CONDITIONED PLACE PREFERENCE AND SPATIAL MEMORY:

CONTRIBUTIONS TOWARDS THALAMUS AND MEMORY

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CHAPTER I Acknowledgements

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CHAPTER II Abstract

Conventional theories of diencephalic amnesia have focused on a single thalamic region as a critical factor in the origins of anterograde amnesia. A more contemporary view is that different thalamic regions might contribute in unique ways to normal diencephalic functioning and therefore provide distinct contributions to the learning and memory. This study directly compared the effects of AT and MT lesions on a spatial pattern separation task, a spatial working memory task and a conditioned place preference task. AT lesions but not MT lesions produces deficits on the spatial working memory task on a cheeseboard. No group AT, MT or control rats acquired a conditioned place preference on the AT/MT lesion conditioned place preference task. Furthermore, this study determined the effect of systematic procedural variations on control rats in a conditioned place preference control task. The only variation that acquired a condition place preference was a separate arms conditioned place preference with one pre-exposure and three training trials. The results of this study provide new information regarding the role of thalamic lesions in spatial memory and suggests a revision of the current theories regarding learning and memory to incorporate the thalamic involvement that has been highlighted

CHAPTER III Introduction

General Introduction

Memory deficits are found in various human disorders: dementia, Wernicke-Korsakoff syndrome, selective amnesia as well as other neurological disorders. Though the symptoms vary, all have impaired memory components in some form. Damage to several different areas of the brain has resulted in a wide range of memory impairments. Principally, memory research has centred mainly on the hippocampus and other medial temporal lobe structures in an attempt to discern its precise functional role in learning and memory (Gilbert, Kesner, & DeCoteau, 1998; Squire & Knowlton, 2000). Although there are several theories surrounding the role of the hippocampus (Aggleton & Brown, 1999; Eichenbaum & Cohen, 2001; Kesner, 1998; McDonald & White, 2002; Squire & Knowlton, 2000), its contribution to different aspects of memory and memory impairment remains unclear. While hippocampal research remains a dominant force in neuroscience, research on the role of the diencephalic structures has also increased. Due to the connections between the medial temporal lobe and the diencephalon, it has been posited that impairments observed in the diencephalon will mimic those observed in the medial temporal lobe. Disruption to this interconnected circuitry is presumed to disrupt processes involved in episodic memory and affect anterograde amnesia (Aggleton & Brown, 1999). Because the medial diencephalon is thought to be involved in new learning and memory, understanding the origins of diencephalic amnesia is important. The focus therefore has been on deficits known to disrupt information processes mediated by the hippocampus and related structures, to determine whether the diencephalon might also have a mediatory role in these processes. The intent of Part One of this thesis was to build upon current knowledge about the structures of the diencephalon, specifically which thalamic nuclei are required to process spatial information versus those thalamic nuclei required to process affect information. Due to an unexpected negative finding in controls on the conditioned place preference task, which may or may not be effected by some of the thalamic injury associated with diencephalic amnesia, Part Two of this thesis employed systematic procedural variations that would be useful for future studies on the thalamic basis of learning and memory.

The medial diencephalon is located within the forebrain and is comprised of several subcortical structures including the thalamus. Research on the thalamus has primarily focused on a single critical thalamic region. The anterior thalamic nuclei (AT), the mediodorsal thalamic nuclei (MT) and the intralaminar nuclei (not investigated in the present study) have all been postulated as important in normal diencephalic function and anterograde amnesia. Previous studies have produced results suggesting that rats with anterior thalamic lesions have a clear impairment on tasks with a spatial element, including T-maze studies (Aggleton, Hunt, Nagle, & Neave, 1996; Warburton, Baird, & Aggleton, 1997), radial maze studies (Byatt & Dalrymple-Alford, 1996; Moran & Dalrymple-Alford, 2003; Sziklas & Petrides, 1999), and Morris water maze studies (Warburton, Morgan, Baird, Muir, & Aggleton, 1999). Recently, the first study to compare AT lesions on a non-spatial hippocampal-dependent memory task (temporal order of odours) found a severe post-operative impairment (Wolff, Gibb, & Dalrymple-Alford, 2006). Mediodorsal thalamic lesions have been found to produce a deficit on tasks that assess visual stimuli and reward association (Gaffan & Murray, 1990; Gaffan & Parker, 2000), impair learning to switch a response from match-to-sample to do not match-to-sample (Hunt & Aggleton, 1998b), and abolish the acquisition of a conditioned place preference in a compartmented box (McAlonan, Robbins, & Everitt, 1993).

In contrast to the premise that there is only one key medial thalamic region, is the suggestion that different regions might contribute in different ways to diencephalic amnesia and therefore, no single region is critical. Aggleton and Brown (1999) suggested that both the anterior thalamic and the mediodorsal nucleus connections are important in amnesia but that they represent two different processes. The anterior thalamic connection (to the hippocampus and mammillary bodies) was suggested to be important for episodic memory and recall; and the mediodorsal thalamic connection (to the amygdala and prefrontal cortex) was suggested to be important in familiarity-based recognition. There is support for this theory in both human and animal research (Aggleton, Neave, Nagle, & Hunt, 1995; Caulo et al., 2005; Hunt & Aggleton, 1998b; Jenkins, Dias, Amin, Brown, & Aggleton, 2002; Wolff, Gibb, & Dalrymple-Alford, 2006), but few studies have directly compared the different thalamic nuclei on the same tasks.

Recent work at the University of Canterbury has investigated the outcome of different medial thalamic lesions on a number of different learning and memory tasks. Mitchell and Dalrymple-Alford (2005) found a severe deficit following AT lesions on a radial arm maze task, a mild and only transient deficit following LT lesions and no deficit following MT lesions. In contrast, on a reward memory task, only the MT lesions showed impairment. On a task of temporal memory for two objects that have previously been observed several hours apart, only the AT lesioned groups showed clear preference for the earlier item. None of the lesions impaired object recognition for novel versus familiar objects. Gibb (2005) found that both AT and LT lesions produced a severe impairment on a odour-place paired-associate learning task, where rats learn an arbitrary association between a spatial location and an odour, but MT lesions had no effect on acquisition. Taken together these dissociations show a clear difference in the contributions of the medial thalamic structures in learning and memory, and provide support for the idea that different medial thalamic structures may vary in their contribution to diencephalic amnesia. Both of these studies employed a more specific approach to the amount of damage produced by the lesions used, which has been continued in the current study. Lesions to the AT consist of damage to all the AT divisions (AV, AM, AD), whilst lesions to the MT consist of damage to the central and medial thalamic nuclei and the intermediodorsal nucleus only (LT lesions were not investigated in the current study).

Although the previously mentioned tasks often have examined spatial memory, currently there is no study directly comparing the different thalamic structures on a spatial pattern separation task. Kesner (1998) proposes that the one facility of the hippocampus is to separate new spatial information into patterns by storing one place as separate to another. This is thought to be accomplished by temporarily separating one spatial event from another. Tasks assessing this have produced hippocampal deficits (Gilbert & Kesner, 2003; Kesner, Gilbert, & Barua, 2002). Presently there is also no study that compares the thalamic nuclei on conditioned place preference in a radial maze. A conditioned place preference is the acquisition of a preference for a place (distal stimuli present at the send of the arm) through its association with a food reward, over a place associated with no food reward. White and McDonald (2002) have produced evidence that both the hippocampus and the amygdala are involved in acquiring a conditioned place preference

in a radial arm maze. Acquisition critically depends on the design of the task itself in terms of the separation between arms and the degree of non-rewarded pre-exposure to the two arms (places).

Initially, Part One of the present study aimed to address this lack by examining the effects of AT and MT lesions on a delayed-match-to-sample task that assesses spatial pattern separation previously found to be impaired by lesions of the hippocampus and dentate gyrus (Gilbert, Kesner, & DeCoteau, 1998; Gilbert, Kesner, & Lee, 2001); then to examine the effects of those same lesions on a conditioned place preference task that assesses the discrimination of unambiguous distal cues previously found to be impaired by the amygdala (McDonald & White, 1993; White & McDonald, 1993). Because of failure to acquire the spatial pattern separation task, animals were instead tested on a simpler match-to-sample task. Furthermore, the failure of rats to show acquisition of a conditioned place preference then provided the direction for to Part Two. Part Two of the present study aimed to determine the influence of different conditioned place preference procedures on control rats, in order to then follow up with the effects of different thalamic lesions on selected procedures (as will be seen, follow up with medial thalamic lesions became impossible in the time frame of the present thesis).

Human Studies

Anterograde amnesia is recognized by the inability to acquire new episodic information following brain trauma (Aggleton & Brown, 1999). Diencephalic trauma can result from vascular accident, cyst, tumour, infarction, and alcohol induced Korsakoff syndrome. The association of diencephalic injury with anterograde amnesia has existed for several decades (Aggleton & Brown, 1999; Kapur, Thompson, Cook, Lang, & Brice, 1996; Parkin, Rees, Hunkin, & Rose, 1994; Schmahmann, 2003; Van der Werf, Witter, Uylings, & Jolles, 2000; Victor, Adams, & Collins, 1971; von Cramon, Hebel, & Schuri, 1985). An example of this arose from post-mortem analysis of the thalamus of 43 patients identified prior to their death to have had Wernicke-Korsakoff syndrome. Five cases did not show a severe memory deficit, and subsequent analysis revealed that the MT was not lesioned, but there was damage to the mammillary bodies (Victor, Adams, & Collins, 1971). This

evidence suggested that damage to MT gives rise to the memory deficits in diencephalic amnesia either by itself or in conjunction with damage to the mammillary bodies, but that damage to the mammillary bodies alone does not. As adjacent areas of the brain were not available for study in this instance, their contribution to the memory impairment seen could not be sufficiently ruled out.

Recent evidence from an investigation into diencephalic damage in alcoholics presenting with Wernicke's encephalopathy and Korsakoff's syndrome (Harding, Halliday, Caine, & Kril, 2000) revealed that patients with Wernicke's encephalopathy (no persisting or severe amnesia) exhibited some neurodegeneration of the MT and mammillary bodies. Patients with Korsakoff's syndrome while showing neurodegeneration of the MT and mammillary bodies also displayed substantial neuronal loss in the AT. This suggests that it is the neurodegredation of the AT that is the critical component of the amnesia found in Korsakoff's syndrome.

In a recent review of the vascular syndromes associated with the thalamus Schmahmann (2003) noted that infarction (interrupted blood supply causing oxygen and glucose deprivation of the neurons) in the tuberothalamic artery which irrigates regions including the anterior nuclei (AD, AM, AV) and the ventral pole of the MT, was associated with impairment in the acquisition of new information and memory alongside other neurological deficits. Infarction of the paramedian artery which irrigates regions that include the mediodorsal nucleus and the intralaminar nuclei however, was also associated with severe anterograde amnesia. It is worth noting that in clinical presentations of infarction damage is seldom confined to specific nuclei and that a plethora of neurological symptoms usually arise subsequent to the damage of which anterograde memory impairment is one.

Due to technological advances it is now possible to observe functional changes following brain injury without having to wait for autopsy. Through magnetic resonance imaging (MRI) Kapur et al (1996) found damage to the medial thalamic regions, mammillary bodies and brain stem in a patient presenting with significant anterograde memory impairment. However, this was only used to identify the locality of the damage, not to

ascertain if there were any functional changes. Functional MRI studies allow the function of lesioned areas to be observed while doing a given task. Using fMRI Caulo et al (2005) found signal changes in the medial thalamic regions, fornix and mammillary bodies during a facial recognition test, in a patient that presented with severe anterograde amnesia. Although the patient had no evident hippocampal damage, fMRI revealed no activation of the hippocampus in contrast to observed hippocampal activation in the control fMRI subjects.

Clearly human studies of diencephalic amnesia can provide us with important evidence and information about the thalamus and its role in anterograde amnesia. Unfortunately the nature of human studies means that they are disadvantaged by their inherent limitations. The causes of damage in the human brain means that the areas affected are not confined to just one region, often the adjacent areas are also damaged to some degree. This can also extend to areas in the brain that are relatively distant due to connecting pathways and fibres. In terms of post-mortem studies there is usually a significant amount of time between onset of symptoms and autopsy. Newer technology like fMRI does reduce this limitation to some degree but lacks the specivity of post-mortem studies. Also in human cases of amnesia there is often no record of memory function prior to injury and onset of amnesia. To alleviate these limitations diencephalic amnesia research has turned toward animal models, where if not eliminated, these problems can at least be controlled. Animal models of amnesia allow the experimenter to assess memory function prior to injury, to control the placement and size of the lesion and to dictate the time between damage and post-mortem analysis of tissue (Aggleton & Pearce, 2001).

Animal Models

The use of animal models in diencephalic amnesia research has meant that specific structures can be targeted and attempts can be made to minimize damage to the surrounding structures. Therefore, experimenters can use animals to study tasks that model aspects of human diencephalic amnesia and then measure the deficits on the basis of specific areas of learning and memory. However, which areas of learning and memory are

important and what the results of these animal model experiments represent when attributed to the human brain is contested.

As stated earlier, studies have predominantly focused on a single specific thalamic region, concentrating on the anterior thalamic nuclei (Aggleton & Brown, 1999; Aggleton, Hunt, Nagle, & Neave, 1996; Aggleton, Neave, Nagle, & Hunt, 1995; Jenkins, Dias, Amin, Brown, & Aggleton, 2002; Moran & Dalrymple-Alford, 2003; Warburton, Baird, & Aggleton, 1997; Warburton, Baird, Morgan, Muir, & Aggleton, 2000), the mediodorsal nucleus (Gaffan & Murray, 1990; Gaffan & Parker, 2000), or the intralaminar nuclei (Burk & Mair, 1998), because it was thought that damage to one of these three thalamic areas was primarily responsible for memory deficits in diencephalic amnesia. The specific contribution of the thalamic structures remains unclear (Schmahmann, 2003). Recent research has begun to compare these thalamic areas (Alexinsky, 2001; Gibb, 2005; Mitchell & Dalrymple-Alford, 2005; Mitchell & Dalrymple-Alford, 2006) but it is still unclear whether one thalamic area is more important in diencephalic memory or whether they pertain to different aspects of memory.

Conceptually, memory used to be thought of as different aspects of a single system. More contemporary theories describe separate multiple systems in the brain that modulate memory in different ways (Kim & Baxter, 2001; Packard & Cahill, 2001; Squire, 2004). There are several competing theories that could be examined with regard to diencephalic amnesia, however only the three most relevant to the present study will be examined here.

The Multiple Attribute Model proposed by Kesner (1998) includes three main memory systems: Event-based, Knowledge-based and Rule-based. Each system is distinct, subserved by different neural structures and at least with respect to event-based memory, different structures process different attributes (different memory representations). There are key attributes (among the many possible); they include space, time, response, sensory perception, affect and language (unique to humans). Interactions among attributes are important, for instance, a cognitive spatial map (important in many animal experiments) is posited to arise from the interaction of the spatial and the sensory perceptual attributes. The specific set of neural structures responsible for each attribute use a number of

processes to generate memory for that attribute. In Event-based memory: language, time and space related information are processed by the hippocampus; affect and reward value information is processed by the amygdala; response information is processed by the caudate and sensory perceptual information (for objects) is processed by the perirhinal cortex. The set of processes required by each attribute for Event-based memory are pattern separation, pattern association, consolidation (pattern completion) and short-term or working memory. Kesner has conducted a series of experiments that provide evidence that the hippocampus and its subregions (specifically the dentate gyrus) are involved in pattern separation, particularly when the process involves spatial or temporal attributes (Gilbert, Kesner, & DeCoteau, 1998; Gilbert, Kesner, & Lee, 2001; Kesner, 1998; Kesner, Gilbert, & Lee, 2002).

White and McDonald (White, 2004; White & McDonald, 2002) propose a theory of multiple parallel memory systems referred to by the anatomical names of their central structures: hippocampus system, dorsal striatum system and amygdala system. Although all three independent memory systems have the same access to information, they each process this information and produce a behavioural output in a unique way. Similar to part of the model proposed by Kesner, different associations are processed by the hippocampus, caudate and amygdala. The hippocampus system (including the fimbria fornix) processes information relating to cognitive information (stimulus-stimulus associations); the amygdala system processes Pavlovian conditioned responses (stimulus-affect associations); and the dorsal striatum (caudate-putamen in rats) system processes reinforced stimulus-response associations. White and McDonald have conducted a series of experiments that have provided evidence that hippocampus and amygdala may both have a role in the acquisition of conditioned place preference, specifically when distal cues are unambiguous (amygdala) or ambiguous (hippocampus) (Chai & White, 2004; McDonald & White, 1993, , 1995a, , 1995b, , 2002; White, 2004; White & McDonald, 1993, , 2002).

The hippocampal-anterior thalamic axis model proposed by Aggleton and Brown (1999) differs from the two previous models in that it accredits more importance to the thalamic nuclei for the normal function of episodic memory. In the models proposed by both

Kesner (1998) and White and McDonald (2002), the thalamic nuclei serve a supportive capacity only within the system as a whole. Aggleton and Brown (1999) ascribe limited value to the distinction between the medial temporal lobe and the medial diencephalon. Importance is instead placed on an extended hippocampal system encompassing the hippocampus, fornix, mammillary bodies, anterior thalamus as well as the connecting pathways and projections. This is postulated to be responsible for the encoding of new information and the subsequent recall of episodic memory but not familiarity recognition. A discrete system comprised of prefrontal cortex, perirhinal cortex and the mediodorsal thalamus as well as the connecting pathways and projections, is thought to be responsible for familiarity judgements and item recognition. As indicated previously there is supportive experimental evidence for the first part of this model (Aggleton, Neave, Nagle, & Hunt, 1995; Caulo et al., 2005; Hunt & Aggleton, 1998b; Jenkins, Dias, Amin, Brown, & Aggleton, 2002; Wolff, Gibb, & Dalrymple-Alford, 2006). The current thesis is relevant to aspects of all three models. It was not designed to test which model is more accurate, but instead use information from each to investigate thalamic lesions, spatial memory and conditioned place preference.

