ANTERIOR AND LATERAL THALAMIC LESIONS IN OBJECT-ODOUR PAIRED ASSOCIATE LEARNING

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Abstract

Diencephalic amnesia is thought to be the result of damage to a single thalamic structure that is responsible for the memory impairment. However, an alternative view is that different thalamic structures contribute to the memory impairment in subtly different ways. Paired-associate learning is one important measure of learning and memory that is highly sensitive to disruption in people with amnesia or dementia. The current study will investigate the influence of lesions to two thalamic subregions, the anterior thalamic nuclei (AT) and the lateral thalamic nuclei (LT) in an object-odour paired associate learning task. Each of these subregions has been suggested by the literature as critical for amnesia after thalamus injury. The current study does not involve a place/ space component. Both AT and LT lesions caused impairments in the object-odour paired associate task, but not in the simple discrimination tasks. The results of this study provide new evidence to suggest that the anterior thalamic region may be responsible for more than spatial memory processing. This result is inconsistent with those of Aggleton & Brown (1999) that consider the AT to be part of an 'extended hippocampal system'. The deficits observed from LT lesions in this study provide new insight into the lateral thalamic region's role in pattern processing.

1. Introduction

1.1 General introduction

It has been widely shown that damage to the medial temporal lobe, particularly the hippocampus, can result in severe amnesia (Gilbert & Kesner, 2002a, 2003; Squire & Knowlton, 2000). More recently, however, increased attention has been paid to the influence on learning and memory processes of the diencephalon, especially the medial thalamus (Aggleton & Brown, 1999; Sziklas & Petrides, 1999). Damage to the diencephalon essentially mimics aspects of the episodic memory deficit observed with damage to the medial temporal lobe (Aggleton & Brown, 1999). The brain damage in clinical cases of thalamic amnesia is problematic because it affects overlapping thalamic regions. The various regions of interest that may be responsible for amnesia are in close proximity to each other and the injury may damage fibres of passage, both of which make it difficult to specify the nature or extent of the injury. The current study addresses this problem of lesion specificity by focusing on the effects of selective lesions to the anterior thalamic nuclei (AT) and the lateral thalamic nuclei (LT), using rat lesion models in which injury is induced using microscopic quantities of a neurotoxin, N-methyl-D-aspartic acid (NMDA) that spares fibres of passage. The AT and LT regions include thalamic nuclei within the medial thalamus that have been implicated in diencephalic amnesia.

The medial thalamus is also known as the limbic thalamus because of its connections with structures of the limbic system. It consists of the anterior and middle regions of the thalamus. The two nuclei, AT and LT make up part of the medial (limbic) thalamus, with each region providing different neural connections with other parts of the brain. By identifying the effects of lesions to these two thalamic nuclei we hope to understand more about memory systems in which they are involved and how the thalamus as a whole is involved in memory processes.

The aim of this study was to extend the work done by Gibb et al. (2006) by examining the effects of AT and LT lesions on a different paired-associate task to that used in their work. In the current study, acquisition of an object-odour paired-associate task examined the rat's ability to learn the arbitrary association between an object and an associated odour. As

paired-associate memory impairment is one important characteristic in amnesia (Eichenbaum et al, 2000; Aggleton & Pearce, 2001; Eacott et al, 2004), the current study used a paired-associate memory task to examine the influence of these thalamic regions in rats with AT and LT lesions. Simple object discrimination and simple odour discrimination tasks were also examined to determine the effects of these lesions on non-paired associate memory and served as a control.

1.2 Human studies of diencephalic amnesia

Amnesia or memory loss is a common feature of many human brain disorders. Amnesia can result from injury to several parts of the brain and is the severe inability to display normal levels of memory. Of particular interest to the present study is the inability to acquire new memory - also known as anterograde amnesia - when brain injury occurs in the diencephalon. Diencephalic amnesia can result from brain injury due to tumours, infarction or from the alcoholic Korsakoff's syndrome.

Findings collated from clinical studies of brain damage in humans have shown that diencephalic amnesia can arise from damage to many structures. Anterograde amnesia has been shown to result from damage to the AT, mediodorsal nuclei (MD) and mammillary bodies and also from damage to the fibre pathways connecting cortical and sub-cortical regions of the thalamus (Harding et al, 2000; Kapur et al, 1996; Mayes et al, 1988; Victor et al, 1971). Previous case studies on Korsakoff syndrome suggest that damage to the MD alone causes severe impairment but damage to the mammillary bodies alone does not cause any major impairment in memory (Victor et al, 1971). This indicates that MD damage on its own is sufficient to cause amnesia. A post mortem examination by Victor et al (1971) revealed that 38 of 43 patients who suffered from the alcoholic Korsakoff syndrome had damage to the MD region. The 5 other patients recovered from their amnesic condition and were found not to have sustained much, if any, damage to the MD. Other studies have shown amnesia arising from damage to the mammillary bodies and paratenial nucleus but with no damage to the MD (Mair et al., 1979; Mayes et al., 1988). Conversely, Burk & Mair (1998) showed that the intralaminar nuclei (ILn) are more critical for amnesia. They showed that lesions to the ILn, but not the MDn, impaired performance of rats on a delay matching to sample task (DMTS). Their findings along with Zhang el al (1998) showed

that the ILn impaired learning on tasks that involved a delay component similar to the amnesic condition observed in humans with damage to the ILn.

Other work supports the view that the AT region may be of particular significance in amnesia. Graff-Radford et al (1990) examined four patients who suffered bilateral medial thalamic damage, two of whom were found to be severely impaired. One of these impaired patients had extensive damage to the mamillothalamic tract and ventroamygdalofugal pathway (an amygdala related neural pathway) and the other had damage to the mamillothalamic tract, the anterior thalamic nuclei and only the superior part of the ventroamygdalofugal pathway. The remaining two patients were not as severely impaired in the memory tasks and had damage to only one of the mentioned regions. Importantly, the size of the damage did not necessarily influence the severity of amnesia, as in the case of patient one who had smaller lesions than the other three patients but had the most severe amnesic condition. Graff-Radford et al (1990) noted that amnesia occurred when damage was in the anterior part of the thalamus. Several other authors came to the same conclusions that the anterior thalamus may be responsible for amnesia. Gentilini et al (1987) reported 8 cases of bilateral medial thalamic lesions (in three cases lesion locations were uncertain). Three cases had the infarction located posteriorly and recovered from their amnesia. Two cases were reported as having severe amnesia and had anterior lesions. Similarly, Kritchevsky et al (1987) reported two patients with thalamic infarctions located posteriorly without damage to mammillary bodies who showed no amnesia. Recent work by Harding, Halliday, Caine and Kril (2000) has shown that while damage to the AT, MD and mammillary bodies are all found in Korsakoff syndrome cases, damage to the AT may be primarily responsible for the amnesic condition. They found that other alcoholic cases, who only experienced the preliminary “Wernicke’s” phase, with neurological symptoms but no permanent amnesic condition, were uniquely characterised by little or no AT damage.

Nevertheless, human studies have key limitations since they cannot address the role of individual regions within the diencephalon. For instance, current imaging tools pose difficulty in specifying the locus of diencephalic injury in human subjects and the delay between the injury and analysis during which the size and structure of lesions may change (Edelstyn et al. 2006). As mentioned above, damage is rarely localized and may involve

fibre-tract connections to various and distant parts of the brain. In addition, the close proximity and the complex structures of the diencephalon make it impossible to allow accurate predictions of the role that these different regions play in memory.

Animal models on the other hand provide a means of overcoming some of the lesion problems that arise in human studies. The location and size of the lesions and the time delay between lesions and analysis can be controlled in animal models. Also, behavioural analysis prior to lesions can be readily assessed and ensure that all subjects begin at the same level of performance for the intended task. In addition, subjects' age and sex can be selected to remove any influence these parameters may have across subjects.

1.3 Animal studies

Animal models have used lesions made by various methods that include the use of electrolytic or radiofrequency signals (Harrison & Mair, 1996; Koger & Mair, 1994;), ablation, excitotoxins (Burk & Mair, 1998; Zhang, Burk, Glode, & Mair, 1998), or the pyrithiamine induced thiamine deficiency method (Mumby et al 1995).

It has been suggested that no single nucleus within the thalamus is responsible for diencephalic amnesia on its own (Aggleton & Brown 1999). Perhaps different regions play a subtly different role in memory and these regions are interconnected to form memory systems that work in parallel. For example, Aggleton and Brown (1999) proposed that anterograde amnesia may be the result of damage to two different memory systems - one system based on recall/ recollection (focused on AT and hippocampal connections) and the second system based on familiarity-based recognition (focused on MD and perirhinal cortex connections). They also presume that damage to any area *within* each system can result in similar impairments.

Previous evidence has focused on three sites within the thalamus: the anterior thalamic nuclei (AT) (Sziklas & Petrides 1999, Moran & Dalrymple-Alford 2003, Byatt & Dalrymple-Alford 1996), the intralaminar nuclei (ILn) (Burk & Mair 1998) and the mediodorsal nuclei (MD) (Gaffan & Parker 2000, Victor et al 1971). It is possible that

damage to each of the cited nuclei can result in some aspects of amnesia, but with subtly different characteristics.

The basis for suggesting involvement of the AT in diencephalic amnesia can be explained by the fact that both the medial temporal lobe and diencephalon neural systems are connected via the fornix, primarily, creating a hippocampus-anterior thalamic axis. In other words, the AT is considered to be a part of an 'extended hippocampal system', since hippocampal efferents to the medial thalamus (particularly the AT) are considered vital for proper hippocampal functioning (Aggleton & Brown 1999). Several studies have shown similar deficits in spatial memory processing in animals with AT and hippocampal lesions (e.g. Gilbert & Kesner 2003, Mitchell & Dalrymple-Alford 2005, Gibb et al 2006). Sziklas & Petrides (1999) investigated the effects of AT lesions in an object-place paired associate task. The task consisted of allocentric and egocentric components. They found that AT lesions impaired performance in allocentric spatial tasks (radial arm maze) but not in the egocentric spatial tasks. It is important to note that in the egocentric task the AT rats performed at a similar rate to the control rats, but in the probe trials the controls performed at a rate higher than 50%. Sziklas & Petrides (1999) suggested that this could be because the controls rats are able to use a combination of allocentric and egocentric cues to solve the task.

The basis for suggesting the LT to be involved in diencephalic amnesia can be explained by experiments conducted by Mair and his colleagues (Burk & Mair 1998). They compared the effects of intralaminar (ILn), mediodorsal (MD) and lateral internal medullary lamina (L-IML) lesions on a delayed match to sample task in an operant chamber. They found that the MD group was only slightly impaired and had an intermediate effect on the task, but the ILn rats were severely impaired in this task. In addition, the effects of ILn lesions were independent of delays. Specific L-IML lesions produced a smaller more transient impairment. In another study Mair and his colleagues (Burk, Mair & Porter 1998) found ILn lesions to produce a general impairment in radial arm maze tasks. They proposed that ILn lesions have a significantly more severe memory impairment that lesions to other regions of the thalamus or the hippocampus. They draw many similarities between ILn lesions impairment and human Korsakoff amnesia.

Lesion studies involving many brain regions have supported the idea of the multiple memory systems in the brain. Consistent with Aggleton and Brown (1999), it is also possible that different regions of the diencephalon have subtly different roles in memory. This has prompted alternative suggestions that a number of different thalamic areas, each underlying a different type of memory or memory related factor, are responsible for intact performance in memory tasks (Aggleton & Brown, 1999; Mitchell & Dalrymple-Alford, 2005; Van der Werf, Jolles, Witter, & Uylings, 2003). Hence, the extent of damage to specific thalamic structures would determine the characteristics or range of memory impairment.

Mitchell & Dalrymple-Alford (2005, 2006) and Gibb et al (2006) conducted studies on rats with AT, LT and MT regions (MT = medial and central MD), based on their anatomical connections in the brain; their LT lesions included the ILn and the lateral MD nucleus. Mitchell & Dalrymple-Alford's studies concluded that different regions of the medial thalamus participate in multiple memory systems, in that only AT lesions produced marked reference and working memory or spatial memory deficits in the radial arm maze, and only LT lesions produced working memory deficits for response memory, and only MT lesions produced working memory deficits for reward magnitude. Memory systems are traditionally classified in terms of the cognitive operations they perform and their underlying cerebral structures (Table 1.1). For example, White and McDonald (2002) proposed that memory systems function simultaneously and independently of each other. They focus on three parallel independent memory systems, each with a central structure: hippocampus, amygdala or dorsal-striatum. All three systems receive the same information (stimuli, response and reinforcer) but their *processing styles* differ for this information. Table 1.2 shows the suggested different associations represented by each of their three memory systems: the hippocampus deals with stimulus-stimulus associations, the amygdala deals with stimulus-reward associations and the dorsal-striatum deals with stimulus-reinforcer associations. The idea is that lesions to any region *within* a system would result in an impaired performance in a memory task whose attributes or elements are coherently represented by the system (White & McDonald 2002).

By contrast, Kesner (1998) proposed multiple parallel memory systems that are primarily based on different memory attributes such as space, time, affect, sensory perception and

language (in humans). He has three over-riding memory systems that process event-based, knowledge-based and rule-based memory. Memory for new information depends on the event-based system, in which processing of space, time, and language, is largely undertaken by the hippocampus, processing of emotional / affect / reward information is undertaken by the amygdala and event-based information about individual objects is processed by perirhinal cortex.

Table 1.1: Standard categorization of memory systems (Squire & Knowlton, 2000; Squire et al, 2004)

Declarative memory (hippocampal dependent)

- Concerns long-term factual or episodic memory that is recalled consciously
- The neural system involved in episodic/ declarative memory is comprised of the hippocampus and the anterior thalamic nuclei; traditionally, aspects of the medial temporal lobes are also included as part of this system.

Non-declarative memory (non-hippocampal dependent)

- Concerns a range of different memory systems, such as procedural memory which in humans is characterised by evidence of memory that occurs without conscious awareness

Working memory

- Short term memory that allows the brain to regularly update and is useful in trial unique or inconsistent (everyday) situations. This type of memory may represent short-term ‘episodic memory’.

Table 1.2: Summary of three different models of multiple neural memory systems based on the different kinds of memory associated with some key brain structures. The difference between systems is presumed to reflect either their 'information processing style' (Aggleton & Brown, 1999; White & McDonald, 2002) or the specific kinds of information that are processed (Kesner, 1998).

