The role of the anterior thalamic nuclei for properties of episodic memory: *what, when, where*

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Summary

Amnesia impairs episodic memory in humans, and can occur following damage to the medial diencephalon. This thesis examines the importance of the anterior thalamic nuclei, a diencephalic structure, for different elements (*what*, *when*, *where*) of episodic memory (or episodic-like memory) in rats. The overall goal was to understand why this region is so critical for normal memory in humans. An initial series of experiments investigated the ability of rats with anterior thalamic lesions to recognise objects and odours with a variety of delays. These experiments found that anterior thalamic lesions spare item recognition (*what*). This same ability was further explored with the use of the immediate early gene *zif268*, and the results again indicated that this thalamic nucleus does not have a direct role in recognition. A related series of studies explored the effects of anterior thalamic lesions on temporal order judgments (*when*) for objects. Two different discrimination procedures were tested, between-block recency and within-block recency. Lesions to the anterior thalamic nuclei selectively impaired performance on within-block recency but spared between-block recency. The ability of rats with anterior thalamic damage to discriminate between two locations (*where*) in both complex and simple environments was also tested. Anterior thalamic lesions significantly impaired place learning compared with control animals, despite the finding that rats with anterior thalamic lesions could sometimes discriminate between the two locations (i.e., could perform significantly above chance). In addition, the effects of anterior thalamic damage on biconditional learning (*what-where conjunction*) were examined. The rats were trained on both item-place and item-context associations. Lesions to the anterior thalamic nuclei disrupted acquisition of the former, but not the latter. The results suggest that the contributions of the rodent anterior thalamic nuclei to episodic memory, as part of the extended-hippocampal system, primarily reflect the involvement of these nuclei in allocentric spatial learning.

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Chapter 1

General Introduction

The research described in this thesis focuses on the importance of one specific brain site (the anterior thalamic nuclei) for learning and memory. The rationale for this research can be traced back to the study of anterograde amnesia - the failure to retain new information in long-term memory. The realisation that amnesia can spare a wide array of cognitive abilities despite the profound loss of memory has meant that the study of this condition has the potential to provide unique insights into brain structures and brain systems specifically required for memory. Damage in several brain regions is associated with anterograde amnesia, one of these being the medial part of the diencephalon. Indeed, it could be argued that the first systematic neuropathological studies on amnesia investigated diencephalic amnesia (Gudden, 1889). Despite over a hundred years of subsequent research, little is still known about the precise causes of diencephalic amnesia. In particular, information is lacking about the particular combinations of structures that are critical for memory and about the roles that these structures normally play in supporting memory.

To address these questions, the present research examines animal models of diencephalic amnesia. This approach is particularly relevant for studies of anterior thalamic function as there is a growing body of human neuropsychological studies implicating these nuclei, but almost all of this evidence is indirect. That is, there is much evidence that damage to the fibre tracts that innervate the anterior thalamic nuclei is associated with amnesia (e.g. Carlesimo, Lombardi, & Caltagirone, 2011; Tsivilis et al., 2008), yet there appear to be no cases of people with selective, circumscribed damage to these nuclei. While there are a small number of cases with relatively localized damage involving the anterior thalamic nuclei (e.g. Mark, Barry, Mclardy, & Ervin, 1970; Clarke et al., 1994), there is a lack of evidence that the pathology is sufficiently selective to ascribe the resultant cognitive changes to just the anterior thalamic nuclei. For these reasons, the

present research uses rats, where it is possible to both create and confirm selective manipulations of these nuclei, and to analyse any impact on learning and memory. In order to do this, it is, however, first necessary to consider the type of memory loss most evident in human amnesia and how this form of memory can be assessed in animal models.

Episodic memory, amnesia, and the "extended hippocampal system"

Episodic memory is defined as memories for particular events, or episodes, of one's life which are bound within a distinct spatial-temporal context that allows the individual to "travel back mentally in time" (Tulving, 1983), and is characteristically affected in amnesia (Kopelman, 1995; Kopelman, 2002). Episodic memory is composed of three properties, which can be individually examined (Aggleton & Pearce, 2001): *what* (e.g., item memory), *where* (spatial memory) did the event occur and *when* (temporal memory) did the event occur. Spatial memory requires remembering the spatial configurations or associations among objects that form a scene; whereas temporal memory is the ability to remember a sequence of events (e.g. which activity occurred first) as well as the ability to locate when an event occurred.

A third component of episodic memory involves "*what.*" This can be examined either through recall (e.g., who did you go to the movies with?) or recognition (e.g., of the following people who did you go to the movies with?). However, it has been argued that recognition memory can be solved by either recollecting the previous encounter of an item (e.g., object, person, word, odour) within a landscape of associated details (e.g., where the item was located) or through a feeling of familiarity in the absence of details associated with the event (often described as the gut feeling of "knowing"; Mandler, 1980; Aggleton & Brown, 1999; Yonelinas, 2002). If episodic memory involves the conjunction of *what* happened *when* and *where*, then it is not surprising that remembering with whom one has experienced a past event is an example of episodic memory, while recognition memory via familiarity alone is not (Aggleton & Brown, 1999, 2006). Some researchers argue that familiarity and recollection are processes within a

single continuum; thus, suggesting the same neural regions are involved in both familiarity and recollection (Dunn, 2004; Squire, Stark, & Clark, 2004, Squire, Wixted, & Clark, 2007). The argument is that familiarity is a weaker neural response, and recollection is a stronger response. In contrast, others have suggested these are two separate and qualitatively different processes, involving different, but interleaving, brain systems (Aggleton & Brown, 1999, 2005, 2006; Brown & Aggleton, 2001; Yonelinas, 2002; Eichenbaum, Yonelinas, & Ranganath, 2007); thus suggesting that some brain regions may be involved in familiarity ("know"), but not recollection ("remember") and vice versa.

Patients with memory impairments have elucidated the critical brain networks involved in episodic memory. In the late 19th century, Sergei Korsakoff described a memory disorder that is characterised by both the loss of previously acquired information (retrograde amnesia) and the inability to form new memories (anterograde amnesia), and is associated with thiamine deficiency (Kopelman, 1995). Since then, numerous cases of Korsakoff's syndrome have been reported, and indicate that damage to the medial diencephalon is critical for the development of amnesia (Victor, Adams, & Collins, 1971; Mair, Warrington, & Weiskrantz, 1979; Mayes, Meudell, Mann, & Pickering, 1988; Harding, Halliday, Caine, & Kril, 2000; Gold & Squire, 2006). A quantitative study of pathology in the brains of alcoholics (Harding et al., 2000) found that the anterior thalamic nuclei were the only region with consistent neuronal loss in cases of Korsakoff's patients with amnesia. However, one problem with Korsakoff's patients is that their pathology can extend beyond the diencephalon (Victor et al., 1971; Kopelman, Thomson, Guerrini, & Marshall, 2009).

The diencephalon can also be affected by other etiologies such as strokes, tumours, or physical insult (e.g., patient BJ who was stabbed through his left nostril with a snooker cue and received damage to his mammillary bodies) which can lead to memory impairments (Squire, Amaral, Zola-Morgan, Kritchevsky, & Press, 1989; Dusoir, Kapur, Byrnes, McKinstry, & Hoare, 1990; Gold & Squire, 2006; Carlesimo et al., 2007, 2011; Cipolotti et al., 2008; Tsivilis et al, 2008). For example, a group of 38 patients that had surgical removal of colloid cysts from the third ventricle were given a battery of recognition and recall memory tasks. The results showed

that the performance of these patients on tests of recall only correlated with mammillary body atrophy (Tsivilis et al., 2008). In addition, mammillary body atrophy did not correlate with measures of recognition. Another example involves the case of a woman who had bilateral radiofrequency lesions to the anterior thalamic nuclei as result of treatment for chronic depression. Although the patient refused to participate in memory tests, she often complained about her recent memory loss. The patient exhibited confusion for both space and time, and often had difficulty locating her hospital room (Mark et al., 1970). While there remains debate regarding which region or regions of the diencephalon are most important for the ability to retain new events, evidence implicates the mammillary bodies (Victor et al., 1971; Mair et al., 1979; Gold & Squire, 2006; Tsivilis et al., 2008), the medial dorsal thalamic nuclei (Mair et al., 1979; Gold & Squire, 2006), and the anterior thalamic nuclei (Mark et al., 1970; Harding et al, 2000; Van der Werf, Witter, Uylings, & Jolles, 2000; Van der Werf, Jolles, Witter, & Uylings, 2003; Gold & Squire, 2006). Other evidence implicates the mammillothalamic tract (Van der Werf et al., 2000; Carlesimo et al., 2011), which directly links the mammillary bodies to the anterior thalamic nuclei.

In the 1950s, evidence for the involvement of the medial temporal lobe in episodic memory grew. For instance, Scoville and Milner (1957) described the unique story of patient H.M., who after falling off his bicycle sustained a head injury, which eventually resulted in the development of epilepsy. Because H.M.'s seizure persisted despite the use of medication, and because his epilepsy appeared to have its origin within the medial temporal lobe, Scoville decided to remove the inner surface of H.M.'s medial temporal lobes bilaterally, including the hippocampus. Following surgery, HM's epilepsy improved, however, he became densely amnesic failing to remember events immediately after they had occurred (Scoville & Milner, 1957; Corkin, 1984; 2002; Bohbot & Corking, 2007). For example, H.M. repeatedly failed to recognise Brenda Milner who studied him for years.

The similarity between memory deficits following damage to either medial temporal lobe region or to the diencephalon (e.g., Gold & Squire, 2006; McKee & Squire, 1992) has led to the hypothesis that a network involving the hippocampus,

fornix, mammillary bodies, and the anterior thalamic nuclei form a reciprocal pathway via the posterior cingulate region that is critical for normal episodic memory, and that diencephalic and medial temporal lobe amnesia are caused by damage to different regions of this system (Delay & Brion, 1969; Aggleton & Brown, 1999). This neural circuit, which was originally described within the "limbic lobe" or "Papez circuit" (Broca, 1878; Papez, 1937), provides a serial set of connections between the medial temporal lobes and the diencephalon (Figure 1.1A). While Papez's original hypothesis that the circuit was critical for emotional processing has been largely disproved (LeDoux, 1993), both neuropsychological and animal studies support an "extended hippocampal memory system" (Aggleton & Saunders, 1997; Aggleton & Brown, 1999; Vann & Aggleton, 2004). Specifically, Aggleton and Brown (1999) proposed that episodic memory relies on hippocampal-diencephalic system by "permitting information to be set in its spatial and temporal context."

Support for the extended hippocampal memory system comes from the intimate anatomical connectivity between the hippocampus (which includes the hippocampal fields CA1-4, the dentate gyrus, and subiculum) and part of the medial diencephalon (e.g., mammillary bodies, anterior thalamus) which suggests that these structures form a functional network, and therefore may contribute to similar processes during learning and memory (see Figure 1.1). The hippocampus proper does not project directly to anterior thalamus, but rather, neurons in the subiculum connect to the anterior thalamic nuclei via the fornix either directly or indirectly by innervating the mammillary bodies (Nauta, 1956; Aggleton, Desimone, & Mishkin, 1986). The mammillary bodies in turn project primarily to the anterior thalamic nuclei through the mammillothalamic tract (Vann, Saunders, & Aggleton, 2007). The importance of this connection is emphasised by the suggestion that every mammillary body neuron might project to the anterior thalamus (Vann et al., 2007; Vann, 2010). The anterior thalamic nuclei can project back to the subicular and post-subicular cortical regions (van Groen & Wyss, 1990b, 1995). Furthermore, there is evidence of an indirect hipppocampal-anterior thalamic pathway via the retrosplenial cortex. Anatomical studies have shown the existence of bi-directional connections between the retrosplenial cortex

and the anterior thalamic nuclei (van Groen & Wyss, 1992, 1995; Shibata, 1998). The retrosplenial cortex also sends and receives projections from the hippocampal formation (Finch, Derian, & Babb, 1984; van Groen & Wyss, 1990a, 1992, 2003). Therefore, the anatomical evidence emphasises a highly interconnected network.

Further support that medial temporal lobe and diencephalic amnesia are caused by damage along a "extended hippocampal" circuit comes from disconnection studies in animals examining components of episodic memory (e.g., *where*; Sutherland & Hoesing, 1993; Warburton, Baird, Morgan, Muir, & Aggleton., 2000; Warburton, Baird, Morgan, Muir, & Aggleton, 2001; Henry, Petrides, St-Laurent, & Sziklas, 2004; Dumont, Petrides, & Sziklas, 2010). A disconnection procedure involves damaging two different neural structures thought to function in conjunction to support a given behaviour or cognitive process, by damaging each structure unilaterally in opposite hemispheres. Provided that the connectivity between the two neural structures is primarily unilateral, the rationale is that the unilateral damage to each structure in opposite hemispheres disconnects the remaining intact regions (i.e., they are unable to communicate to each other). If communication between the two regions is necessary, then damage in opposite hemispheres should impair the behavioural performance more than damage within the same hemisphere. Damage to the two neural regions in the same hemisphere would leave an entire hemisphere intact (i.e., the intact regions would be able to communicate). In this way the functional interaction between the anterior thalamus and the hippocampus has been demonstrated for spatial learning (Sutherland & Hoesing, 1993; Warburton et al., 2001), and for the formation of item-place associations (Warburton et al., 2000; Henry et al., 2004; Dumont et al., 2010).
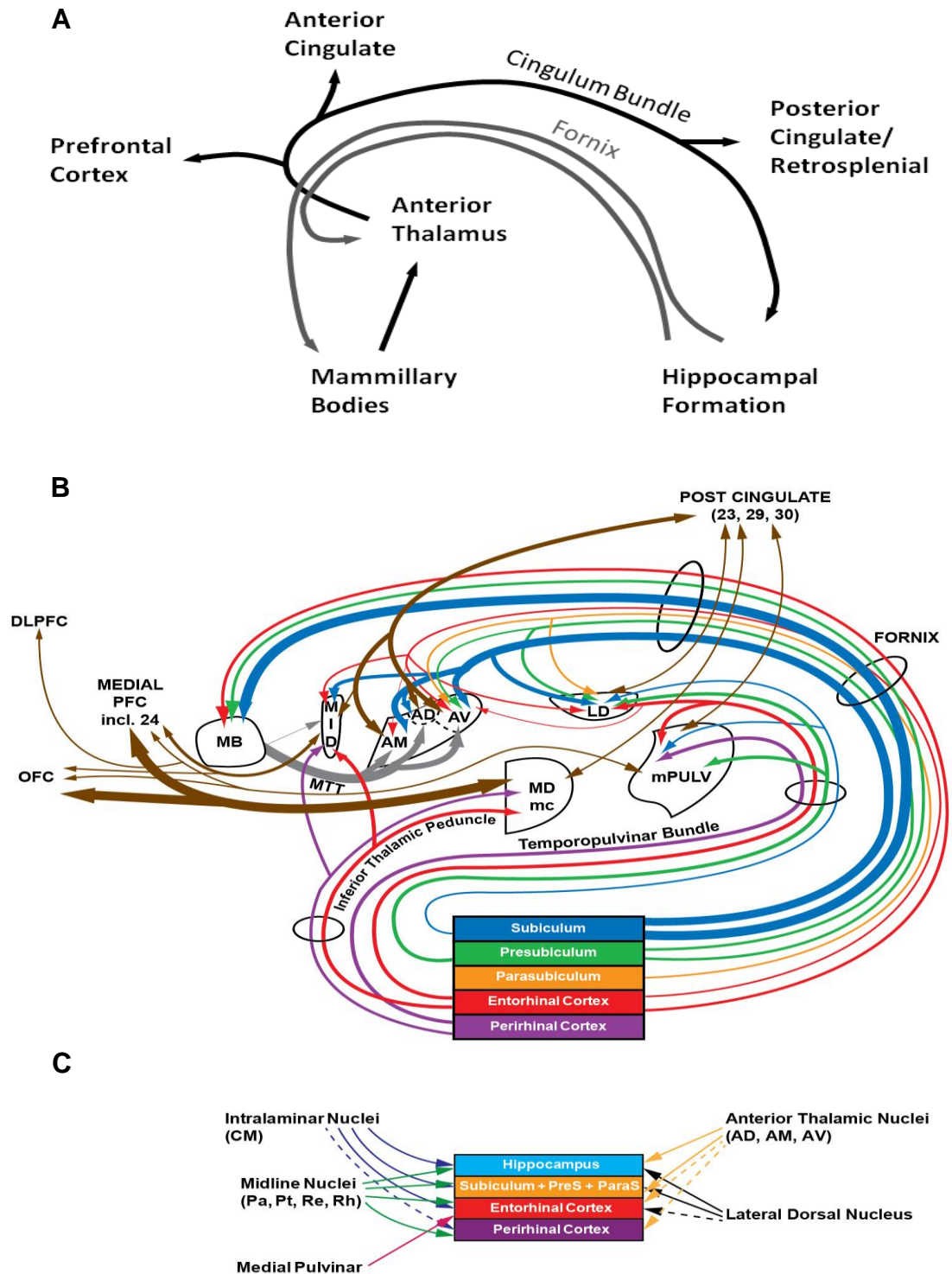
Figure 1.1. **A)** A schematic diagramme of the main connections of the extended hippocampal memory system. **B)** A schematic diagramme showing increased complexity of the main connections between the medial diencephalon, the prefrontal cortex, and medial temporal lobe in the macaque monkey. The thickness of the lines reflects the density of each projection. **C)** Summary of the thalamic projections to the medial temporal lobe. Dashed lines represent light projections. Figure **1.1B** and **C** taken from Aggleton, Dumont, & Warburton, 2011. Abbreviations: AD, anterior dorsal nucleus; AM, anterior medial nucleus; AV, anterior ventral nucleus; CM, center median nucleus; DLPFC, dorsolateral prefrontal cortex; LD, lateral dorsal nucleus; MB, mammillary bodies; MD, medial dorsal nucleus, in pars magnocellular (mc); mPULV, medial pulvinar; MTT, mammillothalamic tract; OFC, orbital frontal cortex; PA, paraventricular nucleus; PFC, prefrontal cortex; Pt, parataenial nucleus; Re, nucleus reuniens; Rh, rhomboid nucleus. The numbers correspond to cortical areas.

Candidate nuclei in the medial thalamus

It has also been suggested that the intralaminar and midline thalamic nuclei may also play an important role in cognition, perhaps as a broad role in attention and arousal instead of learning and memory (Van der Werf, Witter, & Groenewegen, 2002; Aggleton, Dumont, & Warburton, 2011). Van der Werf et al. (2002) had divided these nuclei into four groups each with hypothesised functions: 1) a dorsal group (paraventricular and parataenial) involved in viscero-limbic functions, 2) a lateral group (central lateral, paracentral, and rostral central medial) involved in cognitive processes, 3) a ventral group (reuniens and rhomboid) thought to play a role in multisensory processing, and 4) a posterior group (posteriorcentral medial and parafascicular) with a potential role in limbic motor function. Indeed, many of the midline thalamic nuclei (e.g., reuniens, parataenial, paraventricular) receive and project to the hippocampal formation, perirhinal and entorhinal cortex as well as to prefrontal regions (see Figure 1.1B & C; Amaral & Cowan, 1980; DeVito, 1980; Van der Werf et al., 2002; Hsu & Price, 2007).

Studies of discrete lesions in rats have helped to further elucidate the role of the intralaminar and midline nuclei on learning and memory tasks (Mitchell & Dalrymple-Alford, 2005, 2006; Gibb, Wolff, & Dalrymple-Alford, 2006; Wolff, Gibb, Cassel, & Dalrymple-Alford, 2008; Prasad, Macgregor, & Chudasama, 2012). There is some evidence that these nuclei may be involved in temporal order memory (intralaminar nuclei; Mitchell & Dalrymple-Alford, 2005), behavioural inhibition (e.g., lesions to reuniens lead to impulsivity; Prasad et al., 2012), reward value learning (central and medial parts of the medial dorsal thalamic nuclei; Mitchell & Dalrymple-Alford, 2005). Furthermore, damage to the intralaminar nuclei sometimes, but not always, impairs response-related (e.g., always turn left) behaviours in a cross maze or radial arm maze (Mitchell & Dalrymple-Alford, 2006; Wolff, Gibb et al., 2008). There is little evidence to suggest that these nuclei are involved in the same learning and memory processes associated with damage to the extended-hippocampal system, and clear dissociations between intralaminar/midline nuclei and the anterior thalamic nuclei have been reported (Mitchell & Dalrymple-Alford, 2005, 2006; Wolff, Gibb, et al., 2008). Two notable exceptions are: 1) impaired recency judgments which are also impaired following

lesions to the hippocampus (e.g., Fortin, Agster, & Eichenbaum, 2002; Kesner, Gilbert, & Barua, 2002; Albasser, Lin, Iordanova, Amin, & Aggleton, 2012), and impaired learning of an odour-location biconditional task (see biconditional learning section below; Gibb et al., 2006).

The medial dorsal thalamic nucleus has also been a candidate region in diencephalic amnesia (Mair et al., 1979; Gold & Squire, 2006). However, damage to the medial dorsal thalamic nucleus is not sufficient to cause amnesia (Markowitsch, 1982; Aggleton, Dumont et al., 2011, but see Gold & Squire, 2006). Van der Werf et al. (2000) found that damage to the medial dorsal nucleus was associated with executive memory problems. Furthermore, there is evidence of intact memory in patients with damage to the medial dorsal thalamic nucleus that does not include additional mammillothalamic tract injury (Kritchevsky, Graff-Radford, & Damasio, 1987). Both anatomical evidence that this region is highly interconnected with the prefrontal cortex (see Figure 1.1B; Krettek & Price, 1977; Ray & Price, 1993; Hoover & Vertes, 2011), and animal lesion studies indicate that damage to this region impairs learning about reward contingencies (Chudasama, Bussey, & Muir, 2001; Mitchell, Browning, & Baxter, 2007), and temporal order memory (Mitchell & Dalrymple-Alford, 2005; Cross, Bashir, Brown, & Warburton, 2010), similar to prefrontal lesions (Barker, Bird, Alexander, & Warburton, 2007).

Why study the anterior thalamic nuclei?

Unlike the other candidate thalamic nuclei mentioned in the above section, most studies suggest that the anterior thalamic nuclei are critical for (components of) episodic memory. Given the strong evidence from the patients with damage to the diencephalon (Harding et al., 2000; Van der Werf, 2000, 2003), the anatomical connectivity of the anterior thalamus to other regions implicated in memory (e.g., hippocampus and mammillary bodies; Nauta, 1956, Aggleton et al., 1986, Vann et al., 2007, but see Aggleton, O'Mara et al., 2010), and disconnection studies in animals (Sutherland & Hoesing, 1993; Warburton et al., 2000, 2001; Henry et al., 2004; Dumont et al., 2010), it is increasingly clear that the anterior thalamic nuclei play a pivotal role learning and memory as part of the "extended hippocampal memory system" (Aggleton & Sahgal, 1993; Aggleton & Brown, 1999; Aggleton,

Dumont et al., 2011). The following sections will examine the neuroanatomy of the anterior thalamic nuclei and review the literature on the contribution of the anterior thalamus to individual components of episodic memory: 'what', 'when', and 'where', as well as any combination of these components.

The neuroanatomy of the anterior thalamic nuclei

The rodent anterior thalamus is a subcortical structure composed of three major nuclei – anterior ventral nucleus (AV), anterior dorsal nucleus (AD), and anterior medial nucleus (AM) – with a similar appearance and organization across a large variety of mammalian species (Figure 1.2). In rats a middle interoanteromedial nucleus (IAM) is sometimes recognized (e.g., Shibata & Kato, 1993), but not in the primate brain. Although there is evidence that the interoanteromedial nucleus is part of a unique anatomical pathway (Hopkins, 2005; see Figure 7.1 General Discussion), this subregion is usually regarded as forming part of the anterior medial nucleus. For the purposes of this thesis, lesions of the anterior thalamic nuclei included IAM, and it was considered part of the anterior medial thalamic nucleus.
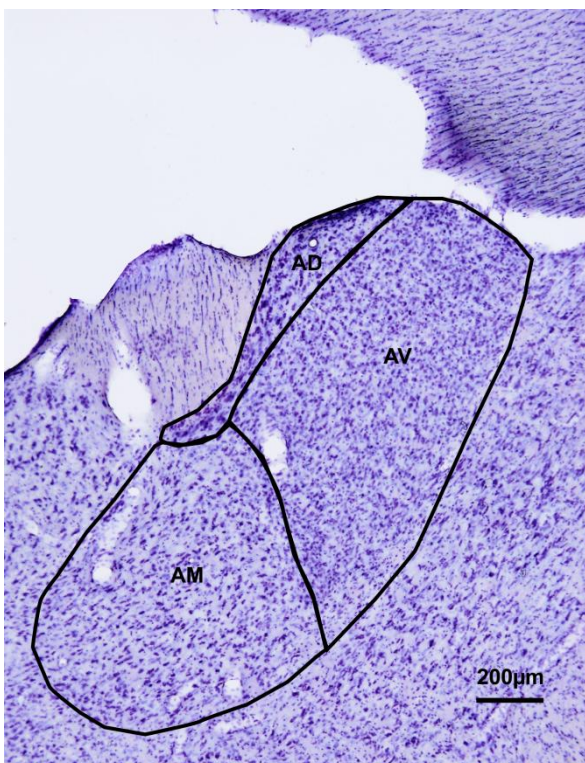
Figure 1.2. A coronal section showing the three anterior thalamic nuclei in the rat brain visualised with a Nissl stain. The bar represents the distance of 200 μm. Abbreviations: AD, anterior dorsal nucleus; AM, anterior medial nucleus; AV, anterior ventral nucleus.

In humans, however, evidence suggests that anterior thalamic nuclei are relatively enlarged (Armstrong, 1986). The lateral dorsal thalamic nucleus (LD) is occasionally considered to be a part of the anterior thalamic nuclei because it shares many similar connections (Bentivoglio, Kultas-Ilinsky, & Ilinsky, 1993) and shares electrophysiological properties with the anterior dorsal nucleus (Taube, 2007). However, one major difference between the lateral dorsal nucleus and the other anterior thalamic nuclei is that the lateral dorsal nucleus does not receive dense projections from the mammillary bodies (Vann et al., 2007). Given this key difference, the lateral dorsal nucleus will not be included with the remaining three nuclei; although, in reality, lesion studies often unintentionally encroach upon this region.

A brief overview of the connectivity of each of the three major nuclei from studies using anterograde and retrograde tracers is discussed below. It is particularly interesting to note that even when these structures are interconnected with the same neural regions, they both receive and project information from different populations of neurons, suggesting that the "extended hippocampal" network is composed of numerous somewhat independent, parallel, neural circuits (Vann et al., 2007; Wright, Erichsen, Vann, O'Mara, & Aggleton, 2010; Yoder & Taube, 2011).

Anterior Medial (AM)

Afferents

The anterior medial nucleus (AM) receives ipsilateral projections from the medial mammillary bodies (pars medialis centralis; Seki & Zyo, 1984).   These projections are also topographically organised so that the ventral and dorsal portions of the pars medialis project to the rostral and caudal portions of AM, respectively (Watanabe & Kawana, 1980). Other subcortical projections to AM include the rostral dorsal portion of the reticular nucleus (Shibata, 1992). AM also receives cortical inputs.   AM receives ipsilateral projections from prelimbic and medial orbital cortices and bilateral projections from the anterior cingulate and secondary motor cortices (Shibata & Naito, 2005).   These connections are organised

topographically with the rostral portion of the secondary motor cortex projecting to the caudal portion of AM, and the caudal secondary motor cortex projecting the rostral part of AM. In contrast, the anterior cingulate cortex maps onto AM in a rostrocaudal fashion (Shibata & Naito, 2005). There is evidence that AM receives projections from the presubicular and postsubicular cortices bilaterally (Seki & Zyo, 1984). Injections of a retrograde tracer into AM also found afferents from layer II of the dorsal subiculum proximal to CA1, and also in layers II of the ventral subiculum (Wright et al., 2010). However, the afferents from the ventral subiculum to AM are much lighter compared with those from dorsal subiculum. There were few labeled cells in the postsubiculum. In addition, cells located in layers II and III of the entorhinal cortex project to AM (Wright et al., 2010).

Efferents

There is evidence that the anterior medial nucleus projects to the medial orbital, frontal polar, retrosplenial granular, entorhinal, perirhinal cortices, as well as to the subiculum, visual cortical area 18b, the lateral and basolateral amygdala (Shibata, 1993a; van Groen, Kadish, & Wyss, 1999). Furthermore, these projections are organised in a topographical fashion, with different neurons projecting to different regions. The ventrolateral portion of the anterior medial nucleus projects to area 18b, whereas a population of neurons located more medially projects to the perirhinal cortex and the amygdala. Anterograde injections into the rostral portion of AM labeled layer I and II of the ventral orbital area and the dorsal olfactory nucleus, and the deep caudal layers of the entorhinal cortex. There was also some label in layers I and V of area TE2 (Shibata, 1993a). Labeled terminals were also found in layer I, V, and VI of the anterior cingulate cortex. In the retrosplenial cortex, rostral AM projects to caudal layers I, V, and VI; whereas caudal AM projects to both layers I and V of the rostral granular and dysgranular retrosplenial cortex (van Groen & Wyss, 1992; Shibata, 1993b). In addition, retrograde tracers injected directly into the hippocampus showed bilateral labeling in the AM (Wyss, Swanson, & Cowan, 1979), thus providing evidence that the AM projects directly to the hippocampus.

Anterior Dorsal (AD)

Afferents

There is evidence that the lateral mammillary bodies project bilaterally to the anterior dorsal nucleus (Watanabe & Kawana, 1980; Shibata, 1992). There is also evidence of a direct hippocampal-anterior thalamic pathway through the parasubiculum. The parasubiculum primarily innervates the rostral portion of the AD (van Groen & Wyss, 1990a). Retrograde tracers injected into AD labeled neurons primarily in layer VI of the parasubiculum. The postsubiculum also projects to AD, and this input is topographically organised: the rostral portion of the postsubiculum projects to the ventromedial AD, whereas caudal postsubiculum innervates the rostrodorsal portions of AD (van Groen & Wyss, 1990b). Retrograde tracers placed in AD further delineated the postsubicular inputs by finding labeled neurons in layer VI of the postsubiculum (van Groen & Wyss, 1990b). There is also evidence of direct retinal inputs to the AD nucleus (Itaya, Van Hoesen, & Jenq, 1981). Anterograde tracers placed in the retinal ganglion cells of monkeys innervated the contralateral AD, primarily on its medial border (Itaya, Van Hoesen, & Benevento, 1986). This direct retinal pathway was also found in the tree shrew (Conrad & Stumpf, 1975) and in the rat (Itaya, Van Hoesen, & Jenq, 1981).

Efferents

Retrograde tracers injected into the parasubiculum labeled a few neurons in rostral AD, and anterograde tracers injected into AD labeled denser terminals in the deep layers (IV-VI) of the parasubiculum (van Groen & Wyss, 1990a). The rostral portion of the AD nucleus also projects to the postsubiculum. Anterograde tracers injected into AD labeled axons in the postsubicular layer I, III-IV (van Groen & Wyss, 1990b). Just like AM, injections of a retrograde tracer into the hippocampus showed labeled cells in the ipsilateral AD located primarily on the superficial surface of the nucleus. However unlike AM, no contralateral labeled neurons were observed (Wyss et al., 1979). There is also evidence that the AD projects to granular region of the retrosplenial cortex (Shibata, 1993b; van Groen

& Wyss, 1990c, 2003), with the ventral portion of AD innervating layers I, III, IV of the rostral retrosplenial cortex, whereas the dorsal part of AD projects to the same layers but in caudal retrosplenial cortex (Shibata, 1993b). However, van Groen and Wyss (2003) report that the topographical organization of the AD projections to granular retrosplenial cortex follow a rostrocaudal map where the caudal portion AD projects to the rostral end of the retrosplenial cortex, and rostral AD projects to caudal granular retrosplenial cortex.

Anterior Ventral (AV)

Afferents

Similar to the AM, the anterior ventral thalamic nucleus (AV) receives ipsilateral projections from the medial mammillary bodies. However, these projections are from the pars lateralis and pars posterior subregions of the medial mammillary bodies (Watanabe & Kawana, 1980), with the dorsal portion of these two subregions projecting to the medial portion of the AV, and the ventral portions of the medial mammillary bodies innervating the lateral AV (Seki & Zyo, 1984). The AV also receives projections from the caudal dorsal reticular nucleus and the lateral dorsal tegmental nucleus (Shibata, 1992). The presubiculum also innervates the AV ipsilaterally with some evidence of bilateral labeling in the rostral and dorsal portion of AV following some smaller injections of an anterograde tracer midway through the septotemporal pole (van Groen & Wyss, 1990a). This finding was further qualified by injecting a retrograde tracer into AV; the retrograde tracer revealed that presubicular projections originate in layer VI (van Groen & Wyss, 1990a). However, Wright et al. (2010) found no labeled cells in the presubiculum following injections of the retrograde tracer, fast blue, into AV. There were, however, afferents to AV from the dorsal subiculum and postsubiculum (Wright et al., 2010). There is also evidence that the retrosplenial granular b cortex innervates AV with the rostral portions of the retrosplenial cortex projecting to the caudal AV, and the opposite pattern for the caudal end of the retrosplenial cortex. Furthermore, these projections originate from layer VI of the retrosplenial granular b cortex (van Groen & Wyss, 2003; Wright et al., 2010).

The retrosplenial granular a cortex also projects bilaterally to AV (van Groen & Wyss, 1990c; Wright et al., 2010).

Efferents

Retrograde tracers placed in the presubiculum revealed labeled neurons in the rostrodorsal AV. However, van Groen and Wyss (1990a) found no evidence of a topographical organization of the thalamic cells projecting to either the dorsal or to the ventral presubiculum (van Groen & Wyss, 1990a). These projections were further qualified by placing an anterograde tracer into the AV. The AV innervates layers I and III of the presubiculum bordering the postsubiculum. In contrast, Shibata (1993a) reports that each subdivision of AV projects to both distinct areas and lamina of the presubiculum with the rostral AV projecting to the ventral layers I and III; the lateral and dorsal midstrocaudal AV innervating layers I, III, and IV-VI of the ventral presubiculum; whereas the ventral and medial quadrants of AV project to layers I, III, and IV-VI of the dorsal presubiculum (Shibata, 1993a). In addition to its projections to the presubiculum, there is evidence that AV innervates the anterior cingulate and the retrosplenial cortex (Shibata, 1993b; van Groen & Wyss, 2003). Again these projections are topographical with the ventral portion of AV projecting to the rostral granular retrosplenial cortex, and the dorsal portion of AV projecting to the caudal granular retrosplenial cortex (Shibata, 1993b; van Groen & Wyss, 2003). There is also some evidence that dorsolateral AV projects to layers I and V of the caudal dysgranular retrosplenial cortex (Shibata, 1993b). Unlike AM and AD nuclei, injection of a retrograde tracer into the hippocampus did not label neurons in AV (Wyss et al., 1979).

Taken together, the anterior thalamic nuclei are situated to receive and integrate information either directly from the hippocampus (through the subiculum; van Groen & Wyss, 1990a, b; Wright et al., 2010), or indirectly primarily through mammillary bodies (Watanabe & Kawana, 1980; Seki & Zyo, 1984), but also the retrosplenial cortex (van Groen & Wyss, 2003; Wright et al., 2010). In turn, the anterior thalamus can project directly to the hippocampus (Wyss et al., 1979; van Groen & Wyss, 1990b, 1995) or indirectly through the retrosplenial cortex (Shibata, 1993b; van Groen & Wyss, 2003). These connections

are highly organised along the dorsoventral and rostrocaudal dimensions with different populations of neurons in each region giving rise to different pathways between the hippocampus and the anterior thalamic nuclei, which suggests that these parallel neural circuits may be involved in different aspects of learning and memory (Vann et al., 2007; Wright et al., 2010; Yoder & Taube, 2011).

Evidence of the importance of the anterior thalamus in memory processes

Lesion Studies in animals

The ability to model episodic memory in animals has proved to be difficult. First, by definition, Tulving (1983) believed that episodic memory requires a conscious recollection, and as a result is unique to humans or at least uniquely demonstrated in humans. Second, the mental life of animals can only be inferred from their behaviour. Nevertheless, animal models are capable of assessing the animal's ability to learn and remember certain components, or combination of components, of episodic-like memory (Clayton & Dickinson, 1998; Clayton, Bussey, Emery, & Dickinson, 2003; Eacott, Easton, & Zinkivskay, 2005; Iordanova, Good, & Honey, 2008): *where* (spatial memory), *when* (temporal order memory), and *what* (recognition memory). One advantage of using animal models of episodic-like memory is the ability to create discrete lesions to particular regions of the brain in order to assess whether the region is necessary for accurate task performance. The following reviews whether the anterior thalamic nuclei are necessary for components of episodic-like memory in animals with either excitotoxic or electrolytic lesions.

Recognition memory: what

The ability to recognise whether an item (e.g., person, object, picture, odour, sound) has been previously encountered is a key component of episodic memory. Early studies in monkeys with lesions to the medial thalamus found deficits in delay non-match to sample tasks using trial unique objects (Aggleton & Mishkin, 1983a,b). In this task, there is both a sample and a test phase. During the sample

phase, the monkey is presented with an object (Object A). Following a delay period, the test phase begins, where the monkey is presented with a copy of Object A (familiar object) and a novel object (Object B). The animal must remember which object it has previously seen during the sample phase (in this example Object A), and select the novel object to receive a food reward. Animals with lesions that targeted the anterior medial thalamus (centred on the anterior thalamic nuclei) and animals with lesions centred on the posterior medial thalamus were both impaired on object recognition (Aggleton & Mishkin, 1983a). However, it was also noted that combined damage of these regions increased the magnitude of the deficit (Aggleton & Mishkin, 1983b). Although these two studies demonstrated the importance of the thalamus for the accurate detection of visual novelty, the surgical procedure involved damage to both the fornix and the mammillothalamic tract, which resulted in atrophy within the mammillary bodies. To test the influence of this additional damage, a single control monkey received a sagittal split of the fornix (which was required during the thalamic surgical procedure), and the mammillothalamic tract was cut in a second control monkey. The monkeys were either unimpaired (fornix damage) or had mild deficits that were far less severe than those observed after thalamic damage (mammillothalamic tract; Aggleton & Mishkin, 1983a, b).

In contrast to the experiments using monkeys, studies in rats with damage to the anterior thalamus have failed to find object recognition impairments (Aggleton, Neave, Nagle, & Hunt, 1995; Warburton & Aggleton, 1999; Wilton, Baird, Muir, Honey, & Aggleton, 2001; Moran & Dalrymple-Alford, 2003; Mitchell & Dalrymple-Alford, 2005). These tasks have relied on normal rats' spontaneous preference to explore novel objects (Ennaceur & Delacour, 1988; Dix & Aggleton, 1999). There are several advantages to using spontaneous object recognition. For example, the task is simple to run, and the animals are not required to learn a rule. In spontaneous object recognition tasks, the animal is placed inside an arena where a pair of identical objects is presented (A, A). After a given amount of time to explore the objects (sample phase), the rat is removed for a delay period. The test phase begins when the rat is returned to the arena and is now allowed to explore a copy of the familiar object (A) and a novel object (B). Normal animals

spend significantly more time exploring the novel object (B) compared with the familiar one (A). Therefore, an animal that explored both objects equally is assumed to have failed to recognise that one of the two objects is familiar (or is novel). Damage to the anterior thalamic nuclei does not impair spontaneous object recognition regardless of whether the objects are small and complex, or much larger painted boxes (Warburton & Aggleton, 1999). Rats with lesions to the anterior thalamic nuclei were also unimpaired on an odour recognition task (Wolff, Gibb, & Dalrymple-Alford, 2006). In this task, the rats had to either dig inside the odourised sand cup that was not presented during the sample phase (i.e., non match-to-sample), or dig inside the cup with the familiar odour (i.e., match-to-sample).

Temporal order memory: when

Although there is evidence that the "extended hippocampal memory system" is important for recency judgments (Fortin et al., 2002; Kesner et al., 2002; Charles, Gaffan, & Buckley, 2004; Kesner, Hunsaker, & Ziegler, 2010), very few studies have examined the role of the anterior thalamus. One study (Mitchell & Dalrymple-Alford, 2005) found that damage to the anterior thalamic nuclei did not impair the normal preference of rats to explore preferentially the old item on a recency judgment task where one pair of items presented two hours prior to the test phase was compared with a different set of objects present one hour prior to the test, i.e. more recently (Mitchell & Dalrymple-Alford, 2005). However, rats with damage to the anterior thalamus were impaired when required to learn which one of two odours was presented earlier in a list of six different odours (Wolff et al., 2006). If the rat correctly dug in the cup containing the odourised sand presented earlier in the list, it would receive a food reward.

A third study (Aggleton, Amin, Jenkins, Pearce, & Robinson, 2011) tested whether rats with anterior thalamic damage could learn the sequence of pairs of stimuli. In this task, the rats were placed inside an operant chamber and were required to learn the sequence of six auditory-visual stimulus compounds that were reinforced if the compound was presented in a particular order (e.g., reinforce if A occurs before B, but not if B occurs before A). Anterior thalamic

damage did not impair sequence learning assessed by this task (Aggleton, Amin et al., 2011). This task differs in many ways from spontaneous recency tests, including the use of multiple test trials and specific reward contingencies.

There are also several spatial memory tasks with high temporal interference, which may also tax order recency. The most common spatial recency task used following damage to the anterior thalamic nuclei is reinforced T-maze alternation.   In this task, the rat is allowed to explore one of the two choice arms of the maze by blocking the other arm (i.e., sample phase). At test, both arms are accessible, however, only the arm not visited during the sample phase contains a food reward (i.e., the rat has to alternate to obtain a reward). Although this task is typically considered to tax spatial learning, the rats are given multiple trials per day where they constantly enter the same two arms.   As a result, the rats are rewarded for selecting the arm experienced less recently in time (i.e., not select the arm during the sample phase). Rats with anterior thalamic damage are severely impaired on this spatial recency task (Aggleton et al., 1995, Aggleton, Hunt, Nagle, & Neave, 1996; Aggleton, Poirier, Aggleton, Vann, & Pearce, 2009; Aggleton, Amin et al., 2011; Warburton, Baird, & Aggleton, 1997; Warburton & Aggleton, 1999). This result is so consistent that the T-maze alternation task is often used to assess the effectiveness of the lesion prior to months of other behavioural testing.

Rats with lesions to the anterior thalamic nuclei were also impaired on a delay non-match to position task in operant chambers (Aggleton, Keith, & Sahgal, 1991). During the sample phase, the rats were presented with one of two levers (one to the left and one to the right of a food pellet dispenser). At test, the rats were rewarded for selecting the lever that was not presented during the sample phase in order to get a food reward. The animals were not impaired when the delay between the sample and the test was 'zero' seconds, reflecting the post-operative retention of the task rules. However, there was a delay dependent impairment.   It is again possible, given the number of trials, that the deficit observed reflects recency processes (Aggleton et al., 1991). However, when earlier trials were compared with later ones, the accuracy of the rats with damage to the anterior thalamus was greater for the later trials; a recency process would predict

a decline in performance. The impairment observed appears, in this experiment, to relate its spatial mnemonic aspects.

These results are supported by a different study (Beracochea, Jaffard, & Jarrard, 1989) where rats with anterior thalamic nuclei lesions were trained on a go/no-go task on a straight alley. When the rats ran along the track in one direction (e.g., left to right), they received a reward; however, when the rats ran along the track in the opposite direction (e.g., right to left), there was no reward. The assumptions is that the rats would run faster on reinforced trials compared with non-reinforced trials as they alternated back and forth along the track (i.e., temporal alternation task). Rats with anterior thalamic lesions required less training trial to reach criterion (discrimination ratio 1.5) compared with sham rats when the intertrial interval between runs was 15 seconds. The mean discrimination ratio of the final three sessions was not significantly different between the anterior thalamic damage group and the control group. However, when the intertrial interval was increased to 45 seconds, the performance of the rats with damage to the anterior thalamus decreased significantly more than the sham rats. The results suggest that anterior thalamic damage impairs the maintenance of information over time (Beracochea et al., 1989). Given that the intertrial intervals were held consistent within a session (i.e., for both 15 and 45 seconds), it is unlikely that recency impairments account for the deficits observed. Recency impairments would predict that rats with anterior thalamic lesions would perform significantly worse under conditions of higher interference (i.e., lower discrimination ratio in 15s delay condition compared with the 45s delay) compared with control rats. Similar to the T-maze alternation and the delay non-match to position tasks described, under certain conditions, the eight-arm radial maze may also reflect some recency memory processes since there is also high temporal interference.

There are numerous differences between all these tasks and studies. Some tasks examine the spontaneous preference of normal rats to explore older items (Mitchell & Dalrymple-Alford, 2005), whereas other experiments require the rats to learn associative rules in order to obtain a food reward (e.g., Wolff et al., 2006). Furthermore, the material presented to rats differs. On some tasks, the rats are

discriminating between objects (Mitchell & Dalrymple-Alford, 2005), light-sound compounds (Aggleton, Amin, et al., 2011), odours (Wolff et al., 2006), and places [usually relying on distal visual cues (e.g., Aggleton et al., 1995), but not always (Aggleton et al., 1991)]. The difficulty of the task as well as the delays between the presentations of the stimuli also varies across all these studies. These methodological differences may account for the inconsistent results following anterior thalamic lesions on recency tasks (e.g. Aggleton et al., 1991; Mitchell & Dalrymple-Alford, 2005; Wolff et al., 2006; Aggleton, Amin et al., 2011). All these different methodological procedures emphasise the need to re-examine the importance of the anterior thalamic nuclei for temporal order memory by designing tasks that systematically manipulates some of these differences one at a time while holding the others constant. For example, testing the rats on the same temporal task, and comparing their performance when objects or when odours are used as stimuli.

Spatial learning: where

The most consistent deficit found following damage to the anterior thalamic nuclei relates to spatial learning. As mentioned above, lesions to the anterior thalamus severely impair T-maze alternation (e.g., Aggleton et al., 1995, 1996, 2009; Aggleton, Amin et al., 2011). Mair, Burk, and Porter (2003) also found that rats with damage to the anterior thalamic nuclei were impaired on a delayed non-match to sample on the eight arm radial maze. The rats were presented with a random arm during the sample phase. At test, the rat was presented with two choice arms: the same arm that was previously visited during the sample phase and a different arm. Because the sample and test arms were chosen randomly, this procedure forced the animals to rely on spatial cues to solve the task, preventing the animals from solving the task with a response strategy (e.g., always going to the adjacent arm on the right; i.e., circling through the maze). Damage to the anterior thalamic nuclei also severely impairs spatial working memory, as examined more conventionally in the radial arm maze (Aggleton et al., 1996; Byatt & Dalrymple-Alford, 1996; Mitchell & Dalrymple-Alford, 2005, 2006; Sziklas & Petrides, 1999, 2007). This ability to remember previously visited arms depends

upon the cholinergic innervations into the anterior ventral nucleus of the thalamus (Mitchell, Dalrymple-Alford, & Christie, 2002).

Furthermore, animals with anterior thalamic lesions have impaired spatial reference memory, i.e., they are impaired at learning spatial problems that are constant across trial, such as navigating to the same location (Sutherland & Rodriguez, 1989; Warburton & Aggleton, 1999; Wilton et al., 2001; van Groen, Kadish, & Wyss, 2002; Loukavenko, Ottley, Moran, Wolff, & Dalrymple-Alford, 2007; Wolff, Gibb, et al., 2008; Wolff, Loukavenko, et al., 2008). For example, rats with anterior thalamic damage have much higher latencies to find a hidden platform in the Morris watermaze task compared with control rats (Sutherland & Rodriguez, 1989; Warburton & Aggleton, 1999; Wilton et al., 2001; van Groen et al., 2002). Rats with damage to the anterior dorsal and lateral dorsal thalamic nuclei were impaired on a watermaze task that could be solved using either extramaze (distal spatial) cues or a landmark located at a fixed distance and position from the platform (Wilton et al., 2001); the results suggest that the animals not only fail to use distal spatial cues, but also are impaired with directional information.

Rats with damage to the anterior thalamic nuclei are also unable to learn about the geometrical properties of a rectangular maze (Aggleton et al., 2009). In this task, the animals had to find one of two hidden platforms located in two of the four corners of the rectangle. The two 'correct' corners with the platform were geometrically identical to each other with, for example, the long wall to the left of the short wall when the animal is facing towards the corner. During a 60 second probe test where the platforms were removed, the rats with anterior thalamic damage spent the same amount of time in the correct compared with the incorrect corners; whereas the sham group spent significantly more time in the correct corners (Aggleton et al., 2009).

The nature of the spatial deficits following anterior thalamic lesions has been further qualified as including an impairment with using allocentric spatial cues. Allocentric spatial learning requires the animals to use the spatial arrangement of distal cues to gain location information, and contrasts with egocentric spatial learning, where direction information is determined relative to

the animal's body; e.g., turn to the right. Lesions to the anterior thalamic nuclei do not impair egocentric learning (Aggleton et al., 1996; Warburton et al., 1997; Sziklas & Petrides. 1999; Mitchell & Dalrymple-Alford, 2006; Wolff, Gibb et al., 2008.).

Not all studies have found severe spatial deficits after anterior thalamic nuclei lesions (Greene & Naranjo, 1986; Beracochea et al., 1989; Beracochea & Jaffard, 1994). One study found that spontaneous alternation in the T-maze was intact in mice with damage to the anterior thalamus, and these animals were only impaired when the mice were given a choice test 6hrs after exploration of the sample arm (Beracochea & Jaffard, 1994). Beracochea et al. (1989) also failed to find spatial working memory impairments in animals with anterior thalamic lesions on a temporal alternation task in a linear alley when the intertrial interval was short (15 seconds), but they were impaired at a longer, 45 second, interval. In another experiment, rats were placed in the centre of a radial maze and allowed free access to all the arms (Beracochea et al., 1989). Rats with anterior thalamic damage did not make significantly more re-entries into a previously visited arm (i.e., an error) compared with control rats. However, on this task, the rats were allowed to freely access all arms simultaneously. It is unclear whether the rats were unimpaired because of a low mnemonic load (i.e., 'zero' delay), or because the rats adopted response strategies (e.g., always go to the adjacent arm on the right of the current arm; Beracochea et al., 1989). A third study using a self-return T-maze, where the animal could leave the choice arms through a one-way door that returned the rat to the start arm of the T-maze, also did not find impaired alternation (Greene & Naranjo, 1989). However, in this last study, lesions were either to the anterior ventral or to the anterior medial nuclei, and it is more likely that impaired performance would have followed damage to all three nuclei (Greene & Naranjo, 1989). van Groen et al. (2002) demonstrated that for severe and long-lasting reference memory impairments in the watermaze, all three nuclei must be damaged; although less severe spatial deficits were observed in rats with damage to the anterior dorsal and anterior ventral nuclei (i.e., intact anterior medial thalamic nucleus; van Groen et al., 2002). In addition, rats alternating in the self-return T-maze may be relying on egocentric strategies (Greene & Naranjo,

1989), and damage to the anterior thalamic nuclei does not impair egocentric spatial learning (Aggleton et al., 1996; Warburton et al., 1997; Sziklas & Petrides. 1999; Mitchell & Dalrymple-Alford, 2006; Wolff, Gibb et al., 2008.).

Additionally, recent research has found that rats with anterior thalamic damage housed in enriched environments are not significantly different from sham animals when required to find a submerged platform from different start locations in a watermaze (Wolff, Loukavenko, Will, & Dalrymple-Alford, 2008). Environmental enrichment also ameliorated spatial working memory impairments on the T-maze alternation task following anterior thalamic damage (Loukavenko et al., 2007); however, enrichment did not improve the performance of rats on a spatial discrimination task in the radial maze where rats were rewarded for choosing one of three arms that differed in the amount of spatial separation (Loukavenko et al., 2007).

Taken together, the literature suggests that anterior thalamic lesions can impair learning about the arrangement of distal spatial cues, i.e., allocentric learning (for example, Aggleton et al., 1996; Byatt & Dalrymple-Alford, 1996; Warburton et al., 1997; Mair et al., 2003; Sziklas & Petrides, 1999, 2007; Loukavenko et al., 2007), geometric properties of environments (Aggleton et al, 2009), and directional information (Wilton et al., 2001). The implication is that more than one spatial process is disrupted. However, under certain conditions environmental enrichment may ameliorate these spatial memory impairments (Loukavenko et al., 2007; Wolff, Loukavenko et al., 2008).

Complex spatial associative learning: what-where

In addition to examining the effects of anterior thalamic damage on components of episodic memory individually, research has also focused on whether rats with lesions to the anterior thalamus are able to form complex associations typically, but not limited to, binding together the *what* and *where* components. When these two components are associated, they potentially create spatial scenes or "mental snapshots" (Gaffan, 1991; Aggleton & Pearce, 2001). It has been argued that "mental snapshots" (or scenes) played in sequence (i.e., in a specific temporal order) underlies episodic memory (Gaffan, 1991, 1994; Aggleton & Pearce, 2001).

Rats with damage to the anterior thalamic nuclei have been studied on two types of tasks that bind *what-where:* associative recognition and biconditional learning.

Associative recognition

Associative recognition refers to the detection of novelty that arises not from novel individual items, but rather from the novel arrangement or configuration of familiar items. The ability of rats with anterior thalamic damage to recognise changes in the arrangement of familiar stimuli has been explored using two different procedures (Gaffan, Bannerman, Warburton, & Aggleton, 2001; Wilton et al., 2001). Wilton et al. (2001) placed rats with damage to the anterior dorsal and lateral dorsal thalamic nuclei (AD/LD) inside an open field arena with four different objects (A1, B1, C1, D1) placed near each corner. The rats were allowed to explore these objects for 5 minutes. During the retention period (6 minutes), the four objects were replaced with identical copies (i.e., all the objects were familiar), but the location of two of the objects swapped (A2, B2, D2, C2); thus, creating novel spatial arrangement of familiar stimuli. Normal animals prefer exploring objects found in the novel location in contrast to the familiar locations; however, rats with lesions to AD/LD spent the same amount of time exploring the objects in familiar locations and those in the novel locations (Wilton et al., 2001). As mentioned above, these animals had no difficulty in discriminating between familiar and unfamiliar objects (Wilton et al., 2001).

In a second experiment, rats with anterior thalamic damage were required to discriminate between scenes composed of three different shapes projected onto computer monitors (Gaffan et al., 2001). One of the scenes was constant (remained the same across 80 trials) and was not rewarded. The second scene, called the variable scene, was always rewarded. There were several different types of variable scenes: 1) where both the position and shapes on the scene were different to the constant scene, 2) where the shapes were the same, but their position on the screen was different to the constant scene, 3) where the shapes were different, but their location on the screen was the same to the constant scene, and 4) where the shapes and the position on the screen was the same, but their arrangement was different to the constant scene. This last manipulation, most

closely resembles associative recognition task in the open arena by Wilton et al. (2001). The results indicate that not only were the rats with anterior thalamic damage able to discriminate between the constant and variable scenes in each of the four conditions compared with a sham control group, but in the condition where the shapes changed (#3) and in the condition where the same shapes were rearranged (#4), they were significantly better than the sham group (Gaffan et al., 2001).

There are several differences between these two tasks. One task relied on the spontaneous preference of normal rats to explore changes in locations of novel items (Wilton et al., 2001), whereas the other task required the animals to learn an associative rule (Gaffan et al., 2001). As a result the task by Gaffan et al. (2001) required numerous training trials which could have influenced behavioural performance.

Intact scene learning in rats with anterior thalamic damage (Gaffan et al., 2001) is also inconsistent with a previous study which found that monkeys with anterior thalamic lesions are impaired on object-in-place memory (Parker & Gaffan, 1997). In this task, monkeys were required to learn which one of two objects was rewarded in a unique scene by pressing the correct (rewarded) object on a touch screen. Given that monkeys with anterior thalamic damage can discriminate between two stimuli (Aggleton & Mishkin, 1983b), these results suggest that the unique scene (context) where these objects are located is encoded along with choice stimuli, and that monkeys with anterior thalamic damage are unable to either: a) ignore the scene, or b) use the scene to help solve the task. This led Parker & Gaffan (1997) to conclude that the role of the anterior thalamic nuclei is "to form a memorial representation of objects and their associated contexts," which they suggest is critical for episodic memory (Gaffan, 1994; Parker & Gaffan, 1997).

However, when rats were tested on their ability to discriminate between visual stimuli that differed in their structure (i.e., structural learning; AB+, BA-, BC+, CB-, CA+, AC-; see Figure 1.3), rats with lesions to the anterior thalamic nuclei were unimpaired (Aggleton et al., 2009). In structural learning, not only must two

separate items (or elements) be associated together, but their spatial (and temporal) arrangement is also critical. As structural learning is a process hypothesised to be critical for the formation of unique representations, scenes, or "mental snapshots" (Aggleton & Pearce, 2001; Sanderson, Pearce, Kyd, & Aggleton, 2006; Aggleton, Sanderson, & Pearce, 2007; Aggleton et al., 2009), it remains unclear under which conditions animals with anterior thalamic lesions are impaired during associative recognition tasks (Parker & Gaffan, 1997; Gaffan et al., 2001; Aggleton et al., 2009). However, one consistent feature of tasks where anterior thalamic lesions do not impair associative recognition is that they require extensive training (Gaffan et al., 2001; Aggleton et al., 2009). In contrast, rapid learning appears more consistently affected (Parker & Gaffan, 1997; Wilton et al., 2001; see Table 1.1).
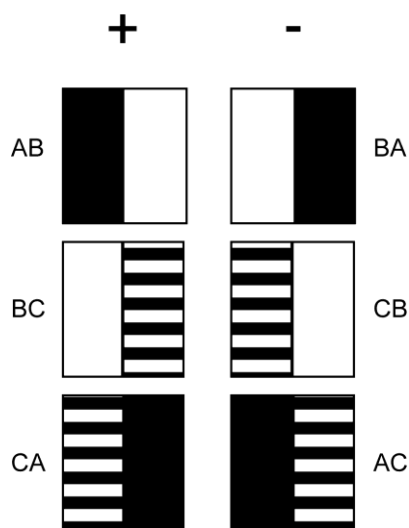
Figure 1.3. An illustration of the stimuli used during a structural learning task in the watermaze (Aggleton et al., 2009). The + indicates the reinforced stimuli (i.e., the stimuli that predicted the location of a submerged platform); whereas the – indicates the non-reinforced stimuli (i.e., no platform). The rats were presented with pairs of stimuli that differed in their structure (e.g., AB+ vs. BA-).

Biconditional learning

Animals with anterior thalamic damage have been tested on a variety of biconditional learning tasks (also called conditional learning tasks, conditional associative learning tasks, and paired-associate learning; Sziklas & Petrides 1999; Chudasama et al., 2001; Ridley, Maclean, Young, & Baker, 2002; Sziklas & Petrides, 2004; Gibb et al., 2006; Sziklas & Petrides, 2007; see Table 1.1). On these tasks rats are required to learn that stimulus A is associated with X, but not Y; whereas stimulus B is associated with Y and not X (i.e. AX+, AY-, BX-, BY+). Lesions to the anterior thalamus impaired learning a *spatial-visual* biconditional task, where the

animals had to learn to choose one of two objects depending on whether they were located at the North end or South end of an open arena (Sziklas & Petrides, 1999); thus demonstrated that the anterior thalamic nuclei are important for learning associations between spatial scenes (or places) and objects embedded within them. Anterior thalamic lesions also prevented rats from learning which odour (cinnamon, cumin) was associated with one of two spatial locations (Gibb et al., 2006). Because these tasks require extensive training, they indicate that behavioural deficits on some types of learning tasks can persist following damage to the anterior thalamic nuclei.

Lesions to the anterior thalamic nuclei did not impair formation of biconditional associations between objects and body turns, e.g., if Object A is presented, turn left; if Object B is presented, turn right (Sziklas & Petrides, 1999, 2004). Similarly, anterior thalamic lesions did not impair the formation of associations between a visual stimuli presented in centre of a touchscreen and a response to either the left or right window, e.g., if Stimulus A, nose poke to the left; if Stimulus B, nose poke to the right (Chudasama et al., 2001). Consistent with these results, when monkeys were required to choose the stimulus on the left when two copies of object A (A1, A2) are presented, but choose the stimulus on the right when two copies of object B (B1, B2) are presented, they were also unimpaired following anterior thalamic damage (Ridley et al., 2002). However, when the anterior thalamic damage was combined with medial dorsal lesions, this group of monkeys were significantly impaired compared with control animals. Anterior thalamic damage also did not impair learning of a visual-spatial biconditional task where an object is associated with a place (i.e., if Object A, go to Place X; if Object B got to Place Y) that cannot be solved by egocentric responses (or body turns; Sziklas & Petrides, 2007); this latter result puts into question the contribution of the anterior thalamic nuclei to the formation of associations between spatial locations (or scenes) and objects embedded within them.

Complex non-spatial associative learning

Other studies have examined whether animals with anterior thalamic lesions are impaired on complex non-spatial associative learning tasks (Ridley et al., 2002;

Ward-Robinson et al., 2002; Moran & Dalrymple-Alford, 2003). For example, Ward-Robinson et al. (2002) examined whether damage to the anterior thalamic nuclei would impair sensory precondition. In sensory preconditioning, the rat is exposed to pairs of stimuli (AX, BY). Then the value of one of the stimuli (e.g., X) changes (usually paired with an aversive unconditional stimulus such as a mild foot shock). If the animal has correctly associated (or integrated) the representation of AX, then the animal's behaviour should change to the presentation of A, but not B. In two experiments Ward-Robinson et al. (2002) presented rats with anterior thalamic damage and sham rats with this type of associative problem: In the first experiment, the rats combined auditory-thermal stimuli (e.g., tone sounds in a warm operant chamber; clicker sounds in a cool operant chamber), and after several presentations the value of one of the auditory stimuli was changed by pairing it with a mild foot shock (e.g., tone followed by shock). Rats with lesions to the anterior thalamic nuclei increased freezing behaviour to the correctly associated thermal operant chamber, and did not differ from the sham group (Ward-Robinson et al., 2002).

In a second experiment, the rats with anterior thalamic damage were also unimpaired compared with the sham group when the stimuli consisted of mixed flavours (Ward-Robinson et al., 2002). In this task, four different flavours in deionised water were used: sucrose, sodium chloride, quinine hydrochloride, and hydrochloric acid. The rats were presented with mixed flavours sucrose-hydrochloride (AX) or sodium chloride-quinine hydrochloride (BY) on separate days. After several days of presenting the mixed flavoured water, the value of one of the flavours was changed by pairing it with the experience of nausea (e.g., hydrochloride, X, is followed by an injection of lithium chloride which induces nausea in rats). Similar to the control group, rats with anterior thalamic lesions drank significantly more of the flavoured water not paired with the revalued flavour (i.e., sodium chloride; B) compared with the flavour that was paired with the revalued flavour (i.e., sucrose; A) during two 60 minute test sessions (Ward-Robinson et al., 2002). These results demonstrate that anterior thalamic lesions do not impair the acquisition of sensory preconditioning.

In a second study (Moran & Dalrymple-Alford, 2003), rats were trained to learn that a food reward was at the end of some of the arms of a radial maze containing different visual-textured floors (e.g., A+, B+, C-, D-). Following initial training, the visual-textured floors were combined (AB, CD), and the rats had to learn that the individual floor cues that were reinforced were not reinforced when combined together (negative patterning; A+, B+, AB-), while the non-reinforced floor cues were reinforced when combined together (positive patterning: C-, D-, CD+). Rats with anterior thalamic lesions were unimpaired on this task.

Overall, the behavioural results from studies with animals with anterior thalamic damage find that they are severely impaired in spatial learning and memory tasks, and when allocentric spatial information is associated other item information (*what-where*) with some exceptions (Sziklas & Petrides, 2007; see Table 1.1). However, damage to the anterior thalamic nuclei does not impair the formation of complex associations involving non-spatial information (i.e., not impaired on *what-what* associations).

What aspects of spatial learning and memory are impaired following anterior thalamic damage?