Anatomical Considerations

As mentioned earlier the thalamus is just one of several subcortical structures in the diencephalon. It can be segregated into distinct aggregates of thalamic nuclei. The thalamic nuclei of importance in this study are found within the medial thalamus: the anterior thalamic nuclei (AT) and the mediodorsal thalamic nuclei (MT). Other thalamic regions of research interest include the intralaminar nuclei and the lateral internal medullary lamina (not investigated in the present study). These aggregates can themselves be further subdivided.

The AT encompasses the anteroventral nucleus (AV), anteromedial nucleus (AM), and the anterodorsal nucleus (AD). There are dense reciprocal projections from the AT to the hippocampus via the retrosplenial cortex (Aggleton & Pearce, 2001; Shibata, 1998; van Groen & Wyss, 1995). Other structures implicated include the fornix (Aggleton & Brown, 1999) cingulate gyrus (Horikawa, Kinjo, Stanley, & Powell, 1988), the entorhinal, perirhinal, presubicular, parasubicular cortices and the subiculum (van Groen, Kadish, &

Wyss, 1999). Other areas of the brain also receive projections from the AT including the prefrontal cortex (Aggleton & Brown, 1999). There are also extensive connections from the mammillary nuclei to the AT (Seki & Zyo, 1984; Shibata, 1992; Vann & Aggleton, 2004), which provides another, indirect influence of the hippocampus on the AT. It is on the basis of these anatomical connections that Aggleton and Brown (1999) postulate a hippocampal-anterior thalamic axis thought to be important in the encoding of new information and episodic memory recall. There are some differences in the projections associated with the regions within the AT. For example limbic cortical areas receive bilateral projections from the AM and AV, while the projections to the AD are ipsilateral; and some infralimbic regions bilaterally project to the AM and AV but not AD (Seki & Zyo, 1984). However, there is little evidence of a possible difference in the contribution of the AM and AV (Aggleton, Hunt, Nagle, & Neave, 1996; Byatt & Dalrymple-Alford, 1996).

The MT (in rats) encompasses the medial, central, lateral and paralaminar segments (Groenewegen, 1988). It is the main subcortical structure with reciprocal projections to the prefrontal cortex (Aggleton & Brown, 1999; Rotaru, Barrionuevo, & Sesack, 2005). Other structures implicated include the perirhinal cortex (Aggleton & Brown, 1999), entorhinal cortex, amygdala and regions in the brainstem. There are also connections from the pallidal, nigral and tectal areas to the MT (Groenewegen, 1988). Its is on the basis of these anatomical connections that Aggleton and Brown (1999) propose a perirhinal-mediodorsal thalamic system that contributes differently to learning and memory than the extended hippocampal system. It has also been suggested that the mediodorsal thalamic-amygdala connection might infer a more critical role in amygdala based memory than previously assumed (Gaffan & Murray, 1990; Mitchell & Dalrymple-Alford, 2005). As mentioned previously, MT lesions in this study only encompass the medial and central segments (as the lateral MT has connections with the dorsal striatum and with different regions of the prefrontal cortex).

Connections of the Anterior Thalamic Region

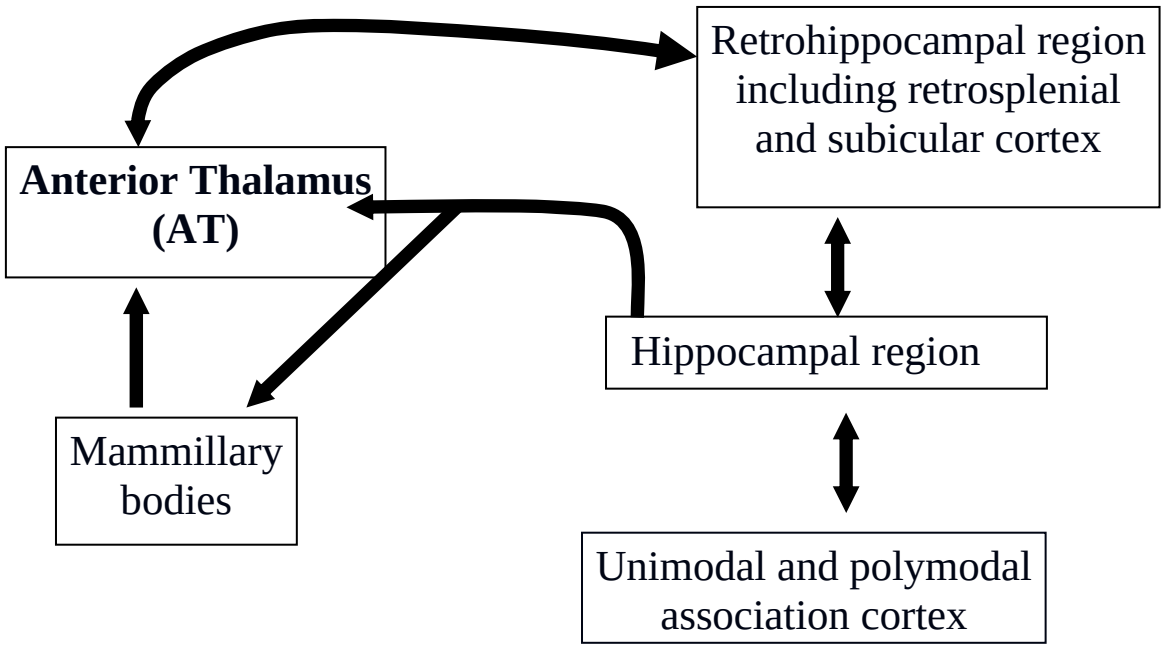

Figure 1 Conceptual diagram illustrating the connections of the anterior thalamic (AT) region. Reproduced with permission from Dalrymple-Alford (2004) and adapted from Aggleton and Brown (1999). Previously depicted in Gibb (2005).

Connections of the Medial Thalamic Region

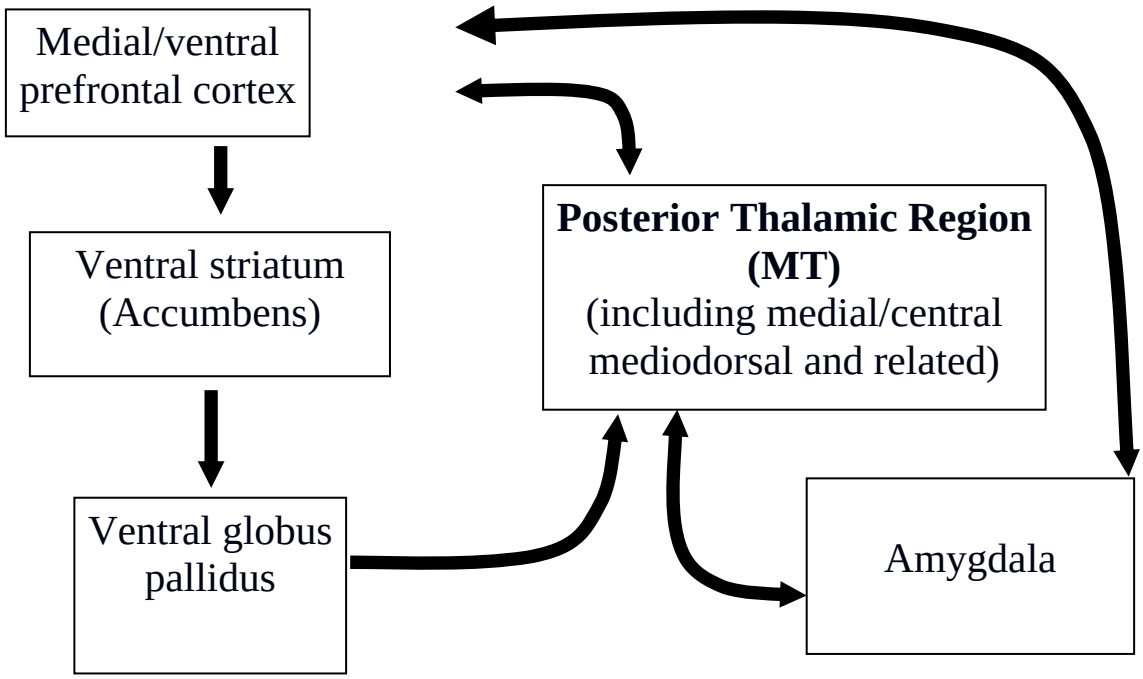

Figure 2 Conceptual diagram illustrating the connections of the posteromedial thalamic (MT) region. Reproduced with permission from Dalrymple-Alford (2004) and adapted from Aggleton and Brown (1999). Previously depicted in Gibb (2005).

Memory Attributes and Experimental Procedures

Memory deficits on a wide range of tasks are observed after brain damage. This variation is thought to be due to the specific factors involved in the task. Any task concerning memory may involve a number of memory systems, attributes and processes, as well as a number of strategies and cues relevant to the task. This is also true for the present study.

Spatial Memory

Anterior Thalamic Nuclei

Aggleton and Brown (1999) propose that the connections between the hippocampus and the AT provide explanation for the wide spread involvement of the AT in learning and memory tasks that pertain to allocentric (cues external to the body) spatial memory. As mentioned previously, AT lesions impair a wide range of tasks with a spatial component (Aggleton, Neave, Nagle, & Hunt, 1995; Aggleton & Saghal, 1993; Gibb, 2005; Sutherland & Rodriguez, 1989; Sziklas & Petrides, 1999; Warburton & Aggleton, 1999; Warburton, Baird, & Aggleton, 1997; Warburton, Morgan, Baird, Muir, & Aggleton, 1999). The most common task used to examine the effects of AT lesions on spatial learning and memory is the radial arm maze task (Aggleton, Hunt, Nagle, & Neave, 1996; Alexinsky, 2001; Byatt & Dalrymple-Alford, 1996; Mair, Burk, & Porter, 2003; Mitchell & Dalrymple-Alford, 2005; Moran & Dalrymple-Alford, 2003; Sziklas & Petrides, 1999), because radial maze tasks are acknowledged as a consistent test of the effects of hippocampal lesions on spatial learning and memory (Jarrard, 1993). Furthermore, only one study is known to have found no effect of AT lesions on a radial maze task (Beracochea, Jaffard, & Jarrard, 1989). This is thought to be because the task could be solved using egocentric (for example, head/body turn) strategies which are not impaired by AT lesions (Aggleton, Hunt, Nagle, & Neave, 1996; Sziklas & Petrides, 1999).

Mediodorsal Thalamic Nuclei

Contradictory evidence has been found in research on the effects of mediodorsal thalamic lesions in spatial learning and memory tasks. In part this may be attributable to lesion selectivity (Mitchell & Dalrymple-Alford, 2005). Traditionally, mediodorsal lesions have

targeted the entire mediodorsal region MD, but recent research at the University of Canterbury has incorporated only the medial and central nuclei in MT lesions, instead including the lateral and paramellar nuclei with the intralaminar nuclei in LT lesions (Gibb, 2005; Mitchell & Dalrymple-Alford, 2005). With these more selective lesions no MT impairment was found on working and reference memory in a radial maze (Mitchell & Dalrymple-Alford, 2005), or the acquisition of odour-place associations (Gibb, 2005).

It is believed that evidence for an involvement of MT lesions in spatial memory could be the result of damage to the adjoining AT nuclei, (Hunt & Aggleton, 1991, , 1998a). This is further supported by evidence that only small lesions producing damage to one of the AT divisions can yield a deficit (Aggleton, Hunt, Nagle, & Neave, 1996; Byatt & Dalrymple-Alford, 1996). No MT impairment is found on a forced alternation in a T-maze task (Hunt & Aggleton, 1991, , 1998a), unless rats are first trained to match-to-place then to not match-to-place (Hunt & Aggleton, 1998b). These results were not attributed to an impairment of spatial memory but rather a general disruption in other processes that impact on task performance, for example perseverative behaviour.

Table 1: Thalamic Lesion Studies with a Spatial Memory Component

Year	Authors	Lesion	Task	Training	Deficits
2006	**Mitchell & Dalrymple-Alford**	AT, LT	SWM - radial maze acquired response - cross maze	Post - op Pre-op	AT LT
2005	**Gibb**	AT, MT, LT	odour-place	Pre-op	AT, LT
2005	**Mitchell & Dalrymple-Alford**	AT, MT, LT	working and reference -radial maze go-no go - reward magnitude Temporal order for objects spontaneous obj recognition	Pre-op Post-op Post-op Post-op	AT, LT MT MT, LT -
2003	**Mair, Burk & Porter**	AT	spatial DNMS- radial maze	Pre-op	AT

2003	**Moran & Dalrymple-Alford**	AT	radial maze spontaneous obj recognition Elemental cue learning Configural cue learning	Post-op Post-op Post-op Post-op	AT - AT AT
2001	**Alexinsky**	AT, MT	reference and working memory in 3/8 baited radial maze New route- pre exposure vs none Contextual light change	Pre-op Pre or post-op Pre-op	AT, MT AT, MT AT
2001	**Wilton, Baird, Muir, Honey & Aggleton**	AD, LD	spatial forced alternation - T-maze Water maze beacon Object-place spontaneous obj recognition	Post-op Post-op Post-op Post-op	AD, LD AD, LD AD, LD -
1999	**Sziklas & Petrides**	AT	Object-place Object-response working memory -radial maze	Post-op Post-op Post-op	AT - AT
1999	**Warburton, Morgan, Baird, Muir & Aggleton**	AT	morris water maze spatial forced alternation - T-maze	Pre-op Pre-op	AT AT
1999	**Warburton & Aggleton**	AT, AT+MT	spontaneous obj recognition morris water maze spatial forced alternation - T-maze	Post-op Post-op Post-op	- AT, AT+MT AT, AT+MT
1998	**Hunt & Aggleton**	MT	working memory -radial maze reference memory - radial maze spatial forced alternation - T-maze	Post-op Post-op Post-op	MT MT -

			spontaneous obj recognition	Post-op	-
1998	**Stokes & Best**	MT	radial maze	Pre-op	MT
1997	**Warburton, Baird & Aggleton**	AT, AT + LD	spatial forced alternation - T-maze	Pre-op	AT, AT + LD
1996	**Aggleton, Hunt, Nagle, & Neave**	AM, AV+AD, AM+AV+AD	spatial forced alternation - T-maze cross maze radial maze	Post-op Post-op Post-op	AM+AV+AD AM+AV+AD AV+AD, AM+AV+AD
1996	**Byatt & Dalrymple-Alford**	AM, AV	working and reference -radial maze	Post-op	AM, AV
1995	**Aggleton, Neave, Nagle & Hunt**	AT	spatial forced alternation - T-maze spontaneous obj recognition	Post-op	AT -
1993	**Aggleton & Saghal**	AT	DNM to position	Pre-op	AT

Spatial Pattern Separation

Episodic memory is often impaired in diencephalic amnesia (Aggleton & Brown, 1999). An episodic memory is formed through the encoding of several attributes, which interact to form a particular memory (Kesner, 1998). Human research has shown that allocentric spatial memory is often more impaired in diencephalic amnesia (Holdstock et al., 2000). In animal research, because of evolution and the consequent difficulty generalizing across species, allocentric spatial memory is regarded as a appropriate test of episodic-like

memory because it combines not just the spatial cues themselves, but also their positions relevant to each other (Aggleton & Pearce, 2001). Part One of the present study compared the effects of AT and MT lesions on a delayed-match-to-sample spatial pattern separation task on a cheeseboard. This required rats to use distal spatial cues to remember a spatial location and then, after a delay, to distinguish between this location and a different location. As the distance between the locations was varied, this required distinguishing between locations with similar or dissimilar spatial cues. Therefore, this task targeted processes involving pattern separation as the rats had to separate incoming spatial information and preserve a unique memory representation of the spatial location they were required to remember. As well as a spatial attribute, this task is presumed to require a temporal (time) attribute as it is thought that to attain a unique memory representation the first spatial location must be encoded as a spatial event that is separate from the second spatial location, reducing spatial interference. Tasks that involve pattern separation are therefore important because they are thought to reflect non-human episodic memory not only because they have been shown to require an allocentric strategy (Gilbert, Kesner, & DeCoteau, 1998) but also because it is presumed that they also require a temporal attribute to separate one spatial event from another (Kesner, 1998).

The spatial pattern separation task is a test of the efficiency of pattern separation in working memory and recall (Gilbert, Kesner, & DeCoteau, 1998), not an acquisition task because of the extensive pretraining employed. However, it has been shown that AT lesions still produce an impairment even when rats are pretrained on the task (Warburton, Morgan, Baird, Muir, & Aggleton, 1999). The task itself was previously used to determine the role of the hippocampus (Gilbert, Kesner, & DeCoteau, 1998) and subregions of hippocampus (Gilbert, Kesner, & Lee, 2001) in spatial pattern separation, but currently there are no studies examining the effects of thalamic lesions on this task. Hippocampal lesions have been found to impair radial arm maze tasks that employ distal cues that are ambiguous, but not when the distal cues were unambiguous (McDonald & White, 1995a), suggesting that similarity between the distal cues can result in a decrease in spatial pattern separation. While there is no evidence as yet that the AT is involved in spatial pattern separation, there is evidence that both the hippocampus (Kesner, Gilbert, & Barua, 2002) and the AT (Wolff, Gibb, & Dalrymple-Alford, 2006) are involved in temporal pattern

separation. Furthermore, there is considerable evidence that the AT is involved in processing spatial memory. Because of the reciprocal connections between the AT and the hippocampus (Aggleton & Brown, 1999; Vann & Aggleton, 2004), AT lesions would be expected to also impair spatial pattern separation in a delayed match-to-sample task. Because of previous disparities regarding the effects of MT lesions on spatial memory and the issue of lesion specivity, it is also important to contrast the effect of the AT lesions with the effects of MT lesions on this task. MT lesions only include the medial and central nuclei in the present study. Recent research using similar lesions reported that the MT is not necessary for spatial memory processing (Gibb, 2005; Mitchell & Dalrymple-Alford, 2005), therefore an MT deficit would not be expected.