Region	Hippocampus	Amygdala	Dorsal striatum	Perirhinal cortex
Aggleton & Brown 1999	Recall / recollection-based			Familiarity/ recognition based
White & McDonald 2002	Stimulus-stimulus associations	Stimulus-reinforcer associations	Stimulus-response associations	
Kesner 1998	Language, time and space	Affect		Sensory perception

The cognitive operations of the memory systems listed in Table 1.2 are based on lesion studies on animals. Lesion data from animals have shown that damage to the medial temporal lobe, particularly the hippocampus, can also result in amnesic conditions that are similar to those that occur in humans (Gilbert & Kesner, 2002, 2003; Squire & Knowlton, 2000). The hippocampus is thought to play an important role in spatial tasks (Kesner 2002, Aggleton & Brown 1999), associative memory tasks (Eichenbaum 1999) and other conditioning tasks (Nadel et al 1997). Lesion experiments in monkeys and rats showed that amygdaloid damage resulted in different memory deficits, particularly in memory tasks that require associations between neutral and incentive stimuli and fear conditioning (Kesner, 1998; White and McDonald, 2002). Damage to the dorsal striatum has been shown to result in memory deficits in tasks that involve a motor response in the presence of a single cue (Kesner, 1998; White and McDonald, 2002). Thus far, the studies by Mitchell & Dalrymple-Alford (2005, 2006) and Gibb et al (2006) provide some support to the idea that different parts of the limbic thalamus are involved in different memory systems, at least with respect to working memory processes.

1.4 The thalamic nuclei used in this study

The location, size and specificity of lesions have varied across previous studies, making it difficult to interpret the role of individual thalamic structures (see section 1.7). The thalamic lesions targeted in this study are the AT (including the anterodorsal, anteromedial and anteroventral nuclei) and LT (including the intralaminar nuclei and the lateral mediodorsal nuclei). This selection was made because studies using lesions of the traditional AT and ILn regions have produced the most consistent evidence of memory impairments in rat models. Both the AT and the LT have different neural connections with respect to the hippocampal system and the striatum, respectively (see Figures 1.1, 1.2), but they also have partially overlapping connections with the prefrontal cortex. It is thought that the confounding results (differential and coincidental effects of lesions) from lesion studies that included AT or ILn regions are due to the fact that the lesions used in previous studies may have overlapped the two regions, resulting in uncertainty as to their role in memory (Mitchell & Dalrymple-Alford 2006). The specificities of the lesions used in this study are based on previous findings from Mitchell & Dalrymple-Alford (2005, 2006) in comparative studies as well as their afferent and efferent connections to other major regions of the brain.

1.5 The Anterior thalamic nuclei

As stated above, disruption of AT in humans is associated with anterograde amnesia (Harding et al 2000, Van der Werf et al 2000). The anterior thalamic nuclei have major connections with the retrohippocampal region and are considered to be part of the 'extended hippocampal system' (Aggleton & Brown 1999, Vann & Aggleton 2004a) (Figure 1.1). For example, Warburton et al (1999) showed that impairments caused by AT lesions in rats were comparable to those caused by fimbria-fornix lesions. This and other evidence mentioned earlier indicates that the AT has a close functional relationship with the hippocampus and is involved in acquisition of spatial memory. Mair et al. (2002) showed that AT and parahippocampal lesions have separate effects on spatial memory but together they can disrupt hippocampal-dependent spatial memory. Dalrymple-Alford's group has shown that selective lesions of the AT, when there is little or no damage to the adjacent ILn

or the MD, produces profound deficits on standard spatial memory tasks (Mitchell & Dalrymple-Alford 2005, 2006).

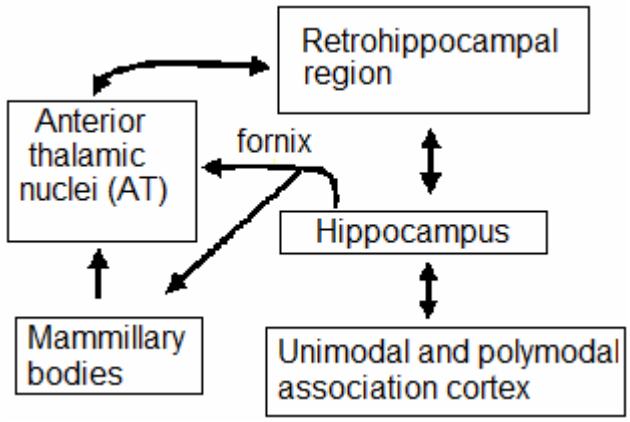

Figure 1.1: Conceptual diagram showing the connections of the Anterior Thalamic (AT) region. Note particularly its connection to the hippocampus via the fornix. Reproduced with permission from Dalrymple-Alford (2005) and adapted from Aggleton and Brown (1999).

As the purpose of the current study was to examine paired-associative memory when object and odour cues were paired, it is relevant to briefly first discuss prior research on simple odour memory, simple object memory and related tasks or the effects of related (i.e., hippocampal) lesions.

1.5.1 The role of the AT region in odour memory

The Gibb et al (2006) study, in which odour-place association memory was markedly impaired by AT lesions, found that acquisition of a simple odour discrimination was only mildly impaired, although this may in part be due to the prior training on the paired-association task. With regard to hippocampal lesions these do not impair performance in an odour-odour (Alvarez et al 2002) nor an object-odour paired association task (Gilbert and Kesner 2002a). However, hippocampal lesions do impair performance in an object-place and odour-place association task, presumably because these tasks involved a spatial component (Gilbert and Kesner 2002a). For this reason it is expected that the AT lesions will not markedly impair acquisition of an object-odour task as there is no spatial component.

1.5.2 The role of the AT region in object memory

Studies with thalamic lesions in rats on spontaneous object recognition have shown no evidence that AT lesions impair memory even if there is a delay component involved in the task (Aggleton et al, 1995; Moran & Dalrymple-Alford 2001).

In terms of associative memory involving objects as one of the component attributes, Sziklas and Petrides (1999) tested the effects of AT lesions in rats in an allocentric object recognition task designed to study the ability of rats to choose between two objects depending on their location in an enclosed arena (one object was correct on one side of the field, whereas the other object was correct on the other side of the field). They found that AT lesions severely impaired performance in this task. However, this may be solely due to the spatial component involved in this task. As mentioned, Gibb et al (2006) reported that AT lesions severely impaired performance on an object-place paired associate task. However, AT lesions did not impair performance in the spontaneous object recognition or simple object discrimination tasks (Gibb et al, 2006).

One study examined the ability of rat to use unique stimulus cues (complex objects, such as multiple small blocks of wood, white wire mesh) in the context of a configural learning task (Moran and Dalrymple-Alford 2001). The rats were required to learn the reward/ non-reward that either appeared alone or in combination along the runway arm of a radial maze. For example, they were rewarded for responding when two complex cues, A and B appeared alone, but they were not rewarded when the two types of cue appeared together (A+, B+, AB-). AT lesions, which impaired spatial memory in a standard (no cues) radial arm maze task, did not impair performance in this cue-based configural task. Acquisition of the configural task, but not the standard spatial memory task, was impaired by perirhinal cortex lesions.

1.6 The Lateral thalamic nuclei

The lateral thalamic nuclei of interest include the rostral intralaminar nuclei (ILn) and lateral mediodorsal nuclei (MDn). Both these regions have strong connections with the dorsal and ventral prefrontal cortex (PFC). The lateral MDn has afferent connections with

lower brain-stem structures including the substantia nigra. As a whole the LT has reciprocal connections with overlapping regions of the striatum as well as the anterior cingulate and precentral cortices of the PFC, which thereby form a fronto-striatopallidal-medial thalamic neural circuit (Berendse and Groenewegen 1991; Van der Werf et al 2002). As stated earlier, LT lesions produce only minor, if any, spatial memory impairments, consistent with the idea that they alter normal functioning between the striatum and frontal cortex, not hippocampal system function (Mitchell & Dalrymple-Alford 2005).

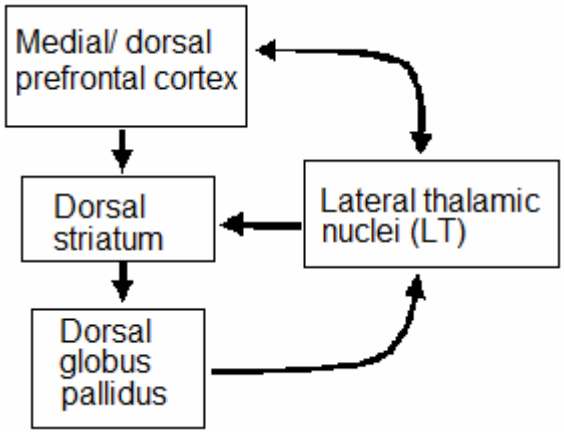

Figure 1.2: Conceptual diagram showing the connections of the Lateral Thalamic (LT) region. Reproduced with permission from Dalrymple-Alford (2005) and adapted from Aggleton and Brown (1999).

1.6.1 The role of the LT region in various memory tasks

Relatively fewer studies have examined the effects of ILn lesions, often in the context of lesions that include the lateral internal medullary laminae (L-IML). L-IML lesions impair olfactory continuous DMS learning but odour discrimination was not impaired (Zhang et al 1998). LT lesions have been shown to produce impairments in the radial arm maze (Mair et al 1998), but this may have been partly due to encroachment of the lesions to adjacent regions (Mitchell & Dalrymple-Alford 2005). For example, localized LT lesions when confined to the ILn and lateral MD produce only minor impairments in working memory in the radial arm maze (Mitchell & Dalrymple-Alford 2005) and no impairments in the Morris water maze (Wolff et al personal communication, June 2007). Mair's group have also shown that LT lesion impairments are also delay independent, leading to the belief that

instead of the LT being involved in odour memory it could be responsible for general learning and memory.

Savage et al (1998) investigated the effects of L-IML and ILn lesions on an object pair discrimination task in rats. Although this task tested the retention of information acquired prior to surgery (retrograde amnesia) it still showed that the ILn had no effects on object memory.

Gibb et al (2006) found that LT lesions severely impaired acquisition of the object-place paired associate task but did not have any effect on the acquisition of simple object or simple place discrimination. This indicates that damage to the LT is sufficient to cause a serious learning deficit. However, since the LT did not impair learning on the simple discrimination tasks or on the spontaneous object recognition tasks it is unclear whether its role involves general learning and memory or a particular memory attribute such as relevant cues, strategies and involve memory systems that the rats may use to solve the tasks.

1.7 Issues of location, method, specificity and analysis of lesions

As stated previously, the thalamus is made up of a very small and complex array of nuclei that are interconnected to other parts of the brain. Due to the complexity of the thalamic nuclei significant methodological issues arise with the location, size and specificity of lesions produced. Small intrusions into other thalamic areas have probably compromised the experimental results of many previous experimental studies using thalamic lesions.

One important issue in such studies is the method used to produce lesions in the thalamus. Methods like electrolytic or radiofrequency (heat-produced) lesions (e.g., Byatt and Dalrymple-Alford, 1996) destroy both cell bodies and fibres of passage, whereas the excitotoxic method employed in this study only destroys cell bodies. The excitotoxic method is used here because of the high density of fibres of passage in the AT nuclei of the thalamus.

However, some unintentional damage to other structures is inevitable due to the close proximity of thalamic nuclei, with previous studies encountering non-target damage. For

example neurotoxic AT lesions can inadvertently damaged LT and MT tissue (Mair et al. 2003), and IL tissue (Warburton et al. 1999). MT lesions damaged tissue in the AT (Hunt & Aggleton 1998), and L-IML tissue (Burk & Mair 1998). IL lesions often damage AT tissue (Savage et al 1998) and MT tissue (Mair et al 1998). Hence there is a need for quantative analyses on lesions, rather that qualitative alone. Our research group at the University of Canterbury have made a detailed quantative analysis of lesions produced and attempted more localised thalamic lesions (Gibb et al., 2006; Mitchell and Dalrymple-Alford, 2005, 2006; Wolff et al, 2006).

The main purpose of the study was to compare the effects of the two thalamic lesions (AT and LT) on an object-odour paired associate task. The task used here was the object-odour paired associate task employed previously by Gilbert and Kesner (2002a) who found that hippocampal lesions had no effect on acquisition of this task, unlike deficits found when the task included a spatial component as part of the association. Their task used a go/ no-go procedure. Rats were required to learn the arbitrary association between an object and an odour to receive a food reward, where two specific pairs of odour and objects signal a reward ("go"), but any mispairing signalled no reward ("no-go"). It is important to note here that while the task involved an odour and an object attribute it did not involve a spatial attribute.

The role of the hippocampus and AT in non-spatial memory is not clear other than memory for the temporal order of events (Fortin et al, 2002; Kesner et al, 2002; Hopkins et al 1995) but considerably evidence exists to show their involvement in spatial memory (Sziklas & Petrides 1999; Gibb et al. 2006; see Kesner 1998 for a review). As the AT nuclei are considered to be part of the 'extended hippocampal system' we do not expect AT lesions to impair performance in this object-odour paired associate task since there is no spatial component to this task.

1.8 Simple object discrimination and simple odour discrimination

Additional go/ no-go simple discrimination tasks followed the main task. In these additional tasks, rats were required to make a simple discrimination between either two different objects or two different odours. The latter tasks show whether the rats'

performance in the main (paired-associate) task was impaired by an inability to discriminate between the objects or the odours used and/or simply the inability to inhibit a response. That is, the simple discrimination tasks did not involve a paired association and thus served as a performance comparison for the main task.

1.9 Summary: Aims of the current study

This study aims to extend earlier work done at the University of Canterbury, on comparative influence of selective AT and LT lesions by examining their effects on Gilbert and Kesner's (2002) object-odour paired-associate task. The formation of an episodic or declarative memory is thought to require not only that a number of attributes of an event are encoded but that they are bound together in a unique way (Aggleton & Pearce 2001).

The study of Gibb et al. (2006) was modelled on Gilbert and Kesner's work but investigated the effect of thalamic lesions, not hippocampal lesions, in an odour-place paired association task. This task included major spatial attributes. They found that the AT lesions severely impaired performance in this task, whereas the LT lesions tended to produce less impairment.

The next logical step from Gibb's (2006) work was to compare the lesions of the thalamic nuclei on an object-odour paired association task. Since there is no spatial component in this task it would be ideal to further investigate the role of the thalamus. It assesses the ability to learn an arbitrary association between one of two objects and the presence of one of two types of odorized sand in which the rat digs for a food reward, without any spatial cues.

We have focused on two of the nuclei to build on these findings and to attempt to identify what roles each region plays. The only previous study to comparatively test the effects of these nuclei of the thalamus was that of Gibb et al. (2006) in an odour-place paired associate task.

It was predicted that the AT would not be impaired in this object-odour paired associate task because of the lack of a spatial or temporal component (which would otherwise make it

sensitive to hippocampal lesions and thus AT lesions). It was predicted that the LT lesions would impair performance in this task because the LT is thought to play a general role in memory and learning (that is, not limited to any specific attribute of the task).

The specific hypotheses were:

a) AT lesions should not affect this type of associate memory task (as this task is unimpaired by hippocampal lesions) (Gilbert and Kesner 2002; Gilbert and Kesner 2003).

b) LT lesions should produce a deficit, on the basis that this region may have a general role in higher order memory and learning tasks, thereby disrupting the acquisition of any arbitrary association

c) None of these lesions should impair, or should only minimally impair, acquisition of simple object discrimination or simple odour discrimination.

