UNIVERSITY OF CANTERBURY
Te Whare Wānanga o Waitaha
CHRISTCHURCH NEW ZEALAND

Enrichment Facilitates Recovery of Spatial Memory but not Retrosplenial Immediate Early Gene Hypoactivation after Anterior Thalamic Lesions

---

A thesis submitted in partial fulfilment of the

requirements for the Degree

of Master of Science in Psychology

in the University of Canterbury

by Stephanie Ann Mercer

University of Canterbury

2015

Acknowledgements

I would like to express my sincere gratitude firstly to Professor John Dalrymple-Alford, whose knowledge, time and experience were invaluable through the course of this thesis. Your input was always appreciated and provided me with skills I would have otherwise never attained. Thank you also to Dr. Juan Canales, whose assistance was much appreciated.

To Neroli Harris, Silvana de Freitas Costa and Kate Freeman, thank you very much for all of your assistance, friendly conversation and advice throughout my time in the animal facility. Many thanks also to Kristin Murphy, Ashlea Dassanayake (do a PhD!) and to the students of the lab – thank you for your support and friendship! To Brook Perry, without whom I would not have been able to acquire the skills and patience (I promise that I will slow down!) necessary to complete this thesis, thank you! A very big thank you also goes to James Perry who provided great comedic relief, a great dose of reality when needed and friendship (often by way of food). I will always be thankful to call you two my friends.

I would also like to acknowledge the University of Canterbury for awarding me a UC Master's Scholarship, which was much appreciated and a financial relief.

Thank you also to my family – notably my mum Rachel, sister Rebecca and my grandparents Joy and John; your support was always timely and I am forever grateful to be surrounded by such wonderful people. Thank you also to Pam for your care and support! Finally, a big thank you goes to Alex, for your love and support throughout the years.

Table of Contents

List of Figures

List of Tables

Abbreviations

ACC	anterior cingulate cortex
AD	anterodorsal thalamic nucleus
AM	anteromedial thalamic nucleus
ANOVA	analysis of variance
A-P/AP	anterior-posterior
ATN	anterior thalamic nuclei
ATN.T	total anterior thalamic lesions
ATN-T	total anterior thalamic lesions
AV	anteroventral thalamic nucleus
B-L	bregma-lambda
BLA	basolateral amygdala
CA1	area CA1 of the hippocampus
Contra	contralateral
CREB	c-AMP response element binding protein
DG	dentate gyrus
dHF	dorsal hippocampal formation
dHPC	dorsal hippocampus
dSub	dorsal subiculum
DTN	dorsal tegmental nucleus
D-V	dorsal-ventral
GAP-43	growth associated protein 43
H	hippocampus
HF	hippocampal formation
HPC	hippocampus
IEG	immediate early gene
IL	infralimbic cortex

ILN	intralaminar thalamic nuclei
Ipsi	ipsilateral
L	lateral mammillary nucleus
LA	lateral amygdala
LD	laterodorsal thalamic nucleus
LT	lateral thalamus
MB	mammillary bodies
MD	mediodorsal thalamic nucleus
M-L	medial-lateral
ML	medial-lateral mammillary nucleus
MM	medial mammillary nucleus
MRI	magnetic resonance imaging
MT	medial thalamus
MTL	medial temporal lobe
MTT	mammillothalamic tract
NMDA	N-Methyl-D-aspartate
pCREB	phosphorylated c-AMP response element binding protein
PH	parahippocampal cortex
PL	prelimbic cortex
PPC	posterior parietal cortex
PRC	perirhinal cortex
PTD	pyrithiamine-induced thiamine deficiency
RAM	radial arm maze
Rdg	dysgranular retrosplenial cortex
Rga	granular a retrosplenial cortex
Rgb	granular b retrosplenial cortex
RNA	ribonucleic acid

RSC	retrosplenial cortex
SE	standard error
Sup.	superficial
TBI	traumatic brain injury
vHPC	ventral hippocampus

The anterior thalamus exists within an ‘extended hippocampal memory system’ and has extensive reciprocal connectivity with regions known to support spatial memory function such as the retrosplenial cortex (RSC). Damage to the anterior thalamic nuclei (ATN) in humans as a result of injury or neurodegenerative disease is associated with severe anterograde amnesia that is not therapeutically manageable. Rat models of ATN lesions have provided potential avenues of treatment through environmental enrichment, to ameliorate some of the lesion-induced deficits. Previously, behavioural recovery after enrichment did not accompany recovery of the striking immediate early gene (IEG) hypoactivation in the RSC found after ATN lesions, but the tasks used may not have been sensitive to RSC function. A modified radial arm maze (RAM) task sensitive to RSC lesions was therefore used to determine whether behavioural recovery was associated with improved expression of zif268, an IEG associated with spatial memory. Initially, water maze spatial tasks were used to establish spatial memory deficits prior to enrichment and to assess memory during the period of continuous enrichment and when overnight enrichment was continued thereafter. There was little or no evidence of recovery from substantial impairments in water maze memory tasks in rats with ATN lesions. However, subsequent testing on the RAM revealed clear, albeit partial, recovery of spatial memory in the enriched rats with ATN lesions. Nonetheless, levels of zif268 expression in the superficial layers of the granular RSC remained at the same level of hypoactivity of standard-housed ATN rats; instead, there was some evidence of recovered CA1 zif268 expression. ATN lesions were also associated with reduced cell counts in the mammillary bodies, which were also not recovered in enriched rats. These findings suggest that IEG expression in the RSC may not always be a critical biomarker for spatial memory function in rats.

1. Introduction

1.1 General Introduction

To acquire, store and retrieve new information is crucial to cognition. Anterograde amnesia represents a substantial deficit in this regard, particularly the formation of new episodic memories, and is often accompanied by partial minor retrograde amnesia. The onset of an anterograde amnesic syndrome typically follows traumatic brain injury, stroke, alcohol abuse or some form of neurodegenerative disease such as Alzheimer's disease or other dementias, which are debilitating for the sufferer and devastating for their families. Such patients often find themselves struggling to recall recent events and being unable to participate normally in daily life. Acceptable therapeutic management of anterograde amnesic syndromes has not yet been achieved, however, environmental enrichment methods applied in animal models of human brain injury show promise for the development of cognitive rehabilitation programs and other ameliorative efforts to counter these deficits.

Episodic memory is often referred to as 'episodic-autobiographical memory', which denotes conscious experience and recall of events in human memory systems (Markowitsch & Staniloiu, 2012). Episodic memory and semantic memory (memories for names and facts), comprise the declarative memory system. Recognition of items, their temporal context and spatial memory ('What', 'When', and 'Where') are different facets of episodic memory that are impaired in anterograde amnesia (Aggleton & Brown, 1999; 2006). By contrast, procedural memories do not rely on the declarative memory system and are largely spared (Aggleton, 2008). Eminent cases of anterograde amnesia such as that of Henry Molaison (H.M.) provide key human examples of severe anterograde and temporally-graded retrograde memory dysfunction, with sparing of procedural memory. Following a childhood accident, H.M. suffered debilitating and frequent epileptic seizures for much of his adolescence and

early adulthood, and received bilateral removal of the medial temporal lobe (MTL) in an attempt to lessen the severity of his epilepsy (Scoville & Milner, 1957). H.M.'s resulting condition focused investigation into the pathology of anterograde amnesia, and clinical research has therefore centred on the hippocampal formation and the adjacent medial temporal cortex (Squire, Stark & Clark, 2004; Markowitsch & Staniloiu, 2012; Aggleton, 2013).

Unlike the focus on the MTL, recent evidence has confirmed earlier studies that suggest anterograde amnesia is associated with widespread damage to multiple regions. Damage to diencephalic limbic system structures, such as the mammillary bodies (MB), anterior thalamic nuclei (ATN), and fibres of the mammillothalamic tract (MTT), alongside other thalamic regions are frequently implicated in cases presenting with anterograde amnesia (Van der Werf, 2003; Vann & Aggleton, 2004; Aggleton & Brown, 2006; Carlesimo, Lombardi & Caltagirone, 2011; Pergola & Suchan, 2013; Aggleton, 2014). This pathology suggests that perhaps anterograde amnesia is the result of damage or dysfunction in multiple interconnected brain regions (McKee & Squire, 1992; Squire & Zola, 1998). Clinical studies of the human brain have uncertain location and extent of injury (Van der Werf et al, 2003). Nonetheless, injury to the hippocampal formation (HF), MB, ATN and fornix have all been associated with human anterograde amnesia (Tsivilis et al, 2008; Aggleton et al, 2010).

Part of the thalamus, the ATN, is one region in which early degeneration or injury is consistently found in various conditions presenting with anterograde amnesia (Markowitsch & Staniloiu, 2012). The ATN has been a particular focus of recent neuroanatomical and behavioural research in animal models (Aggleton, Neave, Nagle & Hunt, 1995; Aggleton, Hunt, Nagle & Neave, 1996; Byatt & Dalrymple-Alford, 1996; see Aggleton, 2014 for review). The ATN is further subdivided into the subgroups of nuclei, the anteromedial (AM), anteroventral (AV) and anterodorsal (AD) nuclei, clearly distinguished in the rat through

histological and immunochemical procedures (Morel, Magnin & Jeanmonod, 1997; see Jankowski et al, 2013 for review). The AD and AV contain densely packed cells and are distinct in their homogeneous cell populations whereas the AM contains slightly larger, comparatively wider-spaced cells (Jankowski et al, 2013, see Figure 1.1). The AV and especially the AD contain head direction cells, which indicate heading direction irrespective of location (Tsanov et al, 2011; Aggleton & Nelson, 2014). The nuclei have connections with numerous cortical and subcortical regions, a large number of which are reciprocal, most notably with the retrosplenial cortex (RSC), hippocampal formation and prefrontal cortex (Van Groen & Wyss, 1990; 1992; 2003; Shibata, 1993; Van Groen, Kadish & Wyss, 1999; Shibata & Naito, 2005; Wright, Erichsen, Vann, O'Mara & Aggleton, 2010; Wright, Vann, Erichsen, O'Mara & Aggleton, 2013; See Jankowski et al, 2013 for review, see Figure 1.1).

1.2 Thiamine Deficiency, Wernicke's Encephalopathy and Korsakoff's Syndrome

'Korsakoff's psychosis' is characterised by severe anterograde episodic deficits with some retrograde memory impairments and dysexecutive syndrome but generally preserved semantic memory, procedural memory and general intelligence (Kopelman, 1995; Harding et al, 2000). Thiamine deficiency (vitamin B1) often arising from chronic alcoholism or malnutrition contributes to acute Wernicke's encephalopathy, a neuropsychiatric syndrome characterised by oculomotor abnormalities, cerebellar dysfunction, nutritional deficiencies, and an altered mental state (Caine, Halliday, Kril & Harper, 1997), which is often followed by Korsakoff's syndrome (Kopelman, 2014). Treatment with parenteral thiamine generally halts the progression of Wernicke's encephalopathy, although progression to Korsakoff's syndrome can occur in approximately 85% of cases if these efforts are delayed or unsuccessful (Kopelman, Thomson, Guerrini & Marshall, 2009).

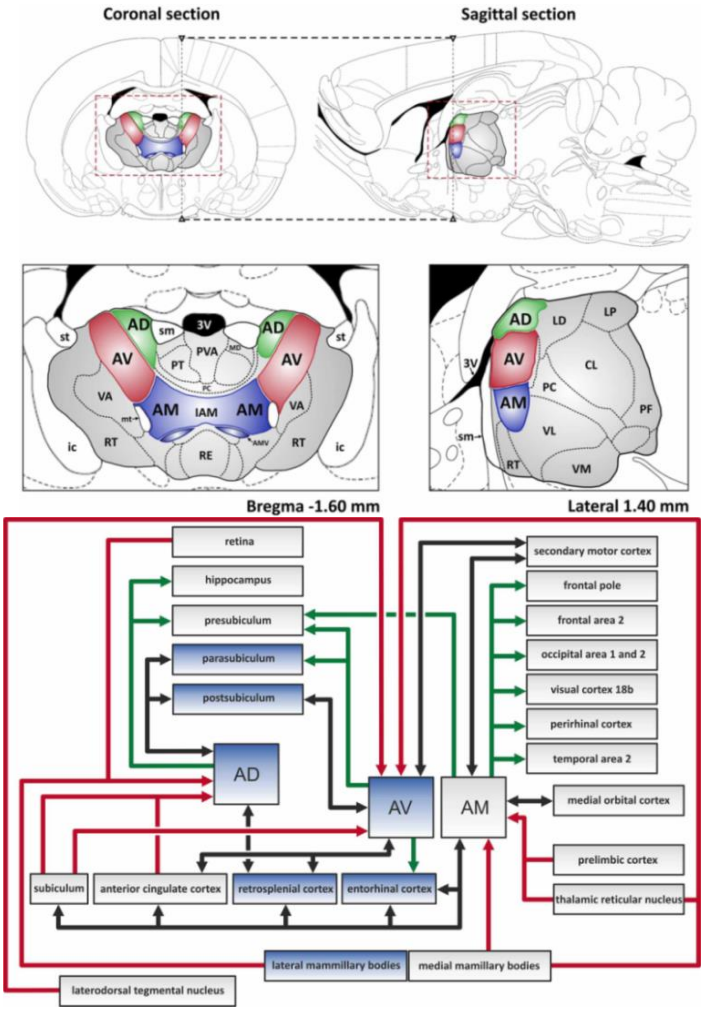


Figure 1.1. Location of the anterior thalamic nuclei (ATN) in coronal and saggital sections of the brain, segmentation of each of the nuclei comprising the ATN (coloured segments), and connections of the anterodorsal (AD), anteroventral (AV) and anteromedial (AM) nuclei. Black arrows indicate reciprocal connections, green arrows indicate efferents, and red arrows indicate afferents of the AD, AV and AM. The AD, AV and AM each have reciprocal connections with the retrosplenial cortex but have different patterns of connectivity with other regions: for example, the AD and AV have reciprocal connections with the para- and post-subiculum, whereas the AM projects to the pre-subiculum. Adapted from Jankowski et al, 2013.

Harding et al (2000) assessed detailed pathology in the diencephalon in healthy controls, alcoholic controls, and alcoholics with Wernicke's encephalopathy or the Korsakoff's syndrome. Widespread atrophy was evident in the latter two conditions with substantial neuronal loss in many brain regions, including the mediodorsal thalamic nucleus (MD). Damage to the ATN was found to be a strong predictor of the Korsakoff's syndrome, although damage and dysfunction in regions outside the diencephalon such as the hippocampal formation (Paller et al, 1997; Sullivan & Pfefferbaum, 2009), frontal lobe (Harper, Rodriguez & Perdices, 1989), and cerebellum (Sullivan, Deshmukh, Desmond, Lim,

& Pfefferbaum, 2000) in other cases suggests that the pathology of Korsakoff's syndrome is widespread. Further, Caulo et al (2005) discovered substantially reduced hippocampal activation in a Wernicke-Korsakoff's patient during encoding and recognition in memory tasks, despite an ostensibly healthy hippocampus. The thalamus is a region in which thiamine turnover is high and thus vulnerable to depletion (Sechi & Serra, 2007), and animal models of Korsakoff's syndrome show that the thalamus is particularly vulnerable to excitotoxic events following pyrithiamine-induced thiamine deficiency (PTD; Savage, Hall & Resende, 2012). Chronic administration of pyrithiamine, a selective antagonist of thiamine pyrophosphate, induces rapid depletion of brain thiamine levels (Langlais, 1995; Zhang et al, 1995). Within the ATN, significant neuronal loss in the AV nucleus in particular occurs in the PTD model in rats (Figure 1.2), with further neuronal loss in the intralaminar thalamic nuclei (ILN) and MB, and functional deactivation of the hippocampal formation, RSC and frontal cortex (Anzalone, Vetreno, Ramos & Savage, 2010; Savage, Hall & Resende, 2012).

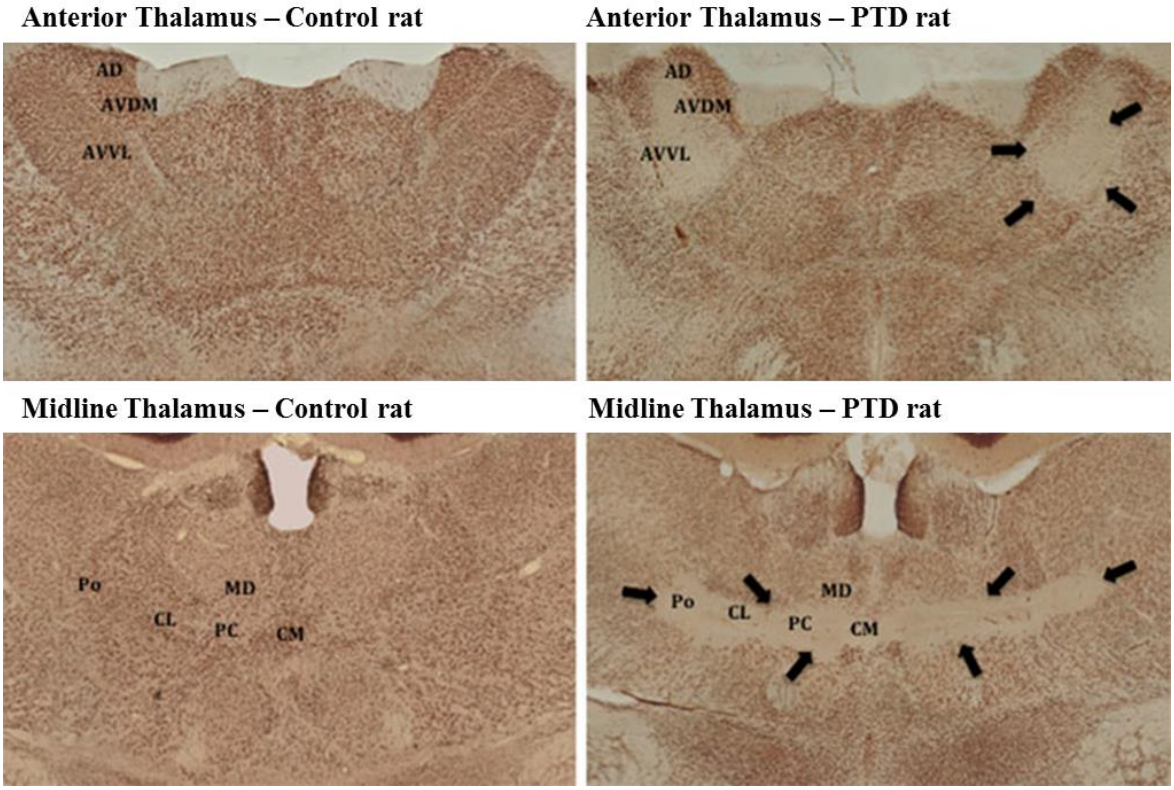


Figure 1.2. Photomicrographs of the ATN and midline thalamus in a control rat (left) and a rat with pyrithiamine-induced thiamine deficiency (PTD; right). Note the substantial cell loss in the anteroventral (AVVL and AVDM) and midline nuclei in particular, with some cell loss in other regions such as the anterodorsal thalamic nucleus (AD). Figure adapted from Savage, Hall & Resende (2012).

1.3 Thalamic Infarcts and Neurodegenerative Disease

In humans, damage to the thalamus occurs most commonly as a result of lacunar stroke, which can be associated with anterograde amnesia (Carlesimo, Lombardi & Caltagirone, 2011; Markowitsch & Staniloiu, 2012). In such cases, occlusion of the tuberothalamic or anterior choroidal arteries which supply the ATN and MTT, can lead to relatively localised lacunar infarcts in one or more of the MTT, MD and ILN as well as the ATN, and can be either unilateral or bilateral (Carlesimo, Lombardi & Caltagirone, 2011). For example, Van der Werf and colleagues (2003) identified the anterior, midline thalamic nuclei and MTT as primary correlates of amnesia in the cases of 22 patients with thalamic infarcts, with attentional impairments occurring irrespective of lesion location. Such evidence of extensive anterograde amnesia, some concurrent retrograde amnesia, executive dysfunction and other behavioural impairments following thalamic infarcts is echoed in a later review where the ATN and MTT were found to strongly predict the amnesic syndrome, with recollective rather than familiarity processes primarily disrupted (Carlesimo, Lombardi & Caltagirone, 2011).

Perren, Clarke & Bogousslavsky (2005) analysed the recovery of 12 patients who suffered infarcts to multiple thalamic nuclei as a result of polar arterial occlusion which can cause injury to the ATN, laterodorsal, dorsomedial, ventroanterior, and ventrolateral thalamic nuclei. Initially, the patients all presented with severe anterograde memory deficits, but those with bilateral infarcts had greater severity of amnesic syndrome. Bilateral lesions of the anterior and dorsomedial thalamus were associated with the worst outcomes, with most patients suffering from severe global memory impairment, behavioural impairments, and oculomotor disturbances, indicating that the severity of deficits may correlate with the size and location of infarcts.

Among neurodegenerative diseases such as Alzheimer's disease, degeneration in the thalamus appears to occur early (Teipel et al, 2007; de Jong et al, 2008). In severe cases of Alzheimer's disease, amyloid plaques have been found to be located in almost every thalamic nucleus, but relatively early in the AD region (Braak & Braak, 1996). Zarei et al (2010), using diffusion tensor imaging and structural magnetic resonance imaging (MRI) methods, found substantial thalamic atrophy in Alzheimer's patients. Additional MRI and neuropsychological testing of Alzheimer's disease patients by Di Paola et al (2007) found significant volume reductions in multiple regions, particularly in the left ATN and cortical regions, which were correlated with episodic memory impairments. Degeneration of the ATN also occurs in individuals with motor neuron disease (Anderson, Cairns & Leigh, 1995).

Clinical evidence of thalamic infarcts and other injury relies heavily upon single case and small participant studies, with lesions extending into multiple regions and potentially disrupting multiple systems. Damage to surrounding structures and fibre tracts such as the MTT, a primary source of hippocampal input to the ATN, as well as the MD and ILN, can also result in deficits, with damage to the MTT in particular providing a strong predictor of amnesic syndrome (Carlesimo, Lombardi & Caltagirone, 2011). Together with evidence that multiple thalamic regions are vulnerable to degeneration in neurodegenerative disease, damage to multiple regions appears to be associated with amnesia.

1.4 Animal Models of Anterior Thalamic Lesions

The introduction of animal models allowed investigators to overcome the frustrations of relying on single case studies with diffuse pathology and examine different types of diencephalic injuries associated with anterograde amnesia. These models also allow the development of therapeutic interventions. Rats are most commonly used, supported by

evidence that there are behavioural and neuroanatomical similarities between rats and primates across an array of neurological disorders (Cenci, Whishaw & Schallert, 2002).

Like human research, animal models of diencephalic amnesia have implicated several regions in 'episodic-like' memory deficits. Lesions targeting the ATN in rats have provided the most reliable assay of such deficits after thalamic brain injury (Aggleton & Brown, 1999; Aggleton, 2010). As previously mentioned, the ATN has dense reciprocal connections with regions such as the RSC and hippocampal formation (Figure 1.1). These connections reinforce the significant role of the ATN in spatial navigation, as the RSC and hippocampal formation are strongly implicated in this function (Maguire, 1998; Ekstrom et al, 2003; Epstein, 2008; Hartley, Lever, Burgess & O'Keefe, 2014). The 'What' 'When' and 'Where' aspects of episodic memory have been assessed through multiple behavioural paradigms in rats with ATN lesions, with perhaps most emphasis placed on spatial memory, that is, 'What' and 'Where' (Aggleton & Brown, 1999). A significant majority of spatial memory paradigms assess allocentric spatial memory which is the ability to navigate through use of external cues that, by their location, inform the rat of its position in space independently of local or internal cues. By contrast, egocentric spatial memory is the ability to navigate using cues such as head direction and body turns, irrespective of external cues. To solve spatial memory tasks, intact rats probably rely on a combination of egocentric and allocentric strategies, such as using head direction and distal cues for navigation on a T-maze (Futter & Aggleton, 2006).

A summary of studies primarily assessing spatial memory performance after ATN lesions is provided in Table 1.1. Of particular interest are the striking and consistent deficits on a range of allocentric spatial memory tasks such as the radial arm maze, t-maze and water maze when compared with a lack of impairment on tasks that require egocentric strategies (Aggleton, Hunt, Nagle & Neave, 1996; Warburton, Baird & Aggleton, 1997; Sziklas & Petrides, 1999; 2007). Modifications to the standard RAM, such as a delayed non-matching

to sample task (Mair, Burk & Porter, 2003), and a version in which rats were required to recall the constant location of baited and unbaited arms throughout training, also reveal significant deficits in rats with ATN lesions (Byatt & Dalrymple-Alford, 1996; Harland, Collings, McNaughton, Abraham & Dalrymple-Alford, 2014). In terms of other tasks, no deficits were found on object recognition tasks (Aggleton, Neave, Nagle & Hunt 1995; Warburton & Aggleton, 1999; Wilton, Baird, Muir, Honey & Aggleton, 2001; Moran & Dalrymple-Alford, 2003) but severe impairments were found on some temporal order memory tasks (Wolff, Gibb & Dalrymple-Alford, 2006). Recent research also found that rats with ATN lesions are impaired when required to make judgements on some object recency tasks (Dumont & Aggleton, 2013).

In contrast to the clear spatial working and reference memory task deficits shown in rats with ATN lesions, conditional and discriminative place learning tasks have shown mixed deficits, with no impairments found on egocentric discrimination and some associative tasks, whereas deficits are found on object in place and object in place conditional tasks (Aggleton, Hunt, Nagle & Neave, 1996; Warburton, Baird & Aggleton, 1997; Sziklas & Petrides, 2004; Dumont, Amin & Aggleton, 2014; Dumont, Wright, Pearce & Aggleton, 2014). In a modification to the spatial reference memory version of the water maze, Moreau and colleagues (2013) used visual cues to guide the rat to a safe platform location. ATN rats were substantially impaired on the spatial discrimination task in which the specific visual cue was redundant, but were not impaired on a visual pattern discrimination task in which spatial location was redundant. Further discrimination and associative learning tasks used by Gibb, Wolff & Dalrymple-Alford (2006) indicated that ATN rats were unable to form associations between odours and spatial locations. It appears, therefore, that the impairments found after ATN lesions may be task dependent, with deficits most strongly arising on tasks that require the use of externally-defined spatial representations.

Table 1.1. Summary of studies that include spatial memory tests after anterior thalamic lesions

Year	Authors	Lesion site/method	Behavioural tasks and training	Behavioural deficits
2014	Dumont, Amin & Aggleton	ATN/NMDA	1. Biconditional discrimination (temperature/auditory) 2. Biconditional learning (context/place) 3. Spatial bi/unidirectional discrimination 4. Place biconditional discrimination	ATN not impaired on 1, impaired on 2 (place only), 3 and 4
2014	Dumont, Wright, Pearce & Aggleton	ATN/NMDA	1. Passive geometric place learning 2. Passive/active place learning (colour arrangement) 3. T-maze alternation 4. Passive/active place learning (cue arrangement)	ATN impaired on 1 and 3, initial acquisition impairment on 2 and 4
2014	Ulrich, Aitken, Abraham, Dalrymple-Alford & McNaughton	ATN/NMDA	1. T-maze spatial working memory i. 1-week training break relearning ii. 15-week training break relearning	ATN impaired on 1, 2 and 3
2013	Dumont & Aggleton	ATN/NMDA	1. T-maze spatial alternation 2. Bow-tie maze object recognition i. Object recency between-block iii. Object recency within-block 5. Odour recognition 6. Odour recency between-block 7. Object recognition open arena 8. Locomotor activity	ATN impaired on 1 and 4 ATN not impaired on 2, 3, 5, 7 ATN showed mild impairment in discrimination between sample phases on 6 ATN showed greater hyperactivity on 8
2013	Mendez-Lopez, Arias, Bontempi & Wolff	ATN/NMDA	1. Radial arm maze (8-arm) spatial discrimination	ATN impaired on 1
2013	Moreau et al	ATN, ILN-LT/NMDA	1. Water maze spatial discrimination 2. Visual pattern discrimination	ATN impaired on 1, not impaired on 2 ILN-LT not impaired on 1 and 2
2011	Aggleton, Amin, Jenkins, Pearce & Robinson	ATN/NMDA	1. T-maze spatial alternation 2. Sequence learning	ATN impaired on 1, not impaired on 2

Year	Authors	Lesion site/method	Behavioural tasks and training	Behavioural deficits
2010	Dumont, Petrides & Sziklas	Fornix-RSC ATN-H-RSC-Ipsi ATN-H-RSC-Contra RF and/or Ibotenic	1. Spatial-visual conditional associative learning 2. Radial arm maze (8-arm) spatial working memory	Fornix-RSC, ATN-H-RSC-Contra severely impaired, ATN-H-RSC-Ipsi impaired on 1 Fornix-RSC, ATN-H-RSC-Contra and -Ipsi impaired on 2
2009	Lopez et al	ATN, ILN-LT/NMDA	1. Water maze spatial reference memory i. Recent (5 days) probe ii. Remote (25 days) probe	ATN impaired on 1, also 2 and 3 due to failure on 1 ILN-LT impaired on 3, not impaired on 1 and 2
2008	Wolff, Gibb, Cassel & Dalrymple-Alford	ATN, ILN/NMDA	1. Water maze spatial reference memory 2. Radial-arm water maze (8-arm) egocentric spatial memory	ATN impaired on 1, not impaired on 2 ILN not impaired on 1 and 2
2007	Sziklas & Petrides	ATN/Electrolytic	1. Visual-spatial conditional associative task 2. Radial arm maze (8-arm) spatial working memory	ATN not impaired on 1, impaired on 2
2006	Frohardt, Bassett & Taube	AD, DTN/NMDA	1. Path integration food carrying, visual/blindfolded	AD mildly impaired on 1 DTN severely impaired on 1
2006	Gibb, Wolff & Dalrymple-Alford	ATN, MT, LT/NMDA	1. Odour-place paired-associate task 2. Odour discrimination 3. Spatial discrimination	ATN and LT impaired on 1, MT not impaired ATN impaired acquisition on 2 & 3, LT and MT not impaired
2006	Mitchell & Dalrymple-Alford	ATN, LT/NMDA	1. Elevated plus maze: response working memory 2. Radial arm maze (8-arm) spatial working memory	LT impaired on 1, not impaired on 2 ATN impaired on 2, not impaired on 1
2006	Wolff, Gibb & Dalrymple-Alford	ATN/NMDA	1. Temporal order memory for odour sequences 2. Odour recognition memory i. Task-reversal	ATN impaired on 1, not impaired on 2 and 3
2004	Henry, Petrides, St-Laurent & Sziklas	ATN-HPC-Contra/Ibotenic	1. Visuospatial conditional associative learning 2. Forced spatial alternation with delay	ATN-HPC-Contra impaired on 1 and 2
2004	Sziklas & Petrides	ATN, HPC, MB/Electrolytic	1. Egocentric visual-spatial conditional associative learning	ATN and MB not impaired on 1, HPC impaired

Year	Authors	Lesion site/method	Behavioural tasks and training	Behavioural deficits
2003	Mair, Burk & Porter	ATN/NMDA, PH/Radiofrequency ATN-PH/NMDA+ Radiofrequency	1. Radial arm maze (8-arm) delayed non-matching task	ATN, PH comparable deficits, ATN-PH delay-dependent deficits on 1 ATN delay-dependent deficits on 1 at 5-6 weeks post-surgery
2003	Moran & Dalrymple-Alford	ATN, PRC/NMDA	1. Spontaneous object recognition 2. Radial arm maze (12-arm) spatial working memory i. Delay task 4. Elemental cue task 5. Configural cue task	ATN and PRC not impaired on 1 ATN impaired on 2 and 3, not impaired on 4 and 5 PRC impaired on 4 and 5, not impaired on 2 and 3
2002a	van Groen, Kadish & Wyss	AD/AV, AD/AV+ AD, AV, AM/Ibotenic	1. Water maze spatial working memory i. Immediate probe	AD/AV mild impairment on 1 and 2 AD/AV+ impaired on 1 and 2 AD, AV AM severe impairment on 1 and 2
2001	Alexinsky	ATN, MD/Ibotenic, RSC, PPC/Excision	1. RAM (8-arm) spatial reference-working memory 2. RAM (8-arm) spatial reference-working memory, new spatial location 3. Contextual change	ATN and MD impaired on 1 ATN, MD and PPC impaired on 2 ATN impaired on 3
2001	Warburton, Baird, Morgan, Muir & Aggleton	ATN-HPC-Contra /NMDA, ATN-HPC-Ipsi/NMDA, HPC+/NMDA	1. T-maze spatial forced alternation 2. Water maze spatial reference memory 3. RAM (8-arm) spatial working memory	ATN-HPC-Contra impaired on 1, 2 and 3 ATN-HPC-Ipsi not impaired on 1, 2 and 3
2001	Wilton, Baird, Muir, Honey & Aggleton	AD-LD/NMDA	1. T-maze spatial forced alternation 2. Water maze spatial working memory 3. Spontaneous object recognition 4. Novel object-in-place	AD-LD impaired on 1, 2 and 4, not impaired on 3
1999	Sziklas & Petrides	ATN/Electrolytic	1. Spatial-visual associations 2. RAM (8-arm) spatial working memory 3. T-maze conditional egocentric task	ATN impaired on 1 and 2 ATN not impaired on 3
1999	Warburton & Aggleton	ATN/NMDA, Fornix/Radiofrequency	1. Water maze spatial reference memory 2. T-maze spatial forced alternation 3. Object recognition	ATN and Fornix impaired on 1 and 2 ATN and Fornix not impaired on 3

Year	Authors	Lesion site/method	Behavioural tasks and training	Behavioural deficits
1999	Warburton, Morgan, Baird, Muir & Aggleton	ATN/NMDA, Fornix/Radiofrequency ATN+/NMDA	1. Water maze spatial reference memory 2. T-maze forced alternation	ATN and Fornix comparable impairments on 1 and 2 ATN+ severely impaired on 1 and 2
1997	Warburton, Baird & Aggleton	ATN/NMDA, ATN+LD/NMDA, Fornix/Radiofrequency	1. T-maze spatial forced alternation 2. Cross-maze allocentric alternation 3. Egocentric discrimination	ATN, Fornix and ATN/LD impaired acquisition on 1 ATN, Fornix and ATN/LD impaired on 2 ATN, Fornix and ATN/LD not impaired on 3
1996	Byatt & Dalrymple-Alford	AV, AM/Radiofrequency	1. RAM (12-arm) spatial reference and working memory	Both AV and AM impaired on 1
1996	Aggleton, Hunt, Nagle & Neave	AV/AD, AM, ATN.T/NMDA	1. T-maze spatial forced alternation 2. Cross-maze allocentric alternation 3. RAM (8-arm) spatial working memory 4. Egocentric discrimination	AM and AV/AD impaired acquisition on 1, not impaired on 2 AV/AD mild impairment on 3, AM not impaired ATN.T impaired on 1, 2 and 3 ATN.T, AM and AV/AD not impaired on 4
1995	Aggleton, Neave, Nagle & Hunt	ATN/NMDA, MB/NMDA and Fornix/Radiofrequency	1. T-maze spatial forced alternation 2. Object recognition	ATN, MB and Fornix lesions impaired acquisition, delay-dependent deficits on 1 ATN, MB and Fornix not impaired on 2

Abbreviations: AD: anterodorsal thalamic nucleus, AM: anteromedial thalamic nucleus, ATN: anterior thalamic nuclei, ATN.T: total ATN lesion, AV: anteroventral thalamic nucleus, Contra: contralateral, DTN: dorsal tegmental nucleus, HPC/H: hippocampus, ILN: intralaminar nuclei, Ipsi: ipsilateral, LD: laterodorsal thalamic nucleus, LT: lateral thalamus, MB: mammillary bodies, MD: mediodorsal nuclei, MT: medial thalamus, NMDA: N-Methyl-D-aspartate (neurotoxin), PH: parahippocampal cortex, PPC: posterior parietal cortex, PRC: perirhinal cortex, RAM: radial arm maze, RSC: retrosplenial cortex.

NB: Additional studies can be found in Table 1.2 and Table 1.3; these studies address the effects of ATN lesions on immediate early gene expression, and recovery of function after environmental enrichment respectively.

Although ATN lesions induce severe and persistent deficits in spatial memory, further examination of the effects on spatial memory after damage to individual nuclei or incomplete ATN lesions has produced mixed findings. Byatt & Dalrymple-Alford (1996) reported that both AV and AM radiofrequency lesions were associated with significant deficits on a spatial working and reference memory task in the RAM, indicating that the integrity of both nuclei was important for the task. To determine whether selective or total damage to the ATN would result in similar deficits on an array of spatial memory tasks and an egocentric discrimination task, Aggleton, Hunt, Nagle & Neave (1996) induced neurotoxic damage to the AM, the AV and AD (AV/AD), and the total ATN (ATN-T). Rats with AM lesions showed impaired acquisition on T-maze spatial forced alternation, which was more pronounced in rats with AV/AD lesions than with AM lesions, while AV/AD rats showed mild deficits on the RAM whereas rats with AM lesions were unimpaired. The most striking deficits arose in the ATN-T group which showed substantial impairments on the t-maze, cross maze, and RAM, indicating that rats with total ATN lesions were severely impaired in their ability to use allocentric cues, whereas rats with less than total ATN lesions were able to use allocentric cues. Van Groen, Kadish & Wyss (2002a) also found that AD and AV combined lesions were associated with less impairment on a spatial working memory task in the water maze than AD and AV lesions that encroached on the AM, and complete ATN lesions.