Spatial Working Memory

Due to an insufficient number of rats reaching the surgery criterion, training on the spatial pattern separation task was discontinued. Instead the rats were trained on a delayed match-to-sample task that assessed spatial working memory but not spatial pattern separation because only one spatial distance was used. Because the task still requires an allocentric spatial strategy, it is still a relevant test of episodic memory. There have been no previous studies using a cheeseboard to test a delayed match-to-sample task, but the basic paradigm has been use for similar tasks using different apparatus that have found an AT deficit of spatial memory. However, because do not match-to-sample is more comparable to the rats innate preference (Hunt & Aggleton, 1998b) and therefore easier for rats to acquire, it is more commonly used. AT lesions have been observed to produce impairment in forced alternation (do not match) tasks in a T-maze (Aggleton, Neave, Nagle, & Hunt, 1995; Warburton & Aggleton, 1999; Warburton, Baird, & Aggleton, 1997), and delayed do not match-to-position in an operant chamber (Aggleton & Saghal, 1993). Due to the consistent evidence of AT impairment in spatial memory tasks, an AT impairment is also expected on the delayed match-to-sample task.

The comparison of the effects of AT and MT lesions on delayed match-to-sample is particularly relevant. Hunt & Aggleton (1998b) found that MT rats were not impaired but significantly slower than sham rats to acquire a match-to-place rule in a T-Maze due to perseveration. However, in 40% of the rats, there was moderate damage to the AT. The

use of selective lesions in the current study is important for determining whether MT lesions do produce impairment or slower acquisition on match-to-sample tasks. Recent evidence suggests that the MT is not necessary for spatial memory processing (Gibb, 2005; Mitchell & Dalrymple-Alford, 2005) when lesions only include the medial and central nuclei, therefore an MT deficit is not expected in the present study.

Conditioned Place Preference

The classic version of the CPP task employs two arms (one food baited, one not) that are widely separated by two other arms (a 135° difference), therefore the distal cues are unambiguous. During training the rats are confined to the end of the arms, only during pre-exposure and testing are the rats allowed to move freely between the central platform and the two arms. Separate arms CPP is generally depicted as an instance of Pavlovian conditioning (Chai & White, 2004; White, 2004). Therefore, to acquire a CPP the distal spatial cues must be associated with the food reward (McDonald & White, 1993). The separate arms CPP version has been found to be impaired by amygdala lesions, but not hippocampal or fornix lesions when rats are given one pre-exposure and four training trials (the level at which control rats normally acquire a CPP in other studies) (McDonald & White, 1993; White & McDonald, 1993). The current presupposition is that separate arms CPP is acquired through both Pavlovian conditioning and spatial discrimination, but that the approach associated (Pavlovian) conditioning contribution is stronger. Part One of the present study utilizes the classic version of CPP. It is an acquisition task therefore all training occurs after surgery. Because of the connections between the amygdala and the MT is it presumed that the MT is part of an amygdala based memory system, and therefore MT lesions will mimic the effects of amygdala lesions (Gaffan & Murray, 1990; Mitchell & Dalrymple-Alford, 2005) and produce a substantive impairment on CPP. However, in a study investigating the effects of MT lesions on this version of CPP Hunt & Aggleton (1998b) found no impairment following MT lesions. Conversely, a study using a compartmented box instead of a radial maze did find an impairment following MT lesions (McAlonan, Robbins, & Everitt, 1993). Lesion specivity is particularly important for this task therefore to determine whether more selective MT lesions also replicate the results found by Hunt & Aggleton (1998b). Traditionally, separate arms CPP involves an affect (reward value) attribute because it requires the rats to acquire a preference for one arm

over another based on an association between a food reward and a spatial location. However, CPP can (depending on the specific procedure used) also invoke pattern separation and therefore utilize the spatial attribute. The hippocampus is thought to process spatial information but not affect or reward value information (Kesner, 1998). By varying the spatial locations used (the difference between the radial maze arms) the hippocampal system (including the AT) and spatial pattern separation can be manipulated. However, the separate arms CPP task employs distal cues that are distinct from each other; therefore a spatial attribute is not required to acquire separate arms CPP. Neither the hippocampus nor the AT (because of the neural connections mentioned previously) is thought to be required for the acquisition of separate arms CPP.

A modified version of the CPP task in the eight-arm radial maze employs two adjacent arms (one food baited, one not) with no arms in between (a 45° difference), therefore the distal cues are particularly ambiguous. Because of the similarity of the distal spatial cues, it is described as a spatial learning version of CPP rather than Pavlovian conditioning (as in the separate arms CPP). Due to the ambiguity of the distal spatial cues, to acquire an adjacent arms CPP the rats must separate the baited arm location from the non-baited arm location using spatial distal cues (Chai & White, 2004). In the separate arms CPP, the cues are unambiguous, so the rats can acquire a CPP by forming an approach association between just the food location (baited arm) and the distal cues present, and not forming an approach association with the non-food location (non-baited arm). Therefore when tested, they approach the distal cues that have become associated with the baited arm, even though the arm itself is no longer baited. However, in the adjacent arms CPP, because of the distal cue ambiguity this behaviour does not produce acquisition of adjacent arms CPP, although the learning itself could still occur (White, 2004). McDonald & White (1995a) found that control rats could not acquire an adjacent arms CPP with only one pre-exposure and up to eight training trials (the level at which control rats normally acquire a separate arms CPP in other studies) when rats were confined in the arms during the training trials. In the standard separate or adjacent arms CPP (and in all the procedures in the present study), the rats are confined to the end of the arm during the training trials (passive place learning). However, in a modified version of the adjacent arms CPP task, rats were

allowed to move between the baited and non-baited arms (active place learning). When allowed this movement, control rats acquired an adjacent arms CPP with one pre-exposure and four training trials (McDonald & White, 1995a; White & Ouellet, 1997). This evidence suggested that movement is required for the rats to form a cognitive map of the maze and separate the distal spatial cues present in order to acquire adjacent arms CPP. The lack of movement might be the reason that control rats fail to acquire an adjacent arms CPP when they are confined to the end of the arm during trials in passive place learning. However, if the number of pre-exposures in the passive place learning adjacent arms CPP is increased from one pre-exposure to three or more pre-exposures, control rats can acquire an adjacent arms CPP (Chai & White, 2004). This suggests that unreinforced exploration of the two arms during three pre-exposure sessions provides enough spatial information for the acquisition of adjacent arms CPP even though the rats are confined in the arms during the training trials. Pre-exposure therefore is posited to allow pure spatial learning. The adjacent arms CPP with three pre-exposures and four training trials (the level at which control rats normally acquire adjacent arms CPP in previous studies) has been found to be impaired by hippocampal and fornix lesions. However, in contrast with separate arms CPP amygdala lesions do not cause impairment (Chai & White, 2004). Presently, there has not been any research conducted into the effects of thalamic lesions on adjacent arms CPP. The current presupposition is that adjacent arms CPP is acquired through both approach associated conditioning and spatial discrimination, but that the spatial learning contribution is stronger. Therefore, due to the considerable evidence of AT impairment in tasks that involve a spatial component and the reciprocal connections between the AT and the hippocampus (Aggleton & Brown, 1999; Vann & Aggleton, 2004), AT lesions would also be expected to produce impairment.

There is evidence that the two systems that underlie the two different strategies operate in competition. The separate arms CPP in addition to being impaired by amygdala lesions (McDonald & White, 1995a; White & McDonald, 1993), has been found to be facilitated (reduce the number of pre-exposure sessions required for acquisition) by fornix lesions (McDonald & White, 1995b; White & McDonald, 1993). Chai & White (2004) postulate this is because the acquisition of pure spatial information mediated by the fimbria fornix during pre-exposure interferes with the amygdala system mediated conditioned approach

response and produces a latent inhibition effect. When the fimbria fornix is damaged, this pure spatial learning does not occur and no latent inhibition effect is produced. Thus pre-exposure in the separate arms CPP inhibits acquisition. This provides an explanation for the ability of control rats to acquire separate arms CPP when given no pre-exposure and two training trials, but not if the rats are given one pre-exposure and two training trials (White & Wallet, 2000). When given one pre-exposure and four training trials, controls rats can again acquire a separate arms CPP (McDonald & White, 1993). This suggests that the latent inhibition effect produced by pre-exposure in separate arms CPP can be overcome by greater opportunity to acquire an approach association with the baited arm (i.e. more training trials). Whether more pre-exposure would again produce interference and prevent control rats from acquiring a separate arms CPP has not yet been investigated. Therefore, the effect of three pre-exposures on separate arms CPP is also investigated in the present study. Adjacent arms CPP in addition to being impaired by hippocampal lesions, has also been found to be impaired by fimbria fornix lesions made prior to pre-exposure but not after pre-exposure. However, amygdala lesions facilitated adjacent arms CPP (Chai & White, 2004). Chai & White (2004) postulate that this is because the acquisition of pure spatial information mediated by the fimbria fornix during pre-exposure improves information about the food location acquired by the hippocampus. Instead of producing a latent inhibition effect (as in separate arms CPP), pre-exposure in adjacent arms CPP produces a latent learning effect. Therefore to investigate the full extent of AT and MT involvement in CPP, separate arms and adjacent arms CPP tasks should be conducted following both AT and MT lesions (outside the timeframe of the present study). It is possible that the lack of impairment following MT lesions seen in the separate arms CPP experiment by Hunt & Aggleton (1998b) was due to facilitation as 40% of the rats had moderate AT damage. Because hippocampal lesions and fimbria fornix lesions produce different effects on both adjacent arms and separate arms CPP it is not currently clear whether AT lesions would mimic hippocampal or fimbria fornix lesions as the AT has neural connections to both.

Control rats failed to acquire a separate arms CPP when given one pre-exposure and four training trials in Part One of the current study in contrast to previous studies. Therefore,

Part Two of the current study investigated this by varying the procedure of both separate arms and adjacent arms CPP to determine what effect this had on the ability of control rats to acquire a CPP. In Experiment One, rats in the separate arms one pre-exposure (S1) and adjacent arms three pre-exposures (A3) conditions were expected to acquire a CPP as had been found in previous studies (mentioned above). Rats in the adjacent arms one pre-exposure (A1) condition were not expected to acquire a CPP because previous studies reported that control rats could not acquire an adjacent arm CPP unless they were given three or more pre-exposures (mentioned above). It was expected that rats in the separate arms three pre-exposure (S3) condition would not be able to acquire a separate arms CPP because the additional pre-exposures would produce a latent inhibition effect due to the additional opportunity to acquire pure spatial information during pre-exposure. Each additional experiment was conducted because the previous experiment did not produce any evidence of CPP acquisition in any of the conditions examined. Although the procedure was varied in each of the four experiments the expectation for each condition remained the same (if the conditioned was examined).

Experiment One: Restricted View used wooden blocks to restrict the distal cues available to the rat from the arm. Panels were attached to the wooden blocks so that they sat just inside the sides of the arm itself parallel to each other, therefore ensuring that the rat could only look out the end of the arm at the specific distal cues for that location. No previous CPP study has used panels to restrict the distal spatial cue to this extent. This restriction was intended to assist in the acquisition of CPP by reducing the ambiguity of the distal spatial cues. Due to the restricted nature of the view from the end of the arm, the distal cues should have been from a smaller area and therefore more distinct from each other than if they were less restricted. The four conditions used varied in the amount of pre-exposure the rat was given and the distance between the arms (separate arms one pre-exposure (S1), separate arms three pre-exposures (S3), adjacent arms one pre-exposure (A1) and adjacent arms three pre-exposures (A3)) and therefore offered different opportunities for the rats to learn the information required as well as differences in cue ambiguity.

Experiment Two: 180° View differs from the previous CPP experiment because of the amount of distal cues available to make a CPP association. Instead of having panels that sat just inside the sides of the arm itself parallel to each other, a single panel was attached perpendicular to the rest of the block. This is the standard CPP apparatus used in previous CPP studies. The single panel allowed the rats a 180° view from the end of the arm, therefore allowing a larger area for the distal spatial cues to come from than in the first experiment. The four conditions which were identical to the conditions used in the previous experiment (S1, S3, A1 and A3).

Experiment Three: Males 180° View differs from the previous experiment in the sex of the rats used. Experiment One and Two used female rats. In all of the previous studies examining CPP, male rats were used. Therefore to ensure that the failure of rats to acquire a CPP in Experiment One and Two was not due to a sex difference, Experiment Three used male rats. The same apparatus and procedure as Experiment Two were used, but only the S1 and A1 conditions were examined (due to a lack of available male rats).

Experiment Four: Female 8 Trials differ from Experiments One – Three in the number of training trials used. The training trials were increased from four to eight training trials. The same apparatus as Experiment Two and Three was used, but S1 was the only condition examined (due to a lack of available rats). This was the only experiment to successfully acquire a CPP.

Table 2: Conditioned Place Preference Studies

Year	Authors	Lesion	Task	Pre-exposure	Training	CPP Acquired
2004	**Chai & White**	None	adj	0,1,2,3,4,6	4	3PE + CPP Acq
		None	adj	3dpe	4	No CPP Acq
		HP Pre-op	adj	3	4	No CPP Acq
		HP Post-op	adj	3	4	No CPP Acq

		FF Pre-op	adj	3	4	No CPP Acq
		Hp Post-op	adj	3	4	CPP Acq
		CN Pre-op	adj	3	4	CPP Acq
		CN Post-op	adj	3	4	CPP Acq
		Amg Pre-op	adj	1	4	CPP Acq
		CN Pre-op	adj	1	4	No CPP Acq
		Amg Pre-op	adj	1dpe	4	No CPP Acq
		CN Pre-op	adj	1dpe	4	No CPP Acq
		Amg Pre-op	adj	-	4	No CPP Acq
		CN Pre-op	adj	-	4	No CPP Acq
		Amg + FFPre-op	adj	1	4	No CPP Acq
2000	**White & Wallet**	None	sep	1	2	No CPP Acq
		None	sep	-	2	CPP Acq
		HP Pre-op	sep	1	2	No CPP Acq
		CN	sep	1	2	No CPP Acq
		None	sep	1	2	No CPP Acq
		FF Pre-op	sep	1	2	CPP Acq
		HP Pre-op	sep	1	2	No CPP Acq
		CN	sep	1	2	No CPP Acq
		HP		-	2	CPP Acq
		HP Pre-op	sep	1	2	No CPP Acq
		CN	sep	1	2	No CPP Acq
1998	**Hunt & Aggleton**	MT Pre-op	sep	1	4	Acq CPP
		CN Pre-op	sep	1	4	Acq CPP
1995	**McDonald & White**	FF Pre-op	adj	1	2	No CPP Acq
		CN Pre-op	adj	1	2	No CPP Acq

		FF Pre-op	adj	1	4	No CPP Acq
		CN Pre-op	adj	1	4	No CPP Acq
		FF Pre-op	adj	1	8	No CPP Acq
		CN Pre-op	adj	1	8	CPP Acq
1993	**McDonald and White**	FF Pre-op	sep	1	4	CPP Acq
		CN Pre-op	sep	1	4	CPP Acq
		CN	sep	1	4	CPP Acq
		Amg Pre-op	sep	1	4	No CPP Acq
		CN Pre-op	sep	1	4	CPP Acq
		CN	sep	1	4	CPP Acq
		DS Pre-op	sep	1	4	CPP Acq
		CN Pre-op	sep	1	4	CPP Acq
		CN	sep	1	4	CPP Acq

Abbreviations: adj=adjacent arms CPP, Acq=acquisition, Amg = amygdala, CN=control rat, CPP=conditioned place preference, dpe= pre-exposure in a different room, DS=dorsal striatum, FF=fimbria fornix, HP=hippocampus, PE=pre-exposure.

Part One

Part One examined the role of the AT and MT in spatial pattern separation, spatial working memory and conditioned place preference.

Method

Subjects

Fifteen naïve female PVGc Hooded rats were used. These rats were aged thirteen to fourteen months and weighed 180g to 220g when the experiment commenced. The rats were housed in groups of three or four rats per cage. The colony room was maintained on a twelve hour reversed light cycle, the lights were off between 8am and 8pm and all testing occurred during this period. Water was freely available throughout testing, but food

was restricted to maintain 80% to 85% of their free feeding weight throughout the experiment other than post surgery recovery.

Apparatus

Cheeseboard

A cheeseboard based on that described by Gilbert, Kesner and DeCoteau (1998) was used. The cheeseboard consisted of a white painted circular piece of wood that was 119cm in diameter and 3.5cm in thickness, with 177 food wells (2.5cm in diameter and 1.5cm in depth) drilled in parallel rows and columns 2cm apart (*See Figure 3:* Cheeseboard apparatus and start box. *on page 35*). A black start box (24cm in length, 15cm in width, and 17cm in height) was placed on the cheeseboard with its rear wall adjacent and perpendicular to the perimeter of the board. The start box contained a removable guillotine door that could be raised and lowered by the experimenter and a hinged lid that allowed the experimenter to transfer rats into and out of the box. The cheeseboard was placed upon a table that stood 65 cm from the floor, in a well lit room (4.6m x 4.1m) with no windows. A beige curtain on one side separated the testing area from the rest of the room. The testing area (3m x 4.1m) of the room contained an additional table, a chair, sixteen pictures of various shapes, sizes and orientations, nine of these were on the walls of the room visible from the cheeseboard and seven of these were attached to the separation curtain. A camera was mounted onto the ceiling directly above the cheeseboard for the purpose of recording behavioural data. During all testing the door was shut and the experimenter remained inside the room.

Figure 3: Cheeseboard apparatus and start box.