2. Methods

2.1 Subjects

Forty PVGc Hooded female rats were bred in the Psychology animal facility and were housed in opaque plastic cages (50cm long, 30cm wide and 23cm high) in groups of four per cage and maintained in a 12 hour light-dark cycle (8am-8pm). All testing was conducted during the dark cycle. Rats received restricted food access to maintain their body weights at 80-85% ad libitum throughout all training. The rats weighted between 150g and 200g at time of surgery and were allowed free food access during the first week of the recovery period before returning to restricted food access. All procedures conformed to the NIH Guide for the Care and Use of Laboratory Animals and were approved by the University of Canterbury Animal Ethics Committee (refer Appendix A for ethics committee approval).

2.2 Apparatus

The large circular wood board used in this task was 119 cm in diameter, 3.5cm thick and was painted white with no wall around the perimeter. A start box (24cm long, 15cm wide and 17cm high) painted black with a hinged lid and manually operated guillotine-like door was placed at the perimeter of the board. A ceiling mounted camera recorded behavioural data. The room also contained a chair, two desks and beige curtains surrounding the board.

Small terracotta pots (6cm wide at top, 6cm high) were painted black and filled with sterilized sand to 1cm below rim. The pots were attached to a platform (25cm X 25cm) to stabilize pot and minimize spillage of sand onto board. Rats were trained to dig for food in these pots. To minimize the use of any food odour cues a wire mesh overlaid inaccessable "Froot loops" cereal placed at the bottom of the pot. Only one of these pots was on the board at any time during training. They always appeared 67.5cm from back edge of start box (Figure 2.1).

Two objects, a yellow plastic spade (22cm high and 4.5 cm diameter at base) and a green bottle (20.5 cm high and 4.5cm diameter at base) were used in this task. During training,

one object was placed directly behind the pot on the platform and was visible to the rats from the start box. The objects always appeared in the same place as the pots did. The main object-odour paired-associate task as well as the simple odour and object discrimination were conducted on the same board, with the same pots, start box and objects.

2.3 Pre-training

Individual rats were placed in a large 1m square box, containing a pot filled with non-odourised sterile sand in the experiment room and were shaped to retrieve "Froot Loops" cereal buried successively deeper in the sand. Subsequently each cage of four rats was placed on the board and allowed to eat "Froot Loops" scattered across the board (15 mins/ day, for 2 weeks). Then each rat was placed in the start box on the board and trained to retrieve "Froot loops" from the pot using 10 trials per day, 5 days a week for 3 weeks.

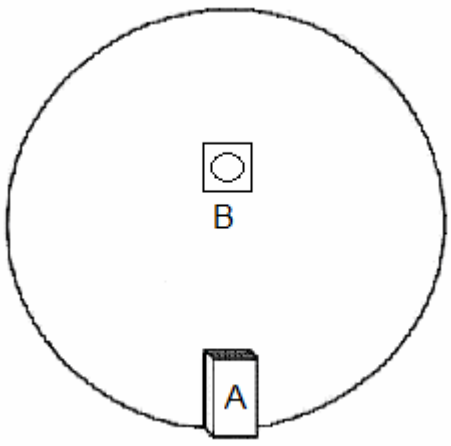

Figure 2.1: Schematic diagram showing the start box (A) shown at one edge of the board, behind which the experimenter stood. The pot and object (B) was 67.5cm from back edge of the start box. On any given trial for the object-odour paired associate task only one pot with odourised sand and object was present on the board. During the simple odour discrimination tasks only one pot containing the odourised sand was on the board (no object present). During the simple object discrimination task only one pot containing non-odourised sand with one object was on the board at any one time.

2.4 Surgical procedures

Rats were randomly assigned to receive either AT, LT or control (sham) lesions. Rats were anaesthetized IP with sodium pentobarbitone (50mg/ ml, at a dose of 1.65ml/ kg) 25 minutes after Atropine IP (0.065mg/ml/kg) supplemented by Mepivacaine (5mg/ml/kg, subcutaneously under the scalp) and Norocarp (5mg/ml/kg, subcutaneously at the nape of the neck). Rats were then placed in the Kopf stereotaxic clamp with incisor bar 7.5mm below interaural line to minimize damage to the fornix via passage of the 1ul Hamilton syringe operated by a Stoelting motorized infusion pump.

Excitotoxic lesions were made using 0.12M of NMDA dissolved in phosphate buffer (pH 7.20). Anterior-posterior coordinates were varied slightly among individual rats based on the distance between lambda and bregma (Table 2.1). Stereotaxic co-ordinates for lesion placements were based on those used previously by Mitchell & Dalrymple-Alford (2005), after further verification and minor improvements in pilot work. Infusions were made by lowering the Hamilton needle slowly to the site and allowing it to rest for 30 seconds prior to infusion; neurotoxin was dispensed at a rate of 0.003ul/ min with 3 min post infusion for diffusion at the site and then slowly retracting the needle. Control lesions (3AT and 2LT sites) received the same surgery but with no neurotoxin in the infusion needle which was lowered to a coordinate 0.3cm above that used for the respective lesion sites (to minimise damage to thalamic structures).

2.4.1 Anterior thalamic lesions

The AT lesions consisted of bilateral infusions aimed at the anteroventral nucleus (AV) using 0.11ul of NMDA, and the anteromedial nucleus (AM) using 0.09ul of NMDA. All measurements were taken from bregma. To ensure accurate placing of lesions the anterior-posterior (A-P) co-ordinates varied between each rat in line with the Bregma-Lambda distance (which ranged from 0.60 to 0.72cm for each lesion type, for clarity see Table 2.1). Hence the AP distances for AT lesions varied from -0.235 to -0.265cm, 0.15cm from the midline and -0.555cm ventral from the dura at the AV site. At the AM site AP distances varied from -0.225 to -0.255cm, 0.12cm from the midline and -0.580cm ventral from the dura. The same basic scheme was used for the LT lesions.

2.4.2 Lateral thalamic lesions

The LT lesions consisted of three bilateral sites (two anterior LT depths/sites and one posterior LT site). At the anterior LT site AP distances varied from -0.345 to -0.375cm at a distance of 0.130cm from the midline and at a depth of -0.560cm and -0.600cm from the Dura using 0.045ul NMDA at 0.03ul/min at each site. At the posterior LT site, the AP distances varied from -0.385 to -0.415cm, and 0.130cm from the midline and at -0.560cm from the dura using 0.05ul NMDA at 0.03ul/min.

Table 2.1: Lesion coordinates and related parameters for individual Bregma-Lambda distances and corresponding anterior-posterior coordinates for AT and LT lesions

	AT		**LT**		
	Anterior (AM)	Posterior (AV)	Anterior (two sites)		Posterior
B-L distance for co-ordinates					
0.60-0.61	-0.225	-0.235	-0.345		-0.385
0.62-0.63	-0.235	-0.245	-0.355		-0.395
0.64-0.66	-0.245	-0.255	-0.365		-0.405
0.67-0.68	-0.255	-0.265	-0.375		-0.415
0.69-0.70	-0.255	-0.265	-0.375		-0.415
0.71-0.72	-0.255	-0.265	-0.375		-0.415
ML	±0.12	±0.15	±0.130		±0.130
DV	-0.580	-0.555	-0.560	-0.560	-0.560
Volume μl	0.09	0.11	0.045	0.05	0.05
Rate μl/min	0.03	0.03	0.03	0.03	0.03

2.5 Object-odour paired associate task

2.5.1 Re-familiarisation

Rats were allowed to recover for three weeks post-surgery and were re-familiarized with the board. Rats were placed in the start box and pots filled with non-odourised sand were placed within 10cm from the start box during re-familiarisation and were allowed to dig in the pot for cereal (12 trials per rat for 4 days).

2.5.2 Object-odour paired associate task

The rats were then required to retrieve "Froot loops" cereal buried 1cm under odourised sand in a pot (1% cinnamon or 0.4% cumin w/w sand). Only one pot paired with only one object (spade or bottle) was present on the board at any one time. When the rat approached the pot it received a food reward if the correct object-odour pairing occurred on that trial (go trial) but no reward for a mis-pairing of an object and odour (no-go trial) (Table 2.2). The reward contingency was counterbalanced across rats within each lesion group and across home cages. There were thus two correct and two incorrect pairings of object and odour per rat and they received 12 trials (six correct pairings and six incorrect pairings) per day. Rats were trained 5 days a week for 16 weeks (until they reached an asymptotical level of performance).

The time taken from when the back feet of the rat exited the start box to when it started digging in the pot was recorded. Rats were given a total of 10s within which to start digging. If they did not dig within this 10s they were returned to the start box for the next trial. Digging was defined as more than 2 consecutive strokes in the sand with one or both paws. Daily average latencies (No-go minus go trials) were used as a dependent measure of acquisition. Optimal acquisition of this object-odour paired associate task required rats to not dig on no-go trials (max of 10s) and to dig quickly on go trials.

Table 2.2: Parings and mispairings of objects and odours in the object-odour paired associate task

		Odour	
		Cumin	Cinnamon
Object	Bottle	DON'T DIG	DIG
	Spade	DIG	DON'T DIG

		Odour	
		Cumin	Cinnamon
Object	Bottle	DIG	DON'T DIG
	Spade	DON'T DIG	DIG

2.6 Simple discrimination tasks

After completion of the main paired-associate task the rats were required to make a simple discrimination between the two objects or between the two odours. One half of the rats were assigned to the simple odour discrimination and the other half to the simple object discrimination. The subjects were balanced across lesion groups, performance and home cages (time of testing during the day).

2.6.1 Object discrimination task

In this task rats were required to dig in a pot of non-odourised sand when the correct object (go trial) was present and not when the incorrect object was present (no-go trial). The 'correct' allocation of objects was balanced across lesions and home cages. Again time was measured when rats back feet exited box to when it began digging in sand. Rats received 12 trials per day for 5 days a week across a period of four weeks.

2.6.2 Odour discrimination task

In this task rats were required to dig in a pot of odourised sand when the correct odour (go trial) was present and not when the incorrect odour was present (no-go trial). No objects were present on the board during this task. The 'correct' allocation of odours was balanced across lesions and home cages. Again time was measured when rats back feet exited box to

when it began digging in sand. Rats received 12 trials per day for 5 days a week across a period of four weeks.

2.7 Histology

After completion of the simple discrimination tasks the rats were injected with an overdose of sodium pentobarbitone and perfused with 0.9% saline and 10% formalin solution. Their brains were carefully removed and stored in 4% formalin before sectioning at 50um using a vibratome. Each section was taken throughout the thalamus and mounted on glass slides. The sections were stained using a standard procedure with cresyl violet (cell bodies, including neurones and glial cells). One in every five sections was stained with NeuN (a neurone-specific immuno-marker; Jongen-Relo et al 2002).

2.7.1 NeuN staining procedure

NeuN is a protein that is expressed in cell bodies of most neuronal cells in rodents, humans and some other animals. The advantage of staining brain sections with NeuN is that this protein is not expressed by glial cells and hence can be used to clearly label lesion sites (Jongen-Relo et al 2002).

For NeuN staining, brain sections were treated with 0.5% H_2O_2 in 0.1M phosphate buffer solution (PBS) for 30 minutes to suppress peroxidase activity. The sections were incubated for an hour in PBS with horse serum and bovine albumin serum and Triton X-100 and then in anti-NeuN serum, visualised using a biotinylated secondary antibody and DAB reaction (for details see Jongen-Relo et al 2002).

3. Results

3.1 Histology

A minimum of 40% damage to the target thalamic areas was used as the inclusion criterion for an acceptable lesion in this study. Examples of lesions are shown in Figs. 3.1 and 3.2, in which schematic diagrams indicate the largest and smallest acceptable lesions from the AT and LT lesion groups. A list of percent bilateral lesion damage for AT and LT lesion rats is shown in Table 3.1. As shown in Table 3.1, seven rats sustained greater than 40% damage to the AT region, with a median value of 70.7% (range, 49.0% to 91.7%), including one rat that had been intended as an LT rat but in which the lesion was anterior and thus in the AT region (the LT damage in this rat was only 17%). In the AT group with acceptable lesions, minimal to relatively minor damage occurred in the adjacent LT region (median, 9.3%; range, 1.0% to 30.4%), minimal damage to the MT region and no damage to midline nuclei except in two cases which had extensive damage to the interanteromedial nucleus. Two AT rats had insufficient AT damage and were excluded from the main behavioural analyses. Five rats had acceptable LT lesions (median 48.9%; range, 41.2% to 58.6%) and three LT rats had insufficient damage and were discarded from behavioural analysis (see Table 3.1). Damage to the AT region was minimal in rats with acceptable LT lesions (median, 48.9%; range, 0.1% to 4.8%) and generally small or absent in other adjacent thalamic nuclei.

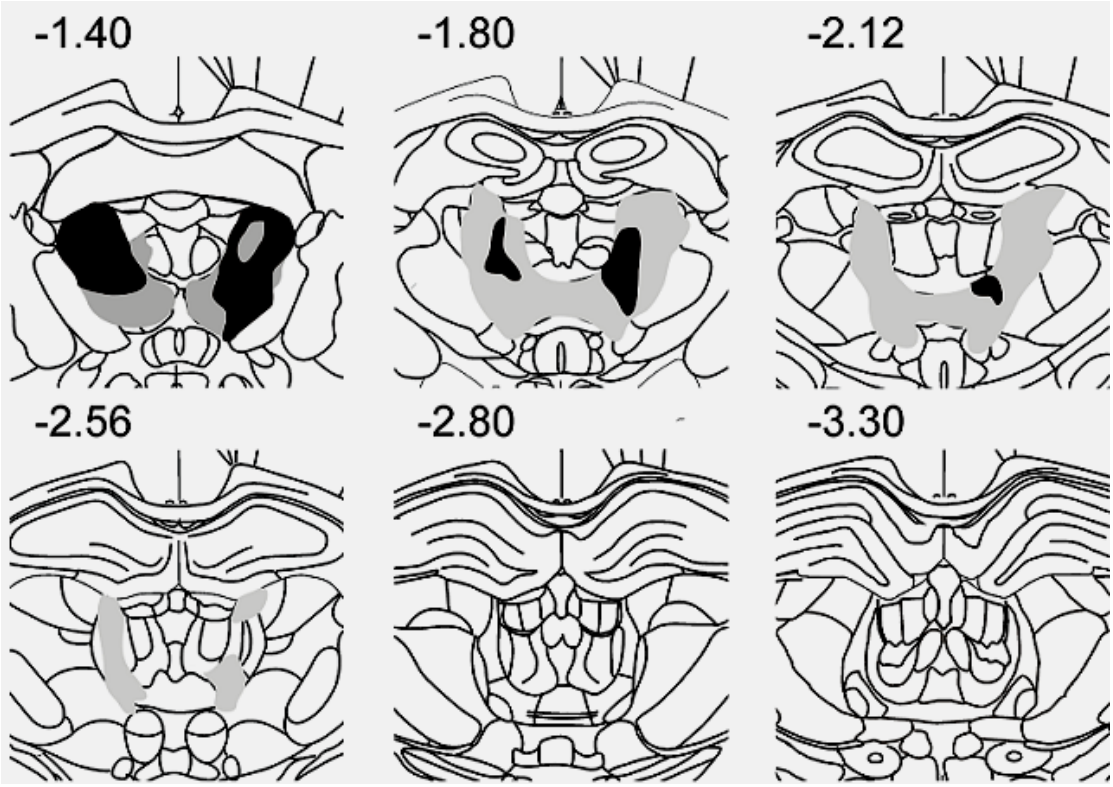

Figure 3.1 Schematic coronal sections through the rat brain showing the locations of the largest (grey) and smallest (black) acceptable lesions in the AT group. Numbers are distances from Bregma per the atlas of Paxinos & Watson (1998).

