals (i.e. a precision analysis) would still provide extremely valuable information irrespective of the outcome of the traditional significance tests (Cohen, 1996).

Log d, Log d_o, b in the Delayed-Non-Matching-to-Position Task

The traditional measure of percent correct for comparing performance across groups will provide the main measure of analysis. However percent correct can be slightly misleading when an animal displays bias. For example if a rat shows bias toward the right lever it is unclear whether an animal (correctly) choosing that lever did so due to its memory of a previous event, or some bias (White, 1986). In addition then, the data were converted to a bias free measure, Log d. Furthermore, where possible, performance across all delays was fitted to an exponential decay function, Log $d = \text{Log } d_o e^{-bt}$. Two measures of performance, Log d_o and b were extracted from this curve fit. Log d_o is an extrapolation of the exponential decay curve to true zero delay, thus giving a delay independent measure of each rats performance. This is important as an inability to perform the task could result from memory impairment or some other impairment, e.g. decreased ability to discriminate between stimuli. The second parameter, b, provides a single descriptor of how performance changes across all delays, i.e. a delay dependant measure (forgetting). Log d is a measure using the equation, Log d=0.5log (C1/E1.C2/E2) where

C1= correct response on the left key

E1= incorrect response on the right key

C2= correct response on the right key

E2= incorrect response on the left key.

Measures of Log d are generated at each delay interval (Log d^t, t=time).

The performance of each of the four groups was equated as far as possible (means, SD) to ensure that there were no group differences before the lesion surgery. This includes measures of; percent performance at each delay, estimated performance at a hypothetical zero delay (Log Do), and estimates of the rate of forgetting by fitting performance to the exponential decay curve and extracting b. In both tasks planned comparisons were intended between the AT and SHAM groups, given previous research, otherwise, post-hoc comparisons were made using Newman-Keuls test.

Object Recognition

On the object task the following measures were used, as in the Aggleton et al. (1995) study;

1. An "exploration index" assessed the amount of time the rats spent exploring the objects in the sample phase.

2. A "discrimination index" was used as a basic estimate of recognition. This involved subtracting the amount of time spent exploring the novel object in the choice phase from the amount of time spent exploring the sample.

3. A "proportional discrimination index" was also calculated by dividing the discrimination index by the total time spent exploring both objects in the choice phase. This measure helps to control for differences in the total amount of time spent in exploring the objects on the choice phase.

Results

Histological Analysis

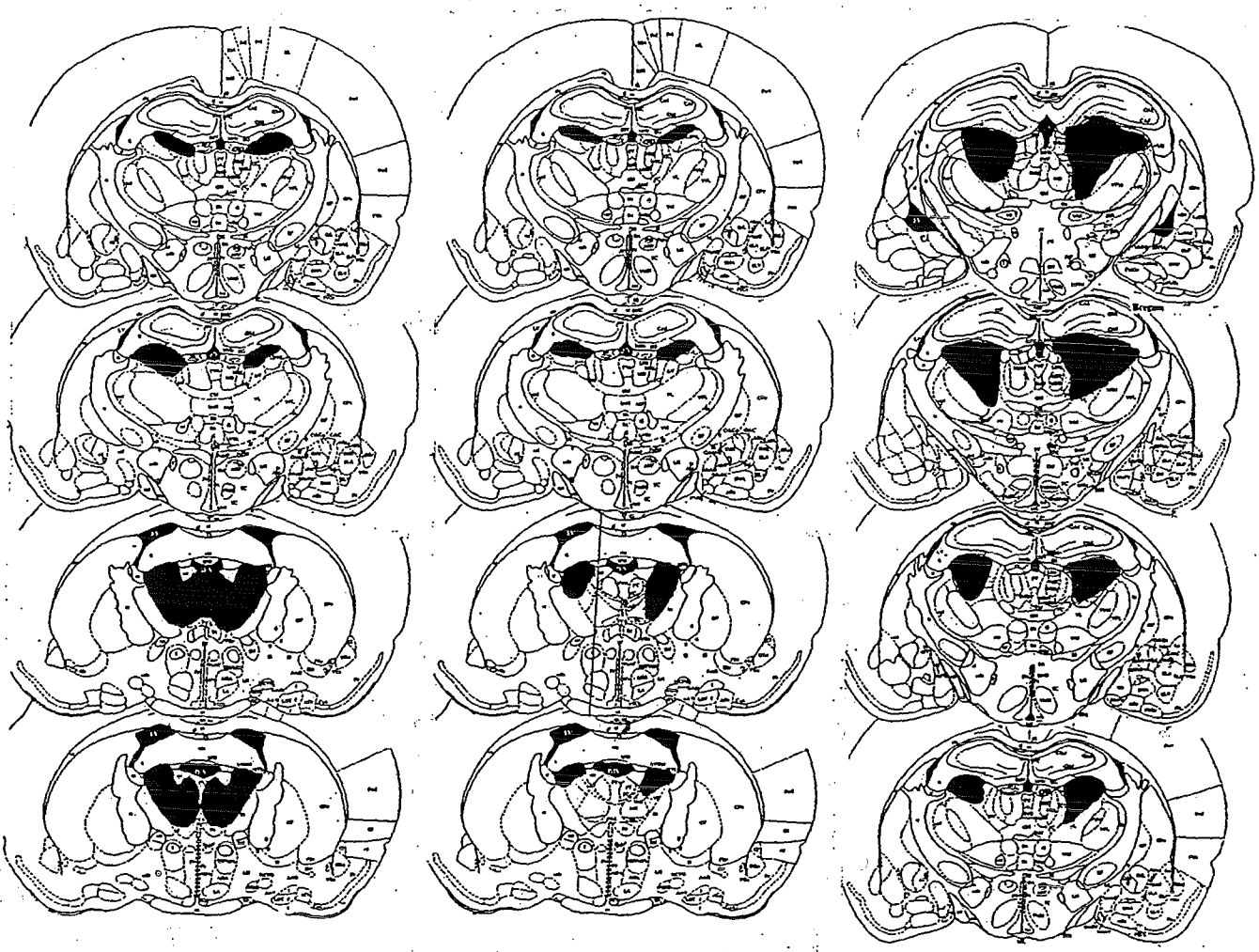
At the end of the experiment rats were perfused transcardially with saline followed by 4% formalin. The brains were later transferred to a sucrose formalin solution, serial frozen 50 u sections taken throughout the extent of the AT and LD regions, and these sections mounted on slides for staining. Alternate sections were stained with cresyl-violet and acetylcholinesterase histochemistry to facilitate identification of the size and location of the lesions. On the basis of histological analyses some rats were excluded due to minimal lesion size or inappropriate lesion location. Representative examples of each lesion are shown in Figure 1.

Overall, all rats in the AT group had lesions of sufficient size and location to be maintained in the analyses. All showed substantial to near total loss of cells in the AV, AM, and the AD region. The anterior, and especially medial anterior regions of the LD were also effected in most cases. In one rat, the anterior region of the MD was also damaged but MD damage was minimal or nonexistent in the remainder.

