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RESEARCH ARTICLE

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# Anterior thalamic nuclei lesions have a greater impact than mammillothalamic tract lesions on the extended hippocampal system

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## Abstract

The anterior thalamic nuclei (ATN), mammillary bodies and their interconnecting fiber tract, the mammillothalamic tract (MTT), are important components of an extended hippocampal circuit for episodic memory. In humans, damage to the MTT or ATN in many disorders is associated with severe anterograde amnesia and it is assumed that their influence on memory is functionally equivalent. The relative influence of these two structures on memory has not, however, been assessed explicitly. Here, a direct comparison found that only ATN lesions impaired spatial reference memory in rats. ATN lesions produced more severe deficits on spatial working memory and reduced zif268 expression to a greater degree and in more corticolimbic sites than did MTT lesions. Conversely, MTT lesions reduced NeuN cell counts in all three subregions of the MB to a greater extent than did ATN lesions, so their relative impact cannot be explained by retrograde neuropathology of the MB. Hence ATN injury causes a more critical dysfunction than would be expected by an emphasis on the indirect influence of brainstem inputs to the extended memory system. The greater ATN lesion deficits found here may represent the consequence of disruption to the direct connections of the ATN with both hippocampal and cortical sites.

**KEYWORDS**

hippocampus, mammillary bodies, retrosplenial cortex, spatial memory, Zif268

## 1 | INTRODUCTION

The mammillothalamic tract (MTT) provides a unique unidirectional path from the mammillary bodies (MB) to the anterior thalamic nuclei (ATN), all of which are considered critical structures within an extended hippocampal-diencephalic circuit responsible for episodic memory (Aggleton, 2014; Child & Benarroch, 2013; Dalrymple-Alford et al., 2015; Vann & Nelson, 2015). The clinical support for this perspective is that injury to the MTT is the most consistent predictor of an amnesic syndrome following thalamic infarction (Carlesimo, Lombardi, & Caltagirone, 2011; Carlesimo, Lombardi, Caltagirone, & Barban, 2015; Van der Werf et al., 2003; Van der Werf, Witter, Uylings, & Jolles, 2000), while ATN pathology may be the critical point of difference between patients with Korsakoff amnesia and nonamnesic patients with alcoholic Wernicke's encephalopathy (Harding, Halliday, Caine, & Kril, 2000; Kopelman, 2014). Other studies suggest that MB pathology is at least as prominent as hippocampal pathology in cases of developmental amnesia and that MB degeneration may be a critical feature of poor recall after fornix injury (Dzieciol, Bachevalier, & Saleem, 2017; Tsivilis et al., 2008). There is, however, ambiguity regarding the boundaries of diencephalic and nondiencephalic pathology that are most relevant for diencephalic amnesia so the contributions to memory of the MB-MTT axis and the ATN remain uncertain (Danet et al., 2015; Duprez, Serieh, & Raftopoulos, 2005; Kopelman, 2014). New experimental evidence suggests a more prominent influence of the MTT and a greater influence of the MB's brainstem connections on the integrity of the extended hippocampal system than was previously recognized (Dillingham, Frizzati, Nelson, & Vann, 2015; Vann & Nelson, 2015). The latter perspective suggests that equivalent deficits after MTT and ATN injury would be commonplace rather than the exception and bolster the call to include more rigorous assessment of the Papez circuit in degenerative conditions such as Alzheimer's disease (Aggleton, Pralus, Nelson, & Hornberger, 2016).

  

While this anatomical and clinical evidence points to functional overlap between the MTT and the ATN, the relative impact of injury to these structures on memory has not been directly tested. Potential anatomical and functional differences between the MTT and ATN have been posited as critical for the impact of clinically-defined lesions that cause diencephalic amnesia (Duprez et al., 2005). Comparisons across separate studies on the effects of experimental MTT or ATN lesions provide some insight to this question. Both lesions consistently produce spatial working memory deficits in the T-maze and radial arm maze (RAM) (Aggleton & Nelson, 2015; Dalrymple-Alford et al., 2015; Frizzati et al., 2016; Vann & Nelson, 2015), but these impairments are sometimes relatively transient in the case of MTT lesions (Vann, 2013; Vann & Aggleton 2003). Of particular relevance is evidence that the effects of MTT and ATN lesions can be dissimilar on other spatial memory tasks. For example, MTT lesions in rats were reported to show weak and transient reference memory impairments in the standard water-maze in one report and did not impair geometric learning in a water-maze in another study (Vann, 2013; Winter, Wagner, McMillin, & Wallace, 2011). In contrast, ATN lesions produce profound deficits on both tasks (Aggleton, Poirer, Aggleton, Vann, & Pearce, 2009; Dumont, Amin, & Aggleton, 2014; Warburton, Morgan, Baird, Muir, & Aggleton, 1999; Wolff, Gibb, Cassel, & Dalrymple-Alford, 2008a; Wolff, Loukavenko, Will, & Dalrymple-Alford, 2008b). Nonetheless, it is possible that some of the differences between the effects of MTT and ATN lesions are a result of subtle procedural variations used in different studies.

One of the most intriguing features of both MTT and ATN lesions is that they produce a dramatic loss of immediate early gene (IEG) neural activity markers in distal regions of the extended hippocampal memory circuit, despite ostensibly normal conventional histology in those regions (Aggleton, 2008; Aggleton & Nelson, 2015; Dillingham et al., 2015). The retrosplenial cortex appears to be particularly sensitive to these two diencephalic lesions and separate laboratories have consistently found substantial hypo-activation of c-Fos and zif268 IEG markers in this cortical structure (Aggleton & Nelson, 2015; Dupire et al., 2013; Dillingham et al., 2015; Frizzati et al., 2016; Loukavenko, Wolff, Poirier, & Dalrymple-Alford, 2016). More variable changes have been observed in the hippocampus across ATN and MTT lesion studies, suggesting that the specific neural marker or the behavioral task may explain these differences (Dumont, Amin, Poirier, Albasser, & Aggleton, 2012; Dupire et al., 2013; Frizzati et al., 2016; Jenkins, Dias, Amin, & Aggleton, 2002a; Jenkins, Dias, Amin, Brown, & Aggleton, 2002b; Loukavenko et al., 2016; Vann, 2013). Similarly, both ATN lesions and MTT lesions reduce the level of the metabolic marker cytochrome oxidase in the superficial lamina of the retrosplenial cortex, but only MTT lesions reduced the expression of this marker in the deep layers of this limbic cortex (Frizzati et al., 2016; Mendez-Lopez, Arias, Bontempi, & Wolff, 2013). Clearly, an understanding of the relative magnitude and specificity of the distal neural changes evident after MTT and ATN lesions would also benefit from direct comparisons within the same study.

The current study therefore compared the relative behavioral and neural effects of MTT lesions and ATN lesions in rats. We examined spatial working memory in the RAM and both spatial reference memory and spatial working memory in the water maze. We then compared zif268 expression in the retrosplenial cortex and hippocampus after the rats had been tested with new extramaze cues and a mid-trial rotation of the RAM as this procedure is considered particularly sensitive to retrosplenial cortex function (Pothuizen, Aggleton, & Vann, 2008; Vann &Aggleton, 2002; Vann, Wilton, Muir, & Aggleton, 2003). Zif268 was used because this marker is associated with spatial memory formation and long-term plasticity whereas c-Fos is a more general measure of neural activation (Farina & Commins, 2016; Jones et al., 2001; Penke et al., 2014). Naturally, postmortem clinical studies have not assessed IEG markers in cases of diencephalic amnesia. Kosakoff's amnesia has, however, been linked with reduced neuron counts and volume in the MB (Harding et al., 2000; Kopelman, 2014); such effects in clinical cases of MTT injury have not been reported. An additional aim therefore was to compare neuronal integrity in the MB after MTT and ATN lesions and determine the association of MB integrity with memory.

## 2 | MATERIALS AND METHODS

### 2.1 | Subjects

Subjects were 55 male PVGc hooded rats bred in our facility and maintained in standard housing of three or four rats per opaque plastic cage (50 cm long × 30 cm wide × 23 cm). Rats were 8–10 months old and 320 to 430 g at lesion surgery. Triplets of rats were randomly allocated to MTT lesion, ATN lesion or Sham surgery groups using matched performance derived from 12 days of preoperative working memory testing in an 8-arm RAM. Individual housing was used for 7–12 days of recovery following surgery. Food and water were available *ad libitum* during surgery, recovery, and initial behavioral tasks. Presurgery testing and later behavioral tasks required food restriction to attain 85% of their free feeding body weight, with water available *ad libitum*. All procedures complied with the University of Canterbury animal ethics committee guidelines and approval. Behavioral testing was conducted during the lights off period (8 am to 8 pm).

### 2.2 | MTT surgery

Rats were anesthetized with an intraperitoneal injection of ketamine (80 mg/kg) and domitor (0.35 mg/kg) and placed in a stereotaxic device with atraumatic ear bars (Kopf, Tujunga, CA). Flat skull was used for MTT lesions, which were made using a Radionics TCZ radio frequency electrode (0.3 mm long, 0.25 mm diameter exposed tip; RFG4-A, Radionics, Burlington, VT). One of four anterior–posterior (AP) coordinates from bregma was used to accommodate different bregma to lambda (B–L) distances: −2.5 for B–L ≥ 6.4; −2.55 for B–L 6.5–6.8; −2.6 for B–L 6.9 to 7.2; −2.65 for B–L < 7.2. The laterality was ±0.9 from the midline and ventrality was −7.2 from dura, with the electrode lowered vertically to the MTT coordinate, where the temperature of the tissue surrounding the electrode tip was raised slowly to 63 °C and then maintained for 60 s. Sham rats received the same procedure

except the electrode was lowered 1.0 mm above the site and the temperature was not raised.