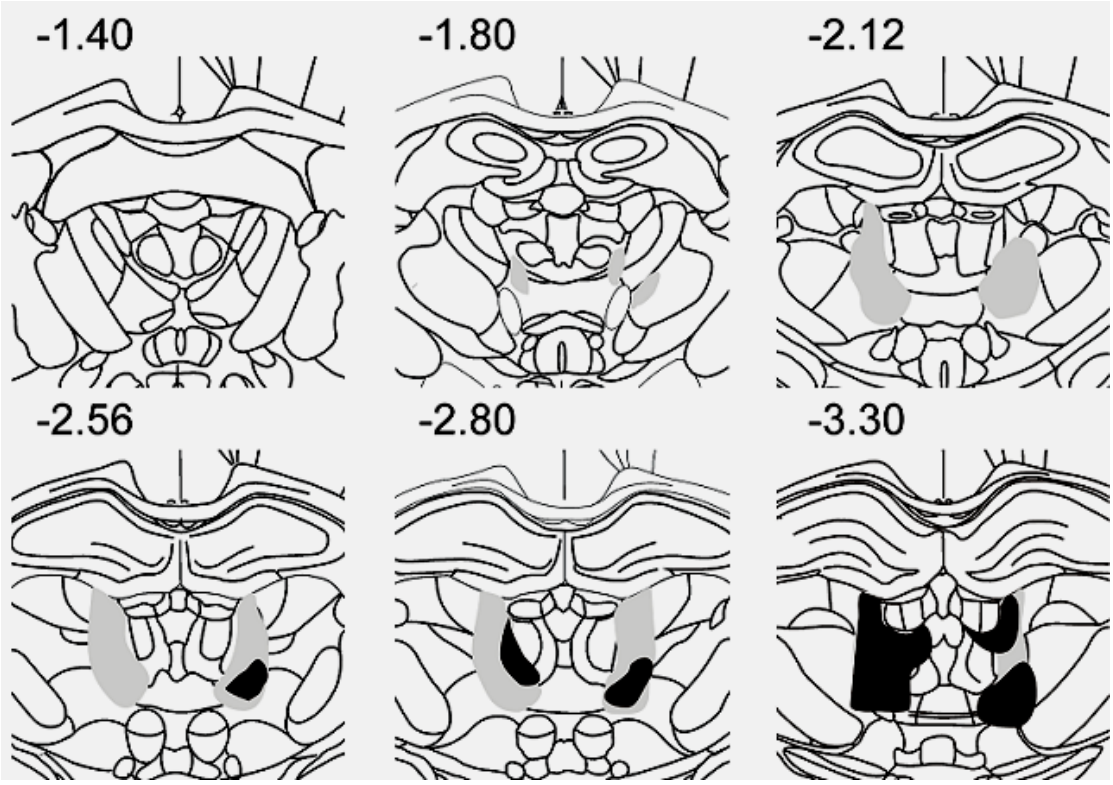

Figure 3.2 Schematic coronal sections through the rat brain showing the locations of the largest (grey) and smallest (black) acceptable lesions in the LT group. Numbers are distances from Bregma per the atlas of Paxinos & Watson (1998).

Abbreviations for Table 3.1: AD= anterodorsal nucleus; AM= anteromedial nucleus; AT= anterior thalamic aggregate; AT median= median percent damage for all included AT rats; AV= anteroventral nucleus; CL= centrolateral nuclei; IAM= interanteromedial nucleus; LT= lateral thalamic aggregate; LT median= median percent damage for all included LT rats; MDl= lateral segment of the mediodorsal nucleus; MDpl= paralamellar segment of the mediodorsal nucleus; PC= paracentral nucleus; PVA= anterior paraventricular nucleus; PV/PVP= paraventricular nucleus/posterior paraventricular nucleus; Re= reunions nucleus; Rh= rhomboid nucleus. * 10G intended to be LT lesion but met criterion for AT lesion. No damage was sustained by the LD (laterodorsal) nucleus in any rat.

Table 3.1: Percent bilateral damage (volume) to the AT, LT and select other nuclei for each rat in this study

	AT and components				LT and components					MT components					Other midline nuclei					
	AD	AM	AV	AT	CL	MDl	MDpl	PC	LT	IMD	MDc	MDm	MD	MT	IAM	CMr	PVA	PV/	Re	Rh
AT inclusions																				
1R	99.8	20.5	83.7	66.6	5.0	3.1	0.0	9.6	5.7	0.0	0.1	0.0	26.6	1.7	0.0	1.2	0.0	0.0	0.0	0.0
2P	98.6	97.2	96.0	91.7	29.8	22.7	55.3	30.2	28.1	0.0	0.6	1.7	11.5	1.9	0.0	10.1	0.0	0.0	0.0	2.0
3B	99.2	49.3	64.4	69.2	34.5	30.3	76.6	22.4	30.4	0.0	0.7	1.4	22.0	2.4	0.0	5.0	0.0	0.0	0.0	0.0
7R	95.6	73.1	72.6	73.2	10.2	4.5	0.0	22.4	11.8	0.0	0.0	2.2	50.3	4.5	57.5	13.0	2.4	0.0	0.0	0.0
8B	40.1	43.6	44.6	51.0	0.0	0.0	0.0	3.6	1.0	0.0	0.0	0.0	2.9	0.2	0.0	0.8	0.0	0.0	0.0	0.0
10P	97.8	45.8	98.5	72.2	7.6	4.1	0.0	8.7	6.8	0.0	0.0	0.0	10.6	0.7	0.0	2.5	0.0	0.0	0.0	0.0
10G*	36.5	87.6	46.3	49.0	17.5	15.7	0.0	18.5	17.0	0.0	1.8	1.9	14.1	2.5	67.7	8.2	0.0	0.0	0.0	0.8
AT median N=7	98.2	47.6	78.2	70.7	8.9	4.3	0.0	16.0	9.3	0.0	0.1	0.7	16.8	1.8	0.0	3.8	0.0	0.0	0.0	0.0
AT exclusions																				
1P	41.5	7.2	44.6	25.9	0.0	0.0	0.0	2.8	0.8	0.0	0.0	0.0	3.4	0.2	0.0	0.0	0.0	0.0	0.0	0.0
7P	32.8	1.8	0.0	5.9	0.0	0.0	0.0	1.0	0.3	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
LT inclusions																				
4G	0.8	10.2	25.9	4.8	72.9	47.2	100.0	48.8	58.6	0.0	11.9	4.7	0.0	6.1	0.0	9.9	0.0	0.0	0.0	0.0
4P	0.0	0.2	0.0	0.6	56.8	57.1	0.0	47.9	53.6	0.0	29.7	8.2	0.0	13.2	0.0	2.2	0.0	0.0	0.0	0.0
5B	0.0	7.8	2.6	2.9	52.3	41.3	57.5	51.8	48.9	0.0	27.5	6.8	0.0	11.7	0.1	8.8	0.0	0.0	0.0	0.0
9G	0.0	0.0	2.1	0.1	43.8	45.8	0.0	34.5	41.2	6.5	31.0	20.9	0.0	21.3	0.0	1.3	0.0	34.1	0.0	0.0
1B	0.0	0.7	9.8	2.6	49.4	43.5	100.0	35.7	44.4	0.0	22.9	7.4	0.0	10.8	0.0	7.2	0.0	0.0	0.0	0.0
LT median N=4	0.0	0.7	2.6	2.6	52.3	45.8	57.5	47.9	48.9	0.0	27.5	7.4	0.0	11.7	0.0	7.2	0.0	0.0	0.0	0.0
LT exclusions																				
2R	0.0	0.0	0.0	0.0	46.0	44.9	32.8	8.8	34.9	0.0	34.4	15.9	0.0	19.0	0.0	0.0	0.0	2.8	0.0	0.0
6R	0.0	0.0	0.0	0.0	15.4	7.3	0.0	5.8	10.0	0.0	0.0	2.6	0.0	1.5	0.0	0.0	0.0	0.0	0.0	0.0
10R	3.4	12.2	18.2	5.6	23.1	39.1	44.8	18.0	26.8	0.0	28.3	11.2	0.0	14.5	2.1	13.9	0.0	0.0	0.0	0.0

3.2 Object-odour paired associate task

A “latency difference” score was used as the dependent variable. For this measure, the average latency to dig in the pot of odourised sand on rewarded trials for each rat on each day was subtracted from the average latency to dig in the pot of odourised sand on the non-reward trials for that day. These scores were then averaged over 2-week blocks of testing. A maximum of 10 seconds on the non-reward trials was allowed, so optimal performance occurred when the rat responded quickly on rewarded trials but withheld responding on non-rewarded trials. That is, higher average latency scores indicated that a rat was making the correct associations between an object and an odour.

Figure 3.3 shows the average latency difference for the three groups, AT, LT and sham lesion groups, over a period of 8 blocks of testing (each block = 2 weeks of testing). There was no difference in performance between the three groups until the fourth block of testing (week 7 and 8) when the control group started to acquire the task. For both the AT and LT lesion groups the task was eventually acquired (block 8) but with impaired performance compared to the control group.

Inspection of Figure 3.3 suggests that there may be some difference in the rate of learning between the two lesion groups. While the average latency scores for the LT group began to increase around block 5 (week 9 and 10), average latency scores for the AT group did not improve until block 6 (week 12 and 13) and at a slower rate. That is, while the final level of performance at week 16 is similar for AT and LT groups, the AT group took longer to start acquiring the task.

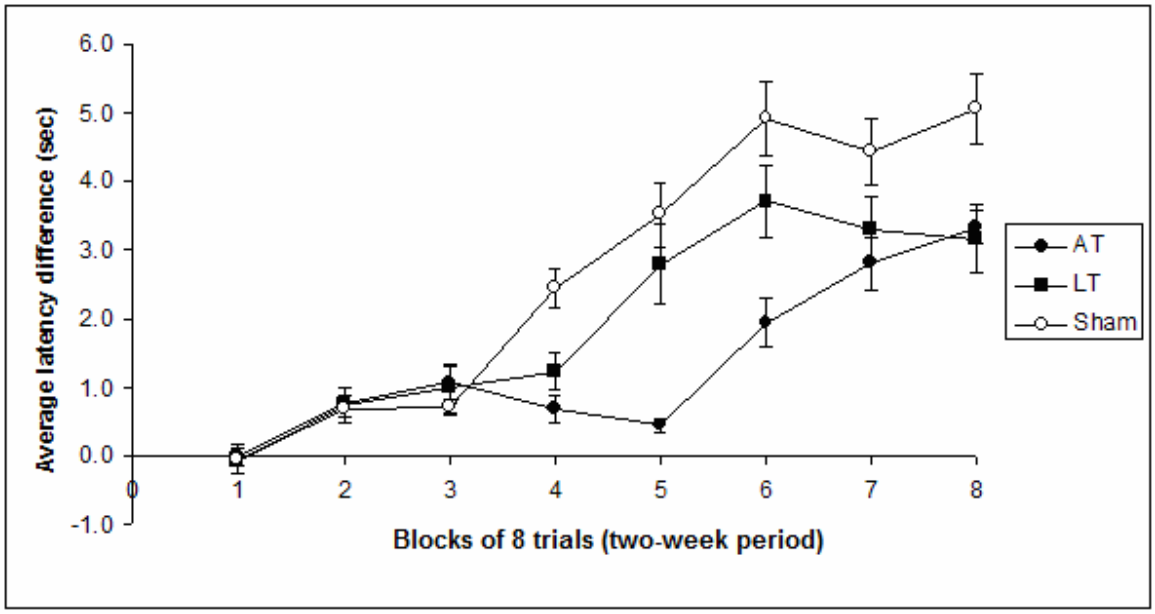

Figure 3.3: Average latency difference (seconds) for the AT, LT and control groups in the object-odour paired associate task over a period of 16 weeks (8 two-week blocks). Vertical bars are ±SEM. The average latency difference scores represent the average difference between the latencies on the go trials and the no-go trials.

These observations were verified statistically in a 3 (lesion: AT, LT and sham) X 8 (block) repeated measures MANOVA (Statistica) analysis, which revealed highly significant effects for Lesion ($F(2,19)=8.939$, $p<0.001$), Week ($F(7,133)=42.979$, $p<0.0001$), and a Lesion by Week interaction ($F(14,133)=4.954$, $p<0.0001$). A Post-hoc comparison (Fisher LSD) performed on the average latency difference scores over the entire testing period, confirmed that both the AT ($p<0.001$) and LT ($p<0.01$) groups were impaired relative to the Control group. However, there was no significant difference overall between the AT and LT groups ($p>0.40$).

When performance within each of the 8 blocks of testing was examined, to examine the simple main effects of Lesion within the significant Lesion by Week interaction, it was found that a significant Lesion effect was only apparent from block 4. There were significant Lesion effects on blocks 4, 5, 6 and 8, with block 7 just failing to reach significance (for p values see Table 3.2). Post-hoc comparisons (Fisher LSD) showed that both AT and LT groups were

significantly worse than control group on blocks 4, 6, 7 and 8. For block 5, the AT group showed poorer performance than the control and LT groups, but the LT group did not differ significantly from controls. The AT and LT groups did not significantly differ for each other on any other block other than block 5. Together with the mean values across blocks, these analyses support a description that the AT and LT groups both showed poorer acquisition (rate and final level of performance) than the sham group and that there was evidence that the rate of acquisition was poorer in AT than in the LT group. For controls, the mean performance did not improve across the last three blocks of testing (last 6 weeks); the same was true of the LT group, but the AT group's mean performance improved from block 5 to block 8.

A 3(lesion) X 2(last 2 weeks of training) repeated measures MANOVA confirmed the significant Lesion effect at the end of training ($F(2,19)=5.99, p<0.01$). A Post-hoc comparison (Fisher LSD) again showed a significant difference between the AT ($p<0.02$) and LT ($p<0.01$) when compared to the Control group.

To provide a clearer picture of the relative degree of learning that occurred over the 16 week training period in the three groups, the difference between the average latencies at Block 8 and Block 1 is shown in Figure 3.4. The mean superiority of the control group is readily apparent. The LT and AT groups also had a similar increase in difference scores over the training period. A one-way ANOVA showed no a marginally non-significant lesion effect ($F(2,16)=2.97$, $p>0.07$). However, a post hoc (Fisher LSD) comparison again confirmed that there was a significant difference between the Control group and LT ($p<0.006$), Control and the AT ($p<0.005$). However there was no significance between the AT and the LT groups ($p>0.50$).

Table 3.2: Fisher LSD scores showing the p values for AT, LT and Sham groups performance in the object-odour paired associate task. Those values in bold indicate a significant lesion effect ($p<0.05$).