1.5 The Extended Hippocampal Memory System

As damage to both the ATN and the hippocampal formation has been noted to be sufficient to induce anterograde amnesia, and in consideration of their interconnectivity, recent research has attempted to address whether these regions function as a system. To examine whether the ATN and hippocampus function cooperatively to support spatial memory, Warburton, Baird, Morgan, Muir & Aggleton (2001) created unilateral lesions to the hippocampus and ATN in either the ipsilateral or contralateral hemispheres in rats, with results indicating that rats with

contralateral lesions were impaired on several spatial memory tasks when the inter-hemispheric hippocampal commissure was also severed.

In 1937, James Papez proposed a neural network which he believed to underlie emotional function, emphasising connections between regions within the MTL such as the hippocampal formation, and regions within the diencephalon, such as the ATN and MB, along with pathways such as the MTT and fornix. The circuit was further investigated by Delay & Brion (1969), who instead implicated the circuit in anterograde amnesia. Until recently, however, this circuit received little attention in research into the pathology of anterograde amnesia, until Aggleton & Brown (1999) observed that the considerable connectivity of the ATN, hippocampal formation, RSC, fornix, subicular complex and the MB, along with recent disconnection and tracing evidence suggested that these regions exist as part of an 'extended hippocampal memory system' supporting episodic memory (see Figure 1.3).

As previously discussed, disconnection studies have noted that the hippocampal formation and the ATN perform in synchrony to support spatial memory function (Aggleton & Brown, 1999; 2006; Warburton, Baird, Morgan, Muir & Aggleton, 2001). Standard disconnection as a different lesion model of other regions known to support spatial memory function using fornix lesions, for example, have occasionally produced only mild deficits, suggesting that the additional connectivity of regions that the fornix innervates (such as the MB, from the hippocampal formation) with other regions known to be important for spatial memory such as the ATN, may allow compensatory support for episodic memory (Vann, Erichsen, O'Mara & Aggleton, 2011). The MB has also been found to contribute to similar memory functions as the ATN (Dillingham, Frizzati, Nelson & Vann, 2014). Within the MB, the medial mammillary nuclei project to the AM and AV, while the lateral mammillary nuclei project to the AD (Jankowski et al, 2013; Dillingham, Frizzati, Nelson & Vann, 2014).

Experimental damage to the MTT, MB projection fibres to the ATN, is also strongly implicated in anterograde amnesia (Dillingham, Frizzati, Nelson & Vann, 2014). Unilateral damage to the AD in cats has been associated with a corresponding unilateral cell loss in the lateral MB (Fry & Cowan, 1972) and damage to the medial thalamus in monkeys was also associated with cell loss in the MB, although damage to the MTT had also occurred which may have caused further disruption to neuronal function in the MB (Aggleton & Mishkin, 1983). The neuron loss in the MB after ATN lesions may be due to the MB cells losing their efferent targets in the ATN, and further supports the proposal of a functional circuit supporting anterograde episodic memory.

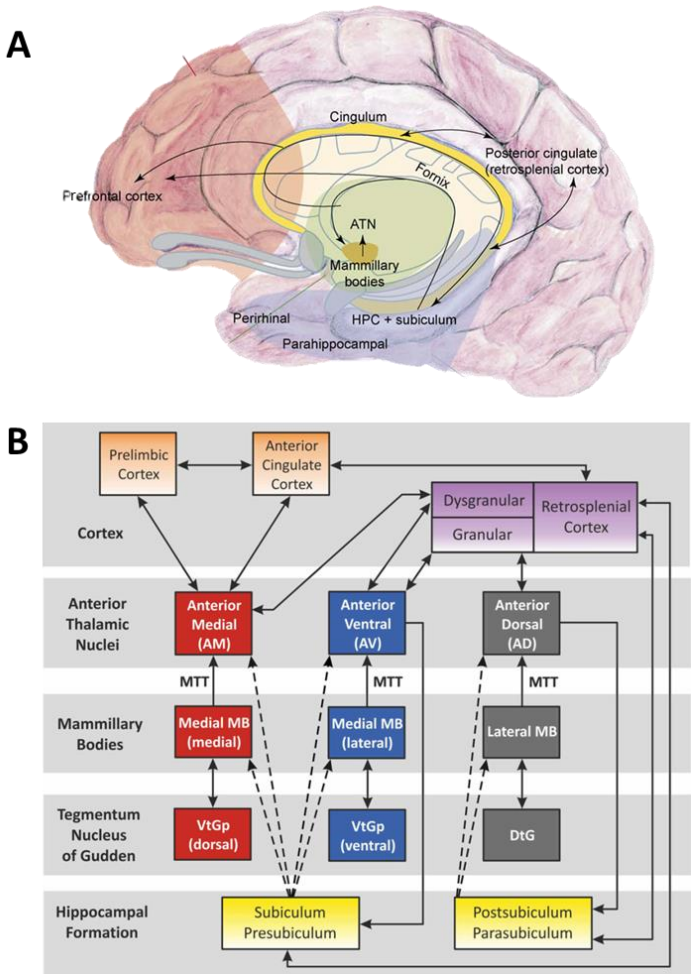


Figure 1.3. The extended hippocampal system outlined by Aggleton & Brown (1999; 2006) in terms of both anatomy (A), indicating the main connections between regions within the system, and connectivity (B): dashed lines indicate connections via the fornix, single-headed arrows indicate unidirectional connections and double-headed arrows indicate reciprocal connections. Figure adapted from (A); Aggleton & Brown 2006, (B); Jankowski et al 2013.

1.6 Covert Pathology and Immediate Early Genes

Additional evidence for the existence of neural networks supporting episodic memory function arose through the discovery of 'covert pathology', specifically immediate early gene (IEG) hypoactivation in the RSC following ATN lesions. In other regions such as the CA1 region of the hippocampus, covert pathology has been found in terms of reduced dendritic spine density after ATN lesions (Harland, Collings, McNaughton, Abraham, & Dalrymple-Alford, 2014), although significant reductions in IEG measures such as c-Fos have not been observed (Jenkins, Dias, Amin, Brown & Aggleton, 2002). The pathology observed in the RSC following ATN lesions is described as covert as there are no direct lesions to the RSC following distal ATN damage, but significant neuronal hypoactivation (Poirier & Aggleton, 2009; see Figure 1.4). The RSC is distal to the ATN, but has direct connections with the AM, AV and AD, as well as with other regions of the extended hippocampal system such as the subicular complex and CA1 (Van Groen & Wyss, 1990; 1992; 2003; Cenquizca & Swanson, 2007; Aggleton, Saunders, Wright & Vann, 2014; see Figure 1.5).

Figure 1.4. Zif268 immunoreactivity in the granular b retrosplenial cortex (Rgb) after unilateral lesions to the ATN at post-lesion intervals of 1 week, 2 weeks and 8 weeks. 'Intact' = Rgb region contralateral to the ATN lesion; Lesion = Rgb region ipsilateral to the ATN lesion. Significant reductions in zif268-positive cells are evident in the superficial layer at the 1-week interval, with this reduction extending to the deep layers at the 8-week lesion interval. Adapted from Poirier & Aggleton, 2009.

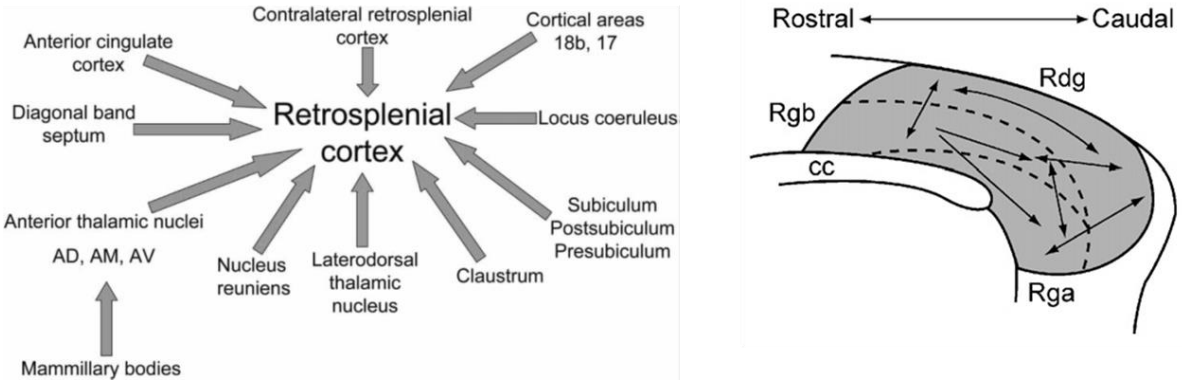


Figure 1.5. Extrinsic inputs (left) and intrinsic (right) connections for the granular (Rga and Rgb) and dysgranular (Rdg) sub-regions of the RSC. Adapted from Aggleton, 2008 (left) and 2010 (right).

The RSC consists of areas 29 and 30, sometimes described as the posterior cingulate cortex, and is comprised of several different sub-regions: the granular RSC consisting of the more caudally-placed granular a (Rga) and the more rostral granular b (Rgb) cortices (area 29), and the dysgranular RSC (Rdg; area 30). As indicated in Figure 1.5, the Rga, Rgb and Rdg are interconnected, although with different extrinsic connections: the granular regions have considerable interoceptive (related to visceral function) connectivity, whereas the Rdg has dense connections with the visual system (Shibata, Honda, Sasaki & Naito, 2009). The significant connectivity of the Rdg with the visual system has implicated the Rdg in visually aided spatial navigation such as extramaze cue use, while the interoceptive connectivity of the granular regions perhaps suggest a role in idiothetic cue use such as path integration and head direction (Aggleton, 2010). Given the considerable intrinsic connections of the RSC, it has been postulated that the granular and dysgranular regions may function as an integrated whole (Aggleton, 2010).

IEGs are a class of gene that are activated in a rapid and brief manner in response to stimuli that induce neuronal activation. Of the known 30-40 neuronal IEGs, 10-15 act as

'regulatory IEGs' (such as zif268 and c-Fos), because their protein products are inducible transcription factors that induce a cascade of changes that can affect downstream neurons (through an increase or decrease of downstream gene expression), which is achieved by encoding proteins. As the presence of IEGs coincides with neuronal activation, it is thought to be a product of this process (Davis, Bozon & Laroche, 2003). Although this observation has not yet been explicitly linked to any changes in behaviour, increases in RNA levels of three IEGs (zif268, Arc and c-Fos) have been observed in the dorsal hippocampus following training in a spatial water maze task (Guzowski, Setlow, Wagner & McGaugh, 2001), and the presence of CREB and c-Fos have been associated with improved spatial memory performance (Czajkowski et al, 2014). Additionally, some IEGs appear to be necessary for the consolidation of long-term memories and, if one of each of these is inactivated, the ability to recall previously learned (24hr+ timeframe) information is disrupted (Davis, Bozon & Laroche, 2003). This suggests that each of the IEGs is important, but none alone may be sufficient for memory consolidation. Given the current evidence that inhibition of gene translation and protein synthesis can disrupt consolidation but not initial learning, IEGs such as zif268 may be important for memory consolidation.

Table 1.2 provides a summary of studies that have assessed the levels of IEG activation in rats after ATN lesions, most of which focus on zif268 and c-Fos. ATN lesions rapidly produce marked reductions of both c-Fos and zif268 immunoreactivity in the Rgb and Rga regions of the RSC (Jenkins, Dias, Amin, Brown & Aggleton, 2002; Poirier et al, 2008). Minor changes in cell morphometry in the RSC have also been observed (Poirier & Aggleton, 2009). Earlier intervals between lesion surgery and sacrifice are associated with a restriction of hypoactivation to the superficial layers (layers II and upper III) of these regions (Jenkins, Vann, Amin & Aggleton, 2004; Poirier & Aggleton, 2009). As the interval between surgery and sacrifice increases, IEG hypoactivation appears to extend into the deep laminae of the

Rgb and Rga, with the Rdg region also showing reduced IEG counts (Jenkins, Vann, Amin & Aggleton, 2004; Poirier & Aggleton, 2009). Additional regions such as the CA1 area of the hippocampus and the subiculum also sometimes show reduction in c-Fos and zif268 after ATN lesions, suggesting that this 'covert pathology' extends to other regions of the extended hippocampal system (Jenkins, Dias, Amin & Aggleton, 2002; Jenkins, Dias, Amin, Brown & Aggleton, 2002; but see Dumont, Amin, Poirier, Albasser & Aggleton, 2012, and Dupire et al, 2013).

Levels of c-Fos and zif268 are generally increased by introducing animals to stimuli prior to sacrifice to reduce the possibility of floor effects. Generally, the induction stimulus includes some form of novelty such as a new cage, new testing or holding room, or novel test cues (Jenkins, Dias, Amin & Aggleton, 2002; Jenkins, Dias, Amin, Brown & Aggleton, 2002; Jenkins, Vann, Amin & Aggleton, 2004; Poirier et al, 2008; Poirier & Aggleton, 2009; Dumont, Amin, Poirier, Albasser & Aggleton, 2012). Comparisons between studies often prove difficult as levels of zif268 increase after spatial memory tasks and zif268 immunoreactivity shows a marked decrease in aged rats (see Knapska & Kaczmarek, 2004 for review) although different rat strains show similar patterns of c-Fos and zif268 expression (Poirier & Aggleton, 2009). In spite of the multiple factors that may influence the degree of IEG immunoreactivity in rats, it appears that c-Fos and zif268 activity is consistently reduced in the RSC after ATN lesions.

Table 1.2. Summary of studies that assess immediate early gene activation after anterior thalamic lesions

Year	Authors	Age at surgery	Duration between surgery and sacrifice	IEG induction procedure	Previous training	Lesion extent and lesion effects	IEG measure and regions analysed	Outcome relative to IEG measure
In press	Loukavenko, Wolff, Poirier & Dalrymple-Alford	5-6 months	Approx. 10 weeks	T-maze with 90 min in dark room after each trial (for 3 trials)	T-maze spatial working memory	Bilateral ATN lesions – assigned to receive enriched/standard housing and cerebrolysin/saline treatments ATN rats with enrichment or cerebrolysin had greater accuracy in the t-maze task than standard-housed or saline ATN rats	c-Fos examined in the PL, IL, ACC, somatosensory and motor cortex, dHF and RSC.	ATN lesions reduced Fos counts in the sup. and deep laminae of the Rgb across all treatment and housing groups. In the Rga, ATN lesions were associated with reduced Fos counts in only the treatment or enriched groups Enrichment associated with c-Fos hypoactivity in the sup. Rga.
2013	Dupire et al	Not stated	Approx. 6 weeks	Delay of 60min between final contextual fear task and sacrifice	Open field Elevated plus maze Contextual fear conditioning	Bilateral ATN lesions – assigned to enriched or standard housing for 25 days For ATN rats: reduced plasma corticosterone, increased exploration of novel arms in elevated plus maze and in the open field and slowed acquisition of contextual fear conditioning	c-Fos and pCREB examined in the amygdala (BLA and LA), HF, subiculum and RSC (sup. and deep).	ATN lesions reduced Fos and pCREB counts in the BLA and sup. and deep laminae of the Rgb. Fos counts were also reduced in the ventral subiculum and sup. lamina of the ACC. pCREB counts also reduced in dCA1 and vCA1. Enrichment was associated with dCA1 Fos hypoactivity.
2012	Dumont, Amin, Poirier, Albasser & Aggleton	4 months	Approx. 7 weeks	**1.** Novel and familiar object exploration **2.** Radial arm maze (8-arm) forced choices in novel room with novel cues	**1.** No previous training **2.** Radial arm maze (8-arm) with forced choices	**1.** Unilateral ATN lesions (split into novel or familiar object groups)- novel group showed improved recency discrimination **2.** Bilateral ATN lesions- forced choices on RAM, thus no group differences	**1.** zif268 in the PL, IL, PRC, HF, dSub and post-sub, Rdg, Rgb and Rga. **2.** zif268, CREB, pCREB and GAP-43 in same regions	**1.** Reduced zif counts in Rgb and post-sub. Novel object task associated with changes in HF zif **2.** Reduced zif counts in Rgb and post-sub, and reduced pCREB counts in Rgb.

Year	Authors	Age at surgery	Duration between surgery and sacrifice	IEG induction procedure	Previous training	Lesion extent and lesion effects	IEG measure and regions analysed	Outcome relative to IEG measure
2009	Poirier & Aggleton	2-3 months	**1.** 1, 2, 4 and 8 weeks **2.** 4 weeks or 1 year **3.** 4 weeks **4.** 3.5-4.5 months	**1.** Novel room and cage **2, 3 and 4.** Novel room with activity cages with beams for locomotor activity	No previous training	**1.** Unilateral ATN lesions **2.** Bilateral ATN lesions- ATN rats were hyperactive when compared with sham rats **3.** Unilateral ATN lesions **4.** Unilateral LD lesions	**1.** c-Fos and zif268 in Rgb (sup. and deep) **2 and 3.** c-Fos in the Rgb and Rdg (sup. and deep) **4.** c-Fos and zif268 in the Rdg and Rgb (sup. and deep).	**1.** Greatest zif and Fos hypoactivity in the 1-week group, primarily in sup. laminae. Deep laminae counts reduced after week 8 in Fos and week 4 (only) in zif. **2.** 4-weeks: reduced counts only in sup. of Rgb. 1 year: reduced counts in sup. Rgb and Rdg, and deep Rdg. **3.** Reduced counts in sup. and deep Rdg and Rgb. **4.** No change in c-Fos and zif counts from above results
2008	Poirier et al	Not stated	6-9 weeks	Novel room and cage with visual stimuli	No previous training	Unilateral ATN lesions	c-Fos and multiple other transcription factors examined in the Rgb region	Reduced c-Fos counts in Rgb on lesion side relative to intact side, as well as in multiple other transcription factors
2004	Jenkins, Vann, Amin & Aggleton	Not stated	**1.** 6-13 weeks **2.** 9-10 months	**1.** Foraging in novel room **2.** Activity box in novel room	No previous training	**1 & 2.** Bilateral ATN lesions **2.** Postrhinal cortex lesions	c-Fos and zif268 examined in both lesion groups, with subregions of RSC analysed	**1 & 2**: ATN lesions: reduced Fos counts in sup. Rga and Rgb **2**: ATN lesions: reduced Fos and zif counts in sup. Rga, Rgb and deep Rgb, also Rdg. Postrhinal lesions: no change in zif or Fos counts
2002	Jenkins, Dias, Amin & Aggleton	Not stated	>15 days	Radial arm maze (8-arm) spatial working memory with dark box after trial	Radial arm maze (8-arm) spatial working memory	Unilateral ATN lesions ATN rats not impaired	c-Fos, with HF, subicular, limbic and PH cortices on the lesion side compared to the intact side.	Lesions associated with reduced counts in post- and pre-sub, DG, dHPC, CA1, and RSC

Year	Authors	Age at surgery	Duration between surgery and sacrifice	IEG induction procedure	Previous training	Lesion extent and lesion effects	IEG measure and regions analysed	Outcome relative to IEG measure
2002	Jenkins, Dias, Amin, Brown & Aggleton	14 weeks	Approx. 5 weeks	Radial arm maze (8-arm) spatial working memory in a novel room for one trial	T-maze spatial alternation Radial arm maze (8-arm) spatial working-reference memory	Bilateral ATN lesions ATN rats impaired on 1 and 2	c-Fos, with HF, subicular and limbic cortices analysed	Lesions associated with reduced counts in PL, ACC, RSC, dHPC and vHPC.

Abbreviations: ACC: anterior cingulate cortex, ATN: anterior thalamic nuclei, BLA: basolateral amygdala, CA1: area CA1 of the hippocampus, CREB: c-AMP response element binding protein, DG: dentate gyrus, dHF: dorsal hippocampal formation, dHPC: dorsal hippocampus, dSub: dorsal subiculum, GAP-43: growth associated protein 43, HF: hippocampal formation, HPC/H: hippocampus, IL: infralimbic cortex, LA: lateral amygdala, LD: laterodorsal thalamic nucleus, pCREB: phosphorylated c-AMP response element binding protein, PH: parahippocampal cortex, PL: prelimbic cortex, post-sub: post-subiculum, PRC: perirhinal cortex, pre-sub: pre-subiculum, RAM: radial arm maze, Rdg: dysgranular retrosplenial cortex, Rga: granular a retrosplenial cortex, Rgb: granular b retrosplenial cortex, RSC: retrosplenial cortex, sup: superficial, vHPC: ventral hippocampus

NB: Numbers in bold in each study refer to separate experiments

1.7 Animal Models of Retrosplenial Cortex Lesions

The 'covert pathology' observed after ATN lesions has been thought to have some influence on behaviour given the aforementioned links between IEGs and spatial memory, although such work has not examined retrosplenial dysfunction and behaviour. Lesions to the RSC in rats are associated with some spatial memory deficits, although these deficits are mild when compared with the severe deficits observed after ATN and HF lesions (Vann & Aggleton, 2002; 2004; Vann, Wilton, Muir & Aggleton, 2003; Pothuizen, Aggleton & Vann, 2008; Pothuizen, Davies, Albasser, Aggleton & Vann, 2009; Pothuizen, Davies, Aggleton & Vann, 2010). A particular aspect of spatial memory that this damage is thought to impair is the use of egocentric cues such as head direction, as approximately 10% of the cells in the rat RSC are head direction cells – cells that function independently of the spatial location of the rat to distinguish the direction that the rat is facing (acts as a 'compass') – and these cells are distributed evenly throughout the granular and dysgranular regions (Shibata, Honda, Sasaki & Naito, 2009). Hence, a significant proportion of recent research into behavioural outcomes after RSC damage has focused on egocentric spatial memory, with deficits found when rats are required to use head direction cues for alternation (Pothuizen, Aggleton & Vann, 2008) and path integration (Whishaw, Maaswinkel, Gonzalez & Kolb, 2001).

A modification to the standard RAM task has elicited clearer deficits than in previous standard water maze and RAM tasks. Rats with RSC lesions show significant impairment when a mid-trial delay and maze rotation of 45° is introduced (Vann, Wilton, Muir & Aggleton, 2003; Pothuizen, Aggleton & Vann, 2008). The rotation of the maze places distal cues and intra-maze cues into conflict, which may disrupt navigation (Vann, Wilton, Muir & Aggleton, 2003). The more mild impairments of RSC lesions on other spatial memory tasks indicate that perhaps the RSC may not be as critical for spatial memory as the ATN or HF, as those lesions cause substantial deficits even in standard tasks. Deficits have also been found

on other aspects of episodic memory after RSC lesions, such as object-in-place memory (Vann & Aggleton, 2002), suggesting that the effects of RSC lesions may extend to aspects of spatial memory other than navigation.

Although direct damage to the RSC may impair similar aspects of memory function to that of ATN lesions, the effects of distal RSC covert pathology after ATN lesions in terms of behaviour is unknown. Recent research focusing on recovery of function after ATN lesions investigations has yet to determine whether recovery of function may extend to a reversal of this pathology in the RSC on tasks that appear particularly sensitive to RSC lesions.

1.8 Environmental Enrichment and Functional Recovery

Animal lesion models of the RSC and ATN provide valuable neural substrates of human brain injury, and allow for the development of therapeutic interventions to assess recovery. Environmental enrichment was developed as one such intervention, and has elicited significant functional recovery in animals following various brain injuries (Will, Galani, Kelche & Rosenzweig, 2004). When provided in an experimental setting, enrichment can be defined as housing that is substantially different when compared to standard housing. Enrichment generally involves access to novel objects in unique configurations for sensorimotor stimulation and informal learning experience and cognitive stimulation; some forms of enrichment include access to running wheels to increase physical exercise beyond that stimulated by the novel objects. The degree of social interaction is also higher than in standard housing, due to a greater number of animals being kept in the larger enrichment cages (Van Praag, Kempermann & Gage, 2000; Will, Galani, Kelche & Rosenzweig, 2004).

In addition to its use as a therapeutic intervention in animal models of brain injury, enrichment has also been applied to transgenic mouse models of early-stage Alzheimer's disease, where enrichment was associated with reduced β-amyloid deposition, a measure of

Alzheimer's-related neuropathology (Herring et al, 2008; Cotel, Jawhar, Christensen, Bayer & Wirths, 2012; Maesako et al, 2012). In animal models of TBI, where pathology is often diffuse and deficits are severe, enrichment has been found to attenuate spatial memory deficits in the water maze (Passineau, Green & Dietrich, 2001). Enrichment also ameliorates behavioural deficits in animal models of stroke, Parkinson's disease and motor neuron disease, as well as various lesion models such as hippocampal, medial prefrontal cortex and sensorimotor cortex lesions (Christie & Dalrymple-Alford, 1995; Galani, Jarrard, Will & Kelche, 1997; Will, Galani, Kelche & Rosenzweig, 2004; Nithianantharajah & Hannan, 2006). It is clear that enrichment affects both behaviour and neurobiology, and shows promise as a therapeutic intervention for animal models of human brain injury and disease and may be of translational benefit for human cognitive rehabilitation and other therapies.

The effects of enrichment appear to be lesion-location and task dependent with certain locations of brain damage appearing to preclude recovery of function to a greater extent than others, and certain behavioural tasks may be more suited to assessing the extent of recovery of function than others for different lesions. Lesions to the fimbria-fornix, septal area and subiculum have shown mixed recovery after enrichment (Dalrymple-Alford & Kelche, 1987; Kelche, Dalrymple-Alford & Will, 1987; Galani, Jarrad, Will & Kelche, 1997). Despite the sensitivity of enrichment effects to factors such as lesion location, the behavioural effects of enrichment are evident to a similar degree in both aged and young adult rats, with recent work by Sampedro-Piquero, Begega, Zancada-Mendez, Cuesta & Arias (2013) demonstrating that both aged (18 months) and young (3 months) adult rats exposed to an enriched environment for a 2-month period in the absence of brain injury had superior performance in a radial-arm water maze reference memory task. Additionally, brief exposure to enrichment of two hours per day has been found to be as beneficial as continuous enrichment (Will, Rosenzweig, Bennett, Hebert & Morimoto, 1977).

Numerous neurobiological effects of enrichment are also associated with functional recovery. Initially, Rosenzweig. Krech, Hebert & Morimoto (1962) discovered that exposure to an enriched environment was associated with greater cortical weight when compared to impoverished (isolated) housing conditions. Subsequent research revealed increased neurogenesis, gliogenesis, synaptogenesis, angiogenesis and dendritic arborisation after enrichment (Passineau, Green & Dietrich, 2000; Van Praag, Kempermann & Gage, 2000; Kempermann, Gast & Gage, 2002; Leggio et al, 2005; Herring et al, 2008).

Analysis of the components of enrichment (social, cognitive, physical and sensorimotor stimulation) has largely been restricted to social stimulation and physical exercise (Will, Galani, Kelche & Rosenzweig, 2004). The extent of neurobiological and functional recovery has been found to be greatly reduced with either component in isolation (Rosenzweig, Bennett, Hebert & Morimoto, 1978; Johansson & Ohlsson, 1996; see Dalrymple-Alford et al, in press for review). As no single component or variable of enrichment can be attributed to the array of effects seen in full enrichment, provision of sensorimotor, social and cognitive stimulation in conjunction appears to provide the best opportunity for recovery of function (Van Praag, Kempermann & Gage, 2000). It has, therefore, been suggested that enrichment is a reversal of the relatively impoverished conditions generally provided in laboratory settings, rather than being an improvement on 'natural' settings (Van Praag, Kempermann & Gage, 2000).

In animal models of ATN lesions, enrichment has been found to reduce spatial working and reference memory deficits on an array of tasks, with enriched-housed ATN rats making fewer errors on RAM and T-maze tasks, and swimming shorter distances in the water maze when compared to standard-housed ATN rats (Loukavenko, Ottley, Moran, Wolff & Dalrymple-Alford, 2007; Wolff, Loukavenko, Will & Dalrymple-Alford, 2008; Dupire et al, 2013; Loukavenko, Wolff, Poirier & Dalrymple-Alford, in press; Harland, Collings,

McNaughton, Abraham & Dalrymple-Alford, 2014, see Table 1.3). Functional recovery after enrichment appears to not be diminished by delays between surgery and onset of enrichment: at post-surgery intervals of either 5 or 40 days before onset of enriched housing, rats with ATN lesions showed reduced deficits at both enrichment intervals on a T-maze spatial working memory task (Loukavenko, Ottley, Moran, Wolff & Dalrymple-Alford, 2007).

Lesion size may affect the extent to which recovery of function is possible in enriched housing. Harland (2013) found that for the enriched group, but not the standard group, there were significant associations between ATN lesion volume and performance on the radial arm maze and cross maze, indicating that enriched rats with larger ATN lesions did not recover to the same extent as enriched rats with smaller ATN lesions. By contrast, Loukavenko, Ottley, Moran, Wolff & Dalrymple-Alford (2007) found no evidence for variation of the beneficial effects of post-surgery enrichment as a function of lesion size, and there was little overlap between the enriched and standard-housed ATN rats in terms of performance as a function of lesion size.

To investigate if recovery of function after ATN lesions also included a reversal of covert pathology in the RSC, Loukavenko, Wolff, Poirier & Dalrymple-Alford (in press) examined c-Fos activation following either ATN or sham lesions in rats with administration of the neurotrophin cerebrolysin or enriched housing. As enrichment has been shown to ameliorate the behavioural impairments associated with ATN lesions, it was expected that c-Fos hypoactivation in the RSC would also be reversed. Although both enrichment and cerebrolysin showed similar efficacy in facilitating functional recovery, neither intervention rescued retrosplenial c-Fos hypoactivation. Harland, Collings, McNaughton & Dalrymple-Alford (2014) showed that ATN lesions produced a significant reduction in the dendritic spine density of neurons in the RSC and CA1 region of the hippocampus.

Table 1.3. Summary of studies that assess spatial memory and neurobiological outcomes after enrichment and anterior thalamic lesions

Year	Authors	Lesion site/method	Enrichment protocol	Behavioural tasks and training	Neurobiological measures	Enrichment outcome
2014	Harland, Collings, McNaughton, Abraham & Dalrymple-Alford	ATN/NMDA	40 days of continuous ENR	1. Radial arm maze (8-arm) spatial reference and working memory 2. T-maze spatial working memory	Retrosplenial and hippocampal CA1 neuronal spine density	Fewer total errors and fewer days until criterion in the ATN and SHAM ENR rats on 1 Higher percentage of correct choices in the T-maze task Low spine density reversed in CA1 but not RSC
2014	Loukavenko, Wolff, Poirier & Dalrymple-Alford	ATN/NMDA	30 days of continuous ENR alone, or combined with neurotrophin cerebrolysin	1. T-maze spatial working memory	c-Fos immunoreactivity in the RSC	Both ENR and cerebrolysin improved t-maze performance in ATN rats No reversal of c-Fos hypoactivation in RSC after ENR or cerebrolysin
2013	Dupire et al	ATN/NMDA	25 days of continuous ENR	1. Open field 2. Elevated plus maze 3. Contextual fear conditioning	c-Fos and pCREB immunoreactivity in the medial prefrontal cortex, amygdala, HF and RSC	Increased exploration time in open arms of elevated plus maze Reduced freezing in contextual fear task No reversal of c-Fos hypoactivation in the RSC
2008	Wolff, Loukavenko, Will & Dalrymple-Alford	ATN/NMDA	40 days of continuous ENR	1.Water maze spatial reference memory with variable start positions 2.Water maze spatial reference memory	No neurobiological measures beyond lesion verification	In the standard reference memory and variable start position tasks, ATN ENR rats swam a shorter distance to the escape platform than standard-housed ATN rats.
2007	Loukavenko, Ottley, Moran, Wolff & Dalrymple-Alford	ATN/NMDA	30 days of continuous ENR, at either 5 days or 40 days post-surgery	1. T-maze spatial working memory 2. Radial arm maze spatial pattern separation	Performance was examined to determine if it varied as a function of lesion size.	Both 5 days and 40 days post-surgery ATN ENR groups: higher percentage of correct choices on 1 No change in performance on 2 based on post-operative enrichment

Abbreviations: ATN: anterior thalamic nuclei, CA1: area CA1 of the hippocampus, ENR: environmental enrichment, HF: hippocampal formation, NMDA: N-Methyl-D-aspartate (neurotoxin), pCREB: phosphorylated c-AMP response element binding protein, RAM: radial arm maze, RSC: retrosplenial cortex, SHAM: sham lesion

In ATN rats housed in enrichment, the reduction of apical and basal dendritic spine density was reversed in the CA1 region of the hippocampus but not in the RSC, suggesting that although behavioural deficits may be rescued, perhaps the 'covert pathology' in the RSC described earlier may not be amenable to recovery after enriched housing. Both studies, however, did not use tasks that are known to be sensitive to retrosplenial dysfunction, such as the modified RAM task (Vann, Wilton, Muir & Aggleton, 2003).

1.9 Aims of the Present Study

The foregoing summaries show that ATN lesions induce severe memory deficits, primarily in spatial memory, and that environmental enrichment ameliorates these deficits. Although enrichment facilitates recovery of spatial memory, the neurobiological effects of enrichment after ATN lesions remain uncertain. A key issue concerns the neuronal hypoactivation in the RSC produced by ATN lesions. The primary aim of the present study was thus to examine spatial memory in rats with ATN lesions after enriched housing using the RAM with mid-trial delay and rotation, which places extra-maze and intra-maze cues into conflict. This variant of the RAM procedure is more sensitive to retrosplenial dysfunction than standard spatial memory tasks (Vann, Wilton, Muir & Aggleton, 2003) and shows an increase in retrosplenial IEG activity in intact rats (Pothuizen, Davies, Albasser, Aggleton & Vann, 2009). It is possible that the failure of enrichment to reverse IEG hypoactivation after ATN lesions (Loukavenko, Wolff, Poirier & Dalrymple-Alford, in press) was the result of using a spatial alternation task, which may be a less reliable indicator of RSC function. Also, while ATN lesions are associated with a reduction in levels of both zif268 and c-Fos in the RSC (see Table 1.2), levels of zif268 have not been examined in enriched rats with ATN lesions. An additional novel aim of this study was to observe the temporal characteristics of recovery of function by testing during the initial enrichment period itself. In addition, the present study

examined the effects of ATN lesions and enrichment on neuron number in the MB, a region that is important for spatial memory and in which almost every neuron projects to the ATN.

In the present study, rats were trained prior to surgery on the standard RAM spatial working memory task to establish a baseline of spatial memory performance relevant to the future RSC-lesion sensitive task, and to assign rats to receive either ATN or sham surgery based on this performance. To examine post-surgery deficits in spatial memory, rats were tested on both the reference and working memory versions of the water maze (Table 1.1). A reference memory probe was added to assess retention of the location of the platform 12 days after completion of reference memory testing. Both water maze tasks were used to confirm post-surgery deficits to match pairs of rats based on this performance prior to transfer to either enriched or standard housing. A modification to the water maze apparatus allowed for testing of performance with minimised allocentric cues, which was expected to exacerbate deficits because previous research shows that rats with ATN lesions are impaired in the use of allocentric spatial memory (Aggleton, Hunt, Nagle & Neave, 1996).

The standardised enrichment protocol of Harland, Collings, McNaughton, Abraham & Dalrymple-Alford (2014) was implemented. This protocol has successfully facilitated recovery of function on spatial memory tasks in rats with ATN lesions. The potential time-course of enrichment effects was examined using the water maze working memory task, conducted at two time-points during the enrichment period, and on the first day of the post-enrichment period. The working memory version of the water maze was used to allow for one day of testing at each time-point to minimise disruption to the continuous housing protocol.

For post-enrichment testing, working memory in the water maze and then reference memory in the same apparatus were re-assessed. Rats were then re-tested on the spatial working memory task in the RAM in preparation for the final test with delay and rotation in

preparation for analysis of the IEG zif-268 in the RSC. Standard-housed ATN rats were expected to exhibit substantial deficits on the post-enrichment water maze and RAM tasks, in accordance with earlier observations of such deficits after ATN lesions (Table 1.1), which were expected to be ameliorated in ATN rats housed in enriched conditions (Table 1.3). Although working memory in the water maze had not been used to assess recovery of function after ATN lesions prior to the present study, this task was expected to elicit recovery due to its similarity in allocentric cue use and navigation to the RAM and t-maze alternation tasks, which have shown sensitivity to recovery of function after ATN lesions (Table 1.3). A substantial reduction in zif268 activation in the RSC and CA1 regions was expected in rats with ATN lesions, as both of these regions share extensive reciprocal connectivity with the ATN (Jankowski et al, 2013). As the RSC is thought to be important for spatial memory function (Aggleton, 2010), zif268 hypoactivation was expected to be associated with deficits on the modified tasks in the RAM, and a reversal of this hypoactivation was expected in accordance with any improvements in behavioural performance on the modified RAM tasks in rats with ATN lesions housed in enrichment. As the CA1 region of the hippocampus is also important for spatial memory function and shows reductions in dendritic spine density after ATN lesions which are reversed by enriched housing (Harland, Collings, McNaughton, Abraham & Dalrymple-Alford, 2014), similar results were also expected for this region in terms of zif268 expression and behavioural performance.