Radial-Arm Maze

An eight-arm radial maze was used for the conditioned place preference (CPP) task. The central platform of the radial maze was 35 cm in diameter, octagonal in shape, made of wood and painted black. Eight aluminum arms (65 cm in length, 9 cm in width and 3 cm high) were attached to the central platform. Clear Perspex rectangle enclosures (45cm long, 21cm high and 8.5cm wide) which allowed the rat to see the distal cues in the room but prevented the rat from escaping were placed in the end of the arm that faced away from the platform (*Figure 4 on page 36*). Each rat was assigned two arms (baited and non-baited) which were always separated by two other arms. During the training trials one of the Perspex enclosures was on each assigned arm. The maze was situated in a second well lit room (3.2m x 3.3m) with no windows. Twelve objects were on the walls of varying size, shape and colour to provide spatial cues additional to those provided by the structure of the room itself. A camera was mounted onto the ceiling directly above the radial maze

for the purpose of recording behavioural data. During all testing the door was shut and the experimenter remained outside the room.

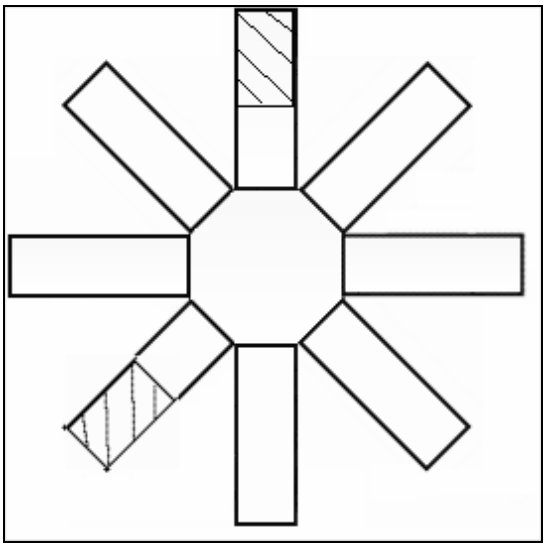

Figure 4: Radial Arm Maze with Perspex enclosures.

General Surgical Procedures

The surgical procedure and AT and MT lesion co-ordinates are based previous exploration studies in the animal lab at the University of Canterbury (Mitchell & Dalrymple-Alford, 2005) and previously described in (Gibb, 2005). 15 rats (AT (n=5), MT (n=5) and sham (n=5)) were anaesthetized with sodium pentobarbitone (50 mg/ml, at a dose of 1.40 ml/kg IP), 25 minutes after atropine (0.13 mg/ml at a dose of 1.5 ml/kg IP). This was supplemented by mepivicaine (2.0 mg/ml at a dose of 2.0 ml/kg) and ketofen (1.0 mg/ml at a dose of 0.50 mg/kg). The incisor bar of the stereotaxic apparatus (David Kopf Instruments, Tujunga) was set at -7.5mm beneath the interaural line to avoid or minimize damage to the fimbria fornix. Lesions were created by the infusion of N-methyl-D-aspartic acid (NMDA: 0.12M dissolved in pH7.2 phosphate buffer) using a motorized infusion

pump (Stoelting) and a 1µl Hamilton syringe. The infusion needle was lowered slowly to a given site, allowed to rest at the site for 30 to 60 seconds prior to infusion of NMDA, left in situ for 3 minutes following infusion and then slowly retracted.

Anterior Thalamic (AT) Lesions

The anterior medial nucleus (AM) and the anterior ventral nucleus (AV) were lesioned at two bilateral sites. The anterior-posterior (AP) coordinates used relative Bregma were based on each rats individual Bregma and Lambda (B-L) distance. The AV Bregma-Lambda distances and coordinates were 0.60-0.61 BL = -0.250cm; 0.64-0.66 BL = -0.270cm; and 0.67-0.68 BL =-0.280cm for the lesions, laterality = 0.148 cm medial lateral distance to the midline and ventrality was -0.555 cm dorsal ventral distance from dura. The volume and rate of NMDA infusion for AV lesions was 0.10µ at 0.03 µl per minute. The AM Bregma-Lambda distances and coordinates were 0.60-0.61 BL = -0.240cm; 0.64-0.66 BL = -0.260cm; and 0.67-0.68 BL =-0.270cm for the lesions, laterality = 0.123 cm medial lateral distance to the midline and ventrality was -0.580 cm dorsal ventral distance from dura. The volume and rate of NMDA infusion for AM lesions was 0.09µ at 0.03 µl per minute. Table 3 shows the coordinates and infusion details (*page 39*). The lesions were always performed in the following order: AV left and right, then AM left and right to improve lesion accuracy.

Medial Thalamic (MT) Lesions

The AP coordinates for the anterior and posterior medial lateral lesions used relative Bregma were based on each rats individual Bregma and Lambda (B-L) distance. The anterior BL distances and coordinates were 0.64-0.66 BL = -0.370cm; and 0.67-0.68 BL =-0.380cm for the lesions and ventrality was -0.560 cm dorsal ventral distance from dura. The volume and rate of NMDA infusion for anterior lesions was 0.16µ at 0.04 µl per minute. The posterior BL distances and coordinates were 0.64-0.66 BL = -0.410cm; and

0.67-0.68 BL =-0.420cm for the lesions and ventrality was -0.570 cm dorsal ventral distance from dura. The volume and rate of NMDA infusion for posterior lesions was 0.18µ at 0.04 µl per minute. Table 3 shows the coordinates and infusion details (*page 39*). The anterior MT lesion was always performed first, followed by the posterior lesion to improve lesion accuracy.

Sham Lesions

The control group also received sham lesions only. In approximately half the cases (*n*=3) the procedure was the same as they lesions surgeries but once the needle was lowered to 3mm above the lesion site no infusion occurred. In the remaining cases (*n*=2) the needle was not lowered into the brain and surgery was terminated after making the hole in the skull.

Table 3: Lesion co-ordinates and parameters for individual Bregma-Lambda distances with AP co-ordinates for AT and MT lesions.

	AT		MT	
B-L distance for AP co-ordinates (cm)	Anterior(AM)	Posterior(AV)	Anterior	Posterior
0.60-0.61	-0.24	-0.25		
0.64-0.66	-0.26	-0.27	-0.37	-0.41
0.67-0.68	-0.27	-0.28	-0.38	-0.42
ML	±0.123	±0.148	0.0	0.0
DV	-0.58	-0.555	-0.56	-0.57
Volume (µl)	0.09	0.10	0.16	0.18
Rate (µl/min)	0.03	0.03	0.04	0.04

Abbreviations: AT=anterior thalamic nuclei; MT=mediodorsal thalamic nuclei AM=anteromedial; AV=anteroventral; B-L=Bregma-Lambda; ML=medial-lateral distance from midline; DV=dorsal-ventral distance from midline.

Experiment One: Spatial Pattern Separation Delayed Match-to-Sample Task

Procedure

Familiarization and Behavioural Shaping

Rats were placed on the cheeseboard in their home cage groups and allowed to freely explore the apparatus for ten minutes on the first two days. Twenty Froot Loop were randomly scattered on the cheeseboard. On the third and fourth days of familiarization individual rats were placed in the start box without the guillotine door and allowed to freely explore the cheeseboard for ten minutes (five Froot Loops on the board). Rats were further shaped to retrieve the Froot Loops placed on the centre row of the board and then adapted to the guillotine door.

Training

Rats were shaped to obtain a ½ Froot Loop in centre well (of centre row) under a small cylindrical object (8cm in height, 4cm in diameter). Once the rats consistently dislodged the object covering the centre well it was randomly placed along the centre row of the cheeseboard until the rats consistently dislodged the object from any well along the centre row.

Spatial Pattern Separation Working Memory (Pre-operative Only)

Note: As animal failed to acquire this task in twelve weeks, a standard match-to-sample task (i.e. no pattern separation) was subsequently used pre-operatively and tested post-operatively.

Initial Training

The sample phase (*see Figure 5 on page 41*) consisted of one of the cylindrical objects either in position one or two (F or K) covering a food well baited with a Froot Loop

quarter. The choice phase (*see Figure 6 on page 42*) consisted of two identical cylindrical objects placed at position one (F) and position two (K) respectively. Rats were required to match to sample. The position of the sample phase object was determined randomly using chance stimulus sequences for discrimination tasks (Fellows, 1967). Each rat completed 16 trials per day across five days and was required to reach a 75% criterion for both positions before the second part began.

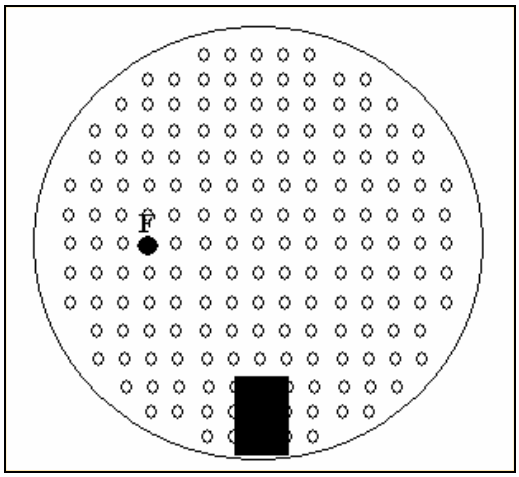

Figure 5: Cheeseboard sample phase example.

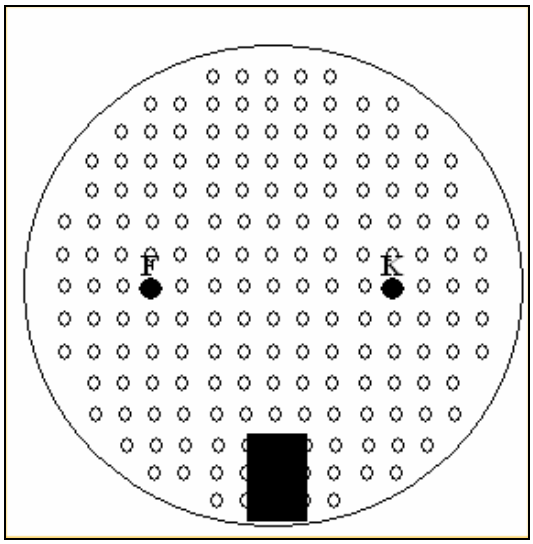

Figure 6: Cheeseboard choice phase example.

In the second part of the pre-operative spatial pattern separation task, five spatial distances were randomly used to separate the correct choice from the incorrect choice on the choice phase (see Gilbert, Kesner, & DeCoteau, 1998). The sample phase is the same as in the previous part, but the location of position one and two is determined by the distance used in the trial. The five distances were 15, 37.5, 60, 82.5, and 105cm (e.g. G/I, F/K, D/L, B/M and A/O respectively; *see Figure 7 on page 43*). Each of these distances remained constant; however the spatial distance did not always correspond to the same well on the centre row. A random determination of the position was made based on the correct object being closer to or further away from the start box, and the correct object being on the left versus on the right was counterbalanced. Each rat completed 16 trials per day across all five spatial distances, five days per week prior to surgery.

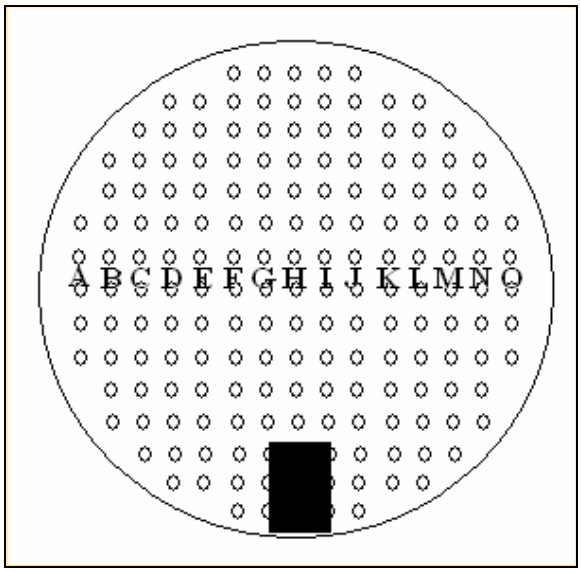

Figure 7: Cheeseboard with start box and labeled centre row.

Experiment One: Match-to-Sample Spatial Working Memory on the Cheeseboard (one distance only)

Pre-operative Training

Rats were trained to match-to-sample using the same objects described above. Only the 60 cm (D/L; *see Figure 7 on page 43*) distance was used. The sample phase consisted of one of the objects either in position one (D) or two (L) covering a baited food well. The choice phase consisted of two new identical objects not used in the sample phase at position one (D) and position two (L) respectively. The baited position was determined randomly using chance stimulus sequences for discrimination tasks (see Fellows (1967)). Each rat completed 12 trials per day and was required to reach a 75% criterion over three days for both positions prior to surgery. 21 rats reached the 75% criterion after four weeks, 7 rats were discontinued because of the limited time frame allowed.

Testing

Following surgery and recovery rats were re-tested for fifteen days. The testing procedure was identical to the pre-operative procedure described above.

Experiment Three: AT/MT Lesion Conditioned Place Preference (CPP) Task

Pre-exposure

One week after the end of post-operative testing of the simpler match-to-sample task, all rats received one pre-exposure session where they were allowed to freely explore the central platform and their assigned unique pair of arms for ten minutes; all other arms were blocked off. Froot Loops were not placed on the maze for this session.

Training

Rats received four training trials; each training trial required two days. Each rat was assigned a unique pair of arms. One of its assigned arms was baited with food and the other was non-baited. Half of the rats in each group were confined in their food arm on day one of the trial for thirty minutes and in their non-baited arms on day two of the trial for thirty minutes. The opposite order was used for the other half of the rats in each group. When in the baited arm, forty Froot Loops were placed at the end of the arm to ensure the rat ate while facing the cues visible from the end of the arm. The entire maze was rotated one arm position to the left at the start of each day to prevent the rats using intramaze cues to acquire a CPP.

Testing

Rats received the test trial twenty-four hours after the last training trial. Testing was identical to pre-exposure except that the rats were on the maze for twenty minutes. The amount of time each rat spent in their baited and non-baited arms was timed using a stopwatch. A rat was considered to be in the arm or to have left the arm once its front feet crossed the threshold between the centre platform and the arm.

Results Part One

Histological Findings

There was bilateral damage to anterior ventral (AV) and anterior medial (AM) thalamic nuclei in all five of the rats in the AT lesion group. The MT lesions showed damage to both the anterior and posterior MT. The slides below are a representative example of the damage cause by the NMDA lesions.

Figure 8 is representative of the anterior bilateral AV and AM NMDA lesions in the AT lesion Group.

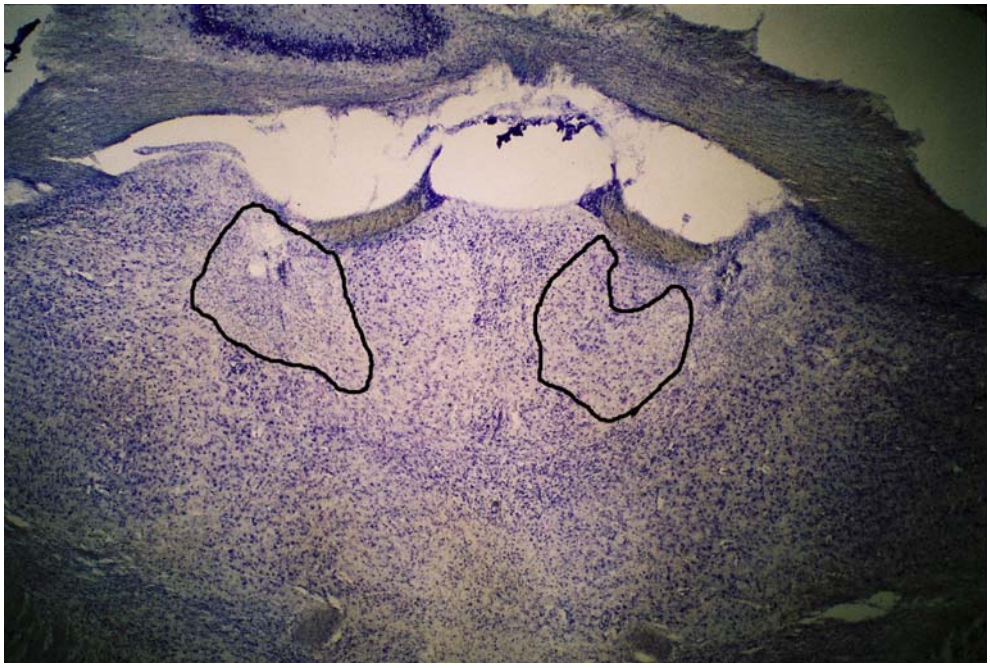

Figure 9 is representative of the posterior bilateral AV and AM NMDA lesions in the AT lesion Group.

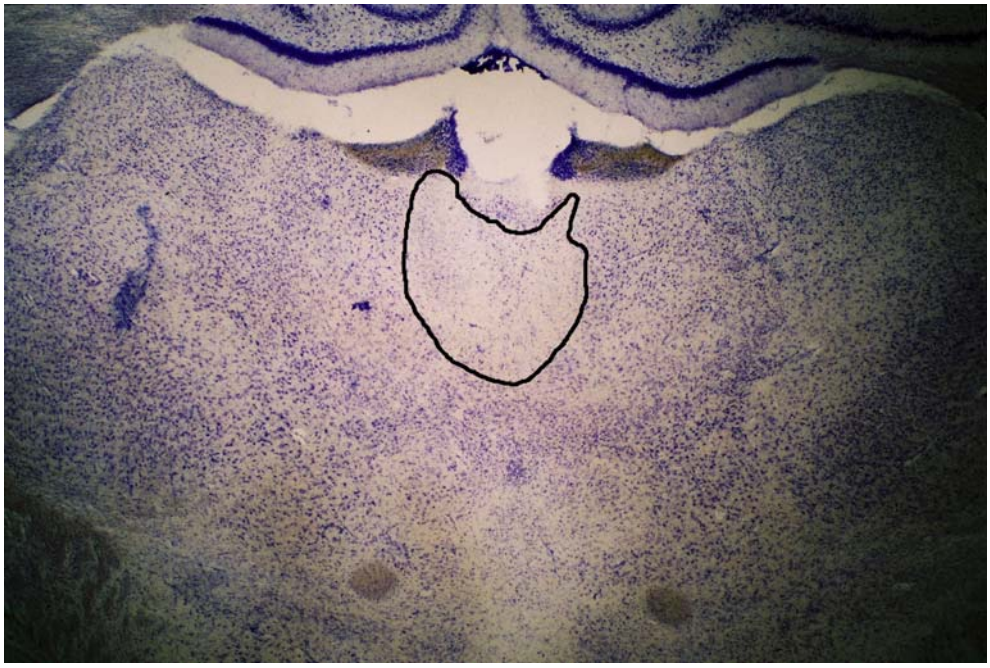

Figure 10 is representative of the anterior MT NMDA lesions in the MT lesion Group.