	Block 1	Block 2	Block 3	Block 4	Block 5	Block 6	Block 7	Block 8
AT vs. LT	0.74	0.69	0.47	0.42	**0.03**	0.23	0.79	0.48
AT vs. Sham	0.77	0.56	0.15	**0.001**	**0.001**	**0.001**	**0.04**	**0.01**
LT vs. Sham	0.93	0.91	0.59	**0.01**	0.08	**0.04**	**0.03**	**0.006**

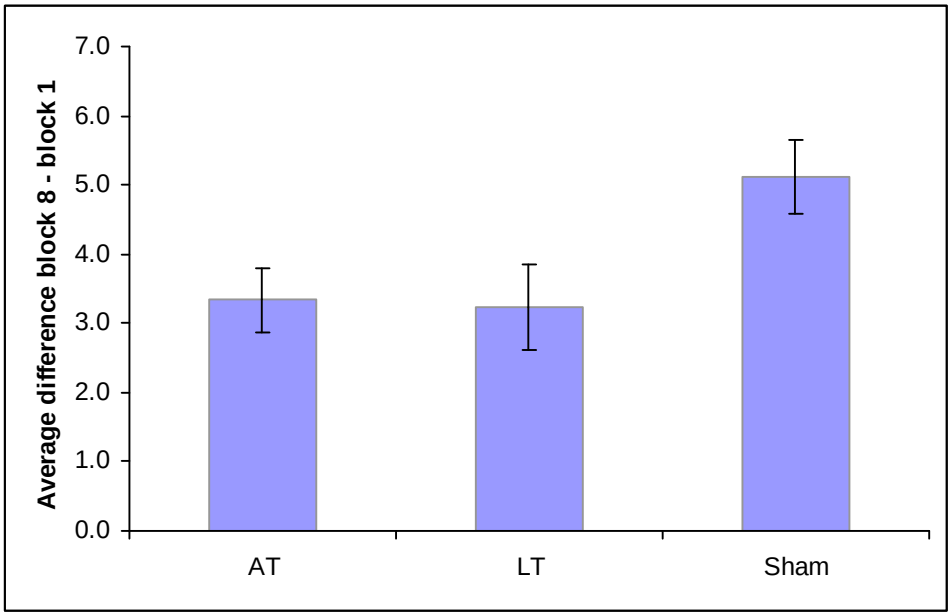

Figure 3.4: Difference in average latencies from Week 16 to Week 1 for AT, LT and control groups. Vertical bars are ±SEM.

3.3 Simple object discrimination task

The simple discrimination tasks were conducted after completion of the object-odour paired associate task. The rats were divided into two groups, balanced across lesion groups and level of performance at the end of training in the main task, and each subgroup was tested on a simple discrimination task. The simple discrimination tasks required them to make a simple discrimination between either two objects or two odours (i.e. no paired-association was required). One group completed the object discrimination task and the other completed the odour discrimination task.

Figure 3.5 shows the average latency difference scores for the three lesion groups over a 4 week testing period in the simple object discrimination task. This figure shows that all three groups rapidly acquired the simple object discrimination at an apparently similar rate. Average latency differences rose steadily from week 1 to week 4 (end of testing). Note that the 4 weeks shown in Figure 3.5 are equivalent to 2 blocks of training shown in Fig 3.3 and that the final level of performance on the simple object discrimination task was high in all rats and that the

means were higher than that shown by even the control group after 16 weeks of training in the object-odour association task, despite the fact that the same objects were used for both tasks. A repeated measures MANOVA showed a significant effect of Week ($F(3,39)=114.8$, $p<0.001$) with a highly significant difference between week 1 performance and week 4 performance (Fisher LSD, $p<0.0001$). However, there was no significant Lesion effect ($F(3,13)=0.24$, $p>0.8$) or Lesion by Week interaction ($F(9,39)=1.05$, $p>0.4$). This suggests that all three lesion groups learned the simple object discrimination task at a similar rate.

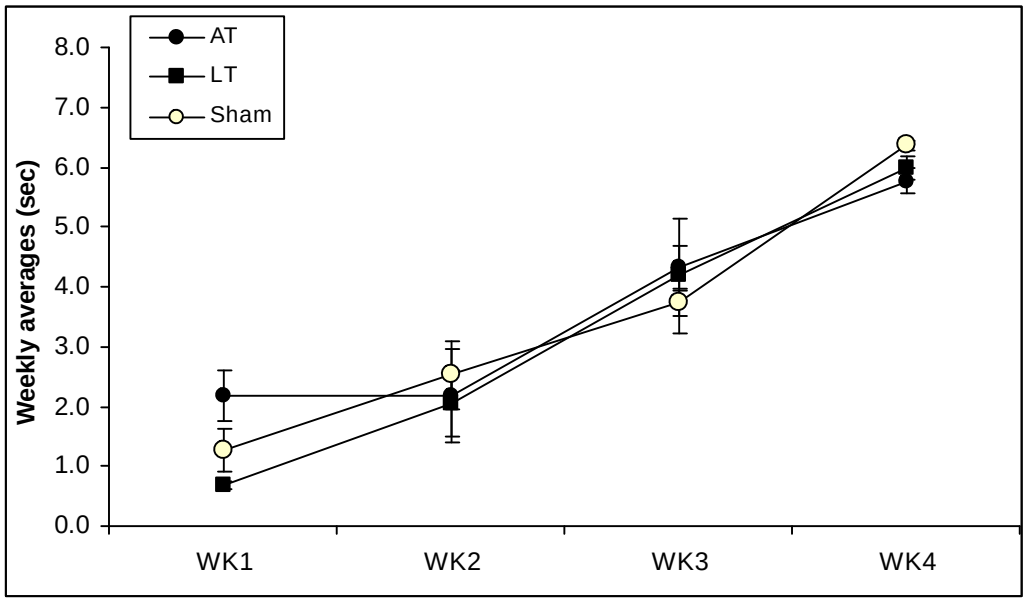

Figure 3.5: Average latency difference (seconds) for the AT, LT and control groups in the simple object discrimination task. Vertical bars are ±SEM.

3.4 Simple odour discrimination task

Figure 3.6 shows the average latency between the three lesion groups over a 4 week testing period in the simple odour discrimination task. This graph shows that the average latency difference for all three groups of rats increased rapidly from week 1 to week 4. Indeed, acquisition of the simple odour discrimination was even more rapid than that of the simple object discrimination. When we compare Figure 3.6 with Figure 3.5 we can see that the mean

latency differences of the AT and control group even at week 1 was above 3 seconds for the simple odour discrimination and were 5 to 6 seconds in all three groups by week 2.

A repeated measure MANOVA showed a significant effect of Week ($F(3,24)=74.94$, $p<0.0001$). However, there was no significant Lesion effect ($F(3,14)=1.71$, $p>0.2$) or Lesion by Week interaction ($F(9,42)=0.61$, $p>0.7$) which confirms that the all three lesion groups learned the simple odour discrimination task to similar levels and at similar rates. A post hoc (Fisher LSD) comparison showed no significant difference between the three lesion groups ($p>0.05$).

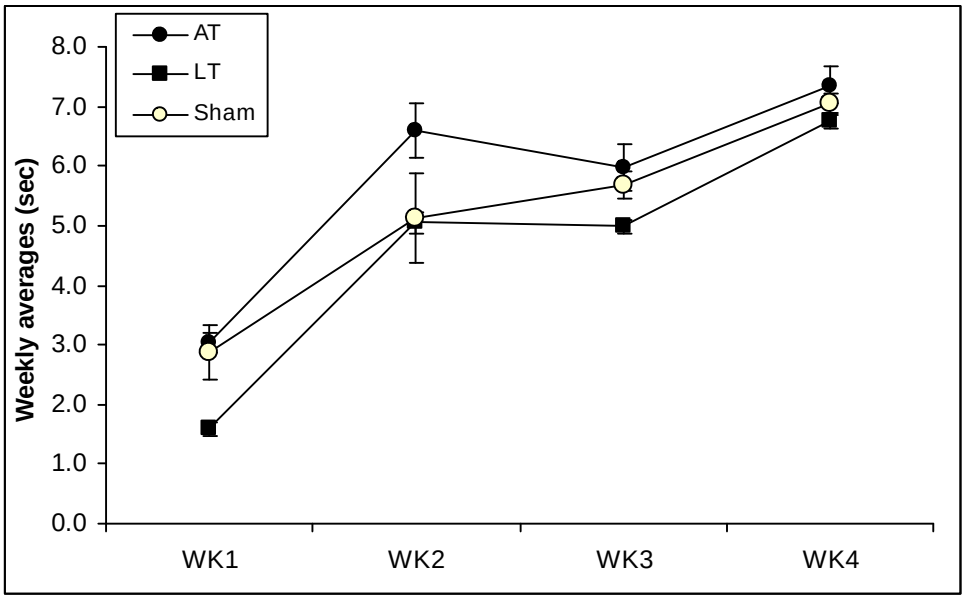

Figure 3.6: Average latency difference (seconds) for AT, LT and control groups in the simple odour discrimination task. Vertical bars are ±SEM.

3.5 Lesion-behaviour correlations

3.5.1 Relationship between lesion damage and performance on the odour-place paired-associate task

Figure 3.7 shows the percent bilateral damage to the AT aggregate for subjects in all three lesion groups, plotted against the latency difference scores for block 8 (weeks 15 and 16) in the

object-odour paired-associate task. This was done to try and distinguish a relationship between amount of damage to the AT and impaired performance. From Figure 3.7 we can see that the level of performance for control rats varies (along the solid line at 0). A few control rats performed at the highest level and achieved an average latency difference above 7 seconds. However, some controls performed at a level similar to the highest performing lesion rats, but the slowest control rat's performance was still well above that of the poorer lesion rats. Figure 3.7 also reveals a rather stable level of performance for AT rats, to the right of the dashed line, independent of the size of the AT lesion. There also appears to be no relationship between the amount of AT damage and the performance for the rats in the LT group, where unintentional AT damage in LT subjects was at a minimum.

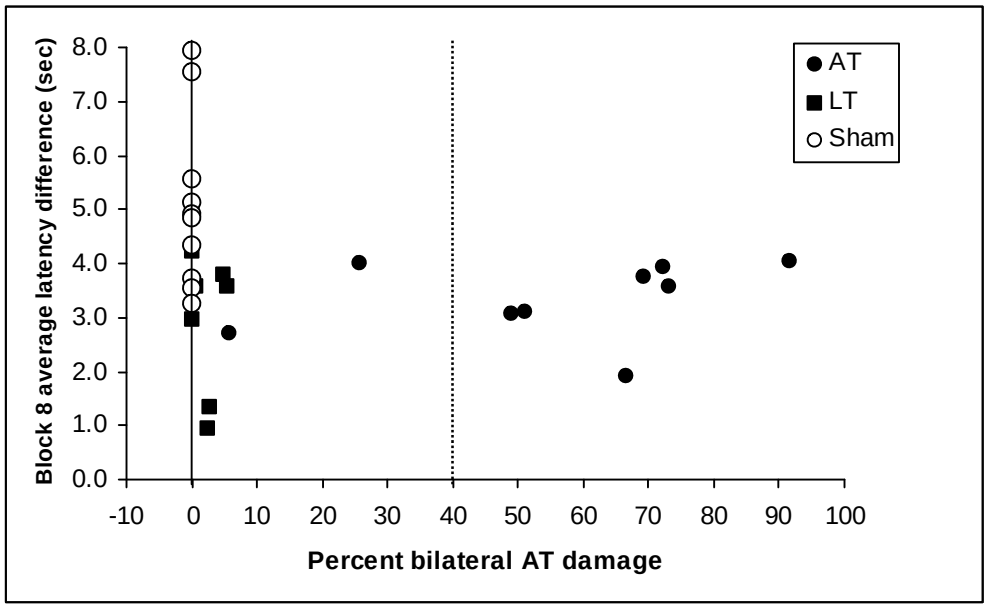

Figure 3.7: Percent bilateral damage to the AT and average latency difference in week 15 and 16 for AT, LT and sham groups. The dashed line indicates inclusion criteria for AT lesions. All data points to the right of the dashed line meet inclusion criteria.

Figure 3.8 shows the rate of learning of the rats that received the biggest and the smallest AT lesions. From this Figure we can see that the level of performance of the AT rats is independent of lesion size, with the biggest AT lesion rat performing at a higher rate than the smallest AT lesion rat.

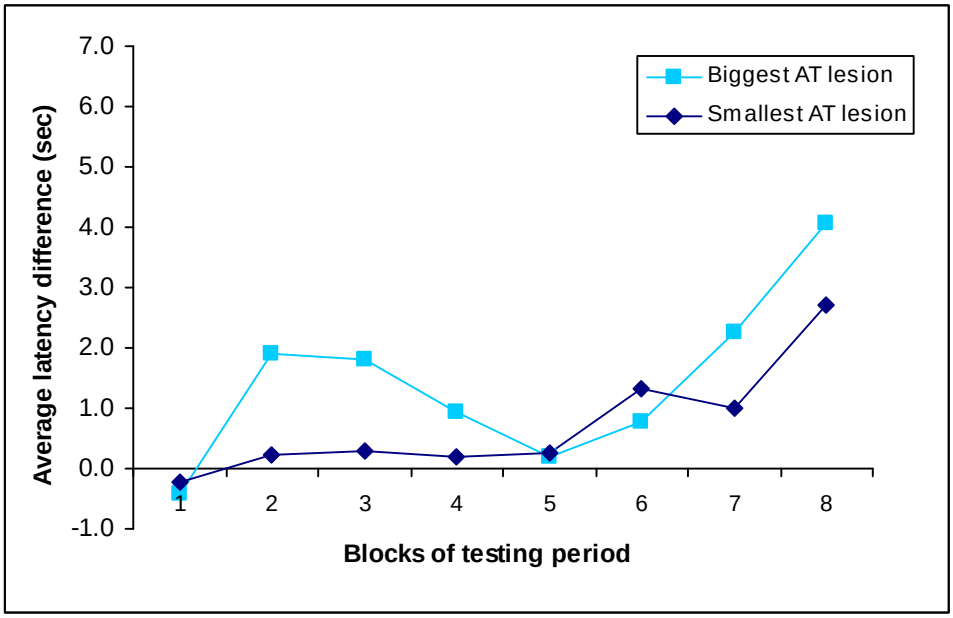

Figure 3.8: Average latency differences of two rats; one that received the biggest AT lesion and the other that received the smallest AT lesion.