## 2.3 | ATN surgery

For ATN surgeries the incisor bar was set at −7.5 mm below the interaural line to minimize fornix damage. To create lesions in all three ATN subnuclei (anterodorsal, AD; anteroventral, AV; and anteromedial, AM), two infusions per hemisphere were directed at an upper and a lower site in the anteroventral nucleus, and an additional infusion was directed bilaterally at the anteromedial nucleus. The four AV coordinates were infused before the two AM coordinates. One of four anterior–posterior coordinates from bregma were used to accommodate different B–L distances. For AV lesions the AP coordinates were: −2.5 for B–L ≤0.64; −2.55 for B–L 6.5 to 6.8; −2.6 for B–L 6.9–7.2; −2.65 for B–L ≥ 7.3. The AV infusions were made at ventrality −5.63 followed by −5.73 from dura at ±1.52 lateral to the midline. The AM infusion was placed 0.1 mm more anterior than the AV coordinates, with laterality ±1.20 and ventrality of −5.86 mm. Lesions were made by infusing 0.15 μL per site of 0.15M *N*-methyl-D-aspartate (NMDA; Sigma, Castle Hill, NSW) in 0.1M phosphate buffer (pH 7.20) at a rate 0.04 μL per minute via a 1 μL Hamilton syringe (Reno, NV, USA) driven by a microinfusion pump (Stoelting, Wooddale, IL). Following infusion the needle was left *in situ* for a further 3 min per coordinate for the neurotoxin to diffuse. Sham surgeries used an identical procedure except that the needle was lowered to 1.50 mm above the dorsal AV and the AM coordinates and no material was infused.

## 2.4 | Behavioral tasks

Prior to lesion surgery, rats were habituated and then tested on a standard radial arm maze task for 12 days, followed by 4 days of habituation to the water maze. Following recovery from lesion surgery the rats were returned to the water maze, first for 12 days of testing in the classic spatial reference memory task, followed by 12 days of spatial working memory testing in the same environment and then 6 days of working memory testing in which the extramaze cues were minimized. Four weeks later, the rats were returned to the standard RAM for a brief period of rehabituation and 12 days of testing before 4 days each of a mid-trial delay and a mid-trial delay plus rotation task. Prior to euthanasia, zif268 expression was stimulated in the rats using a modified RAM procedure.

## 2.5 | Water maze tasks

The water maze was constructed out of white rigid plastic and had an internal diameter of 180 cm with a height of 45 cm with an outer lip protruding 5 cm. It was located off center on the floor of a windowless room (4 m by 4.7 m). It was filled to a height of 30 cm with water that was 21 ± 2°C and made opaque by the addition of acrylic nontoxic paint (Super Tempera, Fine Art Supplies, New Zealand). The water maze arena was divided into virtual segments using eight arbitrarily defined compass points marked on the rim of the pool (i.e., N, NE, E, SE, S, SW, W, NW). A 10 cm circular white Perspex escape platform with a nonslip surface was placed in the pool at various positions and distances from the pool edge and sat 2 cm below the surface of the water. Salient visual cues, when available, included geometric shapes and high contrast visual stimuli in the room or on the walls, e.g., small road cones, sink unit, a computer, tables, and posters. The testing room also contained a beige curtain hanging from the ceiling on a circular track around the pool perimeter that could be opened or closed. A camera fixed to the ceiling above the center of the pool was used to track swim paths (Ethovision XT 5.0.212, Noldus Information Technology, The Netherlands). Measures recorded were path-length, escape latency and swim speed. Three CPUs were placed around the room and left running, one of which was the data recording computer. Lighting was provided by two overhead fluorescent lights and by a large upward facing lamp (300 W) on a stand approximately 180 cm tall and positioned 40 cm from the E sector. One additional lamp (60 W) was located in the corner of the room opposite the SW quadrant and was used to keep the rats warm during testing and provided an additional light source.

## 2.6 | Presurgery habituation in the water maze

All rats were habituated to the water maze after testing in the RAM, with four swims per day for 4 days. On all 4 days, the curtain was drawn closed around the water maze to minimize room cues. For the first 2 days, the platform was raised 1 cm above the water level and thus visible to the rats, and then 2 cm below the surface at the same position for the remaining 2 days. The rats were randomly assigned to one of four fixed platform locations (NE, NW, SE, and SW) with the platform located 45 cm from the edge of the maze. The order of the start locations was randomized across the four cardinal coordinates: N, S, E, and W for the four daily trials.

## 2.7 | Spatial reference memory in the water maze

After recovery from surgery the rats were given 12 days of reference memory testing in which they were trained to swim to a hidden escape platform in a fixed location. For this task the curtain around the pool was gathered open and all extra maze room cues were visible. Rats were randomly allocated to one of four fixed platform locations 45 cm from the edge of the pool, in either the NE, NW, SE, and SW quadrant (Figure 1A). Each rat received four trials per day from the four compass points (N, S, E, and W) in a randomised sequence. This sequence was varied across days and between rats within any day of testing. For each trial the rat was gently lowered into the water facing the pool wall and then released, at which point the tracking software was initiated. A trial finished once the rat had located the platform or after 60 s had elapsed, at which time the rat was guided to the platform by the experimenter's hand; the rat remained on the platform for 15 s. Rats were tested in squads of 3–4, so that there was an intertrial interval of 3–4 min. Between trials, the rat was dried with a towel and kept in a cage under a heat lamp.

## 2.8 | Spatial working memory in the water maze

For this task a total of 12 platform positions varying in distance from the pool perimeter (35 or 55 cm) and 8 release points were used (Figure 1A).

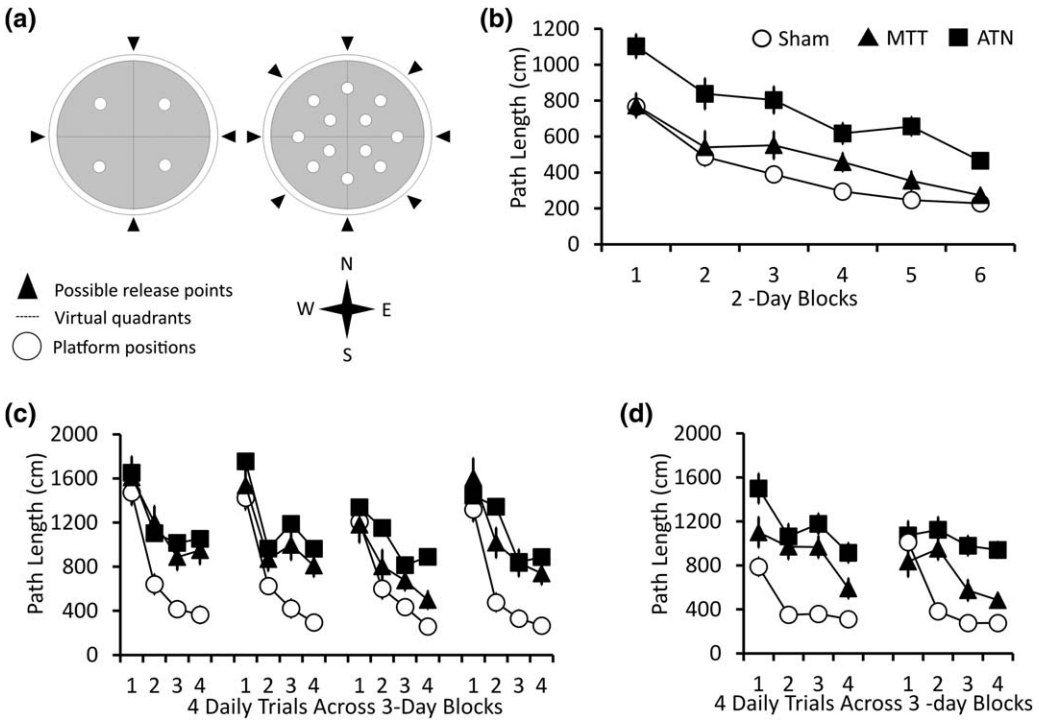


**FIGURE 1** (a) Schematic representation of possible release points and platform locations for the reference memory (left) and working memory (right) water maze tasks. For reference memory, one fixed platform position was used for any single rat, with different platform locations for different rats, and all four starting points were used daily. For working memory, one of the 12 platform positions and three of the eight starting locations was used on any given day. (b) Mean ± SEM path length to the hidden escape platform in the water maze over the 12 days of reference memory testing for the three groups. (c) Mean ± SEM path length to the hidden escape platform across the four-daily trials of working memory testing using the same testing conditions as the reference memory task. (d) Mean ± SEM path length to the hidden escape platform across the four-daily trials of working memory testing when spatial cues were minimized

The platform position remained constant within a session, but varied across the 12 daily sessions so the rats experienced a novel platform location each day. The curtain remained in the same gathered open position and all room cues were visible. Rats were randomly divided into two groups. Each group experienced the same release points and platform locations but in the reverse order; each rat within a group experienced the same four start points each day, but start points varied across days. For any given rat, the same release point was used for trials one and two, but then varied for trials three and four of the session to examine flexibility of the memory representation for the platform location. Each rat received massed trials in which a trial was terminated after it had reached the platform or 120 s had elapsed at which time it was guided to the platform by the experimenter; in either case, it remained there for 30 s, before being dried with a towel and promptly returned to the pool for its next trial resulting in an ITI of ~35 s.

## 2.9 | Spatial working memory in the water maze with minimized spatial cues

We then tested spatial working memory when extra-maze cues were minimized. The curtains were drawn closed around the pool and material was fixed on top of the curtain rail to form a roof over the pool and leave only a small hole for the tracking camera. One salient cue, consisting of four distinct objects suspended from a rectangular wooden frame, was hung from the curtain rail above the N release point. Auditory cues associated with the room computers remained. Rats were trained for 6 days in the same fashion as the standard working memory task.

## 2.10 | Radial arm maze tasks

Spatial working memory was tested using an 8-arm RAM located towards one corner of a large windowless room (4 m by 4.7 m). The maze was raised 67.5 cm off the floor and consisted of a 35 cm wide octagonal wooden hub painted black with 8 detachable aluminium arms (65 cm × 8.6 cm × 3.9 cm). Clear Perspex barriers (19 cm × 25 cm) extended along each arm from the central hub to deter rats from jumping across arms. Entry to each arm was controlled by clear Perspex doors (9 cm by 25 cm) which could be raised singly or together by an overhead pulley system. At the far end of each arm was a wooden block (5 cm × 9 cm × 3 cm) painted black with a recessed food well in the center (3 cm × 1 cm deep) where chocolate pieces (0.1 g) were placed. Inaccessible chocolate was also placed underneath the food wells to provide constant odor cues.