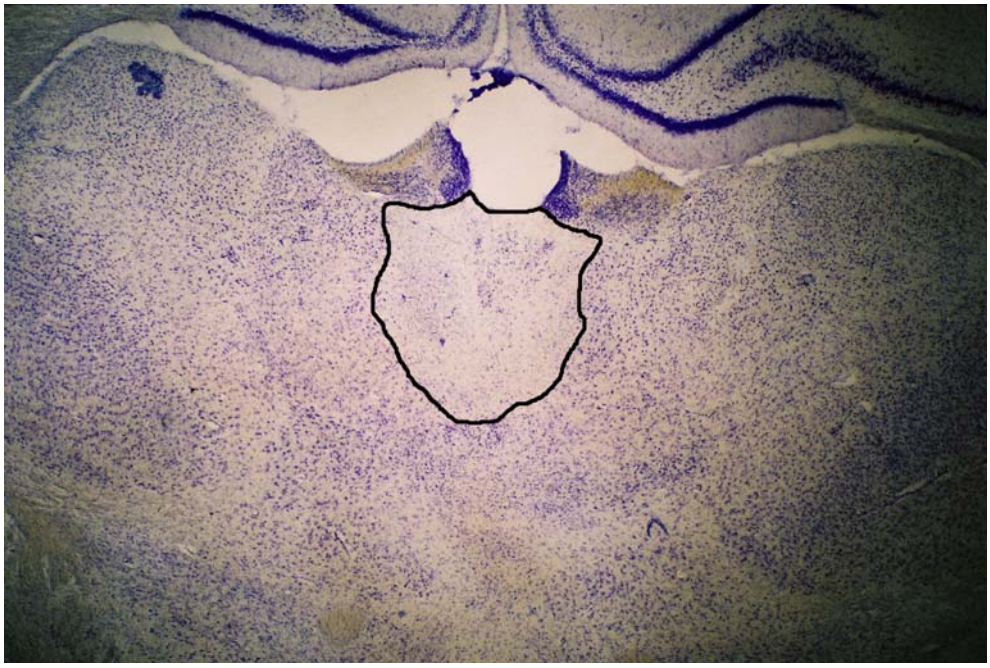

Figure 11 is representative of the posterior MT NMDA lesions in the MT lesion Group.

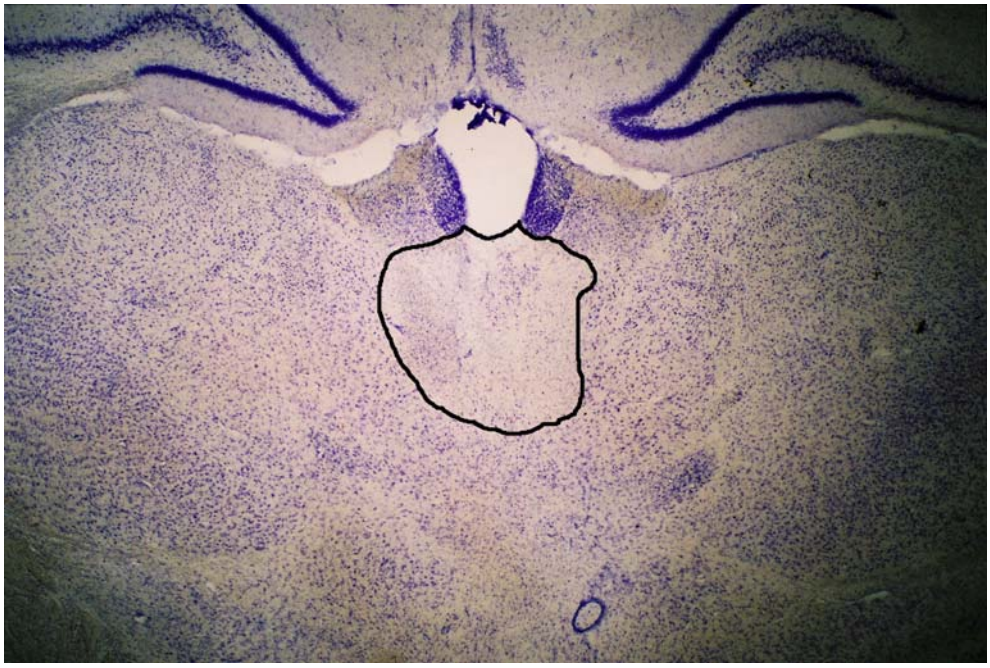

Figure 12 is representative of the posterior MT NMDA lesions in the MT lesion Group

Experiment One: Spatial Working Memory Task (Two consistent places only)

The spatial working memory task required rats to choose between two objects based on a previous sample phase (match to sample). This required rats to hold in working memory spatial information about the position of the object in the sample phase relative to other spatial cues in the room and determine which of the objects presented in the choice phase matched those spatial cues.

Figure 13 shows the initial effects of the lesions in terms of the number of correct choices made the last three days pre-surgery versus the first three days post-surgery for the anterior thalamus (AT), mediodorsal thalamus (MT) and control (CN) lesions. Only the rats with AT lesions showed a post-operative deficit. A 3(Group (AT,MT,CN) x 2(Surgery (PreS, PostS) repeated measures analysis of variance (ANOVA) on these data confirmed a significant main effect of Group ($F(2,11)=24.348$, $p<0.0001$), a main effect of Surgery ($F(1,11)= 39.322$, $p<0.0001$) but also a significant interaction of Group x Surgery ($F(2,11)=15.25$, $p<0.0001$). To further illuminate the effects of surgery on the correct choices made within the three conditions, post hoc comparisons of the correct choices made before and after surgery were examined for each group: AT ($p<0.0001$), MT ($p>0.10$), and CN ($p>0.10$).

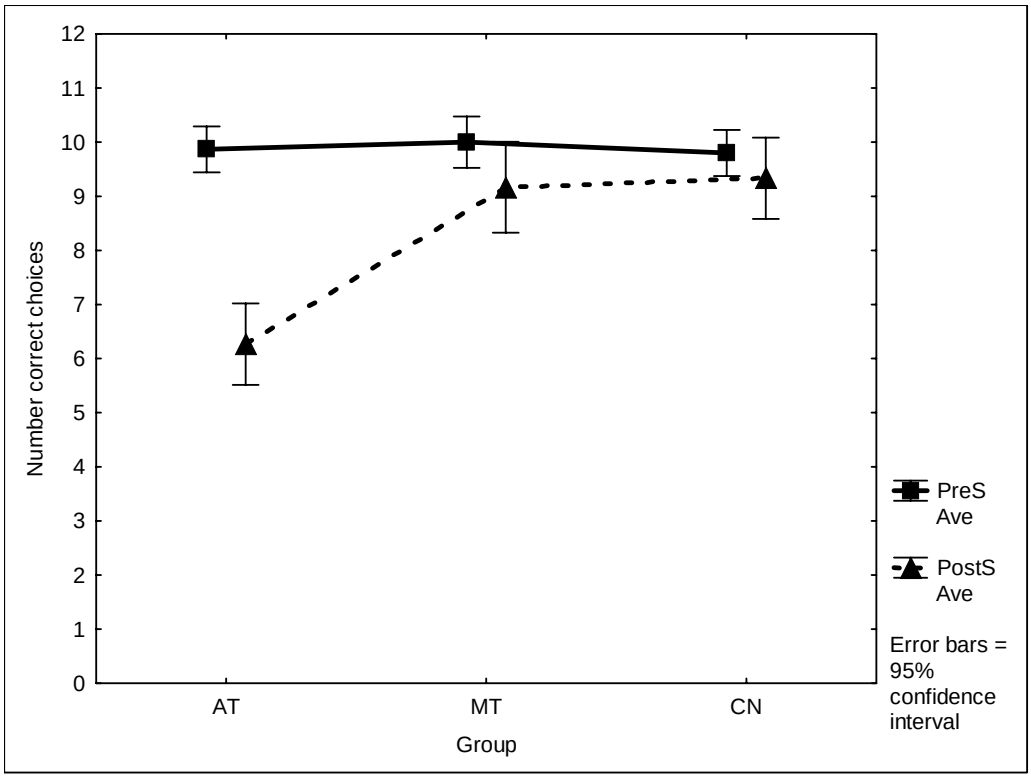

Figure 13: Experiment One: Spatial Working Memory correct choices for the last three days pre-surgery versus first three days post surgery for AT, MT and CN rats (12 trials per day)

Figure 14 shows the number of correct choices made post-surgery for the AT, MT and CN lesions groups averaged across the 15 days of post-operative testing as a function of three day blocks. The rats with AT lesions made fewer correct choices following surgery than the MT lesion and control groups, which did not differ. A 3(Group (AT,MT,CN) x 5(Block 1-5) repeated measures analysis of variance (ANOVA) confirmed a significant main effect of Group ($F(2,11)=12.056$, $p<0.001$), but not Block ($F(4,44)= 1.660$, $p>0.10$) nor interaction Group x Block ($F(8,44)=1.060$, $p>0.10$). Post hoc comparison showed a significant difference between AT versus MT ($p<0.001$) and AT versus CN ($p<0.001$), but not MT versus CN ($p>0.10$).

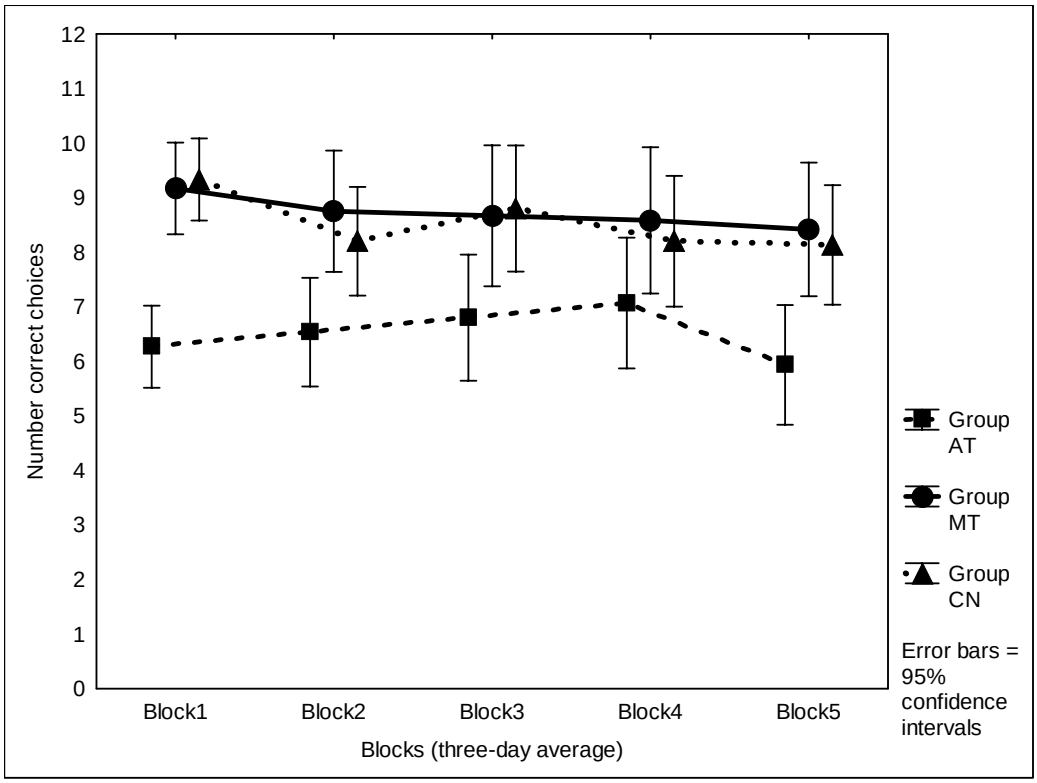

Figure 14: Experiment One: Spatial Working Memory correct choices for 15 days post-surgery averaged across three-day blocks for AT, MT and CN rats

Experiment Two: Conditioned Place Preference (CPP)

The conditioned place preference (CPP) experiment trained rats to acquire an association between the food in a baited arm versus a non-baited arm of an eight arm radial maze and the distal cues that were specific to that arm. The acquisition of this association was assessed by testing a preference for the baited versus the non-baited arms in a choice test when there was no longer any food in the arm. Rats were expected to spend significantly greater amount of time in the baited arm than in the non-baited arm.

Figure 15 shows the time spent in the baited versus non-baited arms for AT, MT and CN rats. There was a clearer mean difference in the AT lesion group but large variances meant that even this group showed no clear preference.

A 3(Group (AT, MT, CN)) x 2(Arms (baited, non-baited)) repeated measures analysis of variance (ANOVA) confirmed there was no significant main effect of Group ($F(2,8)=0.885$, $p>0.10$), arms ($F(1,8)=0.386$, $p>0.10$) nor interaction Group x Arms ($F(2,8)=0.372$, $p>0.10$). Post-hoc comparisons confirmed that there was no significant difference in the time spent in the baited arm versus the non-baited arm for AT ($p>0.10$), MT ($p>0.10$), or control ($p>0.10$).

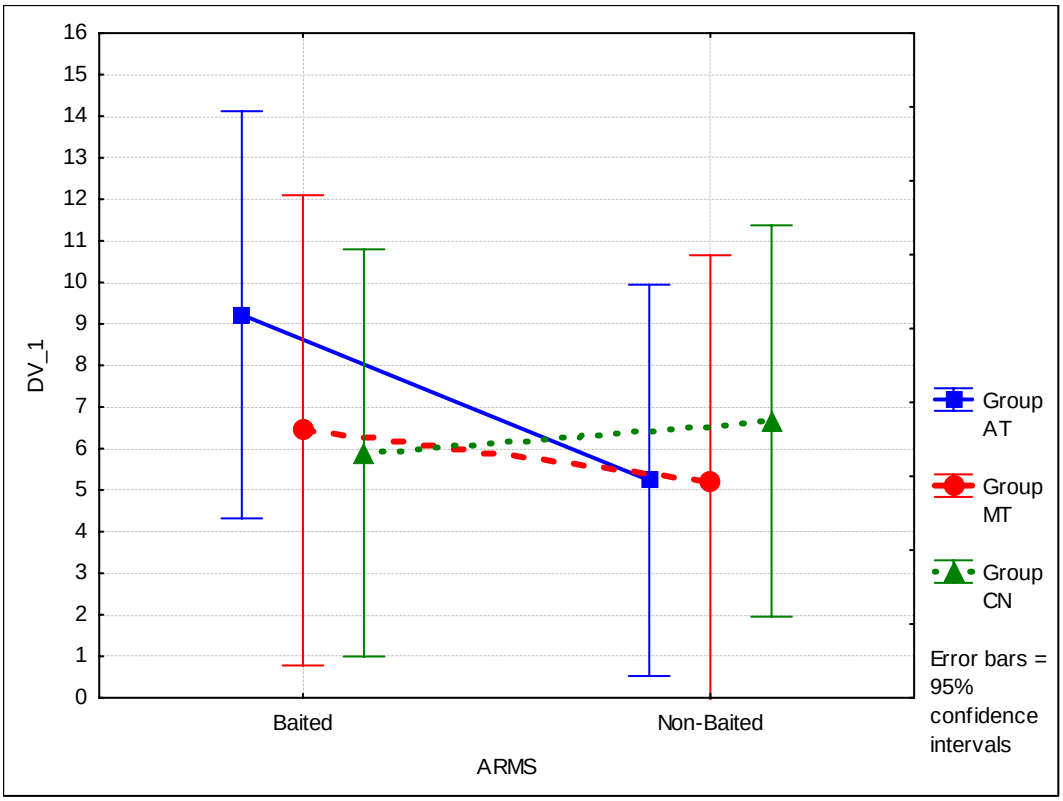

Figure 15: Experiment Two: Conditioned Place Preference CPP time (mins) spent in the baited and non-baited arms for AT, MT and CN rats

Part Two

Part Two of the current study examined the effects of different procedural designs on the ability of control rats to acquire a CPP.

Method

Experiment One: Restricted View

Subjects

Thirty-six naïve female PVGc Hooded rats were used. These rats were aged twelve to thirteen months and weighed 180g to 220g when the experiment commenced. The rats were housed in groups of three or four rats per cage. The colony room was maintained on a twelve hour reversed light cycle, the lights were off between 8am and 8pm and all testing occurred during this period. Water was freely available throughout testing, but food was restricted to maintain 80% to 85% of their free feeding weight throughout the experiment other than post surgery recovery.

The animals were divided into four groups of nine rats. One group was assigned two separate arms (the arms were separated by two arms in between) and one day of pre-exposure (S1). Another group was also assigned two separate arms but with three days of pre-exposure (S3). The remaining two groups were assigned adjacent arms (there were no arms between the two assigned arms), one group with one day of pre-exposure (A1), the other three days of pre-exposure (A3). For all of the rats one of the assigned arms was the baited arm (during training this arm had forty Froot Loops placed at the end facing away from the central platform) and the other was the non-baited arm (this arm never had Froot

Loops placed in it). The first group was randomly assigned a unique pair of arms. Each subsequent group had the same nine pairs of arms assigned randomly.