The ATN receives projections from the medial and lateral mammillary bodies via the mammillothalamic tract (Jankowski et al, 2013; Dillingham, Frizzati, Nelson & Vann, 2014), and earlier research has suggested that ATN lesions induce a corresponding loss of neurons in the MB (Fry & Cowan, 1972; Aggleton & Mishkin, 1983), although such research induced unilateral lesions to the AD only (Fry & Cowan, 1972) and incurred damage to additional regions which may have resulted in the changes observed in the MB (Aggleton & Mishkin,

1983). Lesions to the MB have also been associated with similar deficits as ATN lesions on spatial memory tasks (Dillingham, Frizzati, Nelson & Vann, 2014). Hence, the potential impact of ATN lesions and any effects of enriched housing on the medial, medial-lateral and lateral mammillary nuclei were also examined through NeuN immunofluorescence.

In summary, the novel aims of the present study were to examine: (1) the effects of ATN lesions and enrichment on retrosplenial IEG activation using a task that is especially sensitive to RSC dysfunction; (2) to assess spatial working memory during the enrichment period to determine when recovery of function begins to occur; and (3) to analyse the effects of total ATN lesions and enrichment on cell counts in the MB. Taken together, the findings may provide further insights into the effects of ATN lesions, and of enrichment after ATN injury, on neurobiological and behavioural aspects of the extended hippocampal system.

2. Method

2.1 Subjects

Fifty male PVGc hooded rats bred in the Psychology department of the University of Canterbury were used (an additional two rats were lost during surgery). The rats were randomised to receive ATN lesion (ATN: n = 28) or sham surgery (SHAM: n = 22), at approximately 9 (8-11) months old (350-425g body weight). Food and water were available ad libitum during the surgery and recovery periods, differential housing in enriched or standard cages, and testing in the water maze. For the pre-surgery RAM testing and the post-enrichment RAM tasks the rats received food restriction to maintain 85% ad libitum body weight, with water available ad libitum.

2.2 Surgical Procedure

Aseptic conditions were used throughout surgery. An intraperitoneal (IP) injection of the anti-inflammatory Norocarp (carprofen) was administered one hour prior to surgery (Table 2.1). The rat was then anaesthetised with an IP injection with half of the required ketamine dose followed by an IP injection of atropine to facilitate respiratory function and the remaining half of the ketamine dose that was combined with domitor. Hartmann's saline (sodium lactate, IP injection) was administered, and the rat ear-marked for identification once fully anaesthetised. Methopt Forte eye drops were applied before, during and after surgery, along with moist gauze (tap water) placed above and clear of the eyes. The rat was placed in a stereotaxic frame with atraumatic ear bars (Kopf, Tujunga, CA), with the incisor bar set at –7.5mm below the interaural line to minimise fornix injury. After a midline incision, the scalp was retracted to expose the skull, and local analgesia (mepivacaine) applied. The craniotomy above the target ATN coordinates was varied to accommodate variation in Bregma-Lambda distance (Table 2.2).

Table 2.1. Surgery drug doses

Drug	Concentration	Dose/Volume
Ketamine	50mg/ml	75mg/kg
Domitor	0.5mg/ml	0.35mg/kg
Antisedan	2.5mg/ml	2.5mg/kg
Norocarp	5mg/ml	5mg/kg
Mepivacaine	2mg/ml	0.2ml
Atropine	0.10mg/ml	0.15mg/kg
Hartmann's Saline		2ml (1ml before and1ml after surgery)

Table 2.2. Coordinates for ATN lesions, with anterior-posterior (A-P) varied relative to Bregma-Lambda (B-L). M-L: Medial - lateral, D-V: Dorsal – ventral.

	ATN Co-ordinates		
	AV	**AM**	**Corresponding B-L distance (in cm)**
A-P (cm)	-0.250	-0.240	≤ 0.64
	-0.255	-0.245	0.65 – 0.68
	-0.260	-0.250	0.69 – 0.72
	-0.260	-0.250	≥ 0.73
	± 0.152	± 0.120	**M-L (laterality, cm)**
	-0.565 and -0.575	-0.585 only	**D-V (from dura, cm)**

Bilateral neurotoxic lesions of the ATN at the anteroventral nucleus (AV) and anteromedial nucleus (AM) were made using micro-infusions of 0.15 M N-Methyl-D-aspartate (NMDA; Sigma Chemicals, Australia) dissolved in phosphate buffer (pH 7.20) through a 1 µL Hamilton syringe (needle: 25S, outer diameter 0.51mm; inner diameter 0.13mm) connected to a motorised micro-infusion pump (Stoelting, Reno, USA). Infusions of 0.15 µL were made at a rate of 0.04µL/minute for each of the six coordinates. Lesion location and volume were determined after pilot surgeries. The syringe remained in situ for 3 minutes post-infusion to allow diffusion after which the needle was slowly retracted. For sham surgeries the Hamilton syringe was lowered to 1.5mm above the AM and dorsal AV lesion coordinates and no material was infused.

Throughout surgery, the rat's body was kept warm by overhead lamps, and a thermo-regulated heat pad monitored by a rectal probe maintained the rat's temperature at 37°C (±1°C) (Stoelting Co., USA). Additional ketamine (only) was given while monitoring the rat's condition during surgery. Emla cream (topical analgesic) was applied to the scalp after suturing, followed by an IP injection of the sedative reversal drug Antisedan (atipamezole) and 1.0ml of Hartmann's saline. The rats were monitored closely during the first post-operative week by the researcher and laboratory technicians to ensure that the rats were eating, drinking and quickly returned to being bright, alert and responsive. All procedures complied with the University of Canterbury Animal Ethics guidelines and were approved by the Animal Ethics Committee (AEC).

2.3 Housing Procedures

Prior to surgery, rats were housed in groups of 3 or 4 in standard opaque plastic cages (45cm long by 27cm wide by 22cm high). During the ~7 day post-surgery recovery period the rats were housed individually, after which rats were re-housed in groups of 3 or 4 with a mix of

lesion and sham animals in each cage (see Figure 2.1 for experimental timeline). The rats were transferred to the differential housing conditions approximately 60 days post-surgery, following tests of the effects of lesions on spatial reference memory, working memory and a minimal cues task in the water maze. For all testing procedures, rats were transported from the holding room to the testing rooms in their standard home cages.

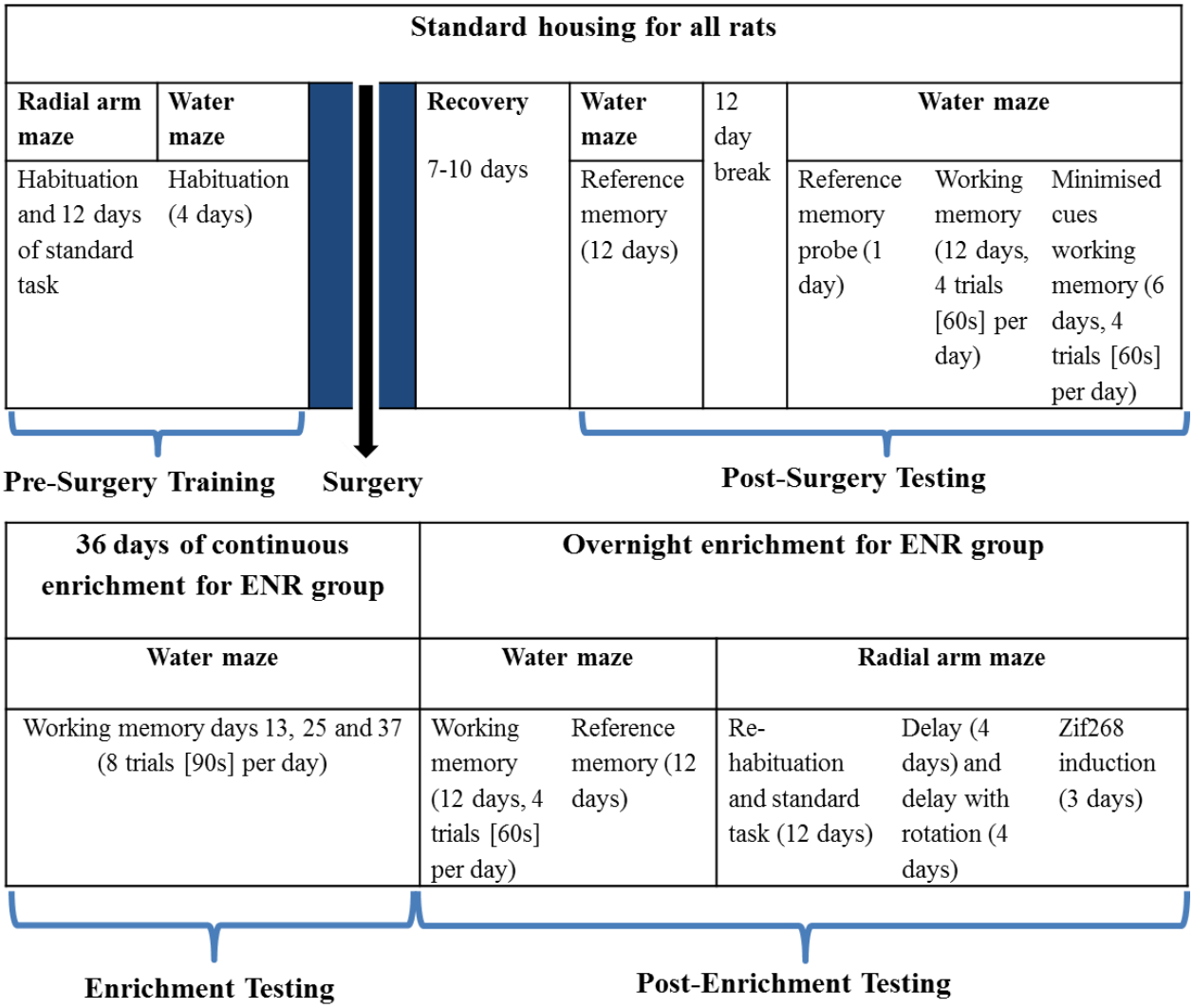


Figure 2.1. Experimental timeline. ENR: rats assigned to enriched housing.

Based on rank-ordered post-operative performance on the spatial working memory task in the water maze, pairs of adjacently-ranked rats were randomised to housing in an enriched environment condition (ENR) or the standard housing condition (STD) for 36 days, with a mix of sham and lesion animals in each cage. All groups were housed in the same colony room, maintained at a temperature of 22ºC. A reversed 12-hour lighting schedule (lights off from 8am-8pm) was used, and all behavioural testing was conducted during the dark (i.e. higher activity) phase of the light cycle. The standardised, University of Canterbury enrichment protocol of Harland, Collings, McNaughton, Abraham & Dalrymple-Alford (2014) was used (http://www.psyc.canterbury.ac.nz/Standardized Enrichment.shtml).

Rats in the ENR group were housed in groups of 12-13 lesion and sham animals, in enrichment cages comprised of wire mesh (85 cm long by 60 cm wide by 30 cm high) with a solid metal floor covered with sawdust (an example of an enriched housing arrangement is indicated in Fig. 2.2). Inside the ENR cages, a daily unique assortment of thirteen different objects was arranged (from 93 total different objects). These objects included PVC tubing, Perspex tunnels, terracotta ornaments, plastic toys, glass cups and plates, metal chains, wooden blocks and boxes; no object was repeated within any five-day period. On every seventh day of enrichment, 13 plastic spouting pieces were arranged in unique configurations to form tunnels, and on every eighth day the enrichment cage was empty save the two wooden blocks (untreated hardwood) always present to provide a preferred material to chew. The ENR-housed rats were briefly moved to standard cages when the enrichment objects were reconfigured, for 30 minutes per day at approximately 10am, and whenever the cage received its weekly clean. The position of the food and water within the cages was varied daily and the location of the enrichment cages in the colony room was also varied every 4 days.

Figure 2.2. Example configuration of enrichment objects in the enrichment cage (Day 35 of the 40-day enrichment protocol), rats in the enrichment cage, and an example of the standard cages in the colony room.

Following the 36 days of differential housing, the ENR group rats were re-housed in standard conditions in groups with 3 or 4 previous ENR group cage-mates such that the group had both lesion and sham animals in each cage; they were returned to enriched housing for 16 hours per day overnight (4pm to 8am). Standard-housed rats remained in their cage-groups from the continuous differential housing period. Following confirmation of suitable lesions (see Lesion Evaluation), the final group numbers were: ATN ENR, *n* = 10, SHAM ENR, *n* = 11, ATN STD, *n* = 9, SHAM STD, *n* = 11.

2.4 Behavioural Procedures

2.4.1 Pre-surgery Training – Radial Arm Maze

The 8-arm radial arm maze (raised 67.5cm above the floor) consisted of a 35cm-wide wooden hub (painted black) and 8 detachable aluminium arms (65cm long by 8.6cm wide, 4.5cm high borders) (Fig. 2.3). A single clear Perspex barrier (19cm long by 25cm high)

placed against the central hub along one side of each arm discouraged the rats from jumping between arms. At the end of each arm was a black wooden block (5cm long by 8.5cm wide by 3cm high) containing a well (2cm in diameter, 1cm deep) for food rewards (dark-chocolate drops ~1g: Foodfirst, New Zealand) and inaccessible food under the well to prevent olfactory cues influencing choices. Clear Perspex guillotine doors (4.5cm wide by 25cm high) controlled access to each arm and were manually operated by an overhead pulley system; the arms could be raised individually or together. The radial arm maze was situated in a windowless room (410.5cm by 471cm) that contained salient visual cues on the walls such as geometric shapes and high contrast stimuli. A curtain was also hung from the ceiling in a corner of the test room, which remained undrawn.

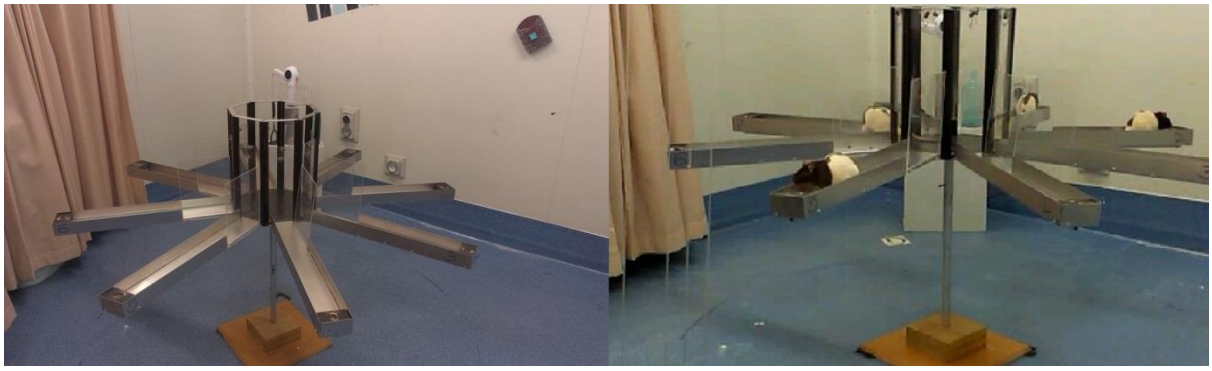


Figure 2.3. Radial arm maze apparatus (L) and rats receiving habituation to the task with cagemates (R).

Rats were reduced to 85% of their free-feeding weight, with water available ad libitum. For 7 days prior to testing, rats were habituated to the food reward in their home cages. Cage groups of 3-4 rats were habituated to the maze for a minimum of 6 days. For the first 3 days, the chocolate drops were scattered down the arms and placed into the food wells. A group of 3-4 rats was allowed to explore the entire maze for 7 mins (day 1) and 5 mins (remaining 2 days), with all doors open. Rats were then placed in the maze individually for 3 mins each and shaped to explore to the end of the arms over 3 days, with the doors open (day 1) and operational (remaining 2 days), with additional habituation trials if necessary.

Formal training on the RAM task for 12 days was used to equate spatial memory across all groups prior to surgery. A standard procedure was used in which the rats were required to retrieve two chocolate drop rewards from each arm without re-baiting. The rat was placed in the maze hub with all doors closed. All doors were opened together and the rat confined to the chosen arm (~10s) and subsequently allowed return to the centre of the maze for a ~5sec delay, after which the procedure was repeated until the rats had entered each of the 8 arms, made 20 total choices, or 10 minutes had elapsed.

2.4.2 Pre-surgery Training - Morris Water Maze

The water maze was a white plastic circular pool, diameter 180cm, height 45cm, with a 5cm rim and filled with water (depth of 30cm), rendered opaque through the addition of non-toxic acrylic water-based paint (Super Tempera, Fine Art Supplies, New Zealand) (Figure 2.4). A 10cm-diameter white Perspex escape platform was used, that was 2cm below the water's surface. The maze was situated in a similar room to that used for the RAM tasks, but with different salient visual cues provided. The water was rendered opaque through the addition of non-toxic acrylic water-based paint (Super Tempera, Fine Art Supplies, New Zealand).

The pool was divided virtually into eight equal segments (the cardinal co-ordinates N, S, W, E, plus NE, NW, SE, SW). Lighting was provided by 3 ceiling fluorescent covered lights and a 60w lamp placed in a corner of the room opposite the maze, which was used to warm the rats during test trials. Two desktop computers without screens were in operation in adjacent corners of the room to distribute auditory cues relative to the third computer used for data recording. A beige curtain was hung from the ceiling in a corner of the test room above the pool, which could be left open or drawn around the water maze. A ceiling-mounted video camera was used to track and analyse performance in terms of path length, swim speed and escape latency (Ethovision XT 5.1, Noldus Information Technology, The Netherlands).

Figure 2.4. Morris water maze location in the room used for the working and reference memory water maze testing procedures.

The rats were habituated to the water maze after the 12 days of RAM pre-surgery training, with four swims per day for four days, with rats from different home cages randomly assigned to swim groups of 3 or 4. The curtain was drawn around the water maze so as to minimise room cues. For the first two days, the platform was raised 1cm above the water's edge so as to be visible to the rats, and 2cm below the surface for the remaining two days. Each group of rats was randomly assigned to one of four fixed platform locations (NE, NW, SE, and SW) with the platform located 40cm from the edge of the maze. The order of the start locations was randomised across the four cardinal co-ordinates: N, S, E, and W for the four daily trials. Each of the rats in a group completed a given trial before the next trial, giving an inter-trial interval of 2-5 minutes. Each trial was terminated when the rat had reached the platform or after 60 sec when the rat was guided to the platform by the experimenter; the rat remained on the platform for 15 sec. The rats were placed in a standard holding cage on a table between trials, and when all trials for each rat had concluded for the day, they were placed in an identical cage which contained towels underneath the 60w lamp to keep warm.

2.4.3 Post-surgery Testing- Water Maze

Spatial Reference Memory

Following recovery, the rats were randomly allocated to two groups with half the rats tested first, and the remaining group beginning after the first group's final day of testing on the reference memory task. The testing order of the rats was randomised, with the rats re-allocated to new swim squads of 3 or 4 lesion and sham animals, with the 3 to 4 rats each completing a given trial prior to the next trial. Reference memory training comprised 4 trials per day for 12 days, with the same procedure used as in pre-surgery training, except the curtain was retracted so that the distal cues were visible. The order of start locations (N, S, W, E) was again randomised across rats and days. Trials were terminated after the rats had located the platform or guided to the platform by the experimenter after 60sec, with the rats allowed 15sec on the platform for spatial orientation.

Reference Memory Probe

The probe trial was given 12 days after the final day of reference memory testing to emphasise long-term spatial memory of the platform location. The platform was removed and rats were released from the quadrant opposite to where the platform had been located for the reference memory task (i.e. released from the SW if the platform had been located in the NE quadrant) and allowed to search the pool for 60sec. The time spent in the target quadrant (the quadrant where the platform had been located for the reference memory task), adjacent left, adjacent right and opposite quadrants was analysed, along with crossings over the annulus, which was a virtual representation of the location of the platform used in the reference memory task enlarged by 20cm (see Figure 2.5).

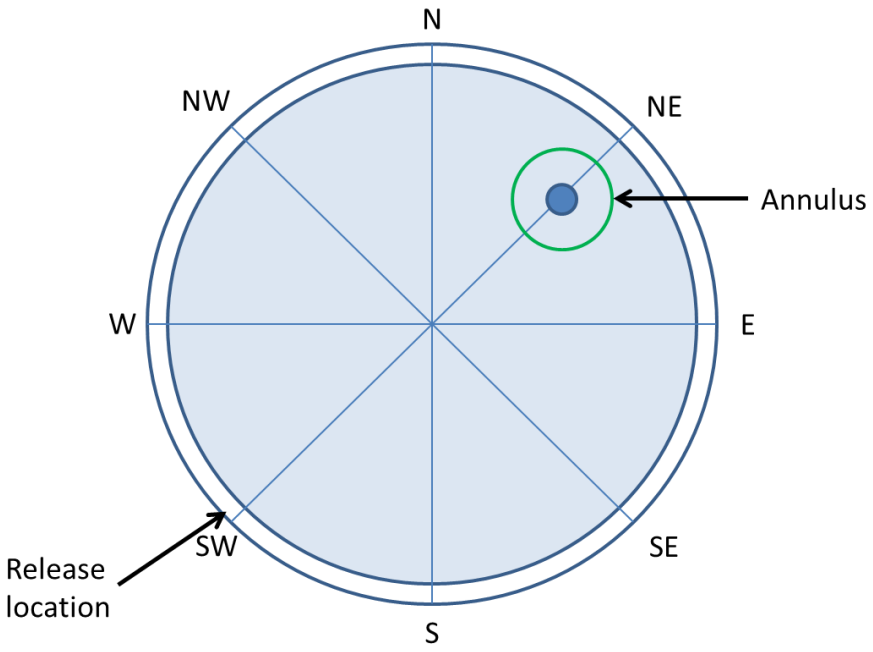


Figure 2.5. Example configuration of the reference memory probe, with location of the annulus (platform removed) in the NE quadrant, with release point from the SW quadrant.

Spatial Working Memory – Delayed Matching to Place

Spatial working memory training in the water maze commenced the day following the probe trial test for 12 days, again with the curtain retracted and distal cues visible. The same groups and testing order were used as in the reference memory training. Massed trials were conducted so that for each day the rats had 4 consecutive swims with the daily fixed platform location (a unique platform location was used each day, with the possible platform locations indicated in Figure 2.6). The four release locations were varied each day, with the first two release locations from the same point and the remaining two varied. The centre of the platform was located either 30cm or 50cm from the water's edge (as shown in Figure 2.6). For each of the four swims per day, trials were terminated when the rat had located the platform, or after 120sec had elapsed when it was guided to the platform by the experimenter; it remained on the platform for 30sec. Massed trials were used with an inter-trial interval of ~15sec.

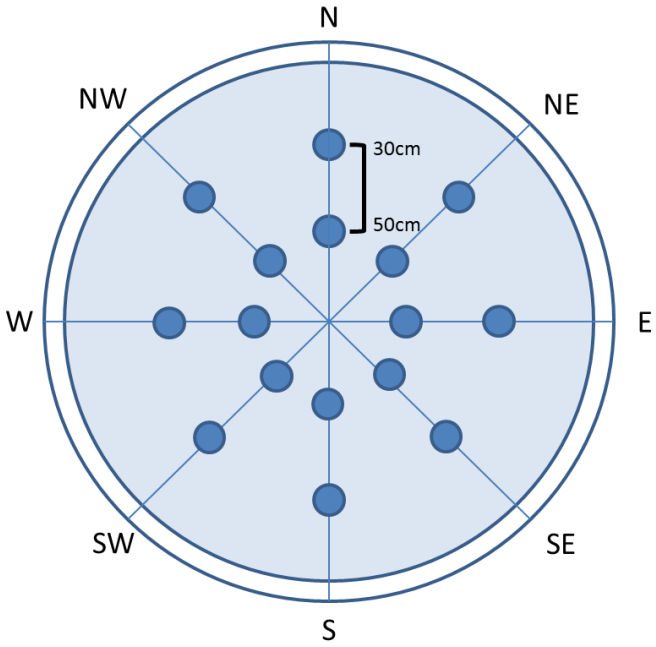


Figure 2.6. Possible platform locations for the working memory task.

Minimised Cues – Spatial Working Memory

A replica of the water maze used for the working and reference memory procedures outlined previously was placed in the room used for the RAM pre-surgery training. To minimise room cues, the curtain encircled the maze for all of the trials. Additionally, a white cloth was placed above and across the curtain rung with a small hole cut for the ceiling-mounted camera so as to render the entirety of the surrounds and ceiling relatively homogenous to the rat. A mobile (depicted in Figure 2.7) consisting of several small objects held in a distinct orientation was hung from the curtain rail of the maze inside the curtain positioned adjacent to the NW quadrant, which served as the sole complex cue.

Two days after completion of the standard working memory task, the rats were assessed on spatial working memory performance with minimised distal cues for six days, with trials conducted as in the standard working memory task.

Figure 2.7. Configuration of the minimised cues water maze depicting the mobile adjacent to the NW quadrant inside the curtain (left) and composition of the mobile complex cue (right).

2.4.4 Testing during Enrichment – Water Maze

Spatial Working Memory

On days 13 and 25 of continuous differential housing and day 1 of the post-enrichment period, the rats were reassessed on standard spatial working memory performance with room cues visible, using eight trials per day each of a maximum 90 seconds' duration. The platform positions used in the previous post-surgery spatial working memory task were rank-ordered for the average path length for both ATN and SHAM rats, with three platform locations within the median range of path length for the ATN rats selected for enrichment testing (to ensure that the platform locations were not too easy or too difficult for the rats to reach over 8 trials). As for the pre-enrichment working memory testing, the first two release locations were the same and for this modified procedure the remaining 6 trials were varied.

2.4.5 Post-enrichment Testing – Water Maze

Spatial Working Memory

On the second day after the continuous enrichment period, the rats were reassessed on the original standard spatial working memory procedure for 12 days, with the daily platform locations newly randomised. The standard working memory task was used prior to the reference memory task for post-enrichment testing, to minimise disruption to the rats as they had completed a similar working memory task during enrichment.

Spatial Reference Memory

Upon completion of the working memory task in the water maze, the rats were given a one-day break before a further 12 days of standard reference memory testing, with random reallocation of each testing group to a new platform location.

2.4.6 Post-enrichment Testing – Radial Arm Maze

Upon completion of testing in the water maze, the rats were gradually reduced to around 85% of their free-feeding weights by restriction of food access. The rats were subsequently re-habituated to the radial arm maze for three days with the first day of habituation in cage lots of 3-4 for 10 minutes and individual habituation for 3 minutes on the remaining two days. Testing on the standard task re-commenced for 12 days as in the pre-surgery training procedure.

Modified Radial Arm Maze with Mid-Trial Rotation and Delay

Following the final day of post-enrichment testing using the standard radial arm maze procedure, the modified task described by Vann, Wilton, Muir & Aggleton (2003) was used. The modified task used the standard procedure for the first four arm choices, but then on return to the centre hub the rat was carefully lifted in a manner to minimise rotation (i.e., the

rat was kept in the same orientation relative to the arm it had been in front of) and placed in a separate cage on the floor in the RAM room for a 60sec delay.

The rats were counterbalanced to first receive one of two conditions: either (1) only the delay of ~60s after the first four arm choices, or (2) a delay during which the maze was rotated 45 degrees clockwise, with the remaining food rewards replaced to their original locations relative to the distal cues. The rat was then returned to the centre of the maze following the rotation or delay, with the trial continued until each of the four remaining food pellets had been retrieved, 20 total trials, or 10 minutes had elapsed.

RAM testing for Zif268 Induction

Following completion of final behavioural testing on the modified RAM task, the rats were divided into squads of four and tested for a further 3 days with a modified procedure so as to maximise zif268 activation from the task. As evidence suggests that novel environments will increase IEG expression in intact rats (Jenkins, Dias, Amin, Brown & Aggleton, 2002) the distal objects (including the curtain) were re-configured for the first day of testing, with several new objects introduced which remained in the same configuration for the second day of testing (as shown in Figure 2.8). For the third day of testing, the configuration of the objects was again changed. Three massed trials were given on each day, with 8 total arm choices allowed. To ensure that rewards were equivalent between trials, the number of errors after each trial were calculated with a reward given to the rat in a food well in its separate cage according to the number of errors: for 0 errors, 2 extra chocolate drops were given, for 1-2 errors, 4 were given, and for 3-4 errors, 6 extra rewards were provided. To allow time to consume the food reward, the rat was returned to its separate cage for 2 minutes after which it was immediately returned to the maze for subsequent two trials which were run in the same manner.

Figure 2.8. Examples of the original configuration used during standard RAM testing (A), the configuration used on days 1 and 2 (B) and day 3 (C) of the Zif268 induction task in the radial arm maze, with each side of the room depicted.

Upon completion of the third trial on the first 2 days of testing, each rat was placed into a dark, quiet room with a towel placed over the cage for 90 minutes to allow for habituation to this procedure that minimises extraneous stimulation, with the time delay corresponding to peak expression of zif268 after the induction task (Dumont, Amin, Poirier, Albasser & Aggleton, 2012). The rats were then returned to their standard home cages for feeding, after which the ENR groups were returned to their enrichment cages overnight. On the third day of the post-test procedure, testing continued as before with the rat placed in the dark room for 90 minutes, after which the rat was immediately euthanized. Each of the four squads were tested separately, so that one squad was euthanized prior to the next squad's first day of testing.

2.5 Histology and Lesion Evaluation

The rats were euthanized with an IP injection of sodium pentobarbitone (125mg/kg) and perfused intracardially with ~150mL chilled (4ºC) saline followed by ~150mL 4%

paraformaldehyde. Brains were post-fixed for a minimum of 2 days and sectioned on a microtome (Thermo Fisher). For ATN lesion and MB analysis, coronal sections were cut in a series of 6 at 25µm, between Bregma -0.80mm and Bregma -5.30mm (encompassing the ATN, MB, dorsal CA1 region of the hippocampus and RSC), with every fourth and seventh section from this region, and every section from Bregma +3.70mm to Bregma +1.60mm (to capture the prefrontal cortex) and Bregma -5.30mm to Bregma -8.30mm (to capture the post-RSC, post-HPC and subicular regions) taken for zif268 analysis. Sections were cryoprotected in a long-term solution (40% 0.1M phosphate buffer [PB], 30% ethylene glycol, 30% glycerol) and stored at approximately -18°C for a minimum of 7 days before zif268, NeuN immunofluorescence or cresyl violet processing.

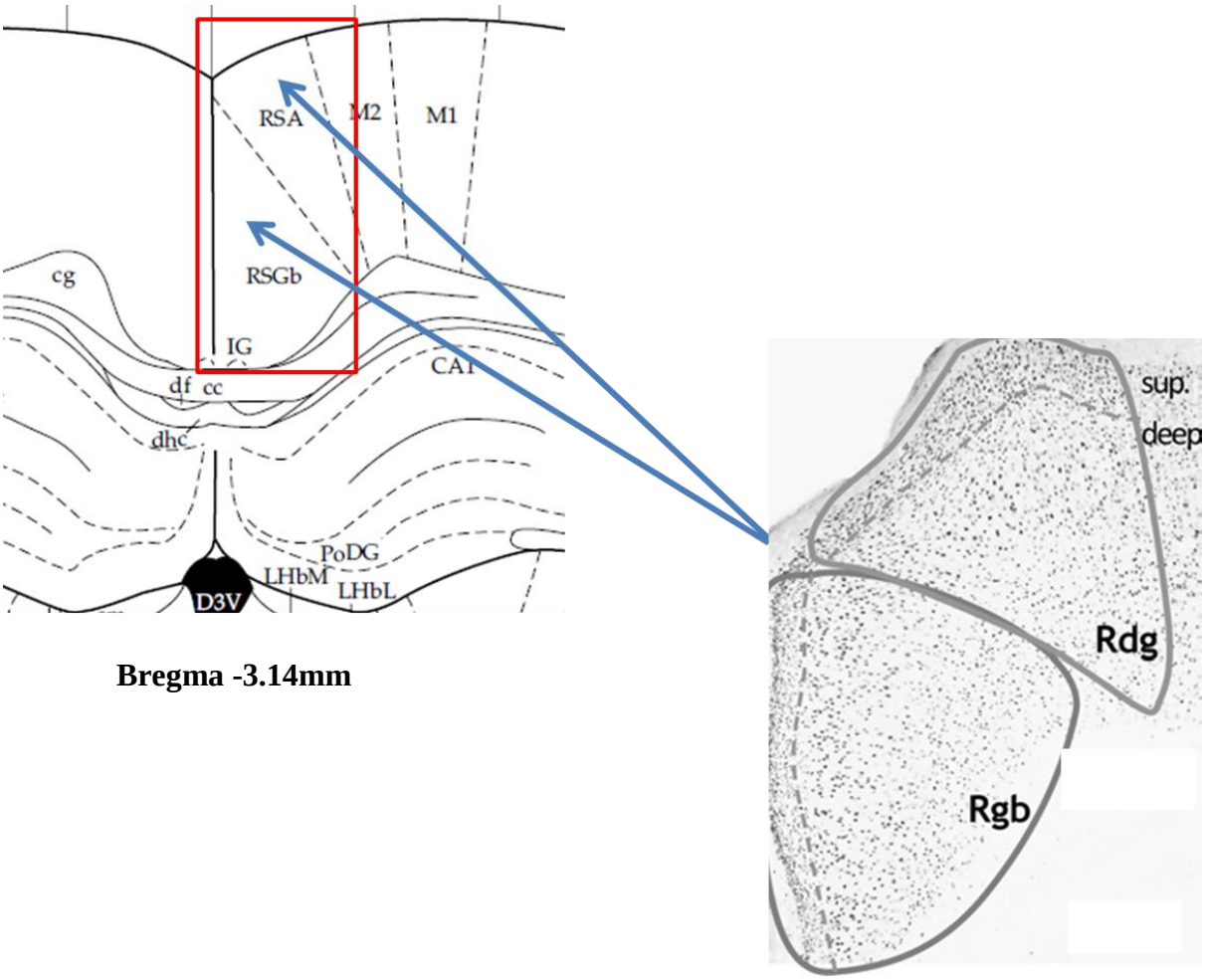
Sections were rinsed with 0.1M PB several times before being mounted on subbed slides and stained with cresyl violet to visualise cell bodies and determine the extent of damage to the ATN and surrounding structures. For cresyl violet staining, slides were first delipidised in 70%, 95% and 100% ethanol for 10 dips each, with a subsequent 5 minutes in 100% ethanol and 10 dips in 95% ethanol, followed by 5 minutes in 70% ethanol. Slides were rehydrated in distilled H_2O for 1 minute, before being placed into 4% cresyl violet acetate solution for staining for 10-14 minutes (depending on the number of previous uses of the solution). Slides were rinsed twice in distilled H_2O for 2 minutes, before dehydration and differentiation: 70% ethanol and 95% ethanol for 2 minutes, followed by 95% acid alcohol (solution of 400ml of 95% ethanol with 1ml glacial acetic acid) for 40 seconds, and 100% ethanol twice for 4 minutes. Slides were subsequently cleared in xylene twice for 5 minutes and cover-slipped with DPX and left to dry. For lesion evaluation, photomicrographs of the extent of the ATN (from -0.92mm to -2.30mm relative to Bregma) were taken at 4x magnification, using a camera (Nikon, DS Fi1) mounted to a microscope (Eclipse E-800, Nikon).

2.6 Zif268 Immunohistochemistry

Free-floating sections were rinsed four times in 0.1M PBS-TX (phosphate-buffered saline with 0.3% Triton-X) and subsequently treated with 3% H_2O_2 (hydrogen peroxide) in 0.1M PBS-TX (0.3%) for 10 minutes for suppression of endogenous peroxidase activity. Sections were rinsed four times in 0.1M PBS-TX before blocking in 10% normal goat serum (NGS; Gibco, NZ) in PBS-TX for 30 minutes. Sections were then rinsed 4 times in 0.1M PBS-TX, prior to incubation in EGR-1 anti-rabbit polyclonal serum (1:3000, Santa Cruz Biotechnology Inc., US) with NGS (1:100) and PBS-TX for 48 hours at 4°C with slow agitation. After four rinses in 01.M PBS-TX, sections were incubated in biotinylated goat anti-rabbit serum (1:400) with NGS (1:100) and 0.1M PBS-TX, for 2 hours at room temperature. Sections were rinsed four times in 0.1M PBS-TX before incubation in an avidin-biotin-horseradish peroxidase complex (6µl/ml, ABC-Elite, Vector Laboratories, US) in 0.1M PBS-TX at room temperature for 1 hour. Sections were subsequently rinsed twice in 0.1M PBS-TX and twice in 0.05M Tris buffer before being placed into a chromagen solution of 0.03% diaminobenzidine (Sigma), Tris buffer and H_2O_2 (13 µl/50ml of H_2O_2 at 50% concentration) for 10 minutes. The reaction was stopped after 10 minutes with rinses of cold 0.05M Tris buffer and 0.1M PBS, and sections were mounted on subbed slides. Slides were dried overnight and dehydrated and differentiated in 70%, 95% and 100% ethanol and cleared with xylene before being cover-slipped with DPX and left to dry overnight prior to analysis.