Problems with the lesions in the AV group resulted in the exclusion of three rats from the analysis. All three of these rats presented with similar types of lesions revealing only minor ventral AV damage, and minor lateral AM damage. In all three of these rats lesions were generally too ventral. All remaining rats revealed bilateral damage to the AV together with some minimal unilateral AM damage in a few cases. The largest AV lesions revealed complete shrinkage of this nucleus in addition to ventral damage.
All LD rats revealed appropriate and extensive bilateral lesions of this nucleus. One rat with particularly large bilateral lesions to the LD revealed some evidence of damage extending to the AV and AM. All rats in the LD group revealed evidence of minor, unilateral, AD damage. The outer layer of the ventral blade of the dentate gyrus was also affected unilaterally in all cases with cell loss in the ventral dentate gyrus layer in four cases. Damage ventral to the LD was present in all cases.

Figure 1: Representative examples of the types of lesions produced in each group.

Figure 1



Representative AT lesion

Representative AV lesion

Representative LD lesion

Filming of Rats on the Delayed Non Matching to Position task

The same procedure that was used preoperatively to identify rats using mediating strategies was carried out postoperatively. Particular care was taken with the four rats found to be "strategy" rats during the preop training period. Postoperatively only two rats were found to be using such strategies, both of which had been preop strategy rats. These two rats belonged to the LD and AV groups respectively. Based on this finding, both these "strategy" rats were removed from the analysis. The two remaining rats that had been preoperative "strategy" rats were observed carefully and deemed to be no longer using a mediating strategy on the task. Consequently these two rats were kept in the analysis.

Final N Sizes For Each of the Groups

Overall the size of the groups was decreased by the above criteria. All rats excluded from the DNMP task by histology were also excluded from the object recognition task. The problems in one of the operant boxes did not obviously effect the object task, and hence these rats were included on the latter task. Similarly the large percent trials missed by two of the rats did not obviously effect the object task and so these two rats were also included on the object task analyses.

The final analysis of the DNMP task consisted of 8 AT rats, 6 AV rats, 9 LD rats, and 10 SHAM controls. On the recognition task 9 AT rats, 7 AV, 11 LD, and 12 SHAM rats were included in the final analysis.

Familiarisation Task

The familiarisation sessions consisted of five six minute trials. During sessions one, three, and five, the behaviour of the rats was observed and filmed. Activity measures were quantified as the number of line crossings occurring on a grid consisting of 20cm by 20cm squares. Total line crossings were taken minute by minute.

Results were analysed using ANOVA which revealed a group effect that just failed to reach significance ($F(3,35)=2.787$, $p<0.055$). Post hoc comparison using the Newman-Keuls test revealed only an effect between the AT and the SHAM controls ($p<0.051$). Figure 2A shows that the AT group had a greater number of line crossings than any other

group over all three recorded sessions. A session effect ($F(2,70)=4.487$, $p<0.015$) led to post hoc comparisons using Newman Keuls which revealed that session one differed significantly from session two ($p<0.009$) and differed also from session three ($p<0.091$). Session one involved more line crossings for all groups than any other session suggesting that overall activity level decreased over the 3 recorded sessions.

Spontaneous Object Recognition

An additional estimate of activity level was assessed comparing the amount of exploration occurring in all sample phases of the object recognition task. The purpose of this was to verify whether any group differences existed in baseline exploration levels. All delay conditions were identical during the sample phase and so were combined to give an overall picture of sample phase exploration. Comparing all four conditions by group revealed only a session effect ($F(3,105)=9.613$, $p<0.001$). Post hoc analysis using the Newman Keuls revealed that the first one minute session differed from all other sessions (15 minute; $p<0.001$, 1 minute; $p<0.006$, and 30 minute; $p<0.002$). No other differences were found. Given this finding the first one minute delay session was treated as a final familiarisation and excluded from the object recognition analysis (see Figure 2B).

To analyse the effects of our lesions on spontaneous object recognition the three alternating delay conditions were compared (the first one minute delay condition was excluded). A discrimination index was calculated which was the difference in time spent exploring the novel object compared to the time spent exploring the sample in the test phase. A positive value on the discrimination index reflected recognition of the sample during the discrimination phase. This occured when more time was spent exploring the novel object as compared with the time spent exploring the sample. No group, delay, or group by delay effects were found (all $F<2.2$ and all $p>0.11$). All groups discriminated at all delays as shown by the positive difference values in time spent exploring the novel object as compared with the sample (see Fig 3).

Figure 2A: Squares crossed during three familiarisation trials prior introduction of the objects.

Figure 2A: Mean Line Crossings
Shown per Group and Session

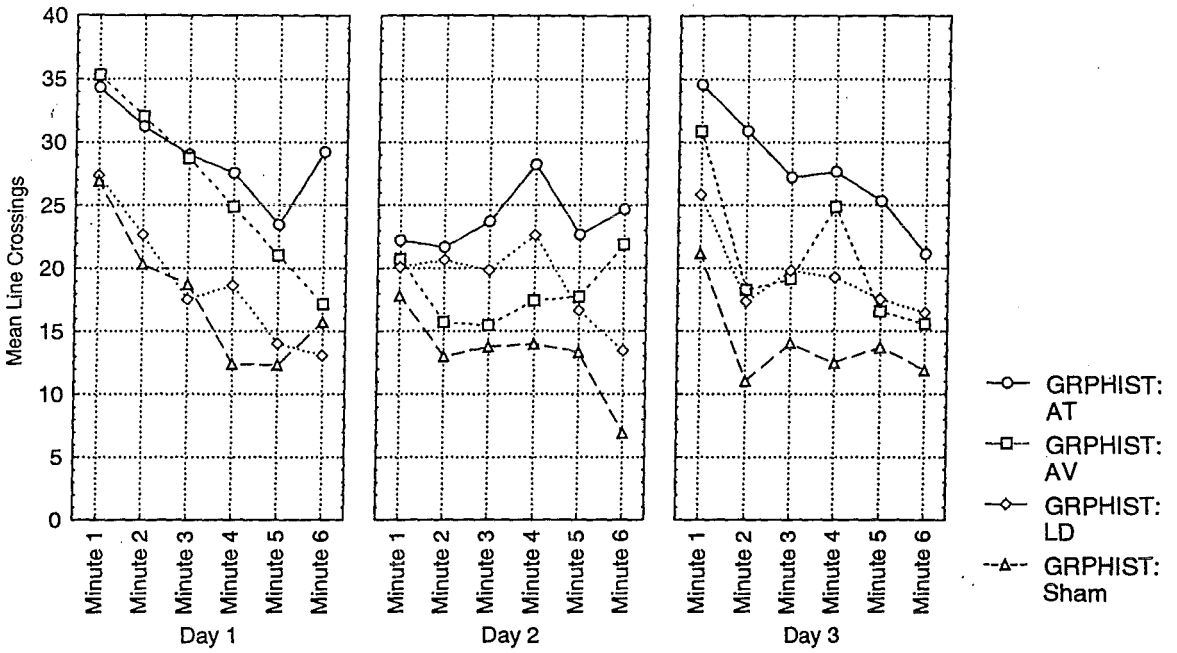


Figure 2B: Overall exploration graph of the spontaneous object recognition task. Mean amounts of time spent in exploring by each group of both sample objects during the sample phase of the object recognition task.