Apparatus

An eight-arm radial maze was used for pre-exposure, training and testing in this experiment. The central platform of the radial maze was 35 cm in diameter, octagonal in shape, made of wood and painted black. The eight arms were 65 cm in length, 9 cm in width and the sides were 3 cm high, all were made of aluminium (this is the same maze that was used in the AT/MT lesion CPP task). Wooden blocks (these differ from the AT/MT lesions CCP task that used Perspex enclosures) were placed in the non-assigned arms were 35 cm in length, 19 cm in height, 9 cm in width, made of wood and painted a light grey (*see Figure 16 on page 58 for a photo of the radial arm maze*). The blocks placed in the assigned arms were the same dimensions as the blocks in the unassigned arms. However, of importance are the two panels 25 cm in length, 28.5 cm in height and 0.3 cm in width attached end of the block that faced away from the central platform and also painted light grey. They were attached so that they sat just inside the sides of the arm itself parallel to each other (*see Figure 17 on page 59; Figure 18 on page 60 for schematic diagrams of blocks with these panels in adjacent arms and separate arms configuration*). These panels restricted the rats view to a very small part of the room external to the maze. The maze was situated in windowless room (the same room as in the AT/MT lesion CPP task) that was artificially well lit. There were twelve objects on the walls of varying size, shape and colour to provide spatial cues additional to those provided by the structure of the room itself. Mounted on the ceiling was a camera to record behavioural data. During all testing the door was shut and the experimenter was outside the room.

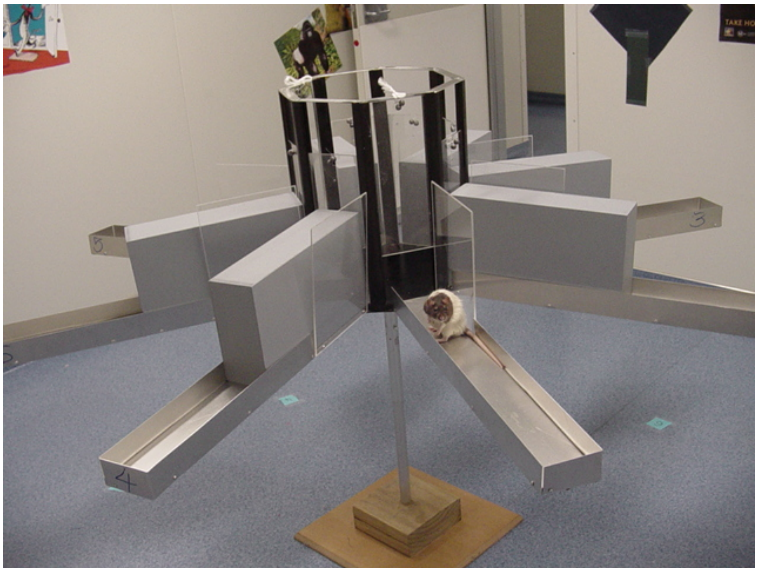

Figure 16: Radial Arm Maze with Wooden Blocks (Pre-exposure/Testing configuration)

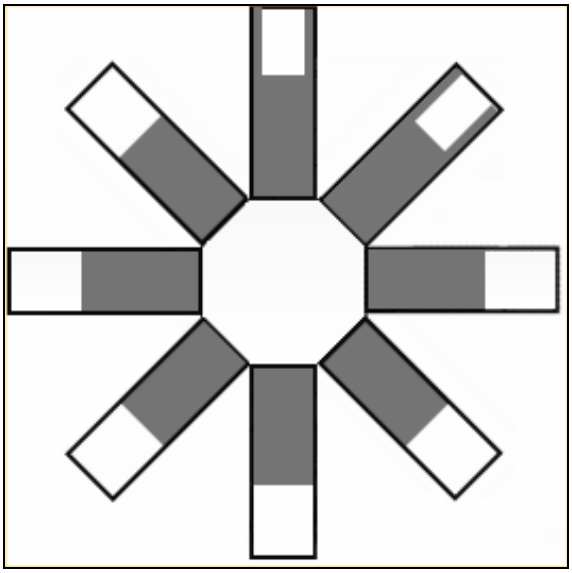

Figure 17: Experiment One: Restricted View – Schematic of Radial Arm Maze Adjacent Arms (Training Configuration)

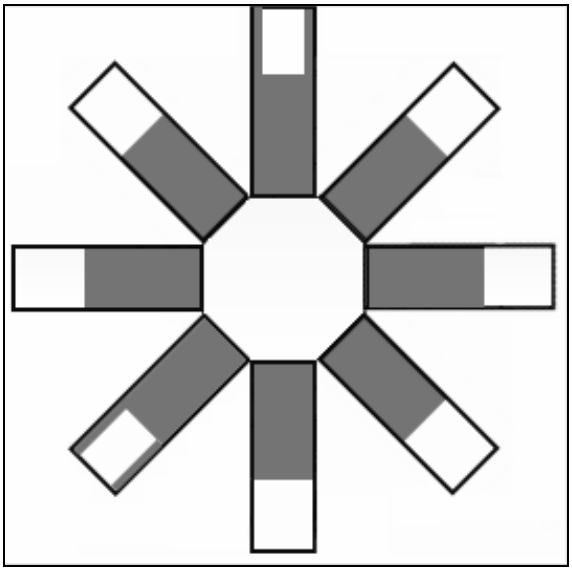

Figure 18: Experiment One: Restricted View – Schematic of Radial Arm Maze Separate Arms (Training Configuration)

Procedure

Handling

Each rat was handled for five minutes a day for four days prior to the start of the experiment. During this time period Froot Loops were added to their rat pellets when fed.

Pre-exposure

During pre-exposure rats were allowed to freely explore the central platform and their unique pair of assigned arms for ten minutes, all other arms were blocked off. Froot Loops were not placed on the maze.

Training

Rats received four training trials; each training trial required two days. Each rat was assigned a unique pair of arms. One of its assigned arms was baited with food and the other was non-baited. Half of the rats in each group were confined in their food arm on day one of the trial for thirty minutes and in their non-baited arms on day two of the trial for thirty minutes. The opposite order was used for the other half of the rats in each group. When in the baited arm, forty Froot Loops were placed at the end of the arm to ensure the rat ate while facing the cues visible from the end of the arm. The entire maze was rotated one arm position to the left at the start of each day to prevent the rats using intramaze cues to acquire a CPP.

Testing

Rats received the test trial twenty-four hours after the last training trial. Testing was identical to pre-exposure except that the rats were on the maze for twenty minutes. The amount of time each rat spent in their baited and non-baited arms was timed using a stopwatch. A rat was considered to be in the arm or to have left the arm once its front feet crossed the threshold between the centre platform and the arm.

Experiment Two: 180° View

The 180° view conditioned place preference (CPP) experiment differs from the previous CPP experiment because of the amount of distal cues available to make a CPP association. In this experiment the rats have a 180° view from the end of the arm, allowing a larger area for cues to come from than in the first experiment. The four conditions which were identical to the previous experiment (separate arms one pre-exposure (S1), separate arms three pre-exposures (S3), adjacent arms one pre-exposure (A1) and adjacent arms three pre-exposures (A3)) offered different opportunities for the rats to learn the information required as well as differences in cue ambiguity.

Subjects

Thirty-six naïve female PVGc Hooded rats were used as subjects. These rats were aged thirteen to fourteen months and weighed 180g to 220g when the experiment commenced. See previous for housing parameters and group assignment.

Apparatus

The same radial maze and room were used as in the previous experiment. The major change from the previous CPP apparatus was the blocks placed in the assigned arms for training trials. The blocks were the same dimensions as the blocks in the unassigned arms;

but on the end of the block that faced away from the central platform one panel was attached perpendicular to the rest of the block. The panel measured 29.5 cm in length, 28.5 cm in height and 0.3 in width (*see Figure 19 on page 63; Figure 20 on page 64*). This panel allowed the rat a 180° view of the room from the arm but prevented it looking back towards the maze itself.

Procedure

Handling, pre-exposure, training and testing is the same as in the previous CPP experiment.

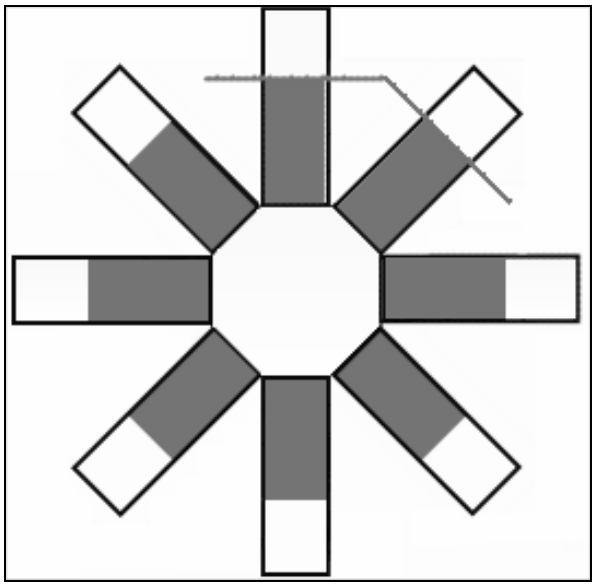

Figure 19: Experiment Two: 180° View – Schematic of Radial Arm Maze Adjacent Arms (Training Configuration)

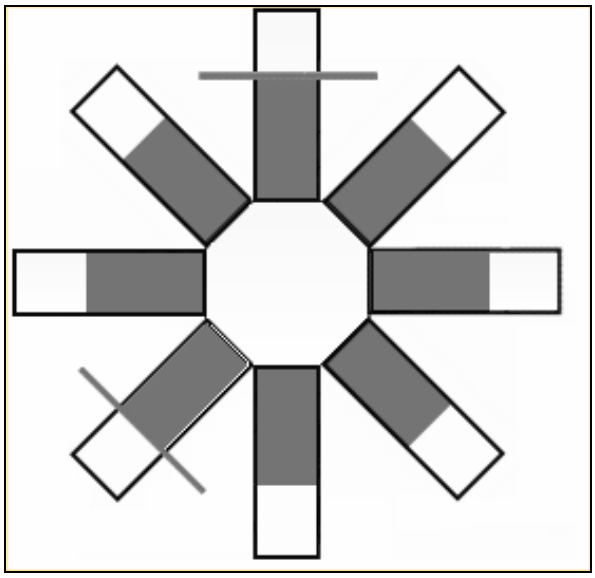

Figure 20: Experiment Two: 180° View – Schematic of Radial Arm Maze Separate Arms (Training Configuration)

Experiment Three: Males 180° View

The Male 180° view conditioned place preference experiment differed from the previous experiment in the sex of the rats (male instead of female) and the group assignment (S1 and A1 only). The rats still have a 180° view from the end of the arm.

Subjects

Eighteen naïve male PVGc Hooded rats were used as subjects. These rats were aged five to seven or twelve months and weighed 240g to 340g when the experiment commenced. See previous for housing parameters. The rats were divided into two groups of nine, one group was assigned two separate arms (the arms were separated by two arms in between),

and the other group was assigned adjacent arms (there were no arms between the two assigned arms). This offered the same opportunities for the rats to learn the information required but differed in the degree of ambiguity of the distal cues present in each condition. To compensate for the age disparity, both groups had the same number of older and younger rats. Both groups received only one day of pre-exposure prior to training. For all of the rats one of the assigned arms was the baited arm (during training this arm had forty Froot Loops placed at the end facing away from the central platform) and the other was the non-baited arm (this arm never had Froot Loops placed in it). The first group was randomly assigned a unique pair of arms. The second group had the same nine pairs of arms assigned randomly.

Apparatus

The apparatus is exactly the same as in Experiment Two.

Procedure

Handling, pre-exposure, training and testing is the same as in the previous two CPP experiments.

Experiment Four: Female 8 Trials

The Female 8 Trials conditioned place preference experiment differed from the previous CPP experiments because rats were given 8 trials instead of four giving the rats more time to learn the location of the food and the distal spatial cues associated with the baited arm. The sex of the rats was changed from the previous experiment (back to female). However, the rats still had a 180° view from the end of the arm.

Subjects

Eight naïve female PVGc Hooded rats were used as subjects. These rats were aged eleven months and weighed 180g to 220g when the experiment commenced. See previous for housing parameters. Only one group, separate arms one pre-exposure (S1) was tested. For all of the rats one of the assigned arms was the baited arm (during training this arm had forty Froot Loops placed at the end facing away from the central platform) and the other was the non-baited arm (this arm never had Froot Loops placed in it). The first group was randomly assigned a unique pair of arms. Each subsequent group had the same nine pairs of arms assigned randomly.

Apparatus

The apparatus was the same as in Experiment Two and Three.

Procedure

Handling and pre-exposure were the same as all the previous CCP experiments.

Training

Training was exactly the same as in the previous CPP experiments except the number of training trials was increased from four to eight.

Testing

Testing was the same as all the previous CCP experiments.

Results

Experiment One: Restricted View (4 training trials)

The restricted view conditioned place preference (CPP) experiment required rats to acquire an association between the food in a baited arm versus a non-baited arm of an eight arm radial maze and the distal cues that were specific to that arm. The acquisition of the association was assessed by testing a preference for the baited arm even when there was no longer any food in the arm. Due to the restricted nature of the view from the end of the arm, the distal cues should have been from a smaller area and therefore more distinct from each other than in a less restricted condition. The four conditions (separate arms one pre-exposure (S1), separate arms three pre-exposures (S3), adjacent arms one pre-exposure (A1) and adjacent arms three pre-exposures (A3)) offered different opportunities for the rats to learn the information required as well as differences in cue ambiguity. For optimum performance on the restricted view conditioned place preference task, the rats were expected to spend a greater amount of time in the baited arm than in the non-baited arm.

Figure 21 shows the time spent in the baited and non-baited arms for each of the four groups (*see page 68)* The figure suggests that there were few differences if any for all groups between time spent in the baited arm and time spent in the non-baited arm. A 4(Group (S1, S3, A1, A3)) x 2(Arms (baited, non-baited)) repeated measures analysis of variance (ANOVA) confirmed there were no significant main effects of Group ($F(3,32)=0.835$, $p>0.10$), Arms ($F(1,32)=0.707$, $p>0.10$) nor interaction Group x Arms ($F(3,32)=0.733$, $p>0.10$) Post-hoc comparisons confirmed that there was no baited/non-baited effect even when each group was analysed separately, S1 ($p>1.0$), S3 ($p>0.10$), A1 ($p>0.10$) and A3 ($p>0.10$).

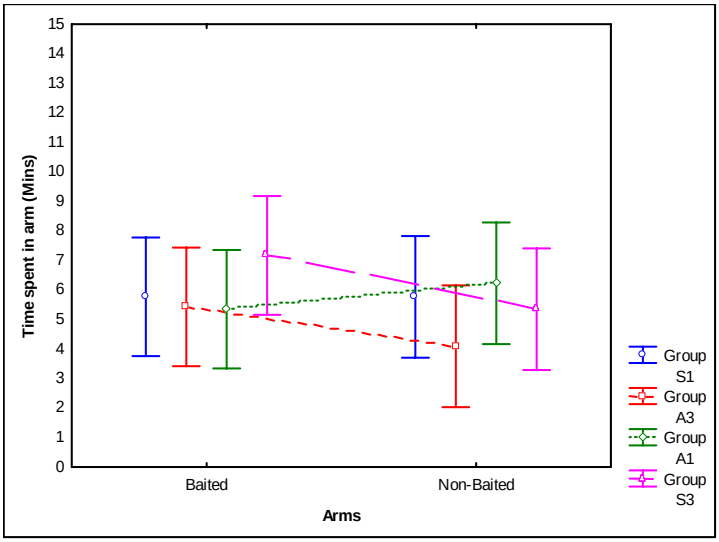

Figure 21: Experiment One: Restricted View CPP time (mins) spent in the baited and non-baited arms for S1, S3, A1, and A3.

Experiment Two: 180° View (4 training trials)

The 180° view conditioned place preference (CPP) experiment differs from the previous CPP experiment because of the amount of distal cues available to make a CPP association. In this experiment the rats have a 180° view from the end of the arm, allowing a larger area for cues to influence the rat than in the first experiment. The four conditions which were identical to the previous experiment (separate arms one pre-exposure (S1), separate arms three pre-exposures (S3), adjacent arms one pre-exposure (A1) and adjacent arms three pre-exposures (A3)) offered different opportunities for the rats to learn the information required as well as differences in cue ambiguity. For optimum performance on the 180° view conditioned place preference task, the rats were expected to spend a greater amount of time in the baited arm than in the non-baited arm.

Figure 22 shows the time spent in the baited and non-baited arms for each of the four groups (*see page70*). The figure suggests a tendency for S3 and A1 to spend less time in the non-baited arm and more time in the baited arm. However, there is no suggestion of S1 and especially A3 having a tendency to spend more time in either the baited or non-baited arm. A 4(Group (S1, S3, A1, A3)) x 2(Arms (baited, non-baited)) repeated measures analysis of variance (ANOVA) revealed only a significant main effect of Group ($F(3,32)=8.73$, $p<0.0001$), but not Arms ($F(1,32)=2.51$, $p>0.10$), nor interaction Group x Arms ($F(3,32)=0.485$, $p>0.10$). Therefore no one group displayed a significant difference in the time spent in the baited and non-baited arms. Post-hoc comparisons confirmed that there was no baited/non-baited effect even when each group was analysed separately, S1 ($p>0.10$), S3 ($p>0.10$), A1 ($p>0.10$) and A3 ($p>0.10$). The group main effect seems to derive from less overall time spent in any arm in A1, and more overall time spent in any arm in S3.

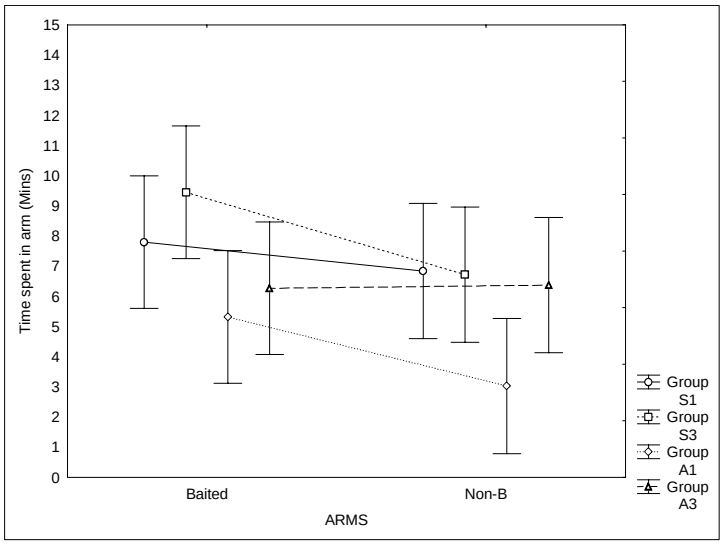

Figure 22: Experiment Two: 180° View CPP time (mins) spent in the baited and non-baited arms for S1, S3, A1, and A3.