Photomicrographs were taken at 4x magnification by an Eclipse E-800 (Nikon) microscope fitted with a camera (Nikon, DS Fi1) of the dorsal CA1 region of the hippocampal formation (between approximately -3.14mm and -4.16mm to Bregma), rostral and caudal Rgb and Rdg subdivisions of the RSC (from -2.52mm and -3.84mm to Bregma, and -4.92mm to -6.30mm to Bregma respectively) the Rga subdivision of the RSC

(approximately -6.12mm to Bregma), and the primary auditory cortex to serve as a control region where zif268 immunoreactivity would not be expected to differ between groups (AudCx: from -4.92mm and -6.24mm to Bregma). Each region of interest was identified and drawn using computer software, and cells were automatically counted for each region with final counts expressed as the number of zif268 cells/area in mm^2 (see Figure 2.9 for example region and layer boundaries). At least three sections from each region of interest were quantified, with no less than two sections used in cases of damage to appropriate regions.



Bregma -3.14mm

Figure 2.9. Example of the cell boundaries used for zif268 immunohistochemistry analysis (R), for the superficial and deep layers of the anterior Rgb and Rdg regions of the RSC, and corresponding atlas diagram for region boundaries (L). Note that the cells within the superficial layer are densely clustered by comparison to the more dispersed cells within the deep layers of both regions. These cell boundaries were observed within the anterior and posterior Rgb and Rdg, and also within the Rga. Atlas image (L) from Paxinos & Watson, 1998; Zif268 image within the Rgb and Rdg (R) from Poirier & Aggleton, 2009. N.B: RSA = Rdg, and RSGb = Rgb. Rgb: Granular b retrosplenial cortex; Rdg: Dysgranular retrosplenial cortex; sup.: superficial layer.

2.7 NeuN Immunofluorescence for Mammillary Body Cell Counts

Free-floating sections were rinsed in 0.1M PBS-TX (0.2%) four times and placed into 10% normal horse serum (NHS; Gibco, NZ) for 1 hour. Sections were rinsed again with 0.1M PBS-TX before incubation in mouse monoclonal anti-NeuN serum (1:1000) with 1:100 NHS and 0.1M PBS-TX for 24 hours at 4°C with slow agitation. Sections were rinsed as above and incubated in anti-mouse dylight 459 (1:1000) with 1:100 NHS and 0.1M PBS-TX in the dark for four hours at room temperature with slow agitation. Sections were subsequently rinsed in 0.1M PB and mounted onto subbed slides and dried overnight. Slides were cover-slipped after re-hydration in distilled water with an aqueous medium (Fluoromount, Sigma) and sealed with varnish (OPI Products, US). Slides were stored at 4°C and protected from light with aluminium foil. Fluorescent photomicrographs of the regions of interest within the MB were taken on a microscope (Olympus BX-51) with a camera fitted (Nikon, DS Fi1) at 4x magnification, at approximately -4.80mm and -4.52mm from Bregma to capture the medial and lateral mammillary nuclei.

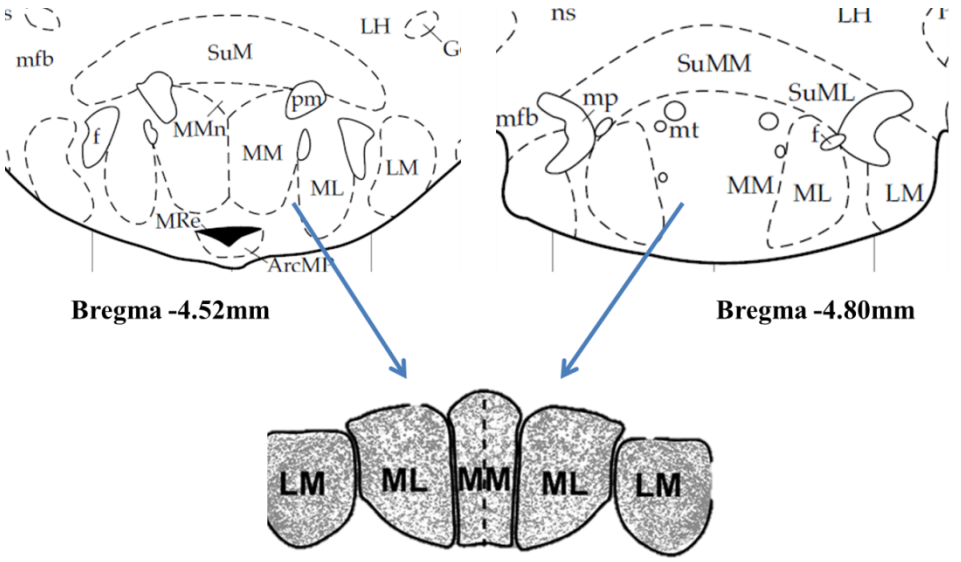


Figure 2.10. Example of the cell boundaries used for NeuN immunofluorescence analysis for the MM, ML and LM regions of the MB, and corresponding atlas diagrams for region boundaries (top). These cell boundaries were observed for both the anterior (-4.52mm from Bregma) and posterior (-4.80mm from Bregma) MB. Atlas image (top) from Paxinos & Watson, 1998; photomicrograph (bottom) from Dillingham, Frizzati, Nelson & Aggleton, 2014. LM: lateral mammillary nucleus; ML: medial-lateral mammillary nucleus; MM: medial mammillary nucleus.

3. Results

3.1 Lesion Verification

Figure 3.1 shows representative photomicrographs of the ATN and surrounding regions in a rat from each of the lesion and housing groups. Due to time constraints, systematic quantification of the ATN lesions was not completed for the present thesis but will be undertaken for future publication of this work. A researcher experienced in ATN lesion analysis and quantification (John Dalrymple-Alford) but blind to the individual performance of the rats in each group assessed all lesions. Inclusion required visual confirmation of approximately 50% bilateral damage of the ATN across the anterodorsal (AD), anteroventral (AV) and anteromedial (AM) nuclei, but exclusions were made if significant damage was observed in surrounding thalamic regions outside the ATN. Nine rats did not meet these criteria and were excluded from subsequent analyses: four ATN ENR rats and five ATN STD rats. The ATN damage observed between rats housed in enriched and standard conditions was highly similar.

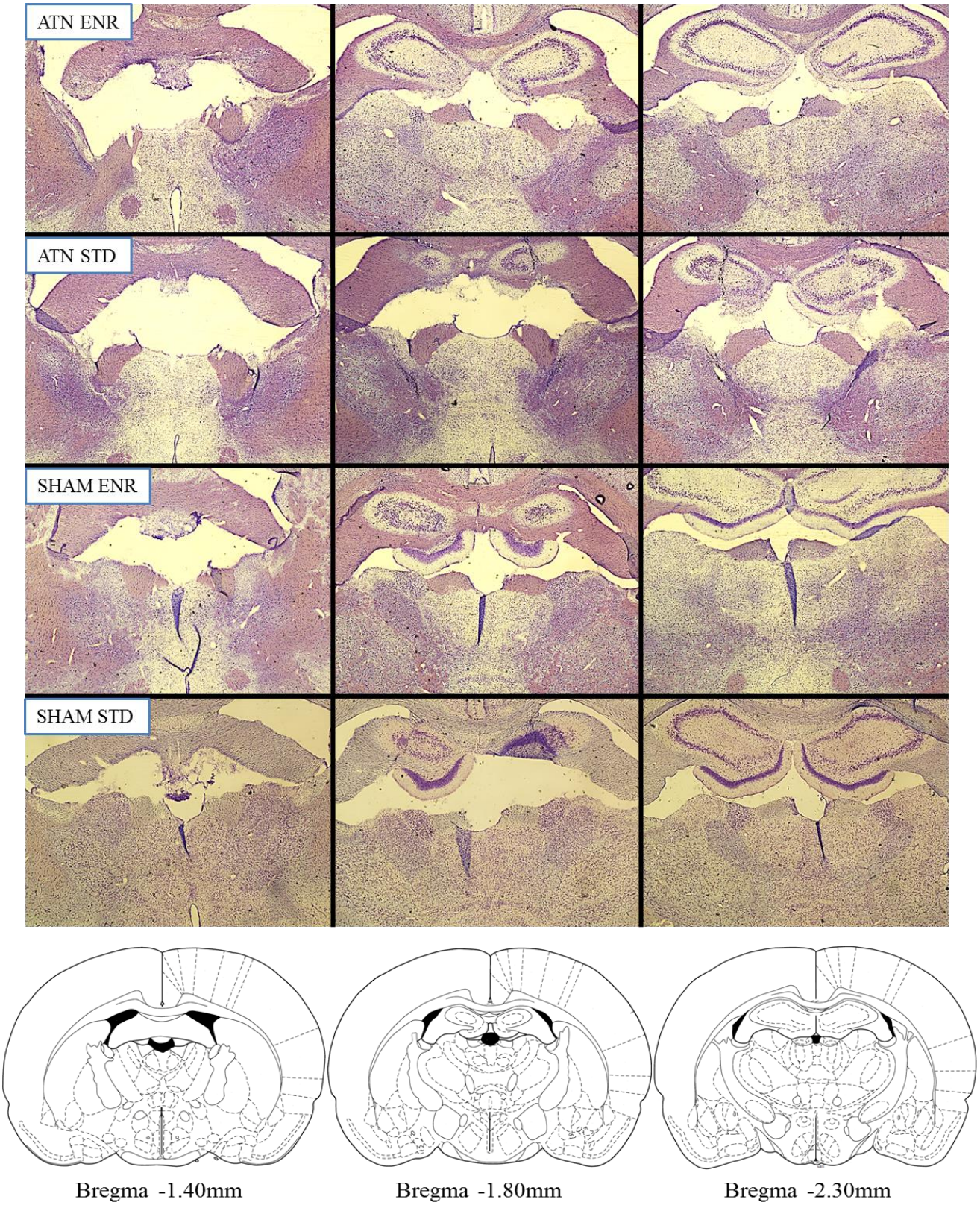


Figure 3.1. Representative photomicrographs at 2x magnification of the ATN with cresyl violet stain in rats with ATN lesions and sham rats housed in enriched and standard conditions, at approximately -1.40mm, -1.80mm and -2.30mm from Bregma (top) and corresponding atlas plates (from Paxinos & Watson, 1998). Each row of images is from the same rat. Substantial cell loss and shrinkage of the ATN can be seen in both ATN rats. ATN: neurotoxic anterior thalamic lesions; SHAM: sham lesions; STD: standard housing condition; ENR: enriched housing condition.

3.2 Pre-surgery Testing

3.2.1 Radial Arm Maze: Spatial Working Memory

For pre-surgery training, only the standard working memory task in the RAM was used. Figure 3.2 depicts errors (total arm revisits) across the 12 days grouped in two-day blocks. As for all statistical analyses in the present study (unless otherwise stated), performance was analysed using ANOVA tests with between-group factors of Lesion (ATN vs. SHAM) and Housing (ENR vs. STD). In this instance, pre-surgery RAM performance was analysed with two-trial blocks as the repeated measure (Block).

Performance was equivalent across all groups (F's all <1; for all analyses in the present study, any F's <1 are treated as p>0.5). All groups rapidly reduced arm revisits over the 12 days of training to one or fewer errors on average, indicating that the groups successfully learned the spatial memory task (Block, $F(5, 185) = 34.34$, $p<0.0001$). This RAM task was used for pre-surgery testing as it would later be the key task, albeit modified, prior to sacrifice for zif268 immunohistochemistry.

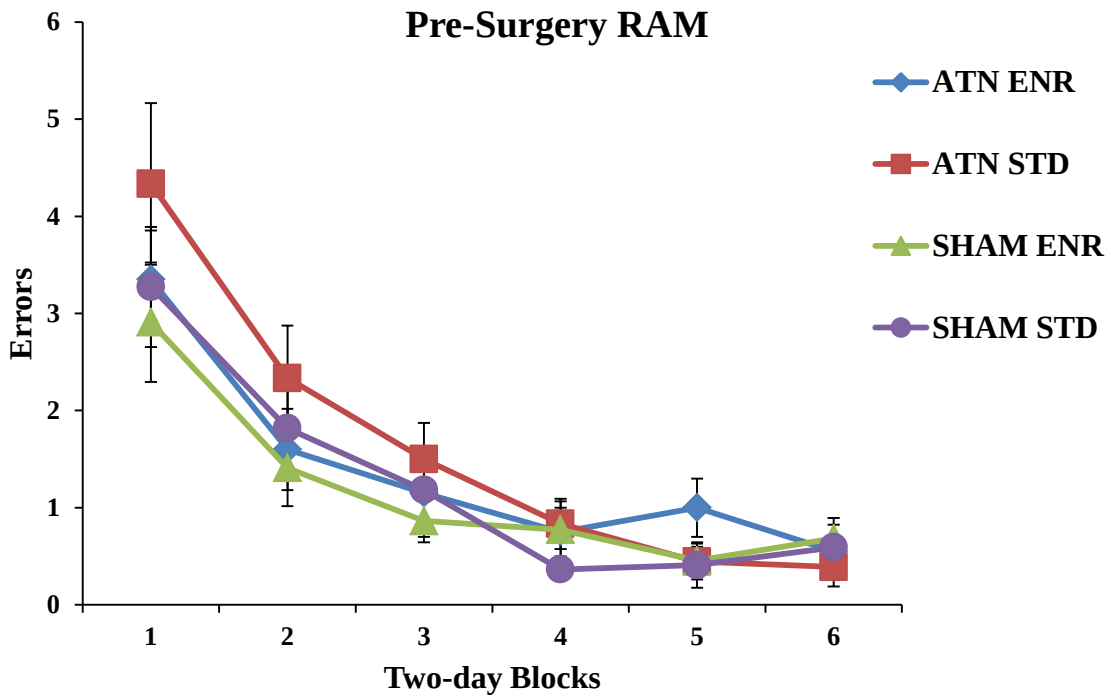


Figure 3.2. Spatial working memory performance in the radial arm maze (pre-surgery): mean errors ± SE for the 12 sessions of pre-surgery training in two-day blocks. ATN: neurotoxic anterior thalamic lesions; SHAM: sham lesions; STD: standard housing condition; ENR: enriched housing condition.

3.3 Post-surgery Testing (prior to enrichment)

For post-surgery testing prior to enrichment, spatial reference memory, working memory and minimised cues working memory tasks in the water maze were used. There were no significant differences in swim speed between the groups in these tasks (mean values across the post-surgery, enrichment and post-enrichment water maze tasks: ATN STD: 23.25cm/s ATN ENR: 21.90cm/s; SHAM STD: 22.9cm/s SHAM ENR: 21.94cm/s). For each of the water maze tasks path length and escape latency were analysed, with only path length analyses reported as results were similar on these measures. The two ATN lesion groups (ATN STD and ATN ENR) had equally severe spatial memory deficits in each of the water maze post-surgery tests prior to enrichment, as described below.

3.3.1 Water Maze: Spatial Reference Memory (prior to enrichment)

Figure 3.3 shows post-surgery performance for the 12 days of spatial reference memory testing in the water maze, which began approximately two weeks post-surgery and prior to enriched housing. The 12 days of testing were analysed with the repeated measure of two-day blocks (Block). As expected, rats with ATN lesions showed severe deficits on this task in terms of taking substantially longer paths to find the fixed platform (Lesion $F(1, 37) = 51.86$, $p<0.0001$). Both ATN and sham groups reduced their path lengths over time, confirmed by a significant effect of Block $F(5, 185) = 63.84$, $p<0.0001$, but there was no Lesion by Block interaction ($F<1$) showing that the ATN groups exhibited a persistent deficit across training. There were no effects of future Housing or interactions in this pre-differential housing task (F's all <1).

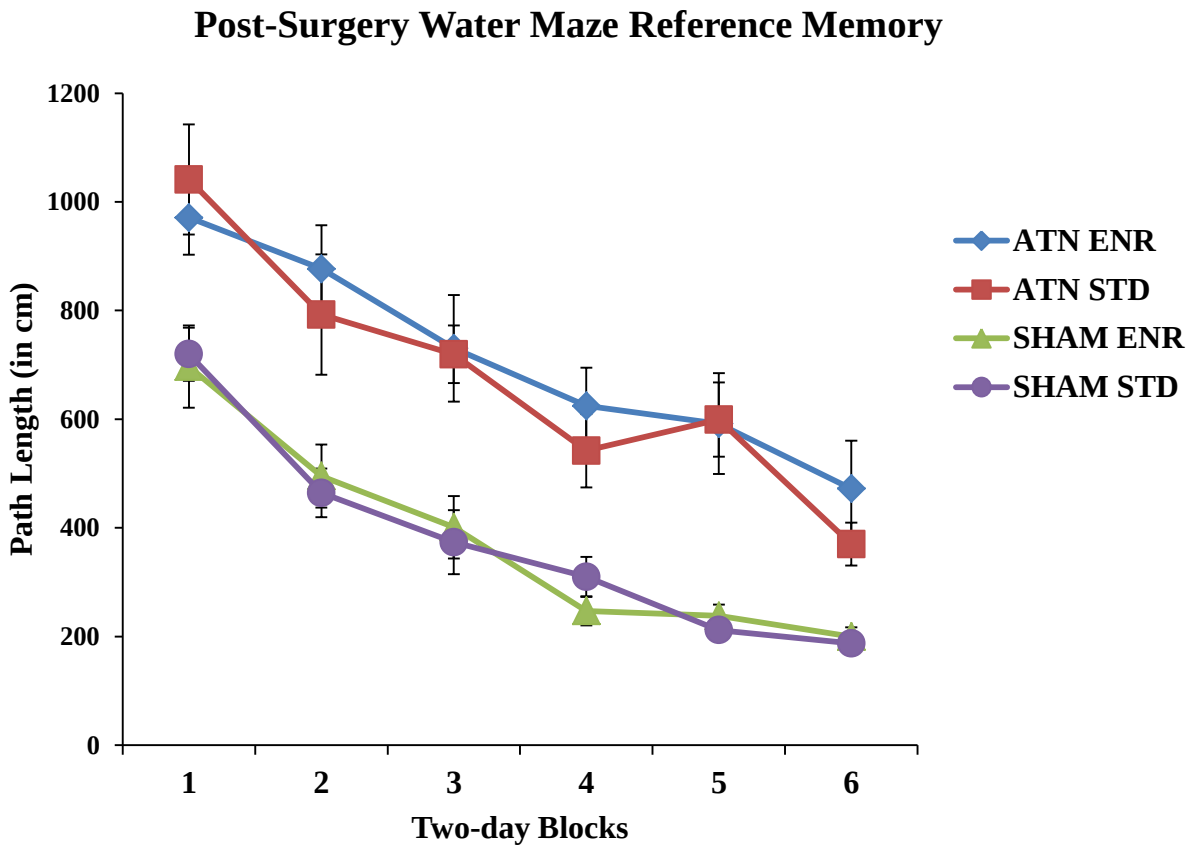


Figure 3.3. Spatial reference memory performance in the water maze (two weeks post-surgery): mean path length in centimetres ± SE for the 12 days of testing. ATN: neurotoxic anterior thalamic lesions; SHAM: sham lesions; STD: standard housing condition; ENR: enriched housing condition.

Reference Memory Probe (prior to enrichment):

Twelve days after the final day of reference memory testing, the rats were tested for recall of the previous platform location. Figure 3.4 shows the swim paths of rats with median performance in the sham and ATN groups. The time spent in the target quadrant (the quadrant in which the platform was located for reference memory testing) and crossings of the annulus (virtual enlargement of the platform area by 20 centimetres) were used as measures of accuracy. The 60-second trial was analysed with the repeated measure of 15-second periods (Time) to determine if there were any group differences over the course of the trial. Figure 3.5 depicts the crossings of the annulus and time spent in the target quadrant for the entirety of the probe trial, in the 15-second sections.

Figure 3.4. Photographs depicting the swim paths of the median rats from the ATN and sham groups on the reference memory probe trial. ATN rats spent a similar amount of time in the target quadrant (mean percentage of time spent in target quadrant: 47.23%) and crossed over the annulus (mean annulus crossings: 7.08) as often as sham rats (mean percentage of time spent in target quadrant: 43.71%; mean annulus crossings: 6.56). ATN: neurotoxic anterior thalamic lesions; SHAM: sham lesions.

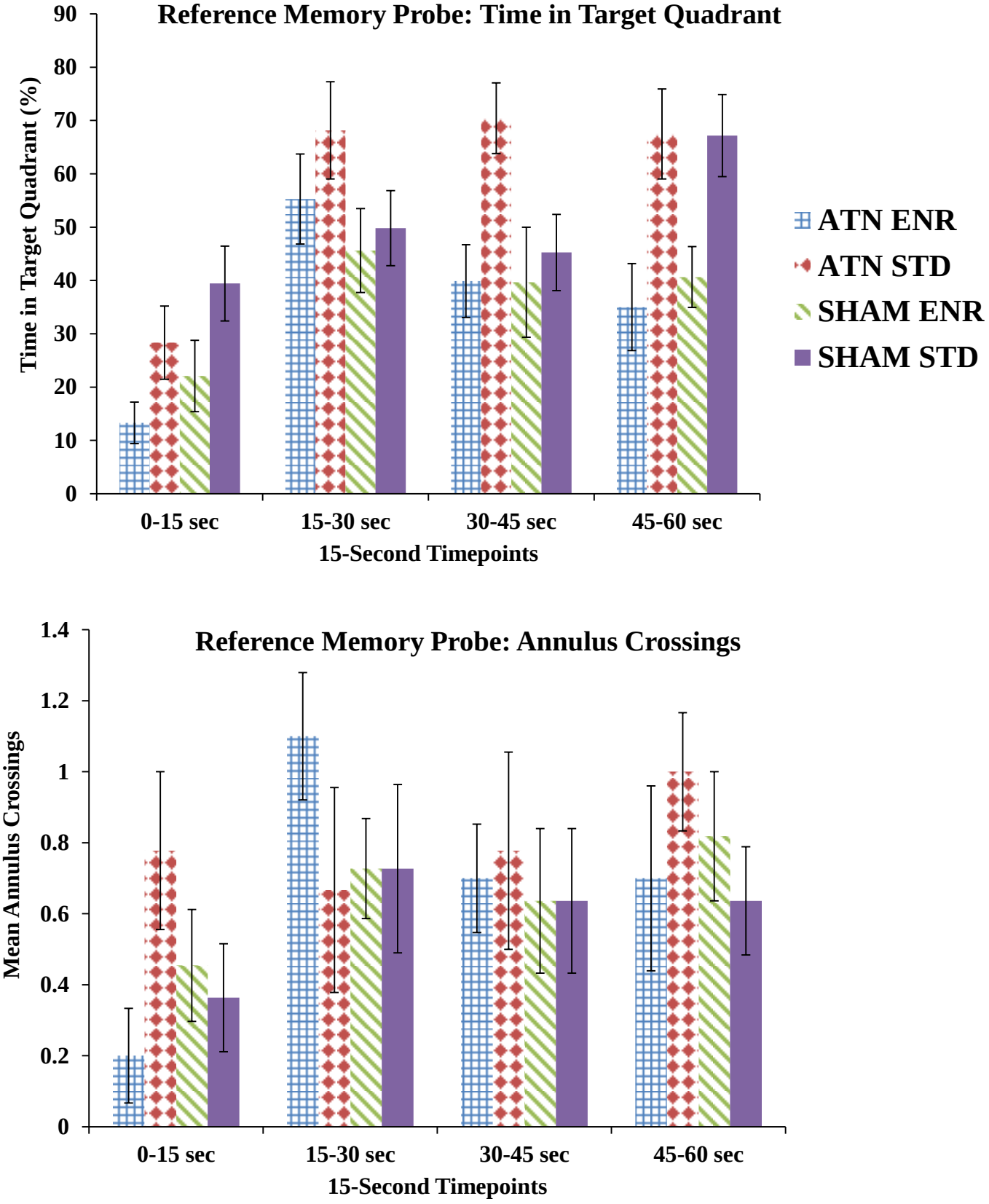


Figure 3.5. Spatial reference memory probe trial performance (post-surgery): mean percentage time spent in the target quadrant ± SE (top) and mean crossings of the annulus ± SE (bottom). ATN: neurotoxic anterior thalamic lesions; SHAM: sham lesions; STD: standard housing condition; ENR: enriched housing condition.

Rats with ATN lesions and sham rats demonstrated similar preferences for the target quadrant, although rats with ATN lesions showed a greater preference for the target quadrant for the entirety of the probe trial (Lesion $F(1, 37) = 5.17$, $p<0.05$). There was no effect of Housing, Time or interactions, suggesting that all groups spent a similar amount of time in the target quadrant for the duration of the probe trial. In terms of annulus crossings, there was no effect of Lesion ($F <1$) indicating that all groups crossed over the annulus a similar number of times. All groups crossed over the annulus a greater number of times after the first 15 seconds (Time $F(3, 111) = 2.74$, $p<0.05$), confirmed by a post-hoc Newman Keuls analysis showing increased annulus crossings from trial one to trial two, and between trial one and trial four (p's all >0.05).

Regression analysis of performance on the final two blocks (last four days) of post-surgery reference memory testing in terms of path length did not predict the number of annulus crossings (b -0.0001, $p>0.5$) or time spent in the target quadrant (b 0.001, $p>0.2$), suggesting that memory for the platform location over the final four days of testing did not predict accurate performance on the probe trial.

3.3.2 Water Maze: Spatial Working Memory (prior to enrichment)

Figure 3.6 depicts spatial working memory performance for rats with ATN and sham lesions. Testing on the post-surgery working memory task began the day following the reference memory probe trial and continued for 12 days, after which testing began on the spatial working memory task with minimised cues. The 12 days of testing were analysed in two-day blocks with Trial and Block as the repeated measures. Rats with ATN lesions demonstrated substantial impairment on this task (Lesion $F(1, 39) = 44.26$, $p<0.0001$) in terms of distance to find the platform location, which changed each day.

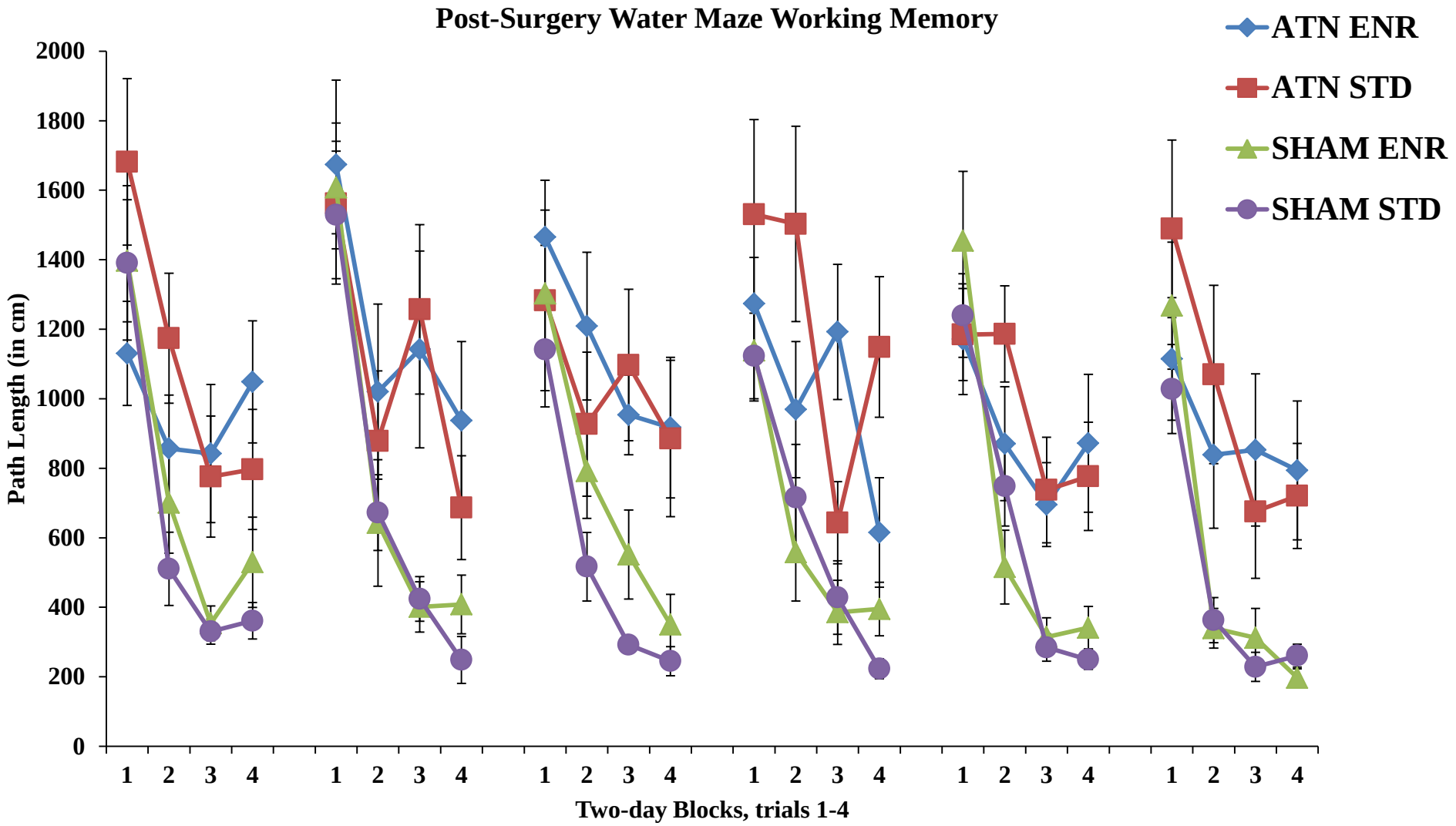


Figure 3.6. Spatial working memory performance in the water maze (post-surgery): mean path length in centimetres ± SE for the 12 days of testing in two-day blocks. ATN: neurotoxic anterior thalamic lesions; SHAM: sham lesions; STD: standard housing condition; ENR: enriched housing condition.

All groups improved their performance within each trial of four trials (Trial $F(3, 111) = 130.34$, $p<0.0001$) but the Trial by Lesion interaction ($F(3, 111) = 13.13$, $p<0.0001$) and post-hoc Newman-Keuls tests showed that although sham rats performed similarly on trial 1 to rats with ATN lesions ($p>0.2$), the subsequent three trials revealed a more rapid improvement in path length (and thus better working memory) for the sham rats (p's all <0.001). By contrast, rats with ATN lesions maintained greater path lengths than sham rats with little improvement between trials 2-4 (p's all <0.001). Performance improved over the 12 days of testing for all groups (Block $F(5, 185) = 2.78$, $p<0.05$). A Block by Trial interaction ($F(15, 555) = 1.69$, $p<0.05$) and post-hoc Newman-Keuls tests (p's all <0.05) suggest that performance for all groups on trial 1 improved over the six blocks of testing. Performance was again equivalent between the groups that were to be assigned to later enriched or standard housing, with no main effects of Housing or interactions (F's all <1).

3.3.3 Water Maze: Spatial Working Memory with Minimised Distal Cues (prior to enrichment)

Figure 3.7 shows spatial working memory performance for the six days of testing when distal cues were minimised. Testing began two days after completion of the post-surgery standard working memory task in the water maze, and continued for six days until commencement of the continuous enrichment period. Performance was analysed with the repeated measures of Day (instead of Block analyses as in previous tasks, as only 6 days of testing were used) and Trial.

Rats with ATN lesions were severely impaired on this task in terms of distance to locate the daily unique platform position (Lesion $F(1, 37) = 44.77$, $p<0.0001$), although all groups were able to learn the location of the platform during each trial (Trial $F(3, 111) = 56.40$, $p<0.0001$). Although all groups improved in their ability to locate the daily unique platform position over the six days of testing (Day $F(5, 185) = 3.77$, $p<0.01$), a Day by Lesion interaction ($F(5, 185) = 3.60$, $p<0.01$) and post-hoc Newman-Keuls tests suggest that rats with ATN lesions were impaired by comparison to sham rats over days 1-5 (p's all <0.05) but there was no difference in path length between the lesion and sham groups on day 6 ($p>0.2$). Sham rats were also able to locate the platform more rapidly over the four trials than rats with ATN lesions (Trial by Lesion $F(3, 111) = 3.80$, $p<0.05$), and post-hoc Newman-Keuls tests showed that sham rats improved over trials 1-3 (p's all <0.001) while rats with ATN lesions only improved their performance between trials 1 and 2, and maintained this performance between trials 2 and 4 (p's all <0.001). Performance was equivalent between the groups assigned to later enriched or standard housing, with no effects of Housing or interactions (F's all <1).

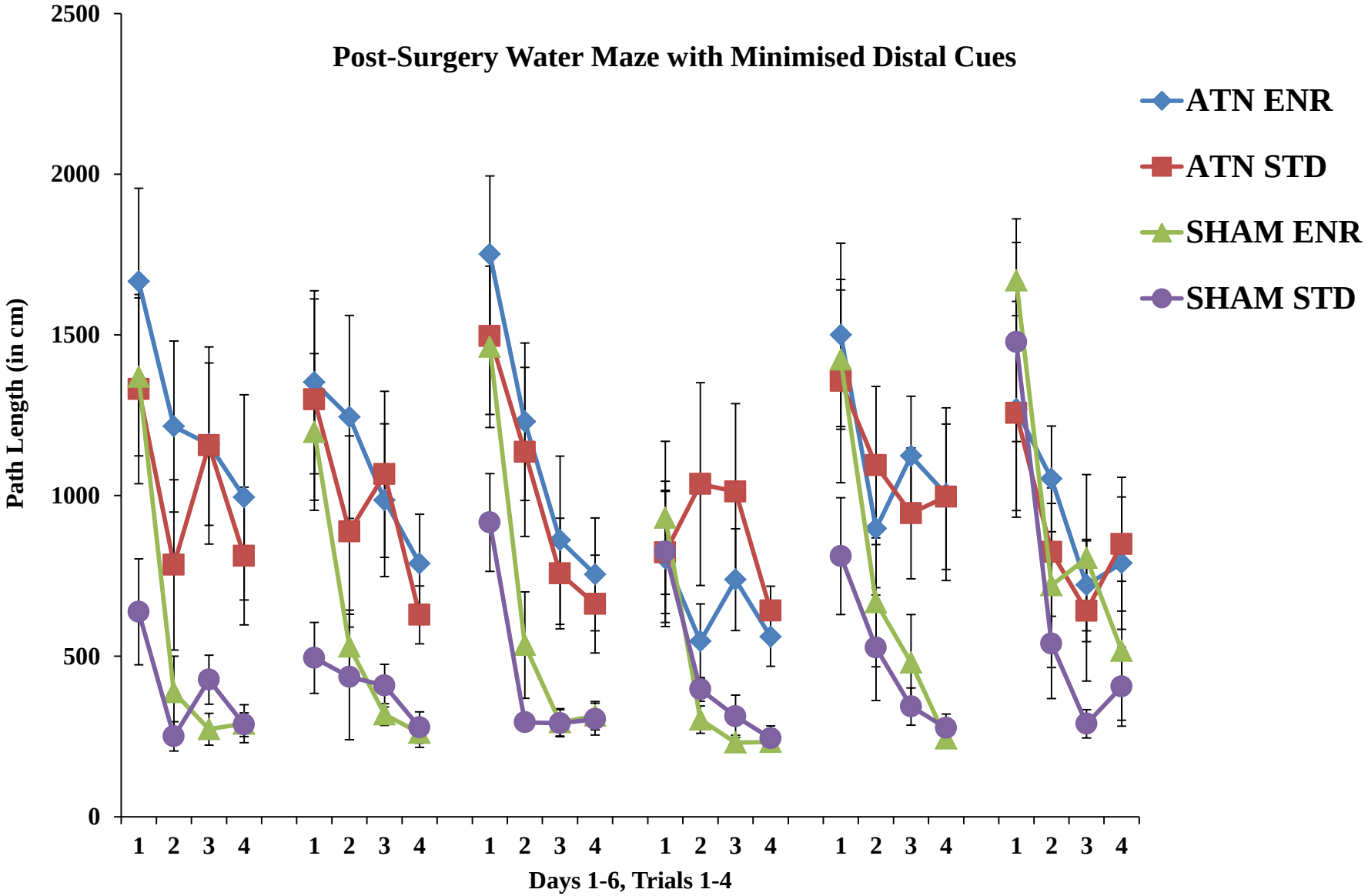


Figure 3.7. Spatial working memory performance when distal cues were minimised in the water maze (post-surgery): mean path length in centimetres ± SE for the 6 days of testing. ATN: neurotoxic anterior thalamic lesions; SHAM: sham lesions; STD: standard housing condition; ENR: enriched housing condition.