One issue that is made more apparent with the study of spatial memory and complex spatial associative learning is the need for a greater understanding of what aspects of spatial processing are disrupted following anterior thalamic lesions. For example, on classical tests of spatial learning and memory (e.g., spatial working memory using the radial arm maze and T-maze; reference memory using the Morris watermaze), animals with anterior thalamic lesions may be impaired for several different reasons: 1) the animals can't remember where the goal location is (i.e., a spatial memory problem), 2) the animal knows where the goal location is but has difficulty navigating to it (i.e., a navigational problem), 3) the animal has difficulty inhibiting a previous response (i.e., a perseverative problem), or 4) the animal is unable to learn where the goal location is (i.e., a spatial discrimination, perceptual, problem). Some studies have found that rats with anterior thalamic lesions have shorter latencies to re-enter a previously visited arm in the radial arm maze (Byatt & Dalrymple-Alford, 1996; Mitchell &

Dalrymple-Alford, 2005) despite evidence that the total amount of time spent in the arms was the same between control and anterior thalamic (AV or AM damage) groups as well as equivalent performance of the groups during the early training in the radial arm maze. This pattern has led these researchers to suggest that anterior thalamic damage does not impair attentional, perceptual or motivational aspects of the task (Byatt & Dalrymple-Alford, 1996); although, group differences in latencies are not always noted (Mitchell & Dalrymple-Alford, 2006). Furthermore, the reduced latencies to revisit an arm may also be indicative of a response inhibition problem (i.e., perseverative problem). There is also evidence that rats with anterior thalamic lesions are hyperactive, which also may influence choice latencies (Byatt & Dalrymple-Alford, 1996; Jenkins, Vann, Amin, & Aggleton, 2004; Poirier & Aggleton, 2009).

The results from complex spatial associative learning tasks have found that anterior thalamic damage does not always impair the formation of *what-where* associations (Sziklas & Petrides, 1999; Gaffan et al., 2001; Wilton et al., 2001; Sziklas & Petrides, 2007; see Table 1.1). The types of spatial cues and processes as well as the *what-where* associative conditions that are impaired by anterior thalamic injury remain unknown. For instance, it is unclear whether anterior thalamic damage impairs the use of distal compared with proximal cues, the ability utilise salient cues to solve the task, or the ability to discriminate between stimuli that share common features (e.g., structural learning; Aggleton & Pearce, 2001). Anterior thalamic lesions may not impair learning about different contexts (knowledge of where the animal is when the features of the environments to be discriminated between are unique), but impaired for knowledge of a location within a context, i.e., distinguishing between places within a test room, where the same set of spatial cues are viewed from different perspective (Gaffan & Harrison, 1989; Aggleton & Pearce, 2001; Dumont, Petrides, & Sziklas, 2007).

Table 1.1. Summary of the studies that examined the effects of damage to the anterior thalamus on complex spatial associative learning tasks. The right column notes whether the animals with anterior thalamic lesions were impaired relative to control animals. All studies were conducted using rats unless otherwise stated.

Study	Task	Details	Extended training	Impaired
Aggleton et al., 2009	Visual structural learning	AB+, BA-, BC+, CB-, CA+, AC-	Yes	No
Chudasama et al., 2001	Visual conditional discrimination	If A, respond left If B, respond right	Yes	No
Gaffan et al., 2001	Scene discrimination: Choose variable scene not constant scene	Objects on variable scene differs from constant by: 1) Shape +position 2) Position 3) Shape 4) Rearranged	Yes	No Performed better than controls on 3) and 4)
Gibb et al., 2006	Odour-location paired-associate (biconditional) learning	If cumin in location 1, go If cumin in location 2, no-go If cinnamon in location 1, no-go If cinnamon in location 2, go	Yes	Yes
Parker and Gaffan, 2007†	Object-in-place	One of two choice items is rewarded on a unique background (scene)	No; the scenes are unique (8 trials/scene)	Yes
Ridley et al., 2002†	Visuospatial conditional task	If A, respond left If B, respond right	Yes	No
Sziklas and Petrides, 1999	Spatial-visual biconditional learning	If objects at North end of arena, choose A If objects at South end of arena, choose B	Yes	Yes
	Visual-motor biconditional learning	If Object A, go left If Object B, go right	Yes	No
Sziklas and Petrides, 2004	Visual-motor biconditional learning	If Object A, go left If Object B, go right	Yes	No
Sziklas and Petrides, 2007	Visual-spatial biconditional	If Object A, go to Place X If Object B, go to Place Y	Yes	No
Wilton et al., 2001*	Object-in-location recognition	Two out of four familiar objects swap locations Sample: A, B, C, D Test: A, B, D, C	No	Yes

Note: * = AD/LD lesion; † = subjects were monkeys

Electrophysiological recordings

A different approach to understanding the role of the anterior thalamic nuclei is through the use of electrophysiological recording of either single neurons or a population of neurons. Studies have made recordings in the anterior thalamus of intact animals (i.e., without lesions) sometimes awake and sometimes anaesthetized (Blair & Sharp, 1995; Taube, 1995; Knierim, Kudrimoti, McNaughton, 1995; Vertes, Albo, & Viana Di Prisco, 2001; Taube & Basset, 2003), but also within the extended hippocampal memory system when different regions of the system have been damaged (Golob & Taube, 1997; Goodridge & Taube, 1997; Blair, Cho, & Sharp, 1998, 1999). Electrophysiological recordings of the anterior thalamus has discovered two main properties: 1) head direction cells located primarily in the anterior dorsal nucleus (AD; Taube, 1995), and 2) theta-rhythmically firing neurons found most commonly in the anterior ventral nucleus (AV; Vertes et al., 2001).

Head direction cells in the anterior dorsal thalamic nucleus

Although head direction cells were first discovered in the postsubiculum (Ranck, 1984; Taube, Muller, & Ranck, 1990), they have also been reported in anterior dorsal thalamic nucleus (Taube, 1995), lateral dorsal thalamic nucleus (LD; Mizumori & Williams, 1993), the lateral mammillary nucleus (Stackman & Taube, 1998), the retrosplenial cortex (Chen, Lin, Green, Barnes, & McNaughton, 1994; Cho & Sharp, 2001), and the entorhinal cortex (Sargolini et al., 2006) among others (see Taube, 2007). Most of these regions form a functionally anatomically linked circuit (see Figure 1.1B and the neuroanatomy of the anterior thalamic nuclei section above; note that LD does not receive dense projections from the mammillary bodies, nor is it connected to any of the anterior thalamic nuclei; however, it does receive projections from the subicular, retrosplenial and the entorhinal cortices).   However, of all the regions containing head direction cells, the anterior dorsal thalamic nucleus may have the greatest proportion of head directions cells. Approximately, 60% of neurons in the anterior dorsal thalamic nucleus are head direction cells (Taube, 1995; 2007). Furthermore, lesions to the AD disrupts head direction signal in the subiculum (Goodridge & Taube, 1997),

whereas lesions to either the hippocampus (Golob & Taube, 1997) or the subiculum (Goodridge & Taube, 1997) do not affect the head direction cell activity in AD. However, head direction cell firing in AD is dependent upon intact lateral mammillary bodies, which in turn rely on the integrity of the dorsal tegmental nucleus of Gudden (Bassett, Tullman, & Taube, 2007); thus emphasising the bottom-up subcortical to cortical generation of head direction activity (Clark & Taube, 2012).

Head direction cells are neurons that fire when an animal is facing a particular direction on a horizontal plane (Taube et al., 1990). These cells fire regardless of the animals' spatial location, trunk position, linear speed, angular head velocity, and ongoing behavioural activity (Taube, 1995). Provided that the animal is facing a particular cell's preferred direction, the cell will continue firing with a progressive and linear decrease in firing rate the further away from this preferred direction (Ranck, 1984; Taube, 1995, 2007). Although, it was initially believed that volitional motoric inputs were necessary for these cells to discharge (Taube, 1995), more recent evidence suggests that both active and passive movement are encoded equally by head direction cells in AD (Shinder & Taube, 2011). Research has also demonstrated that the preferred orientation of the cells can be shifted by moving a salient cue or landmark (Taube, 1995, 2007; Clark, Harris, & Taube, 2012). Anterior dorsal thalamic head direction cells are also influenced by environmental boundaries (Clark et al., 2012). When a trapezoid shaped environment was rotated following an initial recording session where animals were allowed to freely explore the environment, head direction cells recorded in AD shifted their preferred firing direction by the same degree as the environment rotation. It is interesting to note that the results were more variable with the use of a rectangular shaped environment (Clark et al., 2012).

Research suggests that vestibular information is critical for head direction cell firing, whereas proprioceptive (and motor) and landmark (visual cue) information may serve to update the head direction signal (Stackman & Taube, 1997; Taube, 2007). The discovery of head direction cells in regions known to be important for spatial learning and memory (Taube et al., 1990; Chen et al., 1994; Stackman & Taube, 1998; Cho & Sharp, 2001) as well as connected to areas

important for spatial processes, e.g., the hippocampus (O'Keefe & Dostrovsky, 1971; O'Keefe, 1976) has led to the hypothesis that head direction cells may be critical for orientating behaviours during navigation (Taube, 1998; Muir & Taube, 2002; Wiener & Taube, 2005; Taube, 2007).

In addition, evidence that idiothetic cues (i.e., vestibular and proprioceptive) are important for head direction cell firing (Stackman & Taube, 1997; Muir et al., 2009) and that the preferred direction of head direction cells is maintained in the dark (Taube et al., 1990; Mizumori & Williams, 1993; Goodridge, Dudchenko, Worboys, Golob, & Taube, 1998; Yoder et al., 2011), has lead to the hypothesis that the head direction system is important for path integration (McNaughton, Chen, & Markus, 1991; Blair, Lipscomb, & Sharp, 1997; Golob & Taube, 1999; Kubie & Fenton, 2009). Path integration allows an animal to navigate in its environment by monitoring and integrating idiothetic cues (Mittelstaedt & Mittelstaedt, 1980). In other words, the animal uses idiothetic cues to calculate the direction and distance it has moved relative to a starting point. A reliance on self-generated motor cues may be especially important for navigating in conditions with ambiguous distal spatial cues, or when none are available at all (Whishaw, 1998; Yoder et al., 2011).

Theta-rhythmically firing cells in the anterior ventral thalamic nucleus

Theta-rhythmically firing cells in the anterior ventral thalamic nucleus were first recorded in anaesthetised rat (Vertes et al., 2001) and then also found in freely-moving rats (Tsanov, Chah, Wright et al., 2011). Approximately 75% of the cells in AV were found to be synchronous with the hippocampal theta rhythm in the anaesthetised rats (Vertes et al., 2001), whereas this percentage was reduced to approximately 23.7% in freely moving animals (Tsanov, Chah, Wright et al., 2011). Theta rhythmic firing activity has been found in several regions throughout the extended hippocampal system (e.g., Colom, Christie, & Bland, 1988; Kocsis & Vertes, 1994; Bland, Konopacki, Kirk, Oddie, & Dickson, 1995; Kocsis & Vertes, 1997; see also Vann & Aggleton, 2004). Additionally, there is evidence that subcortical regions are critical for generating and modulating theta (Kirk, Oddie, Konopacki, & Bland, 1996; Bassant & Poindessous-Jazat, 2001; Kocsis et al., 2001).

For instance, Kirk et al. (1996) found that inactivating the medial septum abolishes theta activity in the hippocampus and medial mammillary bodies. Others have suggested that ventral tegmental nucleus of Gudden either moderates (Kocsis et al, 2001) or generates (Bassant & Poindessous-Jazat, 2001) hippocampal theta (see Vann & Aggleton, 2004; Vann, 2009). There is evidence that the theta rhythmic firing in Gudden's ventral tegmental nucleus occurs 1-2 seconds prior to the onset of hippocampal theta (Bassant & Poindessous-Jazat, 2001). However, research also shows that the direct hippocampal input via the dorsal fornix, instead of indirect input via the mammillary bodies and mammillothalamic tract, is responsible for modulating AV theta synchrony (Tsanov, Wright et al., 2011). Taken together, the evidence suggests that a complex interaction between interconnected subcortical and cortical structures enable the network to fire rhythmically with hippocampal theta.

It has been repeatedly argued that theta-band activity is important for mnemonic properties, including processes related to episodic memory (Burgess, Maguire, & O'Keefe, 2002; Buzsaki, 2002, 2005). Theta activity may act to bind together neuronal assemblies in their appropriate temporal (*when*) and spatial (*where*) context to give rise to episodic memory. Different assemblies are linked together through theta oscillations which act as a temporal organiser, and together with Hebbian rules of synaptic plasticity may allow for activity from one assembly to jump to the next one in the sequence (Buzsaki, 2005). There is support from studies showing that plasticity between sequentially activated place cells occurs during theta oscillation (Mehta, Quirk, & Wilson, 2000). In addition, the loss of theta activity by temporary inactivating the medial septum impairs both spatial and non-spatial learning (Mizumori, Barnes, & McNaughton, 1990). Indeed Buzsaki (2005) argues that theta may be "the temporal means of navigation in both neuronal space during episodic memory and real space during self-motion."

In addition to neurons firing in the theta band (6-11Hz), there are also reports of head direction cells in the anterior ventral thalamic nucleus (Taube, 1995; Yoganarasimha, Yu, & Knierim, 2006; Tsanov, Chah, Vann et al., 2011), and recent research reports a population of head direction cells in the AV that firing rhythmically in the theta range (Tsanov, Chah, Vann et al., 2011). These cells are

called HD-by-theta, and exhibit the greatest theta-rhythm firing when the animal's head was facing its preferred orientation. These results suggest that AV is integrating information relating to direction and movement (Tsanov, Chah, Vann et al., 2011). Furthermore, stimulation of the dorsal fornix (direct hippocampal pathway) or of the mammillothalamic tract (indirect hippocampal pathway) with high- or low-frequency stimulation protocols found that these two neural inputs have opposing plasticity characteristics within AV; with the dorsal fornix pathway being more involved in long-term depression (LTD), whereas the mammillothalamic tract increased long-term potentiation (LTP; Tsanov, Vann et al., 2011). These findings provide evidence that the anterior ventral thalamic nucleus is not a simple relay system, but is actively integrating inputs from the mammillary bodies and the hippocampus, which are also brain regions known to have cells that oscillate within the theta band (Kocsis & Vertes, 1994; Bland et al., 1995; Kocsis & Vertes, 1997), and are involved in learning and memory (see Sziklas & Petrides, 1993; Aggleton & Brown, 1999; Vann, 2009).

Visualising brain activity ("imaging" studies)

Similar to electrophysiological recordings, "imaging" studies have been used to study the role the anterior thalamus in both the intact (e.g., Vann, Brown, & Aggleton, 2000; Amin, Pearce, Brown, & Aggleton, 2006; Yasoshima, Scott, & Yamamoto, 2007) and damaged brain (e.g., Jenkins, Dias, Amin, Brown, & Aggleton, 2002; Jenkins, Dias, Amin, & Aggleton, 2002; Jenkins, Vann et al., 2004; Caulo et al., 2005; Poirier & Aggleton, 2009). However, unlike electrophysiological recording where either a single neuron or a small population of neurons are recorded in a limited amount of brain regions, imaging studies allows for the activity of neurons in multiple brain areas (and whole brain) to be observed. Imaging is particularly useful for understanding network activity. In animals, activity at a single cell resolution can be observed with the use of immediate early genes (IEG). In humans, visualising the brain and its activity can accomplished through the use of several different magnetic resonance techniques [e.g., volumetric analyses, function magnetic resonance imaging (fMRI), diffusion tensor imaging (DTI)], while metabolic activity can be examined with the use of positron emission

tomography (PET). Research investigating the role of the anterior thalamus using these techniques will be described below.

Animals

What are immediate early genes?

One form of imaging in animals that can give single cell resolution concerns the expression of immediate early genes (IEGs). Immediate early genes are rapidly and transiently induced by neuronal activation without the need for *de novo* protein synthesis (Tischmeyer & Grimm, 1999). Once activated, IEGs can lead to downstream activation of other targets. There are two types of immediate early genes: 1) regulatory IEGs which either increase or decrease gene expression of downstream targets (e.g., *c-fos*, *c-jun*, *zif268*), or 2) effector IEGs which encode proteins that have a direct functional role in the cell, usually at the synapse (e.g., *Arc*, *BDNF*, *Homer 1a*). In addition, the protein products of regulatory IEGs are classified as inducible transcription factors (e.g., *c-fos*) as opposed to constitutive transcription factors (e.g., cAMP response element binding, CREB). Regulatory IEGs have low baseline presence in the cell, and their rapid expression is controlled by the constitutive transcription factors which are present in every cell, and are regulated by post-translational modifications, such as phosphorylation (Herdegen & Leah, 1998).

Two of the most widely studied IEGs are *c-fos* and *zif268*. Research has shown that both of these IEGs are induced by neurotransmitters and neurotropic substances following extracellular stimulation (Ghosh, Ginty, Bading, & Greenberg, 1994; Chaudhuri, 1997; Kovacs, 1998). The result of the extracellular stimulation is an influx of calcium into the cell, either through N-methyl-D-aspartic acid (NMDA) receptor complex or through voltage-sensitive calcium channels (VSCC) after glutamate binding or membrane depolarisation, respectively (see Figure 1.4). Calcium influx through VSCC results in CREB phosphorylation by a calmoduline kinase (CAM) pathway; whereas calcium influx through NMDA channel induces activation of the MAP-kinases pathway (Bading, Ginty, & Greenberg, 1993; Ghosh et al., 1994; Kovacs, 1998). Cyclic adenosine monophosphate (cAMP) can also activate *c-fos* and *zif268* through the phosphorylation of cAMP response element

(CRE) by protein kinase A (PKA; Sheng, McFadden, & Greenberg, 1990, but see Kovacs, 1998).
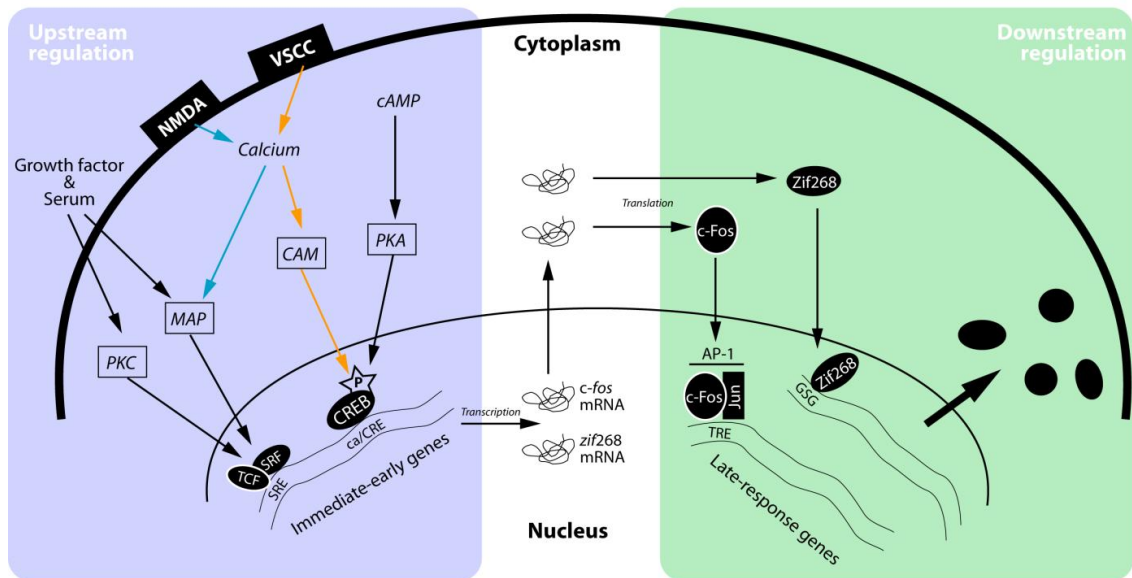
Figure 1.4. A schematic representation of the upstream (blue) and downstream (green) molecular pathways involved in the induction of the IEGs *c-fos* and *zif268* in neurons. Two different pathways both involving increases in intracellular Ca^{2+} levels that can lead to IEG transcriptions are differentiated using orange and blue arrows. This figure has been modified from Albasser (2009) based on Chaudhuri (1997) and Kovacs (1998). Abbreviations: AP-1, activator-protein-1; CAM, calmodulin kinase; cAMP, cyclic adenosine monophosphate; CRE, cAMP response element; CREB, cAMP response element binding; GSG, promoter sequence; MAP, mitogen-activated protein kinase; NMDA, N-methyl-D-aspartic acid; PKA, protein kinase A; PKC, protein kinase C; SRE, specific sequence; SRF, transcription factor; TCF, transcription factor; TRE, promoter sequence; VSCC, voltage sensitive Ca^{2+} channels.

Once *c-fos* and *zif268* are translated, the protein can return to the nucleus and either stimulate or repress other candidate genes by binding to a specific promoter sequence (TRE for AP-1; GSC for Zif269, see Figure 1.4). The protein c-Fos first forms a heterodimeric transcription complex with members of Jun protein family, which can then bind to activator-protein-1 (AP-1, Morgan & Curran, 1991; Kaminska, Kaczmarek, & Chaudhuri, 1996; Chaudhuri, 1997; Kovacs, 1998). The result is that both these IEGs can influence short- and long-term responses by regulating other late-response genes.

Immediate early genes are considered to have a critical role in the transformation of activity in neural circuits into long-term structural changes underlying memories (Lanahan & Worley 1998), and the IEGs *zif268, c-fos,* and *arc* (for example) have been linked to synaptic plasticity, learning, and memory (Cole, Saffen, Baraban, & Worley, 1989; Wisden et al., 1990; Guzowski, 2002; Davis,

Bonzon, & Laroche, 2003; Miyashita, Kubik, Haghigh, Steward, & Guzowski, 2009). For instance, *zif268* is induced in hippocampal neurons by the same stimuli that induce long-term potentiation (LTP). Furthermore, its activation (and that of LTP) is dependent on the activation of NMDA receptors (Cole et al., 1989). In addition, transgenic mice with removal (knockout) of the *zif268* or the *c-fos* gene show spatial memory impairments as well as impaired long-term potentiation (LTP; Jones et al., 2001; Fleischmann et al., 2003). Additionally, infusions of antisense oligodeoxynucleotides, which are short synthetic chains of DNA that bind to their target mRNA (the messenger RNA produced from the target IEG) and prevent its translation, confirm the importance of *c-fos* in learning and memory. For example, infusions of antisense against *c-fos* mRNA into the hippocampus has impaired consolidation of inhibitory avoidance learning (Guzowski & McGaugh, 1997), spatial learning in the water maze (Guzowski, 2002), and socially transmitted food preference (Countryman, Kaban, & Colombo, 2005).

While there is some evidence that *zif268* and *c-fos* can result in the same pattern of activation in certain brain regions (i.e., both IEGs will show increases and/or decreases in the same brain region following the same experimental manipulation; e.g., Jenkins, Amin, Pearce, Brown, & Aggleton, 2004; Albasser, Poirier, Warburton, & Aggleton, 2007; Poirier & Aggleton, 2009), there are many differences between these two IEGs. First, their basal rate is different with the immediate early gene *c-fos* having a lower basal rate compared with *zif268*. This difference is because 1) *c-fos* mRNA is less stable, and 2) c-Fos protein acts as an autoregulator by down regulating its own transcription. Another reason for differences between *zif268* and *c-fos* activity is because inducible IEGs are not present in every cell, and may not be expressed in some brain structures at all; thus IEG activity cannot be used as a general marker of neural activity (Chaudhuri, 1997). These differences had led to the hypothesis that *c-fos* reflects activity caused by novel stimuli (Chaudhuri, 1997), whereas *zif268* may reflect ongoing or the stabilization of activity following stimuli (Davis et al., 2003).

Immediate early gene activity in the anterior thalamus

One advantage of the use of IEG imaging is the ability to examine the changes in brain regions following behavioural manipulations, provided appropriate behavioural controls are used (Shires & Aggleton, 2008). Changes in the IEG activity in the anterior thalamus have been noted on behavioural tasks involving spatial memory (Vann, Brown, & Aggleton, 2000), changes in temporal configurations (Amin et al., 2006), and in an inhibitory avoidance task (Yasoshima et al., 2007). When rats were placed in a radial arm maze and were allowed to explore all eight arms compared with rats that always explored the same arm, all three of the anterior thalamic nuclei were engaged (Vann, Brown, & Aggleton, 2000). Similarly, when rats were tested on a spatial memory task in the eight-arm radial maze in a novel room compared with rats that were tested in the same room, *c-fos* activity was greater in all three anterior thalamic nuclei. In contrast, when animals were presented with different spatial cues in the morning and afternoon, with half the animals (Group Novel) experiencing a temporal mismatch of these cues at test in their familiar location, only the anterior ventral thalamic nucleus showed an increase in *zif268* activity. On this same task, there were no reported *c-fos* changes in the anterior thalamic nuclei (Amin et al., 2006). Similarly, when spatial arrangement of the cues changed for the experimental group (Group Novel), but not the control group (Group Familiar), there were no reported changes in *c-fos* activity in the anterior thalamus (Jenkins, Amin et al., 2004).

Immediate early gene activity in the rat brain following damage to the anterior thalamic nuclei

Another use of IEGs has been to examine the influence of damage to a brain region on activity within related structures. In other words, this allows for the observation of pathological effects away from the lesion site within a neural network. One aspect has been the investigation of covert pathology. The term refers to abnormal neural activity in the absence overt changes to the neural tissue as measured by standard histological procedures (e.g., Nissl stain). Damage to the anterior thalamic nuclei yields striking decreases in IEG activity as measured by *c-*

fos and *zif268* in the retrosplenial cortex (Jenkins, Dias, Amin, Brown et al., 2002; Jenkins, Dias, Amin, & Aggleton, 2002; Jenkins, Vann et al., 2004; Poirier & Aggleton, 2009).   Poirier and Aggleton (2009) further characterized this hypoactivity by demonstrating that the post-operative delay (i.e., the longer the animal lives with the lesion) influences the extent of the reduced IEG activity in the retrosplenial cortex: at short post-operative delays only the superficial layers of the retrosplenial cortex show a reduction in IEG activity; whereas both the superficial and deep layers have significantly reduced activity at longer post-operative delays.   Critically, this reduction in IEG activity occurs without any evidence of overt pathology as assessed by Nissl stain (Jenkins, Vann, et al., 2004; Poirier & Aggleton, 2009).   However, careful analyses of the Nissl stain sections have found increases in the number of cells as well as increases in cell area (i.e., subtle morphological changes) in the retrosplenial cortex following lesions to the anterior thalamic nuclei (Poirier & Aggleton, 2009). In addition to the retrosplenial cortex, damage to the anterior thalamic nuclei also results in reduced *c-fos* activity in the hippocampus (Jenkins, Dias, Amin, Brown et al., 2002; Jenkins, Dias, Amin, & Aggleton, 2002).   Previous reports have not, however, revealed any reductions in *c-fos* activity within the rhinal cortex following lesions to the anterior thalamus (Jenkins, Dias, Amin, Brown et al., 2002; Jenkins, Dias, Amin, & Aggleton, 2002). These results suggest that damage to the anterior thalamic nuclei leads to covert pathology within the "extended hippocampal memory system," but not within more distal regions such as the rhinal cortex, and that some of the behavioural deficits following thalamic lesions may be a result of abnormal activity within the extended hippocampal network (see Aggleton, 2008).

Humans

There are relatively few imaging studies using magnetic resonance techniques in normal participants reporting changes in the diencephalon. One reason is the lack of resolution required to detect a signal from individual nuclei within the thalamus (Montaldi, Spencer, Roberts, & Mayes, 2006; Aggleton, Dumont et al., 2011).

A different approach has been to examine network activity in patients with damage to the diencephalon. One study (Caulo et al., 2005) examined a non-

alcoholic Korsakoff patient using functional magnetic resonance imaging during face encoding, perception, and recognition. Unlike the eight control participants, this patient did not show hippocampal activation during either the encoding or recognition tasks. In addition, the ventrolateral prefrontal cortex had reduced activity during recognition compared with control participants; the results suggest that the recognition memory impairments may be a result of hypoactivity in these two brain areas despite the lack of overt pathology in either the hippocampus or the frontal cortex (Caulo et al., 2005). These results are consistent with findings from PET studies showing cortical hypoactivity following thalamic strokes (Baron et al., 1986; Clarke et al., 1994; Van der Warf et al., 1999) or following alcoholic Korsakoff's syndrome (Joyce et al., 1994; Reed et al., 2003).

Taken together, evidence from imaging studies in animals and humans converge to suggest that anterior thalamic damage can lead to covert pathology throughout the extended hippocampal memory system, and that memory impairments may in part be attributed to functionally abnormal cortical tissue far away from the site of overt pathology. In addition to the reduction in IEGs observed (e.g., Jenkins, Dias, Amin, Brown et al., 2002; Jenkins, Dias, Amin, & Aggleton, 2002; Jenkins, Vann et al., 2004; Poirier & Aggleton, 2009), the evidence for covert pathology within the cortex is also supported by animal studies showing that LTD, a measure of synaptic plasticity, is abolished in the retrosplenial cortex following lesions to the anterior thalamic nuclei (Garden et al., 2009). There is also evidence that damage to the anterior ventral thalamic nucleus impairs the maintenance and retention of discriminative avoidance learning in rabbits, and eliminates the excitatory discharges in retrosplenial cortex to the auditory conditioned stimuli (Gabriel et al., 1983). A microarray study found that anterior thalamic lesions led to lower levels of mRNAs involved in energy metabolism and neural plasticity in granular retrosplenial cortex (Poirier, Shires et al., 2008). Furthermore, anterior thalamic lesions disrupted behavioural increases of acetylcholine in the hippocampus (Savage, Hall, & Vetreno, 2011), and pyrithiamine-induced thiamine deficient rats which suffer damage to the diencephalon, also show cholinergic abnormalities in several cortical regions, including the frontal cortex, hippocampus, and retrosplenial cortex (Anzalone,

Vetreno, Ramos, & Savage, 2010). These results demonstrate electrophysiological, transcriptional, and neurotransmitter abnormalities in distal cortical sites following thalamic damage.

Rationale for experiments

The major objective of this thesis was to further understand and clarify the contributions of the anterior thalamic nuclei in rats to components of learning and memory thought to underlie aspects of episodic memory in humans (i.e., *what*, *when*, *where*). One goal was to understand better the amnesic effects reported following damage to the medial diencephalon.   While temporary inactivations of the anterior thalamic nuclei (i.e., cannulation studies) would provide insight into the acute effects of anterior thalamic pathology, patients with diencephalic damage have permanent atrophy in these regions. For this reason, lesions to the anterior thalamus in rats were used as a model of long-term pathology of this region.

The first part of this thesis re-examined the role of the anterior thalamus in temporal order judgments (*when*; Chapter 2). It was hypothesised that there are perhaps two different types of temporal learning tasks.   The first involves distinguishing the order between two separate events (between-block recency); whereas the second requires discriminating between the temporal order within a single event (within-block recency). In rats, between-block recency was defined as comparing items that occurred on two different lists that were separated by a delay (i.e., the rats were removed from the test arena during the delay); whereas within-block recency compared two items within a single list. In addition, the influence of different type of stimuli (object or odours) was assessed as previous studies differ along these dimensions (e.g., Mitchell & Dalrymple-Alford, 2005; Wolff et al., 2006).

The contributions of the anterior thalamic nuclei to object recognition (*what*) were examined in Chapter 3. Although the literature suggests that damage to the anterior thalamic nuclei does not impair recognition memory in rodents (Aggleton et al., 1995; Warburton & Aggleton, 1999; Wilton et al., 2001; Moran & Dalrymple-Alford, 2003; Mitchell & Dalrymple-Alford, 2005), these findings do not preclude the anterior thalamic nuclei from contributing to recognition memory in

the intact brain. To address this question, rats were given unilateral lesions to the anterior thalamus, and were either presented with a list of novel (Group Novel) or familiar (Group Familiar) objects. This procedure enabled an investigation of whether anterior thalamic damage can modulate immediate early gene activity (*zif268*) associated with object recognition.

The next three chapters (Chapters 4-6) sought to further elucidate the contributions of the anterior thalamic nuclei to spatial memory and complex spatial associative learning. Chapter 4 examined whether lesions to the anterior thalamic nuclei impaired the ability of rats to form complex associations between particular items and spatial or contextual cues (i.e., item-place or item-context associations) using biconditional learning tasks. A review of the literature indicated that rats with damage to the anterior thalamus are not consistently impaired on biconditional learning tasks (e.g., Sziklas & Petrides, 1999; Chudasama et al., 2001; Sziklas & Petrides, 2004; Gibb et al., 2006; Sziklas & Petrides, 2007). The goal of Chapter 4 was to manipulate the availability of certain cues (distal spatial cues and proximal contextual cues) to understand their contributions to performance following anterior thalamic damage.

One question that emerges from a review of a literature and from Chapter 4 is whether anterior thalamic damage impairs the ability of rats to distinguish between spatial locations, or whether the spatial impairments observed following damage to the extended hippocampal memory system reflect navigational problems (e.g., orientating and arriving at the correct location). The effects of anterior thalamic damage on spatial learning tasks that do not tax navigation were, therefore, examined (i.e., the rats were not required to navigate to a goal location). Rats with damage to the anterior thalamic nuclei or to the hippocampus were tested on their ability to discriminate between two spatial locations in a room that contained a variety of spatial cues by being placed in each location (Chapter 5). The rats had to learn to dig inside a cup to retrieve a food reward in Place 1 (go trials), and refrain from digging inside the cup when located in Place 2 (no-go trials). As spatial cues in these rooms were complex, the same cohort of animals with damage to the anterior thalamic nuclei was tested in tasks where the cue dimensions were more closely controlled. In these tasks rats were given place

discrimination tasks that differed along a single stimulus dimension: either the geometrical shape of the environment, or the arrangement of black and white walls of the maze (Chapter 6). In these tasks, the rats were either placed passively in the correct location (one of two identical correct corners) inside a watermaze during training or had to swim actively to the goal location. At test, the time spent swimming in the correct corners was compared to the time spent in the incorrect corners. It was hoped that examining the effects of anterior thalamic damage in simplified environments would provide clues as to the types of stimulus properties or processes these animals cannot resolve.

Chapter 2

Recency Judgments and the Anterior Thalamic Nuclei

Introduction

The anterior thalamic nuclei comprise a key part of the "extended hippocampal system" (Aggleton & Brown, 1999) and are involved in spatial memory processes (Aggleton et al., 1996; Byatt & Dalrymple-Alford, 1996; Parker & Gaffan, 1997; Sziklas & Petrides, 1999; Warburton & Aggleton, 1999; Warburton et al., 1999; Mitchell & Dalrymple-Alford, 2005; Sziklas & Petrides, 2007). For instance, damage to the anterior thalamic nuclei impairs spatial reference (e.g., Warburton & Aggleton, 1999) and spatial working memory (e.g., Aggleton et al., 1995; Mitchell & Dalrymple-Alford, 2006), but does not disrupt the ability of rats to discriminate between novel and familiar objects (i.e., intact recognition memory; Aggleton et al., 1995; Warburton & Aggleton, 1999; Moran & Dalrymple-Alford, 2003; Mitchell & Dalrymple-Alford, 2005, see also Aggleton, Dumont et al., 2011). However, relatively little research has examined whether these nuclei are critical for non-spatial recency judgments, an important component of episodic memory in humans. The role of the anterior thalamic nuclei for recency judgments are of additional interest as hippocampal lesions disrupt object recency judgments in rats (Fortin et al., 2002; Kesner et al., 2002; Kesner et al., 2010; Barker & Warburton., 2011; Albasser et al., 2012).

Previous research has reported conflicting results (Mitchell & Dalrymple-Alford, 2005; Wolff et al., 2006; Aggleton, Amin et al., 2011). For example, one study (Mitchell & Dalrymple-Alford, 2005) assessed recency judgments by allowing the rats to explore two sets of objects separated by a 60 minute delay. Following a second 60 minute delay after the second sample phase, the choice (test) phase began where the rats were presented with copies of the familiar

objects from each of the sample phases. The rats with lesions to the anterior thalamic nuclei, like normal rats, spent more time exploring the older objects; therefore, it was concluded that rats with damage to the anterior thalamic nuclei are not impaired on recency judgments (Mitchell & Dalrymple-Alford, 2005). In contrast, a second experiment (Wolff et al., 2006) presented rats with a list of six odourised sand cups. The rats were rewarded for digging inside the odoured cup that was presented earlier in the list. Lesions of the anterior thalamic nuclei severely disrupted the ability of rats to distinguish the temporal order of odours (Wolff et al., 2006).

Given these different results, it remains unclear whether lesions in the anterior thalamic nuclei have broad effects upon recency judgments as the procedures and tasks differed greatly across studies. One major difference between the two procedures is that in one study, the test items were separated by a delay where the rats were removed from the testing arena (Mitchell & Dalrymple-Alford, 2005); whereas the other study compared items that were presented one after the other (Wolff et al., 2006). The present study used the same behavioural task (the bow-tie maze) to examine the effects of these two different recency judgment procedures: 1) Between-block recency, where the items to be remembered are separated by a large delay and the animal is removed from the arena, creating two separate lists of items in what can be regarded as separate "blocks" (i.e., List 1 vs. List 2), and 2) within-block recency, where the temporal order of a list of items must be remembered (i.e., the order of the items within the same list was examined). In both cases, the rats' ability to recognise earlier compared with later items was examined.

It was hypothesised that the key difference between the conflicting results in the literature was the amount of temporal separation between the items at test (either caused by long delay or by greater number of interleaving items between the test items). Consequently, it was supposed that the greater the temporal separation between items, the easier the task, and the less likely rats with damage to the anterior thalamic nuclei would be impaired. Specifically, it was hypothesised that rats with damage to the anterior thalamus would be impaired on within-block recency tasks, i.e., they will not be able to discriminate which order items were

presented within the same list (Wolff et al., 2006) because of the low temporal separation between the test items, but animals with anterior thalamic damage would be unimpaired on the be between-block recency tasks, i.e., they will be able to discriminate when items occurred between two separate lists of items (Mitchell & Dalrymple-Alford, 2005).

In addition, the delays between the presentation of the two lists of items and the test phase in the between-block recency task were varied in order to determine whether temporal separation (i.e., the delay periods) between the two lists and between List 2 and the test stage could influence task difficulty. This manipulation also allows the examination of whether animals with anterior thalamic damage might be affected significantly more by changes in temporal separation compared with control animals. It was also hypothesised that for the within-block recency task, that rats would more consistently discriminate the older from more recent items when there was a larger number of intervening items between the two test items (i.e., greater temporal separation) compared with small number of interleaving items, and that rats with anterior thalamic lesion would be disproportionately affected by these temporal order judgments with a small number of interleaving items between the two test items. In addition, the type of item (odours or objects) could potentially influence performance as objects differ along several dimensions (shape, colour, texture, etc.), whereas the odours only differ along a single dimension (odour). In order to examine the impact of the type of item (odour or object) on recency judgments, the between-block and within-block recency experiments were conducted using objects and the rats were tested again in separate set of experiments using odours.

The bow-tie maze combines aspects of the delay non match-to-sample procedure (Mishkin & Delacour, 1975; Aggleton, 1985) with spontaneous recognition (Ennaceur & Delacour, 1988), and was originally developed as a spontaneous recognition task with multiple trials (Albasser, Chapman et al., 2010; Albasser, Poirier, & Aggleton, 2010). The bow-tie maze can be modified to examine both within-block (order of items within one list) and between-block (whether items were from List 1 or List 2) recency tasks. The performance of rats with anterior thalamic lesions on the standard recognition task in bow-tie maze

was also examined. In addition, the anterior thalamic damage rats' performance on the standard object recognition in the bow-tie maze was further compared with their spontaneous object recognition memory performance in an open arena (Ennaceur & Delacour, 1988). Finally, the animals were tested on a spatial recency (alternation) task in the T-maze which is known to be sensitive to damage to the anterior thalamic nuclei in order to test the effectiveness of the lesions using a task with high temporal interference (e.g., Aggleton et al., 1995, 2009; Aggleton, Amin et al., 2011).

Materials and Methods

Subjects

The subjects were 25 male hooded rats that weighed 270-320g at the beginning of the experiment (Harlan, Bicester, U.K.). The animals were housed in pairs under a 12-hour light/dark cycle. The animals were given free access to water, but were maintained at 85% of their free-feeding weight for the duration of the experiments. Fifteen rats sustained bilateral lesions of the anterior thalamic nuclei (ATNx1), and 10 control rats received sham (Sham1) surgeries. All animals were habituated to handling before the start of the first experiment. All experiments were performed in accordance with the UK Animals (Scientific Procedures) Act (1986) and associated guidelines.

Surgery

Surgery was performed under pentobarbitone sodium anaesthesia (60mg/kg i.p., Sigma-Aldrich Company Ltd, Dorset, UK). Once anaesthetised, the animal was placed in the head-holder of the stereotaxic apparatus (Kopf Instruments, CA, USA) with the incisor bar adjusted to +5.0 relative to the horizontal plane. Following an incision, the scalp was retracted to expose the skull. A craniotomy was made and the dura cut exposing the cortex above the target location. Lesions to the anterior thalamic nuclei were made by injecting 0.12M N-methyl-D-aspartic acid (NMDA; Sigma Chemicals UK) dissolved in sterile phosphate buffer (ph 7.4) over two separate sites within one hemisphere with the use of a 1-µl Hamilton syringe (Hamilton, Switzerland) that was attached to a moveable arm mounted on the

stereotaxic frame. The lateral and medial sites were infused with 0.22 μl or 0.24 μl of NMDA over a period of five minutes, respectively. The syringe was left *in situ* for an addition four minutes before being retracted. The lesion coordinates relative to bregma were anteroposterior -0.6; mediolateral ± 0.9 and ±1.8 from the midline; dorso-ventral -7.0 and -6.3 from bregma for the medial site and the lateral site, respectively. For the sham surgeries, the syringe was lowered to +0.2 above the target site for a few seconds, and then removed. Critically, no NMDA was injected in these rats. After the removal of Hamilton syringe, the incision was cleaned and sutured. A topical antibiotic powder (Aureomycin, Fort Dodge, Animal Health, Southampton, UK) was applied. The rats also received glucose-saline (5ml s.c.) for fluid replacement, and then placed in a recovery chamber until they regained consciousness (i.e., movement and righting reflex). Rats were given the analgesic Metacam (0.06ml s.c.; 5mg/ml meloxicam; Boehringer Ingelheim Vetmedica, Germany). A respiratory stimulant millophylline (0.1 ml s.c., Arnolds Veterinary Products, Shropshire, UK), an antimicrobial Baytril in their water (2.5%; Bayer Ltd, Animal Health Division, Ireland), and low dose of diazpeman (0.07ml s.c., 5mg/ml; CP Pharmaceuticals Ltd, UK) was administered to facilitate post-operative recovery as needed. All animals were monitored carefully until they had fully recovered.

Histology

Following behavioural testing, the animals were administered with an intraperitoneal injection of a lethal overdose of Euthatal (200mg/ml sodium pentobarbital, Marial Animal Health Ltd., Harlow, Essex, UK) and perfused intracardially with 0.1M phosphate buffer saline (PBS) followed by 4% paraformaldehyde in 0.1M PBS (PFA). The brains were extracted from the skull and placed on a stirrer to postfix in PFA for four hours, after which the brains were placed in 25% sucrose overnight. The brains were frozen on a microtome (Leica, UK) and sectioned at 40μm in the coronal plane. One-in-five sections were mounted and stained with cresyl violet, a Nissl stain. The remaining sections were divided into four (one-in-five sections) series, and were frozen (approximately -20°C) in cryoprotectant for later immunohistochemistry.

Immunohistochemistry for Neuronal Nuclei (NeuN)

One series of frozen sections from every ATNx1 and one Sham1 animal was subsequently used for NeuN immunohistochemistry. Because NeuN only stains neural cell bodies (Mullen, Buck, & Smith, 1992), visualisation of the lesion extent is often improved compared with Nissl stain, which labels both neurons and glial cells. The sections were washed for 10 minutes in PBS, four times. The sections were then rinsed in PBS containing 0.2% Triton X-100 (PBST) for 10 minutes, two times. The sections were then washed in 0.3% hydrogen peroxide in PBST for 10 minutes in order to block endogenous peroxidase activity, and rinsed four times for 10 minutes in PBST. Afterwards, the sections were incubated at 4°C for 48 hours in PBST with mouse anti-neuronal nuclei monoclonal antibody (NeuN; 1:5000, MAB377, lot number: 0703055636, Chemicon International). The sections were then rinsed for 10 minutes in PBST, four times. Following the four washes, the sections were incubated in biotinylated horse anti-mouse secondary antibody (diluted 1:200 in PBST; Vector Laboratories) and normal horse serum for two hours. The sections were washed again in PBST, and incubated for one hour in avidin-biotinylated horseradish peroxidase complex in PBST (Elite Kit, Vector Laboratories). Next, sections were rinsed for 10 minutes in 0.05M Tris buffer (pH 7.4), two times. The reaction was visualised using diaminobenzidine (DAB Substrate Kit, Vector Laboratories), and stopped by washing in cold PBS. Finally, the sections were mounted on gelatine-coated slides, dehydrated through a graded series of alcohols, and coverslipped.

Volumetric Analysis

The size of the lesions in the anterior thalamic nuclei of all 15 rats was estimated. The extent of the lesion was first drawn by hand onto corresponding sections from a rat brain atlas (Paxinos & Watson, 2005) using both cresyl violet and NeuN stained coronal sections [every other section from bregma -1.08 mm until bregma -6.36 mm, a total of 23 coronal plates (three plates through the anterior thalamic nuclei and 18 plates through the hippocampus, with three plates showing both the anterior thalamic nuclei and the hippocampus, to account for any unintended damage in the hippocampus) for each rat; Paxinos & Watson, 2005]. These images were scanned, and the area of damage was quantified using the program

analySIS^D (Soft-Imaging Systems, Olympus). The ability to define borders manually in analySIS^D allows the boundary between the damaged tissue and normal tissue to be drawn precisely. Two regions were quantified: 1) the anterior thalamic nuclei and 2) the hippocampus, as some slight unintended damage occurred in this region. The percent damage to these two regions of interest was quantified by taking the area of damage within the region of interest and dividing it by the total area of that region summed across each drawing (i.e., total area damaged within the region of interest/total area of the region of interest). The volumetric analysis was conducted on each hemisphere separately as well as together. Those animals with anterior thalamic damage that involved less than 50% of the total structure were excluded from the behavioural analysis.

Behavioural Testing

The rats were tested first on a T-maze alternation task at least two weeks after surgery. Following the T-maze, the rats were trained and tested on a series of experiments in the bow-tie maze which assessed both recency and novelty judgments with objects or odours (Table 2.1). Prior to object recognition in an open arena, the rats were re-tested on the T-maze alternation, and were then trained for one month in operant chambers (data of the latter experiment are presented in Chapter 4). Thus, the experiments are not presented in the order that they were conducted, but rather organised by topic; see Table 2.1).

Table 2.1. List of the experiments that the ATNx1 and Sham1 groups performed in the order in which they were conducted (left column). The right column indicates the number of each experiment where the experiments are discussed. The central column provides additional details that distinguish similar experiments.

Experiment order	Details	Experiment number
T-maze alternation	Test	Experiment 2.1
Object recognition	Standard recognition (<1 min delay) Long retention (60 min delay)	Experiment 2.2
Between-block recency	Objects; 'hard' difficulty	Experiment 2.4
Between-block recency	Objects; 'medium' difficulty; first session	Experiment 2.4
Between-block recency	Objects; 'easy' difficulty	Experiment 2.4
Within-block recency	Objects; first session	Experiment 2.5
Within-block recency	Objects; second session	Experiment 2.5
Within-block recency	Odours; first session	Experiment 2.8
Within-block recency	Odours; second session	Experiment 2.8
Odour recognition	Standard recognition (<1 min delay) Long retention (60 min delay)	Experiment 2.6
Between-block recency	Objects; 'medium' difficulty; second session	Experiment 2.4
T-maze alternation	Re-test	Experiment 2.1
Between-block recency	Odours; 'easy' difficulty; first session	Experiment 2.7
Biconditional contextual discriminations	1 month task in operant chambers	Experiment 4.1
Passive placement	Geometrical task in the watermaze	Experiment 6.1
Between-block recency	Odours; 'easy' difficulty; second session	Experiment 2.7
Object recognition	Open arena (15 min delay)	Experiment 2.3
Biconditional learning	Context/Place-Item associations using digging cups	Experiment 4.2
Activity boxes	Measuring locomotor activity for 20 min (number of beam breaks)	Experiment 2.9

Experiment 2.1: T-maze alternation

Apparatus

The T-maze was located in the centre of a room (300 cm x 300 cm x 240 cm) and was elevated 100 cm above the ground with the use of metal supports. The floor of the maze was made of wood and painted white. Each arm was 70 cm long and 10 cm wide with walls made from clear Perspex (16.5 cm high). Sunken food-wells, 3 cm in diameter and 0.75 cm deep, were located at the end of each arm. From the maze, the rats had full view of the distal extra maze cues (e.g., table, posters).

Pre-training

Pre-training began at least two weeks after surgery. During both pre-training and testing, the rats were brought into the room inside a light-tight aluminium carrying box. For each trial, the rats were carried from the carrying box to maze, and subsequently returned to the box at the completion of each trial. The rats were habituated to the apparatus for four days. On the first day, the apparatus was blocked at the junction with the use of a metal barrier. This resulted in two straight alleys: 1) a start arm alley and 2) the choice arms alley (i.e., the top of the "T"). This procedure ensures that the rats are not rewarded for specific arm turns. Each rat was placed for five minutes in both alleys with sucrose pellets (45mg per pellet; Noyers Purified Rodent Diet, Lancaster, NH, USA) scattered along the floor. On the second day, the rats were again placed into the two alleys, but the sucrose was now only located within the food-wells. On day three and four, the food-wells were only baited and re-baited with a single sucrose pellet.

Testing

The rats received six trials per day for six days. Each trial consisted of both a sample phase and a choice phase. During the sample phase, the rat is given access to one of the two arms by blocking an arm with a metal barrier that fitted into the arms at the junction of the maze (Figure 2.1A). At the end of the sample arm, the rat was allowed to consume a single sucrose pellet. The rat was then picked up and confined in the start arm for approximately 15 seconds while the barrier at the

choice point was removed. Following this short 15 second delay, the metal barrier at the start area was removed for the choice phase where the rat had free choice between the two arms of the T-maze (Figure 2.1B). The rat was rewarded with a single sucrose pellet for choosing the arm that was not previously visited during the sample phase (i.e. the rat alternated arms between the sample and choice runs). The rat was deemed to have made a choice when it placed a hind foot down an arm. Following a correct choice, the rat was allowed to eat the reward before being returned to the metal carrying case. When the rat made an incorrect choice, it was allowed to run down the entire length of the arm and see the empty food-well before being returned to the carrying case. The rats were run in squads of 3-4, each rat receiving one trial at a time. Consequently, the intertrial interval was approximately 5 minutes.

Following extensive training in the bow-tie maze (see Table 2.1), the rats were re-tested in the T-maze. Approximately five months have elapsed between the initial test and re-test in the T-maze. They were again tested in the same squads of 3-4, and were given six trials per day for four consecutive days.
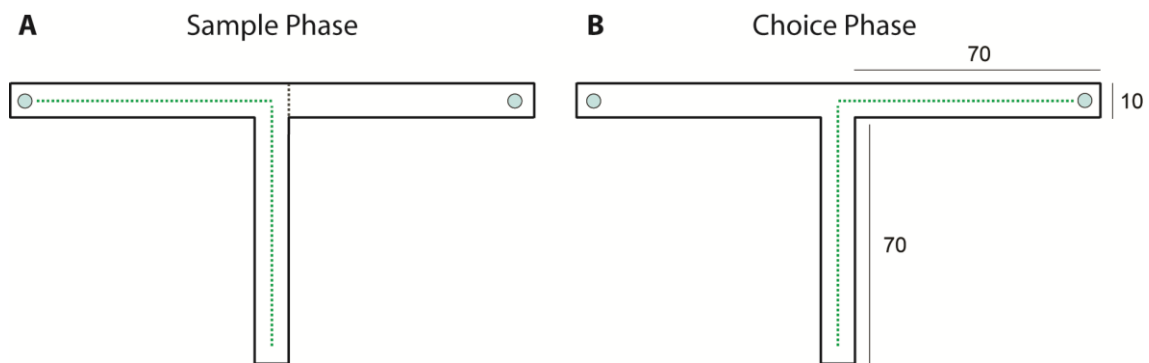
Figure 2.1. An example trial including a sample phase (**A**) and choice phase (**B**) in the T-maze. The green dashed line indicates the path taken by an animal that correctly alternated arms between the sample and choice phases. The grey dashed line in A (sample phase) indicates that the right arm is blocked, preventing the rat from entering the arm during the sample phase. Some of the dimensions (cm) are shown.

Statistical Analysis

The mean percent total correct responses in blocks of two training days for the ATNx1 and the Sham1 groups were analysed using a one between-subject factor (Group: ATNx1, Sham1) x one within-subject factor (Blocks of testing trials) ANOVA. Two different ANOVAS were used: one for the initial acquisition of the T-

maze task and the other for the re-test T-maze sessions. To examine the effect extended training in the bow-tie maze, the mean of all the training sessions during the intital acquisition of the T-maze and those during the T-maze re-test were compared using a third mixed-model ANOVA (between-subject factor: Group and within-subject factor: Test/re-test). The data were also examined to assess the accumulation of proactive interference by re-calculating the correct responses for each of the six trials across the 10 training days (total correct out of 10). This last analysis was examined using a one between-subject factor (Group: ATNx1, Sham1) x one within-subject factor (Trials) ANOVA.

Experiment 2.2: Object recognition in the bow-tie maze

The goal of Experiment 2.2 was to assess the ability of rats with damage to the anterior thalamic nuclei to discriminate novel from familiar objects in the bow-tie maze. This ability was examined using a short retention period (1 minute; standard recognition procedure) and a long retention interval (60 minutes; long retention procedure). Because items from the short retention period (1 minute delay) served as sample items for the long retention interval (60 minute delay), the short and long retentions formed two consecutive test sessions administered on the same day (see Table 2.2A).

Apparatus

The rats were tested in a bow-tie-shaped maze (120 cm long, 50 cm wide and 50 cm high) made of aluminium (Figure 2.2A). Each end of the maze was triangular and joined together at the apices by a narrow corridor (12 cm wide). In the centre of the corridor, an opaque guillotine door could be lowered or raised by the experimenter to allow passage from one end of the bow-tie maze to the other. At the far wall of each of the triangles, two food-wells (3.5 cm in diameter and 2 cm deep) were divided by a short, opaque, wall extending 15 cm from the middle of the end wall. The two food-wells were 25 cm apart. Objects were placed above these two food-wells during the experiment.

A Bow-tie maze
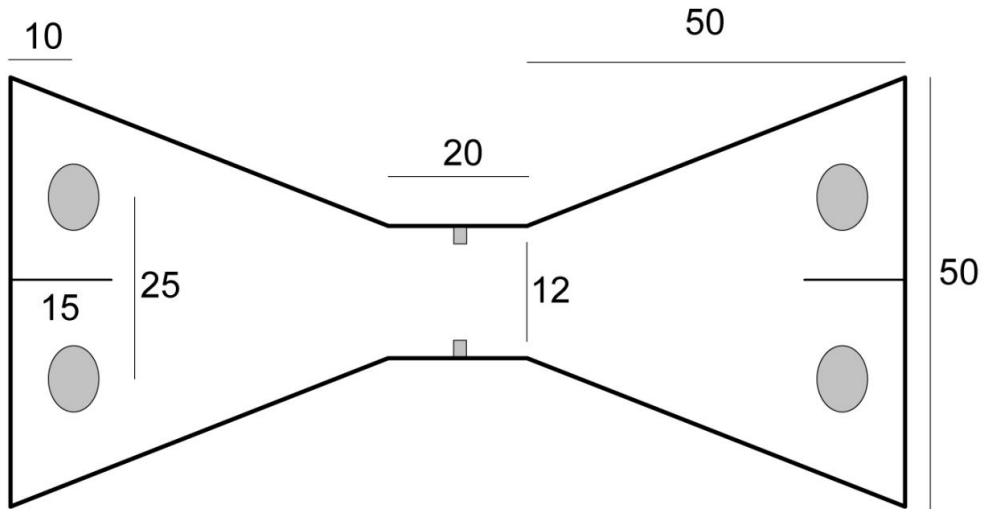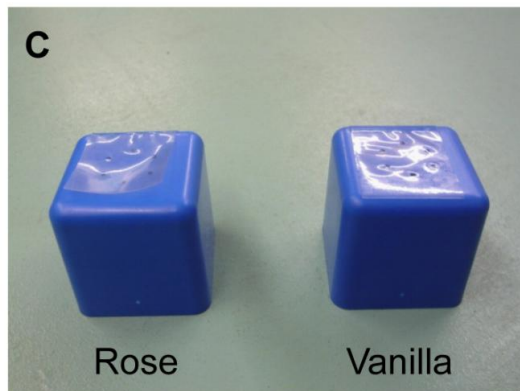
Figure 2.2. A) The shape and dimensions (cm) of the bow-tie maze. Adapted from Albasser, Poirier et al., 2010. **B**) Sample of the objects used for object recognition and recency tasks in the bow-tie maze. **C**) Example of the odour cubes (5cm x 5cm x 5cm) used for the odour recognition and recency tasks in the bow-tie maze. The black line in **B** = 8 cm.

Table 2.2. Presentation order of items in the different bow-tie maze protocols. Letters represent novel (bold) and familiar items. In the recency (test) phase of protocol B and C, older items are in bold face. The vertical line in protocol C indicates the start of the recency (test) phase. Note: Experiment 2.3 was in a square arena.

A) Object Recognition (Experiments 2.2 & 2.6)

	Standard Recognition (<1 minute delay)												Long Retention (60 min delay)									
Trials	0	1	2	3	4	5	6	7	8	9	10	60 min	11	12	13	14	15	16	17	18	19	20
Objects	-	A	B	C	D	E	F	G	H	I	J	delay	A	B	C	D	E	F	G	H	I	J
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**		**L**	**M**	**N**	**O**	**P**	**Q**	**R**	**S**	**T**	**U**

B) Between-block recency (Experiments 2.4 & 2.7)

	Standard Recognition Phase 1 (List A)										Standard Recognition Phase 2 (List B)										Recency (test) Phase 3 (List A vs. List B)							
Trials	0	1	2	3	4	5	6	7	8		0	9	10	11	12	13	14	15	16		17	18	19	20	21	22	23	24
Objects	-	A	B	C	D	E	F	G	H	delay	-	J	K	L	M	N	O	P	Q	delay	J	K	L	M	N	O	P	Q
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**		**J**	**K**	**L**	**M**	**N**	**O**	**P**	**Q**	**R**		**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**

C) Within-block recency (Experiments 2.5 & 2.8)

	Sample Phase																		Recency (Test) Phase							
Trials	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Objects	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**	**N**	**O**	**P**	**Q**	**R**	M	R	K	P	Q	L	O	N
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**	**N**	**O**	**P**	**Q**	**R**	**E**	**B**	**G**	**D**	**A**	**H**	**C**	**F**

Objects

For Experiment 2.2, the study used numerous triplicate sets of identical objects that differed in size, shape, colour, and texture, but without any obvious odour to the experimenter (see Figure 2.2B). The objects were both large enough to cover the circular food-wells and light enough for the rats to displace. The presentation of the objects was counterbalanced, so that half of the rats experienced the list of objects presented in one order (e.g. A-K), whereas the other half experienced the list in the reverse order (e.g. K-A). The relative left and right positioning of the items was also counterbalanced across rats.

Pre-training

Animals were habituated for seven days, so that by the end of pre-training all rats would run from one end of the maze to the other and displace objects covering the food-wells in order to obtain a reward (sucrose pellet). On day 1, pairs of rats were placed in the maze for 20 minutes and allowed to explore and consume sucrose pellets scattered along the floor and in the food-wells. On day 2, rats were trained individually for 10 minutes to run back and forth for a reward located only in the food-wells. From day 3, the rats were introduced to the guillotine door that restricted their movement from one compartment to the other. On day 4, four identical wood blocks were introduced and gradually covered the food-wells, so that by the end of the 10 minutes session, the rats would have to push the blocks in order to obtain the food reward. From day 5, three other pairs of objects were introduced that varied in size, shape, colour, and weight. These three objects were only used during pre-training and were not part of the items used during the experiment proper.

Standard recognition procedure (1 minute delay)

Rats were tested for 11 trials for a single session (Table 2.2A). The session began with the rat being placed in one end of the maze that contained an object (object A_1) and a wood block covering a reward hidden in the wells. The rat was allowed to explore either object for one minute, after which the guillotine door was raised allowing access to the second compartment. Once the rat ran to the opposite side

of the maze, the rat could explore either the novel item (object B_1) or the now familiar item (duplicate of object A_2; trial 1). Both objects covered wells that contained a reward. After a minute, the guillotine door was raised again and the rat ran back to the first compartment of the maze (trial 2) where object C (C_1; novel) and a duplicate of object B (B_2; familiar) were presented. Following one minute the guillotine door was raised again (trial 3), and the rat ran back into the second compartment to explore a copy of object C (C_2; familiar) and new object D (D_1; novel). All items had duplicates preventing the rats from using any cues from a potentially previously marked item. All objects covered only one food pellet. This arrangement motivated the rats to approach the objects, but did not affect the validity of the behavioural test of recognition, which relied on differential levels of exploration between the two baited objects. Animals were video recorded during all sessions.

Long retention procedure (60 minute delay)

Following completion of the standard recognition procedure, the rats were removed from the bow-tie maze and returned to their home cage. After a 60 minute delay from the first trial of the standard recognition procedure, the rat was returned to the bow-tie maze and given a further 10 trials (Table 2.2A). The session began by placing the rat into one of the two compartments of the bow-tie maze with two objects covering the food-well: one object was a third copy of familiar Object A (A_3; seen 60min earlier) and the other novel Object L (trial 1). After a minute, the guillotine door was raise and the rat ran to the second compartment where a copy of familiar Object B (B_3; seen 60min earlier) and novel Object M were presented (trial 2). Similar to the standard recognition procedure, all familiar items were triplicate copies preventing the rats from using any cues from a previously explored and potentially marked item.

Analysis of behaviour

Exploration of an object was defined as directing the nose at a distance of < 1cm to the item and/or touching it with the nose or the paws (including pushing). Sitting on or turning around the item was not included. When the videos were scored, it was noted that the rats spent time chewing, carrying the items in their mouths, and

freezing near or above the items (at a distance of < 1cm). These behaviours were also excluded.

Two discrimination indices (D1 and D2) were calculated (Ennaceur & Delacour, 1988). The D1 index was calculated by subtracting the time spent exploring the familiar item from the time spent exploring the novel item (i.e. time novel – time familiar), and was cumulatively summed across trials. The D2 index is a measure of the differential exploration time (i.e. D1 score) divided by the total time spent exploring both the novel and the familiar item. The D2 score yields a ratio between -1 and +1, where a positive score indicates a preference for the novel item. The D2 score was updated after every trial by recalculating from the cumulative data. The final updated D2 score is equivalent to the mean D2 score of the entire test (not the mean of each D2 score for each trial). Because the bow-tie maze allows the testing of multiple trials, within a session, the cumulative D1 and the updated D2 scores indicate the progression of the rats' cumulative performances throughout the session (see Figure 2.6).

Statistical Analysis

Two-tailed, one-sample t-tests were conducted using the cumulative data (D1 and D2) from the final test trials to assess whether the animals performed significantly above or below chance (showed a preference for the novel or the familiar item, respectively). The discrimination scores (D1 or D2) for the two groups (Sham1 and ATNx1) were analysed using a one between-subject (Groups: Sham1 and ATNx1) x one within-subject (Delays: <1 minute and 60 minutes) ANOVA. Simple effects for each condition were analysed using the pooled error term when significant interactions were found, as recommended by Winer (1971). When the data violated the assumptions of parametric tests (e.g., homogeneity of variance, normality), non-parametric tests were used for statistical analysis (e.g., Mann-Whitney instead of a t-test). When the data violated sphericity (repeated measures designs), the Greenhouse-Geisser correction was applied, as recommended by Fields (2005). Any exceptions to the above are signified in the text.

Experiment 2.3: Object recognition in an open arena (15 minute retention)

Apparatus

The apparatus consisted of a large wooden box (100 cm wide X 100 cm long X 46 cm high) located on the floor in the centre of a room (300 cm x 300 cm x 240 cm; same room as the one used in Experiment 2.1). The walls of the maze were painted grey, and the floor was covered in sawdust. A checkered curtain divider (169 cm high and 242 cm wide) surrounded half of the maze, obscuring the distal room cues. The uncovered half of the room contained posters on a wall and the door to the test room.

Objects

Six identical copies of four objects were used, so that the objects presented at the sample and test phases were not the same and, hence, could not be odour marked. These objects were large and sufficiently heavy to prevent the rats from pushing them. The four objects were: 1) a large tin can, 2) a clear plastic water bottle, 3) a rectangular baking tray, and 4) a large glass Nutella jar (Nutella, Ferrero). All the labels were removed, and the objects thoroughly cleaned prior to their use. Presentation of the objects was counterbalanced so that half the rats saw Set A as the sample objects and Set B as the novel objects, and the other half saw Set B as the sample and Set A as novel.