Figure 2B: Mean time in seconds spent exploring the sample

Over all spontaneous recognition trials

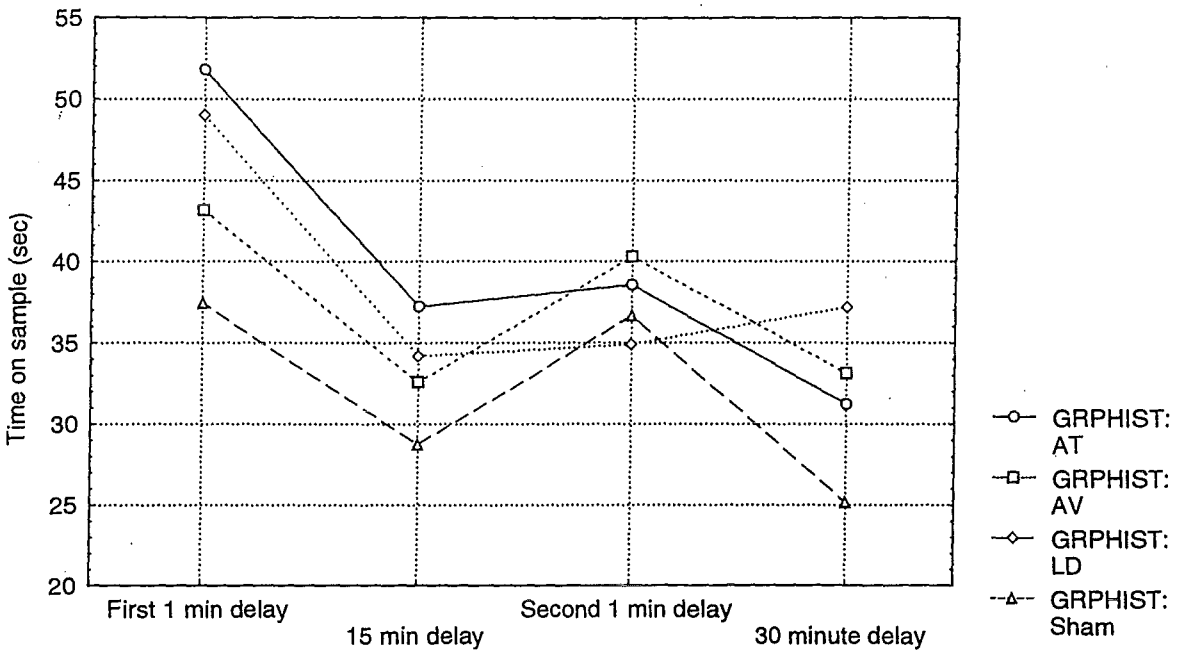
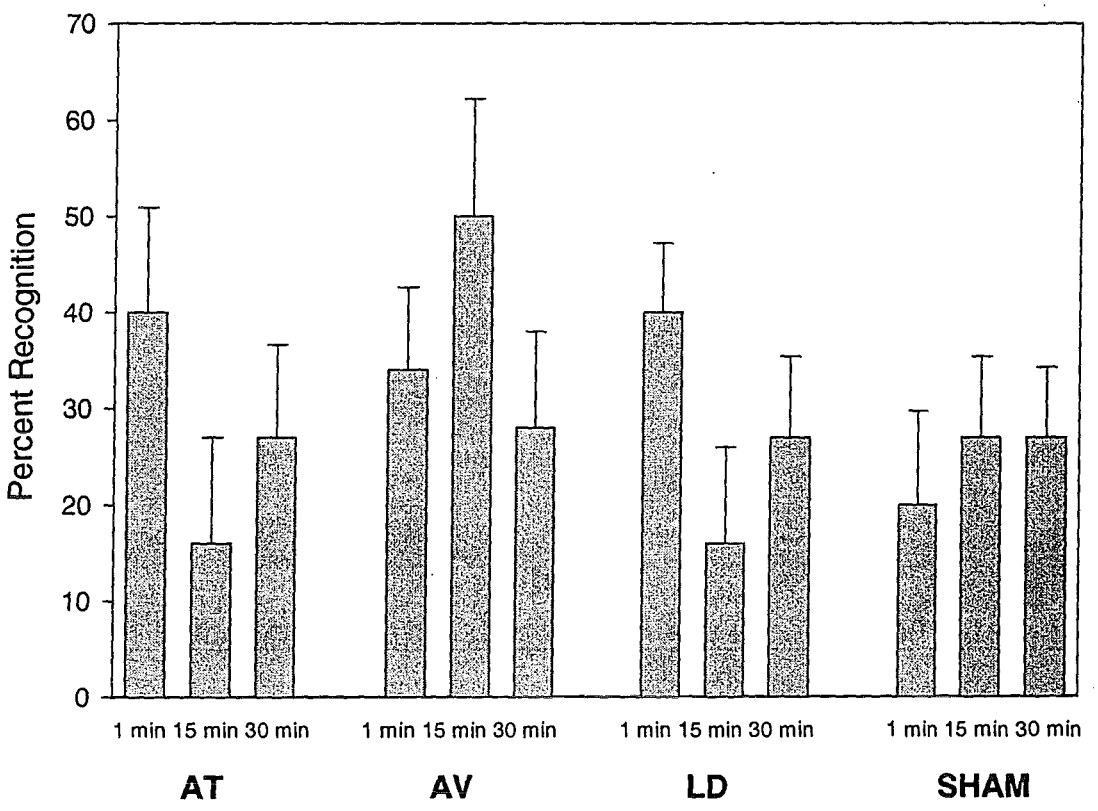


Figure 3: The percent recognition at each delay on the object recognition task. Standard mean errors are shown.

Figure 3:

Percent Recognition and standard mean error for all rats on all delays.



A further analysis using a percent recognition measure was used. This was calculated by dividing the discrimination index by the total time spent exploring the two objects in the sample phase. This measure helps control for total exploration time in the test phase. An ANOVA comparing delay with groups revealed no significant effects, either by group, delay, or interaction (all $F<1.6$ and all $p>0.2$). These results were consistent with the findings of the discrimination index analysis.

Delayed non matching to position, percent correct performance

Prior to analysis of the effects of our various lesions on performance, an initial analysis was undertaken to ensure that all groups were alike on percent performance across all delays at the end of preoperative training. As expected no group, ($F(3,29)=1.077$, $p<0.374$), or interaction effects ($F(15,145)=1.286$, $p<0.336$) were found.

Overall the data consistently pointed to an AT deficit in performance, while the other three groups all performed comparably (see figure 4).

The initial analysis focused on the percent performance of each of the groups. Each condition was contrasted with preop performance. Postop sessions 1-6 were not discussed as these were considered as reflecting postop recovery and reintroduction to the DNMP task. Comparing sessions 7-18 of postop performance with the last twelve days of preop training revealed a significant preop vs postop condition effect ($F=(1,29)=12.316$, $p<0.002$) and group by condition interaction ($F=(3,29)=9.791$, $p<0.001$) (see Figures 4a and 4b). Analysis of the simple main effects revealed that the AT differed significantly from the SHAM controls ($F(1,29)=13.092$, $p<0.001$). This effect was evident despite the fact that the performance of all the groups together differed significantly from pre to postop, as can be seen comparing figures 4a and 4b. The difference between preop and postop suggests a postop impairment across all rats, especially at the shorter delays.

However further analysis of overall performance (preop vs sessions 7-18) revealed that only the AT were performing significantly differently from preop to postop ($F(1,29)=38.193$, $p<0.001$), reflecting a substantial impairment in their performance between these two conditions. No other group effects were found (all $F<2.2$, all $p>0.15$). It is clear that the AT group was significantly impaired at postop compared to preop, unlike all other groups whose performance declined less obviously. There was also a

condition by delay interaction ($F(5,145)=13.000$, $p<0.001$)) but no group by condition by delay interaction ($F<1.0$), which reflects the fact that all groups showed poorer performance at the earlier delays, especially during the postop sessions 7-18 compared to preop performance.