To examine whether performance on the minimised cues task differed from the post-surgery standard working memory task, an ANOVA was performed for the final three days of testing on each task with the within-group factors of Task and Day. As previously shown, rats with ATN lesions were impaired on both tasks (Lesion $F(1, 37) = 42.99$, $p<0.001$). The main effects of Task, Day and Housing were not significant (F's all <1), but there was a significant Task by Day interaction ($F(2, 74) = 9.17$, $p<0.001$). A post-hoc Newman-Keuls test ($p<0.05$) suggests that all groups were unexpectedly less impaired in terms of path length on the third day of testing on the minimised cues task when compared with the first comparison day of standard working memory (Day 9 of testing), whereas results for the two tasks were comparable thereafter.

3.4 Testing during the Main (Continuous) Enrichment Period

3.4.1 Water Maze: Spatial Working Memory

On days 13 and 25 of the continuous enrichment period, and on day 1 thereafter (day 37 of the enrichment protocol), the rats were tested on the standard working memory task in the water maze with all cues available, but using eight massed trials that were each of 90 seconds' duration. Performance on this task was analysed with repeated measures of Day and Trial. Figure 3.8 shows spatial working memory performance during the continuous enrichment period on days 13, 25 and 37 (day 1 of the post-enrichment period).

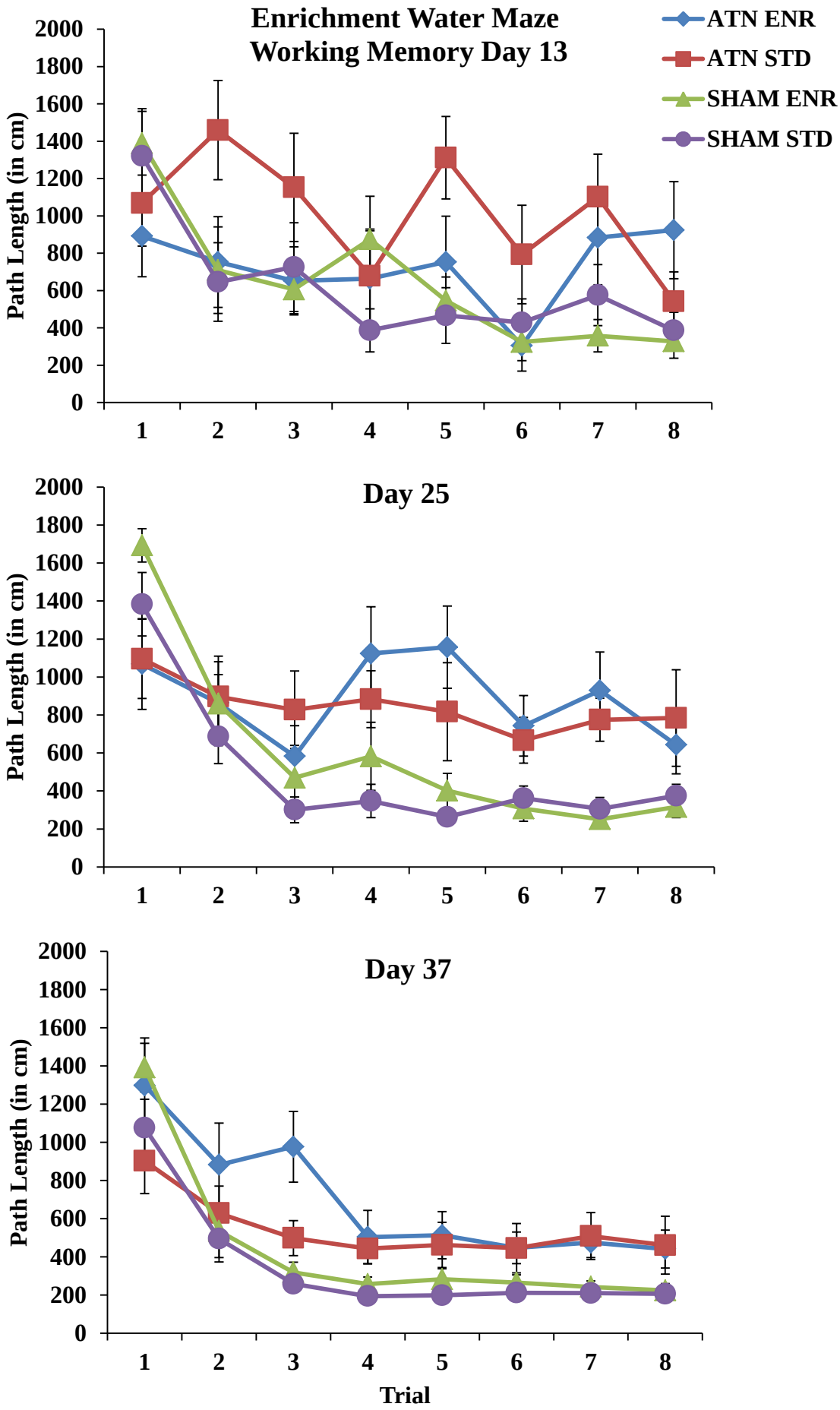


Figure 3.8. Spatial working memory performance in the water maze (enrichment): mean path lengths in centimetres ± SE for testing on days 13, 25 and 37 of the enrichment period. ATN: neurotoxic anterior thalamic lesions; SHAM: sham lesions; STD: standard housing condition; ENR: enriched housing condition.

Rats with ATN lesions housed in both enriched and standard conditions were significantly impaired on this task in terms of path length to locate the daily unique platform position (Lesion $F(1, 37) = 18.59$, $p<0.0001$). All groups improved over the eight trials on each day (Trial $F(7, 259) = 32.19$ $p<0.0001$), although a Trial by Lesion interaction ($F(7, 259) = 8.08$, $p<0.0001$) shows that sham rats again demonstrated better working memory performance than rats with ATN lesions. All groups improved their path lengths over the three trials (Day $F(2, 74) = 10.64$, $p<0.0001$), and post-hoc Newman-Keuls tests revealed that path lengths were shorter for all groups on the third day of testing (Day 37) when compared to the previous two days ($p<0.05$). There was no main effect of Housing or interaction effects.

3.5 Testing after the Continuous Enrichment Period (Overnight Enrichment)

3.5.1 Water Maze: Spatial Working Memory

After the final day of testing for the main (continuous) enrichment period (Day 1 of overnight enrichment), the rats were re-tested on the standard spatial working memory task for 12 days, with four trials per day. Performance was analysed with repeated measures of Block (two days of testing in each block) and Trial. Figure 3.9 depicts post-enrichment spatial working memory performance.

Rats with ATN lesions in both housing conditions were significantly impaired on this task in terms of path length to locate the daily unique platform position (Lesion $F(1, 37) = 50.69$, $p<0.0001$). The Lesion by Housing interaction approached significance ($F(1, 37)= 3.23$, $p<0.08$), suggesting that rats with ATN lesions housed in enrichment had improved performance when compared to standard-housed ATN rats (the two sham groups had equivalent performance). All groups reduced their path lengths over the 12 days of testing (Block $F(5, 185) = 2.91$, $p<0.05$) reflecting improved search strategies. A Block by Housing

interaction ($F(5, 185) = 3.07$, $p<0.05$) and post-hoc Newman-Keuls tests suggested that rats housed in enrichment had substantially improved performance from blocks 2-3 ($p<0.05$), whereas standard-housed sham rats and rats with ATN lesions did not show any improvement across the blocks of testing (p's all >0.05). The Lesion by Housing by Block interaction did not reach significance ($F(5, 185) = 1.69$, $p=0.13$).

All groups improved their performance over the four trials on each day (Trial $F(3, 111) = 148.08$, $p<0.0001$), and a Trial by Lesion interaction ($F(3, 111) = 8.75$, $p<0.0001$) and post-hoc Newman-Keuls test suggested that sham rats had similar performance to rats with ATN lesions on the first trial of each block ($p>0.05$), but the sham rats rapidly improved on subsequent trials in contrast to the relatively impaired performance of the ATN rats (p's all <0.001). A further Block by Trial interaction ($F(15, 555) = 2.42$, $p<0.01$) indicates that all groups reduced their path lengths for the first trial across the blocks of testing. The Lesion by Housing by Block by Trial interaction did not reach significance ($F(15, 555) = 1.15$, $p=0.30$).

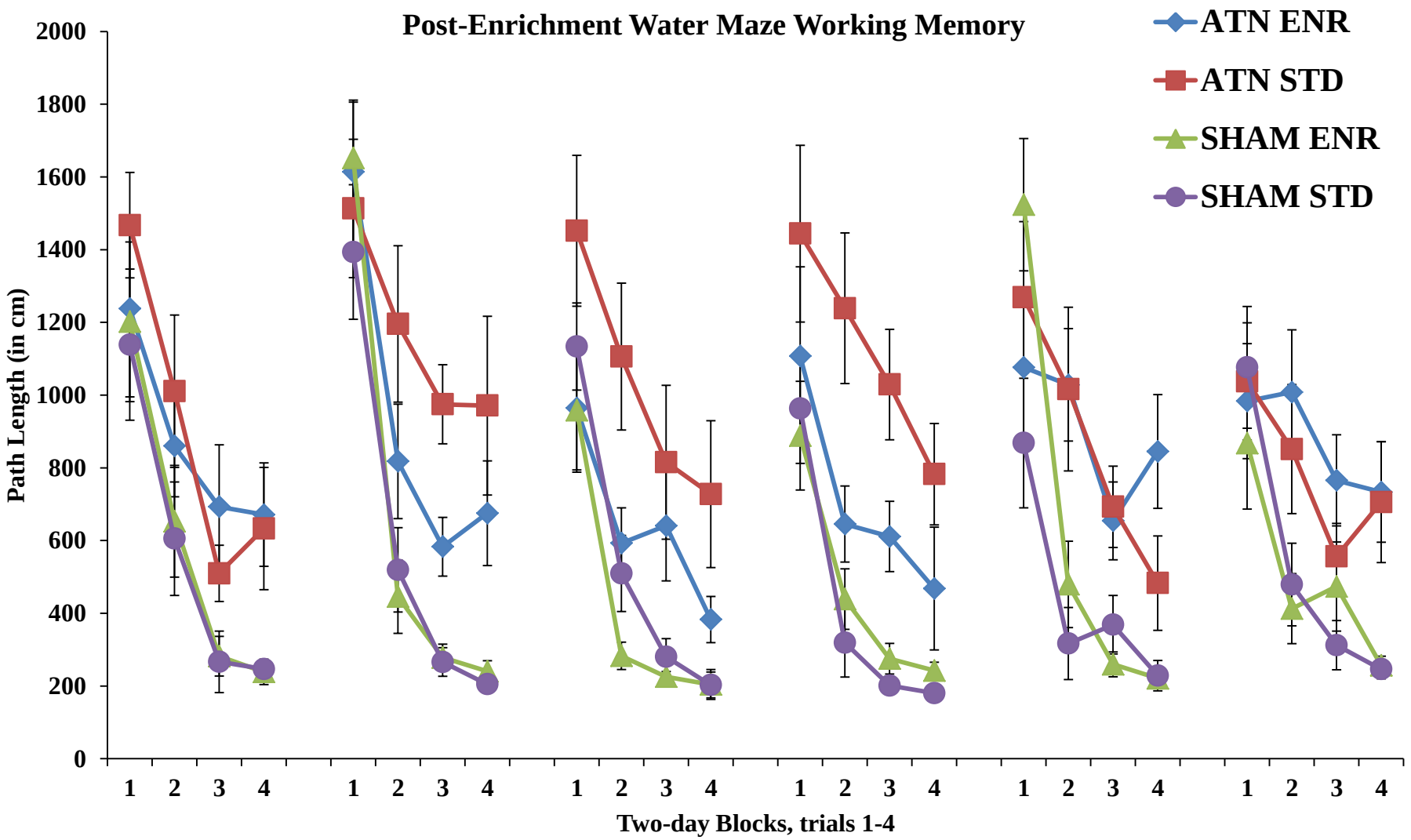


Figure 3.9. Spatial working memory performance in the water maze (post-enrichment): mean path length in centimetres ± SE for the 12 days of testing in two-day blocks. ATN: neurotoxic anterior thalamic lesions; SHAM: sham lesions; STD: standard housing condition; ENR: enriched housing condition.

3.5.2 Water Maze: Spatial Reference Memory

Figure 3.10 shows performance on the water maze spatial reference memory task after the continuous enrichment period. Testing began one day after completion of the prior working memory task and continued for 12 days, the data from which were grouped in two-day blocks for analysis (Block). Rats with ATN lesions, irrespective of housing (Housing, Housing by Lesion, and Housing by Lesion by Block *F*'s all <1) were substantially impaired in terms of path length to locate the fixed platform (Lesion F (1, 37) = 50.29, p<0.0001). All groups showed improved performance over the 12 days of testing (Block F(5, 185) = 13.58, p<0.0001).

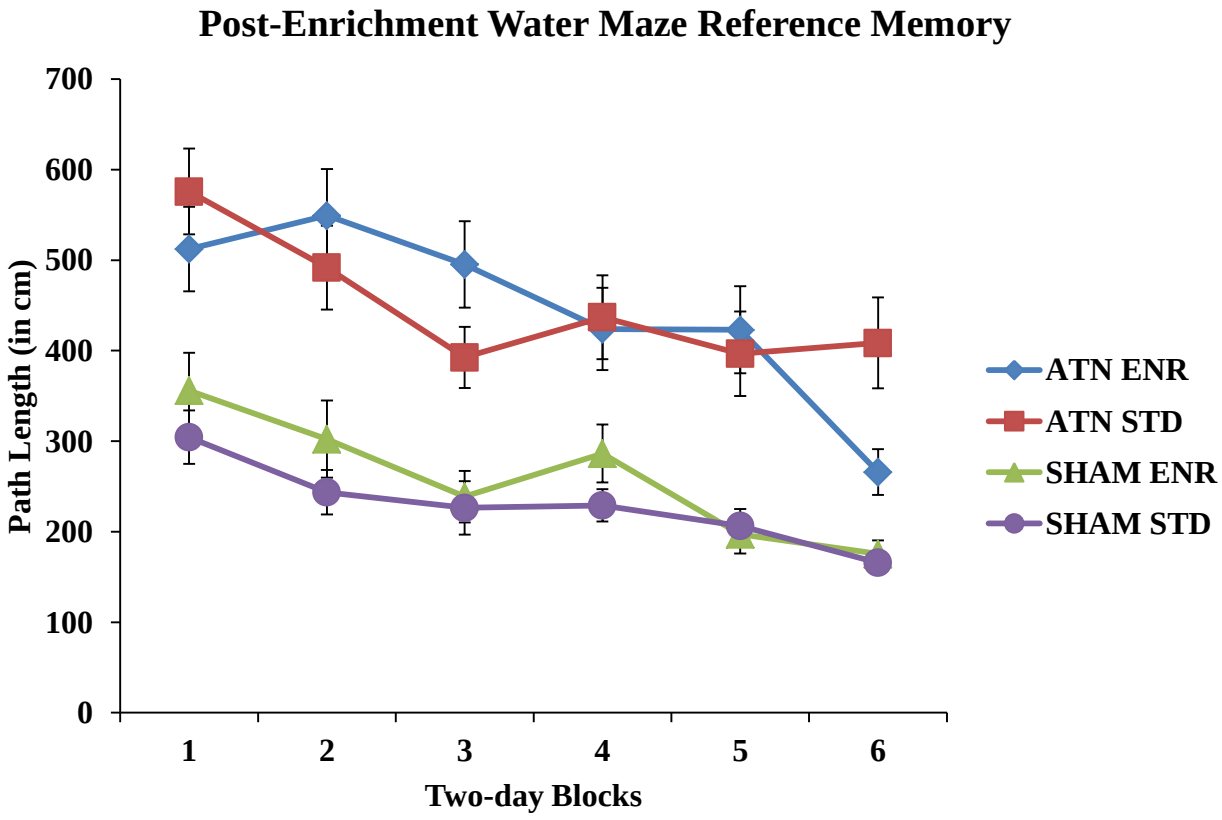


Figure 3.10. Spatial reference memory performance in the water maze (post-enrichment): mean path length in centimetres ± SE for the 12 days of testing in two-day blocks. ATN: neurotoxic anterior thalamic lesions; SHAM: sham lesions; STD: standard housing condition; ENR: enriched housing condition.

As Figure 3.10 suggests, rats with ATN lesions housed in enrichment began to improve on the last block of two trials. A separate analysis was conducted that was restricted to the final block of testing (block 6). This analysis showed a significant Lesion by Housing interaction ($F(1, 37) = 5.37$, $p<0.05$), and a subsequent Newman-Keuls post-hoc analysis suggested that the ATN enriched group had improved performance in terms of path length on the last two days of testing when compared with the standard-housed ATN group ($p<0.01$).

3.5.3 Radial Arm Maze: Spatial Working Memory, Standard Task

Two weeks after the final day of testing in the water maze, the rats were re-tested on the standard spatial working memory task in the radial arm maze on which they had been trained prior to surgery. Figure 3.11 shows the total number of errors across the 12 days of testing, in two-trial blocks. The 12 days of testing were analysed with the repeated measure of two-day blocks (Block). Rats with ATN lesions were substantially impaired on this task in terms of entries to previously visited arms (Lesion $F(1, 37) = 100.50$, $p<0.0001$). Rats housed in enrichment made fewer errors than standard-housed rats (Housing $F(1, 37) = 5.12$, $p<0.05$). The most important finding was that rats with ATN lesions housed in enrichment clearly made fewer errors than standard-housed rats with ATN lesions, whereas there was no difference between sham rats housed in enriched or standard conditions (Lesion by Housing $F(1, 37) = 7.40$, $p<0.01$).

All groups improved their performance significantly over the 12 days of testing. The Block ($F(5, 185) = 3.02$, $p<0.05$), Block by Lesion interaction ($F(5, 185) = 3.42$, $p<0.01$), and post-hoc Newman-Keuls analysis confirmed that sham rats improved over each block (p's all <0.0001) whereas rats with ATN lesions had relatively stable impaired performance ($p>0.05$). The Block by Housing interaction ($F(5, 185) = 3.78$, $p<0.01$) and post-hoc Newman-Keuls test suggested that rats housed in enrichment had more improved

performance between block 1 and block 6 ($p<0.05$) compared to standard-housed rats. The Lesion by Housing by Block interaction did not reach significance ($F(5, 185) = 2.02$, $p=0.07$).

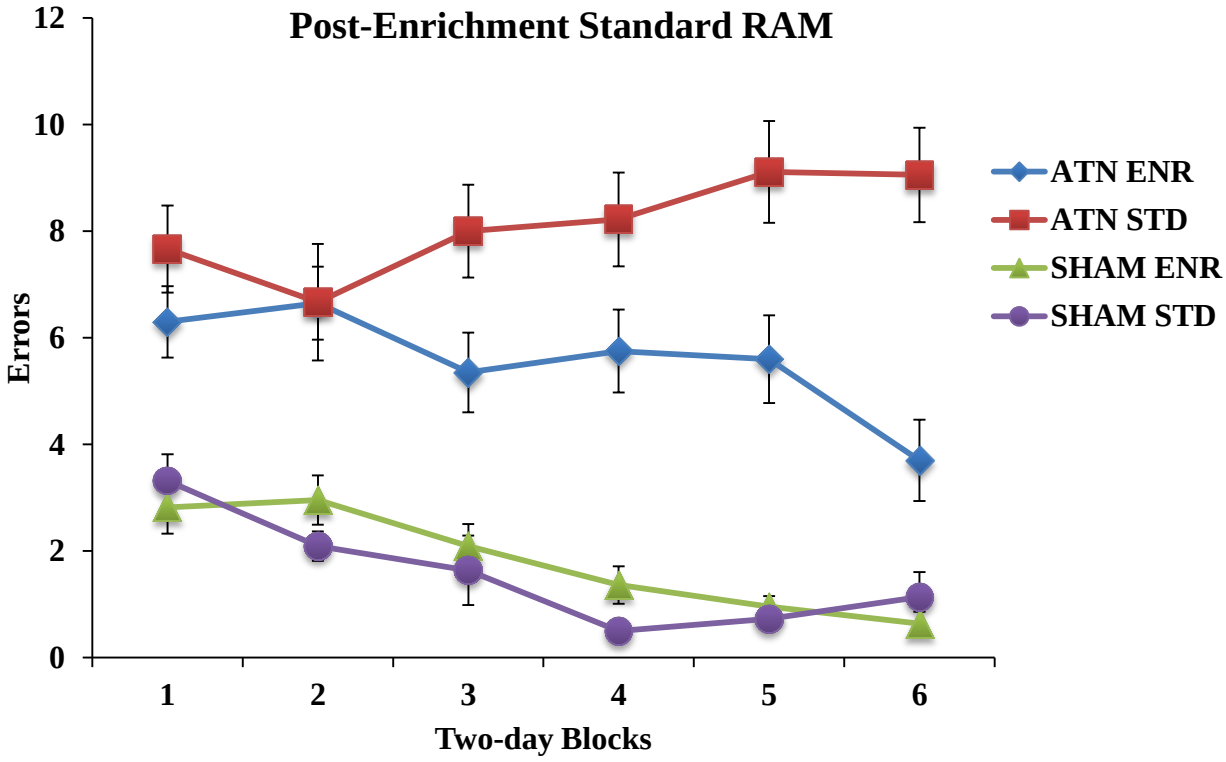


Figure 3.11. Spatial working memory performance in the radial arm maze (post-enrichment): mean total errors (arm re-entries) ± SE for the 12 days of testing in two-day blocks. ATN: neurotoxic anterior thalamic lesions; SHAM: sham lesions; STD: standard housing condition; ENR: enriched housing condition.

3.5.4 Radial Arm Maze: Spatial Working Memory with Delay Only and Delay with Rotation

After the final day of testing on the standard RAM task, the rats were divided into two groups with equivalent housing and lesion numbers and counterbalanced to testing on two new RAM conditions to further examine spatial working memory. After the fourth arm choice, one condition used a delay of 60sec whereas the second condition used a delay of 60sec during which a rotation of the maze by 45° took place. Figure 3.12 shows errors for the four days of testing on these two RAM conditions.

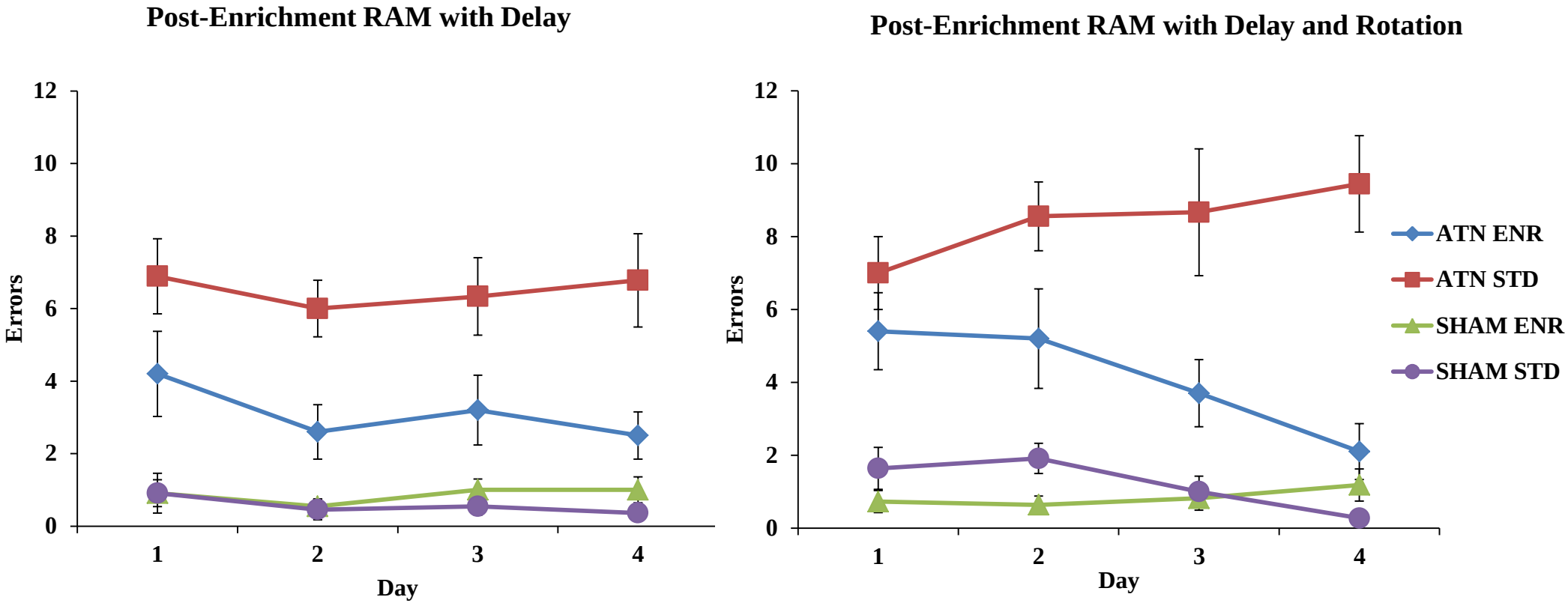


Figure 3.12. Spatial working memory performance in the radial arm maze (post-enrichment) for the delay condition (left) and delay with rotation condition (right): mean total errors (arm re-entries) ± SE for the 4 days of testing for each condition. ATN: neurotoxic anterior thalamic lesions; SHAM: sham lesions; STD: standard housing condition; ENR: enriched housing condition.

To examine whether performance on the delay only and delay with rotation tasks differed in comparison to the final four days of performance on the previous standard task on the RAM, an ANOVA was used with within-group factors of Task (Delay, Delay with Rotation, and Standard) and Day (for the four days of testing on each task). Figure 3.13 summarises performance on the three tasks. Across all three tasks in the RAM, rats with ATN lesions were significantly impaired in terms of arm revisits (Lesion $F(1, 37) = 132.99$, $p<0.0001$) although rats housed in enrichment made significantly fewer errors over the three tasks (Housing $F(1, 37) = 21.49$, $p<0.001$). A Lesion by Housing interaction ($F(1, 37) = 20.90$, $p<0.001$) and post-hoc Newman-Keuls test suggested that rats with ATN lesions housed in enrichment made fewer errors across the three tasks than standard-housed rats with ATN lesions ($p<0.001$).

A significant effect of Task ($F(2, 74) = 6.09$, $p<0.01$) and post-hoc Newman Keuls tests revealed that all rats were generally less impaired on the delay only task in terms of arm revisits when compared with both the delay with rotation ($p<0.05$) and standard (post-hoc $p<0.01$) tasks. However, the Task by Lesion interaction ($F(2, 74) = 4.14$, $p<0.05$) and post-hoc Newman-Keuls tests show that sham rats of both housing conditions performed similarly across the three tasks (p's all >0.5), whereas rats with ATN lesions housed in enriched and standard conditions were more impaired (equally) on the delay with rotation and standard tasks when compared with the delay only task (p's <0.01). The Task by Housing, Task by Lesion by Housing, and Task by Lesion by Housing by Day interactions were not significant (p's >0.10).

A Day by Housing ($F(3, 111) = 3.19$, $p<0.05$) interaction and post-hoc Newman-Keuls tests suggest that rats housed in enrichment made fewer errors than rats housed in standard conditions over days 2, 3 and 4 (days 10, 11 and 12 of the standard task) of testing across all tasks (p's all <0.05). A Day by Lesion by Housing interaction ($F(3, 111) = 6.52$,

p<0.001) and post-hoc Newman-Keuls tests suggest that rats with ATN lesions made fewer errors when compared with standard-housed rats with ATN lesions across all four days of testing (p's all <0.05), whereas the two sham groups did not differ in their performance. The most interesting statistic was that by the final day of testing on the three tasks, rats with ATN lesions housed in enrichment had performance equivalent to that of the two sham groups (p's all >0.05).

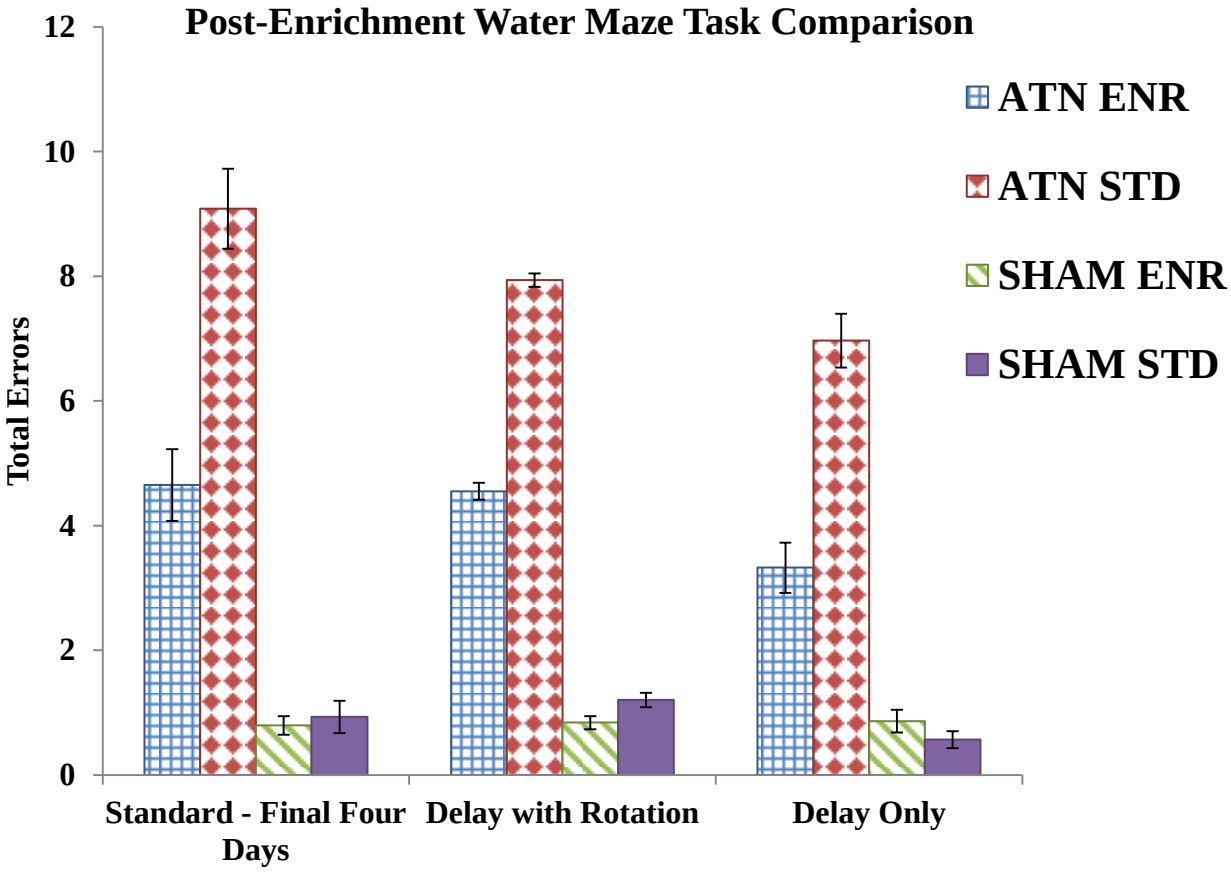


Figure 3.13. Spatial working memory performance in the radial arm maze (post-enrichment): mean total errors (arm re-entries) ± SE for the last four days of testing in standard task, compared with performance on the delay with rotation and delay only tasks. ATN: neurotoxic anterior thalamic lesions; SHAM: sham lesions; STD: standard housing condition; ENR: enriched housing condition.

3.5.5 Radial Arm Maze: Spatial Working Memory, Zif268 Induction Task

Following the final day of testing on the RAM tasks, the rats were introduced to a new delay with rotation procedure where they were restricted to eight arm choices (four choices made prior to the rotation) for the three consecutive trials on each of the three days of testing. The room cues were changed on the first day and again on the third day of testing, to stimulate zif268 induction. Performance was analysed with repeated measures of Trial and Day. Figure 3.14 shows spatial working memory performance on these final three days of testing.

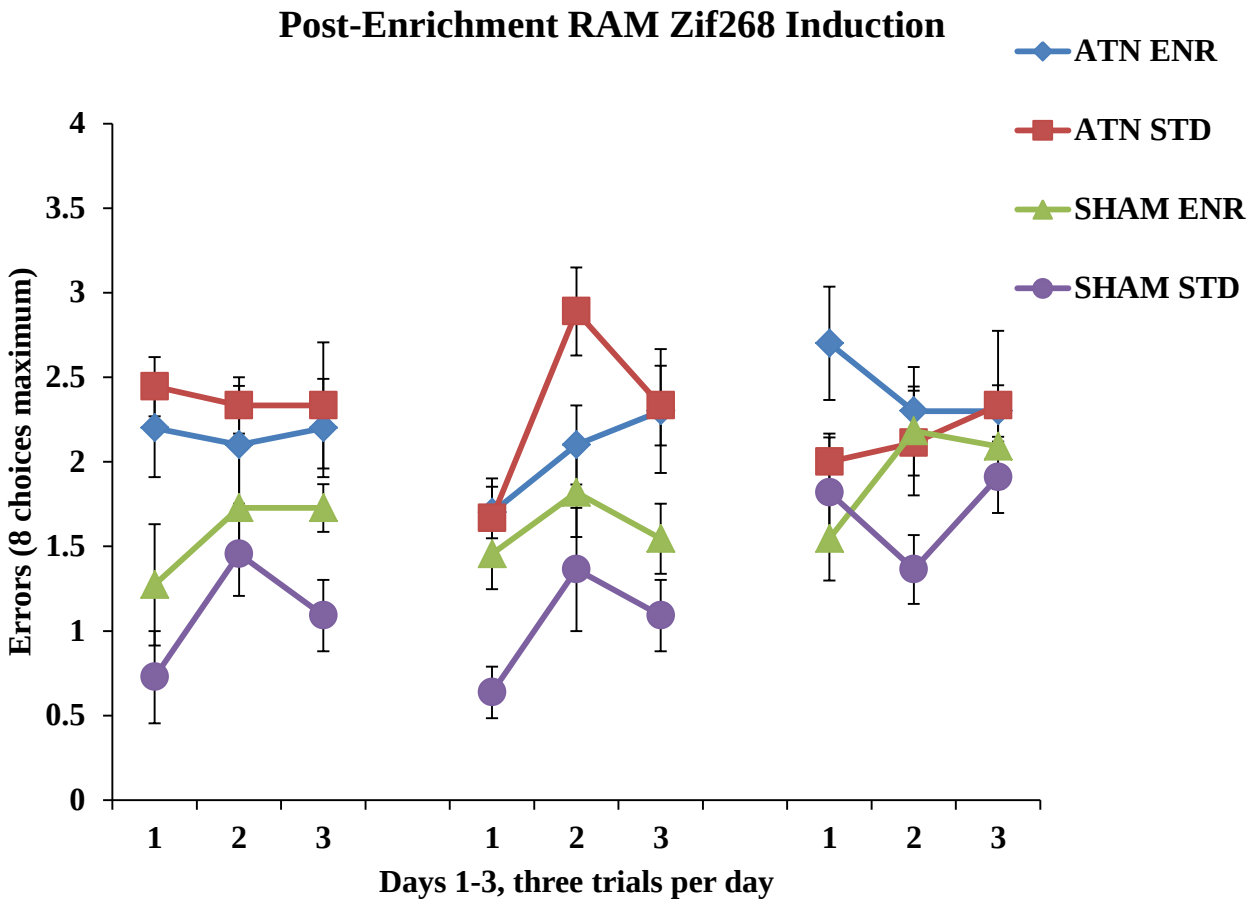


Figure 3.14. Spatial working memory performance in the radial arm maze (post-enrichment zif268 induction task): mean total errors (arm re-entries) ± SE for the 3 days of testing. ATN: neurotoxic anterior thalamic lesions; SHAM: sham lesions; STD: standard housing condition; ENR: enriched housing condition.

Rats with ATN lesions were severely impaired on this task in terms of arm revisits (Lesion $F(1, 37) = 40.21$, $p<0.0001$). There was, however, a Lesion by Housing interaction ($F(1, 37) = 4.36$, $p<0.05$) and a post-hoc Newman-Keuls analysis suggested that sham rats housed in enriched conditions had poorer performance in terms of arm revisits over the three days of testing than sham rats housed in standard conditions ($p<0.05$), whereas both ATN groups did not differ in their performance over the three days of testing ($p>0.5$). Performance for all groups varied over the three days of testing (Day $F(2, 74) = 4.35$, $p<0.05$), most likely due to the change in room configuration on days 1 and 3. Performance differed over the three trials of each day (Trial $F(2, 74) = 5.30$, $p<0.01$), with a post-hoc Newman-Keuls test showing an increase in errors on trials 1 and 2 (p's <0.05) perhaps due to memory retention of arm visits on the previous trials.