Pre-training

The rats were habituated to the open field arena for two days. On the first day the rats were placed inside the arena in groups of two or three for 10 minutes. On the second day, the rats were placed inside the arena one at a time for 5 minutes. Two objects (a blue beer can and a large ceramic figurine of Snoopy, 15 cm high from *Peanuts,* a comic strip by Charles M. Schulz) were placed 10 cm away from the middle of the left and right walls, respectively. These two objects were only used during the two days of habituation and were not present during testing.

Testing

The rats received two test days consisting of both a sample and choice phase. During the five minute sample phase, the rat was placed in the centre of the maze, and was allowed to explore the four identical copies of the sample item (A_1, A_2, A_3, A_4) located in each of the four corners, approximately 10 cm from the walls. Following the sample phase, the rat was placed in a metal carrier for 15 minutes. The rat was then returned to the open arena for the choice phase. During the choice phase, two new copies of the same sample objects (A_5, A_6) occupied two adjacent corners, and two novel objects (B_1, B_2) were placed in the remaining two corners. The location of the pairs of choice (test) objects was counterbalanced between rats as well as between the first and second test days.

Behavioural and Statistical Analysis

The behavioural and statistical analyses are essentially the same as Experiment 2.2. However, instead of an ANOVA, two separate between-sample t-tests compared the discrimination scores (D1 and D2) of the ATNx1 and Sham1 groups after one and five minutes of the choice phase as these data are not independent from one another.

Experiment 2.4: Between-block (List 1 vs. List 2) recency with objects

Apparatus

The experiment used the same apparatus as Experiment 2.2 (i.e. the bow-tie maze). Similar to Experiment 2.2, triplicate copies of objects were also used. These objects were repeated across some of the experiments (Experiment 2.2, 2.4, and 2.5), but no object was re-used within one month. Furthermore, novelty judgments (Experiment 2.2) were carried out before any of the recency judgments (Experiment 2.4 and 2.5) and, therefore, entailed new items for the rats (see Table 2.1).

Testing

The rats were given four sessions of a between-block recency task with varying degrees of difficulty: 'easy', 'medium' (twice), and 'hard'. The four sessions were on separate test days, and used two different lists of nine items for the standard recognition phases (total of six different lists, as the items were repeated for one experiment). Each session comprised three phases. First, nine trials of standard object recognition (<1 min delay; see Experiment 2.2 procedure) were followed in the second phase by a further nine trials of standard object recognition. The third phase comprised eight object recency choice (test) trials (see Table 2.2B). The two standard recognition phases were separated by a delay that varied in duration according to task difficulty. Each standard recognition phase used a different set of objects. After the second standard recognition phase, there was a second delay before the start of the recency phase. The recency phase began by returning the rat inside the bow-tie maze. Copies of the first object from each of the two standard recognition object lists (i.e., Object A_3 and Object J_3; see Table 2.2B) were presented. The rat had one minute to explore the items and retrieve the sucrose pellets under both objects. The guillotine door was then raised, and the rat ran to the second compartment where copies of the second object from both standard recognition phases were presented (i.e. Object B_3 and Object K_3). All the objects used during the recency (test) phase were copies of familiar items. The difference between the two objects presented on any given test trial was the relative recency of the items.

The delays were varied to alter task difficulty (see Table 2.3). It was hypothesised that by increasing the delay between the two standard recognition phases (first delay), and by decreasing the delays between the final standard recognition phase and the recency phase (second delay), the task would become easier. There were three different levels of difficulty: 'easy', 'medium', and 'hard' (Table 2.3). The delays for the 'easy' condition were 30 and 10 minutes for the first and second delay, respectively. The delays for the 'medium' condition were 20 minutes for both delays. The 'medium' condition was tested twice in order to reduce the variance and establish whether performance was above chance (i.e. preference for the older object). The delays for the 'hard' condition were 20 and

60 minutes for the first and second delay, respectively. During the delays, each rat was returned to its home cage, but kept inside the test room.

Table 2.3. The delay periods for the different levels of difficulty of the between-block recency task.

Difficulty	First Delay (between the two standard recognition phases)	Second Delay (between second standard recognition phase and recency phase)
'easy'	30 minutes	10 minutes
'medium'	20 minutes	20 minutes
'hard'	20 minutes	60 minutes

Analysis of behaviour

The D1 and D2 scores were also calculated using the same exploratory behaviours as those described in Experiment 2.2. However, because both of the items were familiar, the time spent exploring the recent item was subtracted from the time spent exploring the older item (i.e. time older – time recent). For the D2 ratio, a positive score indicates a preference for the older item.

Statistical Analysis

A one-way between-subjects factor (Groups: Sham1 and ATNx1) x one within-subjects factor (Difficulty: easy, medium, hard) was used to analyse the discrimination scores (D1 and D2) of the recency phase data. A three-way mixed model ANOVA was used to analyse the standard recognition phases [one between-subjects factor: Group (ATNx1, Sham1) and two within-subjects factors: Difficulty (easy, medium, hard) and Standard recognition phase (Phase 1, Phase 2)]. Because the 'medium' difficulty condition was run twice, the mean D1 and D2 scores of both test sessions was used for these statistical analyses. Two-tailed one-sample t-tests were also conducted in order to examine whether the ATNx1 and Sham1 groups preferred the older or more recent object, i.e., if their scores differed from chance.

Experiment 2.5: Within-block recency with objects

Apparatus

Experiment 2.5 used the same apparatus as Experiment 2.2 and 2.4 (i.e. the bow-tie maze).

Testing

The rats received two test days using two separate sets of objects. Each test day included both an 18 trial sample phase and an eight trial recency (test) phase; all within a single continuous block of trials (see Table 2.2C). The first trial of the sample phase began by placing the rat inside one of the compartments of the bow-tie maze. The rat was given one minute to push aside the two identical objects (A_1, A_2) each covering a food-well, and explore the items. Afterwards, the guillotine door was opened, allowing the rat to run across to the second compartment where two copies of novel Object B (B_1, B_2) were present (Trial 2). The rat had one minute to explore these items and obtain the sucrose pellets. Then the guillotine door was opened and the rat ran back to the first compartment where two copies of novel Object C (C_1, C_2) were presented (Trial 3). Following the final trial of the sample phase (Trial 18), the guillotine door was raised allowing the rat to change compartments and begin the choice (test) phase. Therefore, there was less than a one minute delay between the final sample trial and the first test trial. The rat was not removed from the apparatus nor was the rat handled during this brief period.

For the recency (test) phase, the two objects the rat explored were chosen taking into account; a) the number of interleaving items between the two objects (either 3, 7, 11, or 15), b) the item was experienced in the same compartment (side) of the maze during the test phase and the sample phase (e.g., both Object E and Object M were experienced in compartment 1), and c) the order in which the objects were presented during the recency (test) phase (e.g. not all of the high number of interleaving trials occurred near the start of the test phase). Consequently for Trial 1 of the recency (test) phase the rat was presented with a copy of Object E (E_3) and Object M (M_3). The rat was given one minute to obtain the sucrose pellets and explore the objects. After the guillotine door was raised,

the rat ran across to the other side of the bow-tie maze which contained copies of Object B (B_3) and Object R (R_3; Trial 2; see Table 2.2C for remaining trials). All the objects used during the recency (test) phase were copies of the familiar items experienced during the sample phase. The only difference between the two test objects was the temporal order in which they were presented (i.e. the relative recency of the items).

Behavioural and Statistical Analysis

The behavioural analysis was the same as Experiment 2.4. Mean discrimination scores (D1 and D2) of the two test sessions were used for the statistical analyses. These data were first examined by dividing them into a 'low' (3 and 7) and a 'high' (11 and 15) number of interleaving items categories, and were statistically tested using a two-way mixed factors ANOVA [Group (ATNx1 Sham1) x Number of interleaving items (low, high)]. When a significant effect of number of interleaving items (low, high) was not found, these data were then pooled across all numbers of interleaving items (mean scores), and one-sample t-tests were used to examine whether the rats were significantly above or below chance (i.e., preferred the older or more recent item, respectively).

Experiment 2.6: Odour recognition in the bow-tie maze

Apparatus

Experiment 2.6 used the same apparatus as Experiment 2.2, 2.4, and 2.5 (i.e. the bow-tie maze).

Odours

Experiment 2.6 used 21 triplicate sets of blue plastic cubes (5cm x 5cm x 5cm) containing different aromas (e.g., rose, red apple, popcorn) that were randomly selected from a pool of 36 triplicate sets of odour cubes (Vortex Cubes, Dale Air, UK; Figure 2.2C). All of the cubes, which were identical in appearance, were pierced with six holes on the top dispensing the aromas. The odour cubes were re-used across some of the experiments, but no odour was experienced within the same month. The presentation of the odours was counterbalanced, so that half of

the rats experienced the list of odours presented in order (e.g. A-K), whereas the other half experienced the list in the reverse order (e.g. K-A). The relative left and right positioning of the items was also counterbalanced across rats.

These experiments were conducted in the dark as previous research suggests that rats may use odour cues to guide spatial behaviours in darkness, but not in the light (Lavenex & Schenk, 1995). All sources of illumination were switched off or blocked, resulting in a light intensity of 0.11 lux in the centre of the maze. The experimenter wore night vision goggles (Productive Firm Dipol Ltd., Vitebsk, Belarus) and the sessions were recorded with two infrared cameras (Maplin Electronics, UK) fixed directly above the maze.

Procedure

The same procedure was used as Experiment 2.2, but without the pre-training sessions.

Behavioural and Statistical Analysis

Experiment 2.6 used the same behavioural and statistical analysis as Experiment 2.2.

Experiment 2.7: Between-block recency (List 1 vs. List 2) with odours

Apparatus

The apparatus was the same as Experiment 2.2, 2.4, 2.5, and 2.6 (i.e. the bow-tie maze).

Procedure

The procedure was the same as Experiment 2.4; however, only one of the delay lengths was used: 'easy' (i.e., 30 minute delay between both standard recognition phases and a 10 minute delay between the second standard recognition phase and the recency phase). The rats received two test days using these particular delays. The rats were placed in an adjacent well-lit, quiet, test room during the delay periods. This room did not contain any odour cubes.

Behavioural Analysis

The behavioural analysis was the same as Experiment 2.4.

Statistical Analysis

The same statistical analyses as Experiment 2.4 were used; however, instead of an ANOVA, a between-sample t-test was used to compare the exploratory measures of the Sham1 and ATNx1 groups during the recency (test) phase.

Experiment 2.8: Within-block recency with odours

Experiment 2.8 used the same apparatus and procedure as Experiment 2.5, the same behavioural analysis as Experiment 2.4, and the same statistical analysis as Experiment 2.5.

Experiment 2.9: Locomotor Activity

Apparatus and room

On the west wall of a novel room (272 cm x 135 cm x 240 cm), a 3 x 6 activity test cage rack was located. The cage rack contained 18 activity test cages (Paul Fray, Ltd., Cambridge, UK). The cages measured 56 cm x 39 cm x 19 cm, and contained two photobeams positioned 18 cm from the short walls, 20 cm apart.

Procedure

Each rat was taken into the room and placed individually inside an activity test cage. The room was illuminated and the locomotor activity of the rat was recorded for 20 minutes. The activity period was divided into 20 one minute bins. The number of beam breaks (a single beam being repeatedly broken) as well as beam cross-overs (both the front and back beams broken concurrently) for each bin was recorded.

Statistical Analysis

The mean total beam breaks and the mean total cross-overs for the 20 minute activity period of the Sham1 and ATNx1 groups were analysed using two separate

between-sample t-tests as the two behavioural measures (single beam breaks and beam cross-overs) are not independent.

Results

Histology

Figure 2.3 shows two sections from a Sham1 and ATNx1 rat. The sections on the left are stained with cresyl violet, a Nissl stain, and the sections on the right were reacted using immunohistochemistry for NeuN. As NeuN only labels neurons, the lack of staining indicates cell loss; whereas the Nissl stain labels both neurons and glial cells. Both the Nissl and the NeuN sections were used to determine the extent of the lesion in the ATNx1 group. Three animals were excluded from all analyses due to the small size of the lesions (less than 50% total ATN damage). In the remaining 12 cases, the cell loss was centred on the anterior thalamic nuclei, and as a result these nuclei were the sole common lesion site across all cases. Figure 2.4 shows the extent of the lesions in rats with its smallest and largest amount of tissue loss. Table 2.4 displays the range and mean percent damage to the anterior thalamus for the left and right hemispheres, as well as the total loss in both hemispheres. The total loss to the anterior thalamic nuclei was between 52% - 94% (mean = 76%; median = 76%). The lesions were slightly asymmetrical with most rats having more damage to the left hemisphere (n = 8). Sparing typically occurred caudally and in the most ventral portion of the anterior medial nucleus. However, two rats exhibited the opposite pattern with a more complete lesion at the caudal end of the anterior thalamic nuclei, with sparing occurring rostrally. These two animals had some sparing to the anterior dorsal nucleus. In 11 out of 12 cases, there was partial damage to the rostral and dorsal portions of the laterodorsal nucleus, which in three cases was unilateral. In those rats with larger lesions, there was also some damage to the parataenial nucleus (n = 7; unilateral in two cases), the paraventricular nucleus of the thalamus (n = 3), the reticular nucleus (n = 6; unilateral in three cases), and the nucleus reuniens (n = 7).
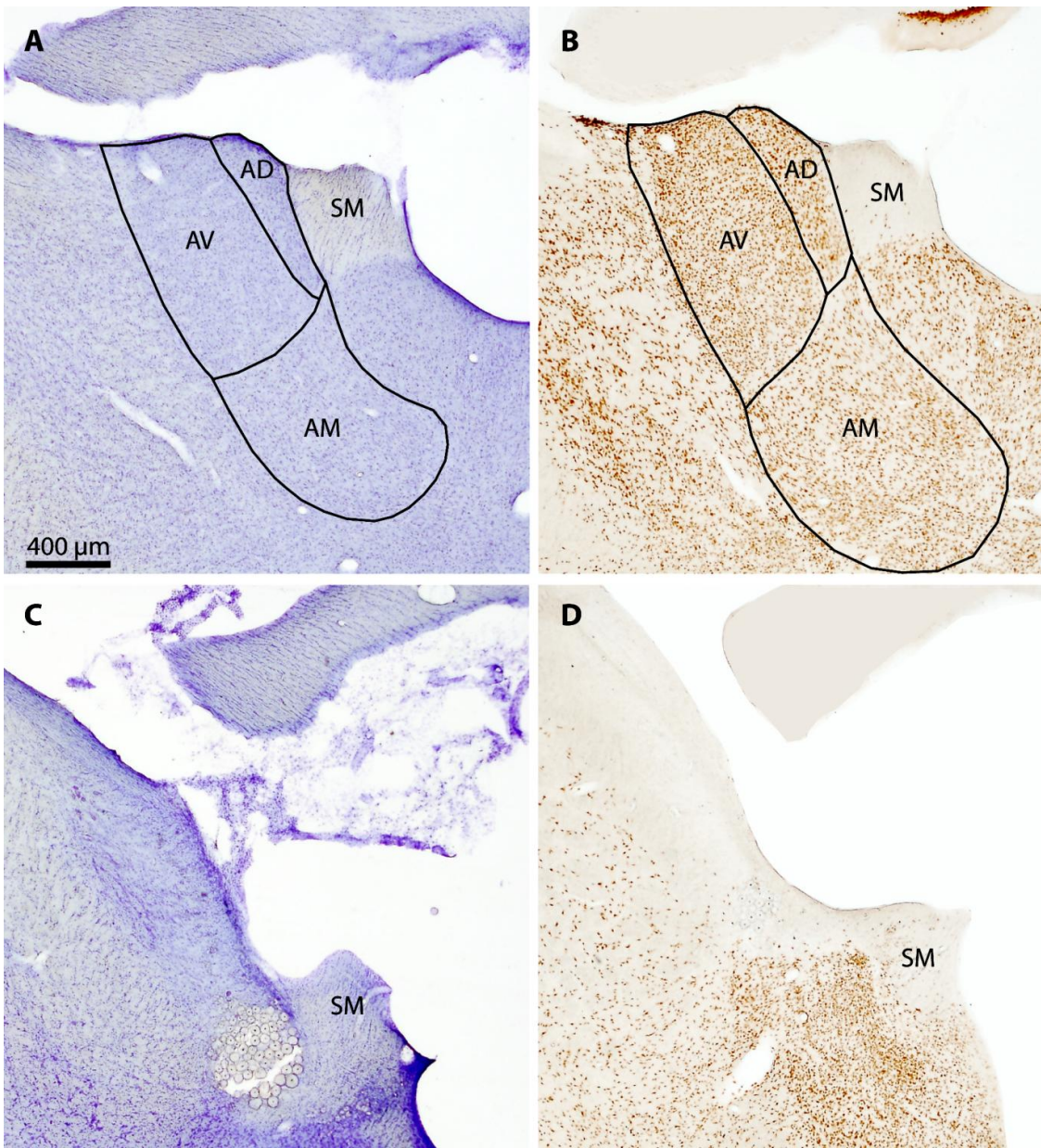
Figure 2.3. (**A**, **B**) Coronal sections of a Sham1 and (**C**, **D**) ATNx1 rat. The left panels (**A**, **C**) show sections stained with cresyl violet, a Nissl stain, and the right panels (**B**, **D**) show the NeuN labeled neurons. Abbreviations: AD, anterior dorsal thalamic nucleus; AM, anterior medial thalamic nucleus; AV, anterior ventral thalamic nucleus; SM, stria medullaris.

In all but one case there was some bilateral cell loss in the hippocampus; one rat had unilateral damage to this region. Table 2.5 shows the range and the mean percent damage to the hippocampus in each of the hemispheres separately as well as together. Most of the damage was restricted to the most rostral part of the ventral (inferior) blade of the dentate gyrus (n = 12; in one case the damage was unilateral), and sometimes the damage extended into the immediately adjacent CA3 (n = 9; although in three cases the damage was unilateral). A mean of 3.3% of the total hippocampus was damaged with a range of 0.2% - 5.8%. In four cases there was potential tract damage to the fornix in addition to the inevitable injection tracts. In one case the fornix damage appeared to be small and primarily unilateral, whereas in the remaining three cases, the fornix appeared both shrunken and distorted. Inspection of the behavioural data indicated that these four rats with additional fornix damage did not perform significantly differently to rest of the lesion group.
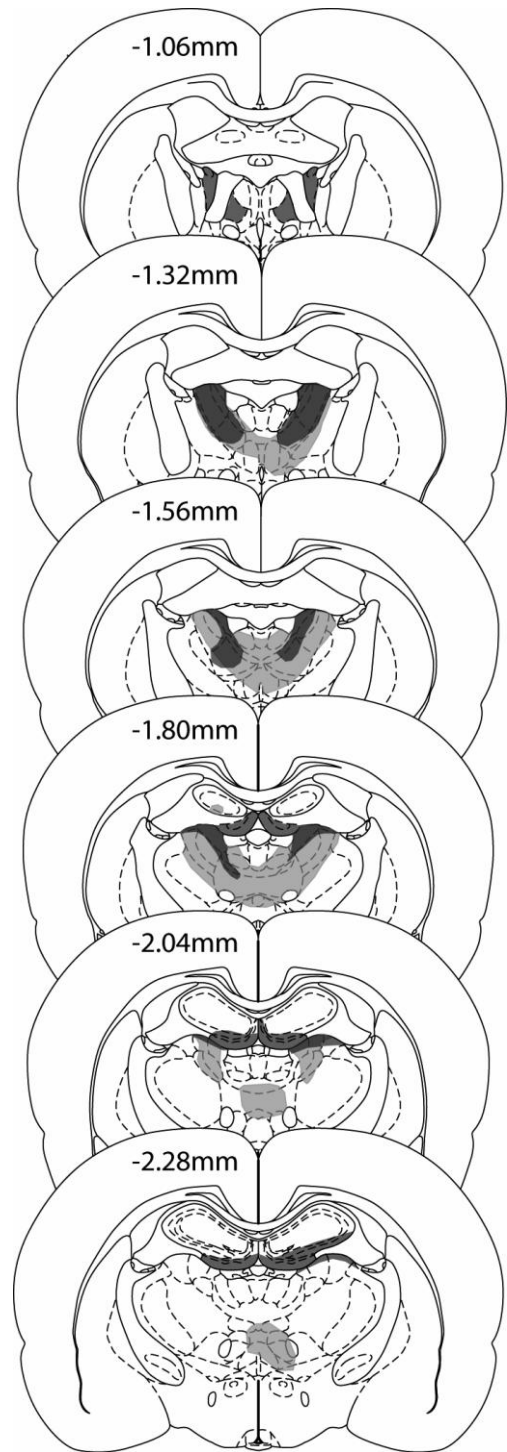
Figure 2.4. The extent of the lesion in the rat with the smallest (dark grey) and largest (light grey) anterior thalamic lesion. Note the rat with the smallest anterior thalamic lesion, did not have the smallest unintended damage in the hippocampus.

Table 2.4. Percent damage to the anterior thalamus

Percent Damage to Anterior Thalamus			
Hemisphere	**Range**	**Mean**	**Median**
Left	39%-99%	80%	86%
Right	44% - 94%	72%	74%
Total	52% - 94%	76%	76%

Table 2.5. Percent damage to the hippocampus

Percent Damage to Hippocampus			
Hemisphere	**Range**	**Mean**	**Median**
Left	0.5% - 5.8%	2.9%	2.7%
Right	0% - 5.9%	3.1%	3.8%
Total	0.2% - 5.8%	3.0%	3.3%

Behavioural Testing

Experiment 2.1: T-maze alternation

Figure 2.5A shows the mean percent correct responses over blocks of training trials for Sham1 and ATNx1 animals. The ATNx1 group was significantly impaired compared with Sham1 animals (test: $F_{(1,20)} = 61.5$, $p < 0.001$; re-test: $F_{(1,20)} = 20.1$, $p < 0.001$). The Sham1 animals made approximately 80% correct responses from the first block of training, and maintained this level of performance throughout blocks of trials, both before and after extensive training in the bow-tie maze. In contrast, the ATNx1 group performed near chance. Taking the mean percent correct responses across all training days for test and re-test indicated that training in the bow-tie maze did not alter performance on the T-maze alternation

task (i.e., no effect of test/re-test; p > 0.1). The ATNx1 group remained as severely impaired during re-test as they were during the initial testing compared with Sham1 animals ($F_{(1,20)}$ = 44.1, p<0.001). There was no significant effect of Testing Blocks (p > 0.01).

The correct responses of the Sham1 and ATNx1 animals were also tabulated for each of the six trials across all 10 testing days (total correct out of 10) to examine the accumulation of proactive interference. As can be seen in Figure 2.5B, the ATNx1 group performed significantly worse than the Sham1 group ($F_{(1, 20)}$ = 50.7, p < 0.001). There was also a significant main effect of Trial indicating that both groups were more likely to alternate to the correct arm of the T-maze on the earlier trials than on the later ones ($F_{(5,100)}$ = 5.36, p < 0.001). The Sham1 group was 91% correct on the first trial but dropped to 80% by the sixth, and the ATNx1 group chose the correct arm of the T-maze 67% of the time on the first trial [which is significantly above chance (t_{11} = 3.63, p = 0.004)] but dropped to chance performance (53%) by the last trial. These results suggest that earlier trials in the T-maze may interfere with later performance.
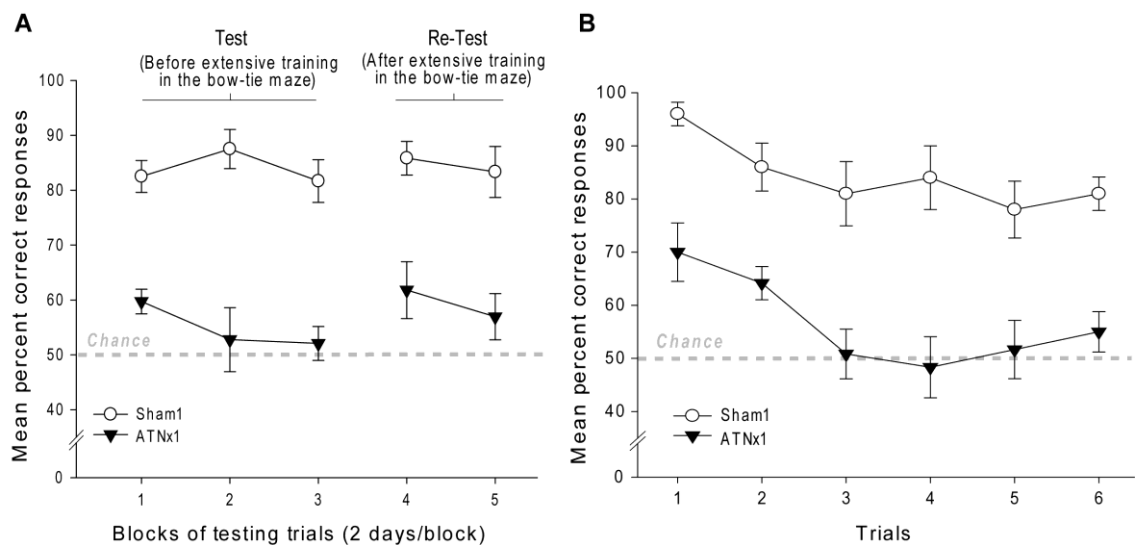
Figure 2.5. A) The mean percent correct responses over blocks of testing trials on initial acquisition of a T-maze alternation task and during re-test. **B**) The same data were re-calculated for each individual trial, and the mean correct responses are displayed. Data shown are group means, and the vertical bars are the standard error of the means (SEM). Fifty percent represents chance (i.e. the likelihood of choosing either arm in the T-maze).

***Experiment 2.2: Object recognition in the bow-tie maze***

The cumulative D1 scores and the updated D2 scores are displayed in Figure 2.6.

D2 Index

The D2 scores indicated that both groups were significantly above chance when the delay period was less than one minute (i.e. standard recognition; Sham1: $t_9 = 18.5$, $p < 0.001$ and ATNx1: $t_{11} = 12.6$, $p < 0.001$), and also when the retention period was 60 minutes long (Sham1: $t_9 = 3.52$, $p = 0.006$, and ATNx1: $t_{11} = 6.65$, $p < 0.001$). The two-way mixed model ANOVA revealed that there was no difference between the Sham1 and ATNx1 groups ($p > 0.1$), nor was there an interaction effect ($p > 0.1$). There was, however, a significant main effect of Delay indicating poorer discrimination between familiar and novel objects at the 60 minute delay period compared with the less than one minute retention of the standard recognition phase ($F_{(1,20)} = 20.1$, $p < 0.001$).

D1 Index & Total Exploration

A similar pattern of results (i.e., no lesion effect) was found for the D1 scores (less than one minute delay, Sham1: $t_9 = 12.1$, $p < 0.001$, and ATNx1: $t_{11} = 8.62$, $p < 0.001$; 60 minute delay, Sham1: $t_9 = 3.18$, $p = 0.01$, and ATNx1: $t_{11} = 6.12$, $p < 0.001$; ANOVA: Significant main effect of Delay only, $F_{(1,20)} = 14.1$, $p = 0.001$). The mean cumulative total exploration of the objects with a 1 min delay was 82.2 s for the Sham1 group and 72.9 s for the ATNx1 group; while the mean cumulative total exploration following a 60 min delay was 81.1 s for the Sham1 group and 70.6 s for the ATNx1 group. There was no significant effect of Group, Delay, or interaction in the amount of time the rats spent exploring both the familiar and the novel objects (i.e., total exploration; $p > 0.1$, all).
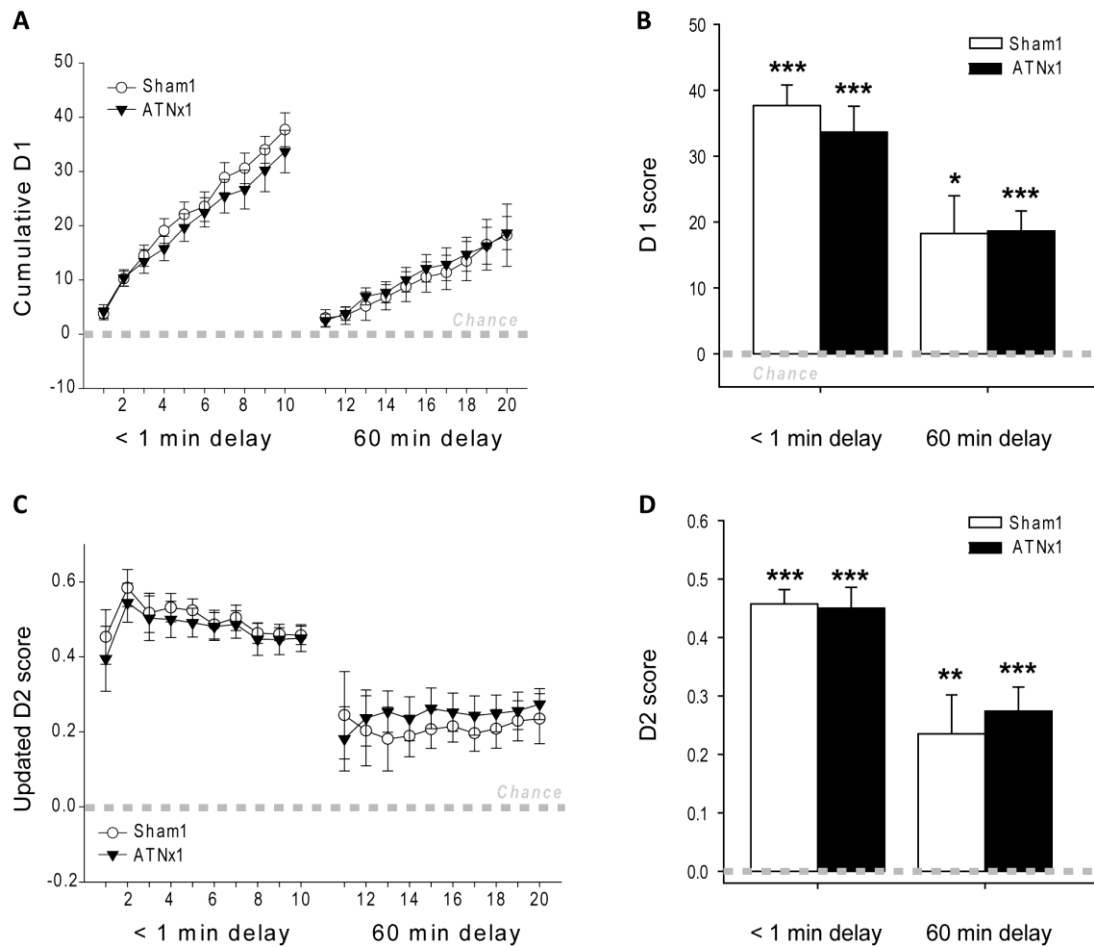
Figure 2.6. (**A, B**) The cumulative D1 scores and (**C, D**) the updated D2 score of Sham1 and ATNx1 rats object recognition performance after a delay of <1 minute or 60 minutes. The left panels (**A, C**) show how performance changes across each trial for the <1 minute and 60 minute delays. The right panels (**B, D**) show only the final (last) trial of the 1 minute and 60 minutes delay for the Sham1 and ATNx1 groups. A score of zero indicates a failure to discriminate novel from familiar (grey dashed line). Data shown are mean scores for each group, and the vertical bars are the standard error of the mean (SEM). Statistical analyses used the final cumulative data point after each block of 10 trials. Whether the groups differed from chance and the significance level is indicated on the right panels (**B, D**); * = P < 0.05; ** = p < 0.01; *** = p <0.001.

Experiment 2.3: Object recognition in an open arena (15 min retention)

D2 Index

The D2 scores of the first minute and the whole five minutes of the choice phase are shown in Figure 2.7. One-sample t-tests indicated that both groups were significantly above chance for both the first minute (Sham1: t_9 = 7.39, p < 0.001; ATNx1: t_{11} = 4.38, p = 0.001) and the whole choice session (Sham1: t_9 = 3.41, p = 0.008; ATNx1: t_{11} = 3.73, p = 0.003); thus the rats showed a preference for the novel objects. Furthermore, the groups did not differ from one another after either the first minute or the whole five minute choice session (both: p > 0.1).

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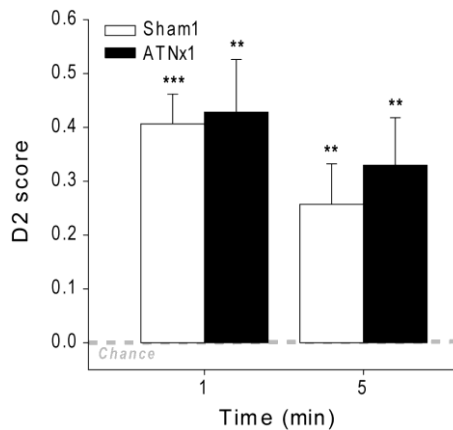
Figure 2.7. The updated D2 scores of the Sham1 and ATNx1 animals for the first or the entire five minute test session of an object recognition task in an open arena. Data shown are group means, and the vertical bars are the standard error of the means (SEM). Zero represents chance (i.e. exploring novel and familiar items equally). Significantly different from chance: ** = $p < 0.01$; *** = $p < 0.001$.

D1 Index & Total Exploration

The D1 scores yielded a similar pattern of results; there was no group difference (first minute, Sham1: $t_9 = 6.08$, $p < 0.001$; ATNx1: $t_{11} = 4.42$, $p = 0.001$; five minutes, Sham1: $t_9 = 3.63$, $p = 0.005$; ATNx1: $t_{11} = 4.31$, $p = 0.001$; no group differences, $p > 0.1$). Although the groups did not differ from one another on either discrimination indices, the Sham1 group spent significantly more time exploring both objects within the first minute of the choice phase ($t_{20} = 2.64$, $p = 0.016$; mean total exploration was 38.6 s and 31.4 s for the Sham1 and ATNx1 groups, respectively), but not for the whole five minute choice phase ($p > 0.1$; mean total exploration was 109.7 s and 101.4 s for the Sham1 and ATNx1 groups, respectively). Finally, a between-sample t-test of the total amount of exploration during the five minute sample phase demonstrated that the Sham1 and ATNx1 groups explored the objects equally ($p > 0.1$; mean total exploration was 104.6 s and 95.2 s for the Sham1 and ATNx1 groups, respectively).

Experiment 2.4: Between-block (List 1 vs. List 2) recency with objects

The final D2 scores for the two standard recognition phases and the recency phases for the three different levels of difficult ('easy', 'medium', and 'hard') are displayed in Figure 2.8a-c.
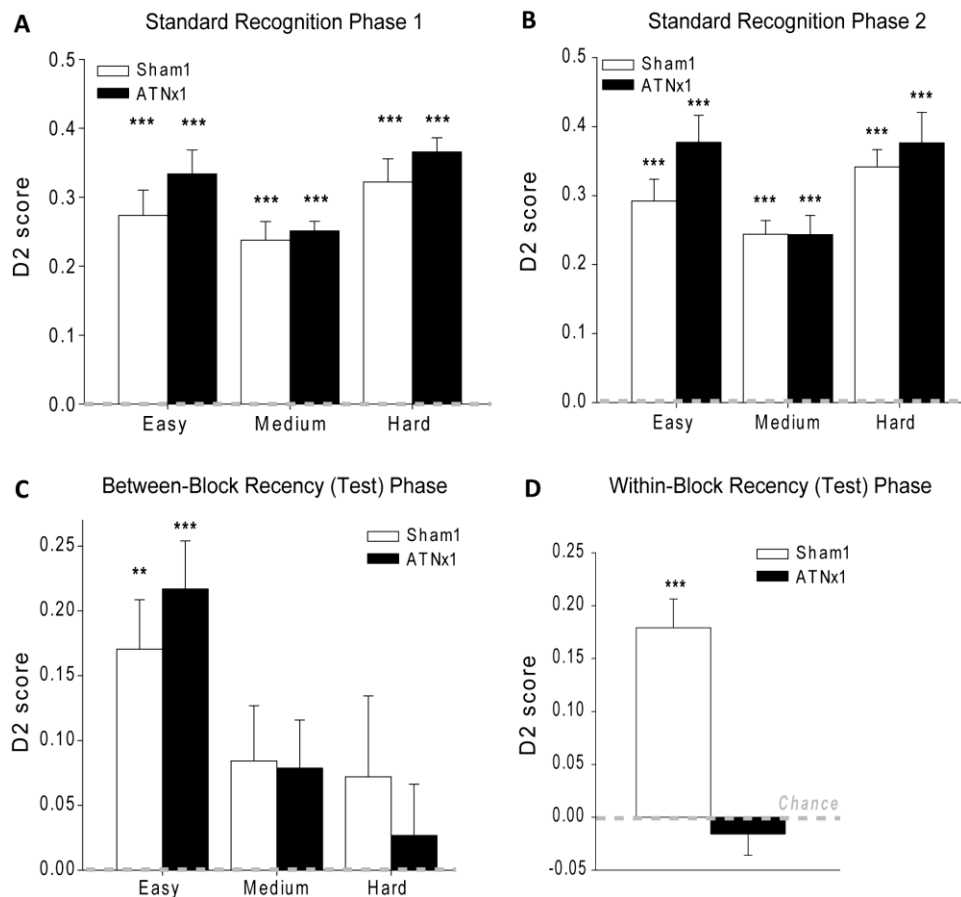
Figure 2.8. A) The final D2 scores for the first standard recognition phase, **B**) second standard recognition phase, and **C**) recency (test) phase of the between-block recency task with objects. The mean D2 scores of the ATNx1 and Sham1 groups for the within-block recency are displayed in **D**. Data shown are group means, and the vertical bars are the standard error of the means (SEM). Zero represents chance (i.e., exploring novel and familiar, or older and more recent, items equally). Significantly different from chance: ** = $p < 0.01$; *** = $p < 0.001$.

Recency Discrimination D2 Index

Both groups were significantly above chance in the 'easy' recency task (Sham1: t_9 = 4.47, p = 0.002; ATNx1: t_{11} = 5.85, p < 0.001), but not when the difficulty was increased to 'medium' or 'hard' (i.e., neither group showed a preference for the older, less recent, objects at the 'hard' condition, both p > 0.1)   Both groups were almost significantly above chance in the 'medium' difficulty condition: Sham1, t_9 = 1.97, p = 0.08; ATNx1, t_{11} = 2.13, p = 0.057 (two-tailed). Further examination of the recency (test) phase using a two-way ANOVA also indicated that there was a significant effect of Task Difficulty ($F_{(2,40)}$ = 5.05, p = 0.011), however, there was no difference between the Sham1 and ATNx1 groups, nor was there a Group x Task Difficulty interaction (p > 0.1 for both). Pairwise comparisons of difficulty levels collapsed across the two groups revealed that the D2 scores of the rats differed

between the 'easy' and 'hard' conditions (t_{21} = 3.56, p = 0.002) as well as the 'easy' and 'medium' conditions (t_{21} = 2.75, p = 0.012), but not between the 'medium' and 'hard' conditions (p > 0.1; all comparisons corrected using Bonferroni).

Recency Discrimination D1 Index

Similar to the D2 index, one-sample t-tests of the D1 scores during the recency (test) phase were significantly above chance at the 'easy' difficulty level (Sham1: t_9 = 3.65, p = 0.005; ATNx1: t_{11} = 4.32, p = 0.001), but not for the 'medium' or 'hard' conditions (p > 0.1 for all with the exception of the 'medium' condition for the ATNx1: t_{11} = 1.90, p = 0.085). An ANOVA of the D1 scores found that the groups did not differ during the recency (test) phase, nor was there an effect of Task Difficulty or Group x Difficulty interaction (all p > 0.1).

Standard Recognition Phase (1 min retention) D2 Index

One-sample t-tests revealed that both groups of rats showed a preference for the novel objects during the standard recognition sample phases at all recency difficulty levels (i.e., a total of six standard recognition phases, two for each of the three difficulty conditions for the Sham1 and the ATNx1 groups; p < 0.001, all). A three-way mixed model ANOVA was used to assess the D2 scores during the two standard recognition phases at the three different difficulty levels (i.e., 'easy', 'medium', 'hard'). There was a significant effect of Group, indicating that the ATNx1 group discriminated novel from familiar objects better than Sham1 rats ($F_{(1, 20)}$ = 6.94, p = 0.016), and a significant effect of Task Difficulty ($F_{(2, 40)}$ = 10.7, p < 0.001). No interactions were significant, nor was there a significant effect of Standard Recognition Phase (i.e., 1, 2; all p > 0.1).

Standard Recognition Phase (1 min retention) D1 Index & Total Exploration

One-sample t-tests of the D1 scores yielded similar results to the D2 scores; both Sham1 and ATNx1 groups were significantly above chance during the standard recognition phases for all three recency difficulty conditions (all p < 0.001) and a three-way mixed model ANOVA examining the D1 scores of the two standard recognition phases across all difficulty levels yielded a significant effect of Task

Difficulty (i.e., 'easy', 'medium', 'hard'; $F_{(2, 40)}$ = 12.0, p < 0.001). There was no effect of Group, Standard Recognition Phase, or interactions (all p > 0.1).

The mean total exploration times per trial for the Sham1 and ATNx1 groups are shown in Table 2.6. There was no difference in the total amount of exploration between the Sham1 and ATNx1 groups during both the standard recognition and recency phases (p > 0.1 for both). However, there was a significant effect of Difficulty for the recency phase (as difficulty increased, so did exploration; $F_{(2, 40)}$ = 6.82, p = 0.003), and a significant effect of Standard Recognition Phase indicating that animals explored the objects of the second standard recognition phase less than the first (F $_{(1, 20)}$ = 9.55, p = 0.006).

Experiment 2.5: Within-block recency with objects

D2 Index

The recency (test) phase was analysed by first grouping the number of interleaving objects into a 'low' (3 or 7 interleaving objects) and 'high' (11 or 15 interleaving objects) categories. One-sample t-tests of the D2 scores indicated that Sham1 rats were significantly above chance (i.e. choosing the older objects) for both the 'low' and the 'high' number of interleaving items (low: t_9 = 4.99, p < 0.001; high: t_9 = 2.59, p = 0.029), whereas the ATNx1 rats were not (low: t_{11} = 1.981, p = 0.073; high: p > 0.1). The ATNx1 rats were significantly impaired compared with Sham1 rats ($F_{(1, 20)}$ = 34.5, p < 0.001). However, there was no effect of Number of Interleaving Items (i.e. no effect of 'high' and 'low' numbers of interleaving items) or Group x Interleaving Item interaction (p > 0.1 for both).

Because of the lack of effect of the number of interleaving items, the data were collapsed across the 'high' and 'low' interleaving items categories (Figure 2.8D). One-sample t-tests of the D2 scores again indicated that the Sham1 group showed a significant preference for the older item (t_9 = 6.59, p < 0.001), whereas the ATNx1 group did not differ from chance (p > 0.1).

Table 2.6. The mean total exploration (in seconds) per trial for the Sham1 and ATNx1 groups at the three difficulty levels (easy, medium, hard) using either objects or odours during the between-block recency task. The standard error of the mean (SEM) is displayed in brackets.

Item	Difficulty	Standard Recognition Phase 1	Standard Recognition Phase 2	Recency (Test) Phase 3
Objects	**'easy'**	Sham1: 15.1 (1.5)	Sham1: 13.9 (1.8)	Sham1: 6.1 (0.6)
		ATNx1: 13.9 (1.1)	ATNx1: 13.6 (1.3)	ATNx1: 6.2 (0.9)
	'medium'	Sham1: 15.0 (0.8)	Sham1: 14.0 (1.0)	Sham1: 7.2 (0.6)
		ATNx1: 14.5 (1.2)	ATNx1: 12.3 (1.3)	ATNx1: 8.0 (0.5)
	'hard'	Sham1: 18.0 (1.4)	Sham1: 14.7 (1.2)	Sham1: 7.5 (0.4)
		ATNx1: 13.9 (0.6)	ATNx1: 13.6 (1.1)	ATNx1: 7.5 (0.8)
Odours	**'easy'**	Sham1: 9.2 (0.6)	Sham1: 7.6 (1.1)	Sham1: 4.9 (0.9)
		ATNx1: 8.9 (0.6)	ATNx1: 8.3 (0.8)	ATNx1: 4.9 (0.6)

D1 Index & Total Exploration

The D1 scores showed a similar pattern as the D2 scores: the Sham1 rats demonstrated a consistent preference for older objects (low: t_9 = 2.53, p = 0.033; high: t_9 = 3.02, p = 0.015). The D1 scores of the ATNx1 rats for the 'low' category resulted in a significant preference for more recent objects (t_{11} = 3.09, p = 0.01), but they showed no significant preference between the older or more recent objects in the 'high' category (t_{11} = 1.81, p = 0.097). A two-way mixed model ANOVA yielded a significant effect of Group (i.e., ATNx1 group impaired; $F_{(1, 20)}$ = 13.4, p = 0.02), but no effect of Interleaving Objects or interaction (p > 0.1 for both).   Because of the lack of effect of Interleaving Objects, the data were then collapsed across 'low' and 'high' interleaving items, and one-sample t-tests of the D1 scores found the same results as the D2 scores: Sham1 rats spent significantly more time exploring the older objects (t_9 = 3.83, p = 0.004), whereas ATNx1 rats did not (p > 0.1).

The mean total amount of time spent exploring the objects during the choice phase did not differ between the Sham1 and ATNx1 groups when the data were divided into 'low' and 'high' categories (p > 0.1). Furthermore, the cumulative total exploration during the sample phase did not differ between the Sham1 and ATNx1 groups (p > 0.1). The mean total exploration times for the Sham1 and ATNx1 groups are displayed in Table 2.7.

Table 2.7. The mean total exploration (in seconds) of the within-block (temporal order) recency task for the Sham1 and ATNx1 groups during the sample and test phases using either objects or odours. The recency (test) phase was also divided into 'low' and 'high' number of interleaving items.

Item	Delay	Sham1	ATNx1
Objects	**Sample**	10.0 (0.9)	9.5 (0.6)
	'low'	3.8 (0.8)	3.7 (0.4)
	'high'	3.6 (0.6)	3.5 (0.5)
	Collapsed ('low' + 'high')	3.7 (0.6)	3.6 (0.4)
Odours	**Sample**	7.5 (0.6)	8.7 (0.7)
	'low'	3.0 (0.4)	3.0 (0.4)
	'high'	3.8 (0.3)	3.8 (0.5)
	Collapsed ('low' + 'high')	3.4 (0.3)	3.4 (0.4)

Experiment 2.6: Odour recognition in the bow-tie maze

D2 Index

Figure 2.9 displays the final updated D2 score for both the standard recognition phase (less than one minute delay) and the 60 minute delay phase. One-sample t-tests demonstrated that the Sham1 group was significantly above chance at delays of less than a minute and 60 minutes (one minute: $t_9 = 4.29$, p = 0.002; 60 minutes: $t_9 = 4.30$, p = 0.002), whereas the ATNx1 group showed a preference for the novel odours only at the 60 minute retention (one minute: $t_{11} = 1.62$, p > 0.1; 60 minutes: $t_{11} = 3.45$, p = 0.005). However, there is no significant effect of Group, Delay, or interaction (p > 0.1 for all). Inspection of Figure 2.9 suggests that the large variance in the performance of the ATNx1 group accounts for the failure to detect a significant preference for the novel odours in the standard recognition task.
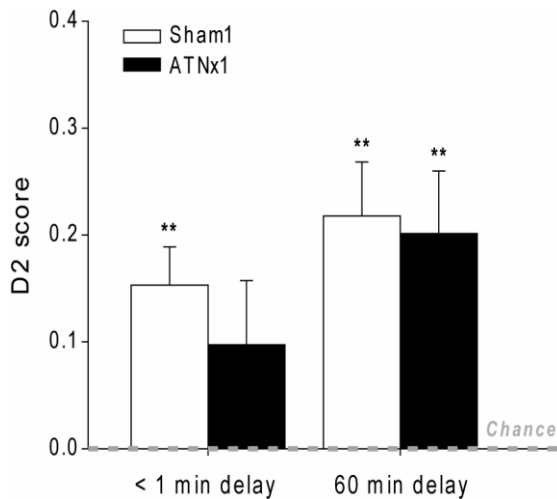
Figure 2.9. The final D2 scores of the Sham1 and ATNx1 animals for an odour recognition task in the bow-tie maze when run in the dark. Data shown are group means, and the vertical bars are the standard error of the means (SEM). Zero represents chance (i.e. exploring novel and familiar items equally). Significantly different from chance: Note: ** = p < 0.01.

D1 Index & Total Exploration

Inspection of the D1 scores demonstrated a similar pattern of performance to the D2 scores (one-sample t-tests: one minute delay, Sham1: t_9 = 3.42, p = 0.008; ATNx1: p > 0.1; 60 minute delay, Sham1: t_9 = 2.99, p = 0.015; ATNx1: t_{11} = 3.89, p = 0.003; ANOVA: all, p > 0.1). The mean cumulative total exploration of the odours with a 1 min delay was 65.8 s for the Sham1 group and 63.4 s for the ATNx1 group; while the mean cumulative total exploration following a 60 min delay was 43.9 s for the Sham1 group and 45.3 s for the ATNx1 group. There was no difference between the Sham1 and ATNx1 groups in the total exploration of the odours (p > 0.1), but both groups spent significantly more time exploring the odours in the standard recognition condition compared with the 60 minute delay condition ($F_{(1,20)}$ = 10.1, p = 0.005).

Experiment 2.7: Between-block (List 1 vs. List 2) recency with odours

The final D2 scores for the two sample phases (i.e., standard recognition) and the choice (test) phase of the between-block recency task are displayed in Figure 2.10A.

Recency Phase D2 Index

During the recency (test) phase, neither the ATNx1 nor the Sham1 group differed from chance (i.e. no preference for the older or more recent odour; Sham1: p > 0.1; ATNx1: t_{11} = 1.99, p = 0.072). Further examination of the test phase also indicated

that there was no difference between the Sham1 and ATNx1 groups (p > 0.1), although this comparison was potentially been affected by floor effects.

Recency Phase D1 Index

Similar to the D2 scores, one-sample t-tests of the D1 index during the test phase indicated that neither group was significantly above chance (Sham1: p > 0.1; ATNx1: t_{11} = 2.03, p = 0.067). Furthermore, the groups did not differ significantly during the recency phase (p > 0.1).
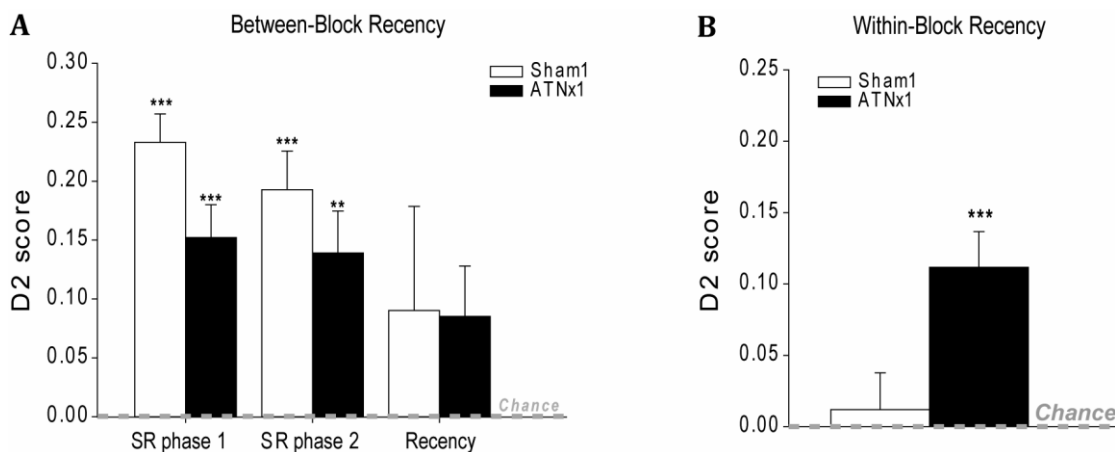
Figure 2.10. A) The final D2 scores for the two sample phases and the choice (test) phase of the between-block recency task, and **B**) the mean D2 score of the ATNx1 and Sham1 animals on the within-session recency task using odours. Data shown are group means, and the vertical bars are the standard error of the means (SEM). Zero represents chance (i.e. exploring novel and familiar items equally). Significantly different from chance: ** = p < 0.01; *** = p < 0.001. SR, standard recognition.

Standard Recognition Phase (1 min retention) D2 Index

One-sample t-tests revealed that both groups of rats showed a preference for the novel odour during the two standard item recognition phases (Sham1 standard recognition phase 1: t_9 = 9.65, p < 0.001; Sham1 standard recognition phase 2: t_9 = 5.87, p < 0.001; ATNx1 standard recognition phase 1: t_{11} = 5.44, p < 0.001; ATNx1 standard recognition phase 2: t_{11} = 3.91, p = 0.002). An ANOVA was used to compare the D2 scores of the ATNx1 and Sham1 groups across the two standard recognition phases. There was no effect of Standard Recognition Phase (i.e. the first or the second) and no Standard Recognition Phase x Group interaction (p > 0.1 for both). There was, however, a significant main effect of Group; therefore,

while the ATNx1 group showed a preference for the novel odour, they were significantly poorer at discriminating between the novel and familiar odours compared with the Sham1 rats ($F_{(1,20)} = 5.81$, $p = 0.026$).

Standard Recognition Phase (1 min retention) D1 Index & Total Exploration

Similar to the D2 scores, one-sample t-tests of the D1 scores demonstrated that both Sham1 and ATNx1 groups were significantly above chance during the standard recognition sample phases (all, $p < 0.001$). However, unlike the D2 scores, an ANOVA examining the D1 scores of the two standard recognition phases did not yield any significant differences in Group ($p > 0.1$), Standard Recognition Phase ($F_{(1, 20)} = 3.10$, $p = 0.094$), or Group x Standard Recognition Phase interaction ($p > 0.1$).

There was no difference in the total amount of exploration between the Sham1 and ATNx1 groups during both the sample and choice phases ($p > 0.1$ for both). However, there was a significant effect of Standard Recognition Phase indicating that animals explored the odours in the second standard recognition phase less than the first ($F_{(1, 20)} = 4.93$, $p = 0.038$). The mean total exploration scores per trial for the Sham1 and ATNx1 groups are shown in Table 2.6.

Experiment 2.8: Within-block recency with odours

D2 Index

The test phase of the within-block recency task with odours was analysed by first grouping the number of interleaving odours into 'low' (3 or 7 interleaving odours) and 'high' (11 or 15 interleaving odours) categories. One-sample t-tests of the D2 scores indicated that the Sham1 rats were not different from chance for both the low and the high number of interleaving items ($p > 0.1$ for both), whereas the ATNx1 rats spent significantly more time exploring the older odours regardless of the number of interleaving items (low: $t_{11} = 2.73$, $p = 0.019$; high: $t_{11} = 4.2$, $p = 0.001$). The ATNx1 rats discriminated between the older and more recent odours while the Sham1 rats did not ($F_{(1, 20)} = 7.64$, $p = 0.012$). There was no effect of Number of Interleaving Items (i.e. no effect of 'high' and 'low') or Group x Interleaving Item Interaction ($p > 0.1$ for both).

Because there was no significant effect of the number of interleaving items, the data were collapsed across the 'high' and 'low' interleaving odours categories. One-sample t-tests of the D2 scores again indicated that the Sham1 group failed to demonstrate a preference for the older item (p > 0.1), whereas the ATNx1 group was significantly above chance (t_{11} = 4.48, p < 0.001; Figure 2.10B).

D1 Index

The D1 scores showed a similar pattern as the D2 scores: the Sham1 rats failed to discriminate between older and more recent odours (p > 0.1 for both). The D1 scores of the ATNx1 rats for the 'low' category resulted in a significant preference for older odours (t_{11} = 2.34, p = 0.004), but they showed no preference for either the older or more recent odours in the 'high' category (p > 0.1). Unlike the D2 index, there was no difference between Sham1 and ATNx1 animals (p > 0.1). There was also no effect of Interleaving Objects or interaction (p > 0.1 for both). Again, in contrast to the D2 scores, once the data were collapsed across 'low' and 'high' number of interleaving items, analyses of the D1 scores found the neither the sham or ATNx1 rats spent significantly more time exploring the older odours (p > 0.1 for the Sham1 group; t_{11} = 1.94, p = 0.079 for the ATNx1 group).

The total amount of time spent exploring the odours during the recency (test) phase did not differ between the Sham1 and ATNx1 groups (p > 0.1) when the data were divided into 'low' and 'high' interleaving odours. However, both groups spent more time exploring the items in the 'high' category ($F_{(1,20)}$ = 8.72, p = 0.008). There was no Group x Interleaving Items interaction (p > 0.1). Furthermore, the cumulative total exploration during the sample phase did not differ between the Sham1 and ATNx1 groups (p > 0.1). The mean total exploration scores per trial for the Sham1 and ATNx1 groups are shown in Table 2.7.

Experiment 2.9: Locomotor Activity

The total number of single and cross-over beam breaks for the Sham1 and ATNx1 groups are displayed in Figure 2.11. Two separate between-subjects t-tests indicated that the ATNx1 group made significantly more single beam breaks (Mann-Whitney U Statistic = 16.0, T = 71.0, p = 0.004; non-parametric test used

because of a violation of normality) and cross-over beam breaks ($t_{20} = 4.28$, p < 0.001) compared to the Sham1 group, signifying that the ATNx1 rats were hyperactive.
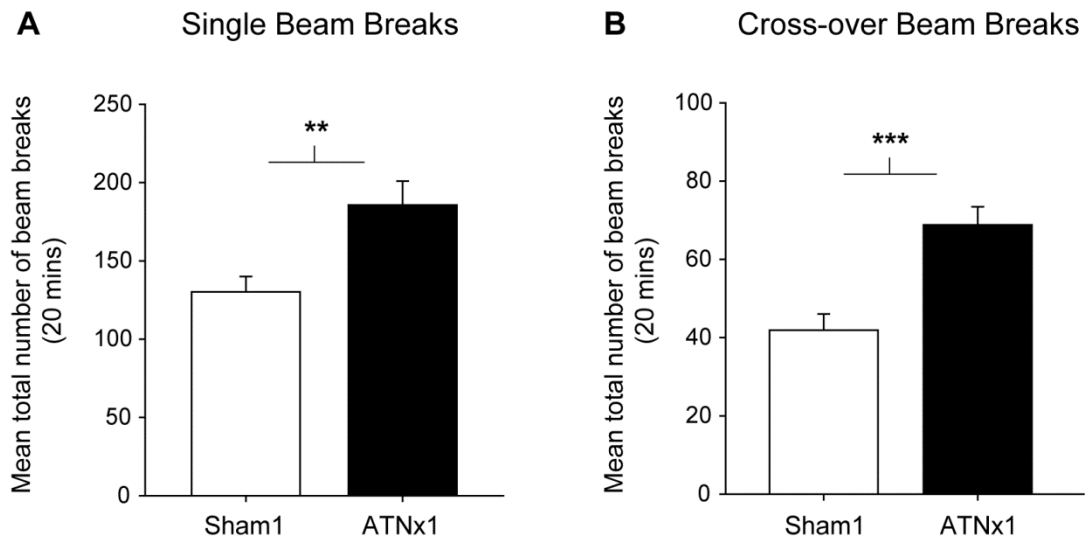
Figure 2.11. A) The total number of single and **B**) cross-over beam breaks for the Sham1 and ATNx1 groups. **Note:** ** = p < 0.01; *** = p < 0.001.

Discussion

The aim of these experiments was to compare rats with lesions to the anterior thalamic nuclei with sham animals on two types of recency judgments: 1) Between-block recency, where long delays and the removal of the animal from the training apparatus creates distinct temporal lists of items (List 1 vs. List 2), and 2) within-block recency, where the animal is asked to distinguish the order of items within a list (e.g., Item 1 vs. Item 7 in a single list of items). Both the between-block and the within-block recency tasks used modified object recognition procedures in the bow-tie maze (Albasser, Chapman et al., 2010; Albasser, Poirier et al., 2010). For comparison, the rats were trained on recognition memory tasks as well as recency tasks using either objects or odours.

Object Recognition

Rats with damage to the anterior thalamic nuclei were unimpaired on object recognition tasks using either the bow-tie maze (Experiment 2.2 & 2.4 sample phases; delays of 1 or 60 min) or an open arena (Experiment 2.3; 15 min delay). These results support previous research also indicating that animals with damage

to the anterior thalamic nuclei have the same spontaneous preference for novel objects as sham animals (Aggleton et al., 1995; Warburton & Aggleton, 1999; Moran & Dalrymple-Alford, 2003; Mitchell & Dalrymple-Alford, 2005, see also Aggleton, Dumont et al., 2011). Although no studies have reported significant differences between rats with anterior thalamic lesions and their controls, in some cases one group is not significantly above chance (i.e., the rats sometimes fail to show a preference for the novel item; e.g., Moran & Dalrymple-Alford, 2003). Traditional spontaneous recognition tasks in an open arena tend to yield large variability in performance as the exploratory preference for the novel items is typically derived from very few trials, in contrast the bow-tie maze. This situation may explain why some previous research has failed to report that either rats with anterior thalamic lesions or their controls are above chance (Aggleton et al., 1995; Moran & Dalrymple-Alford, 2003).

In the present experiment, using either the bow-tie maze or an open arena, both Sham1 and ATNx1 groups were significantly above chance, indicating a preference for the novel objects. In fact, both groups were significantly above chance for the entire five minute choice phase in the open arena. The similarity in D2 scores between the two apparatuses reinforces the validity of the bow-tie maze procedure for tests of object recognition. Furthermore, across both tasks, the ATNx1 rats were unimpaired on object recognition tasks with 1, 15, and 60 minute retention delays, also in line with previous reports finding intact recognition memory in rats with anterior thalamic lesions with up to a two hour delay between the sample and test phases (Mitchell & Dalrymple-Alford, 2005). Across the task, there was a significant main effect of delay, presumably reflecting the increased memory load with longer delays (Experiment 2.2).

Between-block recency with objects

The current study systematically examined the effects of manipulating recency difficulty by changing the length of the delay periods. Manipulating task difficulty significantly affected the ability of rats to discriminate between two lists of objects. When the delay was longer between the sample phases and shorter between the second sample and the test phase (i.e. 'easy'), both ATNx1 and Sham1 rats spent

significantly more time exploring the older object. This preference for the older object decreased and was not above chance for either the 'medium' or 'hard' difficulty. Importantly the performance of both groups was affected by task difficulty, and they did not differ from one another. This result is consistent with another between-block recency task where the rats were presented with two different sets of objects for each of the two sample phases that were separated by a 60 min delay (Mitchell & Dalrymple-Alford, 2005). During the delay periods the animal was returned to its home cage, and then brought back to the testing arena for the second sample phase and the recency (test) phase. Rats with damage to the anterior thalamic nuclei do not differ significantly from control rats when a between-block (List 1 vs. List 2) procedure is used to examine recency memory (Experiment 2.4 and Mitchell & Dalrymple-Alford, 2005).

Within-block recency with objects

Unlike the between-block recency task, ATNx1 rats were significantly impaired on the within-block recency task using objects. The present findings appear inconsistent with the results of very different temporal sequence task (Aggleton, Amin et al., 2011). In this task, the rats were placed inside an operant chamber and were required to learn six auditory-visual stimulus compounds that were reinforced if the compound was presented in a particular order (e.g., reinforce if A occurs before B, but not if B occurs before A). Given that training required 39 sessions of 8-24 trials [divided into 6 learning stages and one probe (test) stage], it remains possible that the lack of impairment in this conditional sequence learning task is simply because of the training procedure: the six stimulus compounds were repeating frequently, and the rule for any given compound remained constant, so that the task may be solved in a qualitatively different way than distinguishing the order of 18 objects (i.e., the procedure used in Experiment 2.5). However, the advantage of the compound sequence learning task is that cannot be solved solely on the basis of trace intensity or short-term habituation, whereas it remains possible in the present temporal order task that the ATNx1 rats have a spontaneous preference for the strong memory trace (more recent object) regardless of the presence of a weak trace; therefore, the rat may not be comparing the two temporal items, but simply spending time exploring the stronger trace (see

Aggleton, Amin et al., 2011). Therefore, any impairment of the ATNx1 animals might be with distinguishing the relative strength of the memory trace and not tagging the temporal order of the objects. However, this explanation seems unlikely for stimuli in the light given that ATNx1 rats performed equally well as Sham1 animals on both the object recognition (in some cases out performed Sham1 rats; see Experiment 2.4 sample phase) and the between-block recency tasks which presumably could be solved on the basis of different memory strengths. More likely, other differences between the two methodologies account for the conflicting results.