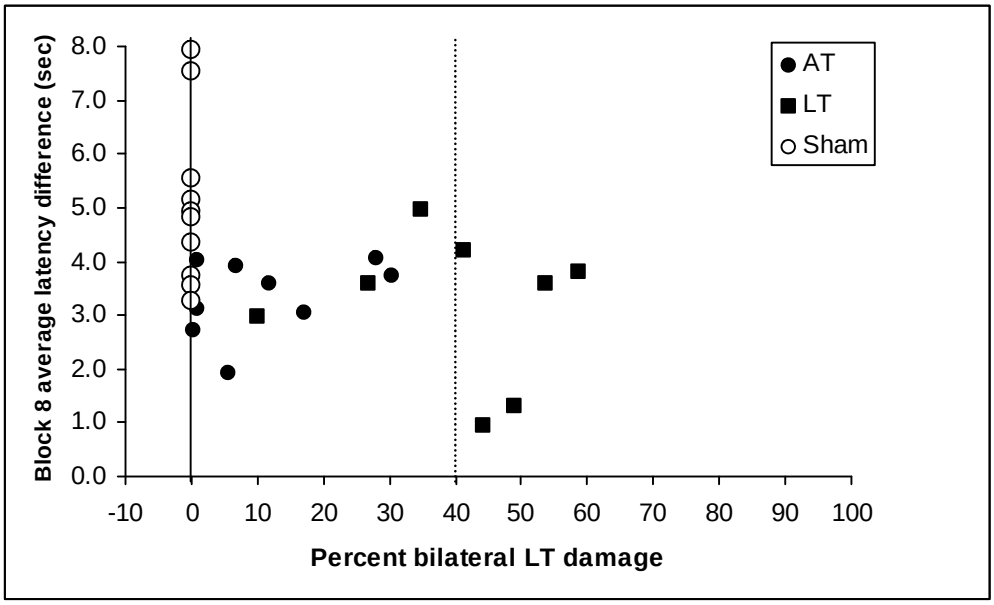

Figure 3.9: Percent bilateral damage to the LT and average latency difference in week 15 and 16 for AT, LT and sham groups. The dashed line indicates inclusion criteria for LT lesions. All data points to the right of the dashed line meet inclusion criteria.

Inspection of Figure 3.9 reveals there is no clear relationship between performance and percent bilateral LT damage. The final level of performance observed for the LT rats may be due to the increased performance of just 3 of the 5 LT rats. Clearly, two LT rats and one AT rat showed very little or no acquisition of the object-odour task. Given the distributions shown in Figure 3.7 and Figure 3.9, Spearman rank correlations were low ($r<0.05$).

Figure 3.10 is a graph of the rate of learning of the rats that received the biggest and the smallest LT lesions. From this Figure we can see that the level of performance of the LT rats is independent of lesion size, with the biggest LT lesion rat performing at a higher rate than the smallest LT lesion rat.

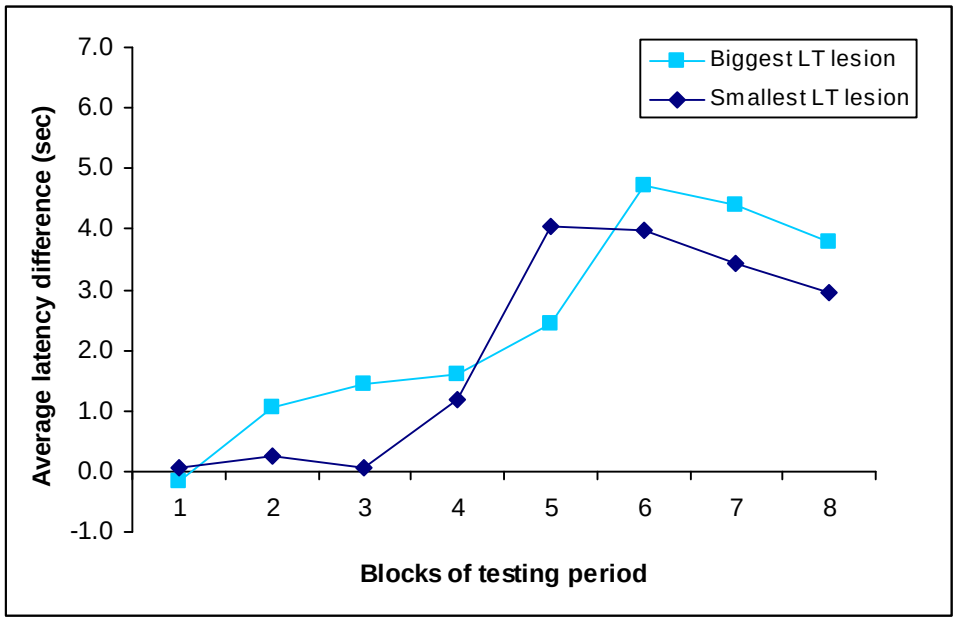

Figure 3.10: Average latency differences of two rats; one that received the biggest LT lesion and the other that received the smallest LT lesion.

A further in-depth analysis of the performance of each rat from the lesion groups was carried out in order to determine whether there was a relationship between the amounts of bilateral percent damage sustained by the *sub*-nuclei and performance of the individual rat during the last 2 weeks (block 8). A Spearman's rank correlation was used to clarify if there is a relationship between the percent bilateral damage of sub-nuclei and the performance in the last

two weeks of training. As before, there was no relationship between bilateral percent damage to sub-nuclei and performance in the last two weeks of training in the object-odour paired associate task.

4. Discussion

4.1 Summary of main findings and issues

This study has provided new evidence that lesions of the AT and LT regions impair paired associate memory. The study revealed for the first time that damage to the AT severely impaired acquisition of object-odour paired-associate memory, which is a particularly novel finding given that no spatial memory attribute was involved in the task and hippocampal lesions do not impair performance on an object-odour conditional discrimination (Gilbert & Kesner, 2002). Similarly, the study provided novel evidence that LT lesions also impair performance in this memory task. Performance of the AT and LT groups in the object-odour paired associate task was not related to the amount of either AT or LT damage caused by the lesions which may suggest that this type of learning is particularly susceptible to thalamic injury. By contrast, neither the AT nor the LT groups were impaired in the simple object and simple odour discrimination tasks. The difference between rapid and unimpaired acquisition on the simple component tasks after AT and LT lesions and the impairments evident on the paired-associate task highlights the specificity of the latter finding and their value in understanding the influence of thalamic lesions on higher-order, more complex memory processes.

The results from this study, when combined with those of Gibb et al's (2006) study, provide interesting new evidence of some similarities between the effects of damage to the AT and LT regions in learning and memory. Gibb et al (2006) found deficits after AT and LT lesions in an odour-place paired associate learning task. In both studies, AT and LT rats showed poor acquisition of the paired associate task, but not in the simple (component) discrimination tasks. The only difference in the two groups observed in the current study was that the LT group acquired the paired-associate task at a faster rate than did the AT group, although both groups achieved a similar mean level of performance at the end of the training period (block 8). Unlike the present study, in which AT and LT rats acquired both simple discrimination tasks at the same rate as did the Sham group, Gibb et al (2006) found a mild acquisition impairment on the simple odour discrimination task in the AT group while no significant effects were evident

in the simple spatial discrimination task. As indicated elsewhere, problems with spatial, rather than odour, cues might be expected in AT rats, but simple spatial tasks have produced little or no memory impairments in rats with hippocampal lesions either (Gilbert & Kesner, 2002). The previous odour discrimination deficit in Gibb et al (2006) more likely reflected an initial problem with the change in procedure from the odour-place association task to the simple odour task. The absence of an odour discrimination deficit in the current study confirms that simple odour discrimination learning is not itself a fundamental impairment for rats with AT lesions.

The hippocampus clearly plays a major role in spatial memory and memory for temporal order (e.g., Gilbert & Kesner, 2002; Kesner et al, 2002), as indeed do AT lesions (Aggleton & Brown, 1999; Mitchell & Dalrymple-Alford, 2005; Wolff et al, 2006). However, when the component attributes of a paired-associate task require odour and object information, but not spatial or temporal information, it is expected that hippocampal and AT lesions would have no effect. The paired-associate learning findings with AT lesions from this study are thus inconsistent with proposals that AT lesions would always mimic the effects of hippocampal system lesions. It is expected the AT are primarily part of an "extended hippocampal system" and thus the effects of AT lesions are expected to be similar to those of hippocampal system lesions. The new evidence presented here thus provides the first difference between the roles of the hippocampus and the AT in learning and memory.

The LT deficit seen in the object-odour paired associate task was less unexpected, as the LT is thought to play a general role in pattern association processing and complex memory processes. When the results of this study are coupled with those of Gibb et al (2006) it appears that the LT has a more general role in pattern association processing rather than a role in memory for specific attributes, at least in terms of odour, objects and place when examined in isolation and not as part of an associative memory task. The reason for this conclusion is that as shown here and elsewhere (Gibb et al, 2006; Mitchell & Dalrymple-Alford, 2005) memories for the latter specific attributes appear to be unimpaired after LT lesions. The extent to which LT lesions generally impair tasks requiring the specific attribute of memory for response (see Mitchell & Dalrymple-Alford, 2006) remains to be determined.

More expanded discussion of the main findings follows.

4.1.1 Object-odour paired associate memory and implications of the current findings

The AT and LT lesion groups reached a similar level of performance towards the end of the training period on the main task. One difference was that the AT group was more severely impaired in terms of a slower rate of acquisition. One possibility to explain the slower acquisition in the AT group may have been that this group had higher percent bilateral AT damage than was the corresponding case for rats in the LT group. However, lesion size alone seems an unlikely explanation because the performance in the paired-associate task was not related to percent bilateral damage of the region or sub-nuclei of the AT or the LT.

The concept of testing for a hippocampal role in paired-associate memory using odour and object component attributes rather than spatial attributes was investigated by Gilbert & Kesner (2002). Their results showed no deficits from hippocampal lesions. Hence, the present results give the first example that AT and hippocampal lesions differ in their effects. However, one possible reason for any differences observed in these two lesions is whether these two tasks were principally the same i.e. were the same strategies, cues and processes involved in both tasks.

Despite procedural aspects of the task being kept as similar as possible, one important difference between this study and that of Gilbert & Kesner (2002) on which the current study was modelled, was the comparative performance of the control groups in the two studies. In Kesner's object-odour task, the control group (and hippocampal lesion group) acquired the task in a period of 6 weeks (60 trials per week as per the current study) and reached a level of performance much higher (average latency > 8 seconds) than the control rats from this current study (average latency $\geq$ 5 seconds after 16 weeks of training). Apart from rat strain differences, and the fact that the current study used female rats whereas Kesner's study used male rats, the difference in rates of acquisition between the two studies might indicate different cues were available to the rats or that the cues were not as salient in the current study.

It is also possible that the differences in time taken to acquire the task between Kesner's study and that presented here indicate that the actual nature of this object-odour paired associate task was not the same between the experiments. It is possible that the task in the current study was less similar to episodic-like memory in humans and instead may have been more semantic-like, where learning is slower and less dependent on event memory. Episodic-like memory is believed to be acquired or operate relatively quickly over time. Alternatively, some researchers believe that very slow learning with numerous repeated trials is more related to stimulus-response memory, which is traditionally believed to depend on the striatum (eg Squire et al, 2004). Thus it is possible that rats used other strategies to solve this task than those used by the rats studied by Gilbert and Kesner (2002) and that these other strategies are more dependent on non-episodic or non-declarative memory systems. In either case, there is still an apparent dissociation between the effects of hippocampal system lesions and AT lesions, because the former lesions would still not be expected to cause any impairment. Gilbert and Kesner (2002) were not so much concerned with whether their task was episodic-like, because for them it was the absence of attributes for which the hippocampus is required, that is space and time (in the rat), that explains their negative findings after hippocampal lesions (although their hippocampal lesions spared the more ventral aspects of the hippocampal formation). Note, however, that Kesner has suggested that the ventral hippocampus may play a role in odour-based memory tasks, so the similarity between AT and hippocampal lesions might vary according to whether dorsal or ventral hippocampal lesions are made if one assumes that AT lesions influence the whole hippocampal system (Kesner June 2007; personal communication via one my supervisors, John Dalrymple-Alford, May 2007).

As mentioned above, Gilbert & Kesner's (2002) procedures were very similar to this current study. However, the rats in Kesner's study also acquired the simple discrimination tasks at a more rapid rate reaching a criterion of 10 correct responses in 10 consecutive trials (in less than 4 days for the odour discrimination and less than 5 days for the object discrimination). Whereas rats in the current study reached criterion of 10 correct responses in 10 consecutive trials at 7 days for simple odour discrimination and 14 days for the simple object discrimination. This provides some evidence that the cues used in the current study were perhaps less salient that those used in the Kesner's study. This provides additional evidence

for the need for caution when comparing results across studies especially when there may be some unsuspected procedural attributes in addition to strain or sex of the animals that differ.

The LT lesion group showed a less impaired rate of acquisition of the object-odour paired associate task but there was very little difference in average latency scores between the two groups (AT and LT) towards the end of the testing period (block 8). Damage to the LT has been shown to impair performance in a variety of tasks than involve both spatial and non-spatial attributes. Results from other studies on the ILn (part of the LT, which itself includes the lateral MDn; e.g Burk & Mair, 1998) provided clear evidence that ILn lesions in rats produced impairments in a DMS task, and were found to be independent of delays (still impaired after 4000 trials). ILn lesions have impaired performance in olfactory continuous DNMS tasks and the radial arm maze (Koger & Mair, 1994; Zhang et al., 1998, Mair et al 1998). This could mean that the LT is involved in general learning and memory. However, such a conclusion would mean that LT lesions would cause impaired performance in all memory tasks like the simple object and simple odour discrimination tasks, but that was not the case in the simple discrimination tasks conducted in this study or in Gibb et al's study (2006).

Also, in the study by Mitchell and Dalrymple-Alford (2005), restricted LT lesions were not found to impair performance in a radial arm maze. They suggested that spatial memory impairments observed after ILn and MD lesions may be due to unintentional damage of the AT nuclei. Once again, then, we have evidence that the effects of LT lesions are not indiscriminate across learning and memory tasks. Nearly all of the previous studies that examined ILn lesions have produced moderate to extensive damage to other thalamic nuclei. By contrast, this study (and similar work; Gibb et al 2006) has provided evidence that restricted LT lesions with minimal damage to other thalamic nuclei impair performance in paired-associate tasks, whereas little or no effects are found in terms of simple discrimination tasks or in terms of spatial memory in the radial-arm maze (Mitchell & Dalrymple-Alford, 2005, 2006).

Mitchell & Dalrymple-Alford's (2005, 2006) studies are in accordance with the view that no single nucleus within the thalamus is responsible for diencephalic amnesia on its own and that different regions play a subtly different role in memory and these regions are interconnected to

form memory systems that work in parallel. They have shown a double dissociation between lesions to the AT and LT nuclei on two different working memory tasks. They found that LT lesions, not AT lesions, impaired performance on a pre-operatively acquired egocentric response-memory task; and only AT lesions, not LT lesions, impaired performance in an allocentric spatial memory task. The neuroanatomical connections of the LT indicate that it is part of the striatum and frontal cortex circuit, which may process information associated with egocentric space and responses or related rules and strategies (White and McDonald 2002). The AT neuroanatomical connections involve the hippocampus via the pre- and parasubiculum and to a lesser extent the subiculum, some interaction via the entorhinal cortex, and a major indirect hippocampal route via the retrosplenial cortex (Shibata 1993; van Groen and Wyss 1995; Aggleton and Saunders 1997). Lesions to either the pre- or parasubiculum or the retrosplenial cortex in rats and monkeys have impaired performance in spatial memory and one-trial memory for object-place associations (Liu et al. 2001; Malkova and Mishkin 2003; Vann and Aggleton 2004a). Based on their neuroanatomical connections and related lesion evidence, and when combined with the findings concerning paired associate learning, it appears that there may be both differences and similarities across both AT and LT lesion groups in learning and memory.