Performance for the final day of testing was of primary importance for the analysis of spatial memory and corresponding zif268 expression as rats were sacrificed 90 minutes after the final trial. This final day was analysed separately across Lesion and Housing with a within-group factor of Trial. Rats with ATN lesions were significantly impaired on this task in terms of arm revisits across the three trials on this day (Lesion $F(1, 37) = 7.65$, $p<0.01$). There were no main effects of Housing or Trial, and no interaction effects (F's all<1).

3.6 Zif268 Immunoreactivity

3.6.1 Retrosplenial Cortex

Figure 3.15 shows mean zif268 cell counts/mm^2 for the superficial and deep layers of the anterior and posterior granular b (Rgb) and granular a (Rga) retrosplenial cortex, Figure 3.16 depicts these counts for the superficial and deep layers of the anterior and posterior dysgranular retrosplenial cortex (Rdg). Figure 3.19 shows representative photomicrographs of the zif268 sections for these regions. To avoid differences in variance across regions, zif268 cell counts in each sub-region (e.g. posterior Rgb, superficial layer) were analysed with a separate ANOVA.

Irrespective of housing condition, ATN lesions reduced zif268 immunoreactivity in the Rgb and Rga regions, in both the superficial and deep layers. ATN lesion effects were associated with substantially reduced zif268 cell counts in the Rgb, anterior superficial ($F(1, 37) = 24.49$, $p<0.0001$), and posterior superficial ($F(1, 37) = 32.57$, $p<0.0001$), and less so in the posterior deep ($F(1, 37) = 15.53$, $p<0.01$) layers. The effect of Lesion was not significant in the anterior deep layers of the Rgb ($F(1, 37) = 2.92$, $p=0.09$). Substantial reductions in zif268 immunoreactivity were also observed after ATN lesions in the superficial ($F(1, 37) = 4.17$, $p<0.05$) and deep ($F(1, 37) = 10.71$, $p<0.01$) layers of the Rga. Housing and Lesion by Housing effects were not observed in any of the retrosplenial regions analysed (F's all <1).

For the Rdg region, significant reductions in zif268 immunoreactivity associated with ATN lesions were only observed within the posterior deep layer ($F(1, 37) = 6.40$, $p<0.05$), but not in the anterior superficial ($F(1, 37) = 1.68$, $p=0.20$), anterior deep ($F(1, 37) = 0.72$, $p=0.39$) or posterior superficial ($F(1, 37) = 0.64$, $p=0.42$) layers of the Rdg.

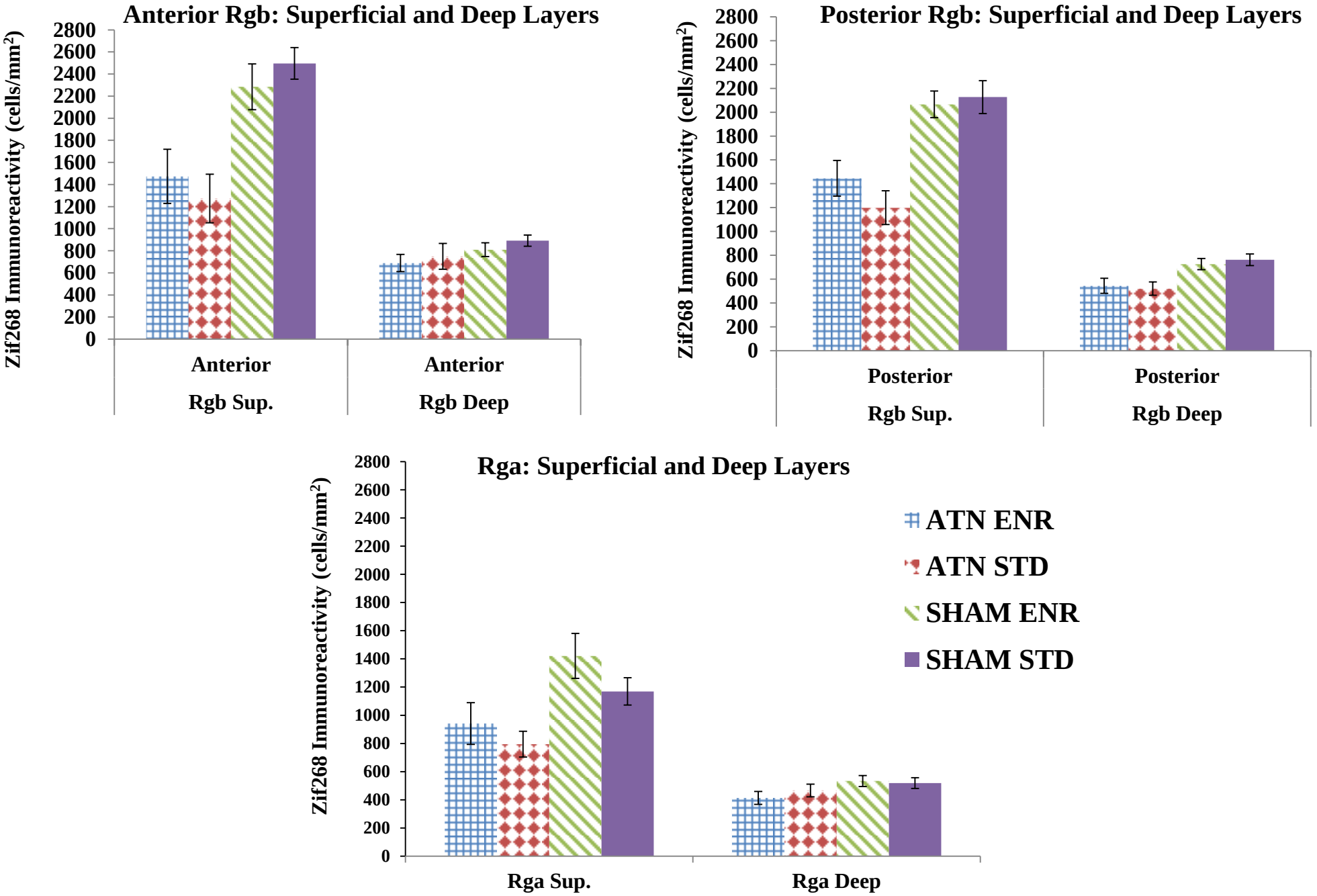


Figure 3.15. Mean (±SE) Zif268 cell counts (cells/mm^2) in the anterior and posterior granular b retrosplenial cortex (Rgb), superficial (Sup.) and deep layers, and the granular a retrosplenial cortex (Rga), superficial and deep layers. ATN: neurotoxic anterior thalamic lesions; SHAM: sham lesions; STD: standard housing condition; ENR: enriched housing condition.

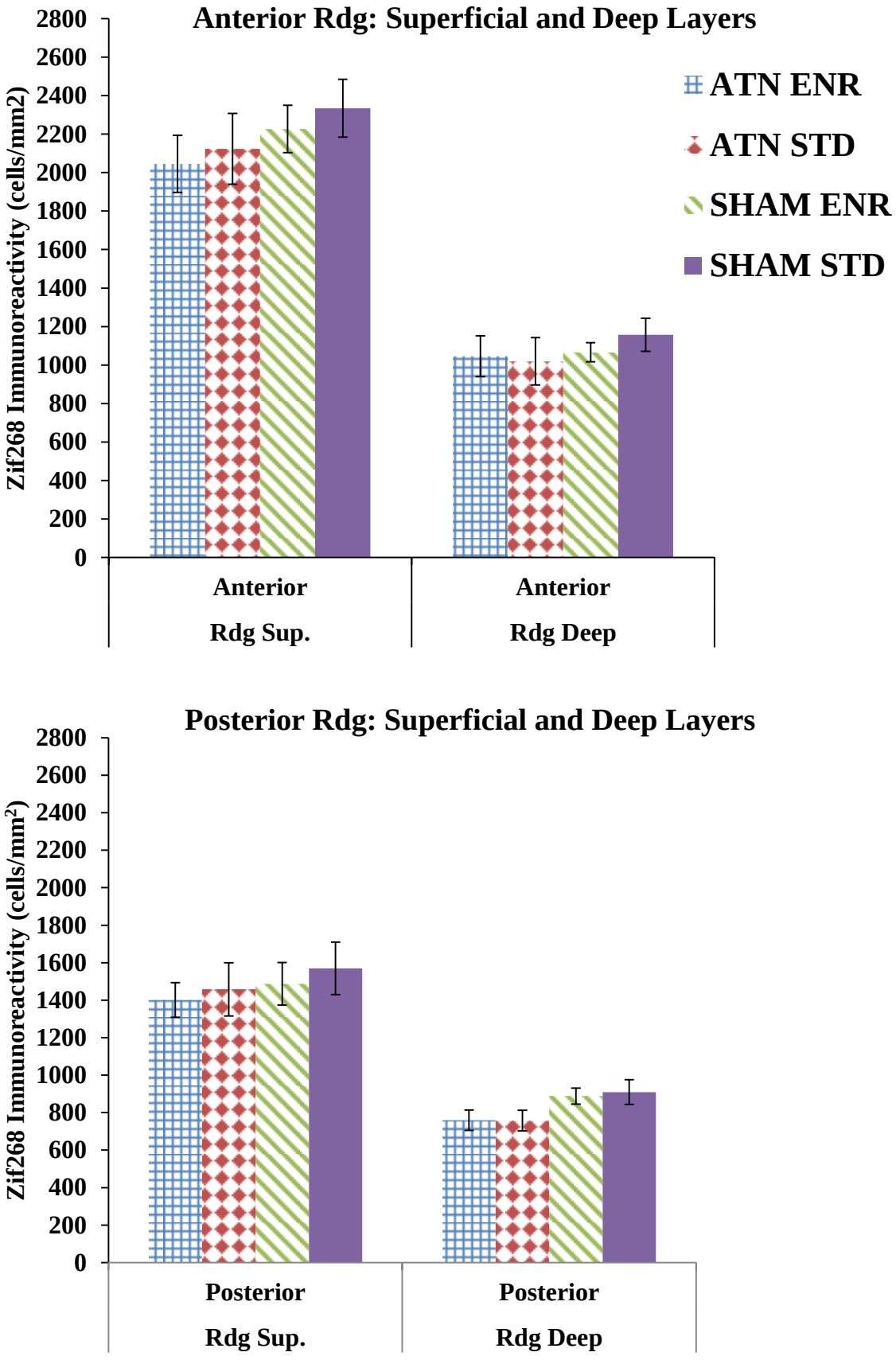


Figure 3.16. Mean (±SE) Zif268 cell counts (cells/mm^2) in the superficial (sup.) and deep layers of the anterior (top) and posterior (bottom) dysgranular retrosplenial cortex (Rdg). ATN: neurotoxic anterior thalamic lesions; SHAM: sham lesions; STD: standard housing condition; ENR: enriched housing condition.

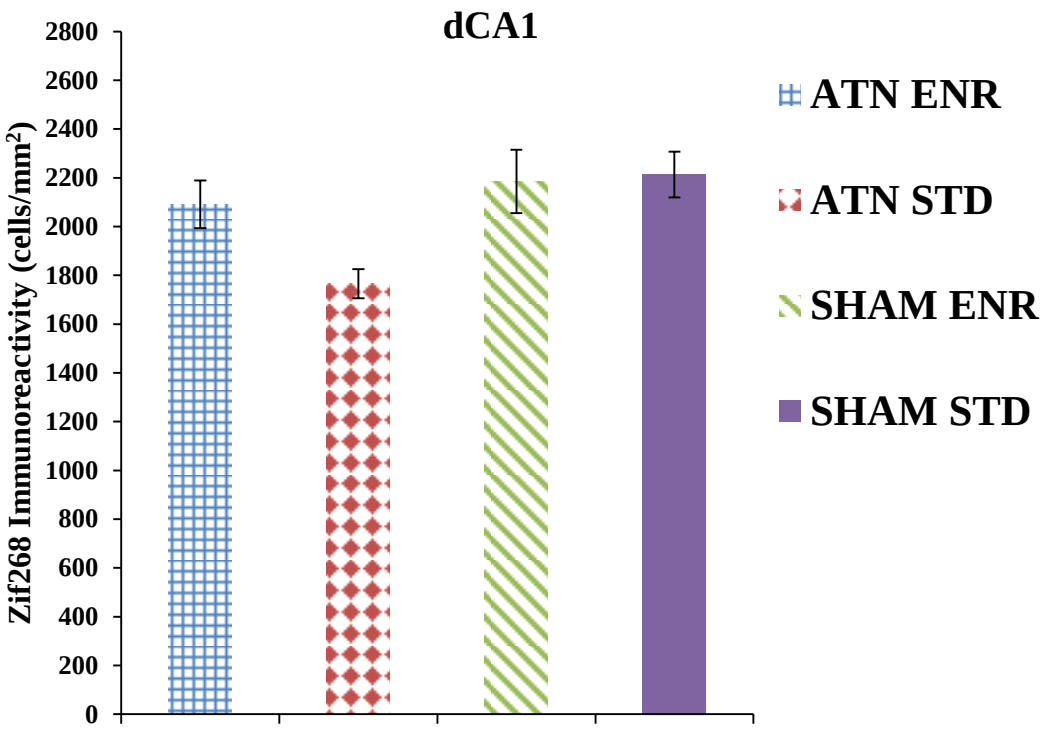


Figure 3.17. Mean (±SE) Zif268 cell counts (cells/mm^2) in the dorsal CA1 region of the hippocampus (dCA1). ATN: neurotoxic anterior thalamic lesions; SHAM: sham lesions; STD: standard housing condition; ENR: enriched housing condition.

3.6.2 Dorsal CA1 Region of the Hippocampus:

Figure 3.17 shows the mean zif268 cell counts for the dCA1 region of the hippocampus. ATN lesions were associated with reduced zif268 cell counts ($F(1, 37) = 6.99$, $p<0.02$), although less dramatically than the superficial region of the granular RSC. The main effect of Housing did not reach significance ($p>0.1$), but a Lesion by Housing interaction approached significance ($F(1, 37) = 2.977$, $p=0.09$). As expected from Figure 3.17, post-hoc Newman-Keuls tests showed that there was higher zif268 immunoreactivity in the dCA1 for rats with ATN lesions housed in enrichment when compared to standard-housed ATN rats ($p<0.05$), and that rats with ATN lesions housed in enrichment did not differ from both sham groups in terms of zif268 cell counts in the dCA1 region ($p>0.5$).

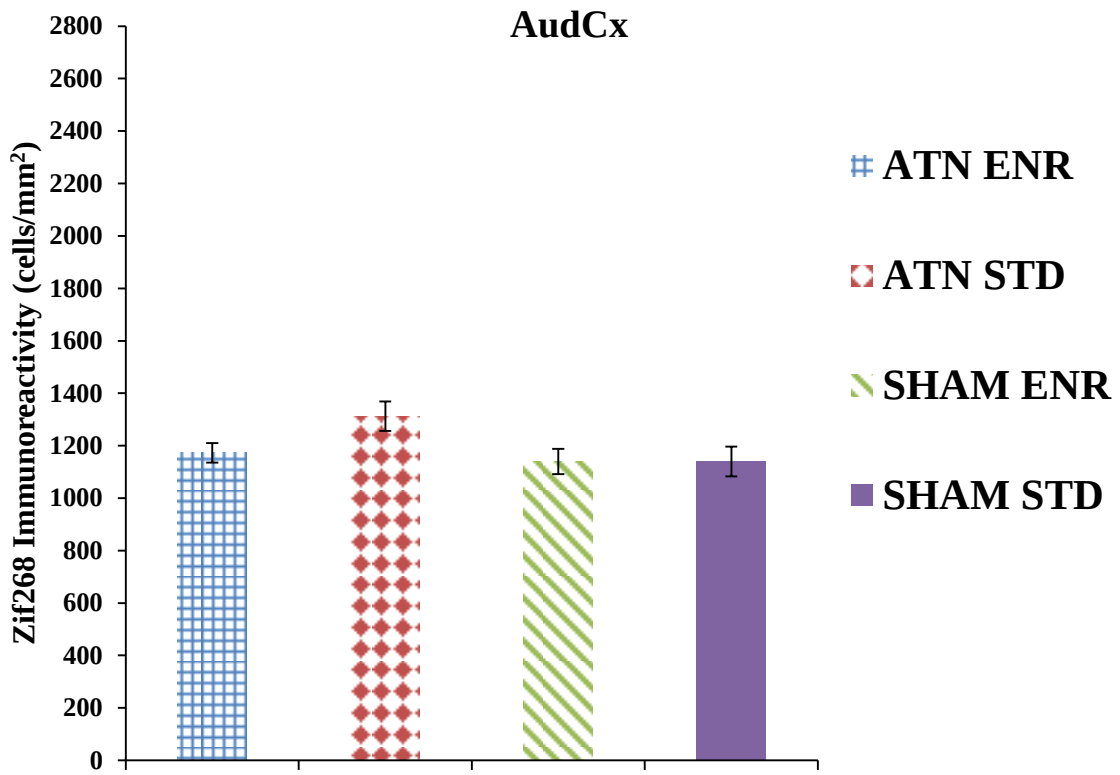


Figure 3.18. Mean (±SE) Zif268 cell counts (cells/mm^2) in the auditory cortex (AudCx). ATN: neurotoxic anterior thalamic lesions; SHAM: sham lesions; STD: standard housing condition; ENR: enriched housing condition.

3.6.3 Primary Auditory Cortex:

The primary auditory cortex (AudCx), which was chosen as a control region (where zif268 expression was not expected to be affected by lesions or training) showed no differences between any of the groups in terms of Lesion, Housing, or interaction effects (*p*'s all >0.10) (Figure 3.18).

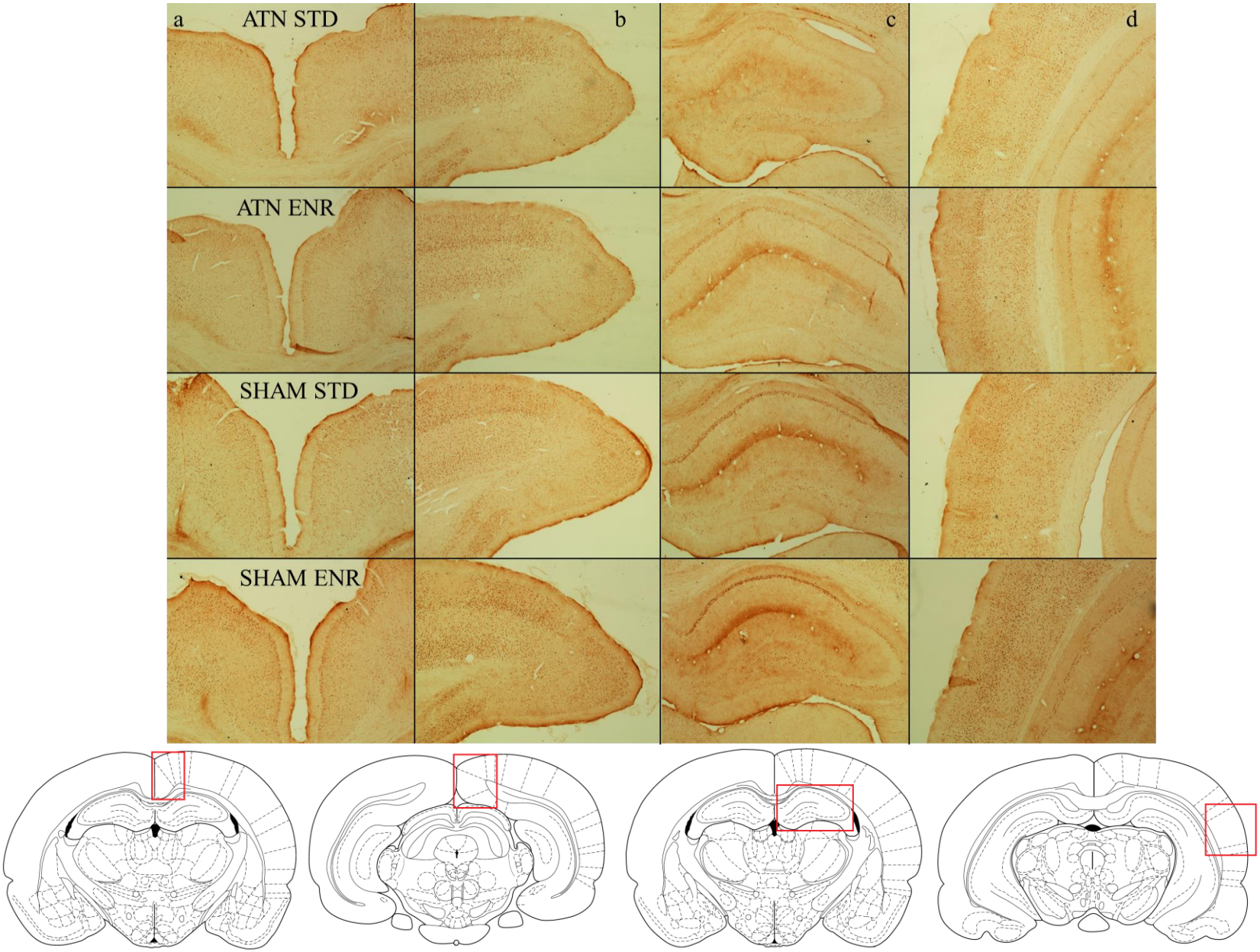


Figure 3.19. Photomicrographs of Zif268 sections (top) in a: the anterior RSC at approximately -3.14mm from Bregma (including the superficial and deep layers of the Rgb and Rdg) and b: posterior RSC at approximately -6.30mm from Bregma (including the superficial and deep layers of the Rgb, Rdg and Rga), c: the dCA1 at approximately -3.30mm from Bregma, and d: the AudCx at approximately -5.20mm from Bregma (control region) for rats in each group (N.B. each row of images is from the same rat in each group), and corresponding rat brain atlas images for each region (bottom; from Paxinos & Watson, 1998). ATN: neurotoxic anterior thalamic lesions; SHAM: sham lesions; STD: standard housing condition; ENR: enriched housing condition. AudCx: auditory cortex; dCA1: dorsal CA1 region of the hippocampus; Rga: granular a retrosplenial cortex; Rgb: granular b retrosplenial cortex; Rdg: Dysgranular retrosplenial cortex; RSC: retrosplenial cortex.

3.6.4 Behavioural Performance and Zif268 Immunoreactivity:

To examine whether performance on the final day of zif268 induction task in the RAM was associated with zif268 activation, correlations were performed between average performance combined over the three trials of the final day of testing for all rats irrespective of housing or lesion, and levels of zif268 activation in each region of interest. In addition, correlations were performed for the four individual groups between zif268 expression and performance on the final day of zif268 induction testing. No associations were found between performance on this final day of behavioural testing in the RAM and zif268 activation in any region of interest (Table 3.1). For the superficial layer of the Rga, in which the association between performance and zif268 expression approached significance (p=0.08), a scatterplot for this association across all rats is shown in Figure 3.20.

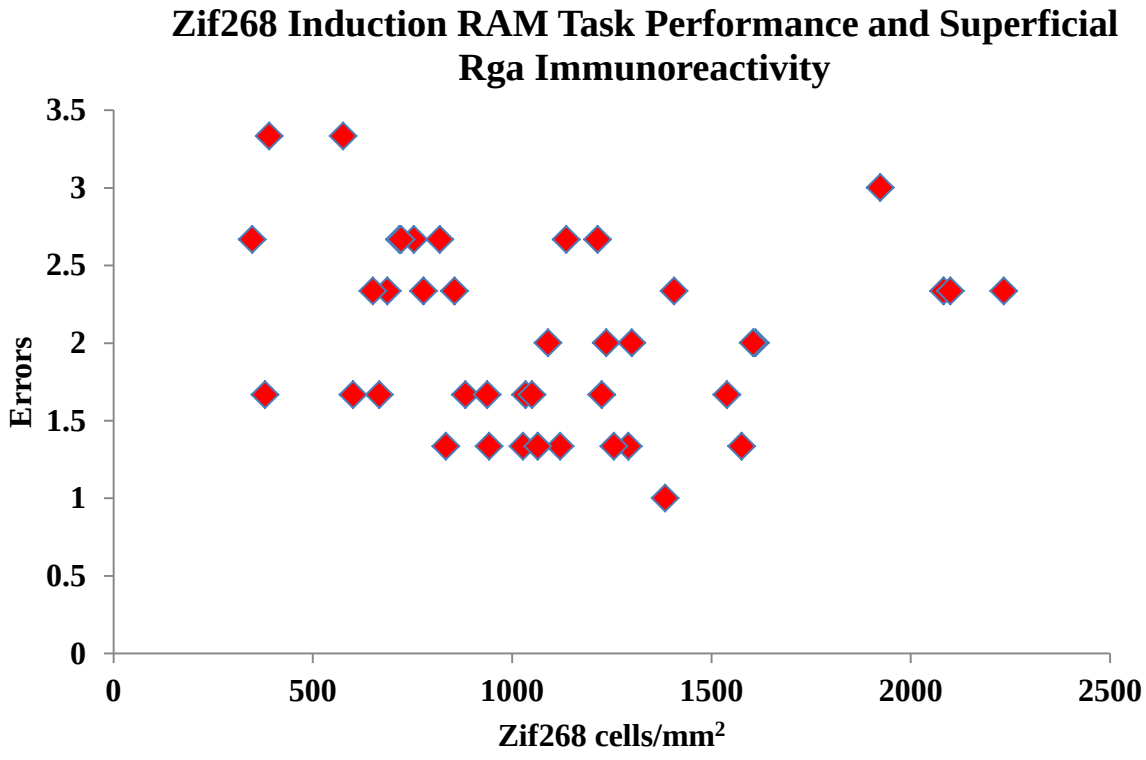


Figure 3.20. Scatterplot showing the correlation for all rats between zif268 expression in the superficial layer of the Rga, and performance on the final day of testing on the zif268 induction task in the RAM in terms of mean errors. This correlation did not reach significance (p=0.08). RAM: radial arm maze; Rga: granular a retrosplenial cortex.

	Zif268 Induction RAM Task Performance (Final Day)				
Region of interest	**All groups combined**	**ATN ENR**	**ATN STD**	**SHAM ENR**	**SHAM STD**
Anterior Rgb Sup.	-0.23	-0.13	0.37	-0.03	-0.29
Anterior Rgb Deep	-0.22	0.11	0.13	0.07	-0.41
Posterior Rgb Sup.	-0.19	0.20	0.58	-0.07	-0.28
Posterior Rgb Deep	-0.14	0.27	0.22	0.11	0.14
Anterior Rdg Sup.	0.01	-0.01	0.13	0.06	0.13
Anterior Rdg Deep	-0.17	-0.22	0.04	0.01	0.14
Posterior Rdg Sup.	-0.01	0.01	0.31	-0.27	0.01
Posterior Rdg Deep	0.06	0.36	0.51	-0.17	0.38
Rga Sup.	-0.27	0.05	0.10	-0.04	-0.22
Rga Deep	-0.19	-0.15	0.58	-0.04	-0.22
CA1	0.05	-0.16	0.28	0.22	0.22
AudCx	-0.08	0.33	-0.31	-0.26	-0.23

Table 3.1. Correlations between performance on the final day of testing on the zif268 induction task (mean errors) and levels of zif268 activation (cells/mm^2) in all regions of interest, for all rats irrespective of lesion or housing group, and across the four groups. No correlations reached significance. ATN: neurotoxic anterior thalamic lesions; SHAM: sham lesions; STD: standard housing condition; ENR: enriched housing condition. AudCx: auditory cortex; dCA1: dorsal region of the CA1 area of the hippocampus; RAM: Radial arm maze; Rdg: dysgranular retrosplenial cortex; Rga: granular a retrosplenial cortex; Rgb: granular b retrosplenial cortex; Sup.: superficial layer.

3.7 NeuN Immunofluorescence for Mammillary Body Cell Counts

Figure 3.21 depicts mammillary body cell counts for the medial (MM), medial-lateral (ML) and lateral (LM) mammillary nuclei, at approximately -4.52mm and -4.80mm from Bregma. Figure 3.22 shows representative sections of the mammillary nuclei at -4.52mm and -4.80mm from Bregma, from rats with the median cell count for each group. An ANOVA was performed with the within-group factors of Sub-Nucleus (for each of three mammillary nuclei) and AP (for regions -4.52mm and -4.80mm from Bregma), and between-group factors of Lesion and Housing.

Rats with ATN lesions had significantly reduced cell counts across each of the mammillary sub-nuclei (Lesion $F(1, 37) = 147.49$, $p<0.0001$; Lesion by Region $F<1$). Higher cell counts were found in the MM and ML when compared with the LM region (Sub-Nucleus effect, $F(2, 74) = 269.17$, $p<0.0001$) confirmed by a post-hoc Newman-Keuls test ($p<0.05$). An effect of AP ($F(1, 37) = 33.87$, $p<0.0001$), Sub-Nucleus by AP interaction ($F(2, 74) = 95.41$, $p<0.0001$) and post-hoc Newman-Keuls test ($p<0.05$) indicated fewer cells in the MM at -4.80mm from Bregma when compared with the MM at -4.52mm. There were also Sub-Nucleus by Lesion ($F(2, 74) = 14.05$, $p<0.0001$) and AP by Lesion ($F(1, 37) = 10.75$, $p<0.01$) interactions and a post-hoc Newman-Keuls test ($p<0.05$), which indicated reduced cell counts in the MM and LM regions at -4.80mm from Bregma for rats with ATN lesions.

The main effect of Housing failed to reach significance ($F<1$), although Sub-Nucleus by Lesion by Housing ($F(2, 74) = 6.22$, $p<0.005$) and Sub-Nucleus by AP by Lesion by Housing ($F(2, 74) = 5.89$, $p<0.005$) interactions and a post-hoc Newman-Keuls test suggest that the source of these effects was that sham rats housed in standard conditions generally had fewer cells in the ML nucleus at -4.80 when compared with sham rats housed in enrichment

(p<0.05). Post-hoc Newman-Keuls tests did not reveal any significant differences between the two ATN groups in terms of cell counts for sub-nucleus or AP (p's all>0.05).

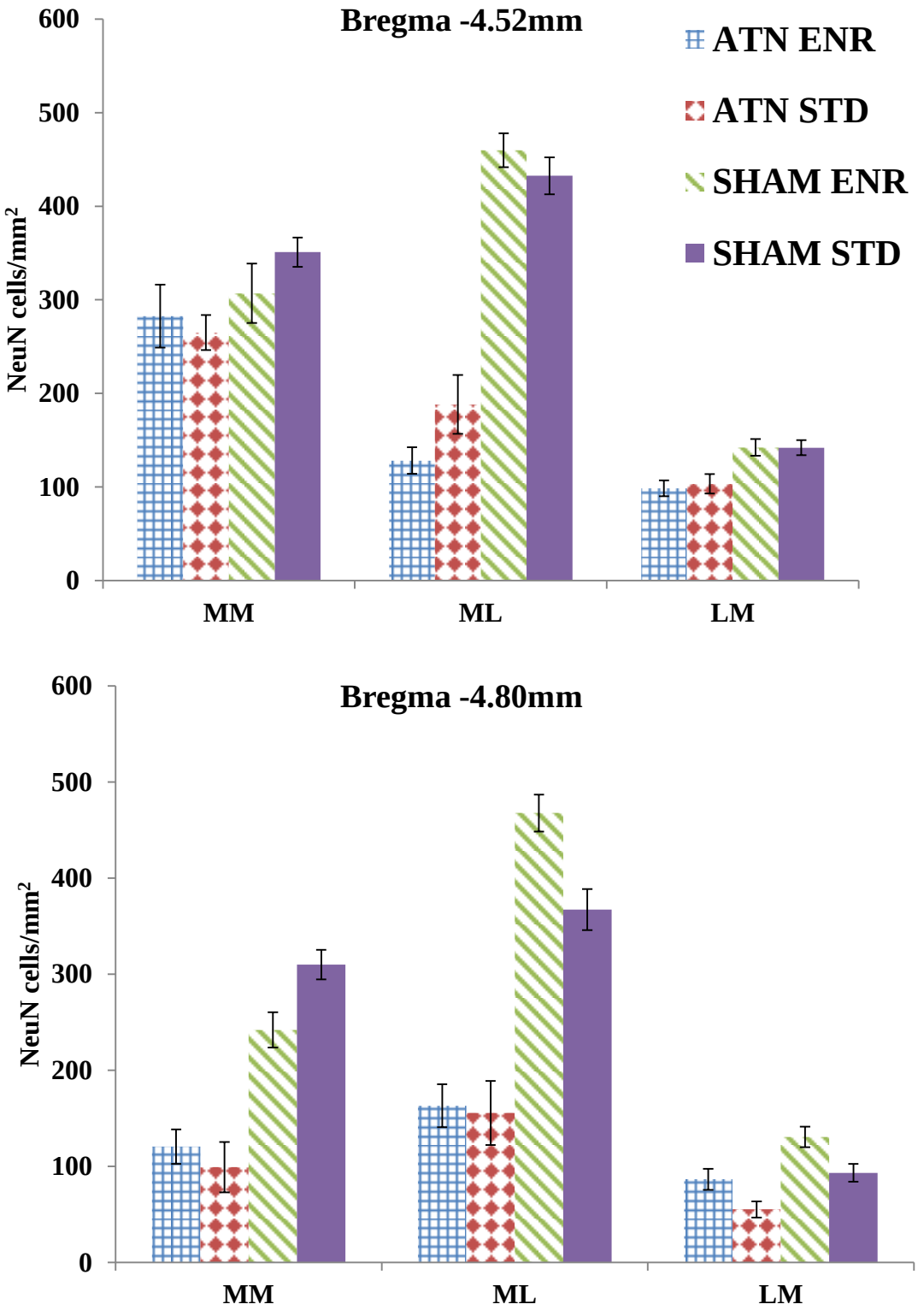


Figure 3.21. Mean (±SE) NeuN cell counts (cells/mm^2) in the MM, ML and LM nuclei, at both -4.52mm and -4.80mm from Bregma. LM: lateral mammillary nucleus; MM: medial mammillary nucleus; ML: medial lateral mammillary nucleus. ATN: neurotoxic anterior thalamic lesions; SHAM: sham lesions; STD: standard housing condition; ENR: enriched housing condition.

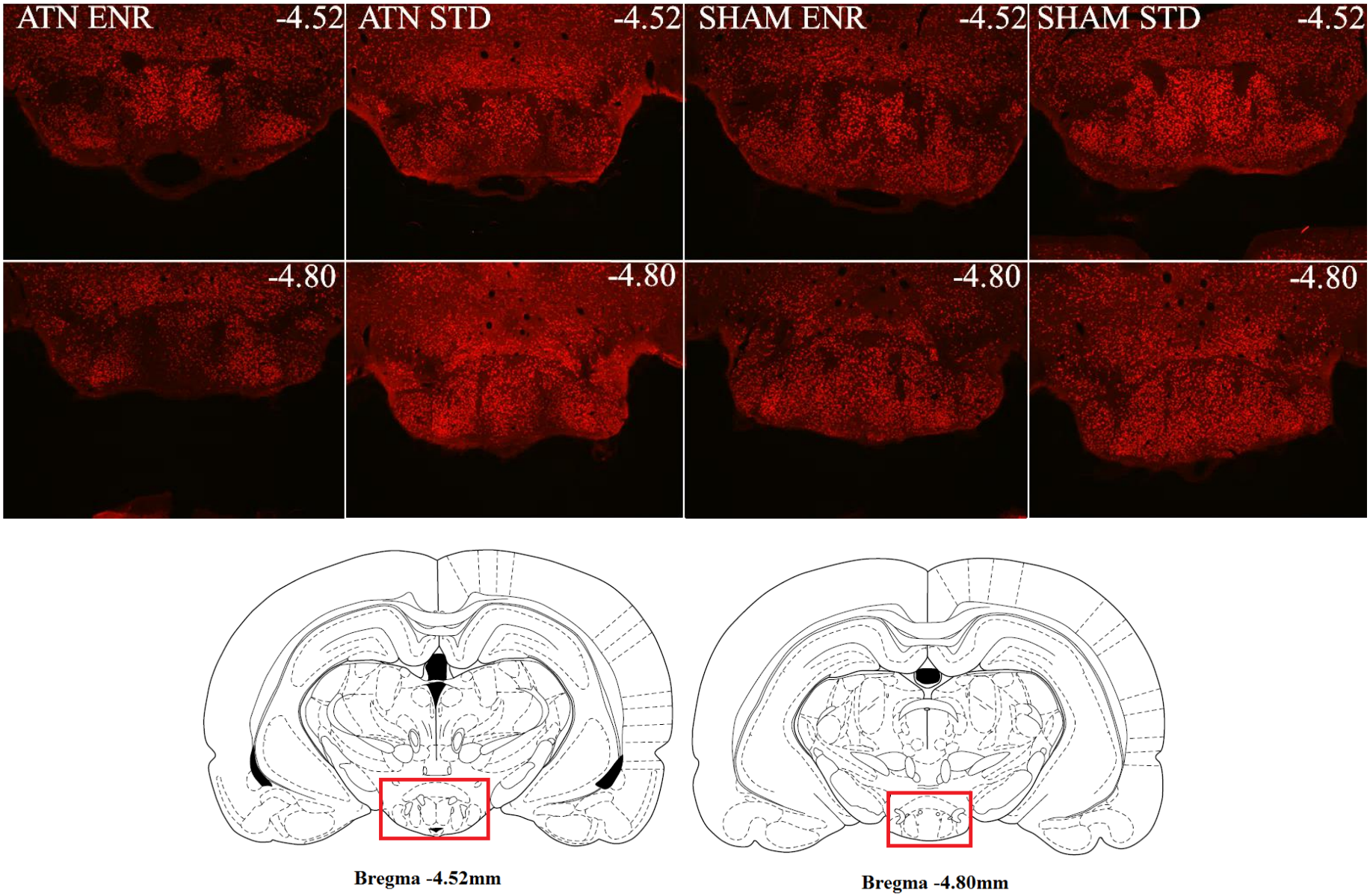


Figure 3.22. Photomicrographs of NeuN MB sections (top) and rat brain atlas images (bottom; from Paxinos & Watson, 1998) at approximately -4.52 and -4.80 from Bregma with each image including the MM, ML and LM regions of the MB for each group (N.B. each image is from the same rat in each group). Substantial bilateral cell loss is evident across the three mammillary nuclei in rats with ATN lesions housed in enriched or standard conditions, at both -4.52mm and -4.80mm from Bregma. LM: lateral mammillary nucleus; MB: mammillary body; MM: medial mammillary nucleus; ML: medial-lateral mammillary nucleus. ATN: neurotoxic anterior thalamic lesions; SHAM: sham lesions; STD: standard housing condition; ENR: enriched housing condition.