The present study did not find a significant effect of the number of interleaving objects. It was hypothesised that animals would distinguish older from more recent objects better when there was a high number of interleaving objects compared with a low number of interleaving objects. This result was not confirmed, despite evidence that the performance of both control animals and those with fornix transections improved with increasing number of interleaving objects (goal boxes) using delay non-match-to-sample procedure (Shaw & Aggleton, 1993).

Odour recognition & between-block recency

This is the first study to examine the effects of ATNx1 damage on a between-block recency task with odours. On the odour recognition task, both the Sham1 and ATNx1 groups spent significantly more time exploring the novel odour when tested with one minute and 60 minute delays (Experiment 2.6 & 2.7 sample phase). These results are consistent with another report of intact odour recognition memory in rats with anterior thalamic damage (Wolff et al., 2006). However, although the rats demonstrated a preference for the novel odour, the ATNx1 rats were significantly impaired compared with the Sham1 rats during the standard odour recognition phases of Experiment 2.7 (but not during the odour recognition in Experiment 2.6). Furthermore, there was no effect of delay (Experiment 2.6), suggesting that the forgetting curve (or trace strength) for odours may be flatter than that of objects. This latter point is particularly relevant as neither group differed from chance during the test phase of the between-recency task (see

Experiment 2.4 & 2.7). The higher variability in performance may in part be due to the limitations of using odours: they are most likely detected at a greater distance than the behavioural threshold set by the experimenter to quantify exploration of the cubes, mixing of the odours most likely occurs, as well as natural preferences and aversions to particular smells (Burn, 2008). Indeed, it may be informative to re-analyse the behaviour of the rats and consider exploratory behaviours at a greater distance from the odour cubes then the threshold currently used in this study.

Within-block recency with odours

The findings that the ATNx1 rats explored the older odours compared to the more recent ones on the within-block recency task, and that Sham1 animals performed at chance seems both surprising and inconsistent with a previous within-block recency task using odours (Wolff et al., 2006). However, the present experiment differed in two important ways: 1) the current study used a list of 18 odours, whereas Wolff et al. (2006) used 10 odours in total and these 10 odours were presented in repeating lists of six sample items, and 2) the measure taken in the present experiment was spontaneous exploration, whereas the rats in Wolff et al. (2006) study were required to learn a particular reinforced rule. It is also unclear why the ATNx1 group could discriminate between two odours, one of which occurred earlier in a list, but failed to do so when objects were used. It should be stressed that the D1 scores did not yield a significant difference between groups, nor were the ATNx1 rats significantly above chance for D1. It is possible that the discrepancy between the D1 and D2 scores was influenced by the low levels of exploration. When the levels of exploration are low, the D1 score may be a better measure because small differences in the D1 scores can result in large D2 ratios when the D1 scores are divided by a low level of total exploration (e.g., if the mean D1 score is 1 s, the D2 scores would be 0.5 if the total exploration is 2 s, but the D2 scores would be 0.1 if the total exploration of the items is 10 s). An examination of the D1 index suggests that neither group could discriminate between older and more recent odours.

The failure of Sham1 and ATNx1 rats to discriminate between the relative recency of the odours could be explained by a loss of sensory information; while the objects differed in shape, size, and texture, the odour cubes differed only along one dimension. Conversely, if trace strength was used to solve the task and odours have a flatter forgetting or trace degradation curve, than the animals may fail at discriminating between the two odours. Arguably, the latter explanation is unlikely given the lack of effect of the number of interleaving odours; unless the degradation of the trace strength is much slower than the time intervals between the two choice odours (i.e., there may be little change in trace strength over the whole 18 minute sample phase). The data from the odour recognition task suggests that this might be the case, given that the discrimination ratio of the animals did not deteriorate with the 60 min delay compared with the one minute delay condition. Sham1 and ATNx1 rats did spend significantly more time exploring odours that had a high number of interleaving items compared with low number of interleaving items, suggesting that while they failed to discriminate which odour occurred earlier in a list, they could both detect difference in the temporal separation between the odours.

Locomotor activity measured by the total number of beam breaks during 20 minutes revealed that rats with damage to the anterior thalamic nuclei are hyperactive. This is consistent with previous findings (Jenkins, Vann et al. 2004; Poirier & Aggleton, 2009). It is striking to note that the increased locomotor activity did not influence the amount of time exploring the items; for all the experiments the Sham1 group and ATNx1 groups did not differ from one another on the total amount of time exploring the objects. The only exception was found during the first minute of the five minute choice phase in Experiment 2.3. During the first minute, Sham1 animals spent more time exploring the objects when compared with ATNx1 rats; however, this difference disappeared when the whole five minutes was considered. One possibility for why this difference emerged only in Experiment 2.3 is that the animals were tested in an open field arena where there was more space to run between objects. It is possible that when ATNx1 rats were less confined, the hyperactivity caused by the lesion resulted in them spending more time running around the arena and less time exploring the objects.

The rats were also tested on T-maze alternation. The ATNx1 rats were significantly impaired compared with Sham1 animals, consistent with previous reports (Aggleton et al., 1995, 2009; Aggleton, Amin et al., 2011). However, the data were also collapsed across training days for each of the six trials in order to examine whether there was a spatial within-block recency effect. It was found that as the trials progressed throughout the session, familiarity of both arms increased making the task more difficult. It was found that both groups performed significantly worse as the trials progressed, suggesting that previous trials interfered with later ones. However, the severe impairment of the ATNx1 group was most likely spatial in nature, consistent with the previous literature (Aggleton et al., 1996; Byatt & Dalrymple-Alford, 1996; Parker & Gaffan, 1997; Sziklas & Petrides, 1999; Warburton & Aggleton, 1999; Warburton et al., 1999; Mitchell & Dalrymple-Alford, 2005; Sziklas & Petrides, 2007).

The goal of this chapter was to explore the role of the anterior thalamic nuclei on recency judgments. The results indicate that anterior thalamic lesions can affect certain classes of recency judgments: within-block recency tasks using objects. In addition, the type of stimuli (objects or odours) influences whether control or lesion animals could discriminate between older and more recent items. The anterior thalamic nuclei are highly interconnected with the hippocampus (Nauta, 1956; Aggleton et al., 1986; van Groen & Wyss, 1995), and hippocampal lesions disrupt both within-block and between-block recency tasks (Fortin et al., 2002; Kesner et al., 2002; Kesner et al., 2010; Albasser et al.,2012). There is also evidence that fornix transection in monkeys impairs both types of recency tasks using a delay-match-to-sample procedure (Charles et al., 2004), but leaves object recognition intact. Taken together, the results suggest that the role of the "extended hippocampal system" can be increased from the spatial domain into the temporal domain as well. In doing so, the effects of anterior thalamic damage do not appear to mimic precisely the impact of hippocampal lesions.

Chapter 3

The effect of anterior thalamic damage on immediate early gene activity following an object recognition task

Introduction

Research investigating the neurobiology of object recognition emphasises the importance of the medial temporal lobe structures. Lesion studies in both monkeys and rats have demonstrated that the perirhinal cortex is necessary for recognising novel objects (Meunier, Bachevalier, Mishkin, & Murray, 1993; Mumby & Pinel, 1994; Aggleton, Albasser, Aggleton, Poirier, & Pearce, 2010; Albasser et al., 2011), whereas the importance of its functional interaction with hippocampus remains much debated (Brown & Aggleton, 2001; Squire et al., 2007). A complementary approach involves imaging at a cellular resolution with the use of immediate early genes (IEGs). Research has shown selective increases in the IEG *c-fos* in the perirhinal cortex following the presentation of novel stimuli (Zhu, McCabe, Aggleton, & Brown, 1996; Zhu, McCabe, Aggleton, & Brown, 1997; Wan, Aggleton, & Brown, 1999), whereas increases in the hippocampus occur when familiar items are presented in a novel configuration (Zhu et al., 1997; Wan et al., 1999; Jenkins, Amin et al., 2004, but see also Aggleton & Brown, 2005; Warburton & Brown, 2010; Aggleton et al., 2012). The next step is to demonstrate the nature of functional links between these two structures. One strategy is to compare activity across many different regions using IEGs.

By combining aspects of delay non-match to sample (Mishkin & Delacour, 1975; Aggleton, 1985) with aspects of spontaneous object recognition (Ennaceur & Delacour, 1988), and by giving multiple trials, Albasser, Poirier et al. (2010) developed a task where behavioural evidence of visual novelty could be measured, and at the same time yield a signal detectable by IEG imaging. Their results found

greater *c-fos* activity in the caudal perirhinal cortex, area TE2, CA3, and CA1, as well as a relative decrease in the dentate gyrus of the hippocampus in rats that were given a list of novel objects compared to those given a list of familiar ones. Furthermore, using structural equation modeling to further analyse the *c-fos* counts, evidence emerged of a functional pathway from the perirhinal cortex to the hippocampus that switched from the temporo-ammonic to perforant pathways when presented with familiar or novel objects, respectively. Thus, suggesting a potential means by which the rhinal cortex can differentially influence hippocampal processing (Albasser, Poirier et al., 2010).

In contrast to the medial temporal lobe structures, the contribution of the diencephalon, in general, and the anterior thalamic nuclei, specifically, during object recognition remains relatively unknown. Monkeys with lesions to the medial thalamus have deficits in delay non-match to sample tasks using trial unique objects (Aggleton & Mishkin, 1983a,b). Aggleton and Mishkin (1983a) compared animals with lesions that targeted the anterior medial thalamus (centred on the anterior thalamic nuclei) and those of the posterior medial thalamus, and found that both lesions impaired object recognition. They also noted that combined damage of these regions increased the magnitude of the deficit. Although these lesions were large and resulted in atrophy in other diencephalic regions (e.g., the mammillary bodies), this study demonstrates the importance of the diencephalon for the accurate detection of visual novelty. In contrast, studies in rats using spontaneous object exploration have failed to find object recognition impairments after anterior thalamic lesions (e.g., Moran & Dalrymple-Alford, 2003; Mitchell & Dalrymple-Alford, 2005; see Chapter 2, Experiment 2.2 & 2.3). However, the lack of behavioural deficits following lesions to the anterior thalamic nuclei in rats does not preclude this neural region from contributing to recognition memory in the intact brain. This possibility could be investigated with the use of IEG imaging provided that there are enough labeled cells within the thalamus, a problem with several IEGs (e.g., *c-fos* and *zif268;* although some studies have successfully counted cells from the thalamus using *c-fos* and *zif268*; see the General Introduction, Chapter 1 for more details).

One way to investigate the potential contribution of the anterior thalamic nuclei on recognition memory is by examining whether damage to the anterior thalamus differently affects activity in other interconnected brain regions when animals explore novel or familiar objects compared with the intact brain. Previous research has shown that damage to the anterior thalamic nuclei results in a dramatic reduction of IEG activity (*c-fos* and *zif268*) in the retrosplenial cortex, and hippocampus (Jenkins, Dias, Amin, Brown et al., 2002; Jenkins, Dias, Amin, & Aggleton, 2002; Jenkins, Vann et al., 2004; Poirier & Aggleton, 2009). However, previous reports have not revealed any reductions in *c-fos* activity within the rhinal cortex following lesions to the anterior thalamus (Jenkins, Dias, Amin, Brown et al., 2002; Jenkins, Dias, Amin, & Aggleton, 2002), suggesting that while both the rhinal cortex and anterior thalamus may influence hippocampal activity, they remain largely independent from one another. However, more research is needed to support this claim, given that one study reported decreased *c-fos* counts in the postrhinal cortex (though not perirhinal cortex) following damage to the fornix, the major axonal fiber connecting the hippocampus with the anterior thalamic nuclei (Vann, Brown, Erichsen, & Aggleton, 2000).

The goal of the present experiment was to investigate the impact of damage to the anterior thalamic nuclei on the activity of the hippocampus and related limbic regions by assaying levels of *zif268* following object recognition. The IEG *zif268* (also known as *egr-1 or krox24*) was chosen for two main reasons: First, the IEG *zif268* was used to visualise active cells because this marker is known to be involved in synaptic plasticity, learning and memory (Cole et al., 1989; Wisden et al., 1990, see also Davis et al., 2003). For example, stimulating the perforant path to induce different decay rates of long-term potentiation (LTP) in the dentate gyrus results in increased *zif268* activity that is correlated with the persistence of LTP (Abraham et al., 1993). Furthermore, Jones et al. (2001) used mutant mice lacking *zif268*, and found that LTP in these mice returned back down to basal levels compared with normal mice after 24 hours, suggesting that *zif268* is important for the long-term maintenance and stability of plastic changes. The second reason for using *zif268* is that it will allow for a comparison with previously published studies using *c-fos* (i.e., would changes in *zif268* activity mirror those found using *c-fos*;

Jenkins, Dias, Amin, Brown et al., 2002; Jenkins, Dias, Amin, & Aggleton, 2002; Albasser, Poirier et al., 2010).

Rats were given unilateral lesions of the anterior thalamus to allow inter-hemispheric comparisons of *zif268* activity levels to be made. These rats were divided into two groups (Group Novel and Group Familiar). One group was given novel objects to explore on the final test session while the other group was given just familiar objects to explore (same protocol used by Albasser, Poirier et al., 2010). Based on previous measures of *c-fos* expression using this protocol (Albasser, Poirier et al., 2010) it was expected that the Group Novel rats would show higher perirhinal and hippocampal IEG expression in the intact hemisphere than Group Familiar, making it possible to detect any impact of anterior thalamic damage on these temporal lobe sites.

Materials and Methods

Subjects

The subjects were 25 male Lister Hooded rats *(Rattus norvegicus)* housed in a 12-hour light/dark cycle and weighing 270-320g at the beginning of the experiment (Harlan, Bicester, U.K.). Water was provided *ad libitum* throughout, but the rats were maintained at 85% of their free-feeding weight for the duration of the experiment. The rats were divided into two groups: Novel (n = 13) and Familiar (n = 12), and where possible housed in pairs of one Group Novel rat and one Group Familiar rat. Every rat received a unilateral anterior thalamic lesion. The experiment was performed in accordance with the UK Animals (Scientific Procedures) Act (1986) and associated guidelines, thereby complying with APA ethical standards for the treatment and care of animals.

Surgery

Surgery was performed as described in Chapter 2. However, one hemisphere was left intact (Sham). In the lesioned hemisphere (ATNx), 0.22μl of NMDA was injected over a period of five minutes in each site, and the syringe was left *in situ* for an addition four minutes before being retracted. The anteroposterior (-0.3),

mediolateral (± 0.9 and ±1.8 from the midline), and the height lesion coordinates [dorso-ventral -7.0 (medial site), -6.3 (lateral site)] were taken relative to bregma.

Behavioural Testing

Apparatus and materials

The rats were tested in a bow-tie-shaped maze (120 cm long, 50 cm wide and 50 cm high) as previously described in Chapter 2.

The study used 147 pairs of objects that differed in size, shape, colour, and texture, but were without any obvious odour to the experimenter. The objects were large enough to cover the circular food-wells but light enough for the rats to displace. The items were divided into seven groups of 21 pairs of objects.

Pre-training

Behavioural training commenced four weeks after surgery. Animals were habituated for approximately seven days as described in Chapter 2. By the end of pre-training all rats would run from one end of the maze to the other and displace objects covering the food-wells in order to obtain a reward (sucrose pellet; 45mg; Noyes Purified Rodent Diet, Lancaster, NH, USA). Objects used during pre-training were not used during the experiment proper (with the exception of a wooden block which was used during Trial 0; see below and Chapter 2 for more details).

Testing protocols

All rats were tested for 13 sessions, each containing 20 trials. For Group Novel each session began with the rat being placed in one end of the maze that contained two items over the baited food-wells, a novel object (Object A_1) and a wood block that was familiar from its repeated use in pre-training. The rat was allowed to explore both objects freely, but after one minute the guillotine door was raised allowing access to the second compartment, so starting Trial 1. The rat typically ran immediately to the opposite side of the maze, where again it could explore two objects that each covered a single sucrose pellet. One of these objects was novel (Object B_1) while the other was familiar as it was a duplicate of Object A (A_2; Table

3.1). After a minute, the guillotine door was raised again and the rat ran back to the first compartment of the maze (Trial 2) where Object C_1 (novel) and a duplicate of Object B (familiar B_2) were presented. Following a further minute of free exploration, the guillotine door was raised again (Trial 3), and the rat ran back into the second compartment to explore a copy of Object C (C_2, now familiar) and new Object D_1 (novel). This sequence continued for a total of 20 trials (Table 3.1).

Table 3.1. Presentation order of objects for Group Familiar (top) and Group Novel (bottom) for Session 1 (left) and Session 13 (final; right). Each of the 13 sessions contains 20 trials. Each trial has two objects depicted by a letter with the exception of Trial 0 which allows the first object to become familiar. For Group Novel, different sets of objects were used throughout training, and novel objects are indicated in bold type. For Group Familiar, the same set of objects was used throughout the 13 sessions, and less recently experienced items are indicated in bold type. The presentation of the objects was identical for Group Familiar and Group Novel on the final session.

Group Familiar

Trials	Session1								Session 13 (Final)						
	0	1	2	3	4	5	6 - 20		0	1	2	3	4	5	6 - 20
Objects	-	A	B	C	D	E	…		-	C	F	B	E	D	…
	A	**B**	**C**	**D**	**E**	**F**	**…**	Session 2-12	**C**	**F**	**B**	**E**	**D**	**A**	**…**

Group Novel

Trials	Session1								Session 13 (Final)						
	0	1	2	3	4	5	6 - 20		0	1	2	3	4	5	6 - 20
Objects	-	Ψ	Ω	α	β	γ	…		-	C	F	B	E	D	…
	Ψ	**Ω**	**α**	**β**	**γ**	**ε**	**…**	Session 2-12	**C**	**F**	**B**	**E**	**D**	**A**	**…**

All test objects covered one food pellet, which motivated the rats to run back and forth across the apparatus and approach the objects, but did not affect the validity of the preferential test of recognition as this relied on differential levels of object exploration. The rats were placed in a dark, quiet room, for 30 minutes prior to testing, and for 60 minutes at the completion of a session to habituate the rats to being placed in the dark as this procedure is required in the final session to help ensure that changes in IEG activity are a result of the behavioural manipulation (experiencing novel or familiar objects), and not other factors (i.e., IEG changes to experiencing other stimuli). After 60 minutes, the rats were returned to their home cages located in the colony room. All rats received 12

sessions, given over 6 days (one session in the morning and one in the afternoon), prior to the last one (Session 13). For Sessions 1-12, Group Novel was offered a pool of 126 items divided into six sets of 21 object duplicates. As new sets were used for each of the first six sessions it was necessary to re-use that same pool of 126 objects for the second six sessions (Sessions 7-12), though the order and pairings of individual objects changed. Animals were video recorded throughout the 13 sessions.

The rats in Group Familiar were trained in exactly the same way as Group Novel, except for one key difference.   Group Familiar explored the same set of 21 objects on every session, i.e., on Sessions 1-13, although the order and individual pairing of objects changed across sessions. Consequently the rats in this second group should become very familiar with the individual objects.

On the final test (Session 13) both groups of rats were allowed to explore the same set of objects, with the order being matched across the two groups. The objects used were those that had been presented repeatedly to the Group Familiar rats (Sessions 1-12), i.e., all were highly familiar. In contrast, Group Novel had never encountered these objects before and so all were novel (see Table 3.1).

Analysis of behaviour

Exploration of an object was defined as directing the nose at a distance of < 1cm to the object and/or touching it with the nose or the paws (including pushing). Sitting on or turning around the object was not included. Likewise, behaviours such as freezing near the object (at a distance of < 1cm), chewing the object, or carrying the object in the mouth were not scored as exploration. Sessions 1, 7, and 13 (final) were scored for their exploration times of novel and familiar objects.

Histology

All rats were perfused 90 min after completion of the final session, the time delay corresponding to the peak of *zif268* protein production after an initiating event (Zangenehpour & Chaudhuri, 2002). At the end of this 90 minutes delay (which the animals spent in a dark, quiet room), the animals were injected with an overdose of sodium pentobarbital (1ml i.p., 200mg/ml; Euthatal, Marial Animal

Health Ltd, Harlow, Essex, UK) and perfused intracardially with 0.1 M phosphate buffer saline (PBS) followed by 4% paraformaldehyde in 0.1M PBS (PFA). The brains were extracted from the skull and placed on a stirrer to postfix in PFA for four hours, after which the brains were placed in 25% sucrose overnight. The brains were frozen on a microtome (Leica, UK) and sectioned at 40μm in the coronal plane. One-in-five sections were mounted and stained with cresyl violet, a Nissl stain. The remaining sections were divided into four (one-in-five sections) series and frozen in cryoprotectant for later immunohistochemistry.

Volumetric Analysis

The size of the lesions in the anterior thalamic nuclei of all 25 rats was estimated from the Nissl stained tissue using the same procedure described in Chapter 2.

Zif268 immunohistochemistry and Zif268-positive cell counts

Sections were removed from the freezer, and washed for 10 minutes in PBS, four times. The sections were placed in 10mM citrate buffer (pH 6) dissolved in deionised water and incubated in a water bath at 70°C for 30 minutes. The sections were then washed in 0.3% hydrogen peroxide in PBS containing 0.2% Triton X-100 (PBST) for 10 minutes in order to block endogenous peroxidase activity, and rinsed for 10 minutes in PBST, four times. Afterwards, the sections were incubated at 4°C for 48 hours in PBST with rabbit polyclonal antibody for *zif268* (1:3000, C-19, Santa Cruz, Insight Biotechnologies, UK). The sections were then rinsed for 10 minutes in PBST, four times. Following the four washes, the sections were incubated in biotinylated goat anti-rabbit secondary antibody (diluted 1:200 in PBST; Vector Laboratories) and 1.5% normal goat serum for two hours. The sections were washed again, and incubated for one hour in avidin-biotinylated horseradish peroxidase complex in PBST (Elite Kit, Vector Laboratories). Next, sections were rinsed in 0.05M Tris buffer (pH 7.4). The reaction was visualised using diaminobenzidine (DAB Substrate Kit, Vector Laboratories), and stopped by washing in cold PBS. Finally, the sections were mounted on gelatine-coated slides, dehydrated through a graded series of alcohols, and coverslipped.

Sections were viewed on a Leica DMRB microscope, and photographed using an Olympus DP70 camera. Automated counts of the stained cells were obtained using the programme Analysis^D (Olympus, UK). Cell counts were taken without knowledge of group assignments and, where possible, without knowledge of lesion hemisphere. Images were greyscaled, and the cell detection threshold was set manually. With few exceptions (e.g., a particularly lightly stained section), the threshold was the same for all sections from a same processing batch, i.e., held constant between hemispheres and between immunohistochemistry pairs. Counts of labelled nuclei in each region of interest were determined by counting those immunopositive cells that were above the detection threshold, and were between 5-20μm in size. Counts were made in a frame area of 0.84 X 0.63mm using 5X magnification. This frame size enabled all laminae to be included in one image. For larger regions (e.g., hippocampus), montages of the whole structure of interest were created in the coronal plane from multiple images. For all brain regions analysed, between two and four sections per hemisphere were captured, depending on the size of the region. These counts were combined to give a mean result.

Regions of interest

The various regions of interest are depicted in Figure 3.1. The position and approximate coordinates of these sites are taken from images by Paxinos and Watson (2005). The hippocampus was divided into its dorsal and ventral parts. Separate counts were made in CA1, CA3, and the dentate gyrus of the dorsal hippocampus from around the AP levels -4.80 to -5.64 from bregma (Paxinos & Watson, 2005). Counts were made in ventral CA1 and CA3 at a similar level. The dividing border between the dorsal and ventral hippocampus corresponded to -5.0 below bregma (Paxinos & Watson, 2005). The most septal portion of the dorsal hippocampus (anterior to AP -4.80) was, however, avoided as some animals had very restricted cell loss in the ventral blade of the dentate gyrus at this level. In addition to these five hippocampal sub-regions, the dorsal subiculum and postsubiculum were counted as both are directly connected to different parts of the anterior thalamic nuclei (Meibach & Siegel, 1977; Wright et al., 2010). The lateral entorhinal cortex (from AP -4.80 to – 6.30) was also included as it provides
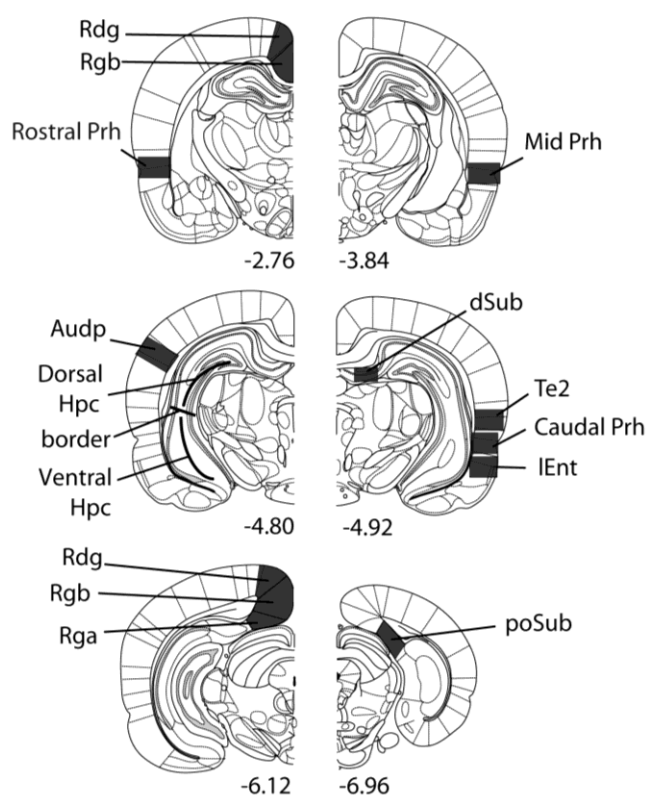
Figure 3.1. Series of coronal sections from Paxinos and Watson (2005) showing the extent of the various areas analysed. The numbers refer to anterior-posterior position relative to bregma. Abbreviations: Audp, primary auditory cortex; dSub, dorsal subiculum; Hpc, hippocampus; lEnto, lateral entorhinal cortex; pSub, postsubiculum; Prh, perirhinal cortex; Rdg, dysgranular retrosplenial cortex; Rga, granular retrosplenial cortex, area a; Rgb, granular retrosplenial cortex, area b.

a key link between the perirhinal cortex and hippocampus (Naber, Witter, & Lopez de Silva, 1999).

Within the parahippocampal region, the perirhinal cortex was examined. The perirhinal cortex was subdivided into three rostro-caudal subregions (see Albasser, Poirier et al., 2010): rostral (from AP -2.76 to -3.84 relative to bregma), mid (AP -3.84 to -4.80), and caudal (from AP -4.80 to -6.30). Multiple counts were taken from each of these three subregions, and their means summed to provide separate totals for areas 35 and 36 (Burwell, 2001). Counts were also taken from the adjacent area TE2 (from AP -4.80 to – 6.30), which has also been repeatedly implicated in novelty detection (Wan et al., 1999; Albasser, Poirier et al., 2010; Ho et al., 2011).

The retrosplenial cortex was first subdivided into granular b (Rgb), granular a (Rga), and dysgranular cortex (Rdg; van Groen & Wyss, 1990, 1992, 2003). Separate counts were made for all three sub-regions, with areas Rdg and Rgb being further subdivided into their rostral (from AP -2.52 to -3.84) and caudal divisions (from AP -4.92 to -6.24). (This extra subdivision was not made for Rga as it is very restricted anterior to the splenium.) The retrosplenial cortex was also divided into superficial (layer II and upper layer III) and deep (lower layers III to

VI) as the effect of anterior thalamic lesions can differentially affect the laminae (Jenkins, Dias, Amin, Brown et al., 2002, Jenkins, Vann, et al., 2004; Poirier & Aggleton, 2009). Finally, the primary auditory cortex was examined to provide a 'control' region, i.e., an area where *a priori* there was not expected to be an effect of condition (Novel versus Familiar) or lesion on *zif268* activity.

Statistics

For the initial analyses, the mean *zif268*-positive cell counts per area were calculated for each animal and separated according to hemisphere. It was then possible to compare these counts in an analysis of variance with one between factor (Group Familiar or Group Novel) and two within factors [surgery (thalamic lesion or intact hemisphere); brain site (region of interest)]. The various regions of interest were grouped, e.g. hippocampal formation, parahippocampal cortex, and retrosplenial cortex, so that each ANOVA consisted of related areas. This procedure resulted in multiple comparisons at the regional level, and so the significance level was further adjusted using the modified Bonferonni test (Keppel & Wickens, 1991) to control further for type I errors. Therefore, for the hippocampus and retrosplenial cortex (both five subregions) the significance level was adjusted to 0.04. The Greenhouse-Geisser correction was applied when the assumption of sphericity of data was violated.

Rats were typically paired (one Novel, one Familiar) so that the rats in each pair were trained one after the other, caged together and perfused one after the other. This pairing made it was possible to normalize data across the two behavioural groups. This normalization consisted of dividing the mean number of activated neurons in a given site of the Group Familiar animal by the combined means of the two animals in each immunohistochemistry pair (Novel and Familiar), and expressing this result as a percentage. Because all normalized scores across pairs sum to 100 it was only necessary to consider the scores from one group (e.g., Group Familiar), i.e., the score for Group Familiar will specify that of Group Novel. Separate normalized scores were calculated for the thalamic lesion hemisphere of each Familiar:Novel pair and the control hemisphere of each Familiar:Novel pair. The effect of the lesion on task condition (novelty or

familiarity) could then be examined using the normalized counts of the intact compared with the lesioned hemisphere for the scores only from Group Familiar in a two-way within subjects ANOVA (within factors hemisphere and region).

Results

Histology

Seven animals were excluded from all analyses due to the small size of their lesions (five from Group Novel and two from Group Familiar). Consequently, Group Novel comprised eight rats while Group Familiar comprised ten rats. In these remaining 18 cases, the cell loss was centred on the anterior thalamic nuclei, which was the sole common lesion site across all cases. Figure 3.2 depicts the lesions in those cases with the smallest and largest lesions within the two groups. The respective mean tissue loss across the anterior thalamic nuclei in these rats was 66.7% (Group Familiar) and 67.1% (Group Novel). The corresponding median scores for the two groups were 65.6% and 69.3%, respectively. In most rats there was some sparing in the caudal parts of these nuclei and in the most ventral portion of the anterior medial nucleus. Importantly, although the NMDA injections created discrete regions of cell loss, these regions did not extend across the midline into the opposite hemisphere. In some cases (n = 9), there was damage to the rostral portion of the lateral dorsal nucleus. There was also restricted cell loss in the medial blade of the septal dentate gyrus immediately dorsal to the anterior ventral nucleus in twelve of the cases, four from Group Novel and eight from Group Familiar. In one case there was some additional damage to the fornix. Involvement of the parataenial and reticular nuclei was observed in only the largest lesions.

Familiar   Novel

-1.06mm

-1.32mm

-1.56mm

-1.80mm

-2.04mm

-2.28mm
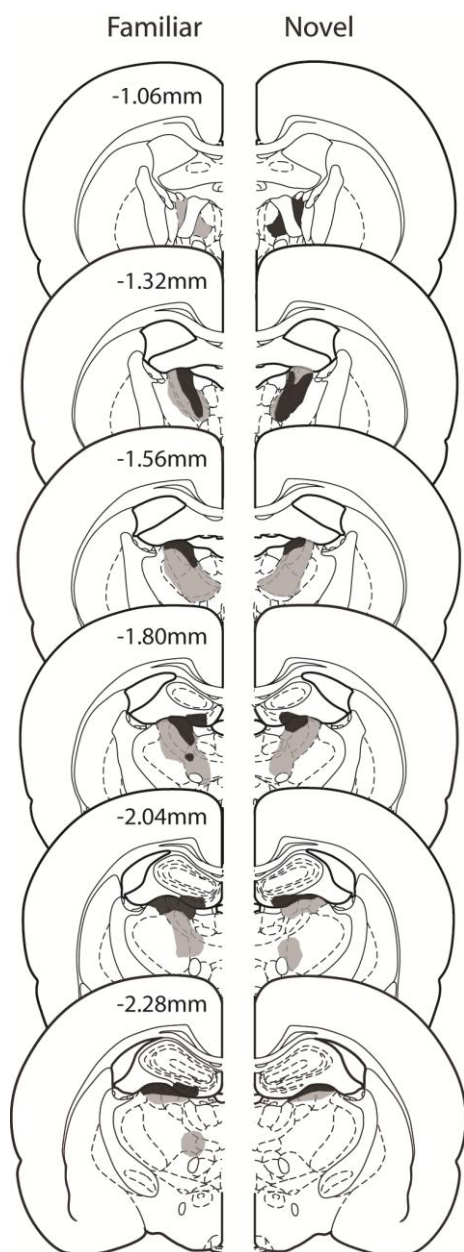
Figure 3.2. Coronal sections depicting the extent of the smallest (dark gray) and largest (light gray) of anterior thalamic nuclei lesions. The numbers refer to the approximate distance of the section in mm caudal to bregma. The sections are modified from Paxinos and Watson (2005).

Behavioural Testing

Object recognition performance – D1 and D2 discrimination indices

Two indices of object recognition were calculated. Index D1 is the difference in time spent exploring the novel object and the familiar object. Index D2 then divides this difference (D1) by the total amount of time spent exploring both objects. Consequently, the D2 index ranges between +1 and -1. The D2 measure has the advantage that it can better compensate for differences in overall amounts of exploration between animals. Index D1 was calculated by adding the difference data from all 20 trials (cumulative D1) while D2 then used the total exploration data from all trials (cumulative D1/total exploration). These indices were calculated for Sessions 1, 7, and 13. For Group Novel, indices D1 and D2 always reflect novelty discrimination, whereas for Group Familiar these same indices reflect recency discriminations for Sessions 7 and 13.

Figure 3.3A shows how the Group Novel rats continued to show a marked preference for novel objects (including Session 13) while the preference shown by Group Familiar diminished over testing as the individual objects became increasingly familiar. Reflecting this pattern, the analysis of variance (ANOVA) with the between subject factor Group (Novel or Familiar) and a within factor Session (1, 7, and 13) revealed significant main effects of Group (D1: $F_{(1, 16)} = 52.2$, $p < 0.001$; D2: $F_{(1, 16)} = 39.0$, $p < 0.001$) and

Session (D1: $F_{(2, 32)}$ = 10.1, p < 0.001; D2: $F_{(2, 32)}$ = 25.8, p < 0.001), and a Group x Session interaction (D1: $F_{(2, 32)}$ = 8.10, p = 0.001; D2: $F_{(2, 32)}$ = 8.06, p = 0.001) for both the D1 and D2 scores. Examination of the simple effects indicated that Group Novel and Group Familiar had comparable D1 and D2 scores for Session 1 (both p > 0.1). By Session 7, Group Novel spent significantly more time than Group Familiar exploring the novel items compared with familiar ones (Figure 3.3A), indicating that objects had become familiar for Group Familiar (D1: $F_{(1, 48)}$ = 33.9, p < 0.001; D2: $F_{(1, 48)}$ = 34.3, p < 0.001). Importantly, this group difference remained on the final session (Session 13; D1: $F_{(1, 48)}$ = 42.4, p <0.001; D2: $F_{(1, 48)}$ = 25.0, p <0.001).
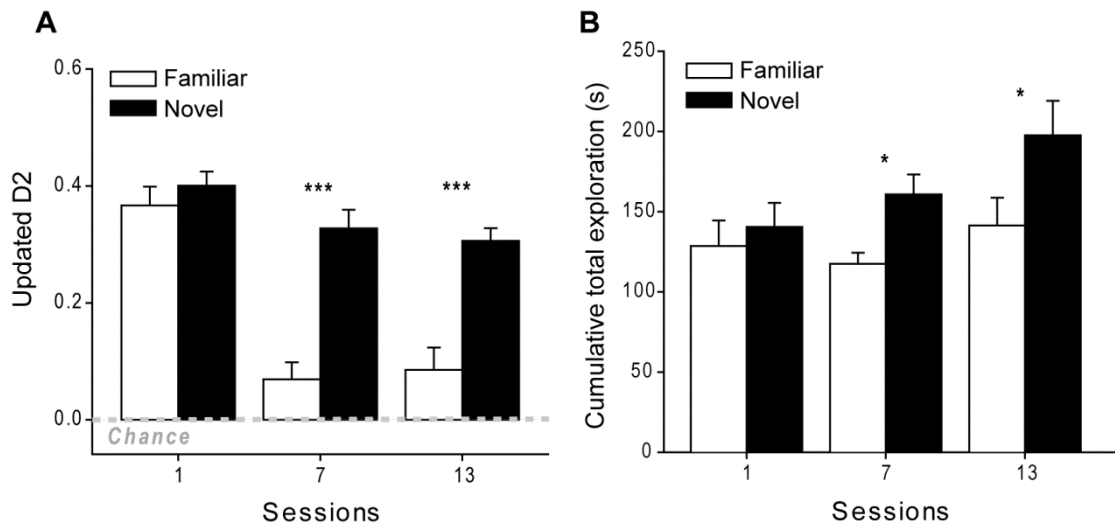
Figure 3.3. Object recognition behaviour: **A**) The final updated D2 score (recognition index) is given for Group Novel and Group Familiar for Sessions 1, 7, and 13. **B**) The cumulative amount of object exploration in each of Sessions 1, 7, and 13. Data shown are mean ± standard error of the mean (SEM). Group differences: * p < 0.05; *** p < 0.001.

One-sample t-tests confirmed that both groups explored the novel objects significantly more than the familiar objects during Session 1 (D1 for Group Novel: t_7 = 8.06, p < 0.001; D1 for Group Familiar: t_9 = 7.66, p < 0.001; D2 for Group Novel t_7 = 16.6, p < 0.001; D2 for Group Familiar: t_9 = 11.4, p < 0.001), i.e., the rats recognised the novel objects. During Session 7, Group Novel continued to explore the novel objects more than the familiar ones (D1: t_7 = 9.46, p < 0.001; D2: t_7 = 10.3, p < 0.001). Group Familiar also spent more time exploring the familiar item

explored on the previous session compared with the highly familiar item explored on the previous trial (i.e., a recency discrimination; D1: t_9 = 2.33, p = 0.045; D2: t_9 = 2.38, p = 0.041). By Session 13 (final) Group Novel still spent considerably more time exploring the novel items than the familiar ones (D1: t_7 = 8.33, p < 0.001; D2: t_7 = 14.1, p < 0.001).   Group Familiar also still showed evidence of a recency discrimination (one-sample t test, D1: t_9 = 3.18, p = 0.011; D2: t_9 = 2.25, p = 0.051), although the level of discrimination was considerably lower than that in Group Novel.

Cumulative total exploration

Although the two groups initially showed comparable total levels of object exploration (Session 1, Figure 3.3B), a difference emerged by the final session, reflecting the increased exploration of the novel objects (Figure 3.3B). An ANOVA examining these cumulative exploration levels yielded a significant main effect of Group ($F_{(1, 16)}$ = 5.38, p = 0.034) and Session ($F_{(2, 32)}$ = 4.73, p = 0.016). Although the Group x Session interaction was not significant (p > 0.1), the simple effects revealed that on the final session Group Familiar explored the objects significantly less compared with Group Novel ($F_{(1, 48)}$ = 5.98, p = 0.018).

Immediate early gene (zif268) results

The *zif268* analyses involved eight Group Novel and ten Group Familiar rats. All rats were included in those comparisons based on raw cell counts (between subjects). For the normalized counts (within subjects) the principal data came from pairs that had been grouped throughout training and were subsequently reacted together. This behavioural pairing was not possible in every case as those lesions that were unacceptable could only be defined post histology. Consequently, one rat from Group Familiar was added to a Familiar:Novel pairing, and all three rats were immunohistochemically reacted together (i.e., as a triplicate). There were two such triplicate groups (two Group Familiar, one Group Novel), alongside the six standard pairings (one Group Familiar, one Group Novel). The data from the triplicate groups were transformed to match the overall totals for the standard pairings (i.e., for the triplicates, when normalization took place each rat from Group Familiar was normalized to the same rat from Group Novel).
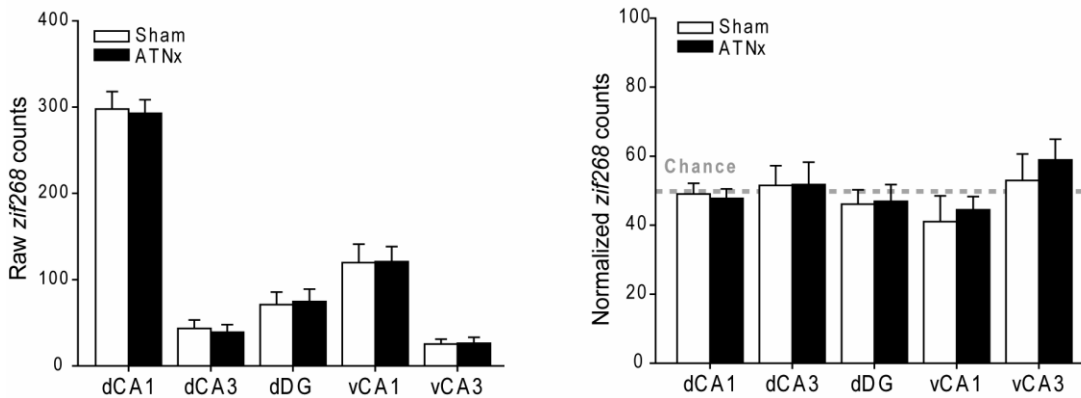
Hippocampal subfields

The initial analyses compared the raw scores of *zif268*-positive cells in five hippocampal sub-regions. Figure 3.4A displays the hippocampal data from the intact and lesioned hemispheres when the recognition conditions (Novel, Familiar) were combined. While there are striking differences in the absolute *zif268* counts from region to region ($F_{(4,60)} = 114.1$, $p < 0.001$), these counts were not differentially affected by the presence of a thalamic lesion ($p > 0.1$). The overall analysis of variance (one between, two within factors) also found no evidence of a task effect (Novel versus Familiar, $p > 0.1$), and no three-way interaction between region, lesion, and task condition ($p > 0.1$).

The next analyses used the data normalized according to their Familiar:Novel pairings (Figure 3.4B). Differential behavioural effects (Novel versus Familiar) will cause the scores to deviate from chance so that a score significantly less than 50 indicates that Group Familiar had lower *zif268* counts than Group Novel. An ANOVA showed that these normalized scores differed across the regions of interest ($F_{(4,32)} = 3.23$, $p=0.025$), i.e., that some sub-regions did show a Familiar:Novel distinction. In particular, ventral (temporal) CA1 and ventral (temporal) CA3 showed differential responses, with relatively increased *zif268* scores with novel objects in CA1, but the opposite pattern in CA3. There was, however, no task by lesion interaction ($p > 0.1$), i.e., this differential response to Novel versus Familiar objects was not modulated by the anterior thalamic lesion. One-sample t-tests showed that none of regions (CA1, CA3) in either the sham or lesion hemisphere differed significantly from the chance score of 50 (for all analyses, $p > 0.1$).
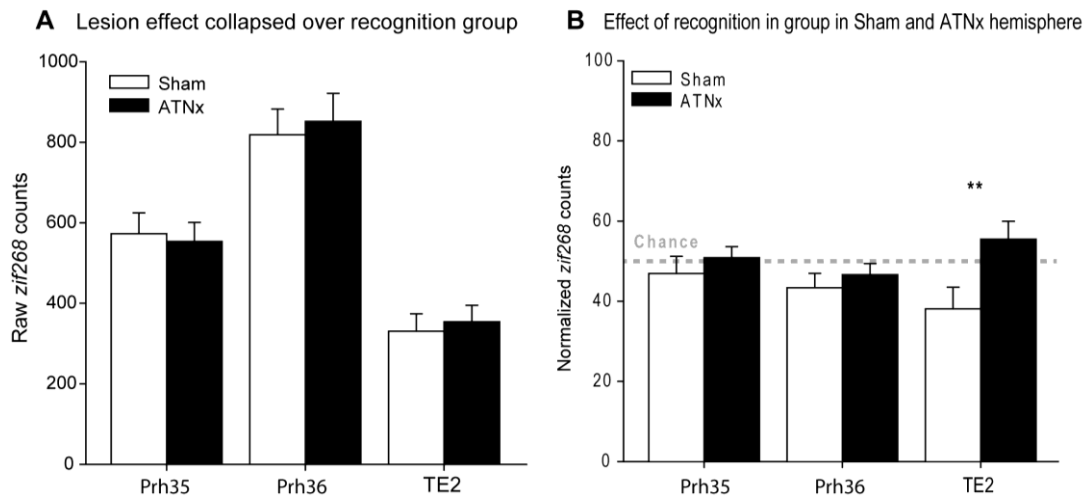
Figure 3.4. Hippocampus: The raw (**A**) and the normalized (**B**) counts of *zif268*-positive cells for the five hippocampal subregions in the sham and anterior thalamic lesion (ATNx) hemispheres. **A**) The raw counts are collapsed across the two recognition conditions (Novel or Familiar). **B**) The scores are normalized in their Familiar:Novel pairings so that a score below chance (dashed line) indicates relatively increased *zif268*-positive cells for those animals exploring novel objects. Data shown are mean ± standard error of the mean (SEM). Abbreviations: dCA1, dorsal CA1; dCA3, dorsal CA3; dDG, dorsal dentate gyrus; vCA1, ventral CA1; vCA3, ventral CA3.

Perirhinal cortex and area TE2

Comparisons across the three sub-regions (TE2 and perirhinal areas 35 and 36) revealed large differences in *zif268* counts ($F_{(2,30)}$ = 68.5, p<0.001), but no overall effect of lesion (p > 0.1) or behavioural condition (Novel versus Familiar, p > 0.1), and no significant interactions between these manipulations (all, p > 0.1; Figure 3.5A). Next, the normalized scores were examined to look at the impact of the test condition and lesion status (as above, scores lower than chance represent a lowering of the *zif268* counts in Group Familiar compared with Group Novel; Figure 3.5B). In the Sham hemisphere there appeared to be a relative reduction of *zif268* in area TE2 and, to a lesser degree, in parts of the perirhinal region associated with exploring familiar objects. One-sample t-tests revealed that only the area TE2 scores appeared to be below chance in the control hemisphere, but this effect was not quite significant (t_8 = 2.22, p = 0.057). These parahippocampal changes yielded a borderline overall effect of lesion ($F_{(1,8)}$ = 4.69, p = 0.062) but no clear site by lesion interaction ($F_{(2,16)}$ = 3.51, p = 0.091; Greenhouse-Geisser correction). However, an examination of the simple effects indicated that in area TE2 there was a greater reduction of *zif268* activity in Group Familiar in the intact hemisphere compared with the lesioned hemisphere ($F_{(1, 24)}$ = 11.5, p = 0.002). It was also possible to divide each of the two main perirhinal regions (areas 35 and

36) into a rostral, mid, and caudal sub-division, but this more fine grained analysis did not reveal any significant changes (all p>0.1).

Figure 3.5. Parahippocampal cortex and area TE2: The raw (**A**) and the normalized (**B**) counts of *zif268*-positive cells for the perirhinal cortices and area TE2 in the sham and anterior thalamic lesion (ATNx) hemispheres. **A**) The raw counts are collapsed across the two recognition conditions (Novel or Familiar). **B**) The scores are normalized in their Familiar:Novel pairings so that a score below chance (dashed line) indicates relatively increased *zif268* for those animals exploring novel objects. Data shown are mean ± standard error of the mean (SEM). Abbreviations: Prh, perirhinal cortex. ** = p < 0.01.

Subicular and entorhinal cortices

Comparisons across the three target regions (dorsal subiculum, postsubiculum, and lateral entorhinal cortex) found raw *zif268* count differences ($F_{(2, 30)}$ = 53.0, p < 0.001), along with evidence that the thalamic lesion reduced some IEG counts ($F_{(1,15)}$ = 4.01, p = 0.064; Figure 3.6A). Simple effects indicated that the lesions reduced *zif268* counts in the postsubiculum ($F_{(1, 45)}$ = 7.16, p = 0.010), although there was no site by lesion interaction (p > 0.1). Finally, analysis of the normalized scores failed to show that the behavioural task affected *zif268* levels in specific sites (p > 0.1) or that this measure was differentially affected by the surgery (p > 0.1; Figure 3.6B).

A Lesion effect collapsed over recognition group **B** Effect of recognition group in Sham or ATNx hemisphere
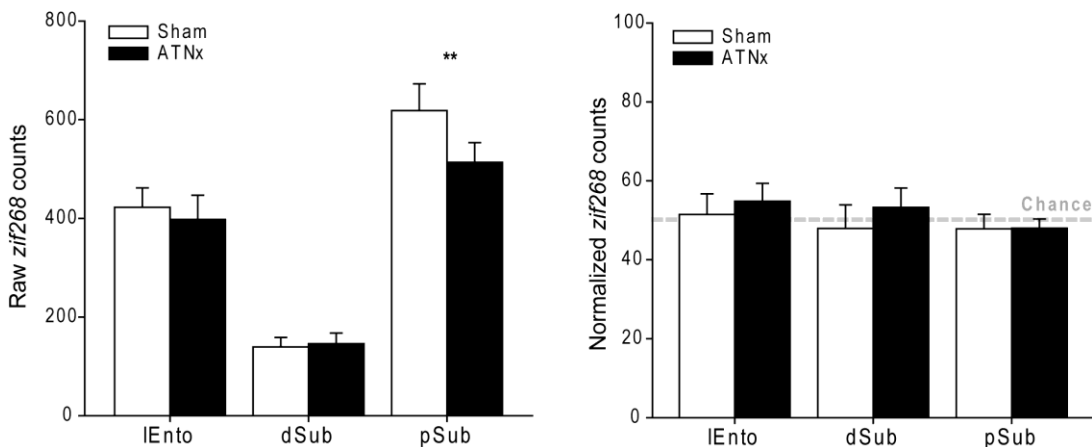
Figure 3.6. Subicular and entorhinal cortices: The raw (**A**) and the normalized (**B**) counts of *zif268*-positive cells for the subicular and entorhinal cortices in the sham and lesion (ATNx) hemispheres. **A**) The raw counts are collapsed across the two recognition conditions (Novel or Familiar). **B**) The scores are normalized in their Familiar:Novel pairings so that a score below chance (dashed line) indicates relatively increased *zif268* for those animals exploring novel objects. Data shown are mean ± standard error of the mean (SEM). Abbreviations: dSub, dorsal subiculum; lEnto, lateral entorhinal cortex; pSub, postsubiculum. ** = p < 0.01.

Retrosplenial cortex

Consistent with previous studies, anterior thalamic lesions reduced *zif268* activity across much the retrosplenial cortex (Figure 3.7). Analyses of the raw scores revealed both an effect of site ($F_{(4,64)}$ = 56.9, p < 0.001) and of lesion ($F_{(1,16)}$ = 7.94 , p = 0.012; Figure 3.7A), but no overall effect of behavioural condition (p > 0.1) or any significant interactions between the above (for all p > 0.1). Simple effects indicated that thalamic lesions significantly reduced *zif268* counts in both rostral Rgb ($F_{(1, 80)}$ = 6.38, p = 0.014) and caudal Rgb ($F_{(1, 80)}$ = 6.99, p = 0.010), but there were no significant differences elsewhere (p > 0.1 for all). These lesion effects in Rgb were further explored by comparing the counts in the superficial and deep layers. The results indicated that for both rostral and caudal Rgb, the reduction of *zif268* activity caused by the lesion was in both the superficial (rostral: $F_{(1, 64)}$ = 6.94, p = 0.011; caudal: $F_{(1, 64)}$ = 6.05, p = 0.017) and the deep layers (rostral: $F_{(1, 64)}$ = 11.2, p = 0.001; caudal: $F_{(1, 64)}$ = 14.4, p < 0.001).

To examine any differential effects of the behavioural conditions the normalized data were considered (Figure 3.7B). First, there was no clear evidence that the behavioural task (Novel versus Familiar) differentially affected some

retrosplenial sub-regions (overall region effect, p > 0.1), and there was no interaction with lesion condition (p > 0.1).
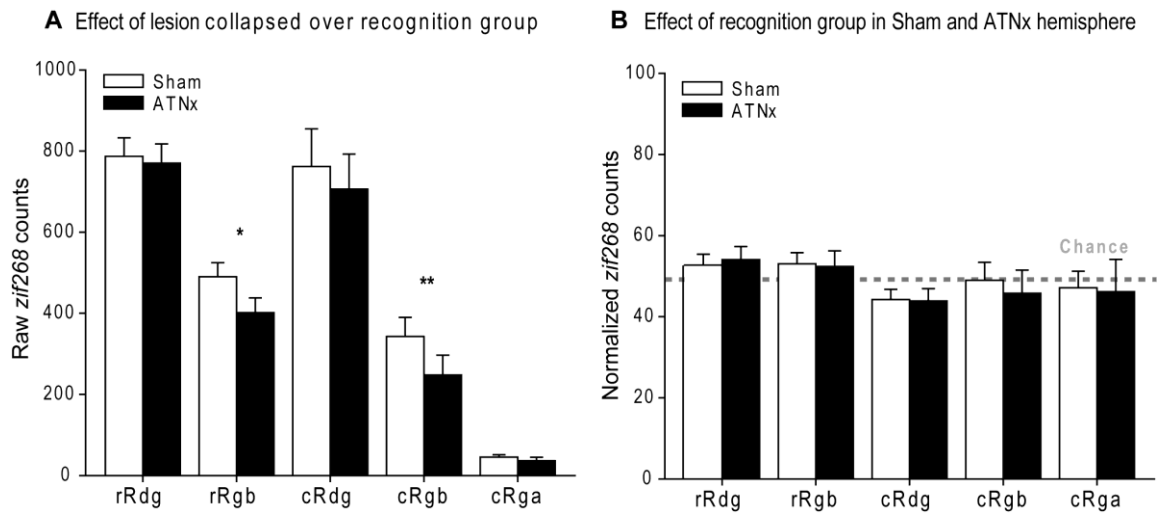
Figure 3.7. Retrosplenial cortex: The raw (**A**) and the normalized (**B**) counts of *zif268*-positive cells for the five retrosplenial subregions in the sham and anterior thalamic lesion (ATN) hemispheres. **A**) The raw counts are collapsed across the two recognition conditions (Novel or Familiar). **B**) The scores are normalized in their Familiar:Novel pairings so that a score below chance (dashed line) indicates relatively increased *zif268* for those animals exploring novel objects. Data shown are mean ± standard error of the mean (SEM). Abbreviations: cRdg, caudal dysgranular retrosplenial cortex; cRga, caudal granular retrosplenial cortex, area a; cRgb, caudal granular retrosplenial cortex, area b; rRdg, rostral dysgranular retrosplenial cortex; rRgb, rostral granular retrosplenial cortex, area b.

Control cortex (primary auditory cortex)

As expected, inspection of the raw counts in the auditory cortex did not yield a significant effect of recognition group, lesion, nor a group x lesion interaction (p > 0.1 for all). Similarly, there was no indication that the anterior thalamic lesions modulated task performance (p > 0.1), nor that the recognition condition influenced normalized *zif268* counts (i.e., the counts did not differ from chance for either the intact or lesion hemisphere; both p > 0.1).

A Effect of lesion collapsed over recognition group **B** Effect of recognition group in Sham and ATNx hemisphere
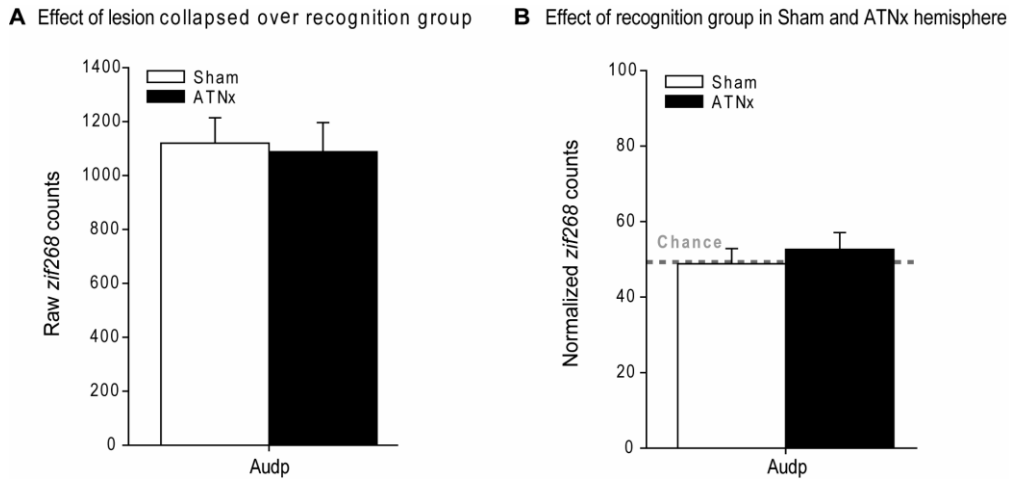
Figure 3.8. Control cortex (primary auditory cortex): The raw (**A**) and the normalized (**B**) counts of *zif268*-positive cells for the primary auditory cortex in the sham and lesion (ATNx) hemispheres. **A**) The raw counts are collapsed across the two recognition conditions (Novel or Familiar). **B**) The scores are normalized in their Familiar:Novel pairings so that a score below chance (dashed line) indicates relatively increased *zif268* for those animals exploring novel objects. Data shown are mean ± standard error of the mean (SEM). Abbreviations: Audp, primary auditory cortex.

Discussion

The purpose of this study was to assess the effects of unilateral anterior thalamic lesions on *zif268* activity following object recognition. Using the bow-tie maze, rats were presented with lists of either novel items (Group Novel) or the same list of objects (Group Familiar) across 13 sessions. On the final session, both groups were presented with the same list of objects: the same list used for the previous 12 sessions for Group Familiar, but never before experienced by Group Novel. Advantages associated with the bow-tie maze protocol included the ability to give each rat multiple trials within a session, yet not need to handle the rat between trials. One potential disadvantage was that the overall times spent exploring objects could not be fully matched on the final session between Group Novel and Group Familiar. This time difference arose as an almost inevitable consequence of repeating the same objects across all previous sessions for Group Familiar, i.e., the resultant decreases in spontaneous exploration confirmed that the rats in this group correctly perceived the repeated objects as familiar. In order to match total exploration times across the two treatment groups it would have been necessary to give Group Familiar extra trials with additional objects, but this would introduce other differences. With these considerations in mind, it can be noted that the

baiting procedure should help to maintain comparable patterns of behaviour across the two groups, i.e., rats approached and manipulated all objects.

This experiment raised three main questions: 1) Would *zif268* activity in the intact hemisphere of Group Novel and Group Familiar yield a similar pattern of results as that found in a previous study using the IEG *c-fos* (Albasser, Poirier et al., 2010)? 2) Does damage to the anterior thalamic nuclei reduce *zif268* activity in related distal neural sites? 3) Would anterior thalamic damage moderate *zif268* activity during object recognition?

Recognition effect (Group Novel compared with Group Familiar in the intact hemisphere)

A previous study using the same behavioural design found that novel stimuli raised c-*fos* activity in caudal perirhinal cortex, area TE2, and hippocampal subfields CA3 and CA1, while the dentate gyrus decreased its c-*fos* activity (Albasser, Poirier et al., 2010). In the present study, this protocol was extended to measure *zif268* responses. Like c-*fos* (Albasser, Poirier et al., 2010), differential hippocampal *zif268* activity was associated with the novel versus familiar stimuli. In particular, temporal CA1 showed a relative increase in *zif268* activity associated with novel objects while temporal CA3 showed a decrease. These changes contrasted with a lack of any differential *zif268* changes in the perirhinal cortex, despite the known importance of this cortical area for object recognition, whether tested in an open arena or in a bow-tie maze (Ennaceur, Neave, & Aggleton, 1996; Winters, Saksida, & Bussey, 2008; Albasser, Chapman et al., 2010; Albasser et al., 2011). In fact, the current null result for *zif268* in the perirhinal cortex is understandable as earlier studies had found c-*fos*, but not *zif268*, activity changes in the perirhinal cortex when rats are shown novel visual stimuli (Brown & Xiang, 1998; Wan et al., 1999; Aggleton, Brown, & Albasser, 2012; see also Romero-Granados, Fontan-Lozano, Delgado-Gracia, & Carrion, 2010). The implication is that *zif268* activity in the perirhinal cortex is not a key process for effective long-term object recognition memory (although see Jones et al., 2001; Bozon, Davis, & Laroche, 2003). In contrast, the functional significance of the perirhinal c-*fos* response for object recognition memory has recently been demonstrated by

blocking c-*fos* activity in this cortical area and impairing recognition memory after extended retention delays (Seoane, Tinsley, & Brown, 2012).

Hippocampal subfields show both differential c-*fos* (Albasser, Poirier et al., 2010) and *zif268* (present study) responses to novel versus familiar stimuli when tested in the bow-tie maze. These findings might suggest that the rodent hippocampus has a direct role in supporting object recognition memory. This conclusion should, however, be treated with caution. The first reason is that extensive hippocampal lesions do not appear to alter object recognition memory when it is tested in the bow-tie maze, even when using a range of retention intervals (Albasser, Chapman et al., 2010; Albasser et al., 2012). A second reason is that on encountering a novel object, rats will spontaneously learn much about its associative features, e.g., its spatial and temporal location. This associative learning is not only hippocampal dependent (Ennaceur, Neave, & Aggleton, 1997; Save, Poucet, Foreman, & Buhot, 1992; Mumby, Gaskin, Glenn, Schramek, & Lehmann, 2002; Piterkin, Cole, Cossette, Caskin, & Mumby, 2008; Warburton & Brown, 2010; Barker & Warburton, 2011) but these forms of spontaneous spatial and temporal learning can selectively engage hippocampal c-*fos* (Wan et al., 1999; Jenkins, Amin et al., 2004; Amin et al., 2006). Given these results, the changes in hippocampal *zif268* found in this behavioural task are not unexpected, even though there was no evidence of moderation after anterior thalamic damage.

Lesion effect (ATNx compared with Sham hemisphere)

The study also sought to extend our understanding of covert pathology following damage to the anterior thalamus by comparing the intact (Sham) and lesion (ATNx) hemispheres. The results indicating no effect of anterior thalamic nuclei lesions on *zif268* levels in the parahippocampal regions are consistent with previous report using the IEG c-*fos* (Jenkins, Dias, Amin, Brown et al., 2002; Jenkins, Dias, Amin, & Aggleton, 2002).

One important caveat is that while the anterior thalamic connectivity is primarily ipsilateral, there is evidence of some contralateral projections (e.g., to the retrosplenial cortex; van Groen & Wyss, 1992, 1995; Shibata, 1998). Therefore, it is incorrect to claim that the hemisphere contralateral to the lesion site is intact

(or completely normal). Despite this caveat, significant and striking reductions in *zif268* activity in the retrosplenial cortex were found, and are consistent with previous reports (Jenkins, Dias, Amin, Brown et al., 2002; Jenkins, Dias, Amin, & Aggleton, 2002; Jenkins, Vann et al., 2004; Poirier & Aggleton, 2009). Similar to other studies, reductions were found in both the rostral and caudal portions of the retrosplenial cortex (Jenkins, Vann et al., 2004; Poirier & Aggleton, 2009). Specifically, there were reductions in superficial layers of the rostral and caudal granular b retrosplenial cortex.

In contrast to other reports (Jenkins, Vann et al., 2004; Poirier & Aggleton, 2009), the present results did not indicate lesion-induced hypoactivity in the ipsilateral hemisphere of the rostral or caudal dysgranular cortex. Possible differences may be the size and extent of the lesion. There is evidence that the anterior medial nucleus projects strongly to the retrosplenial dysgranular cortex, whereas the anterior dorsal and the anterior ventral nuclei project predominantly to the retrosplenial granular b (Shibata, 1993; van Groen & Wyss, 1995; 2003). It is interesting to note that the lesions in the present communication were restricted, and for a large proportion of animals sparing occurred in the ventral portion of the anterior medial nucleus. This sparing partially arose from the need to avoid lesions that crossed the midline to the other hemisphere. This sparing in the anterior medial nucleus may explain the lack of a lesion effect in the dysgranular portion of the retrosplenial cortex. In addition to the retrosplenial cortex, the postsubiculum also showed reduced *zif268* activity in the lesion (ATNx) compared with Sham hemisphere. The postsubiculum is a recipient of anterior thalamic inputs that reliably shares *c-fos* changes following anterior thalamic damage (Dumont, Amin, Poirier, Albasser, & Aggleton, 2012). The postsubiculum is particularly interlinked with the anterior dorsal thalamic nucleus as part of the head direction system (Taube et al., 1990; van Groen & Wyss, 1990b; Taube, 1995; Wright et al., 2010; Taube, 2007; Aggleton, O'Mara et al., 2010).