A similar analysis was carried out comparing the 12 ITI 10 second sessions of the proactive interference manipulation with preop performance. Figures 4a and 4c highlight the differences between these two conditions. Comparing these postop sessions with preop performance revealed a significant group by condition interaction ($F(3,29)=9.835$, $p<0.0001$). Analysis of the simple main effects compared preop and the ITI 10 postop performance. Taking each of the groups alone revealed a significant decline in the AT group, ($F(1,29)=17.690$, $p<0.001$). The LD group ($F(1,29)=8.203$, $p<0.008$), improved but the simple main effects for the AV failed to reach significance ($F(1,29)=3.573$, $p<0.069$). The SHAM group did not differ at the 10 second ITI condition relative to preop performance ($p>0.1$). These results together revealed that the AT were still impaired compared to preop performance, the LD got better, the AV improvement failed to reach significance, and the SHAM's were performing at a level comparable to preop. This analysis confirms a persistent AT deficit, which was maintained after an extensive period of testing.

Comparing preop with the ITI 30 second condition of the proactive interference manipulation revealed a significant condition effect, ($F(1,29)=16.080$, $p<0.004$). Figures 4a and 4d reveal that this effect is clearly an improvement across all delays. A significant group by condition interaction was also found ($F(3,29)=3.566$, $p<0.026$). Analysis of the simple main effects taking all these groups alone, revealed that the AV ($F(1,29)=9.272$, $p<0.005$), the LD ($F(1,29)=10.892$, $p<0.003$), and the SHAM ($F(1,29)=5.852$, $p<0.022$) all improved relative to their preop performance, unlike the AT group which during the 30 second ITI condition had however reached a level similar to their preop performance. It is evident from these results and that all groups barring the AT had regained and surpassed their preop performance, the AT not differing significantly from it.

Further analysis contrasted the various conditions with each other. Comparing the 12 sessions postop (sessions 7-18) with the 12 postop sessions of ITI 10 that occurred during the ITI proactive interference manipulation over sessions 19-42 revealed effects between

these two conditions ($F(1,29)=33.894$, $p<0.001$) and Newman-Keuls tests confirmed that all groups performed significantly better in the latter ITI 10 condition. This revealed an overall improvement in performance in the ITI 10 seconds condition when these sessions were intermixed with the ITI 30 sessions

Comparison of the ITI 10 and ITI 30 conditions which occurred alternately over sessions 19-42 revealed a significant condition effect ($F(1,29)=49.3519$, $p<0.001$), and condition by delay effect ($F(5,145)=2.706$, $p<0.023$). All groups together performed better on the ITI 30 second condition compared to the ITI 10 second condition, suggesting that the ITI 30 condition markedly improved performance, presumably due to reduced proactive interference. Figures 4a and 4d suggest that the condition by delay effect was due to improved performance on the longer delays in the SHAM, LD, and AV groups and across all delays in the AT group during the ITI 30 second condition.

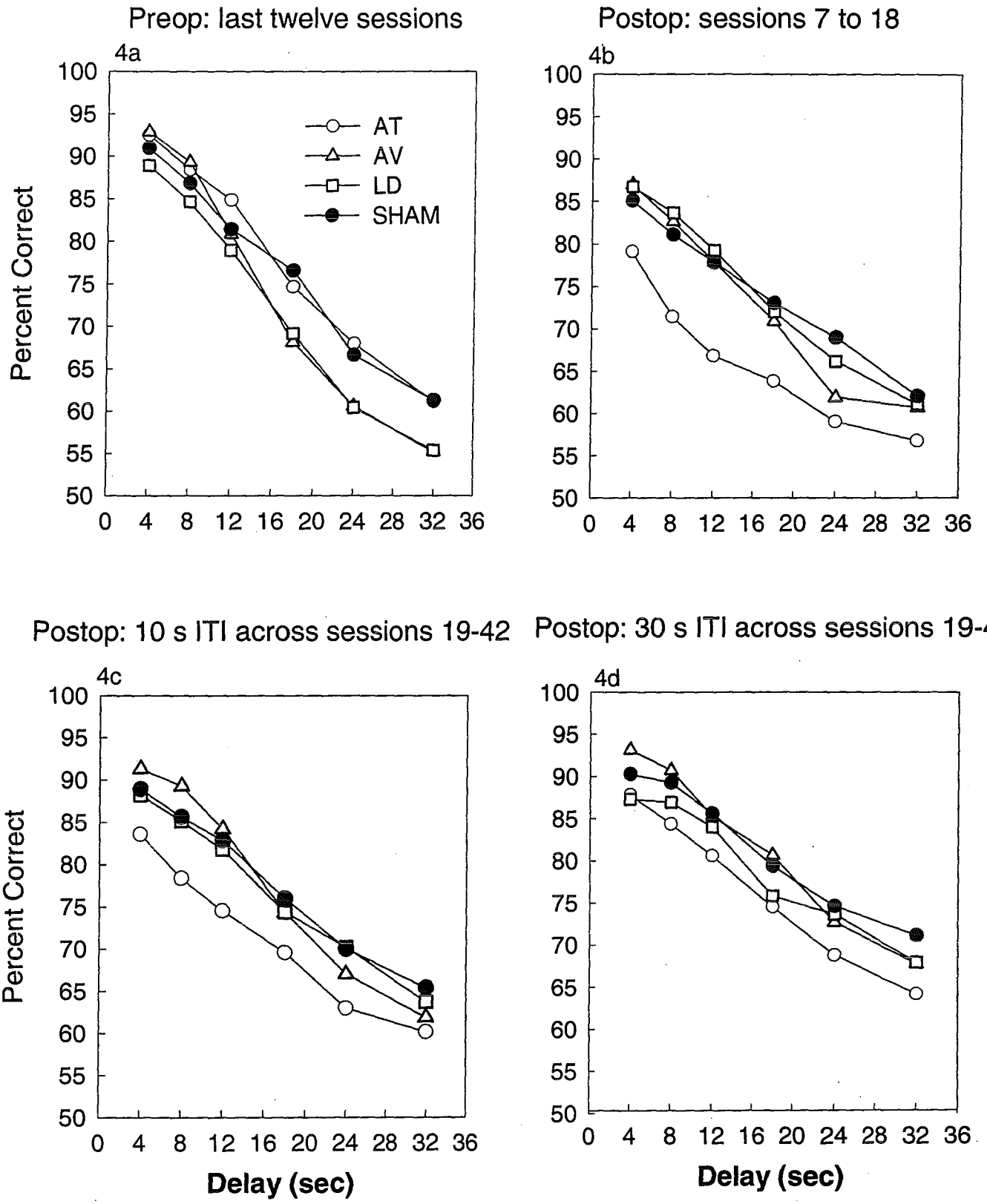
Delayed non matching to position, b scores

A similar procedure was carried out to assess the effects of the lesions on b values. Preop measures of b values confirmed that no group differences were evident prior to surgery ($F(3,29)=0.928$, $p<0.440$). Figure 5 shows Log Do and b values collapsed across rats per group. Note Log Do and b values for each group postoperatively for subsequent analysis.

Comparing preop performance with sessions 7-18 of postop revealed no significant group effects, and a group by condition interaction which failed to reach significance ($F(3,29)=2.401$, $p<0.088$). Planned comparison contrasting the two conditions and comparing the AT with the SHAM group revealed a significant interaction ($F(1,29)=5.249$, $p<0.029$). As shown by figure 6 all groups b values decreased from preop to postop (suggesting an improvement in rate of forgetting) barring the AT group, whose mean b value increased.

Figure 4: Figures 4a to 4d show the percent correct data of all groups by delay. Each of the graphs represents a separate condition. 4a:preop, 4b:postop (sessions 7-18), 4c: postop ITI 10 condition (12 sessions alternating during sessions 19-42), 4d: ITI 30 condition (12 sessions alternating during sessions 19-42)

Figure 4 :Percent Correct Across All Conditions And All Groups



Based on the impression that it was the change in b from one condition to the next that was illustrating the differences between the groups, a "relative change in b" index was calculated as an estimate of change. The "relative change in b" index involved subtracting the postop condition b values for every rat from its preop condition values and dividing these by its preop b value. The outcome was a proportional change in b index. A proportional measure was expected to help account for some of the large variability of the preop b values for all groups given that change was expressed as a proportion of the preop b values. Note that positive values on this measure signify an improvement in b (the rate of forgetting). Using this index, preop performance was contrasted with sessions 7-18 of postop. ANOVA values revealed a significant group effect ($F(3,29)=3.187$, $p<0.038$). Subsequent analysis revealed a significant effect for the AT against the SHAM ($F(1,29)=6.544$, $p<0.016$), the LD ($F(1,29)=7.894$, $p<0.009$), and also against the AV group ($F(1,29)=3.991$, $p<0.051$). Figure 6 reveals that the SHAM, LD and AV improved their b scores whereas the AT b values deteriorated for sessions 7-18.

Analysis of the absolute b values comparing the ITI 10 second condition of the proactive interference manipulation with preop revealed only a condition effect ($F(1,29)=16.357$, $p<0.001$).

Figure six illustrates group changes between these two conditions. Overall, this effect reveals an improvement in b values on this latter condition, as compared with preop. Analysis of the "relative change in b" index also revealed no significant main effect ($F(1,29)=1.79$). The graphed data (figure 5) suggests that for all groups save the AT, b values continued to improve in this latter condition.

Comparing the preop performance with the ITI 30 second condition again revealed a significant improvement in absolute b values ($F(1,29)=39.627$, $p<0.001$). The graphed data, figure 5, reveals that all group b values had improved from preop but less so for the AT group. Using the relative change in b index produced a significant main effect of group ($F(1,29)=3.469$, $p<0.029$). Further analysis revealed that the AT differed significantly from the LD ($F(1,29)=10.070$, $p<0.004$) and the difference with the AV just failed to reach significance ($F(1,29)=3.417$. $p<0.079$), but did not differ with the SHAM

group.

Comparing the two postop ITI 10 second conditions (sessions 7-18 and the ITI 10 second sessions between sessions 19-42) revealed no significant differences or interactions ($F<1$)

Comparing ITI 10 seconds against ITI 30 seconds revealed only a significant condition effect ($F(1,29)=21.837$, $p<0.001$). The LD ($F(1,29)=10.425$, $p<0.002$), the SHAM ($F(1,29)=9.989$. $p<0.003$), and the AV ($F(1,29)=4.421$, $p<0.048$) all differed significantly between the two conditions. The AT did not differ. The graphed data, figure 5, reveals that all groups barring the AT improved on b scores with the ITI manipulation, the AT not benefiting significantly from this.

Delayed-Non-Matching-to-Position and Log Do

A similar overall analysis was conducted for Log Do scores. Comparison of preop scores across all groups revealed no significant differences ($F(3,29)=0.690$, $p<0.566$). Comparing preop with postop sessions 7-18 revealed only a condition effect ($F(1,29)=28.777$ $p<0.001$). Planned comparison revealed no significant difference between the AT and the SHAM group. Figure 5 reveals that all groups Log Do scores dropped markedly and significantly from pre to postop, an effect which appeared to be greater for the AT group but could not be confirmed because of the marked variability in these scores.

Comparing the preop condition against the postop ITI 10 seconds data gathered on sessions 19-42 produced a condition effect ($F(1,29)=16.135$, $p<0.001$). Planned comparison contrasting the two conditions revealed the AT differed significantly from the SHAM controls ($F(1,29)=4.440$, $p<0.044$). Further planned comparisons revealed that only the AT group differed significantly from their preop scores ($F(1,29)=14.933$, $p<0.001$). It can be seen that the AT rats Log Do scores on the ITI 10 second condition were inferior to their preop scores.

Comparing the preop with the ITI 30 second condition revealed similar results, a significant overall effect of condition ($F(1,29)=9.539$, $p<0.004$). Planned comparisons revealed that only the AT differed significantly from their preop scores when taken alone ($F(1,29)=4.962$, $P<0.034$).

Comparing the postoperative condition sessions 7 –18 with the ITI 10 seconds condition that occurred over sessions 19-42 revealed a significant condition effect ($F(1,29)=4.465$, $p<0.043$). Figure 5 shows that all groups improved their Log Do scores between the two conditions.

Comparing the ITI 10 second condition with the ITI 30 condition tested the effects that decreasing proactive interference had on performance at a hypothetical zero delay. No significant overall Log Do effects were found. Planned comparisons contrasting the two conditions and comparing the AT versus the SHAM groups revealed a significant interaction ($F(1,29)=4.541$, $p<0.042$). Graphed data, figure 5, reveals that AT improved their log Do scores between these two conditions as compared with the SHAM controls. Thus these Log Do measures indicated that the ITI 30 second condition improved Log Do for the AT group only, suggesting that decreasing proactive interference improved Log Do for the AT rats.

Figure 5: Log D graphs per group per condition. Also shown are overall Log Do and b values per condition.

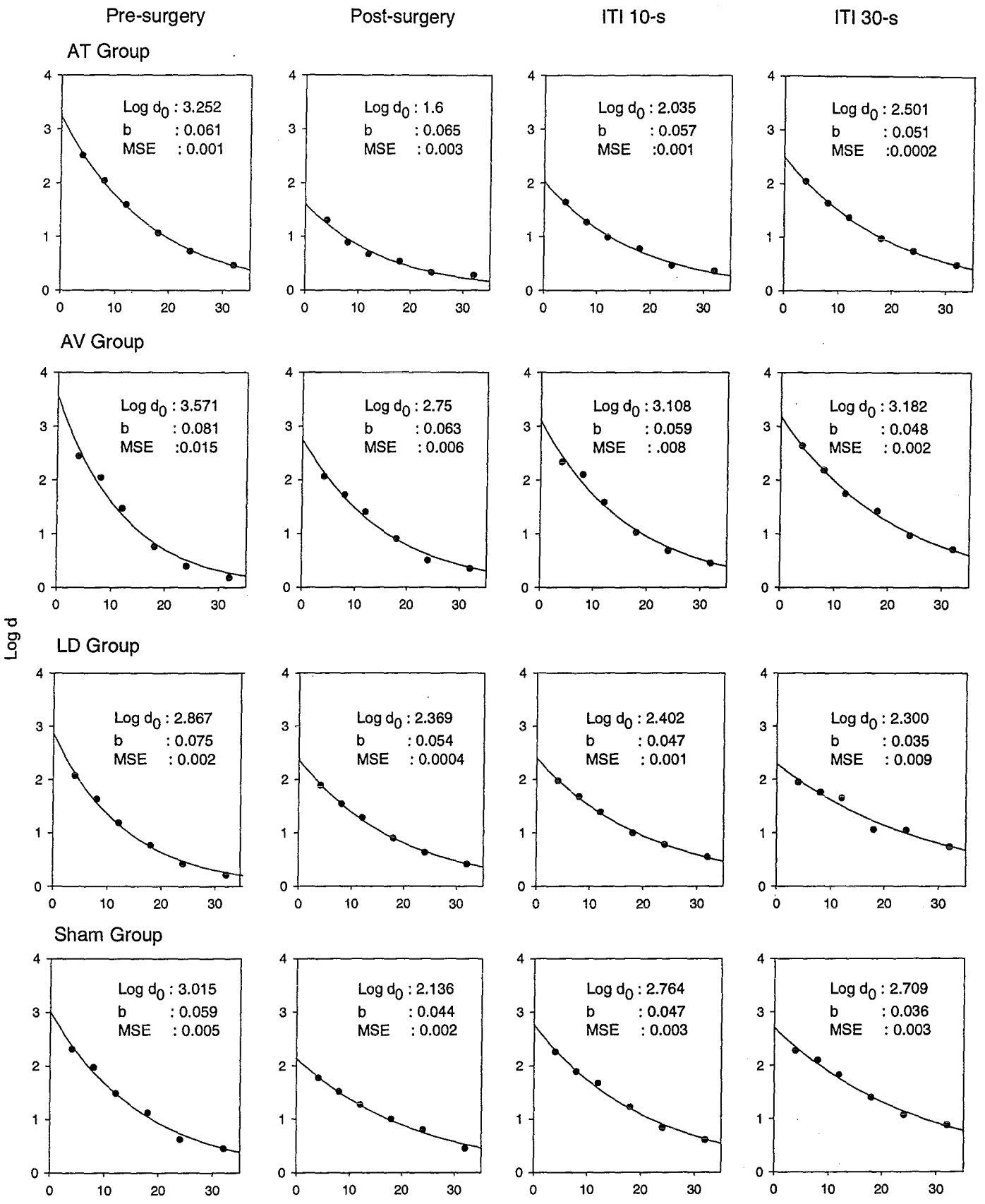
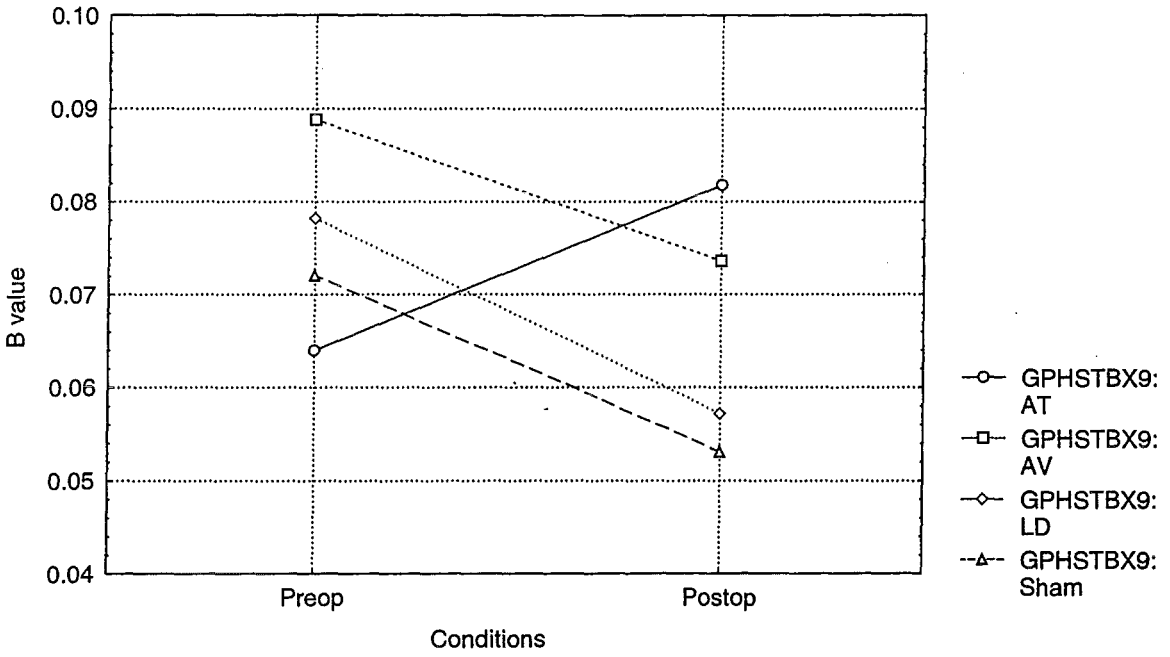

Pre-surgery
Post-surgery
ITI 10-s
ITI 30-s
AT Group
Log d_0 : 3.252
b : 0.061
MSE : 0.001
Log d_0 : 1.6
b : 0.065
MSE : 0.003
Log d_0 : 2.035
b : 0.057
MSE :0.001
Log d_0 : 2.501
b : 0.051
MSE :0.0002
AV Group
Log d_0 : 3.571
b : 0.081
MSE :0.015
Log d_0 : 2.75
b : 0.063
MSE : 0.006
Log d_0 : 3.108
b : 0.059
MSE : .008
Log d_0 : 3.182
b : 0.048
MSE : 0.002
LD Group
Log d_0 : 2.867
b : 0.075
MSE : 0.002
Log d_0 : 2.369
b : 0.054
MSE : 0.0004
Log d_0 : 2.402
b : 0.047
MSE : 0.001
Log d_0 : 2.300
b : 0.035
MSE : 0.009
Sham Group
Log d_0 : 3.015
b : 0.059
MSE : 0.005
Log d_0 : 2.136
b : 0.044
MSE : 0.002
Log d_0 : 2.764
b : 0.047
MSE : 0.003
Log d_0 : 2.709
b : 0.036
MSE : 0.003
Log d
Delay (sec)

Figure 6: Changes in b values between preop and postop for each group, sessions 7 – 18.

Figure 6: Effect of surgery on b values
Comparison Between Preop & Postop (sessions 7-18)



DISCUSSION

Delayed-Non-Matching-to-Position

The role of the AT in several tests of spatial working memory has been well established (see Aggleton & Brown, 1999). Overall the effects of this research point to a clear and profound deficit in the AT rats on the operant spatial memory task used, the DNMP. Despite revealing signs of recovering preoperative levels of function on percent performance and b values during the later stages of the study, the AT rats still revealed significant deficits in comparison to the other groups. This difference was particularly evident against the LD and SHAM groups, but also frequently against the AV group. Clearly, AT insult produces long lasting and persistent deficits on this task as shown by continued impairments during an extensive postoperative testing period involving a total of 42 sessions over a period of eight weeks.

The use of b value analysis is particularly useful in this type of experiment. This is because b values are extracted from Log D values, which are bias free estimates of performance at each of the delays used. In estimating the b value, an exponential decay function, the Log D function, is fitted to the Log D values produced at each delay. From this function the b value can be extracted which describes the steepness of the Log D function. In this way a bias free estimate of the rate of forgetting is produced. Bias free estimates of performance are important given that evidence of increased side bias in AT lesioned rats has been found on the DNMP task (see Aggleton and Hunt, 1991). The presence of side bias makes it difficult to conclude the extent to which DNMP performance reveals spatial working memory capacity as opposed to side bias.

Statistical analysis of the "relative change in b" index from condition to condition produced results consistent with the percent analyses. As mentioned earlier in the methods section, the "relative change in b index" compares b scores from an earlier condition to a later condition, producing a relative change estimate. Positive scores on the "relative change in b index" reveal an improvement in b between the two conditions. This is because positive scores are produced by relative reductions in b between the two conditions analysed. Since b is an estimate of the rate of forgetting, higher scores reveal

faster forgetting. If the b value in the second condition is lower than that in the first condition, a positive relative change score is produced which reveals relatively slower rates of forgetting during the second condition. Overall a persistent AT deficit was revealed which was reflected by lower scores on the "relative change in b" index. This effect was evident comparing the AT with the SHAM controls and the LD in particular, but also frequently in comparison with the AV group. While all groups revealed significant reductions in rate of forgetting (b values) from the first postop condition (sessions 7-18) which continued to decrease throughout the rest of the experimental period, the AT group failed to differ significantly here. Thus the AT group was the only group that failed to improve in their b values from preop to postop.

Log Do estimates are extracted from the same function that produces b values. The Log Do estimates are produced by projecting the Log D function back to a hypothetical zero second delay. Thereby Log Do produces an estimate of true zero second delay performance. The changes produced in the Log Do analysis were less clear cut for the individual groups, although overall revealing an AT deficit. A clear and very significant effect was found comparing preop with the first postop condition, revealing that Log Do scores for all groups had dropped markedly. An AT deficit became clearer during the ITI 10 second condition which occurred during sessions 19-42. Here the AT differed significantly from the SHAM controls, revealing lower Log Do values. The AT impairment was also reflected by the fact that while all groups had lower postop Log Do scores, over sessions 7-18, as compared with preop values, this effect was only statistically significant for the AT group. At the end of the experiment the Log Do scores of all groups barring the AT had returned to preop values, the AT continuing to reveal significant impairments.

Deficits at zero delay are not believed to reflect memory loss but rather a loss of procedural information (e.g. see Warburton et al. 1999). The fact that a deficit at zero second delay was produced postoperatively for all rats suggests that at least some of this Log Do deficit may have been due to the rigors of surgery. However, the AT lesion effect mentioned in the previous paragraph is important, because it is believed that lesions to the HF produce only delay dependent deficits. Given that the AT are believed to be part of the proposed extended HF system, lesions to this nuclei are expected to produce similar

mnemonic deficits on memory as those seen following HF damage (see Aggleton & Brown, 1999). Other studies, including those of DNMP, producing lesions to the AT and other parts of this extended system, have not produced deficits that are evident at the shortest delays (e.g. Aggleton et al. 1991; Aggleton & Sahgal, 1993). These studies produced evidence indicating that AT deficits are not a result of the loss of the procedural aspects of the task, as reflected by unimpaired performance at zero delay, but rather a loss of mnemonic capacity. Of particular relevance here is a recent study by Warburton et al. (1999) revealing that extensive lesions of the AT, which extend into the LD or MD, lead to profound losses of spatial working memory including an apparent loss of procedural information on the Morris water maze.

Delayed-Non-Matching-to-Position and Mediating Strategies

The use of mediating strategies is a source of concern in any study of spatial working memory. On a task such as the DNMP, which is believed to tax spatial working memory, the use of a mediating strategy to solve the task renders the data produced difficult to interpret. This is because it is unclear the extent to which performance reveals working memory capacity as opposed to revealing the use of a mediating strategy that is not working memory based.

The current study took measures to control for the use of such mediating strategies in solving our task of working memory. Chudasama and Muir (1997) found that on the DNMP task rats frequently use mediating strategies and that inclusion of these "strategy" rats significantly influences the results obtained. In their version of the DNMP, rats were required to press a magazine flap in the center of the front wall of the operant chamber during the delay period between the sample and choice phases. In an attempt to minimize the use of mediating strategies, the design of the current study required that rats press a lever on the back wall as opposed to the front wall of the chamber during the delay period between the sample and choice phases. The fact that our postoperative analysis discovered only two rats using mediating strategies to solve the task suggests that the use of a back lever response on the DNMP task may help to prevent rats from using these

strategies. Removal of these "strategy rats" ensured that their data did not contaminate or influence the results.

Proactive Interference and Delayed-Non-Matching-to-Position

The comparison of the ITI 30 second and ITI 10 second conditions allowed assessment of the extent to which proactive interference influenced the results of the current study. Previous research on proactive interference and DNMP has focused on very short ITI's (e.g. 5 seconds; Dunnett & Martel, 1990) and compared these with performance on comparatively longer ITI's (e.g. 15 or 45 seconds). The current study revealed that both percent performance and b scores were better on the ITI 30 second condition for all groups suggesting that all groups found the task easier with the longer ITI and were likely to have been suffering some proactive interference during the ITI 10 second condition.

The recovery of performance in the AT group on all measures, but particularly on percent performance and Log Do, during the ITI 30 second condition suggests that the AT group was particularly sensitive to proactive interference postoperatively. Comparing the ITI 10 sessions with the ITI 30 sessions that occurred alternately revealed that only the AT group's Log Do scores improved significantly with the proactive interference manipulation (Note that the AT group's Log Do scores were still significantly impaired relative to preop). This suggests that the short ITI condition was impairing zero second delay performance for the AT group and possibly impairing the recall of procedural information in this group. It is possible that this type of interference may have been at least partially responsible for some of the lesion deficits found.

Effects of AV Lesions on Working Memory

The lack of AV lesion performance deficits revealed by our study on any of the above measures concurs with Aggleton et al's (1996) results, which revealed a lack of persistent and significant deficits produced by selective lesion to various AT nuclei. In that study the AV/AD lesioned rats produced only an acquisition deficit on T-maze forced alternation, a minor deficit compared to the AT group, which revealed profound and

persistent mnemonic deficits. The current study suggests a lack of deficit in AV damaged rats on the DNMP task. Indeed, on some measures we found that the AV differed significantly from the AT while they did not differ from the SHAM controls.

The results of the current study differ from the positive results revealed by both Byatt and Dalrymple-Alford (1996) and Aggleton et al. (1996) of an AV role in spatial working memory. Byatt and Dalrymple-Alford (1996) conducted a study that revealed AV lesion impairments on the 12 arm radial maze (a test of spatial memory). Aggleton et al.'s (1996) study examined the effects of AV/AD lesion on performance in an eight arm radial maze. The latter study found significant AV/AD deficits on this task which were significant but not as extensive as those produced by AT lesions. Clearly the extent of impairment produced by selective lesion to the AT nuclei remains an open issue, and probably depends on the task used.

An important difference between the current study and that of Byatt and Dalrymple-Alford (1996) is that the latter used radiofrequency lesions, while the current study used NMDA lesions. Radiofrequency lesions produce damage that extends to fibers of passage, while NMDA leave these unaffected. It is possible that the above positive results were a consequence of such additional damage. However, it is probably the task rather than mode of AV lesion that is important in producing these results. It is likely that different task demands exist between the radial maze and the DNMP, which produce a specific sensitivity of the radial maze to detect the effects of AV lesions. Recall that Aggleton at al (1996) used NMDA lesions and provided at least some evidence of a significant AV/AD lesion impairment on the eight arm radial maze. It is likely that full deficits on spatial memory tasks are only produced by complete AT lesions, and no deficits are produced on the DNMP task by small selective AV lesions.