Experiment Three: Males 180° View (4 training trials)

The Male 180° view conditioned place preference experiment differed from the previous experiment in the sex of the rats (male) and the number of groups used (S1 and A1 only). The rats still have a 180° view from the end of the arm. The two conditions (separate arms one pre-exposure (S1), adjacent arms one pre-exposure (A1)) offered the same opportunities for the rats to learn the information required but differed in the degree of ambiguity of the distal cues present in each condition. For optimum performance on the male 180° view conditioned place preference task, the rats were expected to spend a greater amount of time in the baited arm than in the non-baited arm.

Figure 23 shows the time spent in the baited and non-baited arms for the two groups S1 and A1 (*see page 71*). The figure suggests that both S1 and A1 show a tendency to spend more time in the baited arm than the non-baited arm. However, S1 appears to have a greater difference in the time spent in the baited arm versus the non-baited arm. A 2(Group (S1, A1)) x 2(Arms (baited, non-baited) repeated measures analysis of variance (ANOVA) confirmed there was no significant main effects of Group ($F(1,16)=0.841$, $p>0.10$), Arms ($F(1,16)=3.27$, $p>0.05$) nor interaction Group x Arms ($F(1,16)=0.380$, $p>0.10$). Post-hoc comparisons confirmed that there was no baited/non-baited effect even when each group was analysed separately, S1 ($p=0.10$) and A1 ($p>0.10$).

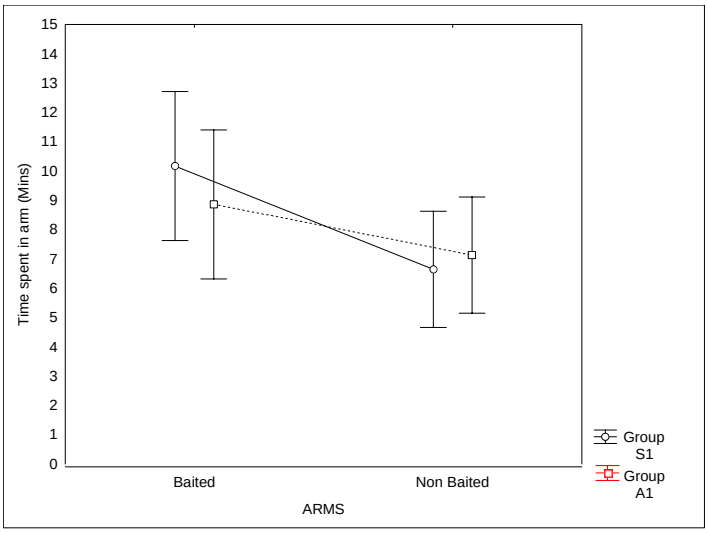

Figure 23: Experiment Three: Males 180° View CPP time (mins) spent in the baited and non-baited arms for S1 and A1.

Due to the disparity of the age of the rats within this experiment the analysis was repeated using only the younger rats (*n*=12) to determine whether the younger rats by themselves would produce a significant result where the combined group of both younger and older rats (*n*=18) had not. Figure 24 suggests a greater difference between the baited and non-baited for S1 if restricted to younger rats only than with combined younger and older rats. There is no difference suggested for A1 (*see page 72*). A 2(Group (S1, A1)) x 2(Arms (baited, non-baited) repeated measures analysis of variance (ANOVA) confirmed there was no significant main effects of Group ($F(1,10)=2.04$, $p>0.10$), Arms ($F(1,10)=2.84$, $p>0.10$) nor interaction Group x Arms ($F(1,10)=1.59$, $p>0.10$). Post-hoc comparisons confirmed that there was no significant difference in the time spent in the baited arm versus the non-baited arm for S1 ($p=0.06$), and A1 ($p>0.10$). However, while there was no significant result of post-hoc comparisons for the S1 younger rats only S1 ($p=0.06$), it was

closer to a significant result than the post-hoc comparison for S1 combined younger and older rats, S1 (p=0.10).

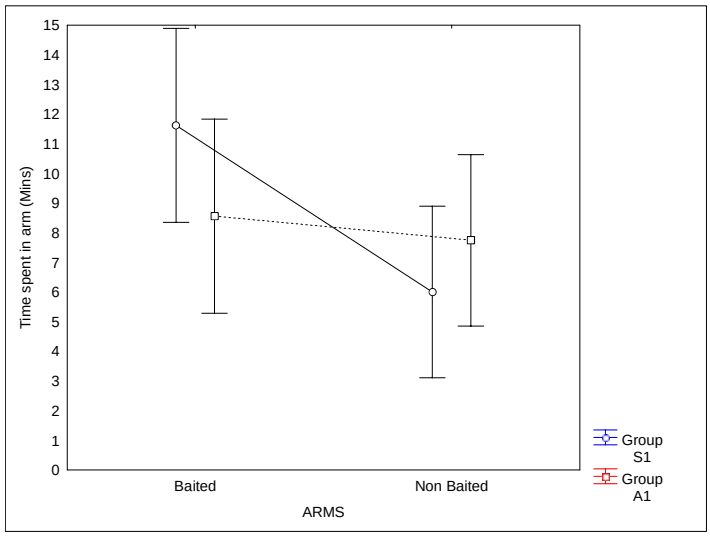

Figure 24: Younger Rats Only - Experiment Three: Males 180° View CPP time (mins) spent in the baited and non-baited arms for S1 and A1.

Experiment Four: Female 8 Trials

The Female 8 Trials conditioned place preference experiment differed from the previous CPP experiments because rats were given 8 trials instead of four giving the rats more time to learn the location of the food and the distal spatial cues associated with the baited arm. Only one group was tested (S1). The rats still have 180° view from the end of the arm. To achieve optimum performance on the rats had to spend a statistically significant greater amount of time in the baited arm than in the non-baited arm.

Figure 25 shows the time spent in the baited and non-baited arms for S1 with eight training trials condition (*see page 73*). The figure clearly shows a marked difference between time spent in the baited arm and time spent in the non-baited arm, suggesting that with an increase of trials from 4 to 8, for separate arms, one pre-exposure a CPP was acquired. All of the rats in this experiment spent more time in the baited arm versus the non-baited arm. A dependant *t*test confirmed there was sufficient difference in the time spent in the baited arm relative to the non-baited arm to produce a statistically significant result ($t(7)=5.81$, $p<0.0001$). Therefore one pre-exposure and eight training trials demonstrated significant acquisition of separate arms conditioned place preference.

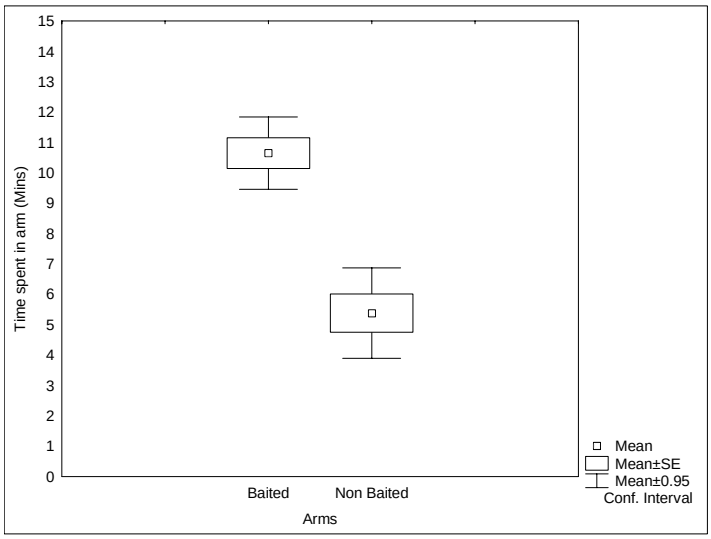

Figure 25: Experiment Four: Female 8 Trials CPP time (mins) spent in the baited and non-baited arms for S1.

Comparison: Female S1 Condition

The separate arms, one pre-exposure condition from Experiments One, Two and Four were compared to place the results of Experiment Four in context with the other experiments and further confirm the significant result obtained in Experiment Four.

Figure 26 shows the comparison of time spent in the baited and non-baited arms for S1 for Experiment One, Two and Four (*see page 75*). The figure suggests that there is a difference in time spent in the baited and non-baited arm for the S1 condition for these three experiments. There is no difference at all in Experiment One. There was a small amount of difference between time spent in the baited and non-baited arms in Experiment Two. There is clearly more time spent in then baited arm than in the non-baited arm for Experiment Four suggesting acquisition of separate arms CPP. A 3(Experiment (Exp1:S1, Exp2:S1, Exp4:S1)) x 2(Arms (baited, non-baited)) repeated measures analysis of variance (ANOVA) confirmed there was a significant main effect of Experiment ($F(2,23)=3.95$, $p<0.05$), but not Arms ($F(1,23)=3.73$, $p<0.10$) nor significant interaction Experiment x Arms ($F(2,232)=2.91$, $p>0.10$). Post-hoc comparisons established that there was no significant difference in the time spent in the baited arm versus the non-baited arm for Experiment One: S1 ($p>1.0$), nor Experiment Two S1 ($p>0.10$). However, they confirmed the previous significant result for Experiment Four S1 ($p<0.001$). This confirmation again implies that to acquire a large enough CPP to be significant one pre-exposure and 8 training trials are needed.

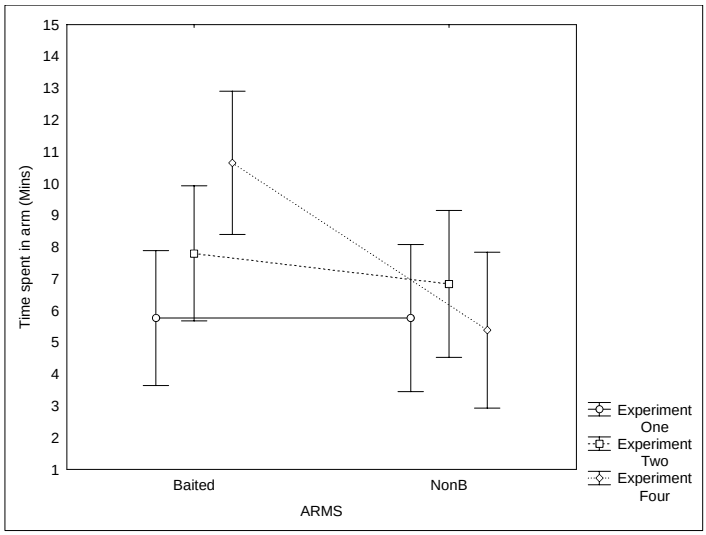

Figure 26: Experiment One, Three and Four CPP time (mins) spent in the baited and non-baited arms for S1.

Discussion

The intent of Part One of the current study was to examine the effects of AT and MT lesions on spatial pattern separation, spatial working memory and conditioned place preference (CPP). AT lesions produced a severe impairment on the match to sample spatial working memory task on a cheeseboard. The AT lesion deficit was still apparent after 15 days of post-operative testing. In contrast, MT lesions had no effect on the match to sample spatial working memory task on a cheeseboard. On the AT/MT lesion conditioned place preference task (separate arms one pre-exposure), AT and MT lesions, as well as control rats failed to acquire a CPP. The intent of Part Two of the present study was to determine the effects of systematic procedure variations on the ability of control rats to acquire both separate arms and adjacent arms CPP following the failure of control rats to acquire a separate arms CPP in the AT/MT lesion CPP task. Of the four experiments conducted only Experiment Four produced evidence of CPP acquisition. In Experiment Four, control rats acquired a separate arms CPP following one pre-exposure and eight training trials. The following sections will address in more detail these findings and the implications of these findings compared with other studies.

Part One

Spatial Working Memory Delayed Match-To-Sample Task on the Cheeseboard

Rats were tested for 15 days following surgery. When compared with the last three days prior to surgery, the first three days of testing after surgery showed a clear AT deficit for delayed matching-to-sample on a cheeseboard. In contrast to this the MT and CN groups showed no performance deficit relative to their performance prior to surgery. When the 15 post-operative testing days were averaged into three day blocks, the AT lesion performance deficit persisted. The severe AT impairment compared to CN rats is consistent with previous evidence that the AT is involved in processing information related to spatial memory (Aggleton, Neave, Nagle, & Hunt, 1995; Alexinsky, 2001; Mitchell & Dalrymple-Alford, 2005; Moran & Dalrymple-Alford, 2003; Warburton & Aggleton, 1999; Warburton, Baird, & Aggleton, 1997). In addition to this, the current findings extend the AT spatial working memory deficit to the cheeseboard, lending further espousal to the proposition that the AT is required for allocentric spatial memory.

Although the basic paradigm is comparable to a delayed match-to-sample task in a T-maze, the availability of intramaze cues is considerably reduced on a cheeseboard than on a T-maze, therefore decreasing the likelihood of an egocentric strategy (navigation by head/body turn). This important because human research has shown that allocentric memory is more impaired than egocentric memory (Holdstock et al., 2000). The current results also support the finding by Warburton et al (1999) that pretraining does not spare the AT deficit found in spatial working memory tasks. The delayed match-to-sample spatial working memory task employed extensive pretraining before surgery. Prior to this the rats were also trained on the spatial pattern separation delayed-match-to-sample task. As AT lesions produced a severe AT deficit on spatial working memory for delayed match-to-sample on the cheeseboard, it is therefore apparent that the pretraining employed did not spare the effects of AT lesions. Hippocampal lesions are also associated with severe impairment in tasks involving spatial memory processing (Jarrard, 1993), especially those involving allocentric spatial memory (Holdstock et al., 2000). The results of this task, combined with previous evidence of the similarity of AT and hippocampal impairment in spatial information processing provides further support for the extended hippocampal-anterior thalamic axis posited to be responsible for episodic memory recall (Aggleton & Brown, 1999; Vann & Aggleton, 2004).

In contrast to the severe AT deficit found, there was no MT deficit evident relative to CN rats. This suggests that the MT is not required for memory involving a spatial component. This finding is confirms and extends recent research at the University of Canterbury that found no MT impairment on a radial arm maze task (Mitchell & Dalrymple-Alford, 2005) or an odour-place paired associate learning task (Gibb, 2005) using selective lesions that encompassed only the medial and central nuclei. No previous study has examined the effects of AT and MT lesions on a match-to-sample spatial working memory task on a cheeseboard. However, it is comparable with a match-to-sample task in a T-maze (Hunt & Aggleton, 1998b) and a match-to-sample task in an operant chamber (Burk & Mair, 1998) which also found no MT deficit. There is contradictory evidence that suggests MT lesions might be involved in spatial information processing (Hunt & Aggleton, 1991, , 1998a), however these studies were found to have collateral damage to the AT region which is known to produce impairment even if the lesions are diminutive (Aggleton, Hunt, Nagle,

& Neave, 1996; Byatt & Dalrymple-Alford, 1996). It is possible that this might also explain the findings by Alexinsky (2001), however as the details regarding any collateral damage were not reported this cannot be definitively determined. Conversely, there are studies that have produced MT impairments on tasks with a spatial memory component that cannot be explained through collateral damage. Stokes & Best (1988) found an MT impairment in a radial maze task. It has been proposed that one explanation for these findings might be the technique used to produce the lesions. Burk & Mair (1998), Gibb (2005), Mitchell & Dalrymple-Alford (2005) and the present study produced lesions using NMDA. This technique destroys the cell bodies but spares the fibres of passage. In contrast to this, Stokes & Best (1988) used electrolytic lesions. This technique destroys both the cell bodies and the fibres of passage. However, this proposition cannot account for all the inconsistencies found in respect to MT lesions and spatial memory because research since then has found MT deficits on spatial memory using NMDA or ibotenic lesions (Alexinsky, 2001; Hunt & Aggleton, 1998a). Another suggestion is that MT lesions may only impair spatial memory if the task is sufficiently demanding (Hunt & Aggleton, 1998a). However, it is difficult to determine what might constitute sufficiently demanding. This task is comparable to a match-to-sample task in a T-maze, therefore comparatively as demanding, and found no MT deficit. This suggestion is also not supported by the lack of MT impairment found on an odour-place conditional association task (Gibb, 2005) which could be described as sufficiently demanding. The arbitrary nature of this suggestion makes it difficult to test and current research does not seem to support this as a viable theory to account for the inconsistency in MT findings on tasks with spatial memory components.

Rats were trained on the match-to-sample spatial pattern separation task prior to being trained on the match-to-sample working memory task. Despite following the same procedure as described in Gilbert, Kesner, & DeCoteau (1998) and Gilbert, Kesner, & Lee (2001), rats in the current study were unable to reach the surgery criterion even though training was continued for eight weeks more than in the previous studies. It is unclear why the surgery criterion was not reached in this experiment.