Interaction effect (recognition effect x lesion effect)

Overall, damage to the anterior thalamus did not appear to modulate *zif268* object recognition. The lesion did not differentially affect *zif268* activity in the

hippocampus, retrosplenial cortex, or subicular and entorhinal cortices. The only effect of the lesion on recognition occurred in the parahippocampal regions (although the interaction effect was not significant). Inspection of the data revealed that there was a greater reduction of *zif268* activity in Group Familiar in the intact hemisphere compared with the lesioned hemisphere in area TE2. It appears as though the lesion may attenuate the increase in *zif268* activity following the presentation of novel objects that is seen in the intact hemisphere. However, *zif268* activity in the Sham hemisphere was not significantly different from chance (i.e., not greater for Group Novel or Group Familiar). Although there is evidence that the anterior thalamic nuclei send projections to the perirhinal cortex and area TE2 (Shibata, 1993a), it is unclear why damage to the anterior thalamus would suppress activity in area TE2, but not modulate activity in other neural region (e.g., the hippocampus) during object recognition.

The major objective of this chapter was to examine whether the anterior thalamic nuclei contribute, though are not necessary (see Chapter 2), for accurate novelty detection. The results from this chapter suggest that the contribution of the anterior thalamic nuclei to recognition memory is either a) limited, b) the use of *zif268* activity as a marker of object novelty is not sensitive enough or c) both.

Chapter 4

The role of the anterior thalamic nuclei in biconditional learning

Introduction

It is well known that the anterior thalamic nuclei, as part of the extended hippocampal system, are critical for spatial learning and memory (Sutherland & Rodriguez, 1989; Aggleton et al., 1996; Byatt & Dalrymple-Alford, 1996; Warburton et al., 1997; Sziklas & Petrides, 1999; Mitchell & Dalrymple-Alford, 2006). For example, anterior thalamic damage impairs the ability of rats to navigate to a goal in a watermaze (Sutherland & Rodriguez, 1989; van Groen et al., 2002). However, the role of the anterior thalamic nuclei for biconditional learning is less clear (Sziklas & Petrides, 1999; Chudasama et al., 2001; Ridley et al., 2002; Sziklas & Petrides, 2004; Gibb et al., 2006; Sziklas & Petrides, 2007; see Biconditional learning, Chapter 1).

Biconditional learning requires animals to learn that stimulus A is associated with X, but not Y; whereas, stimulus B is associated with Y and not X (i.e., AX+, AY-, BX-, BY+). Biconditional tasks are configural in nature (Rudy and Sutherland, 1995); simply learning about one of the stimuli (or element) is not sufficient for the correct performance of biconditional tasks. For example, if an animal always selects stimulus A when A and B are presented, it will only be rewarded 50% of the time: when A is paired with X. The animal will not be rewarded on the other half of the trials for selecting A when it is paired with Y. Therefore, the animal must learn that the conjunction of A and X is rewarded.

Biconditional tasks can be used to examine whether damaging the anterior thalamic nuclei impairs configural learning (Sutherland & Rudy, 1989; Rudy and Sutherland, 1995). The ability to bind different information together (i.e., form configural units) was hypothesised to be the functional role of the hippocampus

(Sutherland & Rudy, 1989; Rudy and Sutherland, 1995), and the conjunction what-when-where associations help form episodic (episodic-like) memory (e.g., Clayton & Dickinson, 1998; Aggleton & Pearce, 2001). If the extended hippocampal system is involved in episodic memory (e.g., Aggleton & Brown, 1999), then the conditions under which damage to neural regions within the extended hippocampal system impairs the formation of biconditional (as a form of configural) associations becomes critical.

There is some evidence that damage to the anterior thalamic nuclei severely impairs learning of a spatial-visual biconditional task (Sziklas & Petrides, 1999). In this task, the rats have to choose one of two objects (Object A) when it is located at the North end of the open field maze, but choose the other (Object B) when the two objects are located in the South end. The correct choice object (A, B) depends on the spatial location (North, South) of the stimuli. While rats with damage to the anterior thalamic nuclei were impaired at learning this rule, they were not impaired when an object (A, B) signaled a location (East, West; Sziklas & Petrides, 2007). In this second, visual-spatial biconditional task, the rats were placed in either the North or South arms of an elevated plus maze. When object A was presented in the centre of the plus maze (i.e., the choice point), the rats had to respond by going East, whereas when Object B was presented, West was the correct location. Rats with anterior thalamic damage were also unimpaired on a visual-motor biconditional learning task, where a correct motor response (turn left, turn right) depended on which object was presented (Object A, Object B; Sziklas & Petrides, 1999).

It is unclear why damage to the anterior thalamic nuclei should impair learning of a *spatial-visual*, but not a *visual-spatial* biconditional learning task (Sziklas & Petrides, 1999, 2007). Is it the case that the anterior thalamic nuclei are critical for biconditional learning, and if so under which conditions? The aim of the present chapter was to examine the performance of rats with lesions to the anterior thalamic nuclei and sham surgery control group on a series of biconditonal tasks where spatial and contextual cues were manipulated. Two very different biconditional protocols were adopted. First, the rats were trained on series of contextual biconditional discriminations in operant chambers, where

Pavlovian associations between contexts that differed either thermally (warm, cool) or visually (spot, checkered) with two auditory stimuli (clicker, tone) were examined. Second, the rats were tested on a series of spatial biconditional learning tasks where the rats were required to dig in a particular cup depending on either the local context (i.e., the box in which the rat was placed), or the location of the rat within the testing room.

Materials and Methods

Subjects

The subjects were the same 25 male hooded rats (ATNx1 = 15; Sham = 10) described in Chapter 2.

Surgery, Histology, Immunohistochemistry for Neuronal Nuclei (NeuN), and Volumetric Analysis

Surgery, histology, immunohistochemistry for NeuN, and volumetric analysis have all been previously described in Chapter 2.

Behavioural Testing

The rats initially completed a series of non-spatial recognition memory experiments as well as a spatial working memory task, T-maze alternation (Chapter 2), prior to the experiments reported below. The experiments below started approximately six months post-operatively.

Experiment 4.1: Contextual Biconditional Discriminations

Apparatus and room

Four operant chambers were customized so that each was uniquely distinct. The four chambers (internal dimensions: 24.5 cm wide x 23 cm deep x 21 cm high; Campden Instruments Ltd., United Kingdom) were arranged in a 2 x 2 layout on shelves at the shorter wall of the room (327 cm x 187 cm x 254 cm) directly opposite the door. The lowest and highest shelves were 92 cm and 142 cm above the floor, respectively. Each chamber had three aluminium walls and ceiling; a

Perspex door formed the fourth wall. The doors of the sound-attenuating boxes for each of the chambers remained open, therefore, each box received ambient light from a brightly lit room as well as local illumination from a single 15-V, 24-W light situated in the centre of each of the chamber ceilings. A speaker mounted above the ceiling of each of the chambers delivered the two auditory stimuli, a 2Hz tone and a 10Hz series of clicks at an intensity of, approximately, 75 dB(A). On the left wall, a transparent plastic flap (6 cm high x 5 cm wide) blocked the entrance to the food-well where food pellets could be dispensed (J. Noyes, Lancaster, NH, USA). The plastic flap was hinged at the top of the food-well aperture, and if the rats pushed the flap more than 2mm, a microswitch was activated resulting in a single response being recorded.

The two chambers on the left of the 2 x 2 layout were designated 'thermal' contexts and the two chambers on the right were the 'visual' contexts (see Figure 4.1). The aluminium walls of the visual contexts were covered with wallpaper protected from the rats by clear Perspex sheets. The top chamber was covered with spotted paper (white background with filled black circles, 1.5 cm in diameter, with a centre to centre distance of 2.5 cm), and the bottom chamber was covered with checkered wallpaper (a series of alternating, 3 cm, black and white squares). The floor of the visual contexts was constructed from stainless-steel rods. In contrast, the floor of the thermal chambers was aluminium with a bracket fixing allowing two Thermos picnic blocks (model no IP400; 9 cm wide x 3.7 cm deep x 16 cm long) to fit underneath and make contact with the floor of the chamber. For the warm context, the Thermos blocks were first heated in a microwave for 2-3 minutes. Placing the heated blocks under the floor for 10 minutes raised the floor temperature to 35 °C which then dropped to 32 °C over the course of 30 minutes. The cool context was created by placing two frozen Thermos blocks below the floor. The temperature of the cool context dropped to 10 °C, and then increased to 12 °C over the course of two hours. The heated Thermos blocks were replaced every thirty minutes, whereas the frozen Thermos blocks were replaced every two hours (for more details see Ward-Robinson & Honey, 2000). The warm/cool contexts changed location from the top operant chamber to the bottom chamber (and vice versa) every two days to reduce the possibility of rats using visual cues

to solve the biconditional rule (e.g., from observation of the test room through the Perspex door). In contrast, the two visual contexts remained in the same location (i.e., the top chamber was always spotted and the bottom chamber was always checkered).
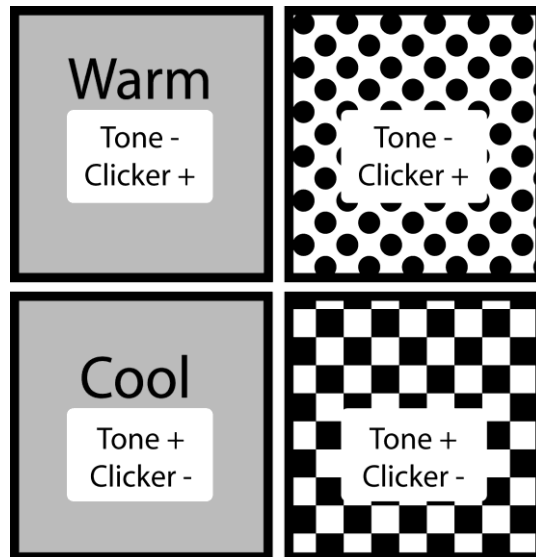
Figure 4.1. A schematic diagramme of the four different contexts (two thermal on the left; two visual on the right) inside the operant boxes. In all contexts, tones and clickers are presented; however only the tone or the clicker is paired with food pellets in a given context. The + sign indicates the reinforced stimulus, whereas the – sign indicates the non-reinforced stimulus. Note: the warm and cool contexts alternated locations (top; bottom). The visual contexts remained in the same place with the spotted pattern on top and the checkered pattern on the bottom.

Pre-training

The rats received two days of pre-training where they were placed in the operant chambers (without the wallpaper or Thermos blocks, and with conventional steel rod flooring) in order to habituate the animals to the chambers, and to train them to push the flap in order to obtain a food reward. On the first day, the flap was fixed and raised allowing the rats to obtain their food reward without having to push the flap. On the second day, the flap was lowered and the rats had to push the flap to obtain their food reward. On each day, the rats were given 20 pellets (two at a time) on a 60 s variable-time schedule (range 30-90 s).

Procedure

Following pre-training, the rats were given 20 days of training, receiving one session per day in each of the four contexts (warm, cool, spotted, checkered). The interval between placing the rats in a different context was approximately two minutes. In each of the contexts, the tone and the clicks were presented 10 times each in a pseudo-random sequence with the following rule: no more than two trial types (i.e., clicker or tone) occurred in succession. The duration of the auditory

stimuli was 10 s, and the presentation of the food reward occurred at the offset of the stimuli. The intertrial interval (i.e., the time between the offset of one auditory stimulus and the onset of another) was 30 s. When rats were placed in one of the thermal and one of the visual contexts (e.g., cool and checkered), the tone was reinforced (i.e., followed by food reward), whereas the clicker was not. In contrast, when those same rats were placed in the remaining two contexts (e.g., warm and spotted), the clicker was reinforced and not the tone (see Figure 4.1). The auditory stimuli that were reinforced in the visual and thermal contexts were fully counterbalanced. The order of presentation of the contexts across the 20 days was also counterbalanced so that every context was presented during the first, second, third, and fourth session of each day. Additionally, placement in any one of the contexts was equally likely to be immediately followed or preceded by placement in any of the other three contexts. The behavioural response measured was the number of food-well entries (i.e., the flap covering the food-wells being pushed more than two millimeters in order to activate the microswitch) during the 10 s duration of the auditory stimuli as well as 10 s prior to their onset for baseline recordings. The delivery of the food reward did not depend upon the rats' behavioural responses; hence, the formation of the contextual biconditional associations was Pavlovian in nature.

Statistical Analysis

The total number of responses during the 10 s presentation of the reinforced and non-reinforced auditory stimuli for the thermal and visual contexts was recorded, and expressed as a discrimination ratio: the number of responses during the reinforced auditory stimuli (e.g., clicker) was divided by the number of responses during both the reinforced and non-reinforced auditory stimuli (i.e., clicker and tone). Using this measure, the scores ranged from 0 to 1, with a score above 0.5 means that responding to the reinforced auditory stimulus was greater than the non-reinforced one. Furthermore, the number of responses during the 10s prior to the onset of the reinforced and non-reinforced auditory stimuli for the thermal and visual contexts was also analyzed in the same manner to examine any possible differences in baseline responding between the two groups.

Experiment 4.2: Biconditional Learning

The aim of Experiment 4.2 was to examine whether rats with lesions to the anterior thalamic nuclei were able to acquire biconditional rules on a task designed to manipulate distal location cues and proximal context cues independently.

Experiment 4.2A: Stage 1 - Simple Discrimination

The rats were first tested on their ability to form simple reward-object associations. In this task the rats had to learn that one of two cups filled with different media was always rewarded.

Apparatus and Room

Animals were tested in a white opaque plastic test box (40 cm long x 20 cm wide x 12.5 cm high; Context 1) and blue semi-transparent plastic test box (33 x 26 x 16.5 cm, Wham Crystal, Whatmore Creative Plastics, 16 LTR, www.whamproducts.co.uk; Context 2). Regardless of the test box, two digging cups were placed in the middle of each of the shorter walls. Each digging cup consisted of a black plastic cylinder with an internal diameter of 7 cm and a height of 6 cm. The base of the cylinder was made of a grey plastic square (9 cm x 9 cm). Velcro secured the cups to the box floor to prevent the rats for tipping them over while digging. The two, black, digging cups were identical during pre-training and contained sawdust. However, during all other testing, both cups and the media inside them differed. One cup remained black and contained shredded red paper, whereas the other cup had white tape surrounding the black cup to create a checked pattern and contained multi-coloured plastic beads. The food reward was half of a single Cheerio (Nestle, UK) that was buried in the digging media at a depth of approximately 3 cm (i.e., half the cup height). To discourage the rats from using odour guided cues, a perforated metal grid was placed inside the cup to create a false bottom. Cereal loops were placed under this grid, where they could not be retrieved by the rats. These cereal loops were replaced with fresh ones twice a week. In addition, cereal crumbs were mixed with the digging medium. The pre-training and testing took place in a room (280 cm long x 280 cm wide x 256 cm

high) that contained a variety of distal cues (e.g., posters, door, shelves fixed on a wall containing various objects). These distal cues were visible from any corner of the room. The room was illuminated with 8 spot bulb lights fixed on the ceiling.

Pre-training

Half of the rats were placed singly in the white opaque plastic test box located on a table (122 cm x 53.4 cm x 70 cm) next to the door of the room half way along a wall (Place 1), whereas the other half was pre-trained in the blue transparent plastic test box located on a second table (102 cm x 56 cm x 76 cm) close to a corner diagonally positioned from the Place 1 (Place 2). The two tables were 180 cm apart, and the long side of the box was always 20 cm away from the walls. Each box contained two identical digging cups filled with sawdust. Initially, the food reward was placed on top of the medium, and was visible to the rats. Then, as pre-training progressed, the reward was buried deeper and deeper into the sawdust forcing the rats to dig into the medium to retrieve the food. Pre-training lasted between four and six days, until all rats were reliably digging to retrieve the rewards.

Procedure

Five rats were simultaneously brought to the test room in an enclosed carrying box made of aluminium. Each rat was in a separate container and could not see the surrounding environment. The rats were then run in spaced trials, i.e., the five rats were run one after the other for trial 1, then the five animals were run for trial 2, and so on. Consequently, there was an intertrial interval of approximately 2-3 minutes.

Animals received 16 trials per day, for three days. A trial began by placing a rat in the middle of the test box that they had previously experienced during pre-training, equidistant from the two digging cups. The rat was then allowed to explore the cups containing either multi-coloured plastic beads (checkered cup) or red shredded paper (black cup). Only one medium was associated with a reward; for half of the rats tested in the white box (Context 1 + Place 1), the beads were correct and for the other half tested in the blue box (Context 2 + Place 2), the paper was correct (Figure 4.2). The left and right position of the correct cup was

counterbalanced pseudo-randomly with the following rules: 1) the correct cup occupied the left and right side of the box equally (i.e., 8 trials each), and 2) the correct cup occupied the left/right side for a maximum of three consecutive trials. A correct choice occurred when a rat dug in the correct cup and retrieved the food. Animals were allowed to put their paws on the medium or to smell the medium before making a choice. An incorrect choice was scored when the rat dug in the unbaited cup, resulting in the removal of the correct cup. The rat was left for an extra 5 s before being taken out of the box. At the end of each trial, the rat was returned to the enclosed aluminium carrying box.
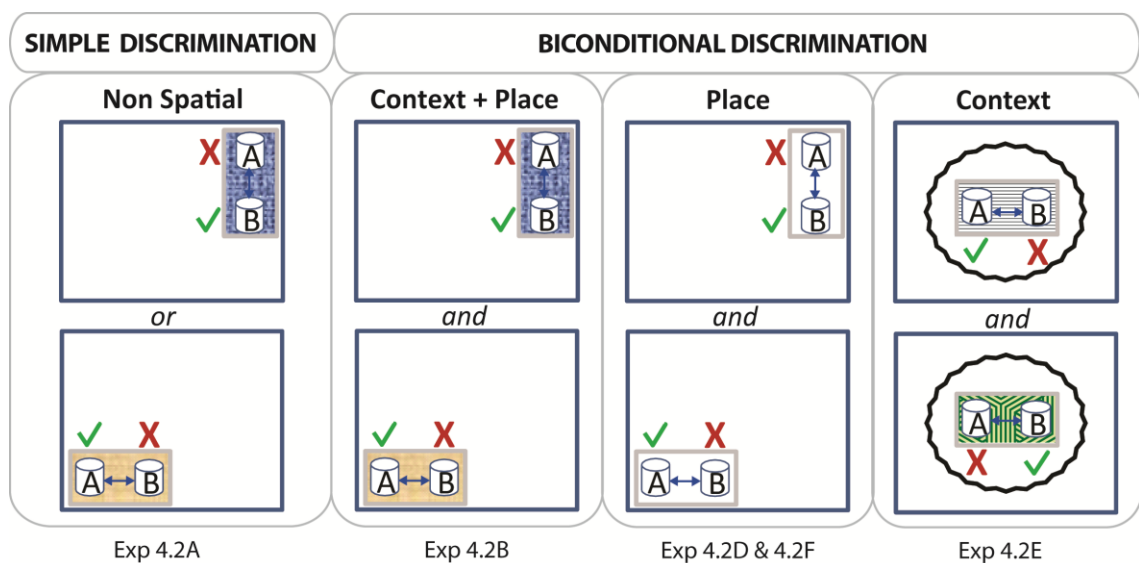
Figure 4.2. A schematic diagramme of the simple discrimination and biconditional tasks. Two different contexts and locations were used for the simple discrimination and the Context + Place biconditional. The Place biconditional task used the same box in the two locations. In the Context biconditional task, the wavy black lines represent the curtain which prevented the use of any distal cues. Also note that two different contexts were used. A green tick indicates the correct (reinforced) cup, and a red "x" indicates the incorrect cup.

Experiment 4.2B: Stage 2 - Place & Context Biconditional Learning

The aim of Stage 2 was to examine whether rats with lesions to anterior thalamic nuclei were able to learn biconditional rules when the items (cups containing different media) were located in two distinct contexts (Context 1 or Context 2) and two different locations (Place 1 or Place 2) in the testing room. To solve this task, the rats could form item-context + place associations (e.g., choose Cup A in Context 1 + Place 1; choose Cup B in Context 2 + Place 2). However, the task could also be solved by ignoring either the place or the context cues, and form item-place or item-context associations.

Apparatus and room

The same apparatus and room as Experiment 4.2A was used in this experiment.

Procedure

The simple discrimination task was transformed into a biconditional one by testing each rat in both boxes (i.e., the same box use for the simple discrimination and another new box; see Figure 4.2 and 4.3). The rats were required to learn that in the white box (Context 1 + Place 1) the beads were correct, whereas in the blue box (Context 2 + Place 2) the shredded paper was correct. The medium that was previously rewarded during the simple discrimination remained the correct medium for the context (and place) in which it was previously correct. However, because there was an equal amount of trials in both contexts (and places) either media was only correct 50% of the time. For example, an animal choosing only the cup containing the beads would be rewarded only 50% of the time (i.e., only when located in the white box: Context 1 + Place 1). The relative left and right position of the digging cups and the box (context + place) were counterbalanced pseudo-randomly with the following restrictions: 1) The correct cup was located equally on the left and right side of the box as well as equally in both the white and blue boxes, and 2) the correct cup was located on the left/right or in the white/blue boxes for no more than three consecutive trials. The contexts (and places) and left/right position of the correct cup inside the box were also counterbalanced within sessions and across groups. To eliminate the use of odour cues made by the rats exploring the cups (e.g., marking the cup with urine), the same two cups were used in both the boxes (i.e., for rewarded and non-rewarded trials). The rats received 16 trials per day until the Sham1 rats reached a mean of 80% correct responses for two consecutive days.

Experiment 4.2C: Stage 3 - Reversal of Place & Context Biconditional Contingencies

The goal of the reversal was to observe whether the rats were primarily attending to the context cues, the place cues, or both. The two context boxes swapped locations, but the biconditional rule remained constant with the place (see Figure

4.3). In this way the place cues directing the correct digging cup remained constant while the local box cues (context) were reversed. It was hypothesised that rats that only relied on the context cues would now perform below chance, whereas rats that relied on the distal cues (i.e. places) would remain above chance.
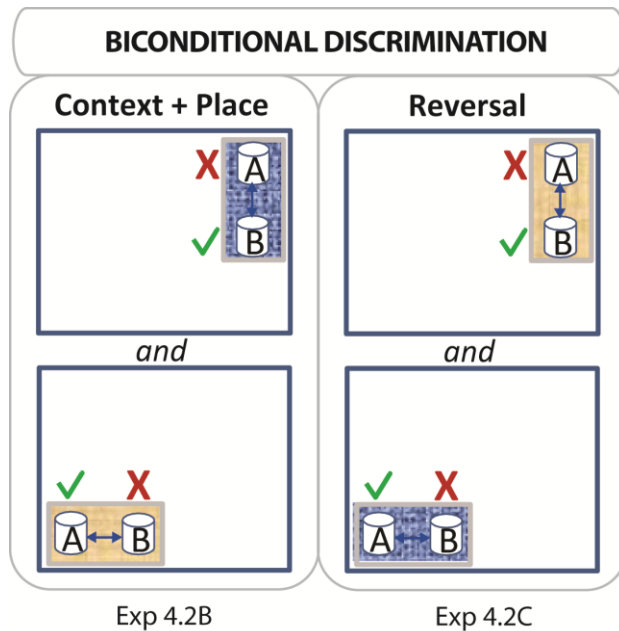
Figure 4.3. A schematic diagramme of showing the original Context + Place biconditional discrimination (from Figure 4.2), and the reversal (Exp. 4.2C). During the reversal the local contexts swapped locations (i.e., Context1 + Place 1 changed to Context 1 + Place 2). The biconditional rule remained with location (Place 1 and Place 2), and not with the contexts. A green tick indicates the correct (reinforced) cup, and a red "x" indicates the incorrect cup.

Apparatus and room

This experiment used same apparatus and room that was used in Experiment 4.2A.

Procedure

The procedure was the same as Experiment 2B, however, the boxes swapped location so that the white box was now in Place 2 and the blue box was placed in Place 1. The correct cup was held constant for the place but not the context. Therefore, the beads were now correct in the blue box, but in Place 1, whereas the shredded paper was correct in the white box, but in Place 2 (i.e., Cup A correct in Context 2 + Place 1, whereas, Cup B is correct in Context 1 + Place 2; See Figure 4.3). The rats were given one session (16 trials).

Experiment 4.2D: Stage 4 - Place Biconditional Learning

The aim of this experiment was to examine explicitly whether rats with lesions to the anterior thalamic nuclei were able to acquire biconditional rules based on spatial locations.

Apparatus and room

The room was the same as Experiment 4.2A. Two identical copies of a novel clear plastic box were used (40 cm x 30 cm x 12 cm; Smartstore, Sweden). The boxes had black opaque handles (20 cm long and 2.5 cm thick) starting 5 cm from the edge of the short walls. The handles were placed facing the inside of the box, so they protruded 1 cm inside the box. For the first day of testing the two identical boxes were used; one at each location (i.e., Box 1 at Place 1; Box 2 at Place 2). From the second day of testing, a single box was transported between the two places to prevent the use of odour markings (e.g., urine) that might otherwise help solve the biconditional task (see Figure 4.4).
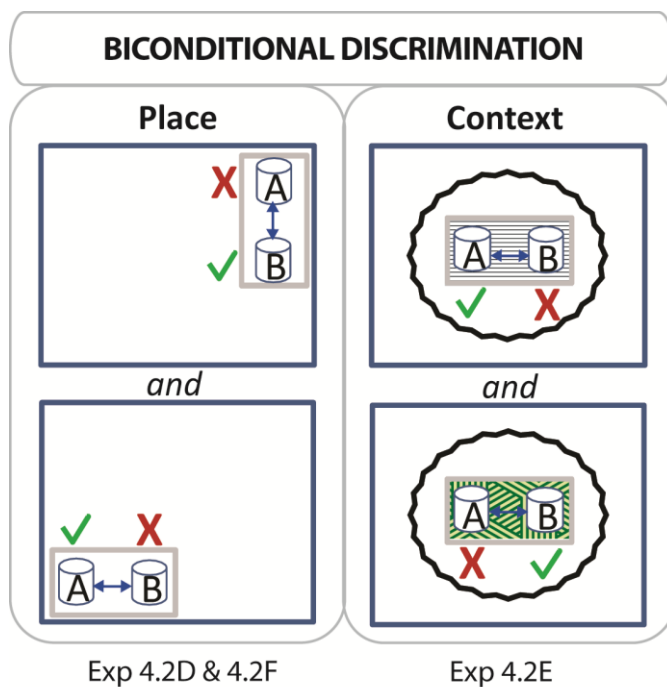
Figure 4.4. A schematic diagramme of showing the Place (Exp. 4.2D and 4.2F) and the Context (Exp. 4.2E) biconditional discrimination (from Figure 4.2). The Place biconditional task used the same box in the two locations. In the Context biconditional task, the wavy black lines represent the curtain which prevented the use of any distal cues. Also note that two different contexts were used. A green tick indicates the correct (reinforced) cup, and a red "x" indicates the incorrect cup.

Procedure

Using the same procedures as in Experiment 4.2B, the rats were trained on a new biconditional association, and were now required to learn explicitly which medium was correct in which place. The biconditional rule was that multi-coloured beads (but not shredded paper) were correct in Place 1, while the cup containing shredded paper (but not coloured beads) was correct in Place 2. Because a single test box was moved between the two locations between trials, it was assumed that the rats could only solve the biconditional rule using the distal cues found in the test room. The location of the box (Place 1 or 2) was determined pseudo-randomly with the following restrictions: 1) The box occupied both locations equally, and 2) the box was placed in one of the two locations for no more than three consecutive trials. The location and left/right positions of the correct cup inside the box were also counterbalanced within sessions and across groups. To eliminate the use of any odour cues made by the rats exploring the cups (e.g., marking the cup with urine), the same two cups were used throughout (i.e., each cup was rewarded for 50% of the trials). Rats were trained for 16 trials per day until the Sham1 group performed at 80% correct.

Experiment 4.2E: Stage 5 - Context Biconditional Learning

The goal of this study was to assess explicitly whether rats with lesions to the anterior thalamic nuclei were able to acquire biconditional rules based on different contexts provided by the test boxes. Although, it was hypothesised that this condition would yield similar results to those found in Experiment 4.1 (Contextual Biconditional Discriminations), there were two potentially important differences between the tasks: 1) in Experiment 4.1 the context-item associations were in different modalities (i.e., auditory-visual or auditory-thermal associations), whereas in the present experiment they are in the same modality (i.e., visual, although the rats could touch and smell the digging cups), and 2) Experiment 4.1 explored Pavlovian associations (i.e., reception of a reward was not contingent on a correct behavioural response), whereas in the current experiment the animals had to make the correct choice to receive reinforcement.

Apparatus and room

For this task, the digging cups were placed in one of two semi-transparent plastic boxes (both 33 x 26 x 16.5 cm, Wham Crystal, Whatmore Creative Plastics). The two boxes could readily be distinguished as one box had laminated wall panels composed of white and red triangles and a green textured Duplo (Lego, UK) base covering the floor (Context 1). The second box had a smooth, checked (black /white) laminated floor, but plain walls (Context 2). The two boxes were placed on a table located in the centre of the test room. Black curtains were placed around the table and box preventing the use of distal cues.

Procedure

The same procedure was used as Experiment 4.2B, but the rats were now explicitly tested on their ability to solve the biconditional rule based on the local context alone. The rats were required to learn that the coloured beads were correct in the Duplo base box (Context 1); whereas the shredded paper was correct when presented in the checkered floor box (Context 2). The boxes (i.e., contexts) and left/right position of the correct cup inside the box were also counterbalanced within sessions and across groups. To eliminate the use of odour cues made by the rats exploring the cups (e.g., marking the cup with urine), the same two cups were used in both the boxes. Animals received 16 trials per session (8 trials in each context) until the Sham1 group reached a criterion of 80% correct responses.

Experiment 4.2F: Stage 6 - Repeat of Place Biconditional Learning

Apparatus, room, and procedure

The apparatus and procedure were identical to Experiment 4.2D. The rats were given one final session (16 trials).

Statistical analysis

For the series of studies in Experiment 4.2, the total correct choice was tabulated for each day (a total out of 16), and the mean percent correct responses for each group was examined. The data for Experiments 4.2A, 4.2B, 4.2D, and 4.2E were

examined using one between-subject factor (Group) x one within subject factor (Days) ANOVA. When there was an interaction, simple effects were examined (Howell, 1982). Between-sample t-tests were used to examine the scores in Experiment 4.2C and 4.2F.

Results

Histology

The histology has been previously reported in Chapter 2. Three animals were excluded from all analyses due to the small size of the lesions (< 50% total ATN damage). Therefore, a total of 12 cases remained in the ATNx1 group.

Behavioural Testing

Experiment 4.1: Contextual biconditional discriminations

The discrimination ratios during the presentation of the auditory stimuli (number of magazine entries during the correct auditory stimulus in a given context divided by the total number of magazine entries during the presentation of both auditory stimuli) for the thermal and visual contextual biconditional discriminations by the Sham1 and ATNx1 animals across blocks of testing trials are displayed in Figure 4.5A. Both groups acquired the task, and there was no evidence of a lesion induced deficit. A three way mixed model ANOVA (Group x Condition x Block) yielded a significant main effect of Block ($F_{(4, 80)}$ = 22.9, $p < 0.001$), indicating that the discrimination ratios increased over testing blocks (i.e., the performance of the rats improved). Both the Sham1 and the ATNx1 groups also performed significantly better on the visual contextual discriminations compared with the thermal ones ($F_{(1, 20)}$ = 37.5, $p < 0.001$). There was also a significant Block x Condition interaction ($F_{(4, 80)}$ = 2.49, $p = 0.05$). Inspection of the simple effects indicated that the rats were significantly better on the visual contextual discriminations compared with the thermal contexts on all blocks ($p < 0.05$) with the exception of second testing block ($F_{(1, 100)}$ = 3.57, $p = 0.062$). No other interactions were significant (all, $p > 0.1$) nor was there a significant main effect of Group ($p > 0.1$).

Figure 4.5B displays the discrimination ratios during the ten seconds prior to the onset of the auditory stimuli for the thermal and visual context of the ATNx1 and Sham1 groups. A three way ANOVA with the between factor Group, and within factors Context and Blocks did not reveal any significant main effects nor interactions (all, p > 0.1). The results indicate that the groups did not differ on baseline magazine entries in both the thermal and visual contexts.
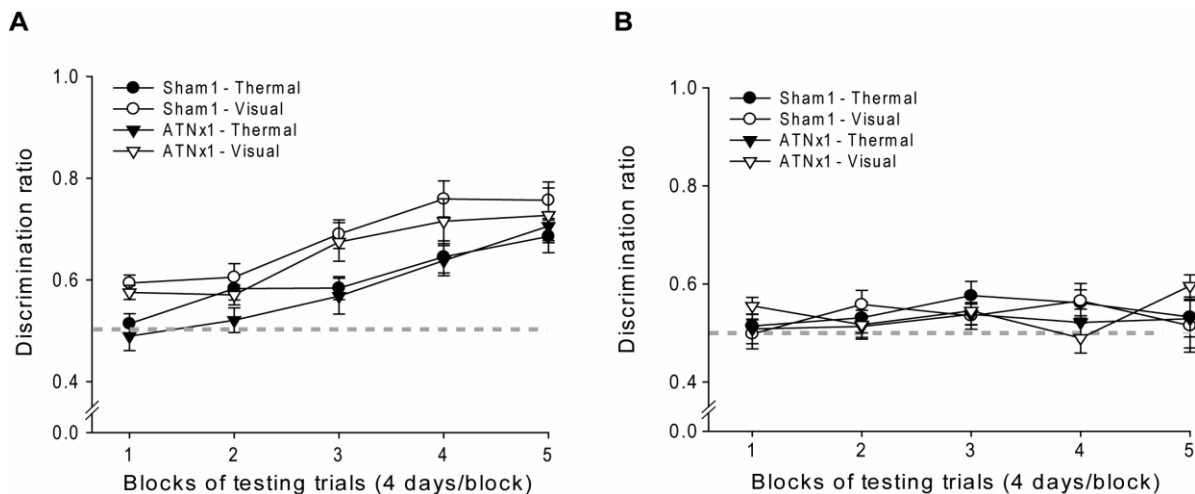
Figure 4.5. The discrimination ratios of the Sham1 and ATNx1 groups for the thermal and visual contexts across blocks of testing trials **A**) during the presentation of the auditory stimuli and **B**) during the ten seconds prior to the onset of the auditory stimuli (i.e., baseline). Note: grey dashed line = chance (0.5).

Experiment 4.2: Spatial Biconditional Learning

Experiment 4.2A: Stage 1 - Simple Discrimination

In this task the rats had to learn that one of the two cups containing different media (beads or shredded paper) was always reinforced, and to dig in that medium for a food reward. The mean percent correct responses over days of training for both the Sham1 and ATNx1 groups are shown in Figure 4.6A. By the second day of testing, both groups were already performing above 80% correct, the criterion. A two-way ANOVA with a between factor (Group) and a within factor (Days) yielded a significant main effect of Day ($F_{(2, 40)} = 33.9$, $p < 0.001$). Neither the main effect of Group nor the Group x Day interaction was significant (both $p > 0.1$).

The mean percent correct responses of both groups were significantly above chance on the first day (Sham1: $t_9 = 2.74$, $p = 0.023$; ATNx1: $t_{11} = 3.82$, $p =$

0.003). It was hypothesised that the simple discrimination was acquired rapidly and that the animals were learning within the 16 trials of the first day. To examine this possibility, the mean percent correct responses of the Sham1 and ATNx1 groups for the first four trials and the last four trials of the first session were compared. As can been seen in Figure 4.6B, both groups had a mean percent correct responses of 52% and 53% during the first four trials for the ATNx1 and Sham1 groups, respectively; however, both groups improved to 79% (ATNx1) and 65% (Sham1) mean percent correct responses for the last four trials. A two way ANOVA with the between factor Group (Sham1, ATNx1) and within factor Trial (first four, last four) yielded a significant main effect of Trial demonstrating that performance was significantly better during the last four trials compared with the first four trials ($F_{(1,20)}$ = 11.7, p = 0.003). There was no significant main effect of Group or interaction. One-sample t-tests confirmed that both groups did not differ significantly from chance performance for the first four trials (both: p > 0.1), but did differ significantly from chance for the final four trials (ATNx1: t_{11} = 4.31, p = 0.001; Sham1: t_9 = 2.25, p = 0.05).
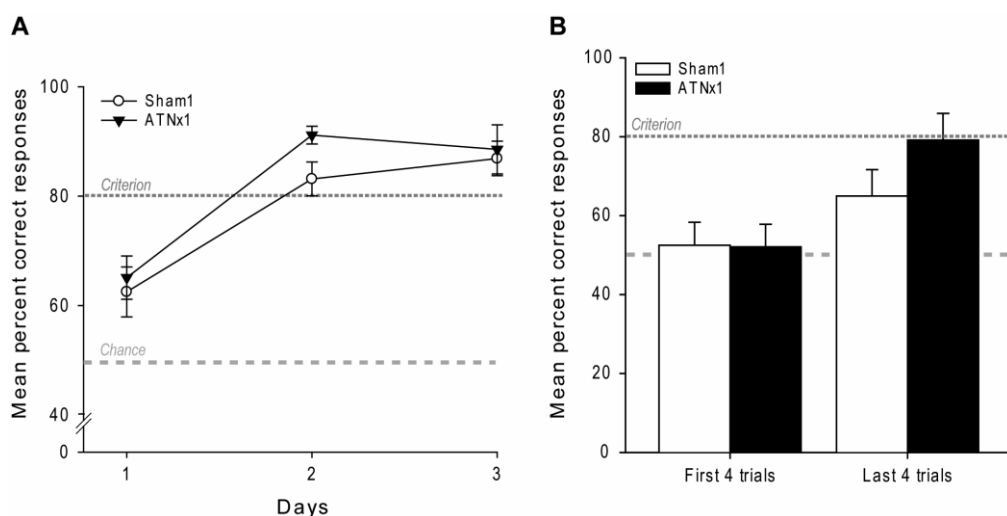
Figure 4.6. A) The mean percent correct responses of the Sham1 and ATNx1 groups across testing days during the standard discrimination task. **B**) The mean percent correct responses of the Sham1 and ATNx1 groups during the first four trials and last four trials of the first day of the simple discrimination. **Note:** light grey long dashed line = chance; dark grey short dashed line = criterion.

Experiment 4.2B: Stage 2 - Place & Context Biconditional Learning

The rats were required to learn that one cup was correct in one context which was also in a particular place in the room, whereas the second cup was correct in a

different context and place (i.e., Cup A correct in Context 1 + Place 1; Cup B correct in Context 2 + Place 2). The mean correct responses over testing days for both groups are displayed in Figure 4.7.

Although both groups could acquire the problem, there was evidence that the anterior thalamic lesions impaired performance (Figure 4.7). A two-way mixed model ANOVA (Group x Day) yielded a significant main effect of Day ($F_{(11,220)}$ = 19.4, p < 0.001) and a significant Group x Day interaction ($F_{(11, 220)}$ = 1.87, p = 0.045). The results indicate that both groups improved over testing days, but inspection of the simple effects indicated that the Sham1 group outperformed the ATNx1 group on Days 9 ($F_{(1, 240)}$ = 3.91, p = 0.049), 11 ($F_{(1, 240)}$ = 11.0, p = 0.001), and 12 $F_{(1, 240)}$ = 7.99, p = 0.005). The main effect of group was marginally significant with Sham1 animals learning the biconditional task better than the ATNx1 group ($F_{(1,20)}$ = 4.01, p =0.059). Despite, the ATNx1 group being impaired compared with the Sham1 group, the ATNx1 group attained a mean of 76% correct responses by the last day, whereas the Sham group had reached a mean of 91% correct responses.
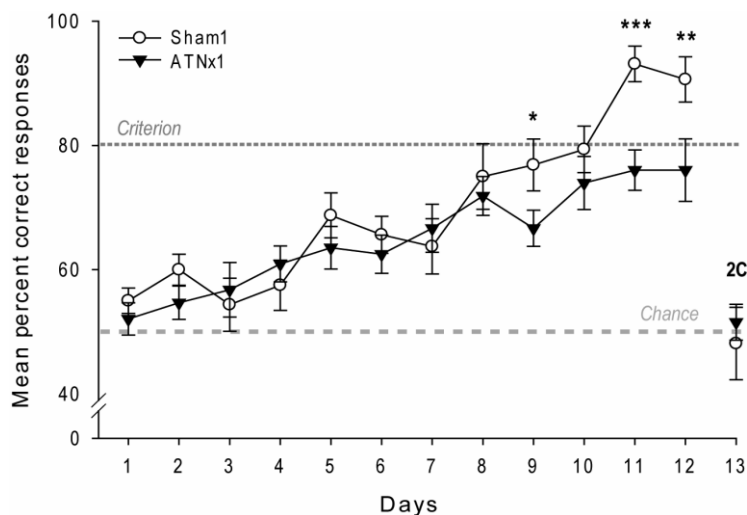

Figure 4.7. The mean percent correct responses of the Sham1 and ATNx1 groups across testing days during the context + place biconditional learning task. **Note:** 2C = Experiment 4.2C (Reversal), where the context (boxes) were swapped creating incongruent context-place information; * = p < 0.05; ** = p < 0.01; *** = p < 0.001.

Experiment 4.2C: Stage 3 - Reversal of Place & Context Biconditional Learning

The goal of the one day reversal was to explore whether the rats were using the context, place, or both cues to solve the task. The two contexts swapped locations, so that Context 1 was now located in Place 2, and Context 2 was now located in

Place 1. The rule remained with the locations (i.e., Place 1 and 2), and did not follow the box. Therefore, Cup A contained the food reward in Context 2 + Place 1, and Cup B contained the food reward in Context 1 + Place 2. Figure 4.7 shows the mean percent correct responses for the Sham1 and ATNx1 rats. Neither the Sham1 nor the ATNx1 group differed significantly from chance performance, and the groups did not differ significantly from one another (all: $p > 0.1$).

Experiment 4.2D: Stage 4 - Place Biconditional Learning

In this task, a single context was used in both locations. The rats were required to learn that in Place 1, Cup A is correct and in Place 2, Cup B is correct. The mean percent correct responses over testing days are shown in Figure 4.8. The ATNx1 rats were clearly impaired as the mean percent correct response for the Sham1 group at the end of training was 82%, whereas the ATNx1 group had a mean of 54% correct responses. A two-way mixed model ANOVA (Group x Day) yielded a significant main effect of group ($F_{(1, 20)} = 70.5$, $p < 0.001$), indicating that the Sham1 group performed significantly better compared with the ATNx1 group. The main effect of Day and the interaction effect were both non-significant (both: $p > 0.1$).
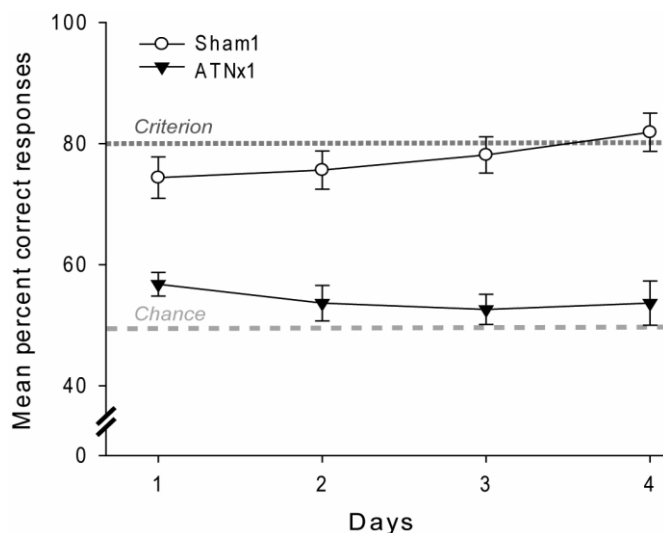
Figure 4.8. The mean percent correct responses of the Sham1 and ATNx1 groups across testing days during the biconditional place learning task. **Note:** light grey long dashed line = chance; dark grey short dashed line = criterion.

Experiment 4.2E: Stage 5 - Context Biconditional Learning

The ability of the animals to use context cues to solve the task was directly tested by using two distinct boxes placed in the same location. Only one box was used at a time, and all other distal cues were hidden with the use of a curtain. Figure 4.9

shows the mean percent correct response of the Sham1 and ATNx1 groups. Now there was no evidence of a lesion effect. It took both the Sham1 and the ATNx1 group eight days to reach 80% correct responses. A two-way ANOVA with a between factor (Group) and a within factor (Day) yielded a significant main effect of Day ($F_{(8,\ 160)}$ =18.4, p < 0.001), demonstrating that the performance of both groups improved over testing days. There was no significant main effect of group, nor interaction effect (both: p > 0.1).
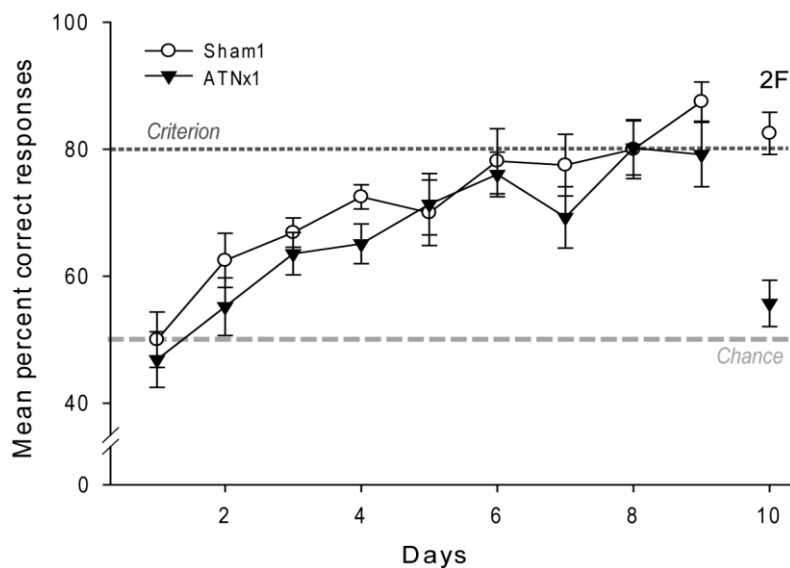

Figure 4.9. The mean percent correct responses of the Sham1 and ATNx1 groups across testing days during the biconditional context learning task. **Note:** 2F = Experiment 4.2F (Repeat), where Experiment 4.2D (Place Biconditional) was repeated for one day only; light grey long dashed line = chance; dark grey short dashed line = criterion.

Experiment 4.2F: Stage 6 - Repeat of Place Biconditional Learning

One final test day (16 trials) using the same, single, box as that in Experiment 4.2D was given to the rats to re-examine the item-place associations (i.e., choose Cup A in Place 1, but choose Cup B in Place 2). The mean percent correct response for the Sham1 group was 83%, whereas the ATNx1 group only obtained a mean 55% correct response (Figure 4.9). Consequently, the Sham1 group chose the correct cup significantly more often than the ATNx1 group (t_{20} = 5.33, p <0.001).

Discussion

This chapter explored whether the anterior thalamic nuclei are necessary for biconditional learning. Rats with lesions to the anterior thalamic nuclei were trained on a biconditional task where both the local context and the location of the room could be used to solve the task. ATNx1 rats were significantly impaired

compared with Sham1 rats by the end of training. However, the ATNx1 rats chose the correct digging cup significantly more than chance performance (Experiment 4.2B). A reversal was performed where the distal room cues (i.e., the two locations; Place 1 and Place 2) and the proximal contextual cues (boxes) were put in conflict. The two distinct boxes swapped locations and the biconditional rule remained with the two places; therefore, continued correct responding would indicate that the rats had formed item-place associations. It was hypothesised that if the ATNx1 rats were only using proximal cues to solve the task that they would drop to chance performance (or even perform significantly below chance), whereas the Sham1 group should stay above chance. However, both groups performed at chance during this reversal, indicating that the groups were 1) either only using the contextual cues and forming item-context associations or 2) had been using both item-context and item-place associations to solve the task which during the reversal were made incongruent. In order to examine these two possibilities, the rats were then trained on two biconditional task that could only be solved using either distal room cues (place) or the proximal context cues. A third possibility is that there was very quick extinction to the use of the distal cues within the 16 trial session, but inspection of the first trial of the probe showed that 58% of the ATNx1 and 60% of the Sham1 chose the correct cup. Therefore the performance of both groups was already near chance from the first probe trial.

Lesions to the anterior thalamic nuclei severely impaired acquisition of biconditional learning tasks when an item (correct digging cup medium) was associated with distal room cues (Experiment 4.2D and 4.2F). This is consistent with previous findings indicating that damage to the anterior thalamic nuclei impairs the formation of spatial-visual and odour-location biconditional associations (Sziklas & Petrides, 1999; Gibb et al., 2006; see Table 4.1). In Sziklas and Petrides' (1999) study, the rats had to choose one of two items depending on whether they were located at the North or South end of an open field maze. In the present experiment, the rats had to dig in a particular cup depending on the location of the cups (placed inside a small plastic box) in the room. However, these findings are inconsistent with the report of a lack of deficit following anterior thalamic lesions when the rats are required to navigate to a particular location

depending on which item is presented (i.e., visual-spatial biconditional learning; Sziklas & Petrides, 2007). This result is particularly surprising given that type of stimuli (elements) needed to form the biconditional associations are the similar (spatial-visual or visual-spatial). In the spatial-visual task, the location of the choice items determines which item is correct, whereas in the visual-spatial task, the presentation of an item determines to which of two locations the rat must navigate (Sziklas & Petrides, 1999; 2007). These results suggest that 1) the nature of how information is associated is an important factor (spatial-visual vs. visual-spatial), and 2) that anterior thalamic lesions are not necessary for binding together all types of information (i.e., all types configural learning), but rather rats with anterior thalamic damage may have difficulty in using place information to guide behavioural choices.

Further support for this hypothesis comes from other biconditional learning tasks where animals were required to form an association between an item and a position (left or right; Chudasama et al., 2001; Ridley et al., 2001; see Table 4.1). For instance, when rats were presented with one of two stimuli on a screen, they had to learn to either nose poke to the left or to the right of the stimulus to obtain a reward. Rats with anterior thalamic lesions were able to learn which stimulus corresponded with the left or right nose poke (Chudasama et al., 2001). Likewise, when monkeys were presented with two copies of the same stimulus (e.g., A1, A2) they had to learn to select the item on the left, and when two copies of stimulus B (B1, B2) were presented, they had to select the item on the right (Ridley et al., 2001). Lesion confined to the anterior thalamic nuclei did not impair performance on this task; however, when the lesions were larger and included the medial dorsal thalamus, the monkeys were impaired compared to control monkeys (Ridley et al., 2001). However, one major difference with these two item-position biconditional tasks compared to the visual-spatial biconditional (Sziklas & Petrides, 2007), is that they could be solved using an egocentric strategy, and anterior thalamic lesions do not impair learning egocentric spatial tasks (Aggleton et al., 1996; Warburton et al., 1997; Sziklas & Petrides, 1999; Mitchell & Dalrymple-Alford, 2006; Wolff, Gibb et al., 2008).

In contrast to the item-place biconditional task, when the rats were required to form item-context biconditional associations (only the proximal contextual cues were available; i.e., the boxes), ATNx1 group performed as well as the Sham1 group (Experiment 4.2E). The lack of a deficit on contextual biconditional tasks following anterior thalamic lesions was also observed in Experiment 4.1 (see Table 4.1).

In Experiment 4.1, the rats were placed in operant chambers and were required to form biconditional associations between contexts that differed either a) visually (spotted, checkered) or b) thermally (cool, warm) with auditory stimuli (clicker, tone). Damage to the anterior thalamic nuclei did not impair contextual biconditional learning compared with Sham1 rats regardless of the type of context. Both groups also learnt the visual contextual biconditional significantly better than the thermal contexts. These results contrast with a previous experiment using the same procedure with rats that had sustained mammillothalamic tract lesions (Vann, Honey, & Aggleton, 2003). Vann et al. (2003) found that damage to the mammillothalamic tract impaired biconditional learning in the visual contexts, but not in the thermal contexts. There are several possibilities accounting for the conflicting results: One possibility stems from the fact that the strains of rats differed. Vann et al. (2003) used Dark Agouti rats, whereas the current experiment uses Lister Hooded rats. Although inspection of the data does not suggest that there were different rates of learning, the control rats in Vann et al., (2007) showed unstable discrimination ratio that fluctuated between chance and 0.6 over the course of four days of testing. When the data were pooled across the four days, there was no difference between the discrimination ratio of the thermal and visual contexts (approximately 0.55; significantly above chance) in the sham group. In the present study, the Sham1 rats (and the ATNx1 group) found the visual contexts easier compared with the thermal ones, even on the first block of four testing days (see Figure 4.5), and the rats did not differ from chance for the thermal contexts.

Table 4.1. Summary of the studies (including the present chapter) that examined the effects of damage to the anterior thalamic nuclei on biconditional associative learning tasks. The right column notes whether the animals with anterior thalamic lesions were impaired relative to control animals. All studies were conducted using rats unless otherwise stated.

Study	Task	Details	Extended training	Impaired
Chudasama et al., 2001	Visual conditional discrimination	If A, respond left If B, respond right	Yes	No
Gibb et al., 2006	Odour-location paired-associate (biconditional) learning	If cumin in location 1, go If cumin in location 2, no-go If cinnamon in location 1, no-go If cinnamon in location 2, go	Yes	Yes
Ridley et al., 2002†	Visuospatial conditional task	If A, respond left If B, respond right	Yes	No
	Visuovisual conditional task	If on Tray 1, choose A If on Tray 2, choose B	Yes	No
Sziklas and Petrides, 1999	Spatial-visual biconditional learning	If objects at North end of arena, choose A If objects at South end of arena, choose B	Yes	Yes
	Visual-motor biconditional learning	If Object A, go left If Object B, go right	Yes	No
Sziklas and Petrides, 2004	Visual-motor biconditional learning	If Object A, go left If Object B, go right	Yes	No
Sziklas and Petrides, 2007	Visual-spatial biconditional	If Object A, go to Place X If Object B, go to Place Y	Yes	No
Experiment 4.1	Contextual Biconditional Discrimination	If warm, clicker reinforced If cool, tone reinforced	Yes	No
		If spot, clicker reinforced If check, tone reinforced	Yes	No
Experiment 4.2D, 4.2F	Item-place biconditional	If Place 1, choose Cup A If Place 2, choose Cup B	Yes	Yes
Experiment 4.2E	Context-place biconditional	If Context 1, choose Cup A If Context 2, choose Cup B	Yes	No

Note: † = subjects were monkeys

Another difference is the deficit noted in Vann et al.'s (2003) study was within the first four days of training. In the present experiment, the rats received much more training (20 days). Perhaps given more training the deficit observed following damage to the mammillothalamic tract would disappear. It is unclear

why training stopped so early, given that the behavioural performance was not asymptotic, and the discrimination ratio itself was very low, and near chance. In contrast, the present study clearly shows that both the ATNx1 and Sham1 group were able to acquire contextual conditional discriminations with a discrimination ratio almost reaching 0.8 by the final block of testing. Furthermore, there is evidence that lesions to the mammillary bodies do not impair spatial-visual biconditional learning that is impaired following anterior thalamic damage (Sziklas, Petrides, & Leri, 1996; Sziklas, Lebel, & Petrides, 1998; Sziklas & Petrides, 1999, 2000, 2004). It is unclear why damage to the mammillothalamic tract should impair biconditional learning when damage to the mammillary bodies does not (e.g., Sziklas et al., 1996); although again the tasks differ significantly. Finally, the lack of a deficit following anterior thalamic damage on the item-context biconditional (Experiment 4.2A) supports the results from Experiment 4.1: the ATNx1 rats can form associations between stimuli and unique contexts.

The results from this chapter suggest that anterior thalamic lesions do impair biconditional learning when selecting an item depends on where (i.e., place) in a distal spatial environment the choice items are located. However, damage to the anterior thalamic nuclei does not impair biconditional learning tasks that can be solved using proximal contextual cues. It is worth noting that anterior thalamic lesions appear to only impair biconditional learning when place information is used to guide choice behaviour (Sziklas & Petrides, 1999; Gibb et al., 2006; Experiment 4.2D). For this reason, the following two chapters try to gain insight into which aspects of place learning rats with anterior thalamic damage cannot resolve, and whether manipulating how the rats approach the digging cups (from a single direction or from two directions) influences their performance. It remains possible that approaching the digging cups from a single direction reduces the amount of overlapping (common) distal cues, and reduces task difficulty by making the locations more distinct from one another (Gaffan & Harrison, 1989).

Chapter 5

A comparison between the anterior thalamic nuclei and the hippocampus on place learning in complex environments

Introduction

The previous chapter found that damage to the anterior thalamus impairs the formation of item-place associations (Experiements 4.2D and 4.2F) which is consistent with previous reports describing the effects of damage to the anterior thalamic nuclei (Wilton et al., 2001; Sziklas & Petrides, 1999; Gibb et al., 2006) and the hippocampus (Save et al., 1992; Sziklas et al, 1996, 1998; Gilbert & Kesner, 2002; Sziklas & Prides, 2002; Henry et al., 2004; Dumont et al., 2007, 2010). However, anterior thalamic lesions spare learning about item-context associations (Experiments 4.1 and 4.2E); the results suggest that rats with anterior thalamic damage are capable of acquiring the biconditional rule (choose Cup A in Context 1; choose Cup B in Context 2). Rats with hippocampal lesions are also unimpaired at acquiring the same item-context task (M. Albasser, personal communication). Given that lesions to either of these two neural regions is sufficient to impair spatial learning with distal spatial cues (Jarrard, 1978; Harley, 1979; Olton & Papas, 1979; Sutherland & Rodriguez, 1989; Aggleton et al., 1996; Byatt & Dalrymple-Alford, 1996; Warburton et al., 1997, 2001; Mitchell & Dalrymple-Alford, 2006), it remains possible that the associative deficits observed on the item-place biconditional tasks are caused by an inability to learn about spatial locations, rather than an inability to combine the spatial and item information together.

The present chapter sought to investigate whether the item-place biconditional impairments could be explained by a failure to discriminate between the two spatial locations. This experiment, therefore, assessed rats with damage to

the anterior thalamic nuclei (ATNx2) and rats with damage to the hippocampus (Hpc), as well as their respective sham groups (Sham2 for the ATNx2 group; ShamH for the Hpc group) on spatial discrimination tasks. The ATNx2 rats were first tested on T-maze alternation (Experiment 5.1) to assess the effectiveness of the surgeries. Next, using the same locations as the item-place biconditional task (Chapter 4), all rats were required to learn to dig for food in a cup in one location (go), but refrain from digging in the *same* cup when placed in a second location (no-go) as it was never baited (Experiment 5.2A). The rats approached the single digging cup from two directions (bidirectional go/no-go) in both locations to encourage the animals to process the whole environment. It was hypothesised that this training procedure would increase the likelihood of the animals processing overlapping (i.e., common) distal cues between the two locations, and that processing overlapping cues may increase task difficulty by making the locations less distinct from one another (Gaffan & Harrison, 1989). The animals were then trained in a different room where they had to discriminate between two spatial locations when approaching the cup from a single direction (unidirectional go/no-go). The unidirectional version (Experiment 5.2B) could potentially make distal cues easier to discriminate, as there should be less overlapping features between the two locations (Figure 5.1), i.e., the problem could be solved using single, salient cues.

This latter point is not trivial given that the rats with lesions to the anterior thalamic nuclei and rats with lesions to the hippocampus were able to form item-context associations (Chapter 4, Experiment 4.1 and 4.2E; M. Albasser, personal communication). Arguably, animals with damage to the extended hippocampal system can solve item-context associations because the contexts are unique (i.e., the cues used are distinct, and have little or no overlapping features); the unique cue (or constellation of unique cues) always predicts which behavioural outcome is correct (i.e., Cue X, choose A; Cue Y, choose B). When the distal spatial cues are not unique (i.e., share similar features), then any given cue does not always predict which behavioural outcome is correct; the animals are now required to take into consideration additional associative features such as the structural relationship (arrangement) of the spatial cues (e.g., if near Cue X, but far from Cue Y, choose A;

Gaffan & Harrison, 1989; Aggleton & Pearce, 2001; Dumont et al., 2007). In other words, the correct behavioural response depends upon the relationship of several distal spatial cues.
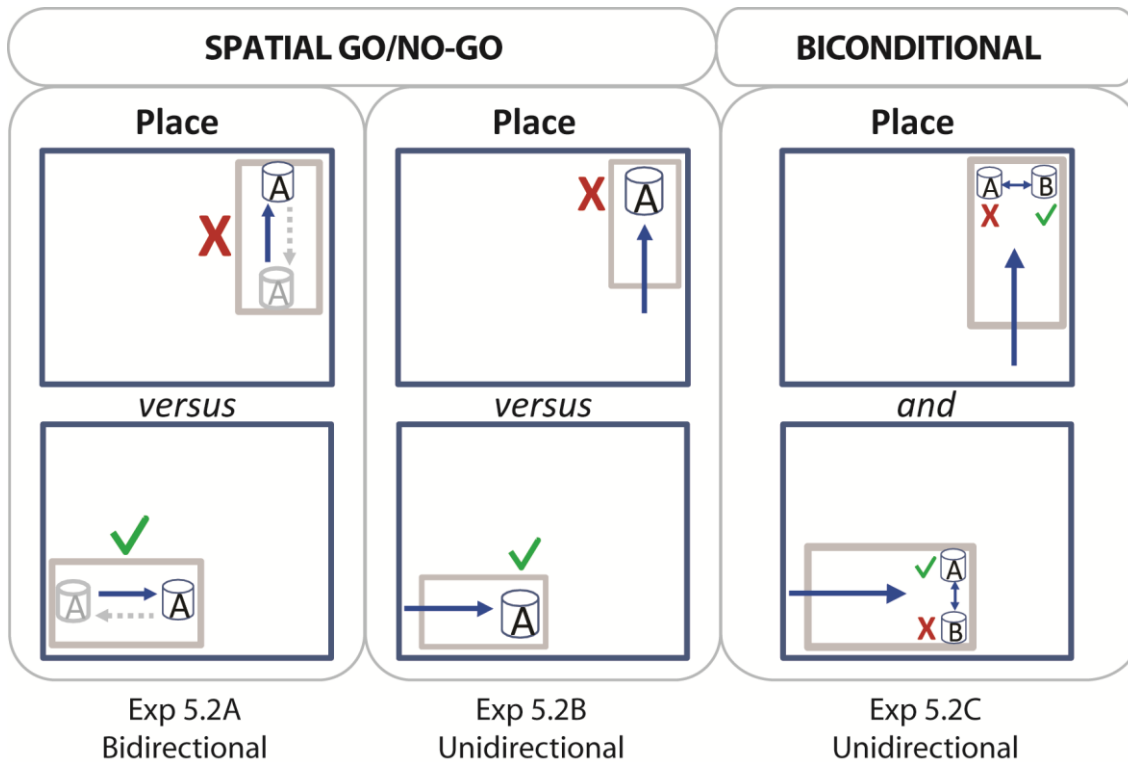
Figure 5.1. A schematic diagramme of the spatial go/no-go discrimination task (Exp 5.2A, B) and the unidirectional place biconditional task. The large blue boxes represent the testing room, and the small grey rectangles represent the plastic boxes where the digging cups were placed. The green ticks indicate the correct location (Exp. 5.2A, B), or the correct digging cup (Exp. 5.2C); whereas the red "X" indicates incorrect responses. The blue arrow shows the direction the animals ran towards the digging cup(s). In Exp. 5.2A, the dashed grey line and cup indicates that for half the trials the rats ran towards the digging cup in the opposite direction to the blue line (bidirectional). The diagramme is not drawn to scale, nor do the locations represent all test room conditions (see text for the placement of the box in the room).

Finally, the rats were tested on an item-place biconditional task (Experiment 5.2C) where the rats were required to associate one of the two choice digging cups with one of the two locations. Unlike the biconditional experiment in Chapter 4, the rats always approached the two digging cups from the same direction in each of the two locations, so using the same places and directions that had been acquired during the unidirectional go/no-go spatial discrimination task (Figure 5.1). This last experiment addresses whether animals could use more salient distal spatial cues and form associations with particular items (i.e., can the animals form biconditional rules when spatial cues or scenes are made more distinct?). Therefore, the animals were first tested on two place discrimination

tasks using a go/no-go procedure, where the animals either approached the goal digging cup from one (unidirectional) or two (bidirectional) directions. Following the place discrimination learning, the ability of animals to form biconditional associations between the same spatial place cues and one of two digging cups was examined.