## 2.11 | Presurgery RAM testing

Prior to RAM habituation rats were food deprived to 85% of their free feed weight, over approximately 2 weeks. During this time each rat was handled for 5 min per day and habituated to the chocolate drops

(0.1 g) that served as the reward for this task. Once the rats approached the 85% target they were habituated to the RAM for 4 days, first in cage groups then singly. Rats then received 12 consecutive days of testing in the standard working memory task with the arms baited only once per session with two chocolate drops each. The rat was placed in the central hub and ~10 s later all eight arms were opened and the rat was allowed to make a choice (both back legs down an arm no back tracking). Once the rat entered an arm all the doors were closed while it ate the food reward if available. After a ~15 s delay the arm door was raised and the rat was allowed back into the central hub, where it was held for ~10 s before all doors were again opened and the rat made another arm selection. A trial concluded when the rat had visited all 8 arms, 20 arm choices had been made or 10 min had elapsed.

## 2.12 | Postsurgery RAM testing (following water maze tasks)

Four weeks after testing in the water maze, rats were re-habituated to the radial arm maze for 3 days with their cage-mates and then singly using a similar procedure to that used presurgery. On the next day, each rat was tested for 12 consecutive days the standard working memory task in the same manner as presurgery testing.

Following the standard task the rats completed 4 days of a mid-trial delay task and 4 days of a delay plus rotation task (order counterbalanced across rats). The initial four choices in both the delay and rotation task used an identical procedure to the standard working memory task. After the rat had made four correct choices it was gently removed from the maze and placed in a separate cage. Care was taken to minimize rotating the rat when removing it from the maze center. In the delay condition the rat was held in the separate cage until 60 s had elapsed at which point it was returned to the central hub in the same spatial orientation it had assumed prior to being removed. The rat was then allowed to carry on making arm choices as per the standard task, until all 8 arms had been visited, 20 arm choices had been made (including those prior to the delay), or 10 min had elapsed. The rotation condition was identical except that when the rat was removed to the separate cage the doors of the maze were disconnected from the overhead rigging and the entire maze was rotated clockwise by 45° so that each arm changed position by one place, for example, arm 4 was now where arm 5 had been prior to rotation. The food pellets in the four remaining arms were moved to retain their relative position in the environment, that is, the food reward in a different arm, but the same room location it occupied prior to rotation.

Following a 2 day break rats were returned to the radial arm maze using a slightly modified procedure. A novel configuration of five cues were hung on the curtain, which now surrounded half the maze, on the first 2 days of testing. These were replaced by a set of seven novel cues for the third day of testing prior to euthanasia. The RAM procedure was also modified to allow three massed trials per day, with a delay introduced after the first four choices per trial but now only four arm choices allowed after rotation, to equate the number of arms visited across all rats on each trial. Each session lasted about 14 min. The novel cue configuration was employed on the basis that novel cues normally stimulate IEG activity in the extended hippocampal system (Jenkins et al., 2002b; Vann & Albasser, 2009). The use of a mid-trial delay plus rotation, which places internal and environmental cues in conflict, was employed on the basis that this procedure has been claimed to be particularly relevant to retrosplenial cortex function within the hippocampal diencephalic circuit (Vann & Aggleton, 2002; Vann et al., 2003; Pothuizen et al., 2008). After the first and the second trials on each day, the rat was removed from the maze and placed in a separate cage for 2 min while the maze was reset. The separate cage contained a wooden food receptacle (4 cm × 5 cm × 1 cm, with a 1 cm diameter food well in the center) to provide a minimum of two chocolate pellets plus an additional two chocolate pellets if one or two arm choices were incorrect on the previous trial or four extra pellets and if three or four incorrect arm choices were made, thereby equating received across rats. After consuming any extra rewards after the third trial, the rat was moved to a quiet dark room for 90 min each day.

## 2.13 | Histology

At 90 min in the dark room after the final RAM trial rats were deeply anaesthetized with sodium pentobarbital (125 mg/kg) and perfused transcardially with ~200 mls of chilled saline followed by ~200 mls of 4% paraformaldehyde in a 0.1M phosphate buffer (PB) solution (pH 7.4). The brain was removed and postfixed in 4% paraformaldehyde solution overnight. Brains were then transferred into a long-term solution (20% glycerol, 0.1M PB, and 0.05% sodium azide) for a minimum of 48 h.

Sections were taken in the coronal plane (25 μm) from approximately +1.3 to −7.30 from bregma using a sliding microtome with a freezing stage (Thermofisher, UK). The coronal sections were collected in two separate series of six 2 mL cryovials containing a cryoprotectant solution (30% glycerol, 30% ethylene glycol, and 40% 0.1M PB). The first series captured the regions of interest for immunohistochemical evaluation. This series included consecutive sections from septal region to the anterior thalamus (approximately +1.6 to −0.80 from bregma) and sections posterior to the mammillary bodies to the presubiculum (approximately −5.60 to −7.80 from bregma). The second series captured regions for lesion verification and included sections immediately anterior to the ATN through to the posterior mammillary bodies (approximately −0.80 to −5.30 from bregma). Sections were stored at −20°C in the cryoprotectant until processed for immunohistochemistry or lesion verification.

To assess ATN lesions, sections were mounted on to gelatinized slides and stained with cresyl violet (Nissl stain). ATN lesion extent was replicated on electronic copies of the Paxinos and Watson atlas (1998; see Mitchell & Dalrymple-Alford, 2006). Automated pixel counts of the estimated damage relative to the relevant intact brain region were used to generate percent lesion volumes by factoring in the pixel areas multiplied by the distances provided in the atlas. Acceptable lesions were defined as having more than 50% bilateral damage to the ATN and minimal damage to the fornix and other adjacent thalamic regions, including the lateral thalamic region (LT, which included the

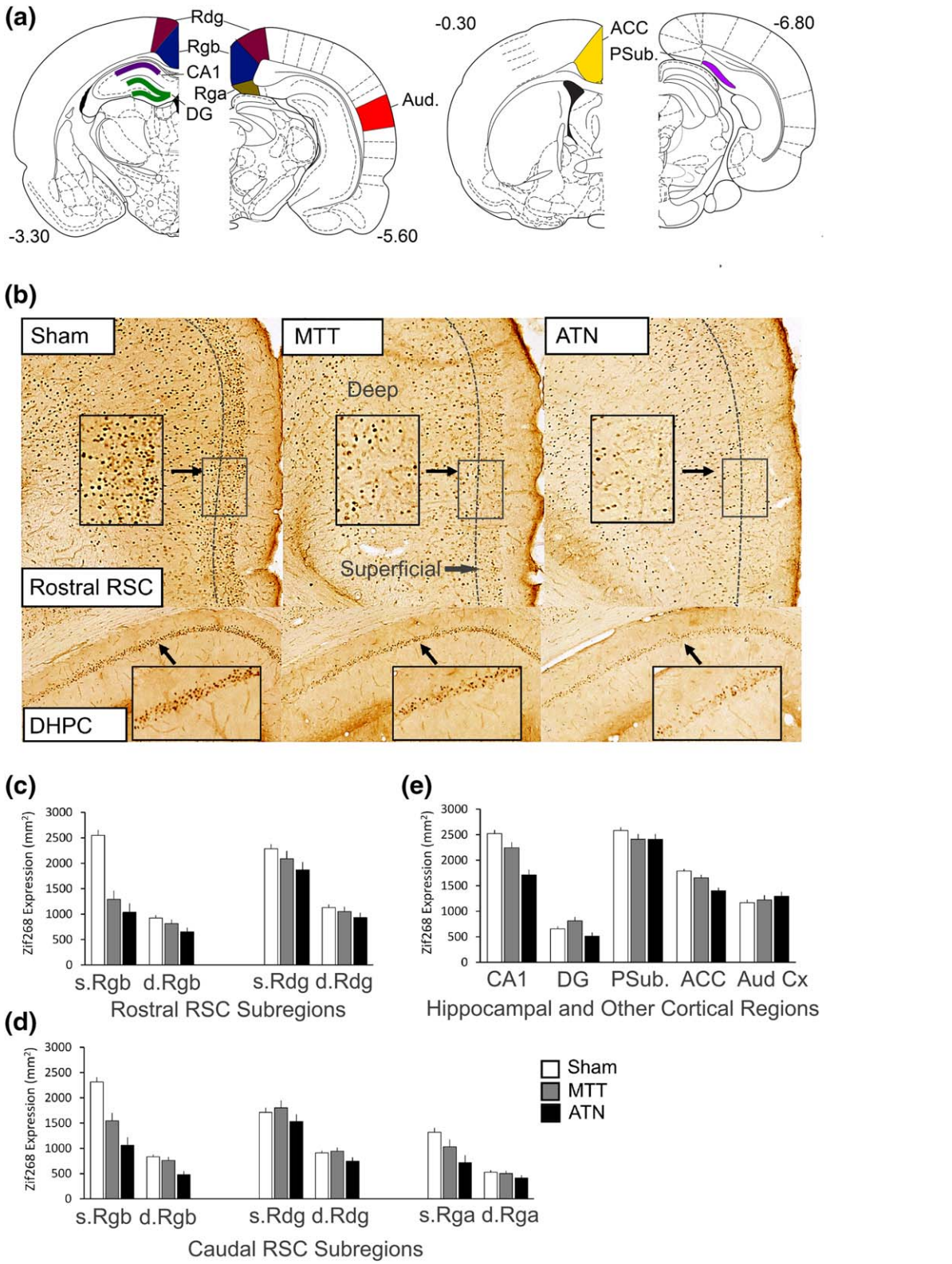


**FIGURE 2** (a) Coronal atlas plates from Paxinos and Watson (1998) indicating the relative location (from bregma) of the cortical and hippocampal regions of interest for zif268 counts (ACC = anterior cingulate cortex; Aud. = auditory cortex; CA1 = CA1 of the hippocampus; DG = dentate gyrus of the hippocampus; PSub = postsubiculum; Rgb = granular b retrosplenial cortex (left, rostral; right, caudal); Rdg = dysgranular retrosplenial cortex (left, rostral; right, caudal); Rga = granular a retrosplenial cortex). (b) Photomicrographs from a sham, MTT lesion and ATN lesion rat showing zif268 staining in the rostral retrosplenial cortex (RSC; primarily Rgb) and CA1 of the dorsal hippocampus (DHPC); inserts show enlarged regions of the superficial layers of the Rgb and CA1 regions. (c)–(e) Mean ± SEM zif268 positive cell counts in the hippocampal and cortical regions [(c) rostral subregions of the RSC; (d) caudal subregions of the RSC; (e) hippocampal and other cortical regions] [Color figure can be viewed at wileyonlinelibrary.com]

intralaminar and lateral mediodorsal nuclei) and posteromedial thalamic region (MT, which included the medial and central mediodorsal nuclei). The latter regions have also been implicated in diencephalic amnesia, although only ATN lesions of more than 50% damage size are consistently associated with severe spatial memory deficits (Bailey & Mair, 2005; Gibb et al., 2006; Mitchell & Dalrymple-Alford, 2006).