Conditioned Place Preference AT/MT Lesion Task

In the AT/MT lesion CPP task rats were tested using the separate arms paradigm with one pre-exposure, four training trials, and one test trial. No group, AT, MT or CN showed evidence of the acquisition of separate arms CPP. No previous study has examined the effects of AT lesions on separate arms CPP. The current presupposition is that separate arms CPP is acquired through both Pavlovian conditioning and spatial discrimination, but that the approach associated (Pavlovian) conditioning contribution is stronger. The hippocampus is thought to process spatial information but not affect information (Kesner, 1998). Because of the connections between the hippocampus and the AT nuclei (Aggleton & Brown, 1999; Vann & Aggleton, 2004), it is posited that AT lesions would produce similar impairments to hippocampal lesions. Research has found that hippocampal lesions do not produce deficits on separate arms CPP tasks (McDonald & White, 1993; White & McDonald, 1993). Therefore it was not expected that AT lesions would impair acquisition of a separate arms CPP. It cannot readily be determined that the AT lesions did not have an effect on the acquisition of a CPP because the control rats did not acquire a CPP for adequate comparison. Therefore the effect of AT lesions on separate arms CPP acquisition in this case in unclear. A graph of the results did visually suggest that AT rats spent more time in the baited arm versus the non-baited arm, however considerable variance within the AT group prevented a significant statistical result. As in the case of the AT lesion group, it cannot definitively be determined that the MT lesions did not have an effect on the acquisition of a CPP because the control rats did not acquire a CPP for adequate comparison. The failure of CN rats to acquire a separate arms CPP means that the effect of MT lesions on CPP acquisition remains unclear as neither the results reported by Hunt & Aggleton (1998b) who found no MT impairment of the ability to acquire a separate arms CPP in a radial maze nor the results reported by McAlonan, Robbins, & Everitt (1993) who found MT lesions did impair acquisition of CPP in a compartmented box can be confirmed or refuted.

The reason for the failure of the CN group to acquire a separate arms CPP in this case is unclear. Previous studies have indicated that control rats can acquire a separate arms CPP if they are given no pre-exposure and two training trials. However, control rats cannot

acquire a separate arms CPP if they are given one pre-exposure and only two training trials (White & Wallet, 2000). Chai & White (2004) posit this disparity is because pre-exposure interferes with CPP acquisition via the amygdala system. This is thought to be due to the acquisition of pure spatial information mediated by the fornix during pre-exposure, which produces a latent inhibition effect on the amygdala system. When the number of training trials is increased from two to four training trials (the number the current task used) control rats can again acquire a CPP (McDonald & White, 1993). Therefore it is suggested the latent inhibition effect produced by pre-exposure in separate arms CPP can be overcome by greater opportunity to acquire an approach association with the baited arm (i.e. more training trials). In the current study, although rats were given one pre-exposure and four training trials the CN rats failed to acquire a separate arms CPP. The lack of a clear explanation for this failure is the motivation for Part Two of the current study.

Part Two

Part Two of the present study aimed to determine the influence of different conditioned place preference procedures on control rats, in order to then follow up with the effects of different thalamic lesions on selected procedures (outside the time frame of the present thesis).

Experiment One: Restricted View Conditioned Place Preference Task

The restricted view CPP task required control rats to acquire an association between the food in a baited arm versus a non-baited arm of an eight arm radial maze and the distal cues that were specific to that arm. Due to the restricted nature of the view from the end of the arm, the distal cues should have been from a smaller area and therefore more distinct from each other than if the distal cues were less restricted. Rats were tested on four conditions, two using the separate arms paradigm and two using the adjacent arms paradigm. Each paradigm was tested with both one pre-exposure and three pre-exposures: S1, A1, S3, and A3. Each of the four conditions had four training trials. Rats in the separate arms one pre-exposure (S1) condition were expected to acquire a CPP because previous studies reported that controls rats could acquire a separate arms CPP with one pre-exposure and four training trials (McDonald & White, 1993). Rats in the adjacent arms three pre-exposures (A3) condition but not the adjacent arms one pre-exposure (A1)

condition were expected to acquire a CPP due to previous studies suggesting that control rats could not acquire an adjacent arms CPP unless they were given three or more pre-exposures and four training trials (Chai & White, 2004). It was expected that rats in the separate arms three pre-exposure (S3) condition would not be able to acquire a separate arms CPP because the additional pre-exposures would produce a latent inhibition effect due to the additional opportunity to acquire pure spatial information during pre-exposure. None of the conditions produced a CPP acquisition.

Experiment Two: 180° View Conditioned Place Preference Task

The 180° view CPP experiment differs from the previous CPP experiment because of the amount of distal cues available to make a CPP association. In this experiment the rats have a 180° view from the end of the arm, allowing a larger area for cues to come from than in the first experiment. This is the most common configuration used in previous studies, and is the configuration used in the AT versus MT lesions study in Part One of the present study. Rats were tested on four conditions, two using the separate arms paradigm and two using the adjacent arms paradigm. Each paradigm was tested with both one pre-exposure and three pre-exposures: S1, A1, S3, and A3. Rats in the separate arms one pre-exposure (S1) condition were expected to acquire a CPP because previous studies reported that controls rats could acquire a separate arms CPP with one pre-exposure and four training trials (McDonald & White, 1993). Rats in the adjacent arms three pre-exposures (A3) condition but not the adjacent arms one pre-exposure (A1) condition were expected to acquire a CPP due to previous studies suggesting that control rats could not acquire an adjacent arms CPP unless they were given three or more pre-exposures and four training trials (Chai & White, 2004). It was expected that rats in the separate arms three pre-exposure (S3) condition would not be able to acquire a separate arms CPP because the additional pre-exposures would produce a latent inhibition effect due to the additional opportunity to acquire pure spatial information during pre-exposure. None of the conditions produced a CPP acquisition. There was some difference between the groups in time spent in the baited versus the non-baited arm, but not enough to yield a significant statistical result. The failure of rats in the S1 condition to acquire a separate arms CPP in this experiment is consistent with the failure of control rats in the AT versus MT lesion task in Part One of the present study. No previous study has examined the effect of three pre-exposures on the separate arms paradigm therefore it was unclear whether the extra

pre-exposures would produce an interference effect or not. However, in this experiment a graph of the results did visually suggest that rats in the S3 condition spent more time in the baited arm versus the non-baited arm. In addition, there appeared to be a greater difference in the S3 condition than in the S1 condition. However, considerable variance within the S3 condition prevented a statistically significant result. A graph of the results visually suggested that the A1 condition spent more time in the baited arm versus the non-baited arm. In addition, there appeared to be a greater difference in the A1 condition than in the A3 condition. The lack of CPP acquisition in any condition however means that nothing definitive can be concluded from the current finidings. Taken together these results suggest that contrary to previous evidence one pre-exposure and four training trials in the separate arms paradigm and three pre-exposures and four training trials in the adjacent arms paradigm, is not enough to produce separate arm CPP acquisition.

Experiment Three: Males 180° View

The Male 180° view conditioned place preference experiment differed from the previous experiment in the sex of the rats, which changed from male to female. Male rats were used as subjects in the previous studies which produced successful acquisition of CPP where this study has not. The conditions also changed, rats were only tested on the S1 and A1 conditions (due to lack of male rats available to the experimenter). The rats still have a 180° view from the end of the arm. The two conditions offered the same opportunities for the rats to learn the information required but differed in the degree of ambiguity of the distal cues present in each condition. Rats in the separate arms one pre-exposure (S1) condition were expected to acquire a CPP because previous studies reported that controls rats could acquire a separate arms CPP with one pre-exposure and four training trials (McDonald & White, 1993). Rats in the adjacent arms one pre-exposure (A1) condition were not expected to acquire a CPP due to previous studies suggesting that control rats could not acquire an adjacent arms CPP unless they were given three or more pre-exposures and four training trials (Chai & White, 2004). Neither of these conditions produced a CPP acquisition. A graph of the results did visually suggest that S1 rats spent more time in the baited arm versus the non-baited arm. Rats in the A1 condition also appeared to spend more time in the baited arm relative to the non-baited arm. However considerable variance within both the S1 and A1 conditions prevented a significant statistical result. There was considerable disparity in age within the rats used in this

experiment (although the same number of older and younger rats was used in each condition). Therefore the results were analyzed again using only the younger rats in both the S1 and A1 conditions. This did not produce a successful CPP acquisition. However, the younger rats in the S1 condition did visually appear on a graph to have a greater difference in the time spent in the baited arm versus time spent in the non-baited arm compared to the younger and older rats together. The younger rats alone produced an almost statistically significant result which was closer to significance than the younger and older rats together. Taken together these results suggest than while sex and age might make a small difference, in this case it was not enough of a difference to produce a CPP acquisition. The age of rats was not generally reported in the previous studies, so the comparative age of the rats used in the present study cannot be contrasted with the age of rats used in the previous studies.

Experiment Four: Female 8 Trials

The Female 8 Trials CPP experiment differed from the three previous CPP experiments because the number of training trials was increased from four to eight. This enabled the rats to spend more time learning the location of the food, and the distal spatial cues associated with the baited arm. The rats still have 180° view from the end of the arm. Only one condition was tested S1 (due to the lack of rats available to the experimenter). The rats in this experiment did successfully acquire a separate arms CPP. This is the only condition where all the rats in a condition spent more time in the baited arm versus the non-baited arm. This suggests that one pre-exposure and eight training trials are required for control rats to acquire separate arms CPP. This is inconsistent with the results of previous studies which report only one pre-exposure and four training trials are required to acquire separate arms CPP (McDonald & White, 1993). The reason for this discrepancy is unclear. However it is impossible to report and therefore replicate the exact distal cues available to the rats in a given experiment. Therefore the discrepancy between previous CPP studies and the present study could be due to a difference in the distal spatial cues in the environment and the level of difficulty they represent in learning the association between a food location and the cues that relate to the baited arm. Another possibility is that the disparity is caused by differences in the particular strain of rats employed. The current study used PVGc Hooded rats; in contrast the previous CPP studies predominantly used Long Evans rats.

Contributions and Future Directions

The current study provided new evidence of the effects of AT and MT lesions on delayed match-to-sample spatial working memory on a cheeseboard. Tasks that involve allocentric spatial memory are presumed to be indicative of critical aspects of episodic-like memory in non-humans. No previous study has investigated spatial working memory via delayed match-to-sample on a cheeseboard. Finding an AT deficit on a novel apparatus is particularly relevant because it provides additional evidence that the AT is impaired in a wide range of tasks that test allocentric spatial memory. This is important because allocentric memory has been found to be more impaired than egocentric memory in humans (Holdstock et al., 2000). Furthermore, this task provided supplementary evidence for the assertion that pretraining does not spare AT impairment on tasks with a spatial component by Warburton et al (1999). A major issue of future research is the need for replication and extension of the delayed match-to-sample spatial working memory on a cheeseboard task. As the particular apparatus used is novel, a replication of the present task would confirm and strengthen the current findings. In addition, the current study should be extended to include LT lesions to determine what effects LT lesions may have on delayed match-to-sample spatial working memory on a cheeseboard as previous studies have indicated that the LT may play a role in memory that is distinct from the other thalamic regions (Gibb, 2005; Mitchell & Dalrymple-Alford, 2005). Furthermore, the delayed match-to-sample spatial working memory on a cheeseboard task was a modification of the original task due to the failure of rats to reach the surgery criterion on the spatial pattern separation delayed match-to-sample on a cheeseboard task. Therefore, it would be interesting to reattempt the spatial pattern separation delayed match-to-sample on a cheeseboard task to divine the possible reasons for this failure to reach surgery criterion, and to fulfill the original objective to ascertain the effects of AT and MT lesions on this task. The original parameters should be extended to encompass LT lesions to also determine the stricture of their role on spatial pattern separation.

The evidence the current study provided on the effects of AT and MT lesions on CPP in a radial maze is not definitive due to the failure of the CN rats to acquire a CPP for comparison. CPP tasks can be used to bridge the gap between episodic memory in humans and episodic-like memory in non-humans because they assess both the spatial and affect

attributes of memory. Tasks involving allocentric spatial memory in particular are presumed to be a good test of episodic-like memory. One strength of the CPP task therefore is the ability to encourage the use of allocentric cues to acquire CPP by rotating entire maze one arm position to the left at the start of each day to minimize intramaze cues. The replication and extension of this task is therefore a plausible direction for future research. The replication of this task would ascertain whether the impairments produced by the AT and MT lesions were legitimate despite the failure of the CN group to acquire a separate arms CPP. The replication and extension of CN separate arms CPP research has already been addressed by Part Two of the present study. Future research into the effects of thalamic lesions on separate arms CPP should be extended to include LT lesions as there is some suggestion from previous studies that the effect of LT lesions may differ from AT and MT lesions (Gibb, 2005; Mitchell & Dalrymple-Alford, 2005).

The current study provided new evidence of the ability of control rats to acquire a CPP in a radial maze using both the separate arms and the adjacent arms paradigm. In particular the finding that one pre-exposure and four training trials does not always produce a separate arms CPP. Due to the lack of available rats Experiment Three and Four did not test all four conditions. One possibility for future research therefore, is the replication and extension of these experiments to include all four conditions. Specifically, as no previous study has attempted to ascertain the effect of three pre-exposures on the ability of control rats to acquire separate arms CPP, the replication of this condition is particularly important both in the experiments that included and didn't include that condition in the current study. The most important future direction for this research however, is the effects of AT, MT and LT lesions on CPP using both the separate arms and adjacent arms paradigm, as current research does not provide definitive evidence.

In addition to the future directions for research suggested by each experiment conducted in the current study there are some general issues of future research that should be addressed. Contemporary research into diencephalic amnesia has posited three separate neural systems underlying normal memory function. However, few of the recent studies have directly compared the different thalamic regions on learning and memory tasks. Fewer still have taken into account or examined the adjacent neural structures and the connecting

pathways postulated to also be important. Therefore, future research should address this lack through currently available techniques including neural tracing and asymmetrical lesions paradigms. Finally, as previously mentioned recent research at the University of Canterbury has used more selective lesions than previous studies. This manipulation of lesion size and the control of collateral damage should be addressed in all future research as when combined with quantitative analysis this practice provides a clearer picture of the relationship between the brain and observable behaviour.

Limitations of the Current Study

It is important to assess the limitations of the current research to enable future research to combat the limitations discussed. The main limitation of the delayed match-to-sample spatial working memory on a cheeseboard task is the assumption that the rats used an allocentric strategy to learn the task instead of an egocentric strategy. This assumption is based on the relatively restricted availability of egocentric cues available. Spatial probes could be used to determine whether an allocentric or egocentric strategy is used. For example, moving the start box to the other side of the cheeseboard after the sample phase (but before the choice phase) would determine whether the rats were indeed using allocentric cues. Another limitation of this task is the small number of rats used in each group. Although the effects of AT lesions can usually be observed with small numbers of rats, a larger number would mean a more robust effect. Furthermore, because the rats were initially trained on the spatial pattern separation delayed match-to-sample on a cheeseboard task, it is impossible to determine the exact amount of training required to learn the delayed match-to-sample spatial working memory on a cheeseboard task.

A major limitation of the AT/MT conditioned place preference in a radial maze task is the failure of CN rats to successfully acquire a separate CPP with one pre-exposure and four training trials as previous studies using control rats have reported that rats can acquire separate arms CPP with those parameters (McDonald & White, 1993). In general, for a robust result CN rats are required for comparison as it presumed that they represent normal function without the lesion. Furthermore, prior to being trained on the separate arms CPP task, the rats were trained on two other tasks. Whether this might interfere with the acquisition of CPP or not cannot be determined. Therefore, studies should start with control rats to establish baseline findings before introducing procedural variations that

may influence the rate of learning. Another limitation as in the spatial working memory task above is the small number of rats in each group. Additional rats might have provided less variance in the data. Therefore, as in the AT group which appeared visually to be spending more time in the baited arm versus the non-baited arm but did not produce a significant statistical result, additional rats might have changed that result.

Although, previously discussed with respect to future research it is important to note that the reduction in conditions in Experiment Three and Four is also a major limitation. The successful acquisition of a separate arms CPP with one pre-exposure and eight training trials (S1) in Experiment Four cannot be compared to control rats in the other three conditions (A1, S3 and A3). Therefore, the information that could be derived from this experiment is incomplete.

In addition to the limitations specific to each experiment mentioned, there are some general limitations of the present study. Recent research conducted at the University of Canterbury has used more selective lesions than in contemporary research (Gibb, 2005; Mitchell & Dalrymple-Alford, 2005). This study employed the same lesion specificity. Evaluation of the lesions in the present study showed that they were highly selective, but analysis could be improved by the use of semi-quantitative methods to specify the extent of the damage and subsequently the extent of the non-intended damage to the adjacent regions.

General Summary

The main contention of research into the origins of diencephalic amnesia surrounds the contribution of the different thalamic nuclei. One proposition is that a single thalamic region is critical for the normal function of learning and memory, therefore damage to this key site is the neural basis for diencephalic amnesia. In contrast, another view is that no single thalamic region is responsible for diencephalic amnesia. Instead, it is posited that the different thalamic structures contribute in different ways to the normal function of learning and memory, therefore each thalamic region provides the neural basis for a different symptom of diencephalic amnesia. The current study provided support for this position by producing further evidence that AT and MT lesions have different effects on spatial working memory tasks. Tasks that include a spatial component, especially those

tasks that assess allocentric spatial memory, are particularly relevant because they are presumed to reflect episodic-like in non-human animals. AT lesions produced a severe impairment in the delayed match-to-sample spatial working memory task, but MT lesions produced no impairment. This supports the view that AT lesions impair tasks that contain a spatial component, but MT lesions do not suggesting that the AT and MT are components of separate memory systems. It does not provide support however, for one theory outright. The findings in the present study, while providing evidence for the hippocampal-anterior thalamic axis proposed by Aggleton & Brown (1999), can also be explained as supportive components of the multiple system models postulated by Kesner (1998) and McDonald & White (2002). The failure of control rats to acquire a CPP on the AT/ MT separate arms CPP task in a radial maze means that this task cannot definitively provide evidence supporting or refuting the theories discussed, because of the subsequent lack of control comparison of the effects of AT and MT lesions found on this task. However, this task did address this situation by comparing the ability of control rats to acquire a CPP on both the separate arms and adjacent arms CPP paradigm. The results suggested that one pre-exposure and four training trials may is not always sufficient to produce a separate arms CPP. Therefore the interference from the acquisition of pure spatial information during pre-exposure in the acquisition of separate arms CPP may be more substantial than posited. This finding highlighted a number of areas for future reference. In summation, the present study combined with previous research supports the view that no single thalamic site is responsible for the underlying neural basis of diencephalic amnesia. Therefore, a revision of the current theories of learning and memory is required to account for the different thalamic involvement that has been highlighted.

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