Materials and Methods

Animals

The subjects were two separate cohorts of male, Lister Hooded rats housed in pairs under diurnal conditions (12 h light/12 h dark). The first cohort was comprised of 27 rats (13 Sham2 and 14 ATNx2). The ATNx2 and Sham2 groups had not received prior behavioural testing (i.e., naïve). The second cohort had 25 rats, where 13 had sustained bilateral lesions of the hippocampus (Hpc) and 12 control rats received sham surgeries (ShamH). The animals had free access to water, but were food-deprived up to 85% of their free-feeding body weight and maintained above this level during behavioural testing. The Hpc rats were approximately 6 months old, and the ATNx2 rats were approximately 8 months old at the start of the study. The Hpc and their match control group (ShamH) had previously been trained on a variety of geometric discriminations in a water tank (Gilroy, Horne and Pearce, unpublished data). All experiments were performed in accordance with the UK Animals (Scientific Procedures) Act, 1986 and associated guidelines.

Surgery

The rats with damage to the anterior thalamic nuclei and the matched controls underwent the same surgical procedures as previously described in Chapter 2. The only difference was that the dorsal-ventral coordinates were deeper at -7.1 and -6.4 from the skull for the medial site and the lateral site, respectively. The ATNx2 and Sham2 groups form a new cohort of rats that have not been mentioned in previous chapters.

The hippocampal cohort and the respective control rats were first anaesthetised using an isoflurane-oxygen mix, and then placed in a stereotaxic

frame (Kopf Instruments, Tujunga, CA), with the incisor bar set at -3.3 mm. The animals were administered with 0.1mg/kg of the analgesic Metacam subcutaneously. A sagittal incision was made in the scalp, and the skin retracted to expose the skull. A dorsal craniotomy was made directly above the target region and the dura cut to expose the cortex. The hippocampal lesions were made by injecting ibotenic acid (Biosearch Technologies, San Rafael, CA) dissolved in phosphate-buffered saline (pH 7.4) to provide a solution with a concentration of 63 mM. The rats received 14 injections of ibotenic acid, in each hemisphere, made through a 2- μL Hamilton syringe held with a microinjector (Kopf Instruments, Model 5000) at an infusion rate of 0.10 μL/min and a diffusion time of 2 min. The coordinates and volume of the ibotenic acid injections are shown in Table 5.1. The control animals received identical treatments except that the dura was repeatedly perforated with a 25-gauge Microlance3 needle (Becton Dickinson, Drogheda, Ireland) and no solution was infused into the brain. Testing for both the Hpc and the ATNx2 cohorts began approximately 4 months after surgery.

Histological Procedures

The histological procedures were almost identical to those reported in Chapter 2 with the only difference that the hippocampal cohort received additional behavioural testing both before and after the experiments reported in this chapter. This additional behavioural testing is not reported in this thesis. The ATNx2 cohort also underwent some additional behavioural training in the watermaze, which is reported in Chapter 6. Futhermore, tissue from the ATNx2 and Sham2 rats was collected for analysis of CREB and phosphorylated CREB counts (not reported in this thesis). Therefore, sodium fluoride (NaF) was added into the every solution used in the histological procedure reported in Chapter 2 as it prevents the dephosphorylation of CREB.

Table 5.1. Stereotaxic coordinates and volume of ibotenic acid for lesions of the hippocampus. The stereotaxic coordinates indicate the distance (mm) from bregma.

AP	ML	DV	Volume (µL)
-5.4	±4.2	-3.9	0.10
-5.4	±5.0	-6.1	0.08
-5.4	±5.0	-5.3	0.08
-5.4	±5.0	-4.5	0.09
-4.7	±4.0	-7.2	0.10
-4.7	±4.0	-3.5	0.05
-4.7	±4.5	-6.5	0.05
-3.9	±2.2	-3.0	0.10
-3.9	±2.2	-1.8	0.10
-3.9	±3.5	-2.7	0.10
-3.1	±1.4	-3.0	0.10
-3.1	±1.4	-2.1	0.10
-3.1	±3.0	-2.7	0.10
-2.4	±1.0	-3.0	0.05

Abbreviations: AP, anteroposterior; DV, dorsoventral; ML, mediolateral.

Volumetric analysis

The size of the lesions in the anterior thalamic nuclei of the 14 rats was estimated using the same procedures described in Chapter 2, but only using Nissl stained tissue. Similarly, the size of the lesions in the hippocampus of all 13 rats in the hippocampal lesion cohort was estimated from the Nissl stained tissue. The extent of each hippocampal lesion was first drawn onto ten, standard sections at different anterior-posterior levels from the atlas Paxinos and Watson (2005; from AP -2.12, -2.80, -3.30, -3.80, -4.30, -4.80, -5.30, -5.80, -6.30, -6.80 relative to bregma). These

drawings were then scanned, and the area of damage was quantified using the program analySIS^D (Olympus, UK). Next, the percent damage to the entire hippocampus was quantified from these same standard sections.

Experiment 5.1: T-maze alternation

Damage to the anterior thalamic nuclei severely impairs acquisition of T-maze alternation (Aggleton et al., 1995, 2009; Aggleton, Amin et al., 2011). To test the effectiveness of the present lesions, ATNx2 and Sham2 rats were first tested on the T-maze alternation task as described in Chapter 2 with some modifications outlined below. The rats with hippocampal damage were not tested on the T-maze alternation task.

Apparatus and room

Pre-training and testing took place in a 304 cm long x 290 cm wide x 239 cm high room that contained a variety of extramaze cues (e.g., posters, tables, door, etc), and illuminated by two fluorescent strip lights (140.8 lux). Two identical cross-mazes were used. The walls of each of the four arms of the two mazes (45.5 cm long x 12.0cm wide x 32.5 cm high) were made of black Perspex. The floor of the two mazes were made of wood and painted white. A sunken food-well (2 cm in diameter and 0.75 cm deep) was located at the end of each arm. By placing an aluminium barrier at the entrance of the arm it was possible to prevent access to the arms. During pre-training the mazes were placed side by side so that the East arm of the left maze (Maze A) touched the West arm of the right maze (Maze B) on a table (74 cm high). However, during the test sessions, only one maze was used and placed in the centre of the table. The maze used (A or B) alternated daily across the test sessions.

Pre-training

Pre-training was identical to that previously described in Chapter 2. The only difference was that over the four days of pre-training each rat was placed in Maze A and in Maze B for two alternating days.

Procedure

The procedure was identical to Chapter 2. However, the rats were only tested for eight consecutive days.

Experiment 5.2A: Spatial go/no go bidirectional discrimination

The purpose of this experiment was to test why the ATNx1 rats were impaired on the biconditional discrimination that taxed the use of room cues (Chapter 4, Experiment 4.2D) but not impaired on the biconditional that relied on local context cues (Experiment 4.2E). One possibility was that the ATNx1 rats could not effectively discriminate the spatial cues; another possibility was that the ATNx1 rats could not integrate that information with the item-place configural rule. The first possibility was tested by giving rats a task that required them to discriminate different spatial room cues. Critically, in this experiment, the two locations to be discriminated were each approached from two directions ("bidirectional"). Furthermore, as a series of similar experiments carried out in rats with damage to the hippocampus found a similar selective pattern of deficits on the biconditional tasks presented in Chapter 4 (M. Albasser, personal communication), a group of rats with hippocampal lesions were also tested on spatial learning.

Apparatus and room

Animals were tested in the same white plastic test box (40 cm long x 20 cm wide x 12.5 cm high) that was previously used in Chapter 4, Experiment 4.2. A single cup filled with sawdust was presented in the centre along the side of a short wall (Figure 5.1). The digging cup consisted of a black plastic cylinder with an internal diameter of 7 cm and a height of 6 cm. The base of the cylinder was made of a grey plastic square (9 cm x 9 cm). Velcro secured the cups to the box floor to prevent the rats from tipping them over while digging. Similar to Chapter 4 Experiment 4.2, the food reward was half a loop of a single Cheerio (Nestle, UK) that was buried in the digging media. To discourage rats from trying to locate the food reward by its scent, a perforated metal grid was placed inside the cup to create a false bottom. Several loops were placed under the metal grid, where the rats could not retrieve them, and replaced by new cereal loops every two days. Several cereal

loops were also ground to a powder and mixed in with the media to ensure that the digging cup always smelt of the food reward.

Pre-training took place in a relatively narrow room (330 cm long x 190 cm wide x 256 cm high; Room A) compared to the other rooms used in this study. Visual cues, such as posters and shelves were fixed on the walls. A table was placed near the back wall of the room. The illumination level where pre-training took place was 430 lux. Testing took place in a different room (Room B; 280 cm long x 280 cm wide x 256 cm high) that contained a variety of distal cues (e.g., posters, door, shelves fixed on a wall containing various objects). This was the same room and the same cues as previously used in Chapter 4, Experiment 4.2, and where anterior thalamic nuclei lesions significantly impaired item-place biconditional learning. (The same is also true of rats with Hpc lesions; M. Albasser, personal communication.) The distal wall cues were visible from any corner of the room, i.e., the centre of the room was kept open. The room was illuminated with 8 spot bulb lights fixed on the ceiling, and the mean luminance from five readings indicated that the illumination in Place 1 was 123.3 lux and in Place 2 was 123.6 lux.

Pre-training

Rats were placed singly in the white plastic test box with two identical digging cups filled with sawdust. First a reward was placed on top of the medium. Then, the reward was buried increasingly deep so the rats had to dig to find the food. Every time the rat found the food, the cup was re-baited, and so on for 10 minutes. Pre-training lasted between four and six days, when all rats were reliably digging to retrieve the rewards.

Procedure

Similar to Chapter 4 (Experiment 4.2), four or five rats were simultaneously brought to the test room in an enclosed carrying box made of aluminium. Each rat was in a separate container and could not see the surrounding environment. The rats were then run in spaced trials, i.e. the 4-5 rats were run one after the other for trial 1, then the 4-5 animals for trial 2, and so on. Consequently there was an inter-trial interval of approximately 2-3 minutes.

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A single cup filled with sawdust was presented in a white plastic box on each trial. The white box could be placed on top of a table in one of two locations of Room B (Table 5.2). Thus, for any given rat, the cup was always baited in one room location (go response), but never baited when located in the other room location (no-go response; Figure 5.1). One table (122 cm x 53.4 cm x 70 cm) was located near the door, whereas the second table (102 cm x 56 cm x 76 cm) was placed by an adjacent wall. The two locations were approximately 130 cm apart. At the start of each trial, the rats were placed at the opposite end of the box, always facing the digging cup. Learning was assessed by comparing the latency of the rat to dig when the box was in the baited location and the latency to dig when the box was in the never-baited position, where the rat should learn to withhold from digging. Each trial had a time limit of 20 s, after which the rat was removed. If the rat dug in the medium before 20 s in the correct location, the rat was removed as soon as it had consumed the cereal reward; but if the rat dug in the incorrect location it was left for an extra 5 s before being taking out of the box. The trial order was counterbalanced pseudo-randomly between the two locations (correct and incorrect) with the following rules: 1) the box was placed in the two locations equally (i.e., 8 trials in the correct and 8 trials in the incorrect location), and 2) the box was not in the same location for more than 3 consecutive trials. In addition, the direction the animal ran to the digging cup (i.e., the start location was either to the North or to the South end of the box) was also counterbalanced across the 16 trials pseudo-randomly with the following rules: 1) the rat ran to the cup from both directions equally (i.e., 8 trials each), and 2) the rat ran towards the digging cup in the same direction for a maximum of three consecutive trials. In order to determine unambiguously when a rat dug, the animals were viewed from the side (Figure 5.1).

Experiment 5.2B: Spatial go/no-go unidirectional discrimination

The purpose of this experiment was to compare directly the performance of the same rats on the spatial go/no-go task when the rats approach the cups in a single direction, and whether this change in procedure simplified the task demands compared to when the rats were required to approach the digging cup from two directions in the two locations they were to discriminate between (Figure 5.1). It

was hypothesised that when the rats approach the cup from a single direction, certain cues may become easier to discriminate, the two locations more distinct, and animals may be able to utilize non-spatial stimulus-response and stimulus-reward strategies. For example, rats with damage to the extended hippocampal system can learn associations between cues and rewards (e.g., conditioned cue preference, visual discriminations where an object is always rewarded; McDonald & White, 1993; Chudasama et al., 2001; Ito, Robbins, McNaughton, & Everitt, 2006, Gibb et al., 2006; Chapter 4, Experiment 4.2A), or between a cue and a behaviour (e.g., operant conditioning, egocentric learning; Aggleton et al., 1996; Warburton et al., 1997; Sziklas & Petrides, 1999; DeCoteau & Kesner, 2000; Ramos & Vaquero, 2000; Mitchell & Dalrymple-Alford, 2005, 2006; Wolff, Gibb et al., 2008.).

Apparatus and rooms

Animals were tested in a larger transparent box (52 cm long x 33 cm wide x 17 cm high, internal dimensions; Crystal, Whatmore Creative Plastics, 45 LTR, www.whamproducts.co.uk) than the ones used in Chapter 4 and Experiment 5.2A (current chapter) and in a different room (Room C and Room D; see Figure 5.2 and Table 5.2). The plastic digging cup was placed in the centre of the short wall of the box (~17 cm away from the long walls). To the left and the right of the digging cup, soft portions of Velcro pieces were stuck to the floor so that two more cups could be attached.

The ATNx2 group and their Sham2 control group were tested in room C (300 cm long x 275 cm wide 239 cm high; Figure 5.2A, B). The test box was placed in one of two locations. In Place 1, the box was placed in the middle of a shelving unit made of metal bars (52 cm between each shelf; Figure 5.2A). The box was 89 cm above the ground, and 6 cm away from the wall along the side of the box. The front of the box (where the digging cups were located) was 69cm away from the wall, and the wall located behind the start end of the box was 154 cm away. Place 2 was in the diametrically opposite corner on top of a trolley (71 cm high; Figure 5.2B). The wall along the side of the box was 11 cm away. The wall behind the start end of the box was 26 cm away. The room was illuminated with eight small light bulbs. However, to match the illumination levels, a small lamp angled from above, down towards Place 1 was used. The heat emitted from the small lamp was

not detectable from within the test box by the experimenter. The mean illuminationfrom five readings for Place 1 was 262.8 lux and Place 2 was 262.2 lux.
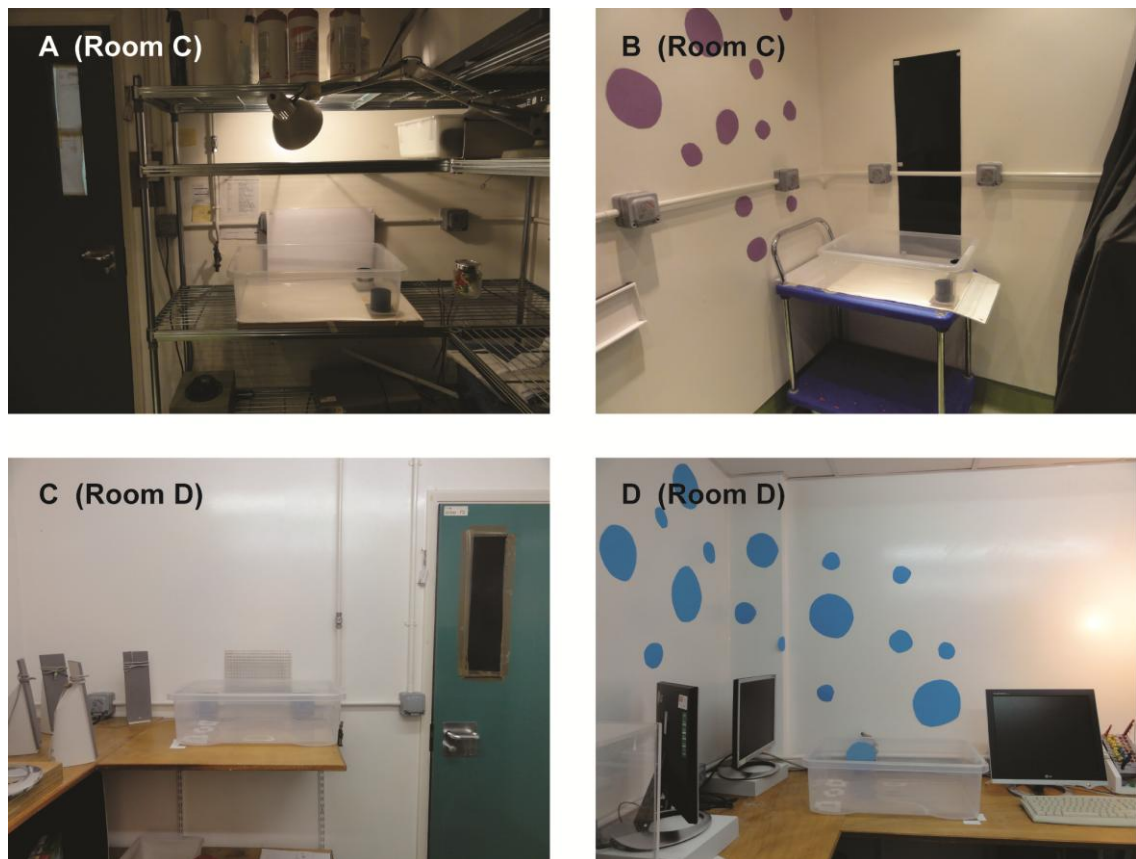
Figure 5.2. Photographs of Place 1 (Left: **A, C**) and Place 2 (Right: **B, D**) of Room C (Top: **A, B**; ATNx2 room) and Room D (Bottom: **C, D**; Hpc room). These two rooms were used during Experiment 5.2B (unidirectional go/no-go) and 5.2C (unidirectional biconditional). Some of the distal spatial cues available to the animals can be seen as well as the large clear plastic box that the rats were placed inside during testing. In photographs **A** and **B**, the two plastic cups used during the biconditional task are visible; whereas the three Velcro squares used to attach the cups in different locations within the box (the two squares adjacent to the walls was used for the biconditional, and the square in the middle was used for the go/no-go) can be seen in **C** and **D**.

The Hpc and their respective ShamH control group were tested in room D (300 cm long x 277 cm wide x 241 cm high; Figure 5.2C and D). The test box was placed in one of two locations on shelves that were 110 cm high. Place 1 was near a door, 17 cm away from the edge of the shelf and 12 cm from the wall along the long edge of the test box (Figure 5.2C). The wall of the room nearest to the digging cup was 80 cm away, whereas the test room wall was 145 cm from the start location of the test box. Four grey speakers (30 cm high x 10 cm wide) were placed between the wall of the testing room and of the box (in front of the cup). Place 2 was 11 cm away from the edge of the shelf and 15 cm away from the wall

along the long edge of the testing box (Figure 5.2D). The goal end of the testing box (i.e., where the digging cups are placed) was 55cm away from the wall. Between the wall and the digging cup there was a 40 cm high x 35 cm wide computer screen, 35 cm away from the box. On the other side of the box (start end), the screen of a computer was placed perpendicular (4 cm wide) and 5 cm away. The testing room wall was 243 cm away from the start end of the box in Place 2. There were other extra maze cues including several operant chambers and shapes stuck on the walls. The room was illuminated with fluorescent strip lights. However, to match the illumination levels, a small lamp angled towards the wall was placed near Place 2. The heat emitted from the small lamp was not detectable from the box by the experimenter. The mean illumination from five readings for Place 1 was 394 lux and Place 2 was 388.8 lux.

Table 5.2. The groups of animals tested in the different rooms for the place discrimination (go/no-go) and item-place biconditional tasks are shown. Only the lesion group are displayed in the table, but the respective control group was also tested in the same room (i.e., ATNx2 and Sham2; Hpc and ShamH). Whether the animals were tested always approaching the digging cup from a single (unidirectional) or two (bidirectional) directions is noted in the column on the right.

Experiment	Description	Group	Room	Direction
Pre-training	Pre-training	ATNx2; Hpc	A	n/a
Exp 5.2A	go/no-go	ATNx2; Hpc	B	bidirectional
Exp 5.2B	go/no-go	ATNx2	C	unidirectional
		Hpc	D	unidirectional
Exp 5.2C	biconditional	ATNx2	C	unidirectional
		Hpc	D	unidirectional
[Exp 4.2D	biconditional	ATNx1	B	bidirectional]

Procedure

The procedure was the same as Experiment 5.2A. The rats were again brought into the testing room in squads of four or five inside the metal carrying box to prevent them from observing the test room. Each rat was run for a total of 16 trials with eight trials counterbalanced in each of the two locations. For half of the animals,

Place 1 was the correct location (go) whereas Place 2 was the incorrect location (no-go). The opposite was true for the other half of the animals. Latencies to dig in both locations were measured, and the rats had a total of 20 s to respond (or withhold responding). Critically, the rats only ran towards the digging cup from a single direction. From the experimenter's point of view, the rats in Room C (ATNx2 and Sham2) always ran from left to right, with the wall always to the animal's left and the edge of the shelf to the right; whereas the rats in Room D (Hpc and ShamH) always ran from right to left, with the wall always to the animal's right.

Experiment 5.2C: Unidirectional place biconditional learning

During the previous place biconditional learning task (see Chapter 4, Experiment 4.2D procedure), the ATNx1 rats (Chapter 4, Experiment 4.2D) and rats with damage to the hippocampus (M. Albasser, personal communication) either had to make a left or right body turn towards the correct digging cup when making a choice. These body turns may increase the ambiguity and difficulty of the task. The goal of this experiment was to assess whether the rats could form item-place associations when the task was simplified by having the rats only approach the two choice digging cups from a single direction (i.e., without a body turn).

Apparatus and room

The same room and testing box as Experiment 5.2B were used in the present experiment (Room C for the ATNx2 and Sham2 groups; Room D for the Hpc and ShamH groups; see Table 5.2). However, instead of a single digging cup filled with sawdust, two digging cups (a black cup filled with red shredded paper and a checkered cup containing beads) were placed along the short wall of the plastic box. There was 15 cm between the two cups, and the soft Velcro that was used to fix the single cup in the go/no-go experiment was located between the two cups. Again, the same box was used in both locations.

Procedure

The procedure was the same as Chapter 4 (Experiment 4.2D), the place biconditional learning task. The rats were brought into the room in their respective squads of four to five inside the metal carrying box. The rat was placed at the start end of the box facing the two choice cups. For half of the animals, the cup filled with the beads contained the half cereal loop reward in Place 1 and the cup filled with the shredded paper was rewarded in Place 2; while for the other half, the beads were rewarded in Place 2 (not Place 1) and the shredded paper was rewarded in Place 1 (not Place 2). The location of the box and the relative left and right positions of the cups inside the test box were counterbalanced across the 16 trials. The critical difference between the present procedure and that of Chapter 4 (Experiment 4.2D) was that the animals were always released facing the cups; therefore, the rats always approached the cups from the same direction (Figure 5.1).

Statistical Analysis

Performances in the T-maze alternation (Experiment 5.1) and the spatial biconditional tasks (Experiments 5.2C) were analysed using a one between-subject factor (Groups) x one within-subject factor (Sessions) ANOVA. For the spatial go/no-go discriminations, the data were first analysed using a three-way ANOVA: one between-subject factor (Groups) x two within-subject factors [1) Sessions and 2) Latency on go/no-go trials]. The data were then also examined as a ratio (no-go/go trials) using a one between-subject factor (Groups) x one within-subject factor (Sessions) ANOVA. When there was an interaction, simple effects were examined (Howell, 1982). Additionally, the Greenhouse-Geisser correction was applied when the repeated measures data violated sphericity (although, in some cases the correction is not mentioned as the p values were not affected, i.e., the data were highly significant or insignificant).

Results

Histology

Anterior Thalamic Nuclei

Figure 5.3A shows the minimum and maximum lesion in the ATNx2 group. Four animals were excluded from further analysis. In three cases, there was excessive sparing of the anterior thalamus (typically with more sparing in the anterior medial and anterior ventral nuclei). In one case, the lesion caused large unintended damage that extended into the medial septal nuclei. Table 5.3 displays the range, median, and mean percent damage to the anterior thalamus in the left and right hemispheres as well as the total loss in both hemispheres for the remaining 10 cases. There was near complete damage to the anterior thalamic nuclei with the total loss between 73% –100% (mean: 93%). In the cases with the smaller lesions, sparing typically occurred in the anterior medial nucleus, and in the right hemisphere. In all 10 cases, the lesion extended posteriorly into the most rostral and dorsal portions of the laterodorsal nucleus and in five cases the lesions included the medial dorsal nucleus of the thalamus (although in three cases, this was only unilateral). Furthermore, there was also some damage to the parataenial nucleus (n = 8), the paraventricular nucleus of the thalamus (n = 5), the reticular nucleus (n = 8, but unilateral in two cases), the nucleus reuniens (n = 9), and the ventral anterior thalamic nucleus (n = 9, but unilateral in four cases).

Table 5.3. Percent damage to the anterior thalamus

Percent Damage to Anterior Thalamus			
Hemisphere	**Range**	**Mean**	**Median**
Left	87%-100%	95%	98%
Right	61% - 100%	91%	95%
Total	73% - 100%	93%	96%

In five cases there was some restricted bilateral cell loss in the hippocampus; three other rats had restricted unilateral damage to this region. Table 5.4 shows the range and the mean percent damage to the hippocampus in the ATNx2 rats in each of the hemispheres separately as well as together. Most of the damage was confined to the most rostral part of the ventral (inferior) blade of the dentate gyrus (n = 8; in three cases the damage was unilateral), but sometimes the damage extended into the immediately adjacent part of CA3 (n = 3; although in two cases the damage was unilateral). In three cases, the damage extended dorsally into CA1; although in two cases the damage was unilateral. A mean of 1.5% of the total hippocampus was damaged with a range of 0% - 5.8%. In one case there was potentially additional unilateral tract damage to the fornix.

Table 5.4. Percent damage to the hippocampus

Percent Damage to Hippocampus			
Hemisphere	Range	Mean	Median
Left	0% - 11.0%	1.9%	0.2%
Right	0% - 4.5%	1.2%	0.4%
Total	0% - 5.8%	1.5%	0.7%

Hippocampus

Four animals were excluded from further analysis. In two of these cases there was excessive hippocampal sparing (less than 40% damage). A further animal was excluded because of widespread cortical damage, while the lesion in the fourth case was largely unilateral. Table 5.5 shows the range, median, and mean percent damage to the hippocampus of the remaining Hpc animals. In the remaining nine Hpc cases, the volume loss for the entire hippocampus was between 42% - 79% (Figure 5.3). The cell loss was greater in the dorsal hippocampus where six cases had greater than 70% damage. In the remaining three cases, the tissue loss in the dorsal hippocampus was less (range: 48% - 53%), with sparing extending into lateral CA3, and sometimes into the medial portion of CA1. The only subfield to

show any consistent partial sparing was the dentate gyrus, but here the subfield was markedly diminished in volume, despite spared granule cells. The dorsal subiculum was lesioned in all cases, often being extensively damaged.

Table 5.5. Percent damage to the hippocampus (excluding the subiculum)

Percent Damage to Hippocampus			
Region	Range	Mean	Median
Dorsal	48% - 84%	68%	71%
Ventral	27% - 69%	48%	52%
Total	42% - 79%	62%	61%

Tissue loss in the ventral hippocampus ranged from 27% - 69%, with any sparing in the most ventral part of the CA1 and CA3, as well as in the dentate gyrus. The ventral subiculum was typically spared. It was, however, evident that in all nine cases the hippocampus was markedly shrunken in all three planes, and so it is likely that the coronal reconstructions underestimated the extent of tissue loss. In eight cases the lesions just encroached into the dorsal thalamus. Five of these cases had partial damage to the laterodorsal nucleus, which in one case included unilateral damage to the most dorsal part of the anterior ventral thalamus. Finally, all rats also had cell loss in cortex dorsal to the hippocampus. The damage involved parts of the primary and secondary motor areas, the primary somatosensory area, and the parietal region of the posterior association area. There was also some restricted damage in dysgranular retrosplenial cortex.
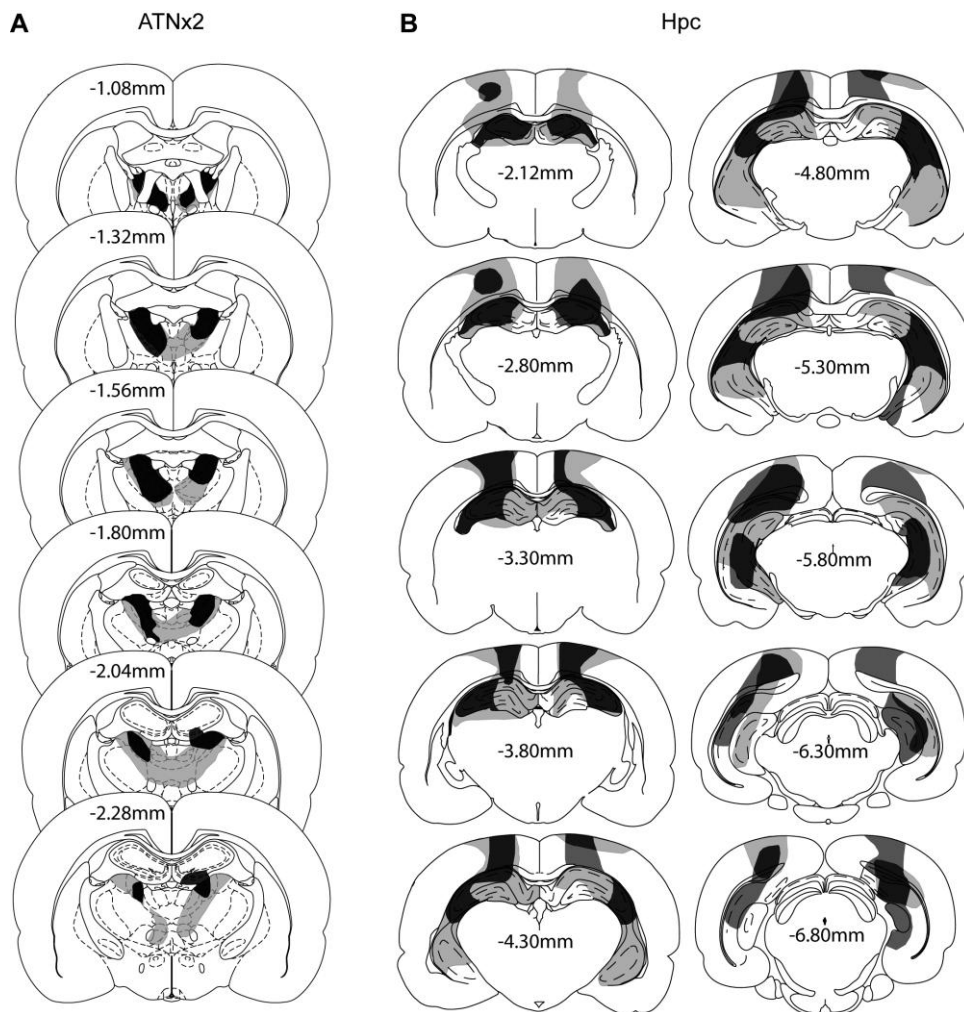
Figure 5.3. The minimum (dark grey) and maximum (light grey) extent of the lesions for the ATNx2 and Hpc groups. The numbers refer to the approximate distance of the section in mm caudal to bregma. The sections are modified from Paxinos and Watson (2005). **Note:** The rat with the smallest anterior thalamic nuclei lesion in the ATNx2 group did not have the smallest amount of unintended damage to the hippocampus. (Two animals with larger anterior thalamic lesions than the smallest lesion shown in A, had intact hippocampi.)

Behavioural Testing

One Sham2 rat was excluded from Experiment 5.2A-C because of injury to his hind paw. This animal was, however, part of Experiment 5.1. Following testing with the digging cups (Experiment 5.2A-C), the ATNx2 and Sham2 animals participated in a series of experiments in the watermaze (see Chapter 6).

Experiment 5.1: T-maze alternation

Figure 5.4 shows the mean percent correct responses across eight testing sessions. As can be seen, the Sham2 rats outperformed the ATNx2 group from the first to the last block of testing. The Sham2 group had a mean of 80% correct responses on the first block, and 82% on the final testing block. In contrast, the ATNx2 group had a mean of 61% correct responses on the first block, and 56% correct responses on the last testing block. A two-way mixed model ANOVA (between factor Group; within factor Blocks) confirmed that there was a significant main effect of Group ($F_{(1, 21)}$ = 52.6, p < 0.001), but no significant effect of Blocks (p > 0.1).
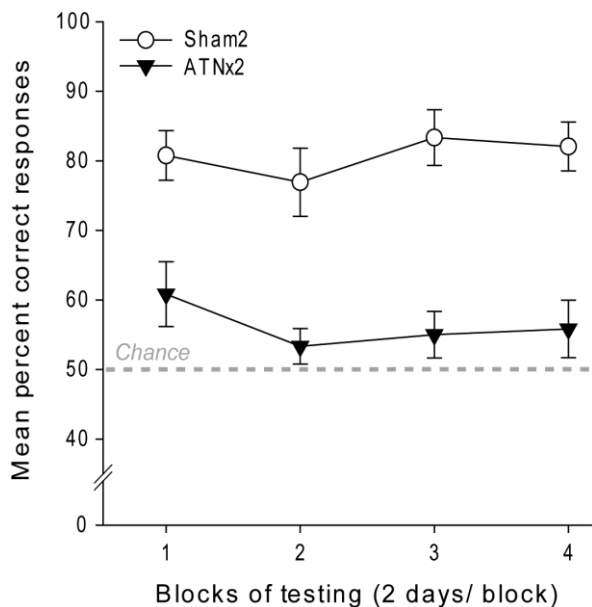
Figure 5.4. The mean percent correct responses across blocks of testing trials on the acquisition of a T-maze alternation task. Data shown are group means, and the vertical bars are the standard error of the means (SEM). Fifty percent represents chance performance.

Experiment 5.2A: Spatial go/no-go bidirectional discrimination

The aim of the spatial go/no-go discrimination was to examine whether deficits following damage to the anterior thalamic nuclei or to the hippocampus on the place biconditional learning task (see Chapter 4; M. Albasser, personal communication) were caused by an inability to discriminate between two spatial locations.

Anterior thalamic nuclei

The latencies to dig (Figure 5.5A) showed that the ATNx2 group was significantly impaired compared with the Sham2 group ($F_{(1, 20)}$ = 5.28, p = 0.033). There was

also a significant Group x Condition (go/no-go) interaction, a Block x Condition interaction, and a three-way interaction between Group x Condition (go/no-go) x Block (p < 0.001 for all), indicating that as testing progressed the Sham2 group were able to withhold responding in the no-go location significantly more than the ATNx2 group. There was also a significant main effect of Block and Condition (go/no-go; p < 0.001 for both), but no Group x Block interaction (p > 0.1).

The data were re-analysed as ratio scores (Figure 5.5B), and yielded a significant effect of Group ($F_{(1,20)}$ = 10.2, p = 0.005), Block ($F_{(4, 80)}$ = 26.6, p < 0.001) and Group x Block interaction ($F_{(4, 80)}$ = 5.02, p = 0.001). Examination of the simple effects showed that the Sham2 group had a significantly higher discrimination ratio compared with the ATNx2 group on the final two testing blocks (p < 0.001 for both).
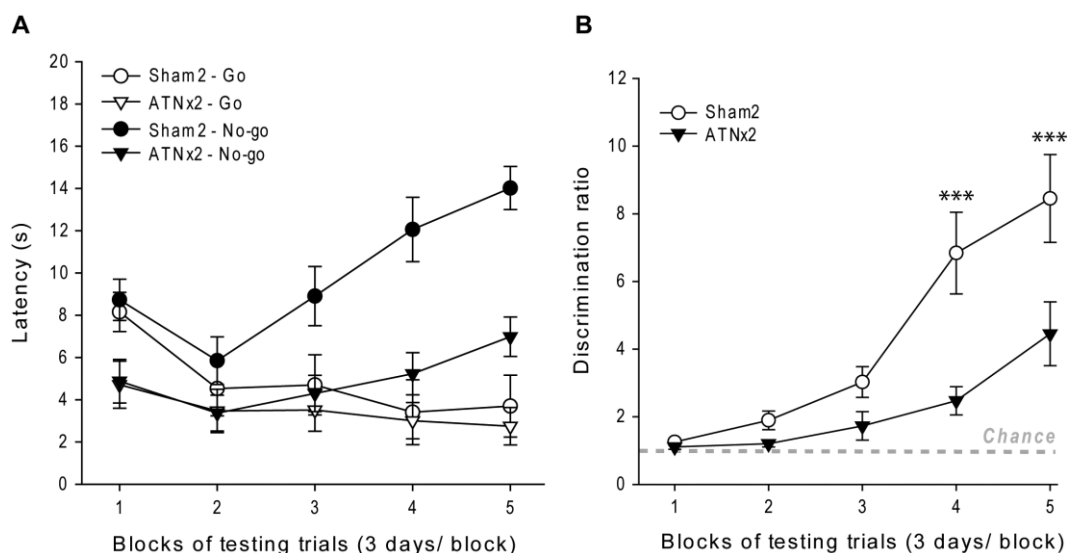
Figure 5.5. **A**) The mean latencies (s) during go and no-go trials, and **B**) the discrimination ratio (no-go/go) of the ATNx2 and the Sham2 groups across blocks of testing during the bidirectional go/no-go spatial discrimination task. Data shown are group means, and the vertical bars are the standard error of the means (SEM). A score of one represents chance (i.e., equal latencies during both go and no-go trials). Significantly group differences: *** = p < 0.001.

Hippocampus

Latencies to dig (Figure 5.6A) showed that the Hpc rats were impaired compared with ShamH rats ($F_{(1,19)}$ = 5.43, p = 0.031). The Group x Condition interaction, however, failed to reach significance ($F_{(1,19)}$ = 3.66, p = 0.071). There was a significant effect of Block, Condition (go/no-go), and Condition x Block interaction (all p < 0.001). None of the other interactions were significant (p > 0.1).

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The data were re-analysed as ratio scores (Figure 5.6B). The Hpc group was significantly impaired (group effect $F_{(1,19)}$ = 6.70, p = 0.018). There was a significant effect of Block indicating that both groups decreased their latencies to dig in the correct location ($F_{(4,76)}$ = 18.4, p <0.001). The Group x Block interaction was not significant (p > 0.1).
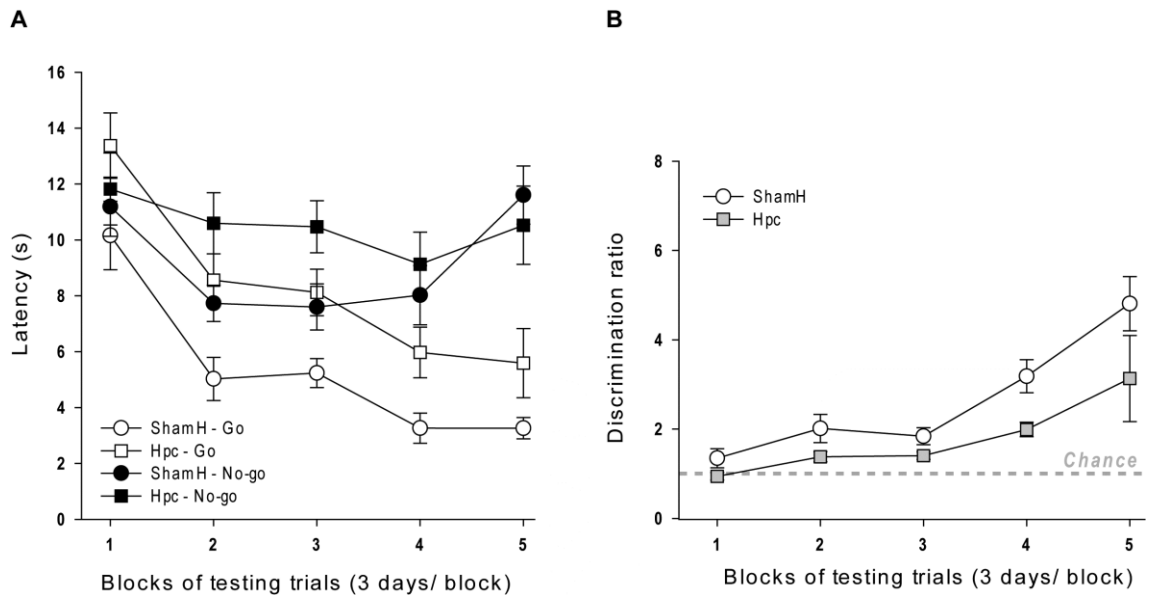

Figure 5.6. A) The mean latencies (s) during go and no-go trials, and **B**) the discrimination ratio (no-go/go) of the Hpc and the ShamH groups across blocks of testing during the bidirectional go/no-go spatial discrimination task. Data shown are group means, and the vertical bars are the standard error of the means (SEM). A score of one represents chance (i.e., equal latencies during both go and no-go trials).

Experiment 5.2B: Spatial go/no-go unidirectional discrimination

In Experiment 5.2A the ATNx2 and the Hpc rats were impaired on a spatial go/no-go task when the trials were run in two different directions in each room location. To determine the significance of the direction manipulation the rats were trained on a spatial go/no- go discrimination when the rats had to approach each digging cup from a single direction.

Anterior thalamic nuclei

Figure 5.7A shows the mean latencies to dig of the ATNx2 and the Sham2 rats. Examination of the latencies to dig yielded a significant main effect of Group ($F_{(1,20)}$ = 10.6, p = 0.004) , Condition ($F_{(1,20)}$ = 184.6, p < 0.001), Testing Day ($F_{(7,140)}$ = 6.08, p = 0.002; Greenhouse-Geisser correction for violation of sphericity). In

addition, there was a Group x Condition ($F_{(1, 20)}$ = 17.2, p = 0.001) and Condition x Day ($F_{(7, 140)}$ = 21.1, P < 0.001) interaction. Again, the results reflect the Sham2 group's greater ability to withhold responding in the no-go location compared with the ATNx2 group. All other interactions were non-significant (p > 0.1; Greenhouse-Geisser correction).

When the data were analysed as a ratio (Figure 5.7B), a borderline main effect of Group was noted ($F_{(1,20)}$ = 3.99, p = 0.06). There was a significant main effect of testing Days, reflecting the improved discrimination between the two spatial locations by both the ATNx2 and the Sham2 groups ($F_{(7, 140)}$ = 8.92, p < 0.001). The Group x Day interaction was not significant (p > 0.1).
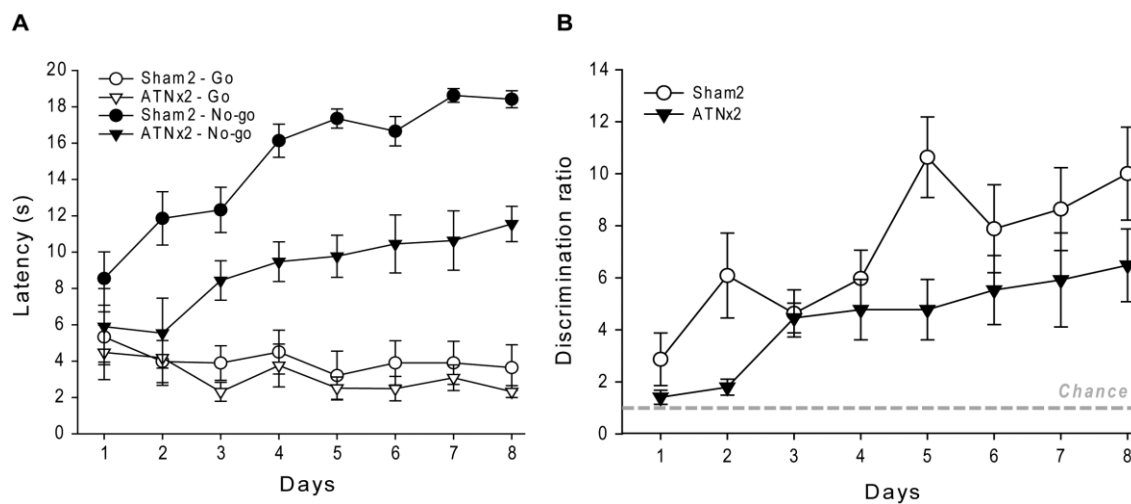
Figure 5.7. A) The mean latencies (s) during go and no-go trials, and **B**) the discrimination ratio (no-go/go) of the ATNx2 and the Sham2 groups across blocks of testing during the unidirectional go/no-go spatial discrimination task. Data shown are group means, and the vertical bars are the standard error of the means (SEM). A score of one represents chance (i.e., equal latencies during both go and no-go trials).

Hippocampus

In contrast to the bidirectional go/no-go task, the Hpc rats now seemed unimpaired. Based on their latencies to dig, the rats could discriminate the correct locations [Condition (go/no-go): $F_{(1,19)}$ = 32.7, p < 0.001]. There was also a significant main effect of Day ($F_{(7,133)}$ = 5.48, p < 0.01; Greenhouse-Geisser correction for violation of sphericity) as the latencies for the incorrect location increased whereas the latencies for the correct location decreased as training progressed. There was no significant Group effect (F < 1; see Figure 5.8A). Re-

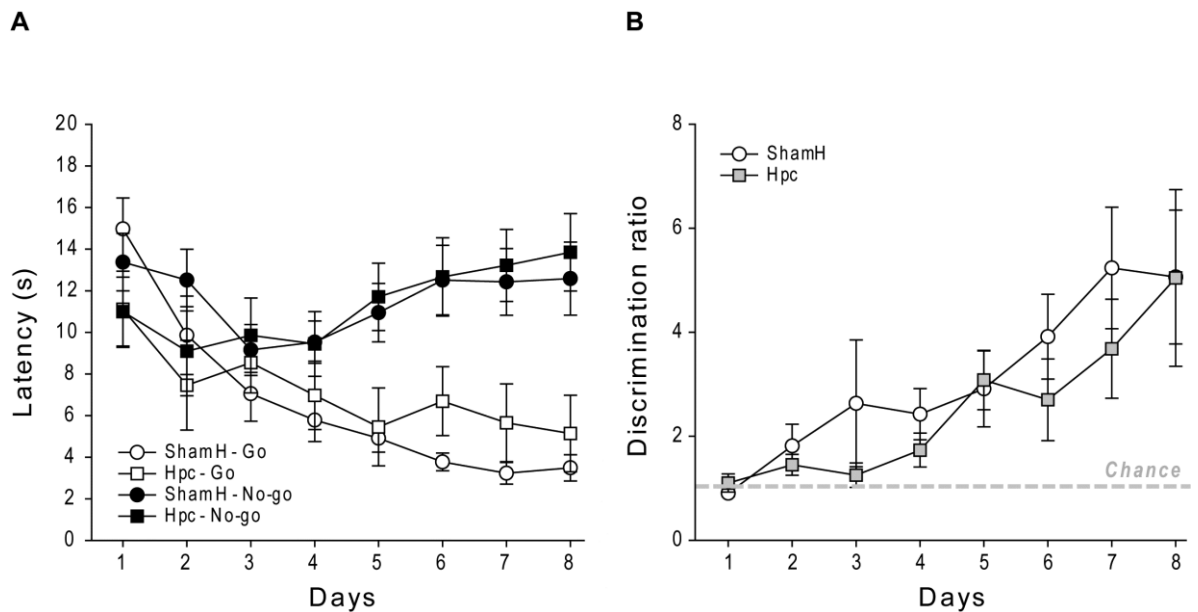analysing the data as ratios (Figure 5.8B) also failed to find evidence of a hippocampal deficit (Group effect: p > 0.1).

Figure 5.8. A) The mean latencies (s) during go and no-go trials, and **B**) the discrimination ratio (no-go/go) of the Hpc and the ShamH groups across blocks of testing during the unidirectional go/no-go spatial discrimination task. Data shown are group means, and the vertical bars are the standard error of the means (SEM). A score of one represents chance (i.e., equal latencies during both go and no-go trials).

Experiment 5.2C: Unidirectional place biconditional learning

Given that the Hpc rats were impaired on the bidirectional go/no-go spatial discrimination (Experiment 5.2A) yet unimpaired on the go/no-go spatial task when they approached the digging cup from a single direction, (i.e., unidirectional), it remains possible that the place biconditional deficit (e.g., M. Albasser, personal communication) arose from the requirement to approach the digging cups from two directions in each location. In contrast to the Hpc group, the ATNx2 group appeared to be impaired compared with the Sham2 group regardless of whether they approached the cups from a single direction or from both directions in the go and no-go locations; although inspection of the latencies to dig and the discrimination ratios (Figure 5.5 and 5.7) suggests that they were better at discriminating between the two locations on the unidirectional compared with the bidirectional go/no-go task. Therefore, it remains possible that the ATNx2 rats performance would be better on an item-place biconditional task where they approached the digging cups from a single direction compared with the

bidirectional item-place biconditional (Chapter 4, Experiment 4.2D). To examine this possibility, the ATNx2 and the Hpc rats were tested on a spatial biconditional task where the rats approached the two digging cups from a single direction in each location using the same spatial cues that the animals used to discriminate between the two locations during Experiment 5.2B.

Anterior thalamic nuclei

Figure 5.9 shows the mean percent correct responses over days for the ATNx2 and Sham2 groups. While on Day 1 both groups were at chance, only the Sham2 group improved and reached the 80% correct responses criterion by the final day. This observation was confirmed by a significant Group x Day interaction ($F_{(13,260)} = 8.40$, $p < 0.001$). The simple effects indicate that the groups differed on Days 5 ($F_{(1, 280)} = 8.33$, $p = 0.004$) and 7-14 (Day 7: $F_{(1, 280)} = 6.41$, $p = 0.012$; Day 8-14: $p < 0.001$); again, reflecting the significantly improved performance of the Sham2 rats over testing day ($F_{(13, 260)} = 22.6$, $p < 0.001$). The ATNx2 rats performance did not improve over testing days ($p > 0.1$). The main effect of Group ($F_{(1,20)} = 34.5$, $p < 0.001$) and Day ($F_{(13,260)} = 15.3$, $p < 0.001$) were also significant.
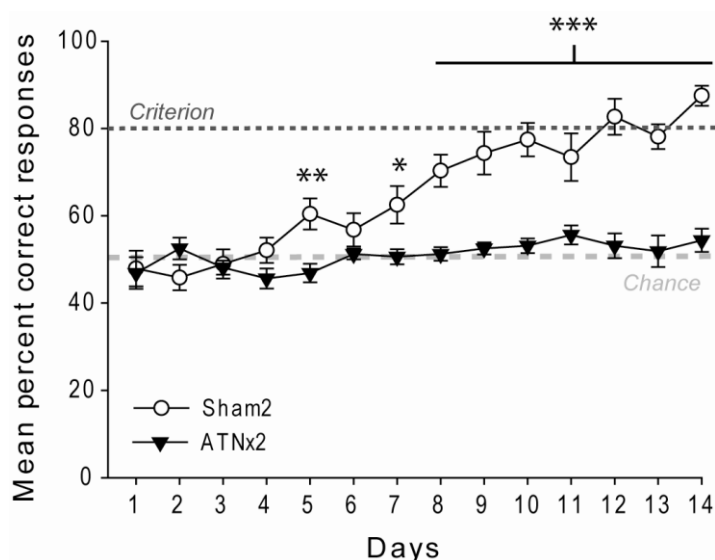
Figure 5.9. The mean percent correct responses of the Sham2 and ATNx2 groups across testing days. Data shown are group means, and the vertical bars are the standard error of the means (SEM). Significant group differences: * = p < 0.05; ** = p < 0.01; *** = p < 0.001.

Hippocampus

While both the ShamH and Hpc groups showed evidence of acquiring the biconditional discrimination (Figure 5.10; main effect of Block: $F_{(4,76)} = 40.6$, $p < 0.001$; main effect of Group $p > 0.1$), there was also a significant Group x Block

interaction ($F_{(4, 76)}$ = 3.48, p = 0.012). This interaction reflected the slower learning by the Hpc rats. Consequently, the simple effects showed that the ShamH group made significantly more correct responses compared with the Hpc group on the final two blocks of training (Block 4: $F_{(1,95)}$ = 5.65, p = 0.02; Block 5: $F_{(1,95)}$ = 7.56, p = 0.007).
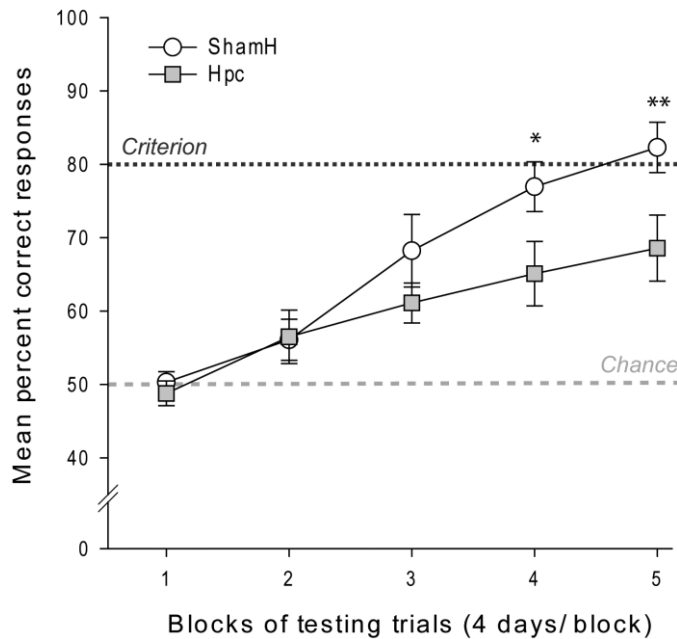

Figure 5.10. The mean percent correct responses of the ShamH and Hpc groups across testing days. Data shown are group means, and the vertical bars are the standard error of the means (SEM). Significant group differences: * = p < 0.05; ** = p < 0.01.

Discussion

Rats with damage to the anterior thalamic nuclei or to the hippocampus were tested on spatial go/no-go tasks in order to examine whether damage to these two neural regions impairs place discrimination. The rats were required to dig inside a cup when they were placed in one location inside the test room (go trials), and withhold responding when placed in a second location (no-go trials).   The rats were tested in the same room where previous cohorts of animals with anterior thalamic damage (ATNx1; Chapter 4, Exp 4.2D) or hippocampal damage (M. Albasser, personal communication) were impaired when acquiring an item-place biconditional learning task compared with sham animals. During the first go/no-go spatial discrimination task, the rats approached the digging cup at each location (Place 1, Place 2) in two directions (bidirectional go/no-go; Figure 5.1). Both the ATNx2 and Hpc groups were significantly impaired compared with their respective sham groups.

These results are supported by previous studies indicating impaired place learning following damage to the anterior thalamic nuclei (Sutherland & Rodriguez, 1989; Warburton & Aggleton, 1999; Wilton et al., 2001; van Groen et al., 2002; Loukavenko et al., 2007) and hippocampus (e.g., Morris, Hagan, & Rawlins, 1986; Whishaw & Jarrard, 1995). These results are inconsistent with a study by Gilbert and Kesner (2002) where rats with damage to the hippocampus were unimpaired on a go/no-go task where the rat had to discriminate between two spatial locations. In this study, the rats with lesions to the hippocampus had to displace an object (that was different on each trial) when it was in one location (Place X), but withhold displacing the object when it was in a second location on a cheese board maze (Place Y; Gilbert & Kesner, 2002, Experiment 4). As the rats always started in the same location (43.5 cm from Place X and Place Y) and approached (or withheld approaching) the two locations which were 67 cm apart, the distal spatial cues shared many common features (i.e., the distal spatial cues during the approach would be extremely similar like the bidirectional go/no-go task). It seems unlikely that the animals would be able to use any unique (non-overlapping) cues to solve this task. It is more likely, given that the start and goal locations were constant, that the animals were using an egocentric response strategy (e.g., always go to the location on left, Place X) which is not impaired following damage to the extended hippocampal system (Aggleton et al., 1996; Warburton et al., 1997; Sziklas & Petrides, 1999; DeCoteau & Kesner, 2000; Ramos & Vaquero, 2000; Mitchell & Dalrymple-Alford, 2006; Wolff, Gibb et al., 2008.). It would be interesting to know whether the rats with hippocampal lesions in Gilbert and Kesner's (2002) study went to the go location (Place X) even when the object was placed in the no-go location (Place Y). In other words, was the rat always approaching the same spatial location (Place X) regardless of whether the object was present at that location? If the rats were habitually approaching the go location (Place X), regardless of whether it was a go or no-go trial, the rats would appear to be unimpaired on the spatial discrimination (go/no-go task) because on no-go trials they would first approach the go the location, which would yield a higher latency score.

In contrast to the bidirectional go/no-go spatial discrimination (Experiment 5.2A), when the rats were trained on a spatial go/no-go task where they always approached the digging cup from a single direction, rats with hippocampal lesions were no longer impaired. However, unlike the Hpc group, the ATNx2 group remained significantly impaired when mean latencies were examined and marginally impaired when the discrimination ratios were considered compared with Sham2 rats on the spatial unidirectional go/no-go task. Inspection of the latencies indicate that the ATNx2 group were just as fast as the Sham2 group on go trials, but the ATNx2 rats were not as good at withholding responding on no-go trials compared with the Sham2 animals. These results suggest that the ATNx2 group may be impulsive. While there is no evidence that damage to the anterior thalamic nuclei increases impulsivity (Beracochea et al., 1989; Chudasama & Muir, 2001), this cohort had consistent unintended damage to the nucleus reuniens (nine out of 10 ATNx2 rats), which can cause impulsive behaviours (Prasad et al., 2012). Furthermore, there is evidence that anterior thalamic lesions can result in hyperactivity, which could also influence latencies (Jenkins, Vann et al., 2004; Poirier & Aggleton, 2009; see also Chapter 2)

Although there is evidence suggesting that damage to the hippocampus can result in problems with inhibiting behavioural responses (e.g., stereotyped and perseverative responding; Jarrard et al., 1964; Stevens & Cowey, 1973; Devenport, Devenport, and Holloway, 1981; Davidson & Jarrard, 2004); the current cohort withheld responding as well as the ShamH rats (Figures 5.5A, 5.7A). In fact, the Hpc group was impaired on the bidirectional go/no-go compared with the ShamH group because they were slower on the go trials. The lack of impulsivity deficits following hippocampal damage in the Hpc group may be a result of large sparing to the ventral hippocampus of the Hpc rats; as research demonstrates that damage to the ventral hippocampus and not the dorsal portion can result in impulsivity (Abela, Dougherty, Fagen, Hill, & Chudasama, 2012).

The ATNx2 group and the Hpc group were also impaired compared with the sham groups on the place biconditional task when they approached the digging cups from a single direction, suggesting that even when the distal spatial cues in the two locations were made more distinct (i.e., the animals can discriminate

between the two locations as well as sham animals in the case of the Hpc group), they still cannot form item-place associations as effectively as shams. These results are consistent with a previous study that directly compared rats with hippocampal lesion on a place biconditional task where half of the animals would view the entire testing room. In contrast, the second half of the rats would only see half the testing room when they approached the choice items from one direction and the other half of the room when the rats approached the choice objects from the opposite direction by covering half the test room with the use of a curtain (Dumont et al., 2007). This study found that the rats with damage to the hippocampus were impaired regardless of whether spatial cues shared common features (overlapped) or not. In addition, Dumont et al. (2007) found that there was no significant effect of condition (i.e., the rats were not better when the distal cues were unique compared with overlapping; Dumont et al., 2007). It is interesting to note that in Experiment 5.2C the Hpc group was significantly above chance despite being significantly worse than the ShamH group.

In contrast to the Hpc group, the ATNx2 animals did not improve over testing trials (see Figures 5.9 and 5.10). The fact that the ATNx2 rats appeared to be more impaired than the Hpc group is surprising; however, as these two cohorts were not directly compared, any interpretation between the behaviour these two groups must be approached with caution. Firstly, for Experiment 5.2B and 5.2C, the two cohorts were tested in different rooms with different spatial cues (see Figure 5.2). It is possible that one room was more difficult than another (i.e., one room used less distinctive locations). Although this possibility seems unlikely given that the Sham2 rats had higher mean discrimination ratios (Experiment 5.2B) and took less time to reach criterion (Experiment 5.2C) compared with the ShamH rats, while the ATNx2 rats were severely impaired. The Sham2 rats also had higher discrimination ratios compared with the ShamH rats on the bidirectional go/no-go task, which was tested using the same spatial cues (Experiment 5.2A), suggesting that the Sham2 group may simply be a superior group.

Secondly, the previous experience of the two cohorts differed. The Hpc group had received prior training on various spatial tasks in the watermaze

(Gilroy, Horne, & Pearce, unpublished observations) whereas the ATNx2 group was naïve prior to the experiments reported in this chapter. T-maze alternation (Experiment 5.1) was the first test for the ATNx2 and Sham2 groups, and was used to assess the effectiveness of the lesions as damage to the anterior thalamic nuclei is well known to impair performance on this task (e.g., Aggleton et al., 1995, 2009). As expected, the results indicated that the ATNx2 group was severely impaired on this task. Lastly, the ATNx2 group was approximately eight months old at the start of the behavioural testing reported in this chapter, whereas the Hpc group was approximately six months old.

This chapter addressed whether the previous impairments following damage to the hippocampus (M. Albasser, personal communication) or to the anterior thalamic nuclei (Chapter 4) during a place biconditional learning task were caused by an underlying impairment in spatial discrimination (i.e., the animals could not discriminate between Place 1 and Place 2). When the rats were tested in the same room, and approached a single digging cup in Place 1 and Place 2 from two directions, both the ATNx2 and the Hpc animals were significantly impaired compared with their respective sham control groups on a spatial go/no-go discrimination task. However, when the rats always approached the digging cups from a single direction in both locations, which is hypothesised to reduce task difficulty by decreasing the overlapping features of particular stimuli, the Hpc group was not different from the ShamH group; whereas there was a marginal group difference between the ATNx2 and Sham2 groups. However, inspection of the data reveals that during both the bidirectional and unidirectional go/no-go tasks, both the Hpc and the ATNx2 groups were significantly above chance, indicating that they could discriminate between the two spatial locations, but that in certain circumstances (e.g., bidirectional go/no-go; Experiment 5.2A) they did not discriminate as well as sham animals. Critically, animals with damage to the hippocampus (e.g., Sziklas et al, 1996, 1998; Gilbert & Kesner, 2002; Sziklas & Prides, 2002; Dumont et al., 2007; M. Albasser, personal communication) or to the anterior thalamic nuclei (e.g.,Sziklas & Petrides, 1999; Gibb et al., 2006; Experiment 4.2D) were severely impaired on item-place biconditional associative learning tasks, and this impairment may not be fully explained by a failure to

discriminate between spatial locations as the biconditional impairments appear to be more severe. The results suggest that there are two underlying problems in Hpc group: 1) the Hpc rats have difficulty in discriminating between locations when the spatial cues are similar (i.e., have overlapping features), and 2) they are also impaired when spatial cues are associated with items (i.e., problems with the biconditional rule). It is possible that the same is true following lesions of the anterior thalamic nuclei. However, the results from ATNx2 group suggest that their difficulty in using spatial cues may underlie their inability to solve item-place biconditional learning tasks. Consequently, the most parsimonious explanation of the selective spatial (but not context) biconditional discrimination deficit is a primary failure to discriminate the locations (but see, Sziklas & Petrides, 2007).

Chapter 6

Place learning in simple environments and the anterior thalamic nuclei

Introduction

Damage to the anterior thalamic nuclei reliably impairs the ability of rats to use distal, allocentric, spatial information in order to navigate to a particular location (e.g., Sutherland & Rodriguez, 1989; van Groen et al., 2002). Consistent with these results, rats with damage to the anterior thalamic nuclei are also impaired on item-place associations (Sziklas & Petrides, 1999; Chapter 4, Experiment 4.2D), but not item-context associations (Chapter 4, Experiment 4.2E). Rats with damage to the anterior thalamic nuclei were also able to discriminate between two spatial locations when they were required to dig in one location (go) and withhold responding in a second location (no-go; Chapter 5, Experiment 5.2A, B), although they were significantly worse than sham rats.

The rooms in which the rats were located to solve the item-place and place discrimination (go/no-go) tasks were complex (i.e., they contained a variety of spatial cues including the shape of the room, shelves, and posters). A resulting problem was the inability to localise the specific cues used to solve spatial tasks. The aim of the present chapter is to further understand the nature of the spatial deficits in rats with damage to the anterior thalamic nuclei by restricting the types of spatial cues available. The current chapter, therefore, focuses on two types of spatial cues: 1) geometrical properties of an environment provided by different lengths of walls, and 2) the visual arrangement of different patterned (black and white) walls.