To assess MTT lesions, sections were mounted on to gelatinized slides and incubated overnight at 55 °C in a 95% ethanol solution containing 0.1% solvent blue (Luxol blue; Sigma, Castle Hill, NSW), a myelin specific stain. The slides were then counterstained with cresyl violet. For MTT lesions, only cases with more than 50% bilateral damage were included in further analysis.

## 2.14 | Zif268 immunohistochemistry

Endogenous peroxidase activity was blocked by washing the tissue sections with 3% hydrogen peroxide in 0.1M phosphate buffered saline containing 0.3% Triton X-100 (PBST) for 10 min. The tissue sections were then washed 4 × 5 min in PBST before nonspecific binding was blocked by incubating sections for 60 min with 10% normal goat serum (NGS; Life Technologies, NZ). Excess blocking solution was removed with 4 × 5 min washes in PBST before sections were incubated for 48 h at 4 °C with a rabbit polyclonal zif268 antibody (also known as Egr-1; 1:3000; Santa Cruz Bio) in PBST with 1:100 NGS added. Tissue was then rinsed in PBST for 4 × 5 min and then incubated in a biotinylated goat antirabbit secondary antibody (1:400; Vector) with 1:100 NGS, followed by avidin–biotin horseradish peroxidase complex in PBST (Vector elite kit). Sections were then rinsed for 4 × 10 min in 0.05 mol Tris buffer (pH 7.4) before immunostaining was visualized with diaminobenzidine (Sigma, Castle Hill, NSW) in 0.05M Tris buffer with 0.00013% hydrogen peroxide added just prior to incubation. Sections were then mounted on gelatinized slides allowed to dry and then run through graded alcohols before being cleared in xylene and coverslipped with DPX.

Zif268 sections were viewed on a Nikon E800 microscope 4× objective, and photographed using a Nikon DS-Fil camera. Automated counts of the stained cells were obtained using the public domain NIH image program (US National Institutes of Health at http://rsb.info.nih.gov/nih-image/). Cell counts were taken blind of group condition, but were not stereological and thus provide relative numbers of cells rather than absolute levels. Images were gray-scaled and the cell detection threshold was set automatically with the built in thresholding algorithms. Labeled nuclei in each region of interest (Figure 2A) were determined by counting those immunopositive cells that were above the detection threshold and between 5 and 20 μm in size. Between two and four sections per hemisphere were analyzed for each brain region. These counts were combined to give a mean result and expressed as cells per $mm^2$.

## 2.15 | NeuN immunohistochemistry

For NeuN staining, sections first received 4 × 5 min washes in PBST before nonspecific binding was blocked with 10% normal horse serum (NHS) in PBST for 1 h. Sections were then rinsed 4 × 5 min in PBST and then incubated for 24 h at 4 °C with a monoclonal mouse anti-NeuN antibody (1:1000, Millipore) in PBST with 1:100 NHS added. Following incubation, sections were washed 4 × 5 min in PBST and incubated in a fluorescent secondary antibody (antimouse dylight 549, Vector, 1:1000) in PBST with 1:100 NHS added, for 4 h in the dark. Finally, sections were rinsed 4 × 5 min in 0.1 mol PB before being mounted on gelatinized slides. The slides were then coverslipped with Fluoromount (Sigma, Castle Hill) and sealed with clear nail varnish. The slides were left overnight to dry and were then stored at 4°c in the dark until NeuN expression was visualized.

Images of NeuN expression in mammillary body subregions were taken from two section at two anterior–posterior coordinates, approximately −4.52 and −4.8 from bregma, and the nonstereological cell counts averaged per rat. NeuN expressing cells were excited with 549 nm light from an Olympus BX51 epifluorescence microscope. Cell counts were taken blind of group condition. Images were gray-scaled, and the cell detection threshold was set automatically. Counts of labeled nuclei in each of the lateral mammillary nucleus (LatMB), the medial aspect of the medial mammillary nucleus (MMB_M) and the lateral aspect of the medial mammillary nucleus (MMB_L) were determined separately by counting immunopositive cells that were above the detection threshold and between 5 and 20 μm in size. Total NeuN positive cell counts were averaged across hemispheres and the two AP coordinates to yield a single value for each MB subregion per rat.

## 2.16 | Data analysis

Statistica (v13; Dell Inc.) was used to conduct ANOVAs for behavioral data, zif268 counts in the cortex and hippocampus, and NeuN counts in the MB. Significant main effects and interactions were further examined with Newman–Keuls *post hoc* tests. The critical alpha value was set at $p < .05$. Counts for zif268 and NeuN immunostaining each region of interest were analyzed using separate ANOVAs for each region to avoid the confound of interpreting complex interactions across multiple sites.

# 3 | RESULTS

## 3.1 | Lesions

Nine of the 16 rats given MTT lesions met the criterion of at least 50% bilateral transection of the tract. The successful lesions were complete or near complete lesions, with a minimum size of 89–100% in five of the nine rats (Figure 3). No association between lesion size in the rats with acceptable MTT lesions and any behavioral effects were found (largest $r = .36$, $p$'s $> .4$). The excluded MTT lesions occurred in two rats that had complete unilateral MTT damage but insufficient damage to the contralateral MTT (100% on one side, but 45 and 30% on the contralateral side, respectively), two rats with partial bilateral damage (30 and 66.6% total damage, respectively) due to the focus of the lesion being too ventral or lateral, and two rats with complete bilateral sparing of the MTT. None of the MTT cases had evidence of any

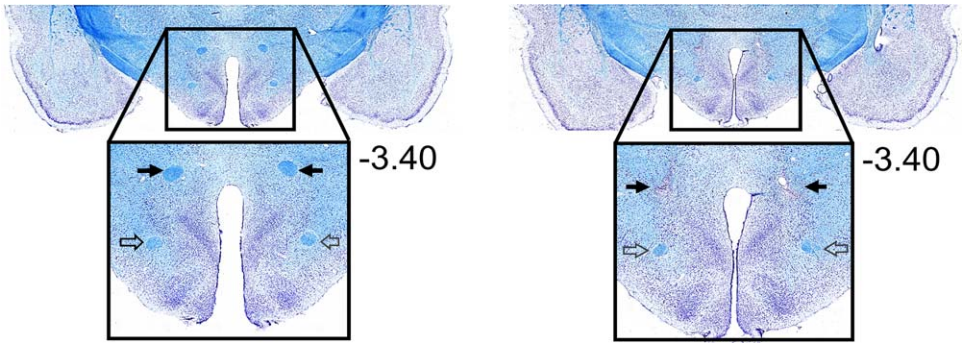


**FIGURE 3** Left, photomicrograph of a coronal section from a sham rat stained with luxol blue (myelin specific) and cresyl violet (Nissl) showing the mammillothalamic tract (black arrows, MTT) and the postcommissural fornix (open arrow, PFx); right, a rat with a bilateral MTT lesion (black arrows) [Color figure can be viewed at wileyonlinelibrary.com]

damage to the postcommissural fornix, the supramammillary nuclei, MB, or mammillotegmental tract. For the 13 rats in the ATN lesion group, 10 met the criterion of >50% bilateral lesions, and minimal damage to the fornix. One excluded ATN rat had substantial fornix damage and two ATN exclusions had lesions below the 50% criterion. The smallest (67.8%) and largest (99.1%) successful ATN lesions are shown in Figure 4 (median = 83%). These produced lesions in all three ATN subregions (AV range = 63–99%, median = 82%; AM range = 47–100%, median = 80%; and AD range = 68–100%, median = 91%). Total number of errors in the RAM was not significantly correlated with total ATN damage ($r = -.48$) due to some rats with smaller lesions having the highest errors. Damage to the MT region (range 0–24%) and the LT region (range 6.6–52%) was minimal and not correlated with total spatial working memory errors in the standard RAM (MT: $r = .2$, $p > .1$; LT: $r < .01$, $p > .5$). The included ATN rat that had 52% LT damage was ranked 5th in terms of errors made on the standard and delay-only versions of the RAM and was ranked 2nd in the delay plus rotation task in the RAM, consistent with prior research that LT lesions do not impair spatial working memory (Mitchell & Dalrymple-Alford, 2006). Note that, in rats with accepted lesions, the percent lesion size for the target ATN region was significantly smaller than the percent lesion size for the target MTT (Mann–Whitney $U = 8.0$, $Z = -3.04$, $p = .001$).

No differences on any behavioral task were found between the MTT-Sham lesion group ($n = 14$) and the ATN-Sham lesion group ($n = 11$) so these rats were combined as a single group for all analyses (Sham, $n = 25$).

## 3.2 | Spatial reference memory in the water maze

Analysis of path length to find the submerged platform revealed a significant group effect (lesion, $F(2,41) = 22.45$, $p < .001$; Figure 1B), which was due to poorer performance by the ATN group compared to both other groups ($p < .001$). The overall mean difference in path length between the MTT group and the Sham group did not reach significance ($p < .10$). Across the six 2-trial blocks, there was an overall reduction in path length [block main effect, $F(5,205) = 51.11$, $p < .001$], but the lesion × block interaction was not significant ($p > .4$).

## 3.3 | Spatial working memory in the water maze

When spatial working memory was tested over 12 days using the same room conditions as used for reference memory testing, a lesion effect was again evident ($F(2,41) = 25.32$, $p < .001$; Figure 1C). Unlike reference memory, however, both MTT and ATN groups showed significantly longer path lengths than the Sham group in the working memory task ($p < .001$ and $p < .001$), and the two lesion groups did not differ ($p > .1$). The typical working memory performance of reduced path length across trials within session was clear in the Sham group, but less evident in both lesion groups (lesion × trial, $F(6,123) = 4.02$, $p < .002$).