As mentioned previously, it is possible that the object-odour paired associate task in the current study was processed in a different way than by the rats in Gilbert & Kesner's study (2002). One possibility is the involvement of the dorsal striatum. Studies investigating acquisition of a place versus response task have suggested that the level of training may be important to the way in which animals solve the task, in addition to the availability of cues. For example, it has been shown that early in the training period place learning tends to dominate, whereas later in training response learning dominates (Packard and McGaugh 1996; Colombo et al. 2003). This could mean that the LT deficit observed in the current study was due to disruption to the dorsal striatum if in fact this task invoked stimulus-response learning. Conversely, it is possible that the effects of the AT lesions were more related to a semantic-like effect, which may depend on the temporal cortex rather than the hippocampus. It is, of course, impossible to know which of these alternative explanations is more correct in terms of the manner in which the rats in the current study attempted to solve the object-odour memory task used here.

Mair and his colleagues (Burk & Mair, 2001) have shown evidence for similar effects of ILn lesions and lesions to the prefrontal cortex and ventral striatum (see also Zhang et al, 2005). The prefrontal cortex has also been implicated in paired associate memory (Browning et al, 2005; Kesner & Ragozzino 2003), so it is possible that disruption of prefrontal cortex activity provides another basis for the current LT lesion effects. Similarly, the AT also have prominent connections with the PFC (Shibata et al, 2005) so there is a possibility that the effects of both AT and LT lesions have a common basis based on their reciprocal connections with the PFC.

The implications derived from animal studies of the effects of different thalamic lesions, to which the work reported here contributes, is that variations in human cases of amnesia may reflect location, size and region of brain damage and damage to other distant brain regions via fibres of passage. Damage to the ILn and thalamic damage in cases of Korsakoff syndrome produces a deficit in response memory while the AT are also clearly implicated in many other kinds of memory deficits in various human examples of diencephalic amnesia (Mair et al. 1998, 2002; Harding et al, 2000; Holdstock et al. 1999; Exner et al. 2001, Van der Werf et al. 2003). In addition, from the evidence mentioned above it is possible that combined lesions have greater deficits on memory tasks that are susceptible to both types of lesions.

Clearly, further research is required to clarify the role of the AT and LT in other types of pattern association e.g. odour-response and place-response paired-associate tasks.

4.1.2 Specificity of lesions

Lesions size, location and specificity in previous studies have varied and many studies targeted one thalamic structure but caused apparently inconsequential damage or ignored damage to an adjacent thalamic region (see Introduction). Inclusion criteria for lesion size and specificity have also been varied. Non-target area damage occurs often and, depending on the amount and location, may have an effect on behavioural deficits observed. In order to analyse lesion specificity it has been standard practice to list the damaged areas and to provide a 'representative lesion' with qualitative reports on the impact of damage on behaviour and the amount of unintentional damage observed.

To avoid the above problems which confound the interpretation of previous lesion studies, this study used highly specific lesions to two thalamic aggregates (AT and LT) and quantified the amount of damage to target and non-target aggregates. AT lesions were highly specific and well localised with a median percent damage to target area above the 40% criterion (median = 70.7%). Damage to the LT aggregate was minimal (median = 9.3%) in the AT group. There was overlapping damage to other nuclei such as the IAM (interanteromedial thalamic nuclei; median = 0.0%) and the MT aggregate but they were also minor (median = 1.8%).

LT lesions were also well localised with the percent bilateral damage above 40% inclusion criterion (median = 48.9%). Damage to the AT aggregate in the LT group was minimal (median = 2.6%). There was little or no damage to the other adjacent thalamic nuclei such as the IAM or the MT aggregate (median = 11.7%).

It is felt that highly localised lesion technique as well as the numerical analysis of damage has afforded a reasonably greater degree of confidence in interpretation of the roles of AT and LT in memory acquisition compared to many earlier studies

4.2 Contributions of this study

The current study has made several valuable contributions to the role of the AT and LT aggregates in learning and memory. A major contribution of the current study is the new evidence of the role of the AT in memory and in particular that some tasks that are ostensibly unimpaired by hippocampal lesions may still be susceptible to the effects of injury to the AT. While the previous study by Gibb et al (2006) demonstrated that the AT and LT groups were impaired in an odour-place paired associate task, it was unclear whether the AT and LT were involved in all pattern association processing or just those that involved a spatial or response (egocentric) attribute. This study has clarified that indeed the involvement of the AT is not limited only to pattern association processing when an egocentric response or spatial attribute is present. In addition, the results from this study have provided important new information on

the role of the LT in learning and memory. The results confirm that the LT is also involved in pattern association memory processing.

Another contribution is the specificity, size and location of lesions produced. This study, along with previous work at the University of Canterbury, produced highly selective lesions and demonstrated that such highly selective lesions are possible with little unintentional damage to several adjacent nuclei within the thalamus. Quantative analysis of the lesions produced provides some clue as to the roles of the thalamic aggregates in memory without confounding by unintentional damage to adjacent thalamic nuclei. The findings suggest that relatively small damage to the AT and LT are sufficient to cause impairments on paired-associate learning.

4.3 Limitations of the current study and future directions

There have been relatively few studies that examine the neural connections of limbic thalamic nuclei in the context of learning and memory tasks. Further research into the neural circuits that underline memory function may provide clues as to how the thalamic nuclei interact with each other and with other parts of the brain. This would clarify whether the AT and LT are part of different neural pathways and this in turn would redefine how we interpret performance deficits in memory tasks such as the paired-associate task used in the present study. It would also be worthwhile to examine the impact of AT and LT lesions on other brain regions using for example c-Fos activity after behavioural testing (see Vann et al., 2000a,b).

Diffusion tensor magnetic resonance imaging (MRI) and white matter fibre tracking have resolutions of up to $1\mu m^3$ and can aide in fibre tract definition (Watts, personal communication, January 2006). A method that could externally scan the rat brain (MRI) would aide in identifying the lesion areas and perhaps help minimise extensive testing of rats with poor or no lesions. Coupling neuroanatomical and neuropsychological analysis along with such imaging techniques will help in identifying which neural pathways the AT and LT belong to. That is, it may be possible to examine microstuctural damage outside the thalamus after lesions to thalamic nuclei using sensitive diffusion tensor imaging MRI techniques.

Further research is needed to determine whether the AT and the LT are involved in all pattern processing or just spatial, object and odour pattern association processing. A replication and extension of the current task using AT, LT, MD, LD and hippocampal lesion rats (and or other areas such as the prefrontal cortex and the striatum) would enable direct comparison of lesion effects on various paired-associate learning tasks. The task could also incorporate a section that examines the effects of lesions to the thalamus and hippocampus using different concentrations of odours paired with the objects. This would further clarify the roles of AT and LT in odour detection and odour thresholds, and would also provide a useful functional comparison between nuclei in the thalamus.

The MD and LD nuclei are particularly important since they share some similar neuroanatomical connections with the ILn and AT respectively and as they too contribute to memory problems after thalamic injury. The LT region used in this study includes the lateral MD so it would be advantageous to isolate deficits caused by the MD lesions from those caused by LT lesions in paired associate tasks thereby providing a clearer picture of the role of the LT in learning and memory, and adding to the findings of Burk & Mair (1998). In addition, the MD has connections with the perirhinal cortex which is thought to be integral to the recognition-based neural system (Aggleton & Brown, 1999). The LD nuclei on the other hand shares many neuroanatomical connections with the AT and it would be advantageous to compare lesion deficits from these two regions on the same paired associate task to further clarify their roles in memory.

More specifically, MD lesions would have been a useful addition because it would have provided a comparative examination of the AT, LT and MD and, in turn, a more complete view of the medial thalamus' role in learning and memory. The MD nuclei have connections with the olfactory regions of the brain and form the olfactory-thalamic-neocortical projection pathway. Hence, the MD is thought to be involved in odour memory, as first shown by Eichenbaum et al. (1980). The MD receives information directly from the olfactory cortex in the rat and project from the MD to the rhinal sulcus (RS) and the anterior medial wall of the neocortex (MW). Eichenbaum et al (1980) showed that the MD lesions did not impair performance in the odour detection and odour threshold tasks but it did impair odour

discrimination. They also showed that lesions to the lateral frontal cortex impaired odour discrimination more than lesions in the medial frontal cortex. This could imply that odour memory may rely on selective connections between the MD and the lateral frontal cortex. Other studies have shown the MD to be involved in odour serial reversal learning and not odour discrimination (McBride and Slotnick 1997). There is evidence to show that lesions in the ventral pallidum, ventral striatum and medial prefrontal cortex, all part of the pallidothalamic system, produce similar results to that of MD lesions (Ferry, Lu, & Price, 2000). It has been speculated that the MD could be part of this system. The inclusion of the MD in future paired-associate studies would thus clarify its involvement in odour memory.

In terms of the laterodorsal (LD) thalamic nuclei Wilton, Baird, Muir, Honey, & Aggleton, (2001) found that combined lesions of the AT and laterodorsal nucleus (LD) impaired recognition of changes in object-place combinations in a spontaneous object recognition task. The LD has connections to the limbic cortex, including projections to the retrosplenial and subicular cortices which is similar to connections from the AT to the limbic cortex (Van Groen et al 2002). Although Wilton et al (2001) did not directly compare AT and LD lesions, one study has shown that neurotoxic LD lesions impair spatial reference and working memory although to a lesser extent than do AT lesions (Van Groen et al. 2002). Future studies that make a direct comparison of these thalamic nuclei would be very valuable in discerning the roles of the AT and LD in memory tasks.

Another limitation of the current study is the duration of testing. Testing lasted a period of 16 weeks, which although considerable was terminated due to time constraints. During the final week of testing the LT rats performance improved slightly and may have increased had the testing period been prolonged. As mentioned above, this task may have had more semantic memory characteristics that episodic-like memory.

Sample sizes for the groups used for the current object-odour paired associate study were small (AT n = 7, LT n = 5, control n = 9). Both the AT and LT groups were impaired relative to the control group but there was no significant difference between the AT and LT groups. A larger

sample size would have given greater power to be able to detect group differences, at least during the intermediate stages of acquisition.

A point to note is the *'pureness'* of this task. White & McDonald (2002) define a 'pure task' as one in which the memory elements involved in the task are processed by only one memory system, or regions within only one memory system. So, components of a task (such as spatial, temporal, odour etc) that do not closely correspond to a processing style of any particular memory system will be represented by more than one system. Hence lesions that affect any one system that processes a major component of the task (object or odour in this case) would severely impair performance. However, most memory tasks are not pure and all attributes are not represented solely by one memory system. Performance in such tasks requires a cooperative input from all neural systems involved, which is the case in this current study. One interpretation for the fact that lesions to the AT and LT caused impairment in this object-odour paired associate task, and which could reflect both regions being involved in different memory systems, is that object and odour components are processed by more than one memory system. However, as mentioned above, the AT and LT also have reciprocal neural connections to the prefrontal cortex (PFC). It is thus unclear whether the effects of the lesions are due to the elements of the task itself or because of disruption to the PFC.

4.4 General summary

This study has provided support to the view that damage to two limbic thalamus aggregates, the AT and the LT, can cause amnesia, with each aggregate playing a role in memory and learning. The similarities in performance observed by the AT and LT lesions can possibly be explained by the fact that both the AT and LT have connections to the frontal cortex. There is evidence to suggest that 'strategy' use in memory tasks involves the PFC (Hirst & Volpe, 1988). Disruption to this system could result in impairment in paired-associate memory tasks. It is also possible that they each affected different procedural aspects of the task, such as the acquisition of conditional stimulus-response learning (LT lesions), or acquisition of semantic-like rule based learning (AT lesions).

The results from the object-odour paired associate task show clear lesion deficits for AT and LT lesion groups, relative to the controls, and also show that the AT and LT groups acquire this task at difference rates. This study is the first to show AT lesions have a deficit in a task that appears to be unimpaired by hippocampal lesions. It provides evidence that the AT may be involved in paired association memory even when there is no spatial attribute involved. The results from this study, along with those of previous research at the University of Canterbury; demonstrate that the LT is responsible for memory processes such as pattern association, perhaps irrespective of the specific memory attributes, but not just learning and memory in general.

This study also highlights the point that comparison of lesion deficits across studies should be done so with caution. Although procedural aspects may be similar between any two studies there could be a difference in the cues, strategies and rules for acquisition of the tasks that may vary. The same is also true for transferring findings from animal lesion models to human cases of amnesia that arise from brain damage.

The current study places an emphasis on the thalamus' role in learning and memory. The results from this study show that selective and small damage to thalamic nuclei are sufficient to cause memory deficits. Although the data from this study do not provide straightforward evidence for the roles of AT and LT in human memory, they do raise a number of possibilities for the lesion deficits observed; and they provide a number of options for future studies.

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Memorandum

Animal Ethics Committee
Telephone: 364 2241
Extension: 6421
Email: Deborah.wekking@canterbury.ac.nz

UNIVERSITY OF CANTERBURY
Te Whare Wānanga o Waitaha
CHRISTCHURCH NEW ZEALAND

To:	Associate Professor John Dalrymple-Alford, Associate Professor Larry Field, Sheree Gibb, Rati Dalal, and Mathieu Wolff
Copy to:	AEC Committee
Date:	7 December 2005
Subject:	HEC Application – 2005/34R

I am pleased to inform you that the Animal Ethics Committee has approved your application entitled: "Comparative influence of three regions of the limbic thalamus on object-odour paired-associate memory".

I enclose a copy of the Animal Welfare (Records and Statistics) Regulations 1999 for your information.

Yours sincerely

Associate Professor Lou Reinisch
Chair, Animal Ethics Committee
Dean of Science

ANIMAL WELFARE (RECORDS AND STATISTICS) REGULATIONS 1999

Regulation 4: Records Required to be Kept (1)

(a) The name of each species of animal manipulated during the year

(b) The number of animals of each species manipulated during the year

(c) The purpose for which each animal was manipulated

(d) The source of supply of each animal manipulated during the year

(e) The status of each animal manipulated during the year according to the following categories:

- (i) normal/conventional
- (ii) specific pathogen free/germ free
- (iii) diseased
- (iv) transgenic/chimera
- (v) protected wildlife
- (vi) pregnant
- (vii) developmental stage (for mammalian fetuses in the last half of development or reptilian/avian pre-hatched young in the last half of development)
- (viii) other

(f) The number of animals of each species manipulated during the year which

- (i) have not been previously manipulated
- (ii) have been previously manipulated

at any time before or during the year

(g) In respect of the manipulation of any animal during the year, the severity of that manipulation according to the following scale (taking into account the effect of any anaesthetic, analgesic, euthanasia technique, or other strategy or practice that is applied or used, or any other step taken, to avoid or alleviate the stress or pain caused to the animal):

- (i) a manipulation that causes no stress or pain or virtually no stress or pain ('no suffering or virtually no suffering')
- (ii) a manipulation that causes stress or pain, of a minor intensity for a short duration ('little suffering')
- (iii) a manipulation that causes stress, or pain, of a minor intensity for a long duration or of a moderate intensity for a short duration ('moderate suffering')
- (iv) a manipulation that causes stress, or pain, of a moderate intensity for a long duration, or of severe intensity for a short duration ('severe suffering')
- (v) a manipulation that causes stress, or pain, of a severe intensity for a long duration, or of a very severe intensity for any duration ('very severe suffering')

(h) The number of animals of each species that died or were destroyed during the year in the course of, or subsequent to, the manipulation of those animals (whether directly or indirectly as a result of their manipulation)

(i) The number of animals manipulated during the year which are still alive at the end of the year

(j) In respect of the number of animals referred to in paragraph (i), the number of such animals

- (i) released from captivity

UNIVERSITY OF CANTERBURY No:

APPLICATION FOR USE OF ANIMALS IN RESEARCH

[Form modified October 2002, modified again April 2003]

This form is required for all experimental work, capture, containment and obtrusive observational work on animals. The Animal Ethics Committee is required to minimise the use of animals as well as reduce, avoid, or ameliorate the pain, suffering, and death of animals used in Teaching and Research at the University of Canterbury. Applicants are advised to refer to the Animal Welfare Act 1999 and Animal (Records and Statistics) Regulations 1999.