Furthermore, despite the limited amount of locomotor activity required in the experiments in Chapter 4 and 5 (i.e., traversing the box), it remains unclear whether the integration of learning spatial locations, and being able to navigate present additional hurdles for rats with anterior thalamic damage. It has been

hypothesised that the animals can learn about spatial locations by either forming a map-like (or global) representation of the relationship of the landmarks in an environment (Tolman, 1948; O'Keefe & Nadel, 1978), or that animals learn a goal location based on a single landmark or a mental snapshot of the goal location (local representation; Gaffan & Harrison, 1989; Aggleton & Pearce, 2001). Electrophysiological studies suggest that locomotor activity may be important for thalamic head direction cell firing (Taube, 1995; Knierim et al., 1995; however, more recent evidence suggests head direction cells in the anterior dorsal thalamus may encode active and passive movement equally; see Shinder & Taube, 2011), and active exploration of an environment is important for the formation of stable representations of the spatial environments in the hippocampus (Rowland, Yanovich, & Kentros, 2011).

There are reasons to suppose that learning an escape platform location by being placed on the platform may be qualitatively different to learning that same location by actively swimming and finding the escape platform. Horne, Gilroy, Cuell, and Pearce (2012) argued that passive placement of animals in the goal location, where the animal cannot move around, would prevent the development of response-based strategies, and an animal that can then successfully navigate to the goal location most likely learnt a global representation of the environment. In contrast, an animal actively swimming to a goal can acquire response-based strategies such as learning to navigate with reference to a single landmark (e.g., navigate to a corner with their right flank adjacent to a black wall; Horne et al., 2012). The rats could also navigate to a goal by using a landmark in conjunction with heading vectors, i.e., path integration (Cartwright & Collett, 1983; McNaughton et al., 1991; Blair et al., 1997).

The hippocampus is thought to be important for forming global associative representations (e.g., a cognitive map; O'Keefe & Nadel, 1978), and so animals with hippocampal lesions should be severely impaired in learning about spatial locations when they are placed passively in the goal location. If the anterior thalamic nuclei are also important for the formation of a map-like representation of the environment, thalamic lesions should also impair learning on a passive placement task. However, anterior thalamic lesions may also impair the ability of

animals to use navigational strategies (i.e., heading vector information) to solve place learning tasks. For example, the anterior thalamic nuclei may be important for orienting and heading towards an appropriate landmark (e.g., Wilton et al., 2001; Wiener & Taube, 2005; Taube, 2007); thus, animals with anterior thalamic damage may be impaired for a variety of reasons when trying to solve place learning tasks.

The aim of the following experiments was to examine the effects of anterior thalamic nuclei lesions on place learning where either only geometrical or visual cues were available to find a correct location. First, the rats were trained on a passive placement task in two different watermazes. The rats were required to learn the location of an escape platform, while they were placed on the platform during the training phase, by relying only on either 1) the geometrical properties of the maze (rectangular) or 2) the spatial arrangement of different walls (black, white). Passively viewing the spatial cues prevented the animals from solving the task by using alternative stimulus-response strategies during training (e.g., swimming with the black/long wall to the left side of the body). Following the passive black-white walls place learning task, the rats were also tested actively (i.e., the rats were required to swim to an escape platform in the goal location) in order to determine whether active navigation could improve performance, and whether the animals were capable of using alternative solutions.

Materials and Methods

Subjects

The subjects for Experiment 6.1 were the same 25 male Lister Hooded rats described in Chapter 2 (Sham1 = 10; ATNx1 = 15), whereas the rats used in Experiment 6.2 were the same 27 male Lister Hooded rats as those described in Chapter 5 (Sham2 = 13; ATNx2 = 14).

### *Surgery, histology, and volumetric analysis*

The same surgery, histology, and volumetric analysis applied as described in Chapter 2 and Chapter 5 for the rats in Experiment 6.1 and 6.2, respectively.

Experiment 6.1: Geometrical passive placement task

The goal of this experiment was to determine whether rats with damage to the anterior thalamic nuclei could learn a particular location based on the geometrical properties of the environment when experiencing the environment passively, i.e., without actually navigating within it during training.

Apparatus and room

A white, circular pool measuring 200 cm in diameter and 60 cm deep was located 60 cm above the floor in the centre of a room (400 cm X 400 cm X 230 cm). The pool was filled with water to a depth of 27 cm and was maintained at a temperature of 25°C (± 2°C). The water was made opaque by adding 0.5 L of white opacifier (Opulyn 303B, Dow, USA; Cat No. 10318500), and changed daily. Throughout the experiment, rats were trained in a rectangular-shaped pool set within the circular pool (Figure 6.1A). This rectangular pool was constructed from two grey, long Perspex boards (180 cm long, 59 cm high, and 2 mm thick) and two grey, short Perspex boards (90 cm long, 59 cm high, and 2 mm thick). Each board was placed vertically in the pool and suspended by bars that extended over the edge of the pool.
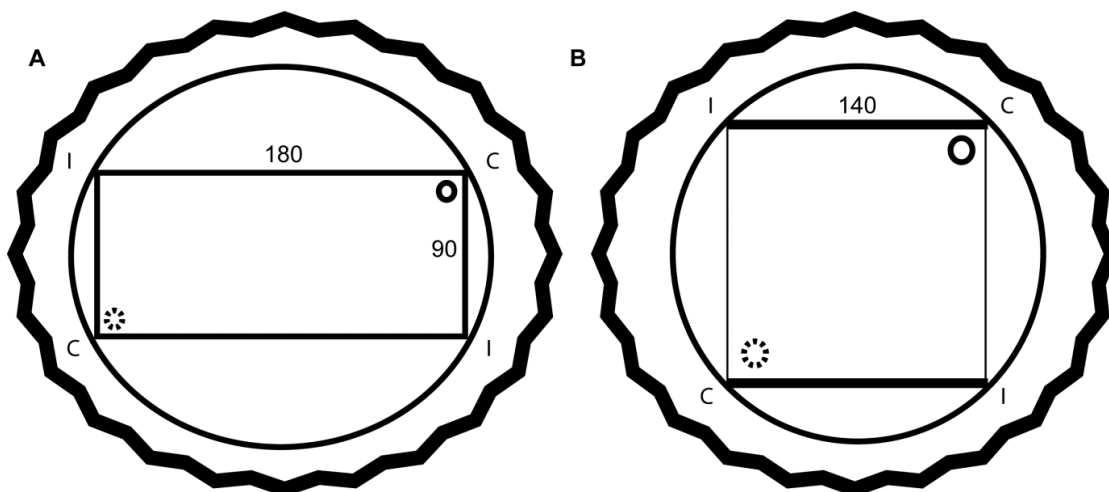
Figure 6.1. Schematic diagramme of the place water maze tasks in **A**) rectangle and **B**) black-white walls square. The large circle represents the watermaze, and the lines inside are the four walls creating the rectangular or square shaped environments. The darker lines in **B** represent the black walls, whereas the thin lines represent the white walls. The small circle represents the platform where a rat would be placed passively. The small dotted circle represents the other identical and, hence, correct corner. The rippled circle represents the curtain used to prevent the use of distal cues. Abbreviations: C, correct corner; I, incorrect corner. Some dimensions (cm) are shown.

A white circular false ceiling (200 cm in diameter) was suspended 175 cm above the floor of the pool. In the centre of the false ceiling was a hole (30 cm in diameter) in which a video camera with a wide-angled lens was situated. The lens of the camera was 25 cm above the hole and was connected to a video monitor and computer equipment in an adjacent room. The rats' movements were analysed using Watermaze software during test trials (Morris & Spooner, 1990). Eight, 45-W lights (22.5 cm in diameter) located in the circular ceiling illuminated the pool. The lights were equidistant from each other in a 160 cm diameter circle whose centre was the same as the centre of the circular ceiling. The training room was also lit by two 153 cm strip lights connected end to end on each of the East and West walls. These lights ran parallel with the floor and were situated 75 cm above the floor. An escape platform (10 cm in diameter) was mounted on a column which rested on the bottom of the pool, and resulted in the platform surface being submerged 2 cm below the surface of the water. The platform, which had a series of concentric ridges on its surface, was used during all training trials. A white curtain, which was attached to the edge of the circular ceiling, was drawn completely around the pool during all training and test trials, so hiding distal room cues. The curtain was 150 cm high and fell 25 cm below the edge of the pool. There was a door (175 cm X 200 cm) in the centre of the South wall connecting the room with the watermaze with the room containing the computer equipment used to monitor the rats' behaviour.

Procedure

The ATNx1 and Sham1 rats completed one session of four training trials each day. For each session they were carried into a room adjacent to the test room in groups of five in a light-tight aluminium carrying box, and they remained in this box between trials. For each trial, the rat was carried from the box to the pool and placed on the platform. The rat was allowed to stay on the platform for 30 s, undisturbed, before being removed, dried and returned to the holding box.

Pre-training

The ATNx1 and Sham1 rats initially underwent three sessions for pre-training in the circular pool. For these sessions the platform was placed in a quadrant (NE,

NW, SW, or SE), with each location used once in a given session. The platform was randomly positioned either 25 cm or 50 cm from the edge of the pool, each for two trials per session. The rats were placed passively on the platform for 30 s (see Procedure above). If the rats fell off the platform, and did not immediately climb back unto the platform, the experiment indicated the location of the platform by tapping on the escape platform. If the rat still failed to return to the platform, the experimenter would guide the rat (they would follow the experimenter's hand through the water) back to the platform where the rat remained for 30 s.

Training

Following the three pre-training sessions, the rats received 12 sessions of training in the rectangle. The platform was positioned 25 cm from a corner on an imaginary line that bisected the corner. The position of the platform was counterbalanced, so that half of the rats from each group had the platform placed in a corner where the short wall was to the right of the long walls (when facing into the corner) and the other half received the platform in the corner where the short wall was to the left of the long wall (when facing into the corner; see Figure 6.1A). Between each trial, the rectangle was randomly rotated 90°, 180°, or 270° clockwise. Four possible orientations were used (North, South, East or West) with each orientation being used once for any given session. Similar to pre-training, the rats were placed on an escape platform. If the rats fell into the pool and failed to climb back onto the platform immediately, the experimenter would remove the rat from the pool and return it to the platform. The experimenter also recorded that the rat had swum.

The first three trials of the final session, Session 12, were conducted in the same manner as previous trials. The fourth trial consisted of a test trial in the rectangle in the absence of the platform. Rats were released into the water from the centre of the pool, facing away from the experimenter, and allowed to swim for 60 s.

Statistical Analysis

Circular search zones in each of the four corners were used to analyse the results from the test trial. Each zone had a diameter of 30 cm with its centre positioned 25 cm from a corner on a line that bisected the corner. The percentage of time spent in the correct zones (i.e., the corner where the platform was located during training, and its geometrically equivalent and diametrically opposite corner – hence, also correct) and incorrect zones (the remaining two corners) of the rectangular pool were analysed using one between-subject factor (Group) by one within-subject factor (Corner: correct; incorrect) ANOVA. Additionally, the first corner the rats approached was recorded (i.e., correct or incorrect corner) as well as the time it took the rats to first swim to one of the two possible correct corners were analysed using the appropriate parametric and non-parametric tests (e.g., t-test, binomial, Fisher's Exact Probability). In addition, the mean swim speed (cm/s) and the mean distance travelled (cm) were examined using t-tests as differences between the ATNx1 and Sham1 groups on these measures could account for any group differences on the latencies to the zones (i.e., if one group swims more slowly, they will arrive at the correct zone later than the other group). The non-parametric Mann-Whitney test was also used instead of a t-test when the data violated the assumptions of parametric tests (e.g., normality).

Experiment 6.2A: Black-white passive placement task

The aim of this experiment was to examine whether rats with lesions to the anterior thalamic nuclei could learn the correct locations that depended on the different arrangement of black-white walls.

Apparatus and room

The rats were tested in a different watermaze and room to Experiment 6.1. A white, circular pool measuring 200 cm in diameter and 60 cm deep (i.e., same as Experiment 6.1) was located 60 cm above the floor in the centre of a room (430 cm X 400 cm X 240 cm). The pool was filled with water to a depth of 30 cm and was maintained at a temperature of 24°C (± 2°C). As in Experiment 6.1, the water was made opaque by adding 0.5 L of white opacifier (Opulyn 303B, Dow, USA; Cat No.

10318500), and changed daily. Throughout the experiment, rats were trained in a square-shaped pool constructed from two white Perspex boards (140 cm long, 50 cm high, and 2 mm thick) and two black Perspex boards (140 cm long, 53 cm high, and 2 mm thick). Each board was placed vertically in the pool and suspended by bars that extended over the edge of the pool, and alternated between black and white walls. This configuration created two pairs of different corners: 1) where the black wall was to the left of the white, and 2) where the white wall was to the left of the black (Figure 6.1B).

As previously described in Experiment 6.1, a white circular false ceiling (200 cm in diameter) was suspended 160 cm above the floor of the pool with lights to illuminate the pool, and a camera to monitor and analyse the rats' movements. Again, the camera was connected to a computer and a video monitor in an adjacent room. The escape platform was identical to the one previously described in Experiment 6.1. However, during the first two sessions of pre-training, a beacon (i.e., a landmark) was attached to the platform. The beacon was a stick (15 cm high and a 4 cm diameter) with alternating black and white stripes (2 cm). A white curtain, which was attached to the edge of the circular ceiling, was drawn completely around the pool during all training and test trials hiding distal cues.

Procedure

Rats completed one session of four training trials each day. For each session they were carried into a room adjacent to the test room in groups of four or five in a light-tight aluminium carrying box, and they remained in this box between trials.

Pre-training

The pre-training in Experiment 6.2A (present study) differed significantly from that used for Experiment 6.1. The rationale was to use the pre-training procedure to assess baseline measures in swimming, orientating, and motivation to escape onto the platform. One potential concern is that the groups might differ because the sham group may be better swimmers or more motivated compared with animals with anterior thalamic nuclei lesions. To examine this possibility, the

animals were given limited prior experience swimming to a submerged escape platform in the circular pool.

All rats (ATNx2 and Sham2) initially underwent four sessions for pre-training in the circular pool. For these sessions the platform was placed in a quadrant (W, E, S, N, NE, NW, SW, or SE), with each location used twice throughout the four sessions, but not within the same session. For pre-training, the platform was randomly positioned either 20 cm or 40 cm from the edge of the pool, each for two trials per session. The rats were also randomly released from a start position (W, E, S, N, NE, NW, SW, or SE), with each location used twice throughout the four sessions, but not within the same session. The rats were required to swim to the platform. For the first two sessions, the platform had a beacon was attached. For Session 3 and 4, the platform had no beacon, and no cues were available to the rat. The rats had a maximum of 120 s to find the platform on the first three sessions, and 90 s during Session 4. If the rats successfully found the platform, they remained on the platform for 30 s before being returned to the carrying box in the adjacent room. However, if the rats did not find the platform, the experimenter showed the rat the location of the platform by tapping gently on it, in the first instance, or guiding the rat (they would follow the experimenter's hand through the water) to the platform where the rat remained for 30 s. This change in pre-training procedure from Experiment 6.1 allows baseline swimming and possible motivation differences (latency to find platform, swim paths, swim speeds) to be examined.

Training

Following the three pre-training sessions, the rats received 12 sessions each with four training trials in the square watermaze (note, it was circular for pre-training). The platform was positioned 25 cm from a corner on an imaginary line that bisected the corner. The position of the platform was counterbalanced, so that half of the rats from each group had the platform placed in a corner where the black wall was to the left of the white wall (when facing the corner) and the other half received the platform in the corner where the white wall was to the left of the black wall (when facing the corner). Between each trial, the square was randomly

rotated 90°, 180°, or 270° clockwise. Four possible orientations were used (North, South, East or West) with each orientation being used once for any given session. As in Experiment 6.1, the rats were placed on the platform for 30s, facing the corner, before being returned to the metal carrying box.

The first three trials of the final session, Session 12, were conducted in the same manner as previous trials. The fourth trial consisted of a test trial in the square in the absence of the platform. Rats were released into the water from the centre of the pool and allowed to swim for 60 s.

Statistical Analysis

The statistical analysis of the 60 s probe was identical to Experiment 6.1. The latency to find the platform during pre-training was also analysed. The mean of all four trials for each session (i.e., Sessions 1 and 2 with the beacon; Session 3 and 4 without the beacon) for the ATNx2 and Sham2 groups were compared using a mixed model ANOVA. The swim speeds (cm/s) of the ATNx2 and Sham2 groups were also examined as difference in swim speeds between the two groups could explain any differences in latencies. The mean distance travelled (cm) was also examined.

Experiment 6.2B: Active black-white place learning task

The goal of this experiment was to observe whether rats with anterior thalamic nuclei lesions could solve the black-white place learning task (Experiment 6.2A) when allowed to actively navigate to the goal location during training.

Apparatus and room

The same room and apparatus was used as Experiment 6.2A. The only difference is that two identical escape platforms (10 cm diameter) were used because there were two correct corners (i.e., two goal locations).

Procedure

The procedure was the same as Experiment 6.2A, with the exception that the rat was released from one of four locations (N, E, W, S) and was given 60 s to swim to

one of the two platforms located in the correct corners (i.e., the same black-white configuration, black-white or white-black, that they had previously experience during Experiment 6.2A). Each of the four start locations was used once per session. At the end of 60 s, if the rat had not located the platform, the experimenter indicated the location of the platform to the rat in the same way as during pre-training (Experiment 6.2A). Having climbed on the platform, the rat remained there for 30 s before being returned to the metal carrying box. The rats received a total of 7 sessions.

On the final trial of Session 7, the rats were given probe (test) trial. The rats were placed in the centre of the pool and allowed to swim for 60 s in the square pool without any platforms being placed in the pool. The time spent in the correct and incorrect corners, the time it took the rat to swim to the correct corner, and whether the first corner visited was correct or incorrect were recorded.

Statistical Analysis

The statistical analysis of the 60 s probe was identical to Experiment 6.1 and 6.2A. The mean latency to find the platform across the four trials of the first six sessions (i.e., active acquisition) was also analysed.

Results

Histology

The histology has been previously reported in Chapter 2 (ATNx1) and 5 (ATNx2). Three and four animals were excluded from all analyses in the ATNx1 and ATNx2 groups, respectively. Therefore, a total of 12 and 10 cases remained in the ATNx1 and ATNx2 groups, respectively.

Behavioural Testing

### Experiment 6.1: Geometrical passive placement task

Pre-training

Because the animals in Experiment 6.1 were placed passively on a platform during pre-training, no baseline measures in swimming (e.g., latency to find platform, mean distance travelled) were recorded. However, the mean distance travelled and mean swim speeds of the two groups were recorded and examined during the 60 s probe (test).

Test (probe)

Rats were placed passively on a platform located in a corner of a rectangular shape pool (see Methods above). Three Sham1 rats each swam once for a single trial during training (although, one rat only swam in a circle near the platform in an attempt to climb back on it). Inspection of the data did not indicate that this one trial where some swimming occurred influenced the performance of the rats. As a result, these three Sham1 rats were not excluded from the following analyses.

During the final test trial, the rats were placed in the centre of the rectangular pool and allowed to swim for 60 seconds. Examples of the swim paths for a Sham1 and ATNx1 rat during the one minute test trial (i.e., without the presence of the platform) are shown in Figure 6.2. Figure 6.3 displays the percent time the Sham1 and ATNx1 rats spent in the two correct corners (where the platform has been located, and its geometrically identical corner) and the incorrect corner. A two-way mixed model ANOVA (Group x Corner) yielded a significant Group x Corner interaction ($F_{(1, 20)} = 8.21$, $p = 0.01$). Examination of the simple effects indicated that the Sham1 group spent significantly more time in the correct compared with incorrect corners ($F_{(1, 20)} = 16.3$, $p = 0.001$), whereas the ATNx1 group did not ($p > 0.1$). Furthermore, the Sham1 group spent significantly more time in the correct zones (corners) compared with the ATNx1 animals ($F_{(1, 40)} = 6.14$, $p = 0.018$), but the groups did not differ significantly in the percent time

spent in the incorrect corner ($F_{(1, 40)}$ = 2.89, p = 0.098). There was also a significant main effect of Corner ($F_{(1, 20)}$ = 8.06, p = 0.01), but not of Group (p > 0.1).

A

B
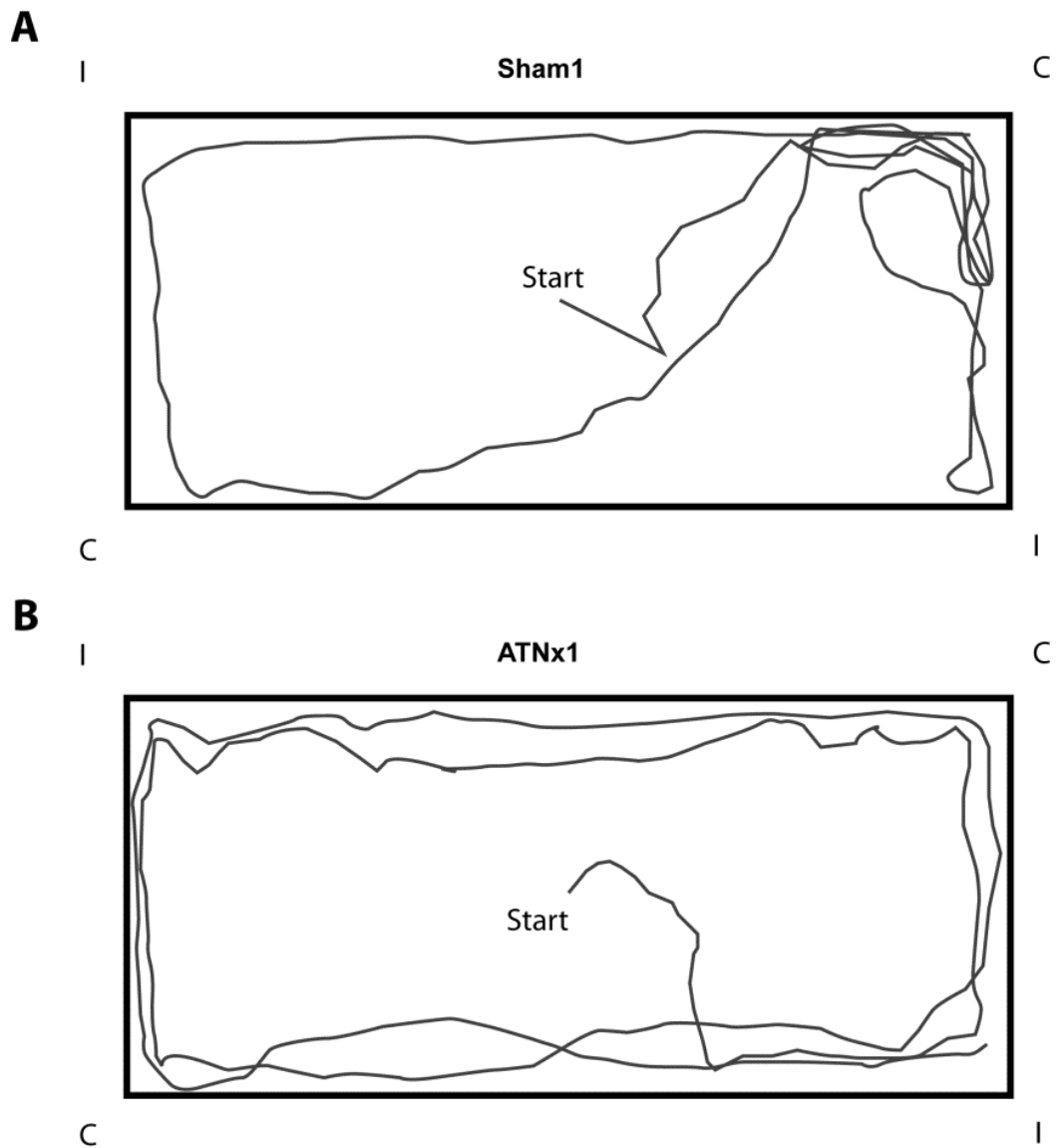
Figure 6.2. Rectangular pool, test trial. Representative swim paths during the 60 second swim test for (**A**) a Sham1 and (**B**) ATNx1 animal. **Note:** at test the platform was removed; C = correct corner; I = incorrect corner.
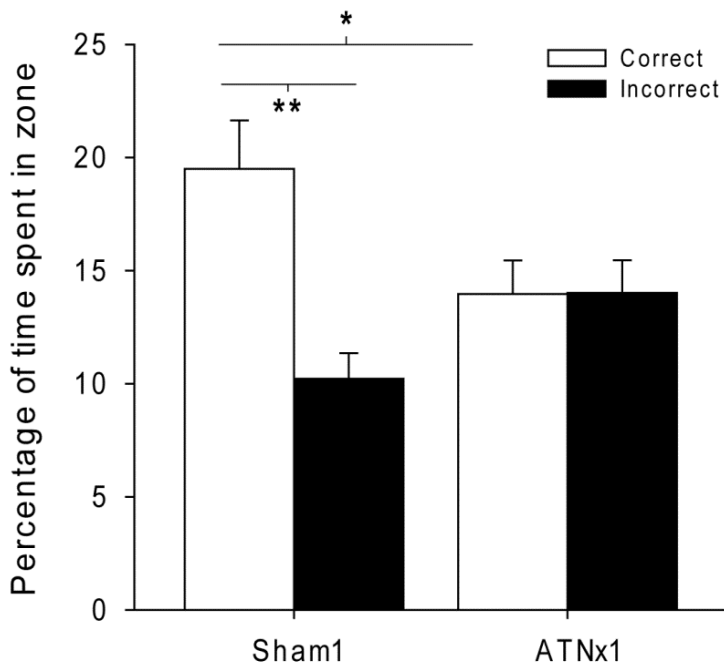
Figure 6.3. Rectangular pool, test trial. Percentage of time spent swimming in the correct and incorrect zones (corners) for both the Sham1 and ATNx1 groups. Data shown are group means, and the vertical bars are the standard error of the means (SEM). **Note:** * = p < 0.05; ** = p < 0.01.

The percentage of rats that swam to the correct corner first is shown in Figure 6.4A. Eighty percent of the Sham1 rats swam to the correct corner first, whereas only 50% of the ATNx1 group went to the correct corner first. Binomial tests indicated that the Sham1 group swam to the correct corner first significantly more often than predicted by chance (p = 0.05; one-tailed), whereas the ATNx1 group did not (p > 0.1; one-tailed). The groups, however, did not differ significantly from one another on this measure (Fisher's Exact Probability, p > 0.1).

The latency to reach the correct zone for both the Sham1 and ATNx1 animals is displayed in Figure 6.4B. The mean time for the Sham1 rats to reach the correct corner was 6.8 s, whereas for the ATNx1 group the mean latency was 13.1 s. Because of a violation of normality, a non-parametric test was used to examine whether the latencies to reach the correct zones differed significantly between the groups. The Sham1 group took significantly less time to swim to the correct corner compared with ATNx1 rats (Mann-Whitney U Statistic = 24.5, T = 79.5, p = 0.02).
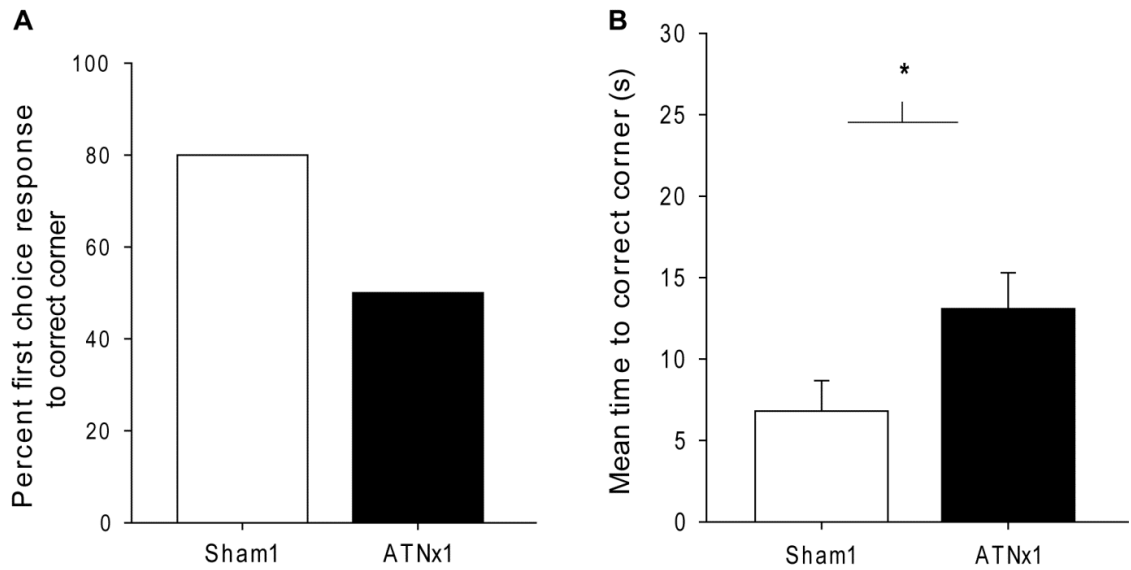
Figure 6.4. Rectangular pool, test trial.   **A**) The percentage of rats that swam to a correct corner (zone) first, and **B**) the time it took rats to first swim to the correct corner (zone). Data shown in **B** are group means, and the vertical bars are the standard error of the means (SEM). **Note**: * = p < 0.05.

Critically, these group difference emerged in the absence of any group differences in the mean distance travelled (cm) and the mean swim speed (cm/s) of the animals (p > 0.1 for both; see Figure 6.5).
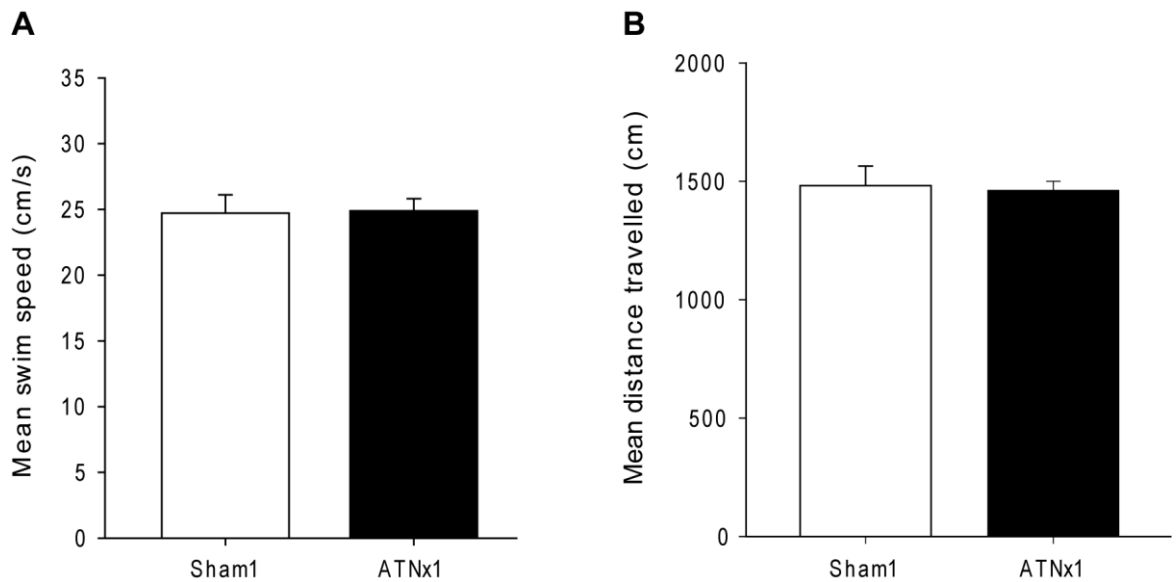
Figure 6.5.   Rectangular pool, test trial.   **A**) The mean swim speed (cm/s) and (**B**) the mean distance travelled (cm) of the Sham1 and ATNx1 groups during the test (probe). Data shown are group means, and the vertical bars are the standard error of the means (SEM).

Experiment 6.2A: Black-white passive placement task

Pre-training

Figure 6.6 shows the mean latency to the platform (A), the mean distance travelled (B), and the mean swim speed (C) for the ATNx2 and the Sham2 rats during pre-training. Statistical analyses revealed a significant main effect of Condition (beacon; no beacon; $p < 0.001$ for all) and Days ($p < 0.001$ for all), but importantly, the groups did not differ from one another on all three measures of baseline swimming activity ($p > 0.1$ for all). The results suggest that both the Sham2 and the ATNx2 swam at a similar speed, and were equally motivated to find the escape platform. There was also a Condition (beacon; no beacon) by Day interaction for the mean swim speed only ($F(1,21) = 26.7$, $p < 0.001$). All other interactions were non-significant [$p > 0.1$ for all with the exception of Group x Condition ($p = 0.075$) and Group x Day ($p = 0.09$) for the mean swim speeds only].
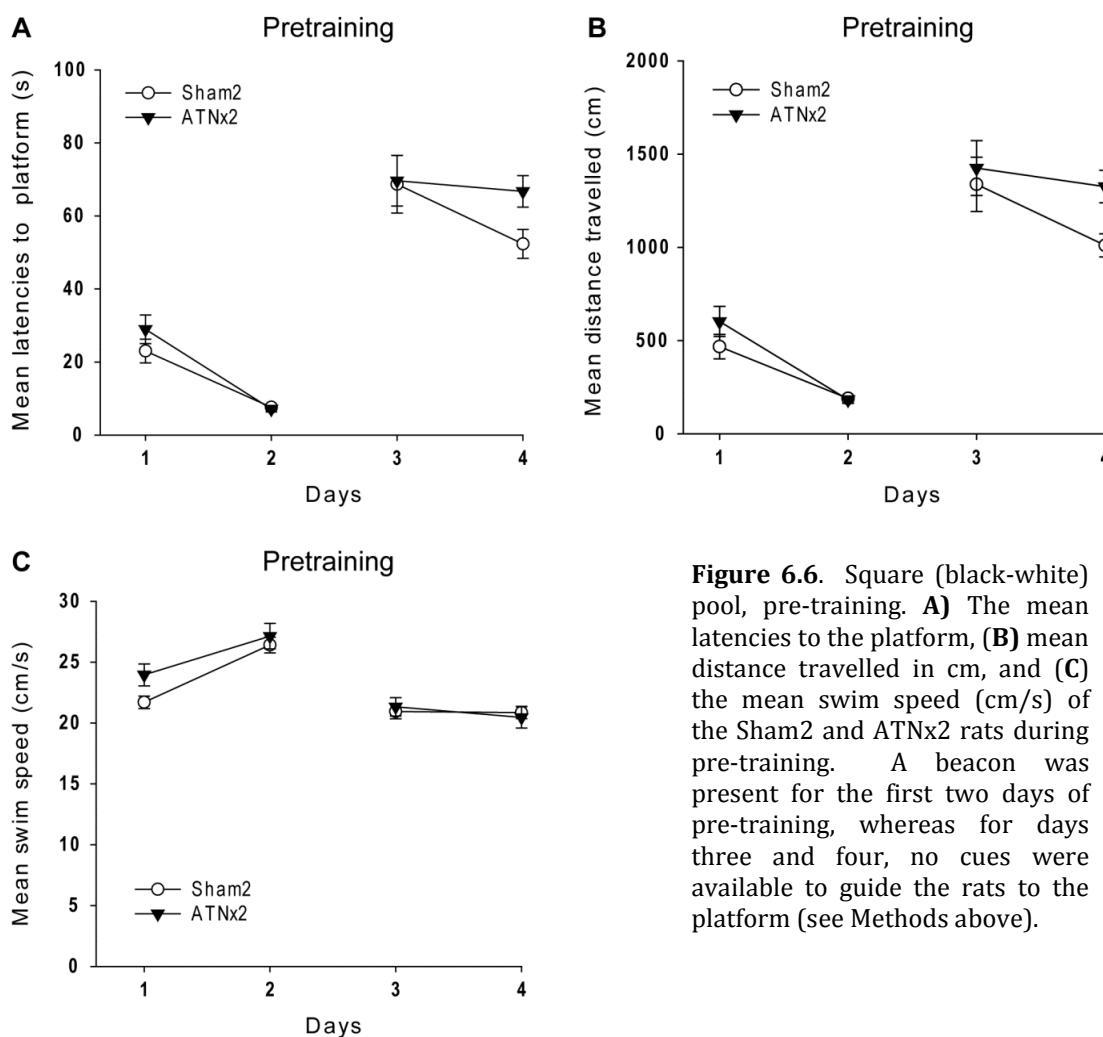
Figure 6.6. Square (black-white) pool, pre-training. **A)** The mean latencies to the platform, **(B)** mean distance travelled in cm, and (**C**) the mean swim speed (cm/s) of the Sham2 and ATNx2 rats during pre-training. A beacon was present for the first two days of pre-training, whereas for days three and four, no cues were available to guide the rats to the platform (see Methods above).

Test (probe)

Similar to Experiment 6.1, rats were placed passively on the platform. However, the platform was located in a corner of a square shaped pool with black and white walls that differed in their arrangement (see Methods above). All of the rats stayed on the platform throughout training (i.e., none of the rats swam). During the final test trial, the rats were placed in the centre of the square pool and allowed to swim for 60 s. Examples of the swim paths for a Sham2 and ATNx2 rat during the one minute test trial (i.e., without the presence of the platform) are shown in Figure 6.7. Figure 6.8 displays the percent time the Sham2 and ATNx2 rats spent in the two correct corners (where the platform was located, and its identical corner) and the incorrect corners. Inspection of the data suggests that the ATNx2 and the Sham2 rats spent a similar amount of time in the incorrect corners (8.81% and 8.62%, respectively), but that the Sham2 rats spent more time in the correct corners compared with the ATNx2 group (ATNx2 group: 18.29%; Sham2 group: 27.47%). Therefore, the results also suggest that the Sham2 group spent more time swimming in all four corners compared with the ATNx2 group. A two-way mixed model ANOVA (Group x Corner) yielded a significant main effect of Group ($F_{(1, 21)}$ = 5.28, p = 0.032) and Corner ($F_{(1, 21)}$ = 21.0, p < 0.001). The Group x Corner interaction was not significant (p > 0.1).
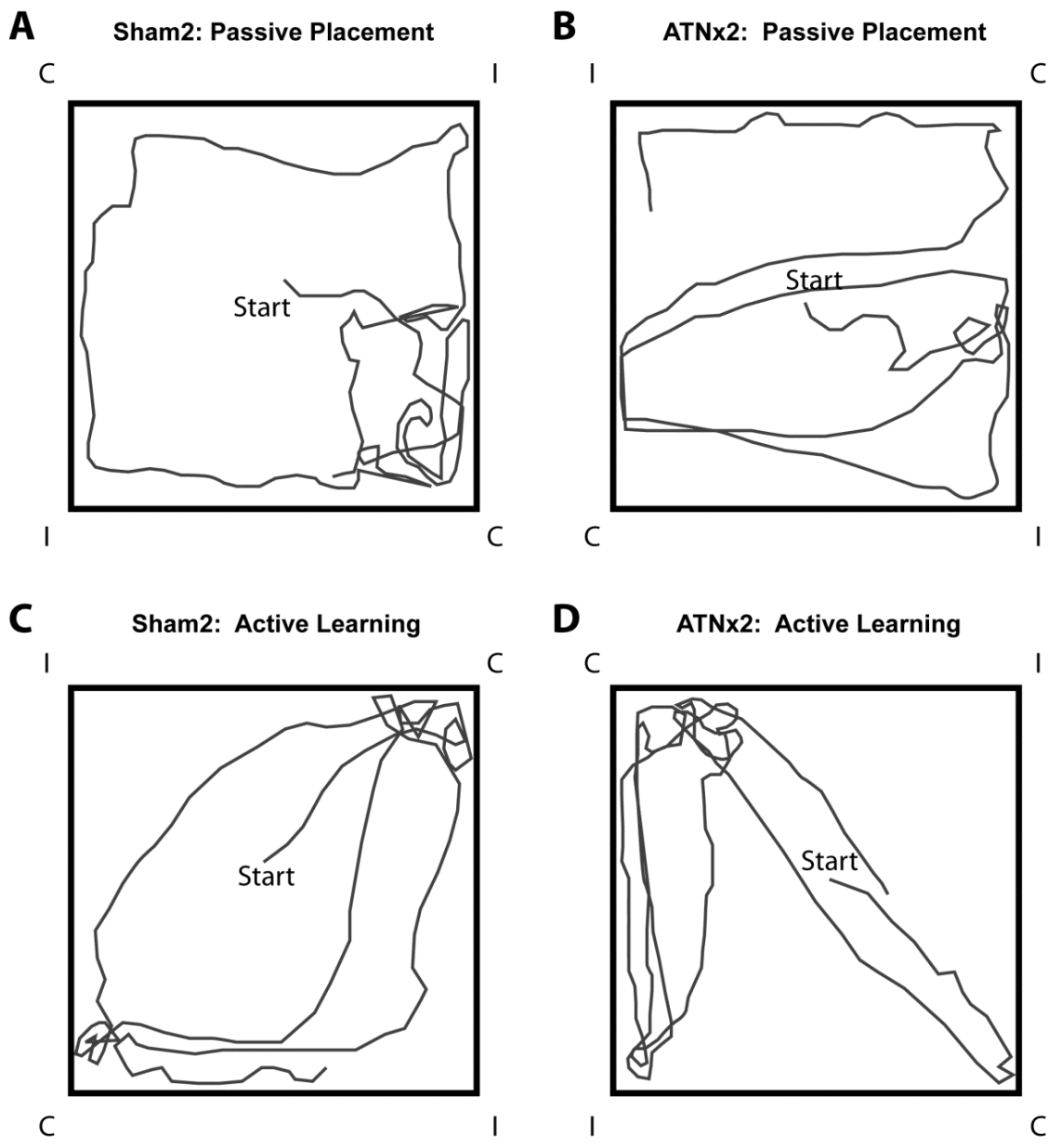
Figure 6.7. Square (black-white) pool, test trial. Representative swim paths during the 60 s swim test following passive training (Top: Exp 6.2A; **A,B**) and after active training (Bottom: Exp 6.2B; **C, D**) for a Sham2 (Left: **A, C**) and ATNx2 (Right: **B, D**) animal. The same Sham2 and the same ATNx2 rats swim paths are shown for comparison. **Note:** At test the platform was removed; C = correct corner; I = incorrect corner.
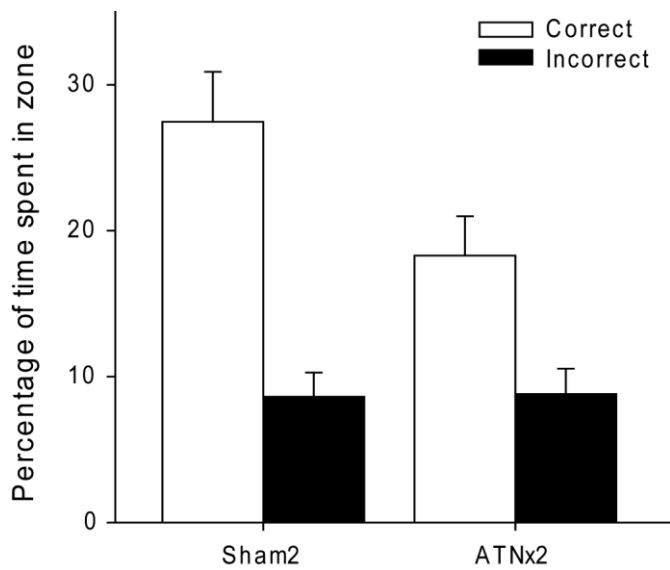
Figure 6.8. Square pool, test trial. Percentage of time spent swimming in the correct and incorrect zones (corners) for both the Sham2 and ATNx2 groups during the 60 s swim test following passive placement learning of the arrangement of the black and white walls. Data shown are group means, and the vertical bars are the standard error of the means (SEM).

The mean swim speed and the mean distance travelled for the ATNx2 and the Sham2 groups are displayed in Figure 6.9. The groups did not differ significantly on either measure (p > 0.1 for both).
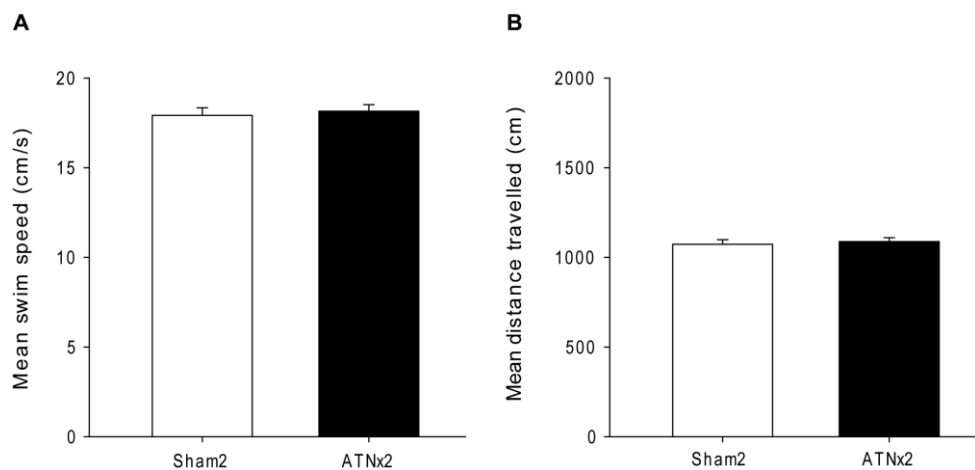
Figure 6.9. Square pool, test trial. **A**) The mean swim speed (cm/s) and (**B**) the mean distance travelled (cm) of the Sham2 and ATNx2 groups during the test (probe). Data shown are group means, and the vertical bars are the standard error of the means (SEM).

Because the total percentage of time in all corners between the Sham2 and the ATNx2 groups differed, and this difference in total percentage of time spent in corners could not be explained by overall difference between the groups' swim speeds and mean distances travelled (see Figure 6.9), the data were re-examined using a discrimination ratio, which allows for the difference between the correct and incorrect corner to be examined while total percentage of time spent in corners is held constant. The ratio score was obtained by taking the percentage of time spent in the correct corner and dividing it by the total time spent in any

corner (i.e., percentage of time in correct corner / the percentage of time in the incorrect + correct corners). The discrimination ratio yields a score between 0 and 1, where chance performance (i.e., spending an equal amount of time in the correct and incorrect corners) is 0.5. Figure 6.10 shows the discrimination ratio for the ATNx2 and the Sham2 groups. Both groups appear to be spending more time in the correct corner (i.e., above 0.5). This observation was confirmed with one-sample t-tests indicating that both groups were significantly above chance (Sham2: $t_{12} = 4.81$, $p < 0.001$; ATNx2: $t_9 = 2.32$, $p = 0.045$). In addition, a between-sample t-test revealed that the groups did not differ significantly from one another ($p > 0.1$).

Figure 6.10. Square pool, test trial. Discrimination ratio [percentage of time spent swimming in the correct zone divided by the total percentage of time spent in the correct and incorrect zones (corners)] for both the Sham2 and ATNx2 groups during the 60 s swim test following passive placement learning of the black and white walls. Data shown are group means, and 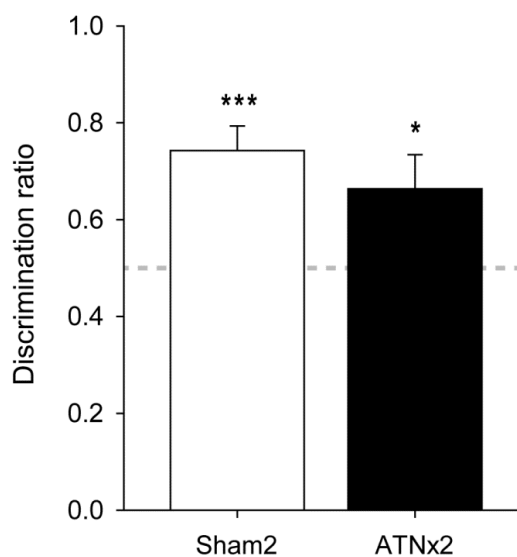the vertical bars are the standard error of the means (SEM). **Note:** grey dashed line = chance; * = p <0.05; *** = p <

Figure 6.11A shows the percentage of rats that swam the correct corner (zone) first. Twelve of the thirteen (92%) of the Sham2 group swam to the correct corner first, whereas only five of the 10 (50%) of the ATNx2 rats did. Binomial tests revealed that the Sham2 group swam to the correct zone significantly more often than predicted by chance ($p = 0.015$; one-tailed), whereas the ATNx1 group did not ($p > 0.1$; one-tailed). There was a marginal difference between the two groups (Fisher's Exact Probability, $p = 0.052$; two-tailed).

The time for the rats to swim to the correct zone is shown in Figure 6.11B. The mean time for the Sham2 rats to reach the correct corner was 12.7 s, whereas for the ATNx2 group the mean latency was 26.2 s. The ATNx2 group took significantly longer to swim to the correct corner compared with the Sham2 group ($t_{21} = 2.82$, $p = 0.01$).
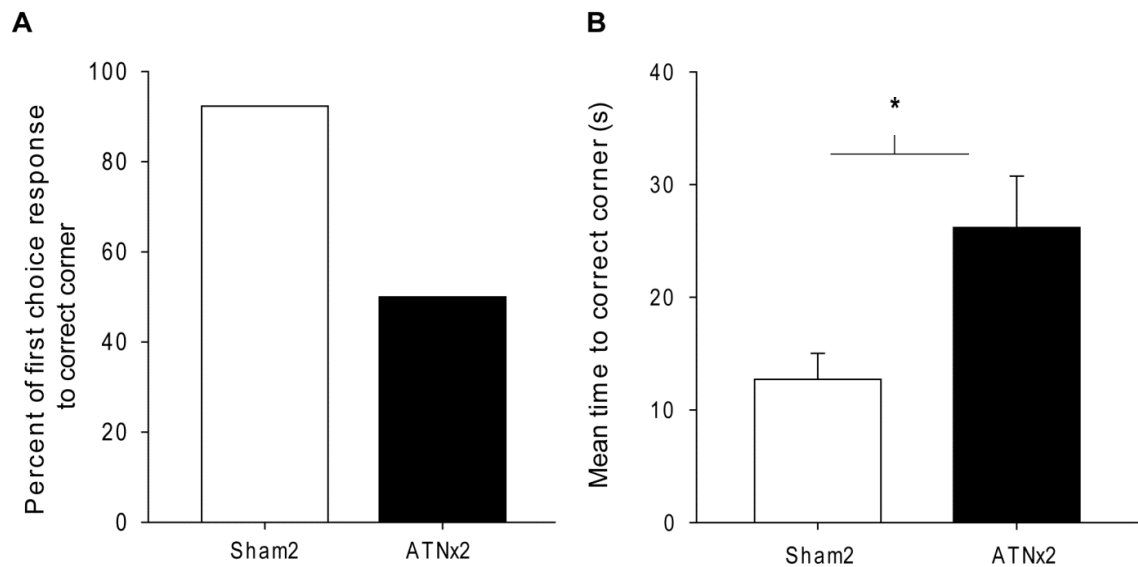
Figure 6.11. Square pool, test trial. **A**) The percent of rats in the Sham2 and ATNx2 group that swam to the correct corner first. **B**) The mean time to the correct corner for the Sham2 and ATNx2 groups. Data shown in **B** are group means, and the vertical bars are the standard error of the means (SEM). **Note**: * = p < 0.05.

Experiment 6.2B: Active black-white place learning task

Acquisition

Figure 6.12 shows the mean latencies to the escape platform by the Sham2 and the ATNx2 groups during the six acquisition days where the rats were allowed to swim actively to the platform. Both the Sham2 and the ATNx2 significantly improved as indicated by a decrease in the mean latencies over days ($F_{(5, 105)}$ = 26.3, p < 0.001). There was also a borderline effect of group ($F_{(1, 21)}$ = 4.21, p = 0.053). Inspection of the figure indicates that the ATNx2 group were slower compared to the Sham2 group on Day 1 (i.e., positive transfer), and that this difference disappeared by Day 6. However, the Group x Days interaction was not significant (p > 0.1).

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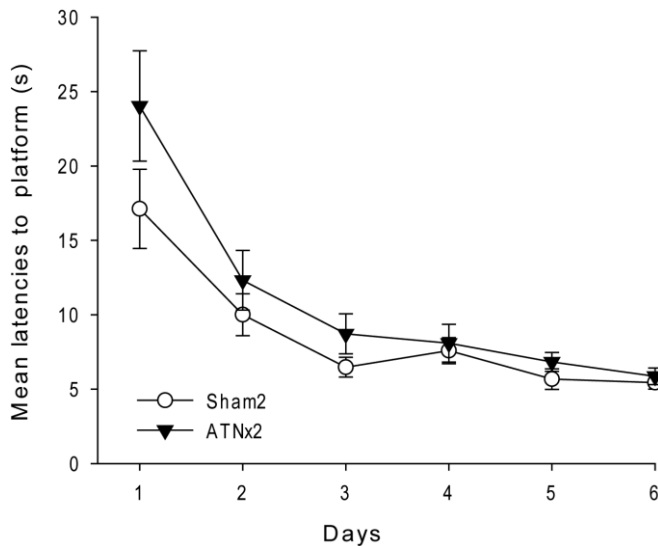
Figure 6.12. Square pool, active acquisition. The mean latencies to one of two platforms of the Sham2 and ATNx2 groups during the acquisition phase of the active colour task. Data shown are group means, and the vertical bars are the standard error of the means (SEM).

Test (probe)

During the 60 s test (i.e., in the absence of the two platforms), the rats were released in the centre of the square maze. Figure 6.7 (C, D) shows the swim paths during the 60 s test (probe) of the same Sham2 and ATNx2 rats from Experiment 6.2A. The percentage of time spent in the correct and incorrect zones of the Sham2 and ATNx2 groups are shown in Figure 6.13. The Sham2 animals spent 46.32% and 3.51% of their time in the correct and incorrect zones, respectively; whereas the ATNx2 group spent 39.19% and 2.94% of their time in the correct and incorrect zones, respectively. These results yielded a borderline main effect of Group ($F_{(1, 21)} = 4.25$, $p = 0.052$) and of Corner ($F_{(1, 21)} = 321.8$, $p < 0.001$), but no Group x Corner interaction ($p > 0.1$).
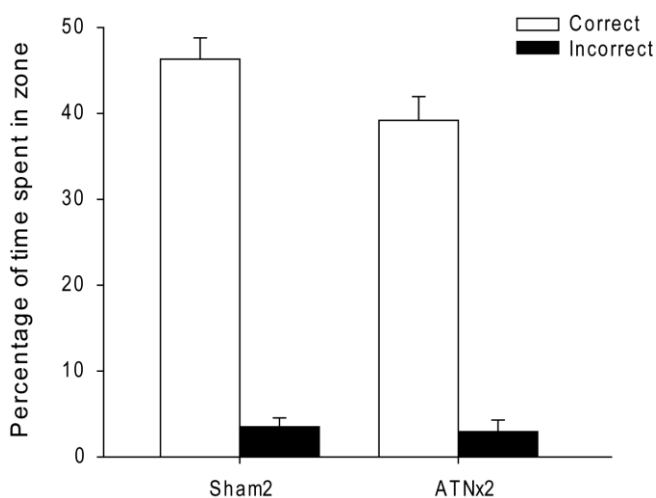
Figure 6.13. Square pool, test trial. Percentage of time spent swimming in the correct and incorrect zones (corners) for both the Sham2 and ATNx2 groups during the 60 swim test following active swimming. Data shown are group means, and the vertical bars are the standard error of the means (SEM).

The percentage of rats that swam to the correct corner first is displayed in Figure 6.14A. In both groups, 100% of the rats swam to the correct zone. Binomial tests indicated that both groups swam to the correct corner more often than predicted by chance (ATNx2: $p = 0.001$; Sham2: $p < 0.001$; one-tailed for both). The groups did not differ from each other (Fisher's Exact Probability; $p = 1.00$).
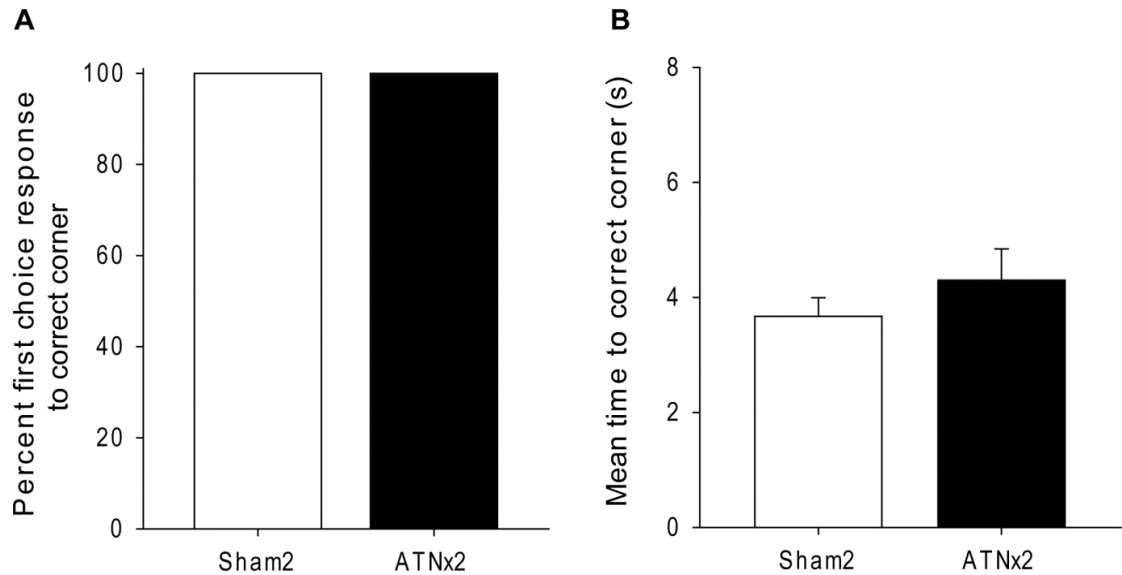

Figure 6.14. Square pool, test trial. **A**) The percent of rats in the Sham2 and ATNx2 group that swam to the correct corner first. **B**) The mean time to the correct corner for the Sham2 and ATNx2 groups. Data shown in **B** are group means, and the vertical bars are the standard error of the means (SEM).

Figure 6.14B shows the mean time to the correct corner of the Sham2 and ATNx2 rats. The Sham2 rats took 3.7 s and the ATNx2 group took 4.1 s to swim to the correct corner. The groups did not differ significantly ($p > 0.1$). Additionally, there was no significant difference between the Sham2 and the ATNx2 groups on the mean swim speeds and on the mean distance travelled ($p > 0.1$; Figure 6.15).
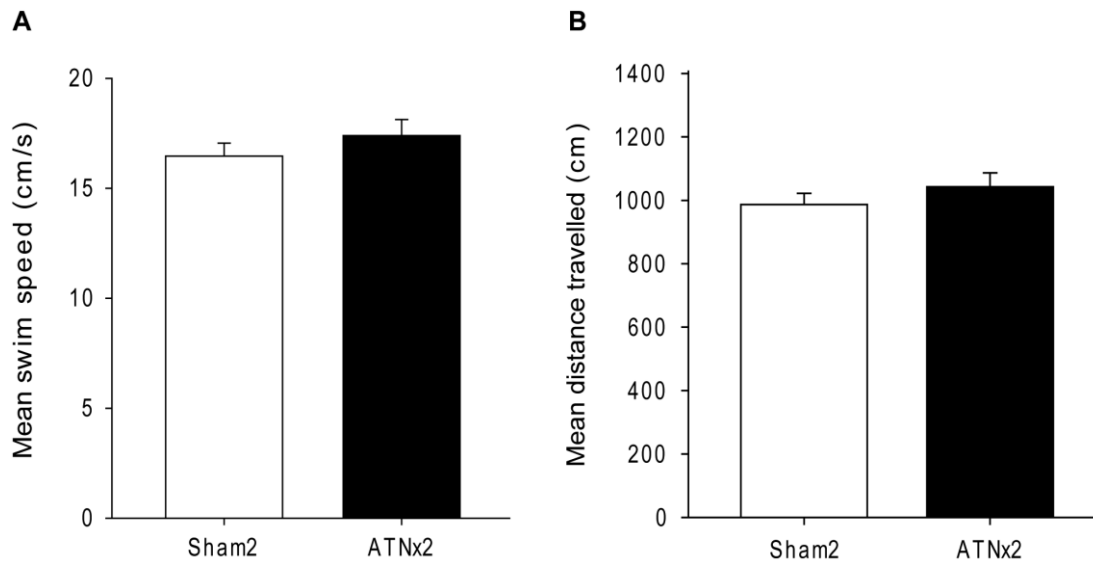
Figure 6.15. Square pool, test trial. **A**) The mean swim speed (cm/s) and (**B**) the mean distance travelled (cm) of the Sham2 and ATNx2 groups during the test (probe). Data shown are group means, and the vertical bars are the standard error of the means (SEM).

Discussion

This chapter examined whether rats with damage to the anterior thalamic nuclei could learn spatial locations using only either the geometrical properties of the environment (long and short walls), or by using the different appearance of the walls (black-white vs. white-black). In Experiment 6.1, the rats were passively placed on a platform located in a corner of a rectangular watermaze (i.e., the correct corner). On the final trial, the rats were given a test (probe), where the rats were placed in the centre of the watermaze and allowed to swim for 60 s. Despite the ATNx1 rats spending a similar total percentage of time in the corners as the Sham1 group, the ATNx1 group failed to distinguish the correct from the incorrect corners. In contrast, the Sham1 rats spent significantly more time in the correct compared with incorrect corners. In addition, the Sham1 rats spent significantly more time in the correct corner compared with the ATNx1 group. The mean latency to the correct corner and the percentage of rats that swam to the correct corner first (instead of the incorrect corner) also demonstrated that the ATNx1 group were impaired compared with the Sham1 rats.

These results are consistent with a previous study that found that rats with damage to the anterior thalamic nuclei were impaired on learning the geometrical properties of the same rectangular watermaze (Aggleton et al., 2009). The rats

were allowed to swim to platforms located in two of four corners of rectangular shaped pool; the two correct and geometrically identical corners (e.g., corners where the short wall is to the left of the long wall). Although the rats with lesions to the anterior thalamic nuclei took less time to swim to the platform as training progressed, they spent an equal amount of time in the correct and incorrect corners during the 60 s test (probe). The results suggest that while the rats with damage to the anterior thalamic nuclei probably did learn that the goal location was in a corner, they had difficulty distinguishing between the geometrically different corners (Aggleton et al., 2009).

In addition, the ability of rats with lesions to the anterior thalamic nuclei to learn a location based on the spatial disposition of different walls (black-white, white-black) was examined. When the ATNx2 rats were trained passively, they were significantly impaired compared with the Sham2 group during the 60 s probe: The ATNx2 rats took significantly longer to swim to the correct corner, and were less likely to swim to the correct corner first compared with Sham2 rats. Although the ATNx2 rats were impaired compared with the Sham2 rats on several behavioural measure analysed, the ATNx2 rats did spend significantly more time in the correct corners compared with the incorrect corners, suggesting that they could discriminate between the corners that differed in the arrangement of the black and white walls. Further examining the percentage of time spent in the correct and incorrect corners as a discrimination ratio did not find that the groups differed significantly.

The same rats were then trained actively (i.e., they were required to swim to the platform located in the correct corner). The ATNx2 rats took longer to swim to the platform on the first day of training compared with the Sham2 group, most likely reflecting better performance of the Sham2 rats on the previously learnt passive placement task (i.e., positive transfer effect). By the end of training, the groups took the same amount of time to reach the platforms. The ATNx2 rats learnt the location of the correct corner: there were no group differences on any of the measures taken during the test (probe) trial.

The results suggest that damage to the anterior thalamic nuclei impairs learning of the geometrical structure of an environment, regardless of whether the animals experienced the environment passively or actively prior to the 60 s tests (Experiment 6.1; Aggleton et al, 2009). In contrast, relatively milder deficits were observed when the ATNx2 rats were required to learn a spatial location using the arrangement of different black and white walls (Experiment 6.2A and 6.2B). One possibility is that loss of the anterior thalamic nuclei impairs learning of distances (i.e., different length walls). It is also possible that the different patterned (black, white) walls created more salient and more distinct corners compared with the walls that differed in length (Pearce, Graham, Good, Jones, & McGregor, 2006). Additionally, in the geometrical maze, the animal must consider the entire length of the wall to determine which corner it is now facing, whereas in the patterned wall maze, the rat only has to face the corner, i.e., the latter task can be solved with more local cues.

The results also suggest that when the correct corner (black-white or white-black) defined by the arrangement of different black-white walls was experienced passively, ATNx2 rats were impaired; whereas the ATNx2 group were unimpaired compared with the Sham2 group when the animals received active training prior to the 60 s test. There are several explanations for the improved performance of the ATNx2 rats following active training. One possibility is that the ATNx2 rats had difficulty changing perspectives: when placed passively the rats always viewed the environment from the same location, whereas at test, the rats are placed in the centre of the maze and viewed the environment from a novel perspective. Active training would have provided the animals with several different views of the correct corner as they approached the platform from different start locations.