The Laterodorsal Thalamic Nucleus

Overall, the LD failed to differ from the SHAM group on any of the tasks or measures analysed. The result implies that LD lesion groups are comparable to SHAM controls on both object recognition, as measured by the spontaneous object recognition task, and spatial memory, as measured by the DNMP. In fact, on some measures, particularly of b

and Log Do, some small evidence emerged that the LD groups performance was if anything, superior to the SHAM controls.

The extent to which the LD should be considered part of the AT complex was not adequately answered in the current study. While the LD differed from the AT by not producing a DNMP deficit on any of the measures used, the AV group also revealed no such deficits, and both the LD and the AV differed significantly in function from the AT on a number of DNMP measures. Given that neither of these nuclei produced any clear DNMP deficit, no comparisons of function could be made between these two nuclei. Inclusion of the LD as part of the AT would have been suggested given a comparable impairment in the LD group as that found in the AV group.

No previous examinations of the LD's role on DNMP have been done. Two studies of spatial working memory have produced mixed results. Mizumori, Miya, & Ward (1994) obtained some evidence of an LD role on an eight arm radial maze by reversibly inactivating this nucleus. Warburton, Baird, and Aggleton (1997) found no significant difference in the impairment produced in AT lesioned rats by adding an LD lesion to an AT lesion on a T-maze. The current results suggest that on at least the DNMP the LD plays no significant role. It is relevant to note that the LD lesions of the current study were well centered and large, destroying large portions of this structure.

Spontaneous Object Recognition

The familiarisation task revealed that the AT lesioned rats had a tendency to be more active in the open field. However, on the object recognition task this did not relate to significantly different amounts of time spent in exploration during the sample phase of the object task. Thus, this difference in behaviour between the groups was not believed to be influential on the object recognition task.

Overall it was found that all groups displayed an ability to recognise familiar objects after delays of up to thirty minutes. Given that object recognition for each of the groups seemed insensitive to delay, as highlighted by the similarities in the recognition index

between the different delays used, it is possible that longer delays would be more sensitive in highlighting any lesion effects that may have occurred.

The current results of the spontaneous object recognition task revealed no differences between any of the groups, and suggest that while all groups were able to recognise a familiar object with delays of up to thirty minutes, no recognition differences existed between the groups. This sits well with the model of item recognition proposed by Aggleton and Brown (1999), which implies no involvement of the AT, the AV, or the LD on tasks of object recognition. It also replicates the findings and conclusions of Aggleton et al.'s (1995) study, which revealed no spontaneous object recognition impairments in AT lesioned rats. An additional result of that study revealed a tendency for the AT group to not improve recognition between a one minute and fifteen minute delay. However, this effect did not reach statistical significance and was in contrast to the other groups in that study (a MB, a fornix, and a SHAM control group), which revealed signs of improved recognition between the two delays used. This tendency was not replicated in the current study.

The results of the DNMP task and the object recognition task taken together add to the weight of evidence suggesting that the AT form part of an extended HF system that subserves spatial working memory which is independent from, and distinct to, an object recognition memory system (Aggleton & Brown, 1999). Recent research has helped to establish the independence of these two systems looking at the various components and the specificity of function of each of these systems (e.g. Aggleton et al. 2000).

Contributions of the Current Study, Limitations and Future Considerations

Our results are a replication of previous studies into the effects of AT lesions (Aggleton et al. 1991; Aggleton & Saunders, 1993) and add to the weight of evidence suggesting that complete AT lesions can produce deficits similar to those observed following fornix and HF lesion. These deficits have been highlighted using both the DNMP (e.g. Aggleton & Sahgal, 1993), and other tests of spatial working memory (e.g. T-maze, Hunt & Aggleton, 1991, Aggleton et al. 1995; and Morris water maze, Sutherland & Rodriguez, 1989). The current results also contribute to the finding that the AT form part of an

extended hippocampal system comprising of the HF, the fornix, the AT, and the MB, (Aggleton & Brown, 1999) which subserves spatial working memory.

The proactive interference manipulation highlighted the fact that ITI of 10 seconds produces proactive interference in all groups but some evidence also emerged for a particular sensitivity in the AT group. The particular sensitivity of the AT group to proactive interference was highlighted by the significant improvements discovered in this group following the proactive interference manipulation on many measures, particularly in Log Do.

The lack of effect of AV lesions on the DNMP supports the school of thought which suggests that complete AT lesions are required to produce complete AT memory deficits. In addition the current study suggests that on the DNMP task the AV play no role (e.g. Aggleton et al. 1996). The findings of Byatt and Dalrymple-Alford (1996) and Aggleton et al (1996) on the radial maze point to at least an important role of the AV on this latter measure of spatial working memory, and highlight the fact that partial AT damage can produce at least discernible memory impairments.

While the current research found some evidence that the LD nucleus is not involved in spatial working memory as assessed by DNMP, the lack of an AV deficit on this task made it difficult to conclude with any certainty that this nucleus should not be considered part of the AT. The fact that our LD lesions were large and frequently destroyed a major part of this nucleus suggests that at the very least the LD, like the AV, is not significantly involved on the DNMP.

The extent to which large AT lesions produce loss of procedural information on tasks of spatial memory is an important issue. Previously no particular sensitivity to procedural information loss has been found with AT lesions but both the current results, and a recent study by Warburton et al. (1999) suggest the possibility that extensive AT lesions can lead to a loss of procedural information, especially if damage extends to the DM or LD. .

The results of the object study help support the view that the two memory systems are independent with regards their neural substrates. A profound AT deficit was found in spatial memory despite a lack of any obvious deficits in object recognition. This clearly highlights the fact that specific lesions to constituent parts of Aggleton and Brown's

(1999) spatial memory system lead to deficits in this type of processing without producing comparable deficits in item recognition. In this latter type of memory task AT lesioned rats perform comparably to SHAM controls, demonstrating the specificity of the type of memory loss associated with these types of lesions.

A major limitation of the current study was the fact that no conclusive evidence, positive or negative, was found for the inclusion of the LD as part of the AT complex. The lack of an AV deficit on this task provided no grounds for comparison against which LD lesion performance could be compared. A study that would help settle this question would involve comparing the effects of lesion to any of the traditional AT nuclei, preferably the AV, with the effects of LD lesions on a task sensitive to partial AT damage. The radial maze task may be the best suited for this comparison given that both Aggleton et al. (1996) and Byatt and Dalrymple-Alford (1996) have demonstrated a significant AV role on this task

Both the current research and a recent study by Warburton et al (1999) have produced results consistent with the possibility that extensive AT lesions can produce losses of procedural information. On the DNMP task it is believed that losses of procedural information are reflected by deficits in performance at zero second delay (e.g. see Aggleton and Brown). This is particularly important given that HF system deficits are believed to produce mnemonic deficits not evident at the shortest delays (see Aggleton and Brown, 1999). The proposed model predicts that lesion to any part of this extended HF system would produce only delay dependant effects. Perhaps future research using a sensitive measure of zero second delay performance, such as Log Do, which tests the effects of various lesions to this extended system will help clarify these results.

It is possible that small effects on the object recognition task were not evident given the relative insensitivity of our groups to the delays used. In the current study it was found that rats recognised comparably at all the delays used. The use of longer delays may help contribute to these results. :LD effects may become clearer with longer, more taxing delays, especially given the comparatively mild effects of entorhinal lesions on object recognition, a structure with which it shares dense neural connections ().

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