4. Discussion

4.1 Summary of General Findings

The aim of the present study was to ascertain the extent of recovery after enrichment in rats with anterior thalamic lesions. Lesions of the ATN are associated with dysfunction in the retrosplenial cortex (RSC), a distal component of the "extended hippocampal system" supporting episodic memory function. Hence the present study aimed to evaluate the expression of the immediate early gene (IEG) zif268 in this cortical region and whether the level of expression was associated with impaired spatial memory performance on a modified radial arm maze (RAM) task thought to be specifically sensitive to RSC damage. As it is unknown when recovery of function begins to occur during enrichment, the present study also aimed to test spatial memory during the initial period of enriched or standard housing. The final novel aim of the present study was to assess the effects of ATN lesions on cell counts in the mammillary bodies (MB), a region in which almost every neuron projects to the ATN.

Spatial reference and working memory tasks in the water maze were used to assess performance after ATN lesions. Consistent with previous research (Van Groen, Kadish & Wyss, 2002a; Wolff, Gibb, Cassel & Dalrymple-Alford, 2008), rats with ATN lesions were substantially impaired on these water maze tasks, swimming further than sham rats to locate the fixed (reference memory task) or daily unique (working memory task) platform location. Rats with ATN lesions were also substantially impaired on an additional post-surgery working memory task in the water maze when distal cues were minimised, and this impairment was comparable between the two working memory tasks.

After completion of post-surgery testing in the water maze, sham rats and rats with ATN lesions were assigned to continuous enriched or standard housing for 36 days. To assess the point in time at which recovery might begin during the continuous enrichment period, the

rats were assessed on spatial working memory performance in the water maze with all cues available, in a single trial on three separate days with a modified procedure. Surprisingly, on all three days of enrichment testing, rats with ATN lesions housed in enrichment did not demonstrate improved performance relative to rats with ATN lesions housed in standard conditions. This trend continued during post-enrichment testing on the spatial working memory and reference memory tasks in the water maze, with rats with ATN lesions housed in enrichment having persistent and similar deficits to their standard-housed counterparts. The final sessions of post-enrichment reference memory testing in the water maze, however, indicated some recovery in rats with ATN lesions housed in enrichment.

Spatial working memory performance on a different task, the standard RAM, revealed a clearer pattern of recovery. Rats with ATN lesions housed in enrichment were significantly less impaired than standard-housed rats with ATN lesions, an effect that became apparent as testing progressed. Post-enrichment recovery was further evaluated using a modified working memory task in the RAM with both a mid-trial delay and delay with rotation. Rats with ATN lesions, irrespective of housing, were more impaired on the delay with rotation and standard tasks by comparison to the delay only task, and performance on the standard task and delay with rotation task was comparable between the enriched and standard-housed ATN rats. ATN rats housed in enrichment, however, made substantially fewer errors on both the delay and delay with rotation tasks by comparison to standard-housed rats with ATN lesions.

A specific and novel aim of the present study was to assess whether recovery of spatial memory function, in terms of performance in the RAM on the mid-trial delay with rotation task, would be associated with retrosplenial IEG activation. As previously mentioned, the delay with rotation version of the RAM task has revealed specifically clear deficits in rats with RSC lesions (Vann, Wilton, Muir & Aggleton, 2003). If neuronal activation in the RSC is important for memory function, it would be expected that poorer

performance on the modified RAM task would be associated with lower levels of zif268 in the RSC. Accordingly, levels of zif268 expression were analysed in the superficial and deep layers of the granular b, granular a and dysgranular RSC regions. Rats with ATN lesions housed in enrichment made substantially fewer errors than standard-housed ATN rats on both of the modified RAM tasks, but zif268 activation in each layer and region of the RSC remained comparable in both ATN groups. In addition, levels of zif268 expression were not associated with spatial memory performance on the final day of testing on the modified RAM task across all groups. ATN lesions clearly produce substantial IEG hypoactivation in the RSC, but its association with spatial memory appears elusive.

With respect to the novel aim of the present study to examine the potential effects of selective ATN lesions and enrichment on neuron number in the MB, ATN lesions were associated with a striking loss in cell counts in each of the mammillary nuclei. This atrophy was most evident in the medial-lateral (ML) and posterior medial (MM) sub-regions but less conspicuous for the lateral (LM) nucleus, and these changes were generally comparable between rats with ATN lesions housed in both standard and enriched conditions.

4.2 Anterior Thalamic Lesions and Spatial Memory

Spatial reference and working memory tasks in the water maze have both revealed deficits in rats with ATN lesions, and hence were used to assess post-surgery deficits (Van Groen, Kadish & Wyss, 2002a; Wolff, Loukavenko, Will & Dalrymple-Alford, 2008). An integral requirement of both water maze tasks is the ability to navigate using allocentric cues as well as egocentric cues such as head direction to locate the hidden platform. As previously mentioned, earlier research suggests that intact rats may rely on both egocentric and allocentric strategies to solve navigation-based tasks (Futter & Aggleton, 2006). ATN lesions generally disrupt the use of allocentric strategies but generally leaving egocentric navigation capabilities intact (Aggleton, Hunt, Nagle & Neave, 1996). Lesions of the anteroventral

nucleus (AV) and anterodorsal nucleus (AD) alone and especially in combination with the anteromedial nucleus (AM) are associated with substantial deficits on spatial memory tasks in the water maze (Van Groen, Kadish & Wyss, 2002a; Wolff, Gibb, Cassel & Dalrymple-Alford, 2008). Here, rats with lesions spanning the three ATN regions were therefore expected to show substantial deficits on both the working and reference memory tasks in the water maze.

Consistent with earlier research (Wolff, Loukavenko, Will & Dalrymple-Alford, 2008) rats with ATN lesions were significantly impaired on the reference memory task but were able to learn the fixed platform location gradually in contrast to the rapid task acquisition of the sham rats. Although the probe trial was planned for 5 days after conclusion of reference memory training to assess memory for the platform location, a 12-day interval was used due to testing schedule conflicts during which no other testing or training took place. Rats' swim paths were analysed to examine quadrant preference and recall of the location of the platform used during reference memory testing. Surprisingly, in spite of the poor performance of rats with ATN lesions on reference memory acquisition, both sham rats and rats with ATN lesions showed a similar preference for the target quadrant (where the platform had been located for testing) and were comparable in terms of crossings of the annulus (platform enlarged by 30cm). Although these findings contrast that of previous research showing that rats with ATN lesions were impaired on both recent (5 day) and remote (25 day) probe trials (Lopez et al, 2009), the results of the probe trial are consistent with recent work showing that rats with complete ATN lesions are eventually able to distinguish the correct quadrant in other water maze reference memory tasks, with this acquisition also evident in probe tests (Dumont, Wright, Pearce & Aggleton, 2014). The lack of deficit in the reference memory probe perhaps also signifies systems level consolidation of memory for the platform location, as the 12-day interval may have allowed for recruitment of memory

systems beyond the ATN (Aggleton & Brown, 1999), although some regions of the thalamus are also thought to have a role in remote memory (Aggleton & Brown, 1999; Moscovitch, Nadel, Winocur, Gilboa & Rosenbaum, 2006; Lopez et al, 2009; Loureiro et al, 2012).

Deficits were also severe in rats with ATN lesions on the working memory task in the water maze. These deficits were more persistent than those observed in the reference memory task with little improvement after continued training. The lack of improvement is partially attributable to the demanding nature of the working memory task, as rats are required to navigate to a new hidden platform location on each day of testing. Interestingly, only one prior study (Van Groen, Kadish & Wyss, 2002a) has assessed spatial working memory in the water maze using a similar standard task after ATN lesions, when rats with significant damage to the ATN showed no improvement across extended training. Van Groen, Kadish & Wyss (2002a) further examined working memory by analysing the differences in escape latencies between trials on each day of testing, finding that sham rats reduced their escape latencies rapidly between trials 1 and 2, whereas rats with ATN lesions swam consistently longer across all trials. These results are consistent with those of the present study, where rats with ATN lesions showed little working memory improvement in terms of path length or escape latency over trials on each day of testing, whereas sham rats demonstrated intact spatial working memory function through rapid path length reductions between trials 1 and 2.

As previously mentioned, rats rely on a combination of allocentric and egocentric strategies to complete spatial memory tasks, and ATN lesions primarily impair performance on tasks that require the use of allocentric strategies (Aggleton, Hunt, Nagle & Neave, 1996; Warburton, Baird & Aggleton, 1997; Sziklas & Petrides, 1999; Wolff, Gibb, Cassel & Dalrymple-Alford, 2008). To examine whether performance on the spatial working memory task would be substantially impaired by the removal of most of the allocentric cues, curtains were drawn around and above the maze thus obscuring most extra-maze cues. Rats with ATN

lesions were substantially impaired on this task, with highly similar path lengths to the standard spatial working memory task. Sham rats also showed comparable performance in terms of rapid trial completion between the standard working memory and minimised cues tasks, although the prior training on the working memory task may have obscured any potential differences in performance on these tasks. These results indicate that the rats may have been able to use other strategies to locate the hidden platform, or were able to navigate using the minimal cues provided. This task was also to be used to examine post-enrichment spatial memory function but time constraints allowed for post-surgery testing only.

4.3 Enrichment and Recovery of Spatial Memory Function

Previous research has shown that ATN lesions are amenable to recovery of function after therapeutic interventions such as environmental enrichment, although such research has generally used tasks such as the radial arm maze and T-maze (Harland, Collings, McNaughton, Abraham & Dalrymple-Alford, 2014). Only the reference memory task in the water maze has been used to assess recovery of function after ATN lesions (Wolff, Loukavenko, Will & Dalrymple-Alford 2008), and spatial working memory has not been used previously in this context. The working memory task, however, was used to assess recovery during the continuous enrichment period as the rats had received training on this task in the post-surgery tests, and particularly as the working memory version allowed a brief examination of spatial memory (necessary to minimise training effects) and would require minimal intrusion to the continuous enrichment protocol. As three single trials were used, the working memory task was modified slightly so that 8 trials of a maximum 90 seconds' duration were provided, allowing extended examination of spatial working memory when compared to the previous protocol of 4 repeated trials.

Thirty-six days of continuous enrichment were provided instead of the 40 allotted in the standardised protocol as this allowed for the three separate, evenly-spaced days of testing

on days 13 and 25 of continuous enrichment, and on the first day after this period (day 37 of the enrichment protocol). Previous research has shown that substantially briefer enrichment protocols than that of the present study are still beneficial after brain injury (Will, Rosenzweig, Bennett, Hebert & Morimoto, 1977). Recent research using 30 days of continuous enrichment elicited similar recovery to another study using 40 days (Loukavenko, Ottley, Moran, Wolff & Dalrymple-Alford, 2007; Harland, Collings, McNaughton, Abraham & Dalrymple-Alford, 2014).

Rats with ATN lesions were substantially impaired on the spatial working memory task in the water maze over the three separate days of testing during enrichment. Although spatial working memory had not previously been used in the water maze, similar working memory tasks in the RAM produced evidence of recovery of function in rats with ATN lesions housed in enrichment (Harland, Collings, McNaughton, Abraham & Dalrymple-Alford, 2014). Surprisingly, in spite of the significantly improved housing conditions offered by enrichment, rats with ATN lesions housed in standard and enriched conditions were similarly impaired and also improved at similar rates on each of the three trials in the water maze during the main enrichment period.

Testing on working memory in the water maze after the period of continuous differential housing elicited similar results. Rats with ATN lesions housed in both standard and enriched conditions were significantly impaired, although both groups showed similar rates of improvement over the 12 days of testing. Subsequent testing on the reference memory task in the water maze revealed a similar pattern of results, with ATN rats of both housing conditions similarly impaired. However, the sixth (and final) block of testing did suggest differences between the two groups of ATN rats, perhaps indicating that the rats with ATN lesions housed in enrichment had recovered some spatial memory function, and further testing may have revealed additional recovery.

In contrast to the generally absent recovery observed from the enriched ATN rats on the water maze tasks, subsequent testing in the standard RAM task revealed evidence of recovery of spatial working memory performance in enriched ATN rats. Rats with ATN lesions housed in standard conditions were significantly impaired on this task, whereas rats with ATN lesions housed in enrichment made significant improvements as training progressed, albeit with poorer performance in comparison to the sham groups. Previous research has also found substantial deficits after ATN lesions on this task (Warburton, Baird, Morgan, Muir & Aggleton, 2001; Mitchell & Dalrymple-Alford, 2006; Sziklas & Petrides, 1999; 2007). The improved performance of enriched rats with ATN lesions is consistent with previous work using a more complicated version of this task (Harland, Collings, McNaughton, Abraham & Dalrymple-Alford, 2014), and suggests that in spite of the lack of clear recovery on the water maze tasks, some recovery of spatial memory had occurred in enriched rats with ATN lesions.

As mentioned previously, although the working memory task in the water maze is similar to the working memory task in the RAM, there is clear aversive motivation provided by the water maze, whereas completion of the RAM task relies upon sufficient food deprivation and successful searching in a relatively safe environment. The two spatial working memory tasks hence motivated rats to complete the tasks by different mechanisms (D'Hooge & De Deyn, 2001). Environmental enrichment is thought to have a stress inoculation effect in rats as it provides complex social, physical, somatosensory and cognitive stimulation in an environment that is frequently changed, hence creating near-constant minor stressors for rats (Crofton, Zhang & Green, 2015). Enrichment may thus reduce the anxiogenic effects of any further tasks or environments that rats experience (Will, Galani, Kelche & Rosenzweig, 2004). ATN lesions are also associated with some anxiolytic effects, with rats exhibiting reduced freezing in contextual fear tasks and increased exploration of the

open arms of an elevated plus maze, with these effects increased after enrichment (Dupire et al, 2013). It is possible therefore that ATN lesions and enrichment may have resulted in reduced escape motivation for the rats on the water maze tasks and thus contributed to the differential performance on the two working memory tasks. This is, however, at best an incomplete answer, as one previous study reported recovery of spatial memory performance in the water maze after ATN lesions and enrichment on reference memory tasks (Wolff, Loukavenko, Will & Dalrymple-Alford, 2008).

After completion of testing on the standard task in the RAM, testing on the modified task previously described by Vann, Wilton, Muir & Aggleton (2003) was commenced. Here, two conditions were introduced after the first four arm choices: a mid-trial delay of 60sec and a mid-trial delay of 60sec with rotation of the maze by 45˚. This modified task, which has elicited clear deficits in rats with RSC lesions (Vann, Wilton, Muir & Aggleton, 2003), was used to examine whether performance would be associated with levels of zif268, an IEG associated with memory function (Davis, Bozon & Laroche, 2003; Toscano, McGlothan & Guilarte, 2006) in the RSC after ATN lesions. The results of the delay and delay with rotation tasks, which both revealed deficits in rats with ATN lesions, were compared to performance on the standard task. All rats were less impaired on the delay task than on the delay with rotation task, perhaps due to the mid-trial rotation disrupting the use of intra-maze cues (Vann, Wilton, Muir & Aggleton, 2003).

Across all three RAM tasks, rats with ATN lesions housed in enrichment made significantly fewer errors than standard-housed rats with ATN lesions. For rats with ATN lesions, irrespective of housing, performance was comparatively worse on the delay with rotation and standard tasks by comparison to the delay only task. The performance across each of the three tasks in the RAM suggests that standard-housed rats with ATN lesions were impaired in their use of both extra-maze and intra-maze cues. The improved performance of

the rats with ATN lesions housed in enrichment on the delay with rotation task in particular suggests that enrichment may have allowed these rats to be more flexible in terms of strategies used to complete the task (Leggio et al, 2005), as the enriched rats with ATN lesions acquired the delay with rotation task at a rapid rate. These rats reached a level of performance comparable with both sham groups by the final trial, which may have persisted if testing had been extended.

Previous research has shown that novel environments or stimuli can increase IEG expression and reduce the likelihood of floor effects in immunoreactivity (Herdegen & Leah, 1998). Hence, the rats were introduced to a new 'RAM with delay and rotation' procedure for three days with three trials per day, and with the room cues substantially altered on the first day and final day of testing to stimulate zif268 expression. Rats with ATN lesions were impaired on this task irrespective of housing condition; sham rats housed in standard conditions tended to make fewer errors than their enriched counterparts. The difference observed between the two sham groups may be due in part to the restriction of all rats to 8 total arm choices (four choices prior to the rotation) creating a ceiling effect in terms of errors, but there was little difference in errors between the two sham groups on the third day of testing in particular. The considerable variation in performance for all groups over the three days of testing is perhaps due to the sudden introduction of the rats to a new test procedure of three trials per day in comparison to the single trials provided previously, and also to the altered room cues on two of the three days of testing which may have disrupted the use of previous allocentric strategies. The groups all differed in performance across the three trials on each day and were more error-prone on trials 1 and 2, with errors on the second trial perhaps due to proactive interference caused by memory for arm visits on the previous trial.

4.4 Zif268 Expression

Ninety minutes after the final day of testing on the zif268 induction task in the RAM, rats were sacrificed and zif268 immunoreactivity was analysed across the superficial and deep layers of the anterior and posterior granular b (Rgb), the granular a (Rga), and the anterior and posterior dysgranular (Rdg) regions of the RSC, as well as the dorsal CA1 (CA1) region of the hippocampus. ATN lesions in rats of both housing conditions were associated with substantial reductions in zif268 immunoreactivity in the superficial and deep layers of the Rgb and the superficial layer of the Rga, although the deep layers of the anterior Rgb did not show any significant changes associated with ATN lesions. The Rdg region showed a significant reduction of zif268 immunoreactivity in the posterior deep layers only. Across all regions analysed in the RSC, no differences in immunoreactivity were observed between enriched and standard-housed rats with ATN lesions, and there were no differences between the two sham groups. Within the CA1, however, ATN lesions were associated with reduced zif268 expression, which was somewhat reversed in rats with ATN lesions housed in enrichment. Sham rats, irrespective of housing, did not show any differences in immunoreactivity. As expected, there were no group differences in the control brain region, the auditory cortex (AudCx), indicating that the results of the immunoreactivity analyses may not be due to systematic errors in histology or cell count procedures.

The pattern of zif268 immunoreactivity results suggest that ATN lesions induce widespread zif268 hypoactivation across the RSC and CA1 regions, with some indication of recovery occurring only in the CA1 after enriched housing. This is consistent with recent research, although with a different neurobiological measure. In that study, basal and apical dendritic spine density was reduced in both the RSC and CA1 in rats with ATN lesions, with recovery of this spine density found only in the CA1 in enriched rats with ATN lesions (Harland, Collings, McNaughton, Abraham & Dalrymple-Alford, 2014).

Previous research into the effects of ATN lesions on IEG immunoreactivity has not utilised behavioural tasks to examine any potential associations between IEG expression and behavioural performance (Poirier et al, 2008; Poirier & Aggleton, 2009; Dumont, Amin, Poirier, Albasser & Aggleton, 2012). The final day of testing in the RAM was used to induce zif268 activation, and performance on this day was therefore expected to correspond to the level of zif268 activation across the RSC and CA1 regions. Accordingly, correlations were used to examine associations between performance for all rats with lesion and housing conditions disregarded and zif268 activation in each region, as well as performance for the rats in each lesion and housing group and zif268 activation. Performance on this final day of testing was not associated with the level of zif268 expression in any of the regions examined, irrespective of lesion or housing condition. These results suggest that there is no clear evidence that zif268 levels in the RSC are linked to performance on the retrosplenial-sensitive modified RAM task.

4.5 NeuN Immunofluorescence for Mammillary Body Cell Counts

The MB is an important component of the 'extended hippocampal memory system' and provides substantial inputs to the ATN, by which both hippocampal and brain stem projections can influence ATN activity. The medial mammillary nucleus (MM) projects to the AM and AV nuclei, while the lateral mammillary nucleus (LM) projects to the AD nucleus (Vann, 2010). As the main outputs of the MB are to the ATN, but only a limited number of additional regions (Vann, 2010), the potential effects of ATN lesions and differential housing on MB cell counts is relevant to the current study. Substantial cell loss in rats with ATN lesions housed in both enriched and standard conditions was observed most significantly in both the anterior (-4.52mm from Bregma) and posterior (-4.80mm from Bregma) ML sub-nucleus, with posterior MM and LM cell counts also reduced to a lesser extent.

No effects of differential housing on each of the mammillary nuclei were observed in rats with ATN lesions, although a small difference in neuron number was found in the posterior ML between enriched and standard-housed sham rats. This small difference may be attributed to variation in cell counts both within and between the two sham groups as it is uncertain that enrichment alone had any effects on neuron number. These findings are consistent with that of previous research showing substantial cell loss and atrophy within the MB after ATN lesions (Fry & Cowan, 1972; Aggleton & Mishkin, 1983) and suggest that this deficit is also not modified by environmental enrichment.

4.6 Limitations

There were limitations in both design and implementation of the present study, as a result of the constraints associated with time-consuming behavioural and neurobiological procedures. These time constraints restricted the length of the initial enrichment period, the number of spatial memory tasks and the number of trials in these tasks, more detailed lesion analysis and the number of brain regions included for IEG analysis.

In terms of behaviour, the reference memory probe was initially planned to take place 5 days after the final day of post-surgery reference memory testing, although schedule conflicts meant that this was extended to 12 days. A 5-day probe examines recent memory for the platform location, whereas later probe trials assess remote memory in rats. It is possible, therefore, that the comparable performance of rats with ATN lesions and sham rats on the probe trial reflected consolidation of memory for the platform location in both groups (Aggleton & Brown, 1999), and analysis of recent memory with an earlier probe may have yielded a different pattern of results. Rats with ATN lesions, however, have shown impairment at both time points in one previous study (Lopez et al, 2009). Time constraints also restricted analysis of performance on the minimised cues task in the water maze to six days of post-surgery testing, and performance on this task after enrichment would have

allowed a more comprehensive analysis of spatial memory in the context of minimised cues. Testing on the delay and delay with rotation RAM tasks was limited to four days on each task, although the ATN rats housed in enrichment reached a level of performance comparable with sham rats on the fourth day of testing on each task.

The time constraints of the present study also limited some of the neurobiological procedures. Systematic lesion quantification could not be completed and as such the results may alter slightly once further lesion analysis is completed. The standard lesion analysis used thus far was completed by a researcher experienced in ATN lesion analysis and quantification but blind to behavioural performance. Lesion quantification would also allow for correlational analysis of lesion size, performance and zif268 immunoreactivity as well as corresponding neuron loss in each of the mammillary nuclei, particularly as each of the mammillary nuclei project to different regions within the ATN.

Other procedural limitations were also evident. Spatial working memory in the water maze to examine the time-course of enrichment effects allowed for three separate, evenly-spaced single days of testing with eight trials (of a maximum 90 seconds' duration), on days 13 and 25 of the initial continuous enrichment period, and on day 1 of the post-continuous enrichment period. Recovery was not evident in enriched rats with ATN lesions on this task, or on the post-enrichment tests in the water maze. As mentioned previously, the potentially anxiolytic effects of enrichment and ATN lesions may have reduced escape motivation for the water maze tasks. Hence, this reduction in escape motivation may have contributed to the lack of improved performance observed on these tasks after enriched housing. Enrichment and post-enrichment analysis of spatial memory performance using a task such as the T-maze or RAM may have elicited a different pattern of results, as these tasks are not aversive and successful completion is not contingent upon adequate escape motivation. It is also possible that recovery had not yet begun to take effect when enrichment testing took place.

Furthermore, as testing during continuous enrichment was completed in single widely-spaced trials, this procedure may not have captured the potential improvement over time afforded by enrichment (Dobrossy & Dunnett, 2001). The change in testing protocol between the post-surgery working memory and enrichment tests may also have been confusing for the rats, and the eight 90-second trials were demanding for many of the rats with ATN lesions.

Some authors have suggested that enrichment may primarily provide an improvement over the relatively impoverished conditions generally given as 'standard' laboratory housing. It has also been argued that enrichment does not provide an improvement on the 'wild-type' settings of rats (van Praag, Kempermann & Gage, 2000). The lack of recovery observed over the enrichment and early post-enrichment tests may therefore be due to insubstantial enrichment conditions relative to standard housing, although this is unlikely given that the same enrichment protocol has been used in previous research with recovery observed in early post-enrichment testing (Harland, Collings, McNaughton, Abraham & Dalrymple-Alford, 2014).

4.7 Contributions of the Present Study

The multiple novel findings of the present study provide substantial contributions to current understanding of the anatomy of memory systems. The trend toward reversal of zif268 hypoactivation after enrichment in rats with ATN lesions in the CA1 region of the hippocampus, but not the RSC, extends similar work showing that neuromorphological recovery occurs in the CA1 region only (Harland, Collings, McNaughton, Abraham & Dalrymple-Alford, 2014). The present study is also the first to examine zif268 immunoreactivity after ATN lesions and enrichment, as previous research in this context has used c-Fos, another IEG linked to spatial memory in rats, with similar findings as the present study (Loukavenko, Wolff, Poirier & Dalrymple-Alford, in press). The lack of reversal of both zif268 and c-Fos hypoactivation and neuromorphological measures in the RSC after

ATN lesions and enrichment provides further evidence suggesting that neurobiological recovery may not extend to the RSC, and that reversal of IEG hypoactivation may not be essential for recovery of spatial memory function.

Rats with ATN lesions were comparably impaired on the standard and minimised cues working memory tasks in the water maze. Hence the removal of most of the distal cues, by which the rats were presumably navigating, did not result in further impairments on the minimised cues task by comparison to the standard working memory task. One previous study found deficits in rats with ATN lesions on the standard spatial working memory task in the water maze (van Groen, Kadish & Wyss, 2002a), but the present study is the first to examine performance on this task after ATN lesions and enrichment.

Although previous research has examined neuron number in the MB after ATN lesions, both studies were limited by either unilateral lesions (Fry & Cowan, 1972) or damage extending to regions outside the ATN including disconnection of the mammillothalamic tract (Aggleton & Mishkin, 1983). The present study is, therefore, the first to examine the effects of bilateral ATN lesions with minimal damage to surrounding regions and enrichment on neuron number in the MB. Completion of detailed lesion analysis will allow further examination of damage to the individual nuclei within the ATN and corresponding cell loss in each region of the MB. Hence, this evidence of cell loss in the MB after ATN lesions is further indication that circuit-wide dysfunction occurs after ATN lesions.

Earlier research has suggested that enrichment induces changes in plasticity, thereby providing opportunities for corresponding behavioural recovery through regular training (Dobrossy & Dunnett, 2001). This is consistent with the findings of the present study, where rats with ATN lesions housed in enrichment had poor performance in early post-enrichment tasks, but made appreciable gains in spatial memory function with repeated testing by comparison to standard-housed rats with ATN lesions. These results suggest that perhaps

some neural effects of enrichment are important but not solely accountable for recovery of function, and that repeated training may be required to achieve functional recovery after enrichment.

As discussed previously by Aggleton & Nelson (2014), an important consideration for analysis of recovery of function and IEG hypoactivation is that enriched rats with ATN lesions remained impaired relative to sham rats even with repeated training. This suggests that although it is possible that spatial memory performance of sham rats is contingent upon sufficient neuronal activation within the RSC (enumerated through zif268 expression), it remains unclear whether variation in zif268 levels in rats as a result of ATN lesions corresponds to variation in behavioural recovery after enrichment. Behavioural performance, however, was not associated with zif268 expression in the RSC and CA1 regions in the present study, which suggests that perhaps recovery of zif268 expression in these regions may not be necessary for recovery of spatial memory function.

4.8 Future Directions

The present study raises some additional questions regarding both behavioural and neurobiological aspects of this research. As comparable post-surgery deficits were found in rats with ATN lesions on the minimised cues and standard working memory tasks, it would be of interest to examine performance on the minimised cues task after enrichment. Re-examination of probe performance on the reference memory task in the water maze at 5 and 25 days would also allow for further examination of the deficits of ATN lesions on recent and remote memory (Lopez et al, 2009).

The implications of 'covert pathology' in terms of IEG hypoactivation in the RSC still remain uncertain. The laterodorsal nucleus (LD) of the thalamus also has dense, reciprocal connections with the RSC, and has some differential lesion effects on spatial memory tasks when compared with the ATN (van Groen, Kadish & Wyss, 2002b). One previous study has

examined the effects of LD lesions on c-Fos expression in the RSC (Poirier & Aggleton, 2009). The study was, however, limited by sample size and only analysed c-Fos expression in the anterior granular b and dysgranular RSC, which is surprising given that the LD also projects to the posterior RSC, including the granular a region (van Groen & Wyss, 1992). It would be of interest, therefore, to compare the effects of LD and ATN lesions and enrichment on IEG expression in both the anterior and posterior regions of the RSC, and to observe performance on the modified RAM, on which rats with LD lesions would be expected to show a lesser deficit than rats with ATN lesions.

Analysis of covert pathology in the RSC has thus far been restricted to morphological and IEG measures. Epigenetic markers such as histone methylation have previously been linked to memory consolidation in the CA1 region (Jarome, Thomas & Lubin, 2014). It would be suitable, therefore, to further examine whether RSC dysfunction after ATN lesions is associated with disruption of other processes associated with memory function. Examination of the effects of enrichment in this context would also provide additional insights into the mechanisms by which recovery of function occurs, particularly as enrichment has been found to increase gene expression in rats (Rampon et al, 2000).

Theta rhythm within mediodorsal thalamus-perirhinal cortex and hippocampal-anterior thalamus networks is associated with learning and memory (Kirk & Mackay, 2003). Hence, measures of theta rhythm in the extended hippocampal system after enrichment and ATN lesions would also allow mechanistic investigations into the implications of ATN lesions and enrichment on extended hippocampal function. An initial study (Ulrich, Spriggs, Abraham, Dalrymple-Alford & McNaughton, in prep), analysed theta oscillations in the prefrontal cortex, dentate gyrus and CA1 region of the hippocampus. ATN lesions were associated with reduced theta coherence between these regions, whereas post-surgical enrichment was found to increase prefrontal-CA1 coherence. However, the behavioural

measures used did not reveal clear evidence of behavioural impairment in rats with ATN lesions. It would, therefore, be of interest to replicate this electrophysiological study with improved behavioural measures such as extended training on the RAM.

The behavioural implications of neuronal loss in the MB after ATN lesions require further examination. ATN lesions induced retrograde degeneration of MB neurons, and the resulting behavioural deficits may be due in part to loss of information from the MB (Vann, 2010). As MB lesions are associated with spatial memory deficits, deficits observed after ATN lesions may also be due to the loss of neurons within the MB (Dillingham, Frizzati, Nelson & Vann, 2014). MB lesions are generally associated with less substantial deficits than ATN lesions on some spatial memory tasks such as T-maze alternation and spatial reference memory in the water maze. Lesions to the lateral MB and AD region of the ATN, however, produce similar deficits on a geometric task (Dillingham, Frizzati, Nelson & Vann, 2014). As such, it would be of interest to compare the effects of lateral MB and medial MB lesions with that of ATN lesions, using memory tasks such as a geometric task where lateral MB dysfunction would be expected to result in deficits. These procedures could perhaps identify whether neuron loss in the MB increases the deficits associated with ATN lesions.

Environmental enrichment was associated with clear recovery on the RAM tasks in rats with ATN lesions. As previously mentioned, however, this therapeutic intervention requires further research to achieve translational efficacy. Provision of modified standard housing to allow for a more 'wild-type' of housing than a relatively impoverished 'standard' housing would perhaps provide an improved baseline for comparison of enriched and standard housing. Although standard housing conditions do not offer the same opportunities for functional recovery as enrichment, the extensive training that the standard-housed rats received may have reduced any potentially deleterious effects of standard housing (Mendez-Lopez, Mendez, Sampedro-Piquero & Arias, 2013).

4.9 General Conclusions

The results of the present study provide novel evidence for the behavioural and neurobiological implications of ATN lesions and enrichment in rats. ATN lesions in rats were associated with substantial deficits on spatial working and reference memory tasks in the water maze, which did not appear to be reversed by 36 days of continuous enriched housing determined by testing both during and immediately after the initial enrichment period. Subsequent testing on a working memory task in the RAM, a task that requires different motivation, confirmed substantial recovery of function in rats with ATN lesions housed in enrichment.

This study is the first evidence of some recovery of zif268 immunoreactivity in the CA1 region of the hippocampus, and is consistent with previous research showing that recovery of neurobiological measures may not extend to the RSC. The immunoreactivity results suggest either that enrichment may provide a selective recovery, or that IEG hypoactivation in the RSC may not be associated with the behavioural impairments observed after ATN lesions.

A further novel element of the present study was the use of the modified RAM task to assess the association between behavioural performance and neuronal activation in the RSC. Performance on this task was not associated with zif268 expression in the RSC and CA1 across rats from all groups irrespective of lesion or housing condition. These results, therefore, suggest that zif268 expression in these regions may not be necessary for spatial memory performance in this context. The NeuN immunofluorescence results in the MB suggest that ATN lesions induce retrograde MB neuronal degeneration, and that this neuron loss is also not reversed by enrichment.

Overall, the findings of the present study suggest that enrichment as a therapeutic intervention after ATN lesions is associated with partial behavioural and neurobiological

recovery in rats, and that retrosplenial IEG hypoactivation after ATN lesions may not be linked to impaired spatial memory function. The present study hence provides a foundation upon which further research is required to explore multiple lines of inquiry. These findings further current understanding of the behavioural and neurobiological effects of enriched environments and allow additional insights into the functional connectivity of the extended hippocampal system and associated behaviour.

5. References

Aggleton, J. P. (2008). Understanding anterograde amnesia: disconnections and hidden lesions. *The Quarterly Journal of Experimental Psychology, 61*(10), 1441-1471. doi:10.1080/17470210802215335

Aggleton, J. P. (2010). Understanding retrosplenial amnesia: insights from animal studies. *Neuropsychologia, 48*(8), 2328-2338. doi: 10.1016/j.neuropsychologia.2009.09.030

Aggleton, J.P. (2013). Understanding amnesia: is it time to forget HM? *The Psychologist, 26*(8), 612-614.

Aggleton, J. P. (2014). Looking beyond the hippocampus: old and new neurological targets for understanding memory disorders. *Proceedings of the Royal Society B: Biological Sciences, 281*(1786). doi: 10.1098/rspb.2014.0565

Aggleton, J. P., Amin, E., Jenkins, T. A., Pearce, J. M., & Robinson, J. (2011). Lesions in the anterior thalamic nuclei of rats do not disrupt acquisition of stimulus sequence learning. *The Quarterly Journal of Experimental Psychology, 64*(1), 65-73.

Aggleton, J. P., & Brown, M. W. (1999). Episodic memory, amnesia, and the hippocampal–anterior thalamic axis. *Behavioral and Brain Sciences, 22*, 425-489.