## 3.4 | Spatial working memory in the water maze with minimized spatial cues

When spatial working memory was subsequently tested for 6 days, but now with the visual room cues minimized, the Lesion main effect remained ($F(2,41) = 45.47$, $p < .001$, Figure 1D), with both lesion groups again impaired relative to the Sham group ($p < .001$, $p < .001$). However, the ATN group was now significantly worse than the MTT group ($p < .001$). Once more, the impairment in the lesion groups was primarily due to their poorer working memory across trials within session (lesion × trial, $F(6,123) = 4.11$, $p < .001$). The increased impairment in the ATN group relative to the MTT group was due primarily to differences in the second block of trials (lesion × block, $F(2,41) = 4.18$, $p < .05$; lesion × block × trials, $F(6,123) = 3.23$, $p < .005$), which appears primarily due to the MTT group showing shorter path lengths than the ATN group on the third and fourth trials for the second half of this test.

## 3.5 | Spatial working memory in the 8-arm radial arm maze (RAM)

The future lesion groups showed similar presurgery acquisition of the standard RAM task. By the last two-trial block of presurgery testing, all groups showed accurate performance with an average of less than one error in the standard RAM task [ATN, = 0.35 (0.52); MTT, = 0.58 (0.82); Sham, = 0.61 (0.58)]. There was no presurgery lesion effect ($F < 1$), a clear effect across the six presurgery blocks

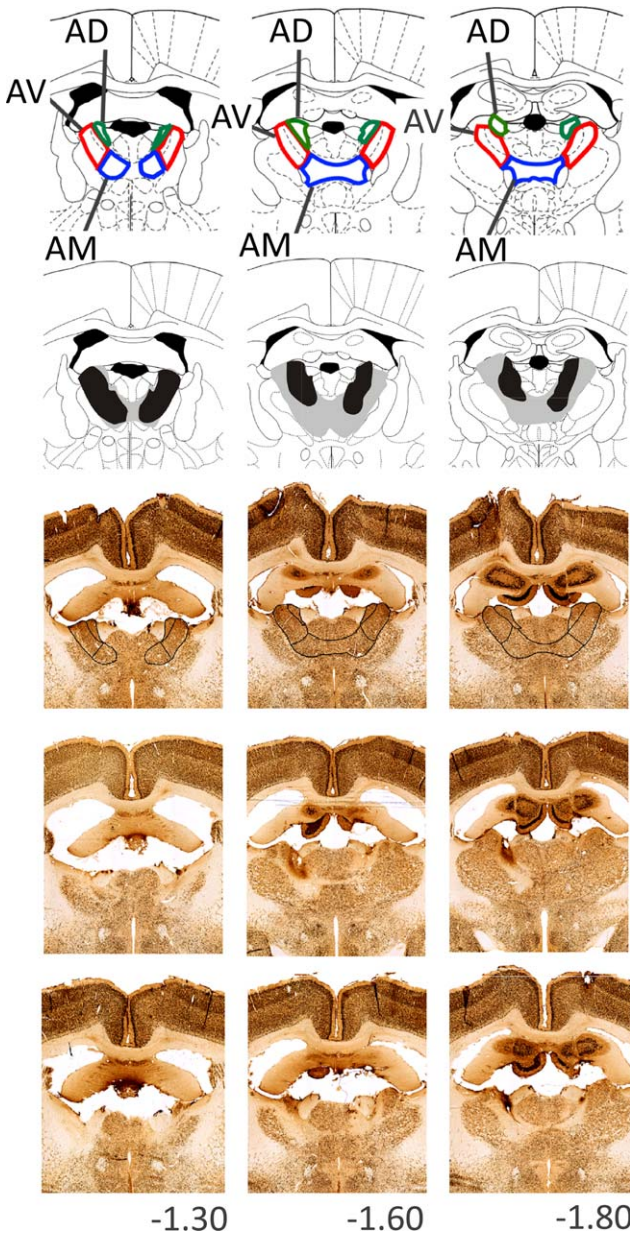


**FIGURE 4** From top to bottom: coronal atlas plates indicating the location of the anteroventral (AV), anteromedial (AM) and anterodorsal (AD) nuclei; smallest (black) and largest (gray) ATN lesions; NeuN stain of sections from a sham rat (third row) and examples of NeuN stain of sections from two rats with ATN lesions (bottom two rows). Compared to the sham, loss of ATN in both examples is clearer on the left side but tissue on the right side shows both collapse of tissue due to the lesion but some posterior dorsal AV intact [Color figure can be viewed at wileyonlinelibrary.com]

($F(5,205) = 23.41$, $p < .001$) reflecting task acquisition, and no lesion × block interaction ($F < 1$).

In contrast, postsurgery testing in the standard RAM task revealed a clear lesion effect ($F(2,41) = 57.93$, $p < .001$; Figure 5A), with the ATN group showing far higher errors than both the MTT group ($p < .001$) and the Sham group ($p < .001$). The MTT group showed a relatively milder but consistent impairment compared to the Sham group ($p < .001$). Both the MTT group and, especially, the Sham group showed improved performance across postsurgery testing, whereas the ATN group maintained poor performance across trial blocks (lesion × block, $F(10, 205) = 2.96$, $p < .01$).

When rats were tested using the 60 s delay after the first four choices and the delay plus rotation condition after the first four

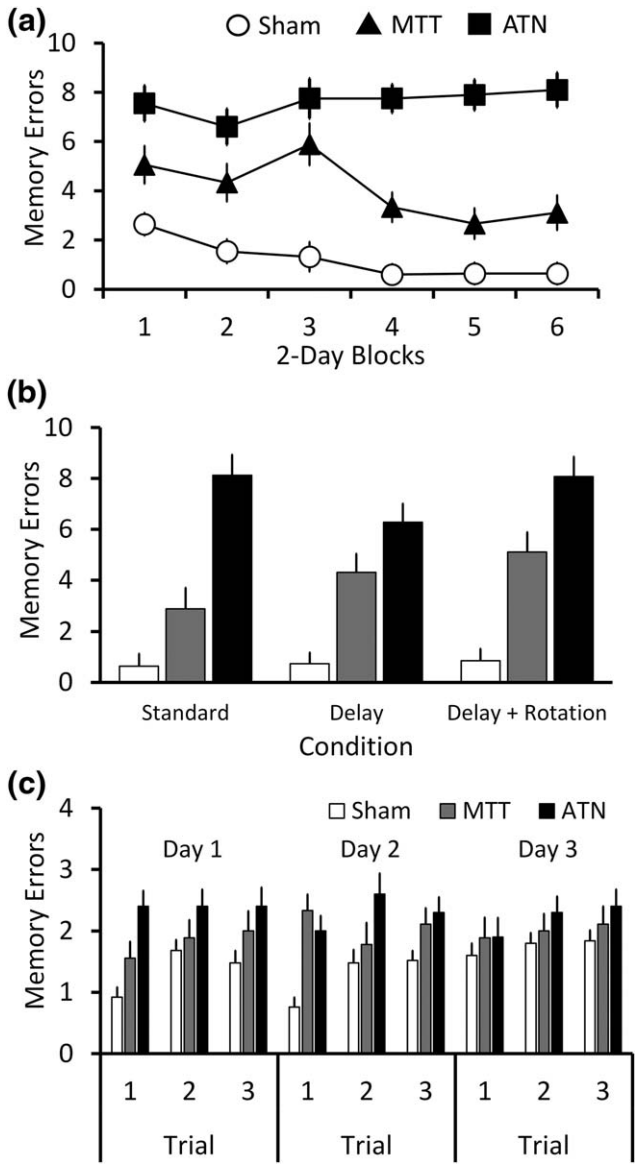


**FIGURE 5** Mean ± SEM spatial working memory errors in the RAM for the three groups. (a) The 12 days of postsurgery testing on the standard RAM task. (b) The last 4 days of the standard postlesion RAM task contrasted with testing on the 4 days of mid-trial delay and the 4 days of mid-trial delay plus rotation. (c) Performance on the three massed daily trials for the 3 days of the final mid-trial delay plus rotation RAM task. Unlike previous testing, only 8 arm entries were allowed for each trial. New spatial cues were used on the first day and repeated on the second day; another novel set of spatial cues was used on the last day, which finished 90 min prior to euthanasia for postmortem examination of zif268 expression

choices, worse performance was displayed by the MTT group compared to their performance on the previous four standard (no-delay) trials, whereas the ATN group appeared to show some benefit of having only a mid-trial delay (Figure 5B). The Sham group was unaffected by these new conditions. These observations were supported by significant Lesion ($F(2,41) = 106.65$, $p < .001$) and lesion × condition ($F(4,123) = 7.01$, $p < .001$) effects. The MTT group showed significantly increased errors for the delay-only trials ($p < .005$) and delay plus rotation trials ($p < .001$) compared to their standard trials, but not between the two nonstandard trials ($p < .10$). The ATN group showed significantly decreased errors for the delay-only condition compared to their standard trials ($p < .001$), but performance across the standard trials and the delay plus rotation trials did not differ in the ATN group ($p > .2$).

Changing the room cues and restricting the number of arm visits to a maximum of 8 per trial reduced the impairment shown by rats with lesions (Figure 5C). Overall, rats tended to make more errors after the first trial (trial main effect, $F(2,82) = 4.06$, $p < .05$). Lesion groups made more errors than the Sham group (lesion, $F(2,41) = 20.07$, $p < .001$; ATN group more errors than the Sham group, $p < .01$ and the MTT group, $p < .05$; MTT group more errors than the Sham group, $p < .01$).

### 3.6 | Zif268 in the retrosplenial cortex (RSC) and hippocampus

Of primary interest was expression in the RSC (Figure 2). Both MTT and ATN lesions resulted in substantially reduced zif268 expression in the superficial layer of the Rgb in both the rostral ($F(2,41) = 53.81$, $p < .001$) and caudal regions ($F(2,41) = 33.22$, $p < .001$). This effect did not differ significantly between the two lesion groups in the rostral superficial Rgb ($p > .1$), but was significantly worse in the ATN group in the caudal superficial Rgb ($p < .01$). However, only ATN lesions significantly reduced zif268 expression in the deep layers of the Rgb (rostral, $F(2,41) = 7.14$, $p < .005$; caudal, $F(2,41) = 14.54$, $p < .001$; ATN vs. Sham, $p < .004$; MTT vs. Sham, $p > .1$). The only other significant Lesion effects for the RSC were in the superficial layer of the rostral Rdg ($F(2,41) = 3.84$, $p < .05$) and the (caudally located) superficial Rga ($F(2,41) = 7.85$, $p < .005$), which in both cases reflected significant zif268 reductions in the ATN group only (ATN vs. Sham, $p < .05$). ATN lesions also reduced zif268 expression in the anterior cingulate ($F(2,41) = 8.01$, $p < .01$), both in relation to the Sham group ($p < .005$) and MTT group ($p < .05$); the MTT group did not differ significantly from Shams in the anterior cingulate ($p > .2$). No effect of lesion was found in the auditory cortex (control region; $p > .1$) or in the postsubiculum ($F(2,41) = 1.9$, $p > .1$). In the hippocampus itself, lesions resulted in differential effects in the dentate gyrus ($F(2,41) = 4.8$, $p < .05$), with the ATN group showing significantly less expression than the MTT group ($p < .005$), but neither lesion group differed from Sham ($p > .06$). There was also a significant lesion effect for the CA1 ($F(2,41) = 23.66$, $p < .001$) where ATN lesions reduced zif268 in the CA1 relative to both the MTT and Sham groups (both $p < .001$), with a weaker effect after MTT lesions (MTT vs. Sham, $p < .05$).

### 3.7 | NeuN expression in the MB

Both MTT and ATN groups produced substantially reduced NeuN positive cell counts in the medial part of the medial MB (lesion, $F(2,40) = 69.76$, $p < .001$), the medial lateral part of the medial MB (lesion, $F(2,40) = 137.90$, $p < .001$), and the lateral MB (lesion, $F(2,40) = 69.48$, $p < .001$; Figure 6). However, the MTT lesions produced a greater reduction than ATN lesions in all three subregions (MTT vs. ATN, $p < .001$).

## 4 | DISCUSSION

This study is the first to provide a direct comparison of the neural and behavioral effects of bilateral lesions to the MTT and the ATN, two key structures within the neuroanatomy of an extended hippocampal system (Aggleton, 2008; Aggleton & Brown, 1999). Neuropathology in both of these regions has been implicated as causal factors in clinically-defined dense amnesia, but their relative influence is uncertain (Danet et al., 2015). The strongest clinical evidence to implicate the ATN comes from patients with Korsakoff's syndrome, which resonates with the view that the ATN complex represents a pivotal structure within the extended brain network responsible for episodic recollection (Aggleton et al., 2010; Dalrymple-Alford et al., 2015; Harding et al., 2000). Korsakoff patients, however, invariably have additional MB and other thalamic pathology and often cortical neuropathology (Harding et al., 2000; Kopelman, 2014; Savage, Hall, & Vetreno, 2012). Injury to the MB and MTT is often associated with amnesia, but surgical injury to the MTT and blockade of MB function may not be sufficient to cause amnesia at least in epilepsy patients (Carlesimo et al., 2011; Van der Werf et al., 2000, 2003; Duprez et al., 2005; Dzieciol et al., 2017). Recent animal work, however, has shown that brainstem structures influence the memory system upstream via their impact on the MB, which suggests that damage to the MTT afferents to the ATN would produce comparable memory impairments to that found after ATN lesions (Dillingham et al., 2015; Vann, 2013; Vann & Nelson, 2015). This latter perspective highlights an association between clinical amnesia and MTT neuropathology.

Our direct comparison, however, showed that ATN lesions clearly resulted in a wider range of spatial memory deficits compared to those of MTT lesions. The greater behavioral deficits after ATN lesions than MTT lesions was not a function of the relative integrity of each lesions region itself, because MTT lesion size tended to be more complete than was the case for ATN lesions. Only ATN lesions impaired the acquisition of spatial reference memory in the Morris water maze. When a task revealed impairments after both lesions, there was often a more severe deficit after ATN lesions. ATN lesions produced more disrupted spatial working memory performance on the standard 8-arm RAM task than was found after MTT lesions, despite the latter lesions producing a greater loss of cells in the MB. The addition of both a delay after the first four arm entries and a mid-trial delay plus rotation condition increased RAM errors made by rats with MTT lesions, but their impairment remained less severe than that of the rats with ATN lesions. Compared to the standard RAM procedure, rats with ATN

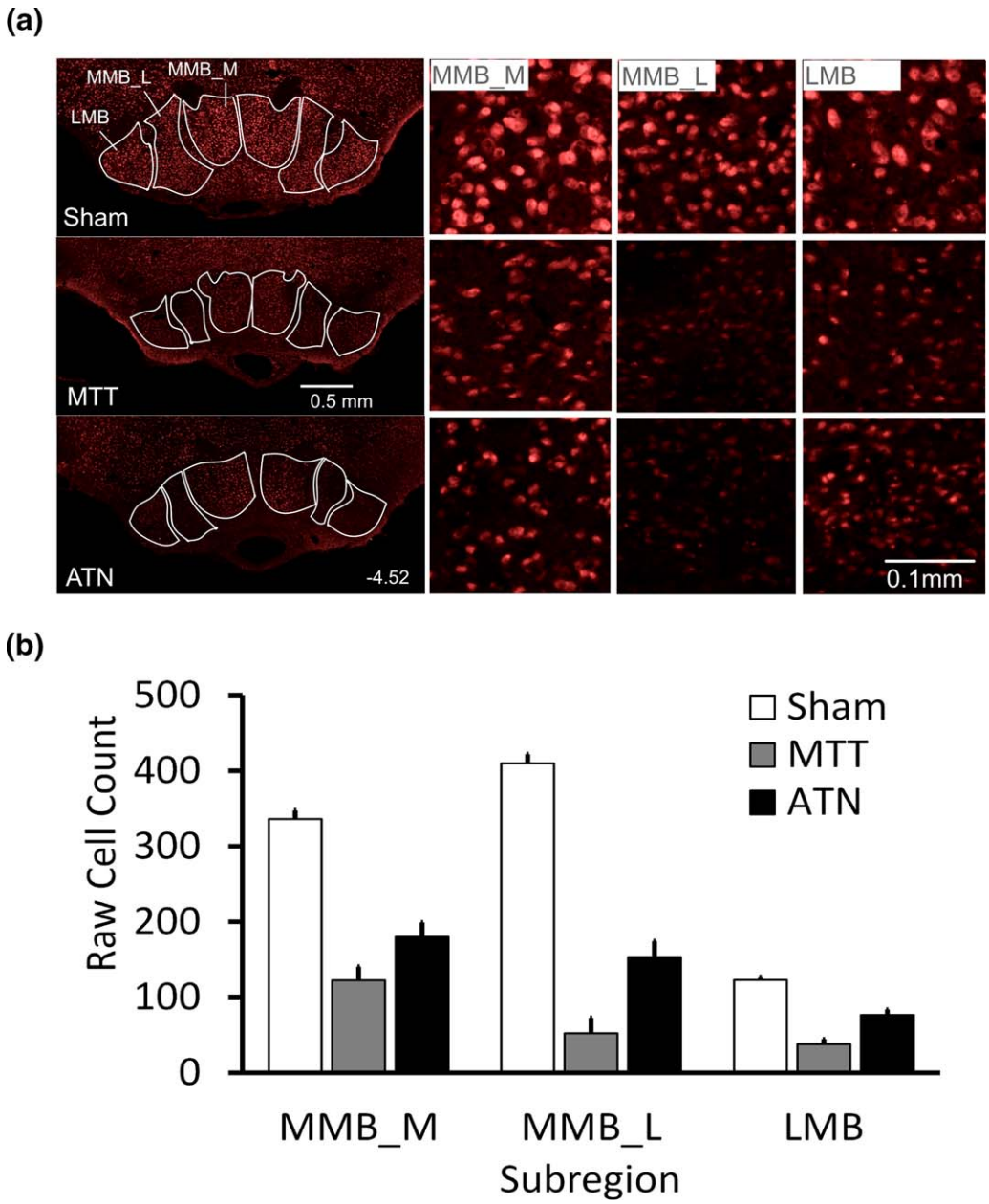


**FIGURE 6** (a) Example photomicrographs showing NeuN-positive flourostain in the two medial subregions and the lateral subregion of the mammillary bodies (MB) in a sham rat, a MTT-lesion rat and ATN-lesion rat; the three sets of panels on the right show magnified areas within each subregion. (b) Mean ± SEM raw NeuNpositive cell counts across the three subregions of the MB. MMB_M = medial mammillary bodies pars medialis; MMB_L = medial mammillary bodies pars lateralis; LMB = lateral mammillary bodies [Color figure can be viewed at wileyonlinelibrary.com]

lesions were if anything helped by the addition of the delay and not made any worse by the introduction of the delay plus rotation condition. While ATN lesions had a greater impact than did MTT lesions on working memory on both RAM and water maze tasks, the latter difference was only evident when the available room cues were reduced and then only with repeated testing when rats with MTT began to improve in their performance. Hence, reduction of the complexity of the available cues to a single salient cue may minimize sources of proactive interference in working memory that benefits rats with MTT lesions but not those with ATN lesions. Previous studies of rats with MTT lesions have also suggested their sensitivity to proactive interference in working memory tasks (Vann & Aggleton, 2003). Conversely, the addition of sources of interference in the RAM, by using a rotation among the arms during the mid-trial delay to place intramaze and extramaze cues in conflict, may have increased the working memory deficit in our MTT lesion group in line with previous reports on MTT lesions (Vann, 2013;Vann & Aggleton, 2003).

Poorer working memory performance in the RAM in the ATN group by comparison to the MTT group may arise because ATN lesions disrupt a greater number of neural circuits across the extended

hippocampal system and the impaired use of multiple strategies may exacerbate their deficit as has been suggested in the context of T-maze performance (Aggleton & Nelson, 2015; Bubb, Kinnavane, & Aggleton, 2017). The ability to learn a single location in the reference memory version of the water maze task appears selectively vulnerable to ATN lesions and not MTT lesions, so the two structures must have different consequences for different aspects of spatial memory. It is possible that MTT rats are better able to use directional cues to master a spatial problem when the target location remains stable and when the problem does not require updating spatial cues in working memory unless these cues, and sources of interference, are minimized. The effects of MTT lesions can only result from the loss of MB input to the ATN and the consequences of such a disconnection, such as downstream effects on the extended memory system. It seems likely that disruption to many pathways between the ATN, prefrontal cortex and hippocampus is less strongly influenced by MTT lesion effects on the ATN than by direct ATN lesions, and thus explains the greater and wider impact of ATN lesions on spatial memory tasks.

The current study also showed that ATN and MTT lesions both affect IEG expression in limbic cortex, but their effects were not always equivalent. Both MTT and ATN lesions resulted in an equally striking loss of zif268 expression in the superficial layers of the Rgb in our study, which replicates previous findings with zif268 after these lesions, whether this histology follows activity in a novel cage (Poirier & Aggleton, 2009) or follows a RAM task in a novel room (Dumont et al., 2012; Frizzati et al., 2016). In the last two of these studies both zif268 and c-Fos, assessed in the same rats, were reduced by about 70% in the superficial Rgb after both MTT (Frizzati et al., 2016; Vann & Albasser, 2009) or ATN lesions (Poirier & Aggleton, 2009). Other ATN lesion studies, however, suggest that in the rostral RSC there can be minimal reduction of zif268 following the RAM but about 90% reduction of c-Fos following contextual fear conditioning, so we do not know whether these differences relate to the IEG marker or the specific task (Dumont et al., 2012; Dupire et al., 2013). An inconsistent pattern of IEG findings also exists for the prefrontal cortex and hippocampus, irrespective of task used (Dumont et al., 2012; Dupire et al., 2013; Jenkins et al., 2002b; Loukavenko et al., 2016; Vann & Albasser, 2009). For example, ATN lesions reduced c-Fos expression in the hippocampus compared to sham rats when tested in the RAM in a novel room but not in a familiar room (Jenkins et al., 2002b), yet this effect after ATN lesions was not found when zif268 expression was examined (Dumont et al., 2012). Such discrepancies highlight the value of the direct comparison of ATN and MTT lesion effects within the same study. We showed that the impact of ATN lesions on zif268 expression after RAM testing is more diverse than that of MTT lesions, at least in the procedures we used here. Without a control task for IEG expression, we cannot be certain that the differential effects we found were due to our specific task immediately prior to euthanasia. However, it was clear that ATN lesions, but not MTT lesions, reduced zif268 expression in the deep Rgb, the superficial Rdg and the superficial Rga subregions of RSC, as well as the anterior cingulate cortex. ATN lesions also had a greater impact zif268 expression in hippocampal CA1. This broader impact of ATN injury on zif268 expression may reflect the direct interconnections between the ATN and the extended hippocampal system. Nonetheless, substantially reduced IEG expression in the superficial layers of the Rgb after MTT lesions supports the view that the MB, which do not have direct afferent connections with distal corticolimbic structures, also have a critical influence on regions upstream to the ATN (Dillingham et al., 2015; Vann & Nelson, 2015).

The relative importance of MB pathology on memory and corticolimbic zif268 expression can be discerned from the impact of MTT and ATN lesions on neuron-positive (NeuN) cell counts in the MB. The influence of ATN lesions on MB cell counts has not been reported previously, but MTT lesions have been shown to reduce MB volume (Vann, 2013; Vann & Aggleton, 2003). There is prior evidence that MTT lesions deafferent the anteroventral and anteromedial nuclei but leave the anterodorsal nuclei afferents from the MB relatively intact (Vann & Albasser, 2009), so we anticipated that large MTT lesions would have less total influence on the MB, and thereby the MB-ATN axis, than would direct ATN lesions that include all three subcomponents of the ATN complex. In fact, we found the reverse. ATN lesions did cause cell loss across all three subregions of the MB complex, but MTT lesions caused greater cell loss in all three of these subregions. Hence, the greater impact of ATN lesions on behavior and zif268 in the upstream corticolimbic system does not co-vary with the relative influence or extent of MB neuropathology or the fact that the MTT lesions were in effect complete whereas the ATN lesions were relatively incomplete. Interestingly, proportional cell loss was greater in the lateral part of the medial MB, which projects primarily to the anteroventral nucleus, after both MTT and ATN lesions. While the behavioral significance of depleted MB neurons in the lateral part of the medial MB is not known, it is possible that neuronal loss here preferentially disrupts theta in the ATN (Dillingham et al., 2015; Jankowski et al., 2013; Kirk & Mackay, 2003).

Previously, only separate studies have been available to estimate the comparative effects of MTT and ATN lesions on memory tasks. The similar effects of both lesion types have been emphasized, in that neither lesion impairs novel object discrimination (Moran & Dalrymple-Alford, 2003; Nelson & Vann, 2014; Warburton & Aggleton, 1999), but both lesions impair temporal memory for a sequence of items (Dumont & Aggleton, 2013; Nelson & Vann, 2016; Wolff et al., 2008a,b) and both lesions impair spatial working memory in radial-arm maze tasks (Aggleton, Hunt, Nagle, & Neave, 1996; Harland, Collings, McNaughton, Abraham, & Dalrymple-Alford, 2014; Mitchell & Dalrymple-Alford, 2006; Nelson & Vann, 2014; Sziklas & Petrides, 1999; Vann, 2013; Vann & Aggleton, 2003). The greater severity of spatial working memory deficits after ATN lesions reported here is reminiscent of the T-maze alternation deficits found previously across separate ATN or MTT lesion studies. That is, rats with ATN lesions generally produce severe impairments in T-maze tasks, whereas MTT lesions often produce mild and sometimes transient deficits unless the availability of intramaze cues is minimized (Aggleton & Nelson, 2015; Vann & Aggleton, 2003; Vann, 2013). On the basis of the current study, it seems unlikely that weaker impairments after MTT lesions found in previous work were due to subtle procedural variations across studies.

The clear difference found between the effects of ATN and MTT lesions on spatial reference memory in the water maze, together with more similar effects in terms of their impact on spatial working memory in the same apparatus, conflicts with the only previous report to examine the effects of MTT lesions on both measures (Winter et al., 2011). The spatial reference memory deficit in that study was at best mild and absent by the final 2 of 5 days of testing. Their failure to find any deficit on spatial working memory after MTT lesions may be due to their use of only two daily trials, and their variation of the platform at a constant distance relative to the pool wall may have encouraged nonspatial solutions in their task. As with the previous report of impaired spatial working memory in the water maze after MTT lesions (Vann & Aggleton, 2003), we used four daily trials, and the difference with sham rats was more apparent with four trials per day, and the distance between the platform and wall was varied across sessions. The current study replicates a single previous report that spatial working memory in the water maze is impaired by ATN lesions (Van Groen, Kadish, & Wyss, 2002). Unlike MTT lesions, ATN lesions consistently result in spatial reference memory deficits in the water maze, which are often reminiscent of the effects of hippocampal lesions (Moreau et al., 2013; Warburton & Aggleton, 1999; Warburton, Baird, Muir, & Aggleton, 2001; Warburton et al., 1999; Wolff et al., 2008a,b).

Differences between the spatial memory effects of ATN lesions and MTT lesions have been reported using other tasks. Rats with MTT lesions are able to discriminate the fixed location in a geometric learning task at the same rate as controls whereas rats with ATN lesions remain at chance levels (Aggleton et al, 2009; Dumont et al., 2014; Vann, 2013). Short-term recognition of object-place memory is impaired by both lesions but long-term memory of an object-place association is, unlike ATN lesions, completely unimpaired by MB lesions (Sziklas, Petrides, & Leri, 1996), and thus probably also unimpaired by MTT lesions. Therefore, together with other lesion studies, albeit separate comparisons of ATN and MTT lesions, the current findings reinforce the view that the ATN constitute a pivotal site within a complex array of interdependent structures now recognized as significant brain regions associated with episodic memory (Aggleton, 2008; Aggleton et al., 2010; Aggleton & Nelson, 2015; Dalrymple-Alford et al., 2015; Dillingham et al., 2015; Jankowski et al., 2013; Vann, 2013; Vann & Nelson, 2015).

It seems likely that the key reason for greater, and more extensive, memory deficits associated with ATN lesions than with MTT lesions is that the ATN is a complex structure with a diverse and extensive set of neural connections. It is known that lesions of the postcommissural fornix projection to the MB fail to replicate the effects of MTT lesions (Vann, 2013; Vann, Erichsen, O'mara, & Aggleton, 2011). Together with the current findings, we consider it possible that direct and indirect connections between the ATN, the subicular cortex of the hippocampal formation, prefrontal cortex and retrosplenial cortex are responsible for the impairments associated with ATN lesions that extend beyond the influence of the unique, unidirectional ATN pathway provided by the MTT (Aggleton & Nelson, 2015; Bubb et al., 2017). It seems also unlikely that any one of these other multiple connections alone can be primarily responsible for all the effects of large ATN neuropathology. For example, an extensive study on the impact of fornix lesions on spatial learning tasks sensitive to ATN-hippocampal processes concluded that fornix pathways may contribute to tasks requiring flexible spatial and temporal cues, but that nonfornix pathways must contribute to spatial memory when tasks require more stable spatial solutions (Dumont, Amin, Wright, Dillingham, & Aggleton, 2015). Based on neuroanatomical and electrophysiological evidence, strong arguments can be made that the components of the MB-MTT-ATN axis work together to support episodic memory and facilitate the acquisition of stable spatial solutions (Aggleton et al., 2010; Aggleton & Nelson, 2015; Dillingham et al., 2015). The problem for a focus on that axis to explain our current findings is that more severe neuropathology across all three components of the MB was produced by MTT lesions than was produced by ATN lesions, yet spatial reference memory was unaffected by MTT lesions. That is, a simple account of additive separate deficits across the different components of the MB-MTT-ATN axis, and perhaps for that matter after other ATN disconnections, may not be sufficient to account for memory impairments after diencephalic injury. A proposal is therefore needed to explain the behavioral impact of ATN lesions and their relative importance to memory.

Many features beyond allocentric cues alone can factor in different spatial memory tasks. Although the use of allocentric cues may sit at the pinnacle of a hierarchy of strategies that rats use to solve spatial memory tasks, some tasks may also be influenced by moderating factors such as proactive interference and cognitive flexibility or be more susceptible to changes in alternate neural pathways that may compensate or exacerbate performance in any given spatial memory task (Dalrymple-Alford et al., 2015). Particularly in view of the impact of MTT and ATN lesions on MB neuropathology, we conclude that our study lends support to Aggleton and Nelson's (2015) proposal that ATN lesions impact a variety of cognitive skills and give rise to a hierarchy of deficits that explain the diverse pattern and severity of deficits produced by injury to this region. The same hierarchy is not necessarily associated with the MTT.

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## REFERENCES

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

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). https://doi.org/10.1098/rspb.2014.0565.

Aggleton, J. P., & Brown, M. W. (1999). Episodic memory, amnesia, and the hippocampal-anterior thalamic axis. *Behavioural Brain Science*, *22*, 425–444.

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., & Nelson, A. J. (2015). Why do lesions in the rodent anterior thalamic nuclei cause such severe spatial deficits? *Neuroscience & Biobehavioral Reviews*, *54*, 131–144.

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*, 2292–2307.

Aggleton, J. P., Poirer, G. L., Aggleton, H. S., Vann, S. D., & Pearce, J. M. (2009). Lesions of the fornix and anterior thalamic nuclei dissociate different aspects of hippocampal-dependent spatial learning: Implications for the neural basis of scene learning. *Behavioural Neuroscience*, *123*, 504–519.

Aggleton, J. P., Pralus, A., Nelson, A. J., & Hornberger, M. (2016). Thalamic pathology and memory loss in early Alzheimer's disease: Moving the focus from the medial temporal lobe to Papez circuit. *Brain*, aww083.

Bailey, K. R., & Mair, R. G. (2005). Lesions of specific and nonspecific thalamic nuclei affect prefrontal cortex-dependent aspects of spatial working memory. *Behavioral neuroscience*, *119*(2), 410.

Bubb, E. J., Kinnavane, L., & Aggleton, J. P. (2017). Hippocampal–diencephalic–cingulate networks for memory and emotion: An anatomical guide. *Brain & Neuroscience Advances*, *1*, 1–20.

Carlesimo, G. A., Lombardi, M. G., & Caltagirone, C. (2011). Vascular thalamic amnesia: Are appraisal. *Neuropsychologia*, *49*, 777–789.

Carlesimo, G. A., Lombardi, M. G., Caltagirone, C., & Barban, F. (2015). Recollection and familiarity in the human thalamus. *Neuroscience & Biobehavioral Reviews*, *54*, 18–28.

Child, N. D., & Benarroch, E. E. (2013). Anterior nucleus of the thalamus functional organization and clinical implications. *Neurology*, *81*(21), 1869–1876.

Dalrymple-Alford, J. C., Harland, B., Loukavenko, E. A., Perry, B., Mercer, S., Collings, D. A., ... Wolff, M. (2015). Anterior thalamic nuclei lesions and recovery of function: Relevance to cognitive thalamus. *Neuroscience & Biobehavioral Reviews*, *54*, 145–160.

Danet, L., Barbeau, E. J., Eustache, P., Planton, M., Raposo, N., Sibon, I., ... Pariente, J. (2015). Thalamic amnesia after infarct The role of the mammillothalamic tract and mediodorsal nucleus. *Neurology*, *85*(24), 2107–2115.

Dillingham, C. M., Frizzati, A., Nelson, A. J., & Vann, S. D. (2015). How do mammillary body inputs contribute to anterior thalamic function? *Neuroscience & Biobehavioral Reviews*, *54*, 108–119.

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. https://doi.org/10.1016/j.neuroscience.2012.08.027

Dumont, J. R., & Aggleton, J. P. (2013). Dissociation of recognition and recency memory judgements after anterior thalamic nuclei lesions in rats. *Behavioural Neuroscience*, *127*, 415–431.

Dumont, J. R., Amin, E., & Aggleton, J. P. (2014). Selective importance of the rat anteriorthalamic nuclei for configural learning involving distal cues. *European Journal of Neuroscience*, *39*, 241–256.

Dumont, J. R., Amin, E., Wright, N. F., Dillingham, C. M., & Aggleton, J. P. (2015). The impact of fornix lesions in rats on spatial learning tasks sensitive to anterior thalamic and hippocampal damage. *Behavioral Brain Research*, *278*, 360–374.

Dupire, A., Kant, P., Mons, N., Marchand, A. R., Coutureau, E., Dalrymple-Alford, J., & Wolff, M. (2013). A role for the anterior thalamic nuclei in affective cognition: Interactions with environmental conditions. *Hippocampus*, *23*, 392–404.

Duprez, T. P., Serieh, B. A., & Raftopoulos, C. (2005). Absence of memory dysfunction after bilateral mammillary body and mammillothalamic tract electrode implantation: Preliminary experience in three patients. *American Journal of Neuroradiology*, *26*(1), 195–198.

Dzieciol, A. M., Bachevalier, J., Saleem, K. S., Gadian, D. G., Saunders, R., Chong, W. K., ... & Vargha-Khadem, F. (2017). Hippocampal and diencephalic pathology in developmental amnesia. *Cortex*, *86*, 33–44.

Farina, F. R., & Commins, S. (2016). Differential expression of immediate early genes Zif268 and c-Fos in the hippocampus and prefrontal cortex following spatial learning and glutamate receptor antagonism. *Behavioral Brain Research*, *307*, 194–198.

Frizzati, A., Milczarek, M. M., Sengpiel, F., Thomas, K. L., Dillingham, C. M., & Vann, S. D. (2016). Comparable reduction in Zif268 levels and cytochrome oxidase activity in the retrosplenial cortex following mammillothalamic tract lesions. *Neuroscience*, *330*, 39–49.

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.

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

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*, *24*(10), 1232–1247.

Jankowski, M. M., Ronnqvist, K. C., Tsanov, M., Vann, S. D., Wright, N. F., Erichsen, J. T., ... O'mara, S. M. (2013). The anterior thalamus provides a subcortical circuit supporting memory and spatial navigation. *Frontiers in Systemic Neuroscience*, *7*, 45.

Jenkins, T. A., Dias, R., Amin, E., & Aggleton, J. P. (2002a). 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. (2002b). Fos imaging reveals that lesions of the anterior thalamic nuclei produce widespread limbic hypoactivity in rats. *Neuroscience*, *22*(12), 5230–5238.

Jones, M. W., Errington, M. L., French, P. J., Fine, A., Bliss, T. V. P., Garel, S., ... Davis, S. (2001). A requirement for the immediate early gene Zif268 in the expression of late LTP and long-term memories. *Nature Neuroscience*, *4*(3), 289–296.

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.

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

Loukavenko, E. A., Wolff, M., Poirier, G. L., & Dalrymple-Alford, J. C. (2016). Impaired spatial working memory after anterior thalamic lesions: Recovery with cerebrolysin and enrichment. *Brain Structure & Function*, *221*(4), 1955–1970.

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. *Behavioral Brain Research*, *250*, 264–273. https://doi.org/10.1016/j.bbr.2013.04.052

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

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.

Moreau, P. H., Tsenkina, Y., Lecourtier, L., Lopez, J., Cosquer, B., Wolff, M., ... 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 & Function*, *218*(3), 657–667.

Nelson, A. J., & Vann, S. D. (2014). Mammilliothalamic tract lesions disrupt tests of visuo-spatial memory. *Behavioral Neuroscience*, *128*(4), 494.

Nelson, A. J., & Vann, S. D. (2016). The importance of mammillary body efferents for recency memory: Towards a better understanding of diencephalic amnesia. *Brain Structure & Function*, *222*(5), 2143–2156.

Paxinos, G., & Watson, C. (1998). *The rat atlas in stereotaxic coordinates*. New York: Academic.

Penke, Z., Morice, E., Veyrac, A., Gros, A., Chagneau, C., Le Blanc, P., ... Laroche, S. (2014). Zif268/Egr1 gain of function facilitates hippocampal synaptic plasticity and long-term spatial recognition memory. *Philosophical Transactions of Royal Society B*, *369*(1633), 20130159.

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.

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.

Savage, L. M., Hall, J. M., & Vetreno, R. P. (2011). Anterior thalamic lesions alter both hippocampal dependent behavior and hippocampal acetylcholine release in the rat. *Learning & Memory*, *18*, 751–758.

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., & Leri, F. (1996). The effects of lesions to the mammillary region and the hippocampus on conditional associative learning by rats. *European Journal of Neuroscience*, *8*, 106–115. https://doi.org/10.1111/j.1460-9568.1996.tb01171.x

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 vs. recognition memory. *Nature Neuroscience*, *11*, 834–842.

Van der Werf, Y. D., Scheltens, P., Lindeboom, J., Witter, M. P., Uylings, H. B., & 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.

Van der Werf, Y. D., Witter, M. P., Uylings, H. B., & Jolles, J. (2000). Neuropsychology of infarctions in the thalamus: A review. *Neuropsychologia*, *38*, 613–627.

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

Vann, S. D. (2013). Dismantling the Papez circuit for memory in rats. *Elife*, *2*, e00736.

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. (2003). Evidence of a spatial encoding deficit in rats with lesions of the mammillary bodies or mammillothalamic tract. *Journal of Neuroscience*, *23*, 3506–3514.

Vann, S. D., & Albasser, M. M. (2009). Hippocampal, retrosplenial, and prefronatal hypoactivity in a model of diencephalic amnesia: Evidence towards an interdependent subcortical–cortical memory network. *Hippocampus*, *19*, 1090–1102.

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*, 945–957.

Vann, S. D., & Nelson, A. J. (2015). The mammillary bodies and memory: More than a hippocampal relay. *Progress in Brain Research*, *219*, 163–185.

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

Warburton, E. C., & Aggleton, J. P. (1999). Differential effects 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., 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. *Journal of Neuroscience*, *21*, 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? *Behavioural Neuroscience*, *113*, 956–967.

Winter, S. S., Wagner, S. J., McMillin, J. L., & Wallace, D. G. (2011). Mammillothalamic tract lesions disrupt dead reckoning in the rat. *European Journal of Neuroscience*, *33*, 371–381.

Wolff, M., Gibb, S. J., Cassel, J. C., & Dalrymple-Alford, J. C. (2008a). Anterior but not intralaminar thalamic nuclei support allocentric spatial memory. *Neurobiology of Learning & Memory*, *90*(1), 71–80.

Wolff, M., Loukavenko, E. A., Will, B. E., & Dalrymple-Alford, J. C. (2008b). 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. https://doi.org/10.1002/hipo.20457

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