Another possibility is that the ATNx2 improved following active training by using different strategies not available during passive training. For instance, it remains possible that damage to the anterior thalamic nuclei impairs the ability of rats to form map-like representations of their environment (e.g., Tolman, 1948; O'Keefe & Nadel, 1978), but does not impair the ability of animals to use other response strategies that include navigating by using heading vectors and a single (or subset) of landmarks (Cartwright & Collett, 1983; McNaughton et al., 1991;

Blair et al., 1997); although others have found that anterior thalamic lesions impair the ability of rats to orientate appropriately to a landmark (Wilton et al., 2001). A related possibility is that the rats could have formed a stimulus-response strategy (e.g., always swim with the black wall along the left side of the body; go to the right when facing the black wall), which is likely to engage other memory systems (Packard & McGaugh, 1992; McDonald & White, 1993; Packard & McGaugh, 1996). This latter possibility appears somewhat unlikely, given that rats with damage to the anterior thalamic nuclei remained impaired on the geometrical watermaze when presumably similar strategies were available to the rats (Aggleton et al., 2009). However, one major difference between the black-white and the geometrical place learning tasks is that if the rats are learning the goal location based on single landmarks, the black (or white) walls provide unambiguous landmarks. In contrast, the "long" walls of the geometrical place learning tasks are only long relative to the shorter walls. Unlike the black (or white) walls, when an animal swims towards a wall, it does not have immediate knowledge of whether it is a long or a short wall. It also remains possible that the improved performance of the ATNx2 rats following active training is a result of the experimental order. By necessity all rats were trained passively first, and then received active training. To avoid the effects of this extended learning regime, different cohorts of rats would be required.

In sum, this chapter provides evidence that the spatial deficits observed following anterior thalamic damage in complex spatial environments, as well as during complex spatial learning tasks, are also evident in simple environments where place learning depends only on either 1) geometrical properties or 2) the arrangement of black and white walls. Furthermore, actively experiencing an environment (i.e., navigating through the environment) compared with passive exploration may reduce (or eliminate) the spatial deficits observed following anterior thalamic damage.

Chapter 7

General Discussion

The main objective of this thesis was to clarify the contributions of the anterior thalamic nuclei to episodic memory and to the amnesic syndrome caused by damage to the diencephalon (e.g., Harding et al., 2000; Gold & Squire, 2006). One limitation is that the original definition of episodic memory involved introspection, conscious recollection that allows for mental time travel, and so may be thought to be unique to humans (Tulving, 1983). Consequently, research has focused on episodic-like memory, which involves examining whether animals can learn what happened when or where (i.e., the formation of *what-where*, *what-when*, or even *what-when-where* associations; Clayton et al., 2003; Eacott et al., 2005; Iordanova et al., 2008). Likewise, research into animal models of amnesia has typically examined the role of its constituent aspects: *what*, *when*, and *where* (e.g., Clayton & Dickinson, 1998; Aggleton & Pearce, 2001). This thesis examined the role of the anterior thalamic nuclei for each component of episodic memory individually as well as the formation *what-where* associations in rats (see Table 7.1 for summary of the main results from this thesis).

Table 7.1. Summary of the experimental results found in this thesis relating to different components of episodic memory (left column): *what, when, where,* and *what-where.* The table indicates the experimental number, and title of the experiment, as well as which cohort of rats with anterior thalamic nuclei lesions were tested. The furthest column on the right indicates whether the lesion group was impaired relative to control group.

Component	Experiment	Title	Group	Impaired
What	2.2 (&2.4)	Object recognition (1 & 60 min delay)	ATNx1	no
What	2.3	Object recognition (15 min delay)	ATNx1	no
What	2. 6 (& 2.7)	Odour recognition (1 & 60 min delay)	ATNx1	no (yes, 1min)
What	3.1	Object recognition and IEG *zif268* activity	ATNx	no modulation of lesion on *zif268* activity
What	4.2A	Simple discrimination	ATNx1	no
When	2.4	Between-block with objects	ATNx1	no
When	2.5	Within-block with objects	ATNx1	yes
When	2.7	Between-block with odours	ATNx1	no
When	2.8	Within-block with odours	ATNx1	no
Where (When?)	2.1 & 5.1	T-maze alternation	ATNx1 & ATNx2	yes
Where	5.2A	Bidirectional spatial go/no-go	ATNx2	yes
Where	5.2B	Unidirectional spatial go/no-go	ATNx2	yes
Where	6.1	Geometrical passive placement	ATNx1	yes
Where	6.2A	Black-white passive placement	ATNx2	yes
Where	6.2B	Black-white active place learning	ATNx2	no
What-where	4.1	Contextual conditional discriminations	ATNx1	no
What-where	4.2B	Item-context + place biconditional	ATNx1	yes
What-where	4.2D	Bidirectional item-place	ATNx1	yes
What-where	4.2E	Item-context biconditional	ATNx1	no
What-where	5.2C	Unidirectional item-place	ATNx2	yes
Other	2.9	Locomotor activity	ATNx1	yes; hyperactive

Item recognition (*what*)

The studies conducted in this thesis have found that anterior thalamic lesions do not impair item recognition (*what*), consistent with previous findings (Aggleton et al., 1995; Warburton & Aggleton, 1999; Wilton et al., 2001; Moran & Dalrymple-Alford, 2003; Mitchell & Dalrymple-Alford, 2005; Wolff et al., 2006; see also Chapter 2, Experiment 2.2, 2.3, and 2.6). In addition, anterior thalamic lesions did not modulate the IEG *zif268* activity in the extended hippocampal system following the presentation of novel objects compared with familiar objects (Chapter 3). These results suggest that the anterior thalamic nuclei are not necessary for item recognition, and that either: 1) these nuclei have a limited role in item recognition in the intact brain, 2) the IEG *zif268* is not a sensitive marker for changes in item recognition, or 3) both. The idea that *zif268* activity is not a sensitive marker for changes in item recognition is supported by studies finding changes in *c-fos* activity but not *zif268* in the perirhinal cortex following the presentation of novel stimuli (Brown & Xiang, 1998; Wan et al., 1999; Aggleton et al., 2012). Another study also failed to find overall difference in *zif268* activity in the hippocampus following training on a spatial memory task in radial arm maze, but found differences in network connectivity between groups of rats depending on the behavioural manipulation when the counts were analysed using structural equation modeling (Poirier, Amin, & Aggleton, 2008).

Furthermore, the rats in Chapter 3 had unilateral anterior thalamic lesions which may have attenuated some differences between the lesion and intact hemispheres as there is evidence of contralateral projections (e.g., van Groen & Wyss, 1992, 1995; Shibata, 1998). This anatomical connectivity also emphasises the need for more studies using a range of IEG markers to examine counts in the anterior thalamic nuclei following item recognition in the intact brain. However, this task is made difficult by the low number of labeled cells within the thalamus using *zif268* and *c-fos*. Thus, additional markers may be needed; although some studies have reported differences in the thalamus using *c-fos* and *zif268* (Vann, Brown, & Aggleton, 2000; Amin et al., 2006; Yasoshima et al., 2007).

The null results for item recognition are consistent with the hypothesis, from dual-processing models, that suggest that item recognition can be solved either through familiarity or recollection, and that different neural regions are involved in these two qualitatively different processes (Aggleton & Brown, 1999, 2006). Aggleton and Brown (1999) suggested that the extended hippocampal memory system is important for recollection ("remember"), but not familiarity ("know"). Because recognition memory tasks can be solved on the basis of familiarity, damage to the extended hippocampal memory system does not impair item recognition. This notion presupposes the existence of other brain sites that are critical for object novelty and familiarity information. One such site is the perirhinal cortex, and research has shown that this region is necessary for object recognition (e.g., Brown & Aggleton, 2001; Buckley, 2005; Albasser, Davies, Futter, & Aggleton, 2009; Aggleton, Albasser et al., 2010; Albasser et al., 2011; Seoane et al., 2012).

The present results from item recognition following anterior thalamic damage in rats are inconsistent with those using monkeys, which found impaired performance on object recognition task (Aggleton & Mishkin, 1983a, b). However, the surgical procedure resulted in damage that extended beyond anterior thalamic nuclei, even when the lesion was centred upon the anterior thalamus (Aggleton & Mishkin, 1983a). Because the lesions were made by using aspiration, there was also additional damage to fibers passing near the thalamus. Given that the monkeys with large lesions to the medial thalamus were more severely impaired compared with those surgeries centred on either the anterior or the posterior portion (i.e., the anterior thalamic nuclei or the medial dorsal thalamic nucleus, respectively), it is possible that combined damage to several diencephalic structures is required to impair item recognition (Aggleton & Mishkin, 1983a, b; see also Aggleton, Dumont et al., 2011). The argument that species different accounts for the differing results is unlikely given evidence from patients that selective damage to the extended hippocampal system impairs recollection, while leaving recognition memory relatively intact (e.g., Carlesimo et al., 2007; Tsivillis et al., 2008; Aggleton, Dumont et al., 2011).

Temporal order memory (*when*)

Chapter 2 found that rats with damage to the anterior thalamic nuclei were impaired on a particular class of temporal order recency task: within-block recency using objects (Chapter 2, Experiment 2.5). In this task the rats were presented with a list of objects, and at test, two objects from the list were presented. Normal rats showed a preference for the less recently presented object, whereas the ATNx1 group explored both objects equally. In contrast, the same ATNx1 rats showed a normal preference for the older object when the two comparison objects were from different lists separated by a delay in which the animal was removed from the test arena (between-block recency; Chapter 2, Experiment 2.4).

Do these results reflect two different temporal processes, or do differences in recency judgments on these tasks simply reflect differences in difficulty with the anterior thalamic lesion rats showing an increased sensitivity to changes in task difficulty? It could be argued that as the delay (or temporal separation) between the two choice items is smaller on the within-block recency task compared with the between-block recency task, the impaired performance of the anterior thalamic lesion group reflects a greater sensitivity to task difficulty. This, however, seems unlikely given that manipulating the delays between the presentation of the two lists as well as the test phase on the between-block recency task, did not affect the performance of the rats with anterior thalamic damage significantly more than the control group. In other words, as task difficulty increased the performance of both groups declined by the same amount during the between-block recency task (Chapter 2, Experiment 2.4). Furthermore, there was no effect of number of interleaving items on the within-block recency task (Chapter 2, Experiment 2.5).

Similarly, there is no evidence to suggest that rats with anterior thalamic lesions are more susceptible to interference, despite evidence that patients with Korsakoff syndrome are more sensitive to interference (Cermak & Butters, 1972). Chapter 2, Experiment 2.1 found that both the sham and the rats with anterior thalamic lesions performed worse on the last trial compared to the first trial of the T-maze alternation task, therefore, the performance of both groups declined as

previous trials influenced the performance of later trials. The effect of interference was not significantly greater in the lesion group. However, this result may have been confounded by both ceiling and floor effects. In addition, there is also evidence that the performance of rats with anterior thalamic damage is better for later trials compared with earlier ones on a delay-non match-to-position task in operant chambers (Aggleton et al., 1991), again suggesting that these animals were not unduly influenced by interference.

Given that there is little evidence to support that anterior thalamic lesions are affected significantly more than control rats by either interference or task difficulty, it remains possible that different processes are involved in between-block compared with within-block recency tasks, and the anterior thalamic nuclei are necessary for those processes needed during within-block recency task. What type of processing does the anterior thalamus do that could be critical for within-block recency, but not between-block recency tasks? One possibility may be that the anterior thalamic nuclei are important for knowledge of a sequence possibly through a temporal tag. There is evidence that theta oscillations may act as a temporal organiser, preventing both spatial and temporal smearing of information by having cells that fire in sequence shift throughout theta rhythm (Buzsaki, 2002, 2005). Given that cells have been found in the anterior ventral thalamic nucleus that oscillate within theta band (Vertes et al., 2001; Tsnaov, Chah, Wright et al., 2011), it remains possible that lesions to that area disrupt theta's role as a temporal organiser for adjacent stimuli. On the face of it this role of the anterior thalamic nuclei for temporal tagging seems unlikely given a previous report that anterior thalamic lesions do not impair explicit tests of sequence learning (Aggleton, Amin et al., 2011), but the sequence learning task used by Aggleton, Amin et al. (2011) required considerable training, so it remains possible that the anterior thalamic nuclei are necessary for rapid sequence learning.

A closely related explanation is that the anterior thalamic nuclei are necessary for the temporal and spatial arrangement of items (e.g., structural learning; Aggleton & Pearce, 2001). Similar to spatial complex associative learning where animals are impaired when the arrangement of common distal cues must be considered, but are unimpaired when learning occurs in a different context which

are composed of unique cues (Chapter 4; but see following sections below), temporal demands may be exacerbated by a fixed spatial environment. Removing the animal from the arena during the between-block recency task caused a break, so that the two lists of items may be considered temporally unique. One way to test this hypothesis is by varying the time between the presentations of stimuli, while the animals remain in the test arena during the delay periods.

In contrast to a temporal order (or sequence) tag, one way animals could solve recency judgments is through the relative strength of a memory trace. If two items have the same forgetting curve, but one item is experienced earlier in time than the second, its trace strength would have decreased relatively more compared with the second item. It is possible that the rate of degradation of a memory trace may be accelerated in animals with anterior thalamic lesions. This hypothesis seems unlikely given the lack of group differences between the control and lesion rats on tasks of object recognition or between-block recency (Chapter 2, Experiments 2.2, 2.3, and 2.4). Furthermore, as mentioned above, there is also no evidence that the memory trace for object recency judgments in the lesion group is affected significantly more compared with the sham group by interference or task difficulty.

It could also be argued that tasks that rely on spontaneous exploration (recognition and recency judgments) reflect simple associative problems, such as a failure of these rats to habituate to the stimuli, instead of reflecting cognitive processes such as familiarity or recency (e.g., Honey & Good, 2000). First, these two explanations may not be mutually exclusive. There is evidence that cells in the perirhinal cortex decrease their firing with repeated presentations of the same stimuli (Fahy, Riches, & Brown, 1993; Xiang & Brown, 1998). Furthermore, decreases in the BOLD signal to familiar stimuli have been noted in the perirhinal cortex of healthy volunteers (e.g., Henson, Cansino, Herron, Robb, & Rugg, 2003; Weiss et al., 2004; Montaldi et al., 2006). It remains possible that habituation at the cellular level may lead to familiarity. However, these cellular changes underlying habituation would have to be long-term (lasting for hours and days) and as a result are not likely explained by short-term habituation to stimuli, or response suppression. Instead, the data are more consistent with long-term plastic

changes, such as weakening of the synapse (Aggleton & Brown, 2006). A recent study indicates that rats with perirhinal lesions show similar decline in exploration as the control group when the familiar objects were repeatedly presented (i.e., normal habituation), but were impaired when novel objects were compared with familiar ones (i.e., impaired object recognition; Albasser et al., 2011). These results support the conclusion that object recognition may not reflect an underlying problem with short-term habituation processes.

Secondly, habituation does not account for the within-block recency impairments found in Chapter 2 as the same rats are unimpaired on between-block recency and recognition memory tasks. It is difficult to imagine why habituation may be selectively impaired on within-block recency, but not on other tasks. Furthermore, previous research not relying on spontaneous exploration has found impaired temporal order memory following anterior thalamic damage (Wolff et al., 2006).

Spatial and complex associative learning (*where* and *what-where*)

The contribution of the anterior thalamic nuclei to spatial and complex associative learning was examined in Chapters 4-6. The results indicated that rats with anterior thalamic damage were unable to form item-place associations using distal cues, but had no difficulty in forming complex associations when contextual (proximal) cues were used to guide responses (Chapter 4 and 5). In addition, when navigation was limited or not necessary, anterior thalamic lesions still impaired place learning compared with control rats (Chapter 5 and 6), despite evidence that the rats with anterior thalamic damage were still able to discriminate the two spatial locations (i.e., they are significantly above chance performance; Chapter 5). In other words, rats with lesions to the anterior thalamic nuclei can discriminate, under certain circumstances (e.g., see Chapter 6), between two different spatial locations, but not as well as control rats.

In addition, making the two locations more distinct [i.e., reducing the overlapping features of particular cues (or scenes)] by having the rats approach

the digging cup(s) in one direction in the two places (unidirectional) as oppose to both directions in the two places (bidirectional) did not significantly influence the performance of rats with anterior thalamic lesions on a go/no-go place discrimination task, nor on an item-place biconditional learning task (Chapter 5). It was hypothesised that making the two spatial locations easier to discriminate by always having the rats approach the digging cup from a single direction, would improve their performance. It was thought that this procedure may help the animals form associations between the go/no-go rule and a distinct sub-set of cues in each location (with little overlap between them), and that animals could possibly treat these cues in a similar way to unique contexts (or a single visual cue) where they do not show impaired performance (e.g., Chudasama et al., 2001; Ridley et al., 2002; Chapter 4, Experiment 4.1 and 4.2E). The implication is that the rats with anterior thalamic lesions may have difficulty with allocentric space because they are required to learn the structural arrangement of the distal cues to solve the task (see section below on structural learning; Aggleton & Pearce, 2001). However, as mentioned, this manipulation did not eliminate the deficit found in place learning when compared with the sham rats. This result is surprising given that the same manipulation influenced the ability of animals with hippocampal damage on these tasks as increasing the distinctiveness of the two locations improved performance (Chapter 5). Chapter 6 found that anterior thalamic lesions also significantly impaired place learning in simple environments when the animals were placed passively in the goal location during training.

As impaired spatial learning is one of the most consistent findings following damage to the extended hippocampal system (e.g., Harley, 1979; Olton & Papas, 1979; Morris, Garrud, Rawlins, & O'Keefe, 1982; Sutherland & Rodriguez, 1989; Sziklas & Petrides, 1993; Aggleton et al., 1996; Byatt & Dalrymple-Alford, 1996), it is not surprising that several theories have tried to explain the pattern of deficits observed (e.g., O'Keefe & Nadel, 1978; Sutherland & Rudy, 1989; Eichenbaum, 2004; Aggleton & Pearce, 2001). While these theories have been applied to the hippocampus, the similar behavioural performance following hippocampal and anterior thalamic damage as well as evidence that they form an interdependent functional network to support these behaviours (Sutherland & Hoesing, 1993;

Warburton et al., 2000, 2001; Henry et al., 2004; Dumont et al., 2010), suggests that these same theories may help explain the contribution of the anterior thalamic nuclei. Is there an underlying process that can account for the pattern of deficits observed following anterior thalamic lesions?

Cognitive map

As mentioned briefly in Chapter 6, animals could learn to navigate to a goal location or discriminate between different locations by using a map-like representation of the environment (Tolman, 1948). This map-like representation of space includes several landmarks and how they relate to one another spatially (i.e., global association among the cues). Following the discovery of place cells in the hippocampus (i.e., cells that fire when an animal is in a particular location within an environment; O'Keefe & Dostrovsky, 1971), O'Keefe and Nadel (1978) proposed that the role of the hippocampus was to form a cognitive map. Therefore, they hypothesised that the hippocampus was particularly important for processing spatial information (O'Keefe & Nadel, 1978). Given the intimate anatomical relationship between the hippocampus and the anterior thalamic nuclei (Nauta, 1956, Aggleton et al., 1986, Vann et al., 2007), it is possible that the anterior thalamic nuclei also have a role in the formation of map-like representations.

Perhaps the most direct evidence is the finding that head direction cells are found in the anterior dorsal thalamic nucleus (Taube, 1995, 1998). Anterior dorsal thalamic head direction cells can come under the influence of environmental boundaries and salient distal cues (e.g., Taube & Burton, 1995; Clark et al., 2012). For example, if a trapezoid environment is rotated 90° following an initial exploratory session in the absence of other cues (landmarks), the head direction cells' preferred firing direction will shift by the same amount (i.e., 90°; Clark et al., 2012). However, if cues are placed in the background (distal), but not the foreground (proximal), the head direction cells' preferred firing will shift with background objects (Zugaro, Tabuchi, Fouquier, Berthoz, & Wiener, 2001). These results suggest that head direction cells are sensitive to the overall shape of an environment as well as distal cues (landmarks). Furthermore, both developmental

studies (Langston et al., 2010; Wills, Cacucci, Burgess, & O'Keefe, 2010) and reversible inactivation studies (Mizumori & Williams, 1993; Mizumori, Miya, & Ward, 1994) suggest that head direction information may give rise and facilitate the gradual development of place cell representations. Therefore, the anterior thalamic nuclei may provide critical directional information that is necessary for the formation of a cognitive map, but the anterior thalamic nuclei are probably not the neural location of map-like representations themselves.

There is also electrophysiological evidence that suggests that not all environmental boundaries reliably produce consistent head direction responses when rotated (Clark et al., 2012). For instance, while rotating a trapezoid by 90° resulted in shift in head direction cells' preferred responding by the same degree, rotating a rectangle resulted in more variable shifts in head direction cells' firing (Clark et al., 2012). The cells' preferred firing direction tended to shift in multiples of 90° (i.e., 0°, 90°, 180°, 270°), suggesting that the environment still did exert some sort of influence on head direction cell activity (Clark et al., 2012).

Unlike electrophysiological evidence, behavioural studies have struggled to demonstrate unequivocally that normal animals form and use a cognitive map (Horne et al., 2012). Animals may use alternative strategies to navigate in their environment (e.g., path integration, template matching, response-based learning; Cartwright & Collett, 1983; McNaughton et al., 1991; Packard & McGaugh, 1992; McDonald & White, 1993; Packard & McGaugh, 1996; Blair et al., 1997; Golob & Taube, 1999). It has been argued that an animal that can travel to a goal location following passive placement training has not had the opportunity to form response-based associations (Horne et al., 2012). As a result animals must learn to navigate to the goal location by learning the relationship among environmental cues. The issue is whether the rat uses global cues (cognitive map), local cues, or both. It could be argued that the anterior thalamic lesions impaired the formation of a cognitive map, which is why animals with damage to the anterior thalamus are impaired on spatial learning tasks that require knowledge of the relationship among distal spatial cues (i.e., allocentric spatial learning; e.g., Aggleton et al., 1996; Warburton et al., 1997; Sziklas & Petrides, 1999; Mitchell & Dalrymple-Alford, 2006; Wolff, Gibb et al., 2008.; Chapter 2, Experiment 2.1; Chapter 4,

Experiment 4.2D; Chapter 5 and 6). Furthermore, rats with damage to the anterior thalamic nuclei also spent the same amount of time in both the correct and incorrect corners that could only be distinguished by the shape of the environment following passive placement training (Chapter 6, Experiment 6.1), again supporting their potential role in the formation of map-like representations.

Although lesion-induced deficits in allocentric space are consistent cognitive map theory, the animals may also be solving the tasks by template matching (Cartwright & Collett, 1983; Collett & Collett, 2002). In other words, the animals may form a mental "snapshot" of the goal location, and navigate to the location that most closely matches the memory of the snapshot (Horne et al., 2012). The use of mental "snapshots" differs from cognitive map because "snapshots" do not form global (map-like) representations of environments, but rather local representations of goal locations. Furthermore, as mental "snapshots" can include several cues, the snapshot also contains information about the structural relationship among these cues (Gaffan & Harrison, 1989; Aggleton & Pearce, 2001). This alternative explanation has lead to the hypothesis that the extended hippocampal system may be important for structural learning (Aggleton & Pearce, 2001; Sanderson et al., 2006).

Structural learning

As briefly mentioned above, one possible explanation is that the extended hippocampal memory system is critical for structural learning, and is thought to underlie the ability of animals to form mental "snapshots" (Aggleton & Pearce, 2001; Sanderson et al., 2006; Aggleton et al., 2007). Spatial structural learning requires the ability to compile individual spatial cues into a unique spatial scene with each item in its relative location. It is not sufficient to simply bind the spatial cues together into a unique group; the structural (spatial or temporal) relationships among the cues must also be recorded. This binding of the relationship between several cues can be seen as prerequisite for the formation of scenes or "snapshots" (Gaffan & Harrison, 1989; Aggleton & Pearce, 2001; George & Pearce, 2003; Aggleton et al., 2007). Therefore, structural learning is important for resolving spatial scenes that contain the same cues that differ in their

arrangement (e.g., the perspective of the animal approaching a goal location from different directions).   In addition, the formation of "snapshots" is thought to be an important process in episodic memory where an event (e.g., object) is remembered within its spatial context (scene) from the viewpoint of the observer (Gaffan & Harrison, 1989; Gaffan, 1991; Gaffan & Hornak, 1997; Hassabis & Maguire, 2007).

The finding that animals with anterior thalamic lesions are impaired in spatial memory tasks when the arrangement of distal cues (i.e., allocentric) is necessary to solve the task could support the hypothesis that these nuclei are involved in structural learning (Aggleton et al., 1996; Warburton et al., 1997; Sziklas & Petrides, 1999; Mitchell & Dalrymple-Alford, 2006; Wolff, Gibb et al., 2008., see also Aggleton & Pearce, 2001).   For example, Chapter 4 (Experiment 4.2D) found that rats with anterior thalamic lesions were impaired when they were required to form item-place associations, which required the animal to associate a particular item with a location within a room possibly based on the structural configuration of the distal cues, i.e., based on the perspective the animal viewed the distal cues.   It also remains possible that the animals could rely on alternate strategies such as only use a unique sub-set of distal cues in each location.   However, the same animals were unimpaired when item-context associations were used (i.e., non-structural task; Experiment 4.2E).   These contexts were distinct, and individual cues (e.g., the different floors or walls of a plastic box) always predicted which item was correct.   Similar to the item-place biconditional task, Chapter 6 found that rats with anterior thalamic lesions were impaired on their ability to learn between two places that could only be distinguished by the arrangement of the walls of the environment.

Although the results from this thesis support the notion that the extended hippocampal system is important for structural learning, there is evidence that anterior thalamic lesions need not always impair learning about the spatial (or temporal) relationships between items.   Critically, one experiment that explicitly test structural learning in a water maze found that rats with anterior thalamic lesions are not impaired (Aggleton et al., 2009), which contrasts with results showing that hippocampal lesions impair this type of learning (Sanderson et al., 2006).   On a visual structural learning task (Sanderson et al., 2006; Aggleton et al.,

2009), the rats were required to learn three concurrent visual discriminations that had the same elements (A, B, C), but the elements were arranged differently (e.g., AB+, BA-). A complex and lengthy training procedure was used in order to prevent the rats from ignoring the spatial relationship between the elements. For example, if the rats were only given a single visual discrimination, the rats could ignore the element presented to the right; reducing the task from AB+, BA- to A+, B-. By using three concurrent visual discriminations composed of combinations of three elements (AB+ vs. BA-, BC+ vs. CB-, CA vs. AC-), the rats were unable to use a simple elemental strategy. The rats were required to swim to a hidden platform which was placed below the correct visual compound in a rectangular watermaze. Rats with anterior thalamic lesions could master this task and were not impaired relative to their controls (Aggleton et al., 2009).

There are several possible explanations for the discrepancies found in experiments that only informally tax structural learning (i.e., are open to alternative solutions) compared with experiments that explicitly address this question. One possibility is that the procedures across tasks are different, and in the case of explicit structural learning task, the lengthy training may have allowed the development of other strategies, or that the anterior thalamic nuclei are only important for more rapid "mental snapshot" scene learning (Gaffan et al., 2001; Aggleton et al., 2009). It also remains possible that the anterior thalamic nuclei are not necessary for structural learning, and it is the case that other features found in spatial memory tasks are impaired following damage to that region (e.g., learning geometrical properties, navigation).

Pattern separation

An alternative hypothesis is that the extended hippocampal system may be critical for pattern separation. Pattern separation is a mechanism for retrieving separate patterns of neural activity from otherwise partially overlapping patterns of activation, and is important for the ability to distinguish between similar memories by reducing interference (Gilbert & Brushfield, 2009). Pattern separation is based on computation models that suggest that the hippocampus has the ability to create distinct, non-overlapping representations (e.g., Rolls & Kesner, 2006; Kesner,

2007). Electrophysiological (Leutgeb, Leutgeb, Treves, Moser, & Moser, 2004; Leutgeb et al., 2005; Leutgeb, Leutgeb, Moser, & Moser, 2007), imaging (Bakker, Kirwan, Miller, & Stark, 2008), and lesion studies (Gilbert, Kesner, & DeCoteau, 1998, Gilbert, Kesner, & Lee, 2001; Lee, Jerman, & Kesner, 2005) have all implicated a role for the hippocampus, particularly the dentate gyrus, in pattern separation. In contrast to pattern separation, pattern completion is the mechanism thought to retrieve an entire representation from partial activation or from a degraded memory signal, and there is some evidence that the CA3 region of the hippocampus is involved in this process (Kesner & Hopkins, 2006). However, given that anterior thalamic lesions impaired learning in environments with a large degree of interference (i.e., the distal cues overlapped in both goal locations; e.g., Chapter 4, Experiment 4.2D), but not when an item is associated with a constellation of unique cues between the two contexts (Chapter 4, Experiment 4.2E), the theoretical implication is that the anterior thalamic nuclei may be involved in processes that separate similar patterns of activity into unique representations (i.e., pattern separation). Perhaps, some case could also be made that the anterior thalamic nuclei also have a role in pattern completion, but this hypothesis would require experiments that use prompting cues thought to aid in the retrieval of the whole memory, or evidence of faster forgetting rate in the lesion group, which was not observed following object recognition memory tests (see Item recognition and Temporal order sections above or Chapter 2) and impossible to test from the spatial tasks used in this thesis.

As briefly mentioned above, the results from Chapter 4 support this hypothesis: rats with anterior thalamic damage are impaired when items are associated with places. The places often differ in the arrangement of the same or very similar distal spatial cues (i.e., the perspective in which the items are viewed) which results in a higher degree of overlapping or similar environmental features. Distinguishing among these features may require pattern separation. In contrast, when a unique context is associated with an item (i.e., pattern separation is not required), anterior thalamic lesions do not impair performance. Likewise in Chapter 6, the ability to learn the correct corner in the simple environments where the correct and incorrect corners are similar could be resolved with pattern

separation using structural features. In fact, it remains possible that pattern separation describes a key process underlying structural learning.

However, when rats with anterior thalamic lesions were tested on a reference memory task in a radial arm maze where the separation between the correct and incorrect arms was either small (i.e., more overlapping features; requiring more pattern separation) or large (i.e., less overlapping features between the arm; requiring less pattern separation), anterior thalamic damage impaired performance regardless of the spatial separation between the arms they were required to choose from, and there was no effect of the spatial separation of the arms (Loukavenko et al., 2007). However, it remains possible that the differences in the separation between the arms in the small (i.e., requiring more pattern separation) and large (i.e., requiring less pattern separation) conditions were not sufficient to observe behavioural changes. Indeed, the major issue with pattern separation is that it is difficult to make *a priori* predictions of when it does and does not occur (e.g., how much separation is required between conditions to observe a change in behaviour?).

A second possibility is that differences in distinguishing between the arms with varying amounts of overlapping features from distal cues were masked by deficits in navigating to the goal location. In other words, it remains possible that rats with anterior thalamic lesions are disporptionately impaired at discriminating between arms in the radial arm maze when more pattern separation was required (i.e., the arms were closer together) compared with discriminations that require less pattern separation (i.e., the arms were further apart), but that anterior thalamic lesions also impair navigation so that behavioural differences on the place discrimination task were not observed. This account seems unlikely, however, given that the performance of rats with anterior thalamic lesions was not rescued when locations were made more distinct by allowing the animals to approach (or withhold approaching) a digging cup from a single direction (i.e., less pattern separation) compared with two directions (i.e., more pattern separation) in the two locations the animals were required to discriminate (see discrimination ratios in Chapter 5). As the navigational demands on the go/no-go task were minimal (i.e., the rat was passively placed in one of two locations, and only had to traverse

the length of the small plastic box), it could be argued that pattern separation need not account for the spatial deficits observed. On the other hand, all the room cues remained visible from the test box, and so the rats may have still attended to several common cues, i.e., require pattern separation. In addition, the go/no-go impairments following anterior thalamic damage in Chapter 5 may have also been influenced by problems with inhibiting responses. Inspection of the data suggests that the animals may have been impulsive.

Path integration and orientation during navigation

It is plausible that lesions of the anterior thalamic nuclei impair spatial learning because damage to this region disrupts the head direction system (Goodridge & Taube, 1997), and head direction information is important for correct orientation during navigation (Wiener & Taube, 2005; Taube, 2007). This hypothesis stems from the finding of head direction cells in the anterior dorsal thalamic nucleus (Taube, 1995). In addition, there is some behavioural evidence indicating that animals with damage to the anterior dorsal and lateral dorsal thalamic nuclei are impaired in locating a submerged platform located at a fixed distance and direction from a landmark (Wilton et al., 2001). Research also suggests that the head direction system influences hippocampal place information (Mizumori & Williams, 1993; Calton et al., 2003). For instance, inactivating the lateral dorsal thalamic nucleus, a region which contains head direction cells (Mizumori & Williams, 1993), both increased the number of errors on a spatial working memory task and disrupted hippocampal place cell activity (Mizumori et al., 1994). Indeed, as mentioned above, it has been hypothesised that head direction cell activity may help give rise to hippocampal place cells, and there is evidence that adult-like directional information is present in the hippocampal and subicular cortices before stable place cell representations in juvenile rats (Langston et al., 2010; Wills et al., 2010).

A second hypothesis is that the head direction system is important for path integration (McNaughton et al., 1991; Blair et al., 1997; Golob & Taube, 1999; Kubie & Fenton, 2009). Path integration is an alternative navigational strategy to using landmarks and map-like representations (i.e., cognitive maps). Path

integration involves monitoring and integrating internal cues such as vestibular, proprioceptive, and self-movement (motor) cues. These cues, also known as idiothetic cues, are constantly updated compared with the starting point allowing an animal to determine its current location as it is moving through space (Mittelstaedt & Mittelstaedt, 1980; McNaughton et al., 1991; Golob & Taube, 1999). Importantly, the starting point and the updating processes are self-contained and do not rely on surrounding cues; although, external cues (landmarks) can be used to correct errors that accumulate over time during the path integration process (Gallistel, 1990). Evidence that the head direction cells preferred firing direction is severely disrupted by damage to the vestibular system emphasise the importance of idiothetic cues in generating head direction firing (Stackman & Taube, 1997; Muir et al., 2009). Furthermore, head direction cells maintain their preferred direction in the dark (Taube et al., 1990; Mizumori & Williams, 1993; Goodridge et al., 1998; Yoder et al., 2011), a condition where navigation should rely upon path integration, so supporting the hypothesis that the head direction system is important in path integration. Head direction cells also maintain their preferred direction when moved from a familiar into a novel environment (Taube & Burton, 1995). As initially there are no familiar landmark cues for the animal to orient towards, the maintained preferred head direction firing may be mediated by path integration processes (Taube & Burton, 1995; Golob & Taube, 1999).

There is some evidence that damage to the head direction system impairs tasks thought to require path integration (e.g., navigating in the dark; Cooper & Mizumori, 1999; Cooper, Manka, & Mizumori, 2001; Frohardt, Bassett, & Taube, 2006; Pothuizen, Aggleton, & Vann, 2008). For instance, when animals were allowed to forage in an arena and return to a sheltered start refuge to consume the food pellets in a light condition and also when blindfolded (which also included training and testing in the dark; i.e. dark condition), damage to the anterior dorsal thalamic nucleus produced mild deficits compared with sham rats, i.e., the rats with AD damage made more error by going to the wrong starting point after retrieval of the food pellet. The AD lesioned rats also had a heading angle that deviated further from the refuge compared with the sham group (Frohardt et al., 2006). There was, however, no main effect of condition, i.e., performance was not

disproportionately affected in the dark. If head direction cells are particularly important for path integration, then the rats should be more impaired in the blindfolded (dark) compared with visual (light) version of the task. As the rats with anterior dorsal thalamic lesions were not significantly worse in the blindfolded condition, it suggests that the deficits found following damage to AD may not relate to path integration, but rather to a more general problem with navigation (e.g., orientating to the appropriate stimuli). This experiment also found that lesions to the dorsal tegmental nucleus produced more severe impairments compared with the anterior dorsal lesions (Frohardt et al., 2006).

There is also evidence from electrophysiological studies examining head direction firing in animals that are navigating to a goal which indicates that choice behaviour can be independent from the cell's preferred firing. This evidence suggests that head direction activity may not be necessary for navigating to a goal (Dudchenko & Taube, 1997; Golob, Stackman, Wong, & Taube, 2001; Muir & Taube, 2002). For example, Golob et al. (2001) trained rats to go to a particular corner in a square arena where the only cue was a white card placed against the wall. When the rats experienced a probe test where the square arena was replaced by a rectangle with the cue held constant, the rats responded correctly by going to the correct corner despite shifts of the head direction cells preferred direction. If a head direction cell's preferred directional firing guided behaviour, the rats should have selected the corner shifted by the same degree as the preferred response of the head direction cell (Golob et al., 2001).

In addition, deficits following damage to the anterior thalamic nuclei on spatial tasks with limited or without navigation cast doubts on the argument that the anterior thalamus is critical for path integration and orientation during navigation (e.g., Aggleton et al., 1991; Chapter 4 and 5). While head direction cells may help learning and navigating to locations, it is difficult to predict why lesions to the anterior thalamic nuclei would impair spatial learning where orientation, navigation, and path integration are not necessary to solve the task (i.e., path integration may not provide a sufficient explanation). Furthermore, lesions to the lateral mammillary bodies which abolish the head direction signal in the anterior dorsal thalamic nucleus (Bassett et al., 2007) and in the postsubiculum (Sharp &

Koester, 2008) have only mild effects on tasks of spatial memory (Vann, 2005, 2009). For example, rats with lateral mammillary lesions were not impaired on the T-maze alternation task, which is sensitive to complete mammillary body lesions (Vann, 2005). The results suggest that the head direction (or orientation during navigation) account cannot fully explain the spatial memory deficits observed following damage to not only the mammillary bodies (Vann, 2005, 2009, 2011), but also to the rest of the extended hippocampal system (e.g., Jarrard, 1978; Harley, 1979; Olton & Papas, 1979; Sutherland & Rodriguez, 1989; Aggleton et al., 1996; Byatt & Dalrymple-Alford, 1996; Warburton et al., 1997, 2001; Mitchell & Dalrymple-Alford, 2006).

Are the anterior thalamic nuclei all involved in the same processes?

At a superficial level, the three nuclei that form the anterior thalamus (anterior dorsal, anterior ventral, and anterior medial) have similar anatomical connections, but more detailed analyses suggest that they may form part of three relatively separate and parallel pathways (Aggleton, O'Mara et al., 2010). Given that information from different populations of neurons innervate different thalamic nuclei, it remains possible that each of the anterior thalamic nuclei contribute differently to learning and memory. Indeed unpublished findings (N. Wright, personal communication) highlight how different cell populations in the subiculum innervate the anterior ventral and the anterior medial nuclei respectively, with only the cholinergic inputs from the lateral dorsal tegmental nucleus showing appreciable collateralization such that individual neurons project to both anterior thalamic nuclei. Consequently, it may not be surprising to find evidence that the functional roles of the three nuclei of the anterior thalamus differ from one another.

Figure 7.1 shows the different patterns of connectivity between the anterior thalamic nuclei and the extended hippocampal system in the rat. First, it is important to note that different populations of subicular inputs arrive at the anterior thalamic nuclei. The subiculum and presubiculum both project directly to the anterior ventral (AV) and anterior medial (AM) thalamic nuclei (van Groen &

Wyss, 1990; Wright et al., 2010). In contrast, the parasubiculum and postsubiculum project to the anterior dorsal nucleus (AD; van Groen & Wyss, 1990a, b; Yoder & Taube, 2011). These direct subicular projections are through the fornix, however, these subicular regions also project to the mammillary bodies, providing an indirect route to the anterior thalamic nuclei (Nauta, 1956; Aggleton, O'Mara et al., 2010). Critically, studies have shown that separate populations of subicular neurons project to the anterior thalamic nuclei or to the mammillary bodies (Wright et al., 2010). Research has also shown that different regions of the mammillary bodies innervate different thalamic nuclei: the medial portion of the medial mammillary bodies project to AM, the lateral portion of the medial mammillary bodies project to AV, and the lateral mammillary bodies innervate AD (Shibata, 1992; Hopkins, 2005; Vann et al., 2007).

The efferents from the three anterior thalamic nuclei also differ from each other. The most notable difference is the discovery that AM both receives inputs and innervates the prelimbic and anterior cingulate cortex, whereas AD does not, and the frontal connections with AV are relatively sparse in comparison to AM (Shibata & Naito, 2005; Shibata, 1993a, van Groen et al., 1999; N. Wright, personal communication). Furthermore, while all three nuclei project to the retrosplenial cortex, there are some differences between the patterns of connectivity. For instance, AD projects to the granular region of the retrosplenial cortex, whereas there is evidence suggesting that AV and AM project to both the granular and dysgranular retrosplenial cortex (van Groen & Wyss, 1992; Shibata, 1993b). Another significant difference is that AM and AD project directly to the hippocampus, whereas AV does not (Wyss et al., 1979).
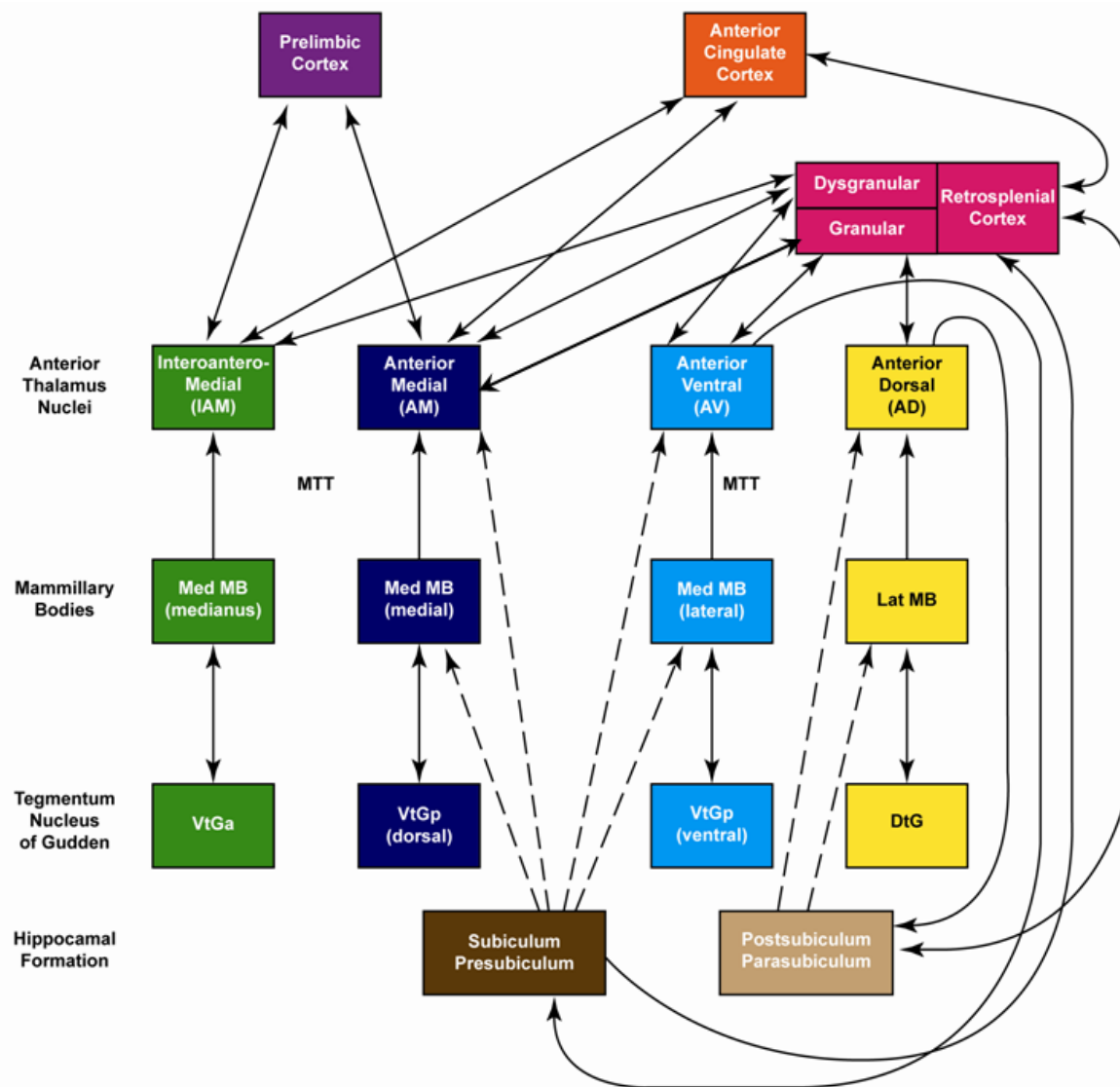
Figure 7.1. Diagramme of the separate anatomical connections to the different nuclei of the anterior thalamus in the rat; taken from Aggleton, O'Mara et al., 2010. The dashed lines convey connections from the fornix, and double arrow heads signify reciprocal connections. The connections of IAM are also shown to highlight that it might make its own unique contribution to learning and memory that is separate to AM; although IAM is not discussed in detail in this thesis (see Chapter 1, General Introduction). Abbreviations: AD, anterior dorsal thalamic nucleus; AM, anterior medial thalamic nucleus; AV, anterior ventral thalamic nucleus; DtG, dorsal tegmental nucleus of Gudden; IAM, interoanteromedial thalamic nucleus; Lat MB; lateral mammillary bodies; Med MB, medial mammillary bodies; MTT, mammillothalamic tract; VtGa, ventral tegmental nucleus of Gudden, pars anterior; VtGp, ventral tegmental nucleus of Gudden, pars posterior.

Other support for the hypothesis that each anterior thalamic nuclei may have a different functional contributions to processes involved in learning and memory comes from the different firing properties of neurons (Aggleton, O'Mara et al., 2010). As noted in the General Introduction (Chapter 1) and in the above sections, electrophysiological studies have found cells in the anterior dorsal thalamic nucleus that fire when an animal's head is facing a particular direction on a horizontal plane (e.g., Taube et al., 1990; Taube, 1995, 2007). These head direction cells are thought to be critical for orientating during navigation (Wiener & Taube, 2005; Taube, 2007) as well as integrating landmark and idiothetic cues during path integration (McNaughton et al., 1991; Blair et al., 1997; Golob & Taube, 1999; Kubie & Fenton, 2009).

In contrast, cells in the anterior ventral thalamic nucleus fire rhythmically with hippocampal-like theta (Vertes et al., 2001; Tsanov, Chah, Wright et al., 2011). Theta rhythm may be important for organising network activity as well as preventing a temporal and spatial smearing of information (Buzsaki, 2002, 2005). For instance, research has shown that the spikes of place cells shift systematically throughout the theta cycle, a phenomenon known as phase precession (O'Keefe & Recce, 1993). When a rat enters a place field, a cell fires at a particular point during the theta oscillation (typically at the peak). As the animal progresses through the place field, the cell's firing activity shifts along the theta cycle allowing sequentially firing cells to advance through the theta cycle as the animal enters different place fields (O'Keefe & Recce, 1993; Buzsaki, 2005). Buzsaki (2005) argues that phase precession allows for a "temporal organising mechanism for bringing cell assemblies together in the time frame critical for neuronal plasticity," and research suggests that plastic changes between sequentially activated place cells occur during theta oscillations (Mehta et al., 2000). Given that theta oscillations are important for plastic changes, it remains possible that the anterior ventral thalamic nucleus is involved in these processes.

These two different neural characteristics, theta oscillation and head direction cells, suggest that each of the nuclei has a different function (Aggleton, O'Mara et al., 2010). There is, however, evidence that there are some head direction cells in AV and that they can be modulated by theta (Tsanov, Chah, Vann

et al., 2011) indicating that there are also overlapping cellular properties that may lead to similar functions. The hypothesised role of the individual anterior thalamic nuclei is discussed below.

Anterior medial (AM): frontal-limbic gateway

Because the anterior medial thalamic nucleus receives and projects to the frontal regions such as the prelimbic cortex and medial orbital cortex (Shibata & Naito, 2005), it is possible that this nucleus acts as a gateway between the frontal cortex and hippocampus (Warrington & Weiskrantz, 1982; Aggleton, O'Mara et al., 2010). In addition, very few cells in AM (approximately 6%) show rhythmic firing with theta (Albo, Viani Di Prisco, & Vertes, 2003). Therefore, it had been suggested that AM may project highly integrated information from the hippocampus and the medial diencephalon (e.g., medial portion of the medial mammillary bodies) to the frontal cortex. As the medial prefrontal cortex is involved in executive function, planning, organising, and cognitive flexibility (e.g., Seamans, Floresco, & Philips, 1995; Dias & Aggleton, 2000; Delatour & Gisguet-Verrier, 2000; Delay et al., 2004), it seems likely that AM aids in these processes possibly by bridging the information to be retrieved with the mechanism needed to monitor its retrieval (Aggleton, O'Mara et al., 2010). Furthermore, as these projections are reciprocal (Shibata & Naito, 2005), they provide a way by which the frontal cortex can influence the extended hippocampal system, and possibility exert an active "top-down" role in memory retrieval.

This proposal would lead to the hypothesis that lesions to AM could possibly disrupt the retrieval of appropriate limbic signals (e.g., retrieval of the wrong memory, or errors with components of memory), and that deficits may emerge on tasks that are also sensitive to frontal (prelimbic, anterior cingulate) functioning. Future studies could test this hypothesis by selectively damaging AM in rats and examining their performance on tasks typically associated with frontal functions. An example would be the performance of rats with AM lesions on a cue and response competition task analogous to the Stroop task in humans (Haddon, George, & Killcross, 2008). In this task, rats were trained on two biconditional tasks each in a different context. In one context, a left or a right lever press is

associated with one of two auditory stimuli (A1L1; A2L2), whereas in the second context, the behavioural response (lever press) is associated with visual stimuli (V1L1; V2L2). At test, the animals are given auditory-visual compounds. These compounds can either be congruent (the auditory-visual compound is associated with the same lever during training; A1V1, A2V2) or incongruent (the auditory-visual compound signal a response to different levers during training; A1V2, A2V1). To resolve the conflicting response information in the incongruent trials, rats have to use the context cues. Studies have shown that this task is sensitive to damage of the prefrontal cortex (Haddon & Killcross, 2006; Marquis, Killcross, & Haddon, 2007). However, there is evidence showing that anterior thalamic lesions do not disrupt performance on a 5-choice serial reaction task which is also sensitive to prelimbic damage (Chudasama & Muir, 2001), suggesting that the role of the prefrontal cortex can still be dissociated from that of AM.

Anterior dorsal: orientation and navigation

As previously mentioned, the anterior dorsal thalamic nucleus forms part of a neural circuit containing head direction cells (i.e., cells that fire when the animal is facing a particular direction regardless of its location in the environment; Taube, 1995). These cells are thought to be important during navigation as they may help orientation (Wiener & Taube, 2005; Taube, 2007) and help the integration of landmark and idiothetic cues during path integration (McNaughton et al., 1991; Blair et al., 1997; Golob & Taube, 1999; Kubie & Fenton, 2009).

However, studies reporting impaired performance following anterior thalamic lesions on tasks that do not tax navigation (e.g., Aggleton et al., 1991; Chapter 4 and 5) weaken this hypothesis. Likewise, studies failing to find impaired performance on tasks that require the animals to navigate [e.g., object recognition in large open field maze (e.g., Aggleton et al., 1995; Wilton et al., 2001; Moran & Dalrymple-Alford, 2003; Mitchell & Dalrymple-Alford, 2005; Chapter 2, Experiment 2.3); discriminating between scenes in a Y-maze (Gaffan et al., 2001); egocentric spatial learning (e.g., Aggleton et al., 1996; Warburton et al. 1997; Mitchell & Dalrymple-Alford, 2006; Wolff, Gibb et al., 2008.)], also questions the validity of this hypothesis. For example, a previous study has found that damage to

the anterior thalamus impairs spatial working memory task without navigation (Aggleton et al., 1991). In this task the rat had to select between a left or a right lever during the test phase the one not pressed during the sample (i.e., delay-non matching-to-position). As mentioned above, these results suggest that the anterior thalamic nuclei may not be critical for navigating; although it remains possible that the distance between the two levers is far enough apart to require different head direction cells (i.e., the rats are facing in slightly different directions to the left compared with the right lever). The impaired performance on the delay-non matching-to-position task may also be a result of damage to the other two nuclei of the anterior thalamus. Therefore, it would be interesting to compare the performance of rats with discrete lesions to each of the three anterior thalamic nuclei on spatial memory tasks that require navigation, or orienting in a particular direction, and those that do not.

Anterior ventral: optimizing synaptic plasticity

The anterior ventral thalamic nucleus both receives and projects to the subiculum and retrosplenial cortex (van Groen & Wyss, 1990; 2003 Shibata, 1993a, b). Furthermore, cells in AV fire rhythmically with theta (Vertes et al., 2001; Tsanov, Chah, Wright et al., 2011). There is also evidence that the convergent inputs from the fornix and the mammillothalamic tract have opposing plasticity effects in AV (Tsanov, Vann et al., 2011; see Chapter 1). Therefore, it seems likely that AV integrates information from both mammillary bodies and the hippocampus before projecting back to the subiculum and the retrosplenial cortex (the latter acting as an indirect route to the hippocampus), and so influences activity in regions that also oscillate in the theta band (Kocsis & Vertes, 1994; Bland et al., 1995; Kocsis & Vertes, 1997). Given the link between theta activity and plasticity (Mehta et al., 2000), it is possible that AV is ideally placed to influence and optimize synaptic plasticity (Aggleton, O'Mara et al., 2010). It is also known that lesions to the anterior thalamic nuclei impair long-term depression in electrophysiological slice recordings of the retrosplenial cortex (Garden et al., 2009). Future studies should examine whether damage to AV is sufficient to impair measures of synaptic plasticity (e.g., long-term potentiation, long-term depression, IEG activity, transcription factor activity such as CREB and phosphorylated CREB) in the

retrosplenial cortex and hippocampus, as well as theta rhythmic firing in these regions both using slice work and freely behaving animals. For instance, there is evidence that anterior thalamic nuclei lesions reduced levels of phosphorylated CREB in the hippocampus, but when or how damage to the anterior thalamic nuclei disrupts the phosphorylation of CREB remains unknown (Dumont et al., 2012).

Although, the separation of the major nuclei of the anterior thalamus may be useful in exploring how they each may contribute to different processes involved in episodic (episodic-like) memory, there remains an important question: where might this closely related information converge and integrate? One obvious candidate region is the retrosplenial cortex as all three of the nuclei send dense projections to this region. However, as mentioned previously, there are differences in the pattern of their connections to the retrosplenial cortex (see Figure 7.1; van Groen & Wyss, 1992; Shibata, 1993b). Similarly, the anterior thalamic nuclei innervations of the subicular cortices differs between nuclei (van Groen & Wyss, 1990a, b; Shibata, 1993a), suggesting that these parallel pathways may only converge in later, more distal, sites out in the neocortex, or perhaps in the hippocampus. However, different sub-regions of the retrosplenial cortex project to other retrosplenial sub-regions (i.e., the retrosplenial cortex is highly interconnected; Shibata, Honda, Sasaki, & Naito, 2009); perhaps, allowing for convergence to take place within the retrosplenial cortex before projecting back out to different neural sites (Wyss & van Groen, 1992; van Groen, Vogt, & Wyss, 1993; van Groen & Wyss, 2003).

Future Directions

Some potential future studies were mentioned above, as the different anterior thalamic nuclei may be involved in different processes. Discrete lesions to each of the nuclei individually could be used to examine the above hypotheses. For example, does selective damage to AD impair orientation or path integration? And, importantly, do lesions to AM and AV spare this type of learning? In addition, finding a neural marker of activity (i.e., IEG activity) with greater numbers of labeled anterior thalamic nuclei cells may allow for differences to be detected between the three nuclei following different behavioural manipulations in the

intact brain. Some studies have found changes in anterior thalamic IEG activity in normal rats following spatial learning (Vann, Brown, & Aggleton, 2000), changes in the temporal order (Amin et al., 2006), and in an inhibitory avoidance task (Yasoshima et al., 2007). It is interesting to note that when stimuli were in familiar locations, but were experienced at a different time, changes in *zif268* were only found in AV, and not in either AD or AM (Amin et al., 2006). If, as hypothesised, AV has a role as a temporal organiser because of the oscillatory properties of its neurons (i.e., theta rhythm), then one would predict to see changes in IEG activity in behavioural tasks with temporal manipulations.

Similar to research in animals, developments in human imaging at both a structural and functional level are important. Better visualisation of the damage to each of the nuclei in patients would help clarify which regions of the medial diencephalon are damaged, and this could guide the type of behavioural testing given to the patients. For instance, if damage is primarily to AM, one might expect a different pattern of deficits than if the damage was primarily involving AD. However, damage to the diencephalon in patients does not follow anatomical borders (i.e., damage is non-selective). For example, one common cause of thalamic pathology is stroke, and the vascular supply to the thalamus also does not follow nuclear borders (Castaigne et al., 1981). Furthermore, damage following stroke is typically asymmetric (often unilateral; see Aggleton, Dumont et al., 2011), therefore, even with improved MRI resolution, it will be difficult to find patients with selective damage. Another issue is that tasks would need to be sensitive enough to test the different hypotheses derived from the animal literature. Perhaps a better approach will be to improve the resolution during functional magnetic resonance imaging (fMRI) to allow observation of blood oxygen level dependent (BOLD) changes in the anterior thalamic nuclei in healthy volunteers. Unlike cortical areas that have benefited from fMRI studies (e.g., hippocampus, frontal cortex, retrosplenial cortex, parahippocampal regions), our knowledge of the medial diencephalon lags behind due to resolution limitations.

In addition, there are several more specific questions that could be examined following the results from previous chapters in this thesis. First, the results during the object recency tasks indicated that damage to the anterior

thalamus impairs within-block object recency judgments, but spares between-block recency judgments. However, it remains unclear what specific process is impaired by anterior thalamic damage (see above section, Temporal order memory: *when*). It is unclear whether difference in time between the two procedures (i.e., the temporal separation) or the removal of the animals from the arena in the between-block recency influenced the performance of the lesion group. To test this hypothesis, it would be possible to design the task so that the animal remained in the arena during the delay. This could be done in two ways: First, because the bow-tie maze has two compartments, opening the barrier following the final sample trial would allow the animal to enter an empty compartment. The animal could stay in an empty compartment of the bow-tie maze during the delay. Second, the rat could alternate between trials where items were presented and trials without items as the rat alternates compartments in the bow-tie. This latter procedure would increase the temporal separation between items during the within-block recency task in order to examine how temporal separation influences recency judgments.

Differences in performance might also result from other procedural differences. For example, in the within-block recency task, the animals were tested continuously on items that differed in the number of interleaving items between them. It is possible that because the testing procedure was continuous, the items being compared earlier in the test phase interfered with later test trials. Although more time consuming, future studies could eliminate this potential confound by testing the rats on only a single pair of items per testing session. It could also be interesting to examine the influence of list length in comparison to number of interleaving items. For example, would the performance of animals with anterior thalamic damage be affected significantly more compared to shams on items during the test phase that differed by three interleaving items on a list of 5 compared with 7 items?

One advantage of using spontaneous recognition tasks is that the animal does not have to learn an associative rule, thus, it reflects fast one-trial learning (Ennaceur & Delacour, 1988; Dix & Aggleton, 1999). However, one caveat is that the exploratory behaviour of animals with lesions may be abnormal (i.e., animals

may be able to correctly recognise items, but may appear impaired because of other behavioural problems). There is sufficient evidence indicating that damage to the anterior thalamic nuclei can result in hyperactivity (Jenkins, Vann et al., 2004; Poirier & Aggleton, 2009; Chapter 2, Experiment 2.9) and this hyperactivity is present even after approximately a year following surgery (Chapter 2). However, there is no research characterizing the effects of hyperactivity on exploration. For example, while Chapter 2 has found that both Sham1 and ATNx1 rats had the same total amount of item exploration (with the exception of Experiment 2.3 where the Sham1 group spent significantly more time exploring the objects compared with the ATNx1 group), it is possible that they explore the items differently. It would be interesting to observe if there are any differences in the number and duration of exploratory bouts between these two groups.

Another result that merits further investigation is the fact that rats with anterior thalamic damage explore both correct and incorrect corners of a rectangular shaped watermaze equally regardless of whether they received passive or active training (Aggleton et al., 2009; Chapter 6, Experiment 6.1). In contrast, the deficits observed when the animals had to differentiate between two corners that had different arrangement of the colour of the walls (i.e., black to the left of white vs. white to the left of black) were more mild; the anterior thalamic lesion animals spent more time in the correct compared with incorrect corner (Chapter 6, Experiment 6.2). One possibility is that the different colour (black-white) walls provided a more salient cue than the geometric properties; there is evidence that the colour (black, white) of the walls can overshadow learning of geometric cues (Pearce et al., 2006). In other words, learning about the wall colours impaired learning about the geometrical shape of the maze. However, another possibility is that rats with anterior thalamic damage cannot discriminate between walls of different lengths. It remains possible that rats with anterior thalamic damage exhibit spatial memory deficits because they cannot monitor distances (or lengths).

In summary, this thesis has shown that damage to the anterior thalamic nuclei impairs properties of episodic (episodic-like) memory. However, these deficits are specific to within-block recency with objects (*when*), spatial memory

(*where*) and complex spatial associative learning tasks where the animal has to have knowledge of distal spatial cues to guide its behavioural choices (i.e., place, not context; *what-where*). This final chapter has examined the results from this thesis in the light of those in the literature, and has found that there is no single theory to date capable of explaining the complex pattern of deficits. One possibility is that each of the three individual anterior thalamic nuclei may be contributing differently to learning and memory (Aggleton, O'Mara et al., 2010). Furthermore, as behavioural tasks are typically complex and so may tax two or three of these processes simultaneously, more severe deficits would be found following complete anterior thalamic damage (e.g., Aggleton et al., 1996; Byatt & Dalrymple-Alford, 1996; van Groen et al., 2002). This conclusion highlights the importance of developing behavioural tasks that only tax one of the hypothesised processes, so making it possible to isolate the potential effects of discrete lesions to each of the anterior thalamic nuclei.

The anterior thalamic nuclei have been conceptualised as part of an extended hippocampal memory system. Lesion studies have found that hippocampal and anterior thalamic damage typically impair performance on the same behavioural tasks (e.g., spatial memory; Morris et al., 1986; Sutherland & Rodriguez, 1989; Whishaw & Jarrard, 1995; Warburton & Aggleton, 1999; Wilton et al., 2001; van Groen et al., 2002; Loukavenko et al., 2007), and disconnection studies have shown how these two neural regions function in an interdependent manner to support allocentric spatial learning (Sutherland & Hoesing, 1993; Warburton et al., 2000, 2001; Henry et al., 2004; Dumont et al., 2010). Furthermore, a growing amount of research has shown that damage to the anterior thalamus leads to covert pathology in the retrosplenial cortex and the hippocampus (e.g., Jenkins, Dias, Amin, Brown et al., 2002; Jenkins, Dias, Amin, & Aggleton, 2002; Jenkins, Vann et al., 2004; Poirier & Aggleton, 2009; Anzalone et al., 2010; Savage et al., 2011; Dumont et al., 2012). For example, decreases in the IEGs *c-fos* and *zif268* following anterior thalamic lesions have been reported in the retrosplenial cortex and hippocampus (Jenkins, Dias, Amin, Brown et al., 2002; Jenkins, Dias, Amin, & Aggleton, 2002; Jenkins, Vann et al., 2004; Poirier & Aggleton, 2009). There is also evidence from a microarray study that anterior

thalamic nuclei lesions reduce levels of several mRNAs involved in neural metabolism and neural plasticity (Poirier, Shires et al., 2008). Similarly, anterior thalamic lesions decrease levels of CREB phosphorylation in the hippocampus, which may influence neuronal plasticity by reducing immediate-early gene expression (Dumont et al., 2012). In addition, lesions to the anterior thalamic nuclei disrupt behavioural increases in acetylcholine levels in the hippocampus (Savage et al., 2011), and abolish a measure of synaptic plasticity, long-term depression (LTD), in the retrosplenial cortex (Garden et al., 2009). Therefore, while this thesis has examined the effects of lesions to the anterior thalamic nuclei on components of episodic (episodic-like) memory, it remains unclear to what degree the behavioural deficits following anterior thalamic damage are a result of the overt pathology to these thalamic nuclei, are caused by covert abnormalities to the rest of the extended hippocampal system, or a combination of the two.

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