Aggleton, J. P., & Brown, M. W. (2006). Interleaving brain systems for episodic and recognition memory. *Trends in Cognitive Sciences, 10*(10), 455-463. doi: 10.1016/j.tics.2006.08.003

Aggleton, J. P., Hunt, P. R., Nagle, S., & Neave, N. (1996). The effects of selective lesions within the anterior thalamic nuclei on spatial memory in the rat. *Behavioural Brain Research, 81*, 189-198.

Aggleton, J. P., & Mishkin, M. (1983). Memory impairments following restricted medial thalamic lesions in monkeys. *Experimental Brain Research, 52*(2), 199-209.

Aggleton, J. P., Neave, N., Nagle, S., Hunt, P. R. (1995). A comparison of the effects of anterior thalamic, mamillary body and fornix lesions on reinforced spatial alternation. *Behavioural Brain Research, 68*, 91-101.

Aggleton, J. P., & Nelson, A. J. (2014). Why do lesions in the rodent anterior thalamic nuclei cause such severe spatial deficits? *Neuroscience & Biobehavioral Reviews*. doi: 10.1016/j.neubiorev.2014.08.013

Aggleton, J. P., O'Mara, S. M., Vann, S. D., Wright, N. F., Tsanov, M., & Erichsen, J. T. (2010). Hippocampal-anterior thalamic pathways for memory: uncovering a network of direct and indirect actions. *European Journal of Neuroscience, 31*(12), 2292-2307. doi: 10.1111/j.1460-9568.2010.07251.x

Aggleton, J. P., Saunders, R. C., Wright, N. F., & Vann, S. D. (2014). The origin of projections from the posterior cingulate and retrosplenial cortices to the anterior, medial dorsal and laterodorsal thalamic nuclei of macaque monkeys. *European Journal of Neuroscience, 39*(1), 107-123. doi: 10.1111/ejn.12389

Alexinsky, T. (2001). Differential effect of thalamic and cortical lesions on memory systems in the rat. *Behavioural Brain Research, 122*(2), 175-191.

Anderson, V. E. R., Cairns, N. J., & Leigh, P. N. (1995). Involvement of the amygdala, dentate and hippocampus in motor neuron disease. *Journal of the Neurological Sciences, 129*, 75-78.

Anzalone, S., Vetreno, R. P., Ramos, R. L., & Savage, L. M. (2010). Cortical cholinergic abnormalities contribute to the amnesic state induced by pyrithiamine-induced thiamine deficiency in the rat. *European Journal of Neuroscience, 32*(5), 847-858. doi: 10.1111/j.1460-9568.2010.07358.x

Braak, H., & Braak, E. (1996). Evolution of the neuropathology of Alzheimer's disease. *Acta Neurologica Scandinavica, 165*, 3-12.

Byatt, G., & Dalrymple-Alford, J. C. (1996). Both anteromedial and anteroventral thalamic lesions impair radial-maze learning in rats. *Behavioral Neuroscience, 110*(6), 1335-1348.

Caine, D., Halliday, G. M., Kril, J. J., & Harper, C. G. (1997). Operational criteria for the classification of chronic alcoholics: identification of Wernicke's encephalopathy. *Journal of Neurology, Neurosurgery, and Psychiatry 62*, 51-60.

Carlesimo, G. A., Lombardi, M. G., & Caltagirone, C. (2011). Vascular thalamic amnesia: a reappraisal. *Neuropsychologia, 49*(5), 777-789. doi: 10.1016/j.neuropsychologia.2011.01.026

Caulo, M., Van Hecke, J., Toma, L., Ferretti, A., Tartaro, A., Colosimo, C., Romani, G. L. & Uncini, A. (2005). Functional MRI study of diencephalic amnesia in Wernicke-Korsakoff syndrome. *Brain, 128*(Pt 7), 1584-1594. doi: 10.1093/brain/awh496

Cenci, M. A., Whishaw, I. Q., & Schallert, T. (2002) Animal models of neurological deficits: how relevant is the rat? *Nature Reviews Neuroscience, 3*, 574-579.

Cenquizca, L. A., & Swanson, L. W. (2007). Spatial organization of direct hippocampal field CA1 axonal projections to the rest of the cerebral cortex. *Brain Research Reviews, 56*(1), 1-26. doi: 10.1016/j.brainresrev.2007.05.002

Christie, M. A., & Dalrymple-Alford, J. C. (1995). Behavioural consequences of frontal cortex grafts and enriched environments after sensorimotor cortex lesions. *Journal of Neural Transplantation & Plasticity, 5*(4), 199-210.

Cotel, M. C., Jawhar, S., Christensen, D. Z., Bayer, T. A., & Wirths, O. (2012). Environmental enrichment fails to rescue working memory deficits, neuron loss, and neurogenesis in APP/PS1KI mice. *Neurobiology of Aging, 33*(1), 96-107. doi: 10.1016/j.neurobiolaging.2010.02.012

Crofton, E. J., Zhang, Y., & Green, T. A. (2014). Inoculation stress hypothesis of environmental enrichment. *Neuroscience & Biobehavioral Reviews*.

Czajkowski, R., Jayaprakash, B., Wiltgen, B., Rogerson, T., Guzman-Karlsson, M. C., Barth, A. L., Trachtenberg, J. T. & Silva, A. J. (2014). Encoding and storage of spatial information in the retrosplenial cortex. *Proceedings of the National Academy of Sciences, 111*(23), 8661-8666. doi: 10.1073/pnas.1313222111

Dalrymple-Alford, J. C., & Kelche, C. R. (1987). Behavioral effects of differential postoperative housing after septal lesions made in weanling rats. *Psychobiology, 15*(3), 255-260.

Dalrymple-Alford, J. C., Harland, B. C., Loukavenko, E. A., Perry, B. A. L., Mercer, S. A., Collings, D. A., Ulrich, K., Abraham, W. C., McNaughton, N., & Wolff, M. (In press). Anterior thalamic nuclei lesions and recovery of function: Relevance to cognitive thalamus. *Neuroscience and Biobehavioural Reviews, 49*.

Davis, S., Bozon, B., & Laroche, S. (2003). How necessary is the activation of the immediate early gene zif268 in synaptic plasticity and learning? *Behavioural Brain Research, 142*(1-2), 17-30. doi: 10.1016/s0166-4328(02)00421-7

de Jong, L. W., van der Hiele, K., Veer, I. M., Houwing, J. J., Westendorp, R. G., Bollen, E. L., de Bruin, P.W., Middelkoop, H. A. M., van Buchem, M. A. & van der Grond, J. (2008). Strongly reduced volumes of putamen and thalamus in Alzheimer's disease: an MRI study. *Brain, 131*(Pt 12), 3277-3285. doi: 10.1093/brain/awn278

Delay, J., Brion, S. (1969). Korsakoff's syndrome. Oxford, England: Masson Et Cie. 170 pp.

D'Hooge, R., & De Deyn, P.P. (2001). Applications of the Morris water maze in the study of learning and memory. *Brain Research Reviews, 36*, 60-90.

Di Paola, M., Macaluso, E., Carlesimo, G. A., Tomaiuolo, F., Worsley, K. J., Fadda, L., & Caltagirone, C. (2007). Episodic memory impairment in patients with Alzheimer's disease is correlated with entorhinal cortex atrophy. A voxel-based morphometry study. *Journal of Neurology, 254*(6), 774-781. doi: 10.1007/s00415-006-0435-1

Dillingham, C. M., Frizzati, A., Nelson, A. J., & Vann, S. D. (2014). How do mammillary body inputs contribute to anterior thalamic function? *Neuroscience and Biobehavioural Reviews*. doi: 10.1016/j.neubiorev.2014.07.025

Döbrössy, M. D., & Dunnett, S. B. (2001). The influence of environment and experience on neural grafts. *Nature Reviews Neuroscience, 2*(12), 871-879.

Dumont, J. R., & Aggleton, J. P. (2013). Dissociation of recognition and recency memory judgments after anterior thalamic nuclei lesions in rats. *Behavioral Neuroscience, 127*(3), 415-431. doi: 10.1037/a0032750

Dumont, J. R., Amin, E., & Aggleton, J. P. (2014). Selective importance of the rat anterior thalamic nuclei for configural learning involving distal spatial cues. *European Journal of Neuroscience, 39*(2), 241-256. doi: 10.1111/ejn.12409

Dumont, J. R., Amin, E., Poirier, G. L., Albasser, M. M., & Aggleton, J. P. (2012). Anterior thalamic nuclei lesions in rats disrupt markers of neural plasticity in distal limbic brain regions. *Neuroscience, 224*, 81-101. doi: 10.1016/j.neuroscience.2012.08.027

Dumont, J. R., Petrides, M., & Sziklas, V. (2010). Fornix and retrosplenial contribution to a hippocampo-thalamic circuit underlying conditional learning. *Behavioural Brain Research, 209*(1), 13-20.

Dumont, J. R., Wright, N. F., Pearce, J. M., & Aggleton, J. P. (2014). The impact of anterior thalamic lesions on active and passive spatial learning in stimulus controlled environments: geometric cues and pattern arrangement. *Behavioral Neuroscience, 128*(2), 161-177. doi: 10.1037/a0036280

Dupire, A., Kant, P., Mons, N., Marchand, A. R., Coutureau, E., Dalrymple-Alford, J., & Wolff, M. (2013). A role for anterior thalamic nuclei in affective cognition: interaction with environmental conditions. *Hippocampus, 23*(5), 392-404. doi: 10.1002/hipo.22098

Ekstrom, A. D., Kahana, M. J., Caplan, J. B., Fields, T. A., Isham, E. A., Newman, E. L., & Fried, I. (2003). Cellular networks underlying human spatial navigation. *Nature, 425*(11), 184-187.

Epstein, R. A. (2008). Parahippocampal and retrosplenial contributions to human spatial navigation. *Trends in Cognitive Sciences, 12*(10), 388-396.

Frohardt, R. J., Bassett, J. P., & Taube, J. S. (2006). Path integration and lesions within the head direction cell circuit: comparison between the roles of the anterodorsal thalamus and dorsal tegmental nucleus. *Behavioral Neuroscience, 120*(1), 135.

Fry, F. J., & Cowan, W. M. (1972). A study of retrograde cell degeneration in the lateral mammillary nucleus of the cat, with special reference to the role of axonal branching in the preservation of the cell. *Journal of Comparative Neurology, 144*(1), 1-23.

Futter, J. E., & Aggleton, J. P. (2006). How rats perform spatial working memory tasks: limitations in the use of egocentric and idiothetic working memory. *Quarterly Journal of Experimental Psychology, 59*(1), 77-99. doi: 10.1080/02724990544000068

Galani, R., Jarrard, L. E., Will, B. E., & Kelche, C. (1997). Effects of postoperative housing conditions on functional recovery in rats with lesions of the hippocampus, subiculum, or entorhinal cortex. *Neurobiology of Learning and Memory, 67*, 43-56.

Gibb, S. J., Wolff, M., & Dalrymple-Alford, J. C. (2006). Odour-place paired-associate learning and limbic thalamus: comparison of anterior, lateral and medial thalamic lesions. *Behavioural Brain Research, 172*(1), 155-168. doi: 10.1016/j.bbr.2006.05.017

Guzowski, J. F., Setlow, B., Wagner, E. K., McGaugh, J. L. (2001). Experience-dependent gene expression in the rat hippocampus after spatial learning: a comparison of the immediate-early genes Arc, c-Fos and zif268. *The Journal of Neuroscience, 21*(14), 5089-5098.

Harding, A., Halliday, G., Caine, D., & Kril, J. (2000). Degeneration of anterior thalamic nuclei differentiates alcoholics with amnesia. *Brain, 123,* 141-154.

Harland, B. C. (2013). Recovery of function after lesions of the anterior thalamic nuclei: CA1 neuromorphology. Unpublished thesis.

Harland, B. C., Collings, D. A., McNaughton, N., Abraham, W. C., & Dalrymple-Alford, J. C. (2014). Anterior thalamic lesions reduce spine density in both hippocampal CA1 and retrosplenial cortex, but enrichment rescues CA1 spines only. *Hippocampus*. doi: 10.1002/hipo.22309

Harper, C., Gold, J., Rodriguez, M., & Perdices, M. (1989). The prevalence of the Wernicke-Korsakoff syndrome in Sydney, Australia: a prospective necropsy study. *Journal of Neurology, Neurosurgery, and Psychiatry, 52*, 282-285.

Hartley, T., Lever, C., Burgess, N., & O'Keefe, J. (2014). Space in the brain: how the hippocampal formation supports spatial cognition. *Philosophical Transactions of the Royal Society B: Biological Sciences, 369*(1635), 20120510.

Henry, J., Petrides, M., St-Laurent, M., & Sziklas, V. (2004). Spatial conditional associative learning: effects of thalamo-hippocampal disconnection in rats. *Neuroreport, 15*(15), 2427-2431.

Herdegen, T., & Leah, J. D. (1998). Inducible and constitutive transcription factors in the mammalian nervous system: control of gene expression by Jun, Fos and Krox, and CREB/ATF proteins. *Brain Research Reviews, 28*(3), 370-490.

Herring, A., Yasin, H., Ambree, O., Sachser, N., Paulus, W., & Keyvani, K. (2008). Environmental enrichment counteracts Alzheimer's neurovascular dysfunction in TgCRND8 mice. *Brain Pathology, 18*(1), 32-39.

Jankowski, M. M., Ronnqvist, K. C., Tsanov, M., Vann, S. D., Wright, N. F., Erichsen, J. T., Aggleton, J. P. & O'Mara, S. M. (2013). The anterior thalamus provides a subcortical circuit supporting memory and spatial navigation. *Frontiers in Systems Neuroscience, 7*, 45. doi: 10.3389/fnsys.2013.00045

Jarome, T. J., Thomas, J. S. & Lubin F. D. (2014). The epigenetic basis of memory formation and storage. *Epigenetics and Neuroplasticity – Evidence and Debate,* in Progress in Molecular Biology and Translational Science, 128, 1-27.

Jenkins, T. A., Dias, R., Amin, E., & Aggleton, J. P. (2002). Changes in Fos expression in the rat brain after unilateral lesions of the anterior thalamic nuclei. *European Journal of Neuroscience, 16,* 1425-1432.

Jenkins, T. A., Dias, R., Amin, E., Brown, M. W., & Aggleton, J. P. (2002). Fos Imaging Reveals that Lesions of the Anterior Thalamic Nuclei Produce Widespread Limbic Hypoactivity in Rats. *Neuroscience, 22*(12), 5230–5238.

Jenkins, T. A., Vann, S. D., Amin, E., & Aggleton, J. P. (2004). Anterior thalamic lesions stop immediate early gene activation in selective laminae of the retrosplenial cortex: evidence of covert pathology in rats? *European Journal of Neuroscience, 19,* 3291-3304.

Johansson, B. B., & Ohlsson, A. (1996). Environment, social interaction, and physical activity as determinants of functional outcome after cerebral infarction in the rat. *Experimental Neurology, 139,* 322-327.

Kelche, C., Dalrymple-Alford, J. C., & Will, B. (1987). Effects of postoperative environment on recovery of function after fimbria-fornix transection in the rat. *Physiology & Behavior, 40,* 731-736.

Kempermann, G., Gast, D., & Gage, F. H. (2002). Neuroplasticity in old age: sustained fivefold induction of hippocampal neurogenesis by long-term environmental enrichment. *Annals of Neurology, 52*(2), 135-143. doi: 10.1002/ana.10262

Kirk, I. J., & Mackay, J. C. (2003). The role of theta-range oscillations in synchronising and integrating activity in distributed mnemonic networks. *Cortex, 39*(4), 993-1008.

Knapska, E., & Kaczmarek, L. (2004). A gene for neuronal plasticity in the mammalian brain: Zif268/Egr-1/NGFI-A/Krox-24/TIS8/ZENK? *Progress in Neurobiology,74*(4), 183-211. doi: 10.1016/j.pneurobio.2004.05.007

Kopelman, M. D. (1995). The Korsakoff syndrome. *The British Journal of Psychiatry, 166*(2), 154-173.

Kopelman, M. D. (2014). What does a comparison of the alcoholic Korsakoff syndrome and thalamic infarction tell us about thalamic amnesia? *Neuroscience & Biobehavioral Reviews.* doi: 10.1016/j.neubiorev.2014.08.014

Kopelman, M. D., Thomson, A. D., Guerrini, I., & Marshall, E. J. (2009). The Korsakoff syndrome: clinical aspects, psychology and treatment. *Alcohol and Alcoholism, 44*(2), 148-154. doi: 10.1093/alcalc/agn118

Langlais, P. J. (1995). Pathogenesis of diencephalic lesions in an experimental model of Wernicke's Encephalopathy. *Metabolic Brain Disease, 10*(1), 31-44.

Leggio, M. G., Mandolesi, L., Federico, F., Spirito, F., Ricci, B., Gelfo, F., & Petrosini, L. (2005). Environmental enrichment promotes improved spatial abilities and enhanced dendritic growth in the rat. *Behavioural Brain Research, 163*(1), 78-90. doi: 10.1016/j.bbr.2005.04.009

Lopez, J., Wolff, M., Lecourtier, L., Cosquer, B., Bontempi, B., Dalrymple-Alford, J.C., & Cassel, J. C. (2009). The intralaminar thalamic nuclei contribute to remote spatial memory. *The Journal of Neuroscience, 29*(10), 3302-3306.

Loukavenko, E. A., Ottley, M. C., Moran, J. P., Wolff, M., & Dalrymple-Alford, J. C. (2007). Towards therapy to relieve memory impairment after anterior thalamic lesions: improved spatial working memory after immediate and delayed postoperative enrichment. *European Journal of Neuroscience, 26*(11), 3267-3276. doi: 10.1111/j.1460-9568.2007.05879.x

Loukavenko, E. A., Wolff, M., Poirier, G. L., & Dalrymple-Alford, J. C. (In press). Impaired spatial working memory after anterior thalamic lesions: Recovery with cerebrolysin and enrichment.

Loureiro, M., Cholvin, T., Lopez, J., Merienne, N., Latreche, A., Cosquer, B. Geiger, K., Kelche, C., Cassel, J. C., & de Vasconcelos, A. P. (2012). The ventral midline thalamus (reuniens and rhomboid nuclei) contributes to the persistence of spatial memory in rats. *The Journal of Neuroscience, 32*(29), 9947-9959.

Maesako, M., Uemura, K., Kubota, M., Kuzuya, A., Sasaki, K., Asada, M., Watanabe, K., Hayashida, N., Ihara, M., Ito, H. Shimohama, S., Kihara, T., & Kinoshita, A. (2012). Environmental enrichment ameliorated high-fat diet-induced Abeta deposition and memory deficit in APP transgenic mice. *Neurobiology of Aging, 33*(5), 1011 e1011-1023. doi: 10.1016/j.neurobiolaging.2011.10.028

Maguire, E. A. (1998). Knowing where and getting there: A human navigation network. *Science, 280*(5365), 921-924. doi: 10.1126/science.280.5365.921

Mair, R. G., Burk, J. A., & Porter, M. C. (2003). Impairment of radial maze delayed nonmatching after lesions of anterior thalamus and parahippocampal cortex. *Behavioral Neuroscience, 117*(3), 596-605. doi: 10.1037/0735-7044.117.3.596

Markowitsch, H. J., & Staniloiu, A. (2012). Amnesic disorders. *The Lancet, 380*(9851), 1429-1440. doi: 10.1016/s0140-6736(11)61304-4

Mendez-Lopez, M., Arias, J. L., Bontempi, B., & Wolff, M. (2013). Reduced cytochrome oxidase activity in the retrosplenial cortex after lesions to the anterior thalamic nuclei. *Behavioural Brain Research, 250,* 264-273. doi: 10.1016/j.bbr.2013.04.052

Mendez-Lopez, M., Mendez, M., Sampedro-Piquero, P., & Arias, J. L. (2013). Spatial learning-related changes in metabolic activity of limbic structures at different posttask delays. *Journal of Neuroscience Research, 91*(1), 151-159. doi: 10.1002/jnr.23134

Mitchell, A. S., & Dalrymple-Alford, J. C. (2006). Lateral and anterior thalamic lesions impair independent memory systems. *Learning & Memory, 13*(3), 388-396. doi: 10.1101/lm.122206

McKee, R. D., & Squire, L. R. (1992). Equivalent forgetting rates in long-term memory for diencephalic and medial temporal lobe amnesia. *The Journal of Neuroscience, 12*(10), 3765-3772.

Moran, J. P., & Dalrymple-Alford, J. C. (2003). Perirhinal cortex and anterior thalamic lesions: comparative effects on learning and memory. *Behavioral Neuroscience, 117*(6), 1326-1341. doi: 10.1037/0735-7044.117.6.1326

Moreau, P. H., Tsenkina, Y., Lecourtier, L., Lopez, J., Cosquer, B., Wolff, M., Dalrymple-Alford, J. C., & Cassel, J. C. (2013). Lesions of the anterior thalamic nuclei and intralaminar thalamic nuclei: place and visual discrimination learning in the water maze. *Brain Structure and Function, 218*(3), 657-667.

Morel, A., Magnin, M., & Jeanmonod, D. (1997). Multiarchitectonic and stereotactic atlas of the human thalamus. *Journal of Comparative Neurology, 387*(4), 588-630.

Moscovitch, M., Nadel, L., Winocur, G., Gilboa, A., & Rosenbaum, R. S. (2006). The cognitive neuroscience of remote episodic, semantic and spatial memory. *Current Opinion in Neurobiology, 16*(2), 179-190.

Nithianantharajah, J., & Hannan, A. J. (2006). Enriched environments, experience-dependent plasticity and disorders of the nervous system. *Nature Reviews Neuroscience, 7*(9), 697-709. doi: 10.1038/nrn1970

Paller, K. A., Acharya, A., Richardson, B. C., Plaisant, O., Shimamura, A. P., Reed, B. R., & Jagust, W. J. (1997). Functional neuroimaging of cortical dysfunction in alchoholic Korsakoff's syndrome. *Journal of Cognitive Neuroscience, 9*(2), 277-293.

Papez, J. W. (1937). A proposed mechanism of emotion. *Archives of Neurology & Psychiatry, 38*(4), 725-743.

Passineau, M. J., Green, E. J., & Dietrich, W. D. (2001). Therapeutic effects of environmental enrichment on cognitive function and tissue integrity following severe traumatic brain injury in rats. *Experimental Neurology, 168*(2), 373-384. doi: 10.1006/exnr.2000.7623

Paxinos, G., & Watson, C. (1998). The rat brain in stereotaxic coordinates. Vol. 4. Academic Press, San Diego.

Pergola, G., & Suchan, B. (2013). Associative learning beyond the medial temporal lobe: many actors on the memory stage. *Frontiers in Behavioral Neuroscience, 7*, 162. doi: 10.3389/fnbeh.2013.00162

Perren, F., Clarke, S., & Bogousslavsky, J. (2005). The syndrome of combined polar and paramedian thalamic infarction. *Archives of Neurology, 62*(8), 1212-1216.

Poirier, G. L., & Aggleton, J. P. (2009). Post-surgical interval and lesion location within the limbic thalamus determine extent of retrosplenial cortex immediate-early gene hypoactivity. *Neuroscience, 160*(2), 452-469. doi: 10.1016/j.neuroscience.2009.02.021

Poirier, G. L., Shires, K. L., Sugden, D., Amin, E., Thomas, K. L., Carter, D. A., & Aggleton, J. P. (2008). Anterior thalamic lesions produce chronic and profuse transcriptional de-regulation in retrosplenial cortex: A model of retrosplenial hypoactivity and covert pathology. *Thalamus & Related Systems, 4*(1), 59-77.

Pothuizen, H. H., Aggleton, J. P., & Vann, S. D. (2008). Do rats with retrosplenial cortex lesions lack direction? *European Journal of Neuroscience, 28*(12), 2486-2498. doi: 10.1111/j.1460-9568.2008.06550.x

Pothuizen, H. H., Davies, M., Aggleton, J. P., & Vann, S. D. (2010). Effects of selective granular retrosplenial cortex lesions on spatial working memory in rats. *Behavioural Brain Research, 208*(2), 566-575. doi: 10.1016/j.bbr.2010.01.001

Pothuizen, H. H., Davies, M., Albasser, M. M., Aggleton, J. P., & Vann, S. D. (2009). Granular and dysgranular retrosplenial cortices provide qualitatively different contributions to spatial working memory: evidence from immediate-early gene imaging in rats. *European Journal of Neuroscience, 30*(5), 877-888. doi: 10.1111/j.1460-9568.2009.06881.x

Rampon, C., Jiang, C. H., Dong, H., Tang, Y. P., Lockhart, D. J., Schultz, P. G., Tsien, J. Z., & Hu, Y. (2000). Effects of environmental enrichment on gene expression in the brain. *Proceedings of the National Academy of Sciences, 97*(23), 12880-12884.

Rosenzweig, M. R., Krech, D., Bennett, E. L., & Zolman, J. F. (1962). Variation in environmental complexity and brain measures. *Journal of Comparative and Physiological Psychology, 55*(6), 1092.

Rosenzweig, M. R., Bennett, E. L., Hebert, M., & Morimoto, H. (1978). Social grouping cannot account for cerebral effects of enriched environments. *Brain Research, 153*(3), 563-576.

Sampedro-Piquero, P., Begega, A., Zancada-Menendez, C., Cuesta, M., & Arias, J. L. (2013). Age-dependent effects of environmental enrichment on brain networks and spatial memory in Wistar rats. *Neuroscience, 248C*, 43-53. doi: 10.1016/j.neuroscience.2013.06.003

Savage, L. M., Hall, J. M., & Resende, L. S. (2012). Translational rodent models of Korsakoff syndrome reveal the critical neuroanatomical substrates of memory dysfunction and recovery. *Neuropsychology Review, 22*(2), 195-209. doi: 10.1007/s11065-012-9194-1

Scoville, W. B., & Milner, B. (1957). Loss of recent memory after bilateral hippocampal lesions. *Journal of Neurology, Neurosurgery, and Psychiatry, 20*(1), 11.

Sechi, G., & Serra, A. (2007). Wernicke's encephalopathy: new clinical settings and recent advances in diagnosis and management. *The Lancet Neurology, 6*(5), 442-455.

Shibata, H. (1993). Efferent projections from the anterior thalamic nuclei to the cingulate cortex in the rat. *Comparative Neurology, 330*, 533-542.

Shibata, H., Honda, Y., Sasaki, H., & Naito, J. (2009). Organization of intrinsic connections of the retrosplenial cortex in the rat. *Anatomical Science International, 84*(4), 280-292. doi: 10.1007/s12565-009-0035-0

Shibata, H., & Naito, J. (2005). Organization of anterior cingulate and frontal cortical projections to the anterior and laterodorsal thalamic nuclei in the rat. *Brain Research, 1059*(1), 93-103. doi: 10.1016/j.brainres.2005.08.025

Squire, L. R., Stark, C. E., & Clark, R. E. (2004). The medial temporal lobe. *Annual Reviews Neuroscience, 27*, 279-306. doi: 10.1146/annurev.neuro.27.070203.144130

Squire, L. R., & Zola, S. M. (1998). Episodic memory, semantic memory, and amnesia. *Hippocampus, 8*(3), 205-211.

Sullivan, E. V., & Pfefferbaum, A. (2009). Neuroimaging of the Wernicke-Korsakoff syndrome. *Alcohol and Alcoholism, 44*(2), 155-165. doi: 10.1093/alcalc/agn103

Sullivan, E. V., Deshmukh, A., Desmond, J. E., Lim, K. O., & Pfefferbaum, A. (2000). Cerebellar volume decline in normal aging, alcoholism, and Korsakoff's syndrome: relation to ataxia. *Neuropsychology, 14*(3), 341.

Sziklas, V., & Petrides, M. (1999). The effects of lesions to the anterior thalamic nuclei on object–place associations in rats. *European Journal of Neuroscience, 11*, 559–566.

Sziklas, V., & Petrides, M. (2004). Egocentric conditional associative learning: effects of restricted lesions to the hippocampo-mammillo-thalamic pathway. *Hippocampus, 14*(8), 931-934. doi: 10.1002/hipo.20044

Sziklas, V., & Petrides, M. (2007). Contribution of the anterior thalamic nuclei to conditional learning in rats. *Hippocampus, 17*(6), 456-461. doi: 10.1002/hipo.20286

Teipel, S. J., Born, C., Ewers, M., Bokde, A. L., Reiser, M. F., Moller, H. J., & Hampel, H. (2007). Multivariate deformation-based analysis of brain atrophy to predict Alzheimer's disease in mild cognitive impairment. *Neuroimage, 38*(1), 13-24. doi: 10.1016/j.neuroimage.2007.07.008

Toscano, C. D., McGlothan, J. L., & Guilarte, T. R. (2006). Experience-dependent regulation of zif268 gene expression and spatial learning. *Experimental neurology, 200*(1), 209-215.

Tsanov, M., Chah, E., Vann, S. D., Reilly, R. B., Erichsen, J. T., Aggleton, J. P., & O'Mara, S. M. (2011). Theta-modulated head direction cells in the rat anterior thalamus. *The Journal of Neuroscience, 31*(26), 9489-9502.

Tsivilis, D., Vann, S. D., Denby, C., Roberts, N., Mayes, A. R., Montaldi, D., & Aggleton, J. P. (2008). A disproportionate role for the fornix and mammillary bodies in recall versus recognition memory. *Nature Neuroscience, 11*(7), 834-842.

Ulrich, K., Aitken, P. N., Abraham, W. C., Dalrymple-Alford, J. C., & McNaughton, N. (2014). Effects of thalamic lesions on repeated relearning of a spatial working memory task. *Behavioural Brain Research, 261C*, 56-59.

Ulrich, K., Spriggs, M. J., Abraham, W. C., Dalrymple-Alford, J. C. & McNaughton, N. (2015). Anterior thalamic disruption of frontal and hippocampal theta rhythmicity is rescued by environmental enrichment. Manuscript in preparation.

Van der Werf, Y. D., Scheltens, P., Lindeboom, J., Witter, M. P., Uylings, H. B. M., & Jolles, J. (2003). Deficits of memory, executive functioning and attention following infarction in the thalamus; a study of 22 cases with localised lesions. *Neuropsychologia, 41*(10), 1330-1344. doi: 10.1016/s0028-3932(03)00059-9

Van Groen, T., & Wyss, J. M. (1990). Connections of the retrosplenial granular a cortex in the rat. *The Journal of Comparative Neurology, 300*, 593-606.

Van Groen, T., & Wyss, J. M. (1992). Connections of the retrosplenial dysgranular cortex in the rat. *Comparative Neurology, 315*, 200-216.

Van Groen, T., & Wyss, J. M. (2003). Connections of the retrosplenial granular b cortex in the rat. *Journal of Comparative Neurology, 463*(3), 249-263. doi: 10.1002/cne.10757

Van Groen, T., Kadish, I., & Wyss, J. M. (1999). Efferent connections of the anteromedial nucleus of the thalamus of the rat. *Brain Research Reviews, 30*, 1-26.

Van Groen, T., Kadish, I., & Michael Wyss, J. (2002a). Role of the anterodorsal and anteroventral nuclei of the thalamus in spatial memory in the rat. *Behavioural Brain Research, 132*(1), 19-28.

Van Groen, T., Kadish, I., & Wyss, J. M. (2002b). The role of the laterodorsal nucleus of the thalamus in spatial learning and memory in the rat. *Behavioural Brain Research, 136*, 329-337.

Vann, S. D. (2010). Re-evaluating the role of the mammillary bodies in memory. *Neuropsychologia, 48*(8), 2316-2327.

Vann, S. D., & Aggleton, J. P. (2002). Extensive cytotoxic lesions of the rat retrosplenial cortex reveal consistent deficits on tasks that tax allocentric spatial memory. *Behavioral Neuroscience, 116*(1), 85.

Vann, S. D., & Aggleton, J. P. (2004). Testing the importance of the retrosplenial guidance system: effects of different sized retrosplenial cortex lesions on heading direction and spatial working memory. *Behavioural Brain Research, 155*(1), 97-108.

Vann, S. D., Erichsen, J. T., O'Mara, S. M., & Aggleton, J. P. (2011). Selective disconnection of the hippocampal formation projections to the mammillary bodies produces only mild deficits on spatial memory tasks: implications for fornix function. *Hippocampus, 21*(9), 945-957. doi: 10.1002/hipo.20796

Vann, S. D., Wilton, L. A., Muir, J. L., & Aggleton, J. P. (2003). Testing the importance of the caudal retrosplenial cortex for spatial memory in rats. *Behavioural Brain Research, 140*(1), 107-118.

Van Praag, H., Kempermann, G., & Gage, F. H. (2000). Neural consequences of enviromental enrichment. *Nature Reviews Neuroscience, 1*(3), 191-198.

Warburton, E. C., & Aggleton, J. P. (1999). Differential deficits in the Morris water maze following cytotoxic lesions of the anterior thalamus and fornix transection. *Behavioural Brain Research, 98*, 27–38.

Warburton, E. C., Baird, A. L., & Aggleton, J. P. (1997). Assessing the magnitude of the allocentric spatial deficit associated with complete loss of the anterior thalamic nuclei in rats. *Behavioural Brain Research, 87*, 223-232.

Warburton, E. C., Baird, A., Morgan, A., Muir, J. L., Aggleton, J. P. (2001). The conjoint importance of the hippocampus and anterior thalamic nuclei for allocentric spatial learning: evidence from a disconnection study in the rat. *The Journal of Neuroscience, 21*(18), 7323-7330.

Warburton, E. C., Morgan, A., Baird, A. L., Muir, J. L., & Aggleton, J. P. (1999). Does pretraining spare the spatial deficit associated with anterior thalamic damage in rats? *Behavioral Neuroscience, 113*(5), 956.

Whishaw, I. Q., Maaswinkel, H., Gonzalez, C. L. R., & Kolb, B. (2001). Deficits in allothetic and idiothetic spatial behavior in rats with posterior cingulate cortex lesions. *Behavioural Brain Research, 118*, 67-76.

Will, B., Galani, R., Kelche, C., & Rosenzweig, M. R. (2004). Recovery from brain injury in animals: relative efficacy of environmental enrichment, physical exercise or formal training (1990-2002). *Progress in Neurobiology, 72*(3), 167-182. doi: 10.1016/j.pneurobio.2004.03.001

Will, B. E., Rosenzweig, M. R., Bennett, E. L., Hebert, M., & Morimoto, H. (1977). Relatively brief environmental enrichment aids recovery of learning capacity and alters brain measures after postweaning brain lesions in rats. *Journal of Comparative and Physiological Psychology, 91*(1), 33.

Wilton, L. A. K., Baird, A. L., Muir, J. L., Honey, R. C., & Aggleton, J. P. (2001). Loss of the thalamic nuclei for "head direction" impairs performance on spatial memory tasks in rats. *Behavioral Neuroscience, 115*(4), 861-869. doi: 10.1037//0735-7044.115.4.861

Wolff, M., Gibb, S. J., Cassel, J. C., & Dalrymple-Alford, J. C. (2008). Anterior but not intralaminar thalamic nuclei support allocentric spatial memory. *Neurobiology of Learning and Memory, 90*(1), 71-80. doi: 10.1016/j.nlm.2008.01.007

Wolff, M., Gibb, S. J., & Dalrymple-Alford, J. C. (2006). Beyond spatial memory: the anterior thalamus and memory for the temporal order of a sequence of odor cues. *The Journal of Neuroscience, 26*(11), 2907-2913.

Wolff, M., Loukavenko, E. A., Will, B. E., & Dalrymple-Alford, J. C. (2008). The extended hippocampal-diencephalic memory system: enriched housing promotes recovery of the flexible use of spatial representations after anterior thalamic lesions. *Hippocampus, 18*(10), 996-1007. doi: 10.1002/hipo.20457

Wright, N. F., Erichsen, J. T., Vann, S. D., O'Mara, S. M., & Aggleton, J. P. (2010). Parallel but separate inputs from limbic cortices to the mammillary bodies and anterior thalamic nuclei in the rat. *Journal of Comparative Neurology, 518*(12), 2334-2354. doi: 10.1002/cne.22336

Wright, N. F., Vann, S. D., Erichsen, J. T., O'Mara, S. M., & Aggleton, J. P. (2013). Segregation of parallel inputs to the anteromedial and anteroventral thalamic nuclei of the rat. *Journal of Comparative Neurology, 521*(13), 2966-2986. doi: 10.1002/cne.23325

Zarei, M., Patenaude, B., Damoiseaux, J., Morgese, C., Smith, S., Matthews, P. M., Barkhof, F., Rombouts, S., Sanz-Arigita, E. & Jenkinson, M. (2010). Combining shape and connectivity analysis: an MRI study of thalamic degeneration in Alzheimer's disease. *Neuroimage, 49*(1), 1-8. doi: 10.1016/j.neuroimage.2009.09.001

Zhang, S. X., Weilersbacher, G. S., Henderson, S. W., Corso, T., Olney, J. W., & Langlais, P. J. (1995). Excitotoxic cytopathology, progression, and reversibility of thiamine deficiency-induced diencephalic lesions. *Journal of Neuropathology & Experimental Neurology, 54*(2), 255-267.