"Animals" means:

(a) any live mammal including a marine mammal (but does not include human being);
(b) any live bird;
(c) any live reptile or amphibian;
(d) any live fish, octopus, squid, crab, lobster, or crayfish;
(e) any other animal that is declared by the Minister to be an animal by notice in the Gazette.

Please send SEVEN printed or typed copies of the completed form, appropriately signed by the applicant and supervisor and the Head of Department, and any relevant documentation to the Secretary of the Animal Ethics Committee, Level 6, Registry.

Name(s) of applicants: John Dalrymple-Alford, Rati Dalal, Larry Field, Sheree Gibb, and Mathieu Wolff
Position(s): Associate Professor, MSc student; Associate Professor, PhD student and Post-doctoral fellow.
Department: Psychology and Biological Sciences

I(We) the undersigned **have read and understood the Code of Ethical Conduct and the appendices** under which the University of Canterbury Animal Ethics Committee operates.

If the application is approved I (We) agree to

(i) *If a protocol needs to be changed the application will be resubmitted to the Animal Ethics Committee for their approval.*
(ii) *Inform the AEC immediately in writing if unanticipated problems eventuate that could be an offence under the Animal Welfare Act 1999.*
(iii) *Furnish annual returns to my Departmental Representative on the Animal Use Statistics form. These records are to be retained by the University for ten years after the year to which they relate. The Department will be contacted by the Secretary of the Animal Ethics Committee when these statistics are required for collation and reporting to MAF.*
(iv) *Obtain approval from DOC, Ngai Tahu or other iwi as appropriate if the work involves protected indigenous species*

Please note that in some circumstances applicants may be required to appear before the AEC to answer questions

Signature of applicant(s): J. D-Alf Date: 8/11/05
Sheree Gibb
See note in #3 for Rati Dalal.

HOD Support: Signature of the Head of Department: Date: 11/10/05

Office use: Date received: Date approved/declined:

UNIVERSITY OF CANTERBURY **No:**

APPLICATION FOR USE OF ANIMALS IN RESEARCH

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Signature of applicant(s): Date:

See note in #3 for Rati Dalal.

HOD Support: Signature of the Head of Department: Date:

Office use: Date received: Date approved/declined:

1 **Name(s) of applicant(s) and Supervisors :** a) John Dalrymple-Alford and b) Larry Field
Position(s): Associate Professors, a) Department of Psychology and b) Biological Sciences
Contact Phone: a) 364 2998 / ext 6382 or 6998 and b)
Email: john.dalrymple-alford@canterbury.ac.nz; larry.field@canterbury.ac.nz

Name(s) of applicant(s): Rati Dalal
Level of Study/Degree Sought: MSc
Contact address: School of Biological Sciences
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2 **Title of project:** Comparative influence of three regions of the limbic thalamus on object-odour paired-associate memory

3 **Purpose and Importance of the Research (scientific, commercial value)**
(The purpose and importance is to be in lay/non-technical language and the justification may be in technical language, if necessary.)

Special note: The proposed study will contribute towards a MSc by Ms Rati Dalal, a student in Biological Sciences, but will be undertaken in Psychology (under agreement between the two departments). Ms. Dalal has agreed the proposed research but will sign the form on her return at the end of November (she has had to visit India because of a family bereavement). The reason that we are submitting this application in advance of her return is that she is required, due to regulations in the School of Biological Sciences, to commence her MSc project in December 2005. Available rats have been set aside for this purpose and would in any case probably be too old if she had to wait until next year. Ms Sheree Gibb (current PhD student) and Dr. Mathieu Wolff (Post-doctoral fellow) will also assist in this research. If anyone on the committee wishes to discuss this issue, please contact John D-A (by email and / or phone).

The current application:
This research constitutes an important part of our overall research programme at the University of Canterbury, for which similar, previous applications have received Ethics approval. Essentially, the programme under the direction of John Dalrymple-Alford is investigating the influence of damage to the brain's thalamic regions on memory function.

Unmasking the neural regions and systems responsible for memory, and reversing or reducing memory deficits associated with brain impairment, are among the most important goals in the brain sciences. Memory deficits are usually associated with the brain's temporal lobe, but profound amnesia can also result from injury to the very centre of the brain, called the limbic thalamus. The current research will enhance our understanding of the role of different areas within this complex region of the thalamus. As with our other work, it is envisaged that the results of the present study may influence the development of treatment models that may provide the basis for future therapies in the human domain, relevant to dementia, acute injury (such as stroke or cyst removal) and alcohol-induced injury to the thalamus. Previous research from our lab has provided evidence that the different small subregions of the thalamus may each contribute to different memory circuits in the brain (reflecting multiple memory systems), which is an entirely new approach to the theoretical basis of memory and to understanding the variability associated with thalamic injury in clinical (human) cases. Understanding this variability is essential to any scientific basis for potential future therapies.

The current study will investigate the influence of lesions to three thalamic subregions, the anterior thalamic nuclei (AT), the posteromedial thalamic nuclei (MT) and the lateral thalamic nuclei (LT). Each of these subregions has been suggested by the literature as critical for amnesia after thalamus injury. In the paired-associate learning task to be used in this study, rats learn that one object (X) will provide a reward (the rat digs in sand in a small pot that is partially inside the large object) if one odour (A) is present, but not if a second odour (B) is present, whereas a second object (Y) is associated with reward when it is paired with the second odour (B) but not the first odour (A). Paired-associate learning is one important measure of learning and memory and is highly sensitive to disruption in people with amnesia or dementia. The study described here represents entirely novel work, but builds on a related study conducted by Sheree Gibb for her Masters in 2004, which was highly successful and is currently being submitted to an international neuroscience journal. The reason that this is novel work is that our previous study examined paired-associate memory for odour-place associations, which involves spatial memory and in which both AT and LT, not MT, lesions produced substantial deficits. Different paired-associate

tasks are sometimes differently sensitive to different kinds of brain injury; by contrast to our previous study, the task in the current study does not involve "place / space".

4 Justification for the Research

(If this research proposal constitutes to some extent repetition of work that is already in the literature, please justify.)

The proposed study has never been attempted before.

Objective: To examine the comparative effects of separate lesions to nuclei in the anterior thalamus (AT), nuclei of the posteromedial thalamus (MT) and nuclei in the lateral region of the thalamus (LT) on paired associate memory for object-odour associations.

Our lab has produced the first clear evidence from rat models that different sites of thalamic injury may produce different effects on memory tasks and is attracting considerable interest from colleagues elsewhere. Our evidence suggests that, rather than either the anterior, lateral or medial nuclei of the limbic (medial region) thalamus being the sole critical locus responsible for human cases of thalamic amnesia, each sub-region may make separate contributions to different types of learning and memory. We are currently engaged in mapping these similarities and differences and the proposed study will thus advance knowledge in this field.

5 Species to be used (and numbers):

This work is technically demanding and the regions of interest are so small that each researcher must define their own specific procedures to find the correct targets, which in turn may vary slightly with the age of the rat. It is difficult to cause restricted cell loss in any one of these tiny parts of the thalamus, and to ensure minimal damage to the adjacent structures. It is estimated that about 10 female PVGc Hooded rats will be required to define the optimal procedures for producing the lesions.

The main experiment will involve four groups of rats (AT lesions, LT lesions and Shams), with 12 rats per group to ensure that we have sufficient animals with accurate experimental lesions for the two lesion sites. The number of animals required is the minimum number required to achieve 80% statistical power, and has been calculated based on the results of similar past research. The number of animals required is comparable to previous numbers required in the literature and in our previously approved research. As the three thalamic regions are closely adjacent, we have derived criteria for selective lesions which means that some rats in each group are found to have insufficiently selective injury. On average we find that approximately 15% of our animals are excluded due to inaccurate lesion placement. We will also first use 5 other rats to establish that the objects can be easily discriminated (simple discrimination, no pairing with odours; we know that rats readily learn to discriminate odours per se). Thus the total number of rats will be 63.

6 A Short Description of the procedure(s) requiring Committee's approval:

Behavioural testing: temporal order task (Codes i-a, ii-O)

The association memory task essentially requires that the rat learns that one stimulus (e.g., one object and not another) is associated with another type of stimulus (i.e. one, not another, type of

odourised sand in a small container; rats readily learn to dig in sand). Testing will take place on a "hole-board", which is a large circular field with numerous holes that help define the location of object for the rat (we expect that this part of the experiment will take about 2-3 months). The same rats will then be tested for the simple discriminations (odours only; objects only) to show that it is the association memory that was important and then checked for spatial memory using a standard task (either a standard radial arm maze or a water maze per previously approved procedures, to provide a comparative behavioural assay for AT lesions which, we predict, will not affect memory for object-odour associations). To motivate the rats to perform the paired-associate and discrimination tasks, their weight will be gradually reduced and maintained at just under 85% of ad libitum body weight (under free-food conditions, the rats are probably slightly heavy already) and this will need to be introduced prior to surgery when the rats are familiarized with the cheeseboard and the basic procedure of digging for food reward. This regime of restricted food access will be maintained throughout behavioural testing, except that free food is available for post-surgery recovery. Rats are monitored to ensure that the appropriate weight is maintained. Water will be freely available at all times throughout the study.

Surgical procedure: (Codes: i-a, ii-B)

Stereotaxic surgery will be conducted as per current standard procedures, using aseptic conditions, while the animal is held under deep anesthesia (atropine followed by 80mg/kg pentobarbitone). Prior to incision the animal is given an injection of ketophen (1.0 mg/ml, 0.1 ml subcutaneous, nape of neck) and the scalp is locally anesthetized with a subcutaneous injection of mepivicaine (2.0 mg/ml 0.2 ml subcutaneous under the scalp). During the surgery the animal is given injections of Hartmann's solution for hydration. Lesion surgeries involve bilateral microinfusion of sub-microlitre amounts of a neurotoxin, called NMDA, made directly via a thin one-microlitre needle syringe to produce cell loss in specific thalamic subregions of interest. The control group experiences only a sham surgery procedure (needle is lowered into the brain to slightly above the target depth but no substance is infused; the surgery procedure is otherwise identical to the lesion group), to ensure that the experimental findings are due to the lesion effect, rather than a consequence of general surgery. For all surgeries SOOV analgesic and antiseptic cream will be applied to scalp area for post-operative pain relief and previously approved procedures will be followed. All animals will have food and water ad libitum during the surgery period and recovery.

(i) Include a-e descriptor for each procedure:

a Animals to be treated under anaesthesia, or administered or deprived of materials or subjected to unusual conditions all with recovery for subsequent investigation.

b Animals to be treated under anaesthesia and killed without regaining consciousness.

c Animals to be killed in preparation of tissues.

d Animals to be used for antisera production.

e All other uses e.g. safely returned an end of trials.

(ii) Also indicate in respect to each procedure its likely severity according to the following scale (taking into account the effect of any anaesthetic, analgesic, euthanasia technique, or other strategy or practice that is applied or used, or any other step taken, to avoid or alleviate the stress or pain caused to the animal):

The grading scale is:

No suffering	O (a manipulation that causes no stress or pain or virtually no stress or pain)
Little suffering	A (a manipulation that causes stress or pain, of a minor intensity for a short duration)
Moderate suffering	B (a manipulation that causes stress, or pain, of a moderate intensity for a short duration)
Severe suffering	C (a manipulation that causes stress, or pain, of a moderate intensity for a long duration, or of severe intensity for a short duration)
Very severe suffering	X (a manipulation that causes stress, or pain, of a severe intensity for a long duration, or of a very severe intensity for any duration.

7 Reasons why alternative methods (non-invasive, not involving the death of an animal, etc) are not available, or suitable:

This type of research is essential for researchers to accumulate knowledge on the neural basis of memory and mechanisms of memory recovery after brain injury. Human evidence is valuable, but localised damage is non-existent in these structures.

8 Where are the animals to be held and the experiments performed?

In the Department of Psychology animal facility.

9 Relevant qualifications and experience of applicants:

John Dalrymple-Alford has over 20 years experience in behavioural neuroscience. Mathieu Wolff also has extensive experience in behavioural neuroscience. Sheree Gibb has completed a Masters thesis in behavioural neuroscience that provided her with experience in animal training, surgical and histological techniques and is currently engaged on her PhD. These individuals will primarily undertake the surgery and help train Ms Dalal in the required procedures.

10 Details of anaesthetic procedure and post-operative care and/or method of euthanasia:

Atropine is administered prior to injection of the anaesthetic agent to ensure rats do not have breathing difficulties during surgery. The volume of pentobarbitone (the anaesthetic agent, 80mg/kg) is determined by individual rat's weight prior to surgery. The surgical procedure commences once it is determined that the rat is under deep anaesthesia. Ketophen (1.0 mg/ml, 0.1 ml subcutaneous, nape of neck) and Mepivicaine (2.0 mg/ml 0.2 ml subcutaneous under the scalp) are administered for local pain relief prior to incision. Hartmann's solution is administered throughout surgery to maintain hydration. The rat's condition is monitored carefully throughout surgery and after surgery. SOOV analgesic cream is applied to the sutured scalp area following surgery. Post-operatively, especially during the first week, both the researcher and laboratory technicians monitor the rats to ensure full recovery. Rats have access to food and water ad libitum during the post-surgery recovery period.

To verify the placement of lesions at the conclusion of the behavioural testing, rats are given an overdose of pentobarbitone (0.8 ml of 100mg/ml or more as required), perfused transcardially

with saline and a fixative (for example 4% formalin), and brains are prepared for detailed histology. If time permits, the rat brains will also be processed for C-fos immunohistochemistry, which stains brain cells for a protein that may help reveal the pattern of active, and inactive, brain regions associated with the lesions.

11 What will happen to the animals once the project has been completed?

They will be euthanased as described in 10 above.

12 Duration of the project

8-10 months

Approval:

Approved (Chair, Animal Ethics Committee) **Date:**

Any Special conditions applying: