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ORIGINAL ARTICLE

# Lesions of the anterior thalamic nuclei and intralaminar thalamic nuclei: place and visual discrimination learning in the water maze

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**Abstract** Medial thalamic damage produces memory deficits in humans (e.g., Korsakoff's syndrome) and experimental animals. Both the anterior thalamic nuclei (ATN) and rostral intralaminar plus adjacent lateral thalamic nuclei (ILN/LT) have been implicated. Based on the differences in their main connections with other neural structures, we tested the prediction that ATN lesions would selectively impair acquisition of spatial location discrimination, reflecting a hippocampal system deficit, whereas ILN/LT lesions would impair acquisition of visual pattern discrimination, reflecting a striatal system deficit. Half the rats were first trained in a spatial task in a water maze before switching to a visual task in the same maze, while the remainder were tested with the reverse order of tasks. Compared with sham-operated controls, (1) rats with ATN lesions showed impaired place learning, but normal visual discrimination learning, (2) rats with ILN/LT lesions showed no deficit on either task. Rats with ATN lesions were also hyperactive when their home cage was placed in a novel room and remained more active than ILN/LT or SHAM rats for the subsequent 21 h, especially during the nocturnal phase. These findings confirmed the influence of ATN lesions on spatial learning, but failed to support the view that ILN/LT lesions disrupt striatal-dependent memory.

**Keywords** Anterior thalamic nuclei · Hippocampus · Intralaminar thalamic nuclei · Spatial memory · Striatum · Visual pattern memory · Water maze

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## Introduction

Injury to the medial thalamus is a major cause of severe memory dysfunction in humans (Kopelman 2002; van der Werf et al. 2003). The specific thalamic substrates of this diencephalic amnesia are uncertain, with some clinical studies attributing memory deficits in a non-specific manner to many medial thalamic nuclei (Gold and Squire 2006), others that focus on particular thalamic nuclei (Harding et al. 2000), and some on the possibility that separate nuclei primarily influence different aspects of cognition beyond memory (van der Werf et al. 2000; Jodar et al. 2011). Variability in lesion locus and close proximity of the thalamic nuclei and their related pathways permit only tentative conclusions from the clinical evidence. Experimental animal lesion studies to address this issue,

however, have also generated conflicting views (Aggleton et al. 2011; Mair et al. 1998; Bailey and Mair 2005; Savage et al. 1998). The anterior thalamic nuclei (ATN) and the rostral intralaminar thalamic nuclei plus the adjacent lateral thalamic region (ILN/LT) have been most frequently emphasised, but evidence for their relative influence on memory after injury is mixed, with many (Lopez et al. 2009; Mair et al. 2003; Mitchell and Dalrymple-Alford 2005; Mitchell and Dalrymple-Alford 2006; Wolff et al. 2008a) but not all studies (Gibb et al. 2006; Savage et al. 1998) suggesting dissociations. The need to clarify the relative role of these thalamic nuclei is emphasised by recent evidence that thiamine deficiency in the rat, a model for the Wernicke–Korsakoff syndrome, depletes neurones in both of these thalamic regions (Anzalone et al. 2010).

It is possible that ATN and ILN/LT nuclei each influence different memory processes and that impairments associated with their injury vary as a function of the memory task (Lopez et al. 2009; Mitchell and Dalrymple-Alford 2005, 2006; Wolff et al. 2008a). One promising perspective is that the influence of each thalamic region reflects its involvement in different neural systems and, by implication, an involvement in different memory systems in the brain. An influential view in the literature is that the ATN are an important part of an extended hippocampal memory system (Aggleton 2008; Aggleton and Brown 1999; Vann and Albasser 2009). There is ample evidence that selective ATN lesions often impair performance on many, although not all, hippocampal-dependent memory tasks (Aggleton et al. 2009, 2011; Byatt and Dalrymple-Alford 1996; Mair et al. 2003; Moran and Dalrymple-Alford 2003; Sziklas and Petrides 1999, 2007; Warburton et al. 2001; Wolff et al. 2006, 2008b). In contrast, the rostral ILN/LT region has prominent connections with the dorsal striatum (Berendse and Groenewegen 1991; van der Werf et al. 2002), so that this thalamic region may influence memory processes that depend on the functional integrity of the dorsal striatum and its network interactions with other brain structures, especially the prefrontal cortex (Mair et al. 1998).

Some support for this dissociation comes from evidence that ATN, but not ILN/LT lesions impair acquisition of allocentric spatial memory in both the radial arm maze and water maze (Bailey and Mair 2005; Mair et al. 2003; Mitchell and Dalrymple-Alford 2005, 2006; Wolff et al. 2008a). Stronger support was provided by evidence of a double dissociation between the effects of ATN and ILN/LT lesions on two memory tasks. Mitchell and Dalrymple-Alford (2006) reported that ILN/LT, but not ATN lesions impaired a preoperatively acquired egocentric working memory task, while only ATN lesions impaired allocentric spatial memory. Egocentric spatial memory impairments have often been reported after dorsal striatum lesions (Cook and Kesner 1988; DeCoteau and Kesner 2000; Kesner et al. 1993; McDonald and White 1993, 1994; Mitchell and Hall 1988; Packard and Teather 1998). A recent study, however, failed to observe an egocentric spatial reference memory deficit in a radial-arm water maze after ILN/LT lesions, which questions the degree of overlap between the effects of ILN/LT and striatal lesions (Wolff et al. 2008a).

The separation between the effects of ATN and ILN/LT lesions was extended here by comparing their effects on the classic water maze tasks designed by Packard and McGaugh (1992). These authors demonstrated a double dissociation between the effects of fornix lesions and dorsal caudate lesions, in which only fornix lesions impaired acquisition of a spatial reference memory task for locations tagged by irrelevant visual cues, whereas only dorsal caudate lesions impaired acquisition of a visual pattern discrimination task between cues tagged by irrelevant spatial location. It was predicted that the effects of ATN and ILN/LT lesions on these tasks would correspond to the previous effects of fornix and dorsal caudate lesions, respectively. The comparable nature of the two tasks means that any differential deficit between them would be unlikely to be due to differences in motivational, sensory or motor characteristics. Visual discrimination learning is relatively spared in Korsakoff's amnesia (e.g., Oscar-Berman and Zola-Morgan 1980), so a dissociation across these two tasks after ATN and rostral ILN lesions in rats would support the suggested importance of ATN dysfunction in human amnesic cases (Harding et al. 2000; van der Werf et al. 2003).

Based on their anatomical connections, an additional comparison between the two thalamic lesions examined their effects on home cage activity measured in a novel room over a 24-h period. Indeed, hippocampal lesions consistently increase this measure of home cage activity (e.g., Galani et al. 1997). Moreover, the intralaminar nuclei may influence arousal and one study has suggested that striatal lesions increase nocturnal locomotor activity (Mena-Segovia et al. 2002; Van der Werf et al. 2002).

## Methods

### Subjects and housing conditions

Fifty-eight adult male Long Evans rats (3 months old at the time of surgery; Centre d'Elevage R Janvier, Le Genest-St-Isles, France) were used. They were housed individually in opaque Makrolon cages (42 × 26 × 15 cm) under controlled temperature (21 °C) and 12/12 h light/dark cycle (light on at 7:00 h). Food and water were provided ad libitum. The study adhered to the regulations specified by the

European Committee Council Directive of November 24, 1986 (86/609/EEC) and the French Department of Agriculture (personal authorization license no. 67-215 for J-C.C. with co-authors operating under his authority).

## Surgery

Surgeries were conducted under aseptic conditions by authorized personnel. Rats received either ATN ($n = 22$) or ILN/LT ($n = 21$) lesions after being anaesthetized with sodium pentobarbital (50 mg/ml i.p., 20 min after 0.15 mg/ml atropine at 1.5 ml/kg, i.p.). The lesions were made via slow infusions of sub-microlitre volumes of *N*-methyl-D-aspartate using coordinates adapted to different Bregma-to-Lambda distances (see Table 1 for coordinates) and the incisor bar set at −7.5 mm. The needle was left in place for 3 min after each infusion before being slowly retracted. A third group (controls, $n = 15$) received sham lesions with the infusion needle lowered to the ATN (about half of the group) or the ILN/LT sites.

## Water maze, apparatus

The Morris water maze (diameter 160 cm, height 60 cm) was filled 32 cm deep with 22 °C water made opaque by the addition of powdered milk. The pool was located in the colony/experimental room and contained numerous extra maze cues (e.g., cages and racks; a desk; lights; two pillars; pictures on the wall, etc). The pool was divided into four virtual quadrants with four start points identified as north (N), east (E), south (S), and west (W). Following Packard and McGaugh (1992), the visual cues were provided by rubber balls (8 cm in diameter) that were painted with one of two different visual patterns. One pair of rubber balls was painted white with three black horizontal stripes (1 cm width) while the second pair was painted with three black vertical stripes (1 cm width). One of each pair was attached to a 5 cm round Plexiglas support that rested 1 cm below the water surface (i.e., fake platform) and one of each pair provided an escape because it was attached to one corner of a rectangular platform (11 × 14 × 0.6 cm; Fig. 1). About half of the rats from each surgery group were chosen randomly and tested first on the spatial discrimination task followed 3 days later by training on the visual pattern discrimination task, whereas the remaining rats were tested with the reverse order of tasks.

## Spatial discrimination task

In the place learning task, rats had to swim to the appropriate location to escape the water on to a platform, with the correct location and one of the three remaining locations cued by the irrelevant visual cues. On the morning, before training, the rats were placed on a platform with no cue, positioned in one quadrant (NW or SE), and replaced on the platform if it failed to remain there for 10 s (unless 60 s in total had elapsed). On the afternoon, before training, the rat was released from the opposite quadrant and allowed to find the platform and climb onto it. When a rat failed to find the platform within 60 s, it was gently guided to it by the experimenter's hand and allowed to stay there for 10 s.

For training, an escape platform was located at the centre of the "NE" quadrant, regardless of the horizontal or vertical striped cue used to mark that location (see Fig. 2). For any three successive trials, one of the two cues marked the location of the escape platform while the alternate cue (with no escape) was located in the centre of one of the three other quadrants, using one of each position for the three trials. The particular cue associated with the correct

**Table 1** Infusion volumes, infusion rates and coordinates for the NMDA lesions of the anterior (ATN) or lateral (ILN/LT) thalamic aggregates

| ATN | | | ILN/LT | | |
|---|---|---|---|---|---|
| | Ant | Post | Ant | Ant | Post |
| AP coordinates for B–L distance (cm) | | | AP coordinates for B–L distance (cm) | | |
| 0.60–0.63 | –0.22 | –0.20 | –0.31 | –0.31 | –0.35 |
| 0.64–0.66 | –0.23 | –0.21 | –0.32 | –0.32 | –0.36 |
| 0.67–0.72 | –0.24 | –0.22 | –0.33 | –0.33 | –0.37 |
| ML distance | ±0.15 | ±0.08 | ±0.13 | ±0.13 | ±0.13 |
| DV distance | –0.45 | –0.45 | –0.54 | –0.56 | –0.55 |
| Volume (µl, 0.12 M) | 0.09 | 0.11 | 0.06 | 0.06 | 0.06 |
| Infusion rate (µl/min) | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 |

*Ant* anterior site, *Post* posterior site, *AP* anterior–posterior coordinates from Bregma, at three B–L (Bregma–Lambda, see Paxinos and Watson 1998) distances, *ATN* anterior thalamic aggregate comprising the anterodorsal, anteromedial, and anteroventral thalamic nuclei, *DV* dorsal–ventral distance from dura, *ILN/LT* lateral medial thalamic aggregate comprising the intralaminar nuclei (centrolateral, paracentral, and rostral central medial nuclei) and lateral mediodorsal thalamic nuclei (lateral and paralamellar nuclei), *ML* medial-lateral distance from midline, *Post* posterior AP site

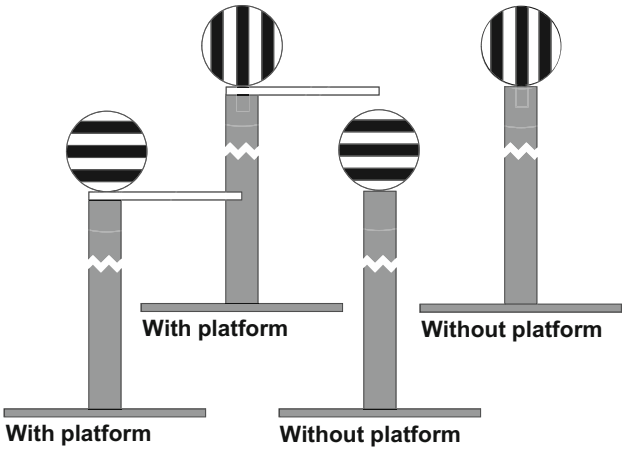


**Fig. 1** Drawing showing the real (*left*) and fake (*right*) escape platforms used for the spatial and visual versions of the water maze learning tasks. The size of the escape platform associated with the cue (*left*) was 11 × 14 × 0.6 cm. The height of the pedestal was 31 cm. The diameter of the rubber ball was 8 cm. The cues (the white rubber ball painted with *black vertical* or *horizontal stripes*) protruded 7 cm above the water surface. The rubber ball with the *vertical stripes* was used as the cues in the visual discrimination task

(escape) location remained the same for either one or two trials on a pseudo-random basis. Across every four trials, the rat was placed into the pool, facing the wall, at one starting point designated pseudo-randomly among four possibilities (N, E, S or W) and given a maximum of 60 s to reach the correct platform, with guidance after 60 s. When the rat had climbed onto the platform, it was allowed to stay there for 10 s. A random half of every four trials used a start point that was close to the platform location. As per Packard and McGaugh (1992), the primary-dependent measure was the number of errors made (when a rat touched the incorrect target). The latency to reach the correct location (i.e., the escape platform) was also recorded.

The rats that were tested first for spatial learning were trained over 10 blocks of 8 trials each. These rats were given two trials per day for the first 2 days and four trials on day 3. These eight trials were considered the first block. For the subsequent 9 days (i.e., days 4–12), they were given eight consecutive daily trials. The rats that were first tested in the visual discrimination learning task (see below) were already familiarised to the general water maze procedures and were thus given eight trials per day from day 1 onwards for the spatial discrimination task, using the same methods.

## Visual pattern discrimination task

Rats that began first with the visual discrimination task were given the same pretraining familiarisation as those starting with the spatial task. For the visual discrimination task, rats had to swim to the correct visual pattern

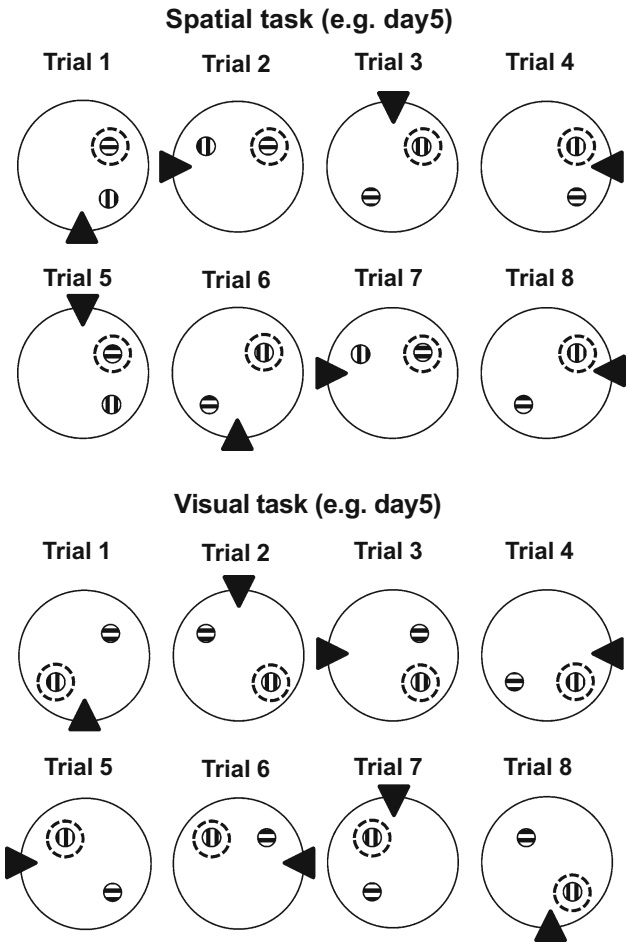


**Fig. 2** Training protocol in the water maze used on day 5 of either the spatial learning (*top*) or the visual discrimination tasks (*bottom*). The *solid triangles* indicate the location from where the rats were released on each trial (1–8). The *two circles* with *horizontal* or *vertical stripes* indicate the location of the cues in the pool. For each trial, the *stippled circle* surrounding one of the four cue locations points to the cue associated with the escape platform. In the spatial task, rats had to swim to a constant location regardless of the cue (*horizontal* or *vertical stripes*). In the visual discrimination task, rats had to swim to the correct cue (*vertical stripes*) regardless of its location

(i.e. vertical stripes), which was consistently associated with the escape platform regardless of its location in the pool, and ignore the alternate visual cue (i.e., horizontal stripes) with no escape platform located at the centre of one of the other quadrants (see Fig. 2). The general methodology was the same as that used for the spatial learning task, with four different start positions used randomly across every four trials, and the same measures were collected. From the first trial onwards, the location of the correct cue remained in the same position for three consecutive trials and was changed on every fourth trial to a new randomly designated location; the incorrect cue was placed in one of the three other quadrants on each of the three consecutive trials. Each of the four possible locations

for the correct cue was used across every 12 trials. The rats that were tested first on the visual discrimination task received two trials per day for the first 2 days and four trials for day 3, followed by eight trials per day for the remaining eight blocks of eight trials. The rats that had been tested first for spatial learning (see above) received eight daily trials from the start of training over seven blocks of trials when trained on the visual discrimination task.

### Home cage locomotor activity

Spontaneous locomotor activity was assessed for 24 h 2 weeks after the end of the water maze experiments. Clean transparent Perspex home cages were placed on shelves located in a new room. Two photocells outside each cage, located 4.5 cm above floor level and 28 cm apart, recorded horizontal locomotor activity. As per Galani et al. (2001), the first 3 h "habituation" period is the time when rats generally adapt to the novelty of the test situation. The remaining 21 h provided a diurnal period of 9 h and a nocturnal period of 12 h.

### Histology

Rats were deeply anaesthetized with an overdose of sodium pentobarbital (200 mg/kg, i.p.) and perfused transcardially with 4 % paraformaldehyde (PFA, in 0.1 M PBS; 4 °C). The brains were post-fixed for 2 h in 4 % PFA (4 °C), cryoprotected by a 48-h immersion (at 4 °C) in a 20 % sucrose solution (in PBS), snap-frozen in isopentane (−40 °C) and kept at −80 °C until coronal sections (30 µm) were made (Reichert Jung cryostat and Frigocut 2800 microtome). Verification of the location and extent of thalamic damage was performed as described previously (Mitchell and Dalrymple-Alford 2005; Wolff et al. 2008a) using sections stained with cresyl violet. Briefly, the area covered by the lesions in each rat was replicated on electronic copies of the Paxinos and Watson (1998) atlas, which were then used to generate automated pixel counts of the percent damage and hence lesion volume of the brain region of interest. Additional sections through the dorsal and ventral hippocampus were stained for acetylcholinesterase (AChE) histochemistry and optical density (OD) was used to examine the cholinergic innervation of the hippocampus.

### Statistical analyses

All data were analyzed by a mixed factor analysis of variance (ANOVA)—(Group: SHAM, ATN, ILN/LT vs. within subject: trial blocks for water maze performance, hours for habituation in the activity test). Newman–Keuls tests provided post hoc comparisons (Winer 1971). The association between lesion extent and performance was examined using Spearman's correlation.

## Results

### Lesion analysis

Representative lesions are shown in Fig. 3. Lesion extent and location are illustrated in Figs. 4 and 5. Five ATN and three ILN/LT rats had minor damage and were discarded from the statistical analysis. The rats retained for behavioral analyses exhibited highly selective, but subtotal damage, comparable to previous work (Mitchell and Dalrymple-Alford 2006; Wolff et al. 2008a). Rats first tested for spatial learning and those first tested for visual learning exhibited comparable lesion extents. Lesions in the ATN group (median of 75.8 %; range 50.0–94.5 %) produced little damage in the ILN/LT region (9.9 %; range 1.3–29.4 %) or the remaining mediodorsal (MD) region (3.8 %; range 0.6–13.7 %; the MD region is of interest because damage to this region may give rise to some memory impairments; Mitchell and Dalrymple-Alford 2005). Lesions in the ILN/LT group were also highly selective (median of 63.7 %; range 50.8–74.3 %), with minor ATN damage (3.3 %; range 0.0–20.0 %) and little MD region damage (22.2 %; range 5.2–33.5 %). The damage to other thalamic structures including midline nuclei was generally minimal to modest, with the exception of the interanteromedial nucleus (in ATN rats: median 24 %, range 1.7–90.3 %; in ILN/LT rats: median 0.0 %, range 0.0–35.8 %) and the parataenial nucleus (in ATN rats: median 34.4 %, range 9.9–70.8 %; in ILN/LT rats: median 0.0 %, range 0.0–25.0 %). Little damage occurred to the laterodorsal nucleus: ATN rats, 5.9 % (range 1.4–13.2 %) and ILN/LT rats, 4.7 % (range 0.2–18.4 %). The median damage was <1.0 % in both groups for each of the following: paraventricular and posterior paraventricular nuclei, anterior paraventricular nucleus, reuniens nucleus, and rhomboid nuclei. The maximum value for any of these regions was just below 20.0 % (anterior paraventricular nucleus in both groups).

Visual inspection of the other AChE-stained slides did not reveal obvious cholinergic denervation. Owing to a technical problem, sections from three rats did not show sufficient AChE stain for reliable optical density (OD) measurements. OD measurements showed a weak, but significant lesion-induced AChE reduction, irrespective of hemisphere, in the ventral hippocampus (lesion effect, $F(2,45) = 3.7$, $p < 0.05$; Table 2), with reduced OD (−23.3 %) in ATN when compared with either ILN/LT or SHAM rats ($p < 0.05$), which did not differ from each other. The group differences on AChE measures for the

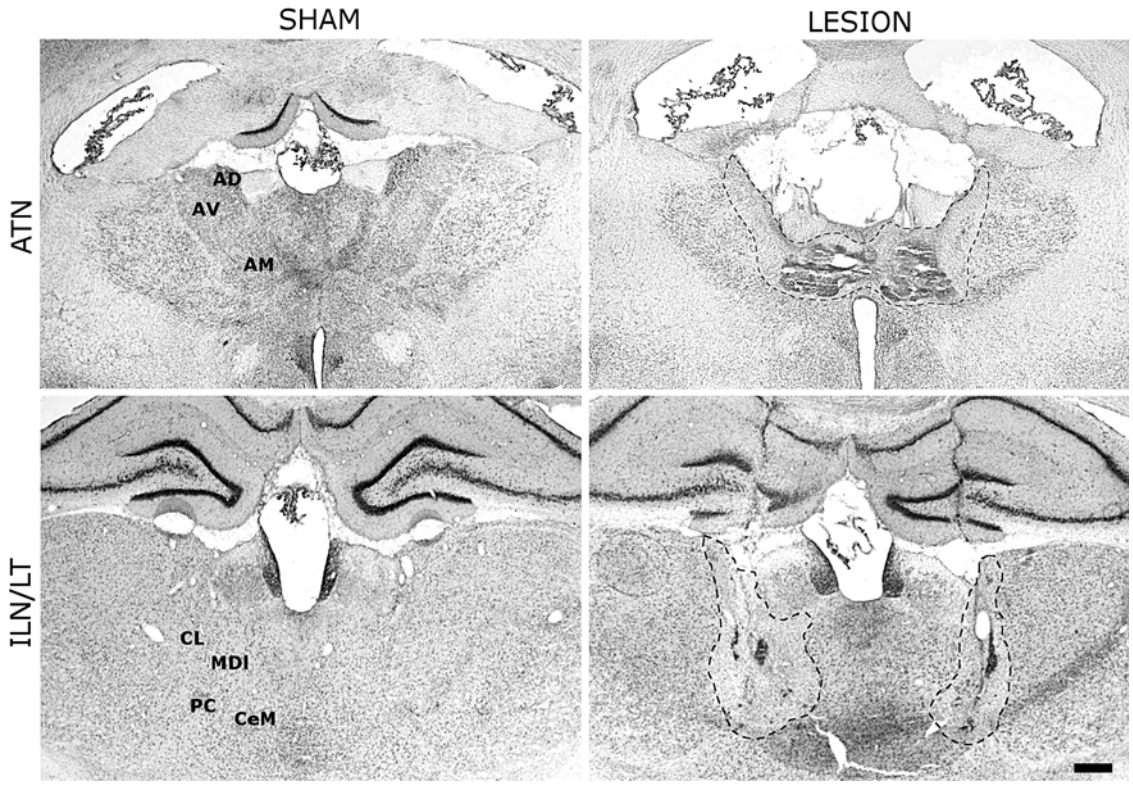


**Fig. 3** Photomicrographs showing a representative example of an ATN lesion (*top*, *right*) and an ILN/LT lesion (*bottom*, *right*) on coronal brain sections stained with cresyl violet. The lesion is delimited by the interrupted line in each case. This ATN lesion encroached onto 75.9 % of the ATN, 15 % of the ILN/LT and 3 % of the medial thalamus. This ILN/LT lesion encroached onto 63.4 % of the ILN/LT, 0 % of the ATN and 19.4 % of the medial thalamus (compare with data shown in Fig. 5). Note that the lesion, especially in the ATN case, has caused shrinkage of the tissue. Comparative locations of the main nuclei targeted by each lesion are shown on the corresponding left sections, illustrating two sham controls. *AD* anterodorsal nucleus, *AM* anteromedial nucleus, *AV* anteroventral nucleus (the AD, AM and AV constitute the ATN, i.e. anterior thalamic nuclei); *CL* central lateral nucleus, *MDl* lateral mediodorsal nucleus, *PC* paracentral nucleus, *CeM* central median nucleus (the CL, MDl, PC and rostral CeM constitute the rostral ILN/LT, i.e., intralaminar/lateral thalamic nuclei; the caudal part of CeM are regarded as posterior ILN; van der Werf et al. 2002). *Scale bar* 500 μm

dorsal hippocampus did not reach significance, both in the anterior ($F(2,45) = 2.6$, $p = 0.087$) and posterior regions ($F(2,45) = 2.5$, $p = 0.096$).

## Groups trained on spatial learning, followed by visual discrimination learning

The SHAM and ILN/LT groups showed acquisition of the spatial task, whereas there was little evidence of spatial learning in the ATN group (Fig. 6a, left panel). There were significant overall Lesion ($F(2,23) = 13.0$, $p < 0.001$) and Block ($F(9,207) = 9.6$, $p < 0.001$) effects, but the interaction between the two factors was not significant ($F(18,207) = 1.41$, $p > 0.10$). Newman–Keuls tests confirmed that ATN rats produced significantly more errors than both SHAM ($p < 0.001$) and ILN/LT groups ($p < 0.01$); there was no significant difference between the two latter groups. The analysis of the latencies to reach the correct platform (data not shown) also produced a significant Block effect in the spatial learning task ($F(9,207) = 74.0$, $p < 0.001$), due to improvement over the six first test days primarily, and a significant Lesion effect ($F(2,23) = 6.1$, $p < 0.01$), due to longer latencies in the ATN group as compared to SHAM ($p < 0.01$) and ILN/LT ($p < 0.05$) rats. The Lesion by Block interaction for latency was not significant.

The switch to the visual discrimination task resulted in an increase of the number of errors in all groups. Subsequently, there was a marked improvement of performance in all three groups, resulting in a significant Block effect ($F(6,138) = 14.9$, $p < 0.001$; Fig. 6a, right panel) irrespective of lesion status (Lesion effect, $F(2,23) < 1.0$, $p > 0.75$; Lesion × Block interaction, $F(6,138) = 1.52$, $p > 0.10$). The analysis of latencies to reach the correct platform led to the same conclusions, with a reduction in latencies over time (data not shown) being the only significant effect (Block, $F(6,138) = 9.6$, $p < 0.001$).

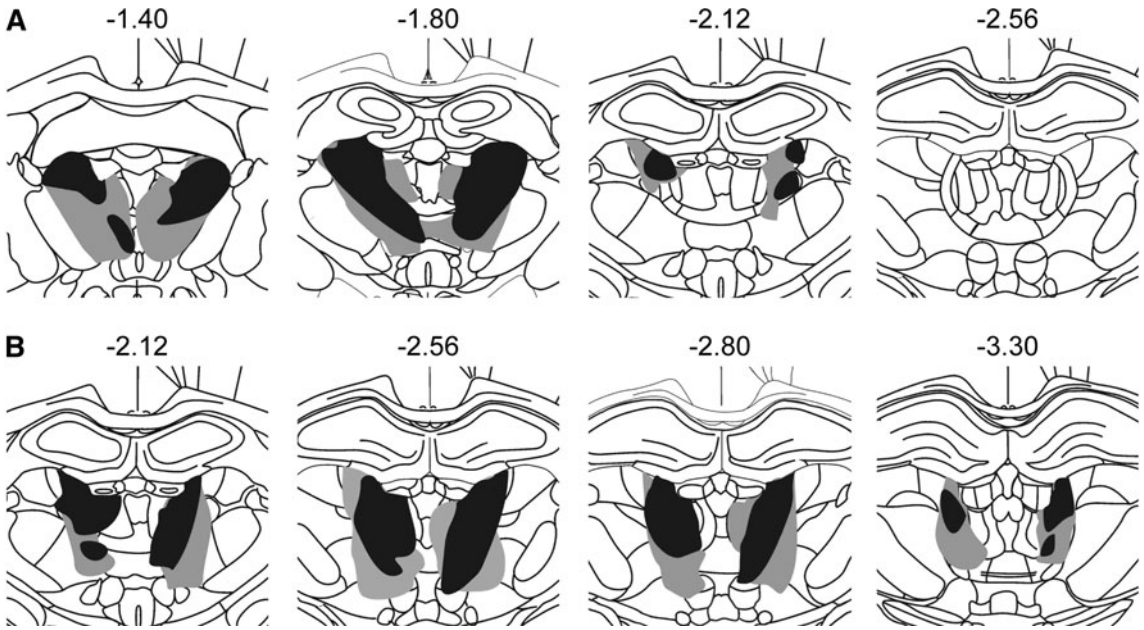


**Fig. 4** Schematic representation of the smallest (*black*) and largest (*gray*) thalamic lesion extents in the rats retained for statistical analyses. **a** Anterior thalamic nuclei group (ATN), **b** intralaminar nucleus/lateral thalamic lesion group (ILN/LT). *Numbers above the drawings* indicate the distance (mm) of each section from bregma (according to Paxinos and Watson 1998)

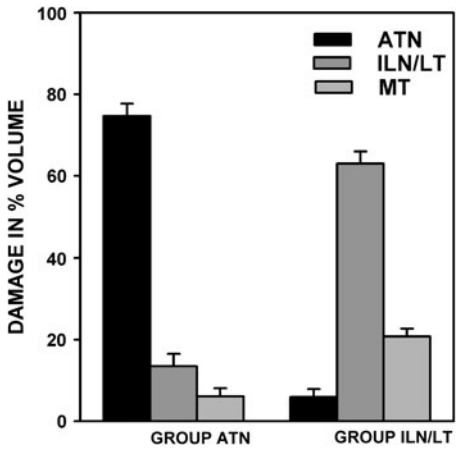


**Fig. 5** Mean lesion extent +SEM for the two lesion groups (expressed as a percent bilateral volume of damage) in three regions of the medial thalamus: ATN (anterodorsal, anteromedial and anteroventral nuclei), the ILN/LT (centrolateral, rostral centromedian, lateral mediodorsal, paralamellar and paracentral nuclei) and MT (intermediodorsal, mediodorsal, central mediodorsal and medial mediodorsal nuclei)

## Groups trained on visual discrimination learning, followed by spatial learning

The pattern of results found in the groups that were trained with the reverse order of tasks replicated the previous findings. In the visual discrimination task (Fig. 6b, left panel), all rats acquired the task, irrespective of group (Block effect, $F(8,176) = 15.2$, $p < 0.001$; Lesion, $F(2,22) = 1.5$, $p > 0.20$); Lesion × Block interaction, $F(16,176) < 1.0$, $p > 0.85$). The analysis of the latencies for the visual task (not shown) also produced only a significant Block effect ($F(8,176) = 60.0$, $p < 0.001$).

The switch to the spatial task resulted in an increase in the number of errors in all groups (Fig. 6b, right panel). As before, the Lesion ($F(2,22) = 7.1$, $p < 0.01$) and Block ($F(7,154) = 12.0$, $p < 0.001$) effects were significant, but not the interaction ($F(14,154) < 1.0$, $p > 0.95$). Once again, ATN rats were significantly impaired as compared to SHAM ($p < 0.001$) and ILN/LT ($p < 0.01$) rats, which did not differ. Similarly, the ANOVA of the latencies (not shown) produced significant Block ($F(7,154) = 25.2$, $p < 0.001$) and Lesion effects ($F(2,22) = 6.9$, $p < 0.01$).

## Correlations between lesion extent and performance

No association was evident between ATN or LTN lesion extent and behavioral performance on either the spatial or visual water maze tasks (ATN lesion extent: respectively, $r = 0.13$ and $-0.24$; LTN lesion extent: respectively, $r = 0.10$ and $-0.13$). Thus, the minimal acceptable ATN lesions were sufficient to impair spatial memory acquisition. Similarly, there was no evidence that the extent of damage to adjacent nuclei influenced the level of impairment found in ATN rats (no correlation approached significance).

**Table 2** Optic density measurements (±SEM) of ATN and ILN/LT rats expressed as a percentage of the average density measured in controls (SHAM)

| Lesion | Anterior dorsal hippocampus (Bregma −2.8 mm) | Posterior dorsal hippocampus (Bregma −3.6 mm) | Ventral hippocampus (Bregma −4.8 mm) |
|---|---|---|---|
| SHAM | 100.0 ± 6.9 | 100.0 ± 6.8 | 100.0 ± 6.3 |
| ATN | 80.7 ± 5.9 | 78.0 ± 7.1 | 76.7 ± 7.4 * |
| ILN/LT | 85.8 ± 5.9 | 94.0 ± 7.0 | 97.1 ± 5.8 |

Values given between brackets are levels of anteriority defined according to Bregma (Paxinos and Watson 1998). Statistics: * $p < 0.05$ versus SHAM

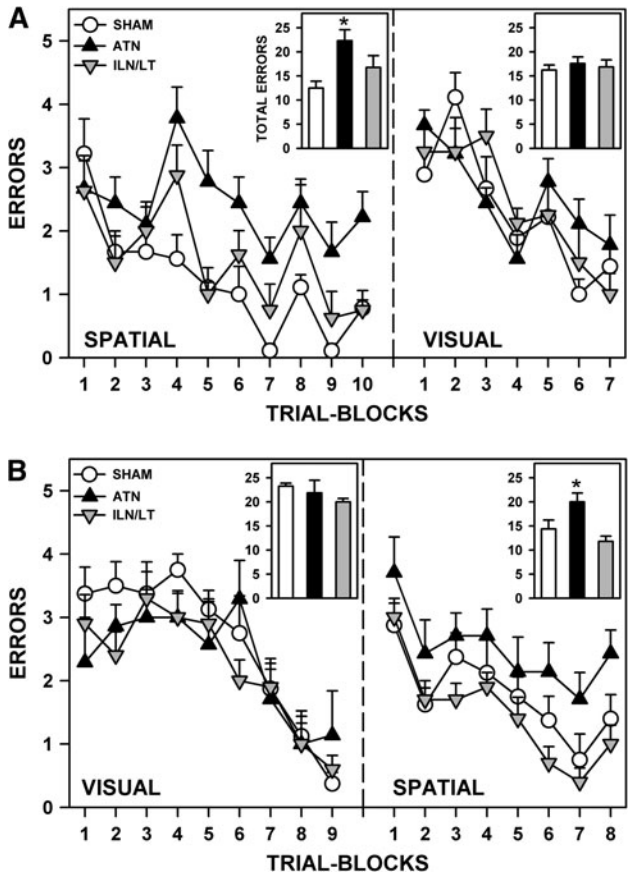


**Fig. 6** Effect of ATN and ILN/LT lesions on the acquisition of the spatial task and of the visual discrimination task in the water maze. **a** Rats were first trained in the spatial task and, after ten 8-trial blocks, in the visual discrimination one for seven additional 8-trial blocks. **b** The order of testing was inversed: these rats were first trained for visual discrimination over nine 8-trial blocks, then for spatial learning over eight additional 8-trial blocks. All data are mean +SEM. In the *inserts*, the *bar graphs* indicate the average total number of errors (+SEM) found in each group, illustrating the overall Group effects. Statistics: *asterisks* significantly different from SHAM, $p < 0.05$

## Home cage locomotor activity

As shown in Fig. 7, ATN lesions produced hyperactivity, which was particularly marked during the first hour of the habituation period and during the nocturnal phase of the cycle. The ANOVA of the activity scores recorded during the habituation period showed significant Lesion ($F(2,48) = 11.4$, $p < 0.001$), Hour ($F(2,96) = 27.9$, $p < 0.001$), and Lesion × Hour interaction ($F(4,96) = 12.0$, $p < 0.001$) effects. Newman–Keuls tests confirmed that the activity recorded in ATN rats over the first hour was significantly higher than in both other groups ($p < 0.001$). Nocturnal hourly activity was about four times higher than activity during the diurnal phase ($p < 0.001$, irrespective of group). The Lesion effect was also significant across the whole diurnal and nocturnal phases ($F(2,48) = 4,7$ and 10,3, respectively, $p < 0.05$ and 0.001). In both phases, the ATN group once again showed significantly higher activity levels than the ILN/LT group ($p < 0.01$) and the SHAM group ($p < 0.05$), which did not differ.

## Discussion

The current findings did not confirm the expected double dissociation of the effects of ATN and ILN/LT lesions

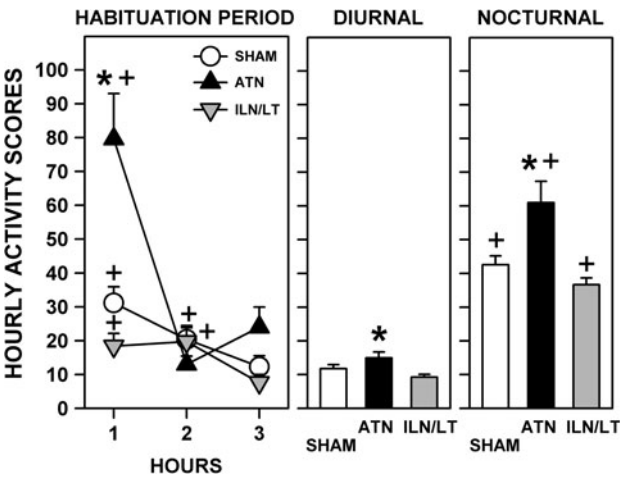


**Fig. 7** Effect of ATN and ILN/LT lesions on locomotor activity recorded in the rats' home cages during a 3-h habituation period (*left*), the remaining 9-h diurnal phase (*middle*) and the entire, uninterrupted 12-h nocturnal phase (*right*) of a 24-h cycle. All data are hourly mean +SEM. Statistics: *asterisks* significantly different from SHAM, $p < 0.05$; *plus* significantly larger than the average hourly diurnal activity found in each group, $p < 0.05$

across the two water maze tasks. As expected, however, ATN lesions severely disrupted the ability of rats to learn the allocentric spatial task when the correct location was tagged with an irrelevant visual cue, but had no effect on the acquisition of the visual discrimination task when spatial location was irrelevant. ILN/LT lesions had no effect on either task. Thus, a single dissociation was demonstrated on one task only.

Evidence that ATN lesions impaired acquisition of the allocentric spatial learning task, but not the visual discrimination task used here, adds further support considering that the ATN constitute an important region of an extended hippocampal memory system (Aggleton 2008; Aggleton and Brown 1999; Vann and Albasser 2009). In addition, the pattern of increased activity in the home cage is also consistent with similar findings using the same conditions in rats with hippocampal lesions (e.g., Cassel et al. 1998; Galani et al. 1997). It would be interesting to know whether the activity changes after ATN lesions are, like after hippocampal lesions (e.g., Wilkinson et al. 1993), the consequence of an increased dopaminergic activity in the basal ganglia due to a reduced inhibitory influence on the nucleus accumbens.

The selective deficit exhibited by ATN rats in the water maze cannot be explained by differences in motivational or sensory-motor demands as they were similar in the two tasks (spatial vs. visual discrimination). In addition, this deficit did not reflect proactive (or any other kind of) interference between the two memory procedures, as it was found regardless of the order in which the two tasks were performed. It is possible, however, that reduced cholinergic activity in the ventral hippocampus contributed to this deficit (see Table 2). The reduced AChE staining was not a consequence of mechanical damage to the fornix or adjacent regions by the infusion needle used to make the ATN lesions, because sham rats also had similar surgery. Related studies have demonstrated an alteration of ventral hippocampal cholinergic function in the pyrithiamine-induced thiamine deficiency model of diencephalic amnesia (Anzalone et al. 2010; Savage et al. 2007). It seems unlikely, however, that the weak ventral hippocampal cholinergic depletion observed played a major role in the deficits found after ATN lesions because rats with an almost complete cholinergic denervation of the hippocampus are still able to acquire a spatial task (e.g., Lehmann et al. 2002; Parent and Baxter 2004). Furthermore, non-specific functional blockade of the ventral hippocampus by lidocaine infusions before acquisition of a spatial version of the water maze task does not prevent spatial learning (Loureiro et al. 2011).

The hypothesis that the effects of rostral ILN/LT lesions generally mimic those of dorsal caudate lesions appears thus far to have limited predictive utility (Mitchell and Dalrymple-Alford 2005, 2006). The current prediction was based on the neuroanatomical data that the central lateral thalamic nucleus projects densely to the dorsolateral striatum and the paracentral and rostral central medial nuclei to the dorsomedial region (Berendse and Groenewegen 1991; Van der Werf et al. 2002), which overlap with the same site of dorsal caudate lesions that severely impair the visual pattern discrimination used in the current study (Packard and McGaugh 1992). The current failure to find visual discrimination learning deficits after rostral ILN/LT lesions adds to other evidence that these lesions did not produce an egocentric spatial memory deficit (Wolff et al. 2008a) or increased visuospatial reaction time (Hembrook and Mair 2011), both of which would be expected if disruption occurred to critical striatal pathways (Bailey and Mair 2006; Mitchell and Hall 1988; Packard and McGaugh 1992; White and McDonald 2002, review). It is nevertheless worth mentioning that Kato et al. (2011) recently reported that mice that received recombinant immunotoxic lesions of the parafascicular nucleus, depleting the primary caudal ILN projections to the lateral and dorsolateral striatum, showed impaired visually guided attention. Functional heterogeneity within the striatum and across ILN regions is, however, one complication that is neglected by a simple ILN-striatal perspective (Mair et al. 2002). Thus far, the primary evidence for a striatal-like effect after rostral ILN/LT lesions relies on impaired egocentric working memory (Mitchell and Dalrymple-Alford 2006). It is clear that working memory per se is not impaired after ILN/LT lesions, because allocentric working memory is only transiently affected by these lesions (Mitchell and Dalrymple-Alford 2006).

Nonetheless, other evidence supports the idea that the rostral ILN/LT region and the prefrontal cortex functionally interact to influence several different functions, including memory consolidation, motor planning, temporal coding, and motor working memory (Kesner 2000; Bailey and Mair 2004; Bailey and Mair 2007; Harrison and Mair 1996; Koger and Mair 1994; Lopez et al. 2009; Mair et al. 1988, 2011; Mitchell and Dalrymple-Alford 2005). The extent to which these latter effects require an involvement of the striatum, or conversely that the lack of some expected effects of ILN/LT lesions such as the visual discrimination task examined here can be supported sufficiently by cortico-striatal pathways alone, will require investigation in future studies, perhaps using a unilateral lesion-disconnection approach. The extent of the rostral ILN/LT lesion may also be an issue, because it is difficult to make large selective lesions without encroaching onto other adjacent nuclei. Indeed, the use of large rostral ILN lesions and their encroachment onto the ATN nuclei or other central nuclei in particular seems likely to be responsible for earlier reports of working memory or

acquisition deficits in allocentric memory in rats with ILN injury (Savage et al. 1998; Hembrook and Mair 2011).

In conclusion, based on the neural connectivity and evidence from existing behavioral studies, we expected that ATN lesions would impair allocentric, but not egocentric memory functions and that rostral ILN/LT lesions would yield an opposite picture. We could not establish such a double dissociation. Our current data, however, consolidate the existing evidence showing that the ATN constitute a crucial node in an extended hippocampal system subserving spatial memory processes in particular. They also confirm that the ILN/LT, which in previous studies has been shown to contribute to egocentric working memory and remote memory processes, and is not implicated in visual discrimination memory and thus may not be crucial to striatum-dependent functions. Therefore, it seems that damage to the ATN is the major contributor to the classical neuropsychological symptoms of diencephalic amnesia.

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