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Towards therapy to relieve memory impairment after anterior thalamic lesions: improved spatial working memory after immediate and delayed postoperative enrichment

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Abstract

Injury to the anterior thalamic nuclei (ATN) is associated with severe amnesia in humans. To test the principle that these deficits may be amenable to intervention, the behavioural effects of postoperative housing in an enriched environment were examined in rats that received neurotoxic lesions of the ATN. As expected, rats with ATN lesions maintained in standard group-housing showed severe, and in this case long-term, deficits in a preoperatively trained non-matching-to-sample spatial working memory task. Thirty days of enriched housing, introduced either at 5 days (Experiment 1) or delayed until 40 days post-surgery (Experiment 2) markedly reduced this working memory impairment, including evidence of improved utilization of spatial cues. The treatment gains found in Experiment 2 were maintained at 4 months post-surgery despite no further enrichment. ATN lesions also retarded the postoperative acquisition of spatial discrimination problems in Experiment 1, irrespective of the separation between target arms, but this impairment was not ameliorated by the prior enrichment. This study provides the first evidence of substantial recovery of severe, and otherwise long-lasting, spatial working memory deficits after ATN brain injury and suggests that further investigation on the extent of possible recovery of function in animal models of diencephalic amnesia is warranted.

Introduction

Considerable clinical evidence shows that the integrity of the thalamus is crucial for normal memory (Schmahmann, 2003; Van der Werf *et al.*, 2003). Thalamic infarcts and alcohol-related disorders suggest that disruption to the anterior thalamic nuclei (ATN) makes a significant contribution in many cases of diencephalic amnesia (Ghika-Schmid & Bogousslavsky, 2000; Harding *et al.*, 2000; Van der Werf *et al.*, 2000). These clinical observations receive support from experimental reports that severe deficits in spatial and temporal order memory exist after ATN lesions, including localized subtotal lesions that minimize confounds from damage to adjacent thalamic structures (Aggleton *et al.*, 1995, 1996; Byatt & Dalrymple-Alford, 1996; Parker & Gaffan, 1997; Warburton *et al.*, 1997; Sziklas & Petrides, 1999; Van Groen *et al.*, 2002; Mair *et al.*, 2003; Moran & Dalrymple-Alford, 2003; Mitchell & Dalrymple-Alford, 2005, 2006; Gibb *et al.*, 2006; Wolff *et al.*, 2006). This lesion evidence, its similarity to many effects of hippocampal lesions, and the ATN's various direct and indirect neural connections with the hippocampal system support an influential proposal that the ATN constitute a critical nodal point in an extended brain system responsible for normal episodic memory (Aggleton & Brown, 1999; Vann & Aggleton, 2004).

The current study provides an examination whether memory deficits produced by ATN lesions are amenable to experimental intervention. Recovery of function in robust animal models would encourage the possibility for therapeutic intervention in clinical cases of diencephalic amnesia. This study employed postoperative enriched housing as a therapeutic tool because enrichment enhances brain plasticity with beneficial effects after many types of brain injury, including hippocampal system lesions (Dalrymple-Alford & Kelche, 1985; Kolb & Whishaw, 1998; Will *et al.*, 2004). The primary interest was spatial working memory using a preoperatively trained T-maze non-matching-to-place task, as this alternation task has revealed severe memory deficits after ATN lesions (Aggleton *et al.*, 1995; Aggleton & Brown, 1999; Warburton & Aggleton, 1999; Warburton *et al.*, 1999; Ward-Robinson *et al.*, 2002), especially when a cross-maze procedure is used that emphasizes the use of spatial cues and minimizes the benefits of an egocentric (response-based) strategy (Aggleton *et al.*, 1996; Warburton *et al.*, 1997).

Thirty days of enrichment was introduced either immediately after surgery (Experiment 1) or delayed until 40 days post-surgery when the spatial working memory deficit induced by ATN lesions had already been characterized by postoperative testing (Experiment 2). Experiment 1 also examined the ability to discriminate between fixed locations in a radial-arm maze, using problems with different spatial pattern separations. Fornix lesions spare the acquisition of easy spatial discriminations (widely separated arms), but impair acquisition of difficult discriminations (close/adjacent arms; McDonald & White, 1995), a difference that has not been tested with ATN lesions. In addition to post-enrichment testing, Experiment 2 re-examined spatial working memory at 4 months post-surgery to determine any potential long-term benefits of enrichment.

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Materials and methods

Experiment 1 – enrichment starting 5 days after ATN surgery

Animals, surgery and housing conditions

Forty-six female PVGc hooded rats were used (6–7 months old, between 148 and 171 g at surgery). Testing occurred during the dark phase of their reversed 12 h light cycle. Body weights were restricted to 85–90% of free-feeding weight during testing, with free food access for the surgery and recovery period, and during subsequent differential housing in enriched vs standard cages. All protocols in this study conformed to the NIH Guide for the Care and Use of Laboratory Animals, and were approved by the Animal Ethics Committee University of Canterbury.

Rats were anaesthetized with sodium pentobarbitone (75 mg/kg) 20 min after atropine (0.18 mg/kg), supplemented by mepivicane (3.0 mg/kg) and ketofen (0.50 mg/kg), and placed in a stereotaxic frame with atraumatic ear bars (Kopf, Tujunga, CA, USA). The incisor bar was set at −7.5 mm below the interaural line to minimize damage to the fornix. Two bilateral lesion sites were used to maximize ATN damage, directed at the anteroventral nucleus (AV) and the anteromedial nucleus (AM). To improve target accuracy, one of five anterior–posterior coordinates was used based on an individual rat's bregma to lambda (B–L) distance (in mm). For the AV lesion, the AP coordinates from bregma were: −2.2 for B–L = 6.0 and 6.1; −2.3 for B–L = 6.2 and 6.3; −2.4 for B–L = 6.4, 6.5 and 6.6; −2.5 for B–L = 6.7 and 6.8; −2.6 for B–L = 6.9 and 7.0; and −2.7 for B–L = 7.1 and 7.2. The AV lesion was ± 1.65 mm lateral from the midline and −5.50 mm ventral from dura. The AM lesions followed an identical scheme, except that lesions were placed ± 0.90 mm lateral from the midline, −5.80 mm ventral from dura, and AP was 0.1 mm more anterior than for the AV lesion. Either 0.12 μL (AV lesion) or 0.09 μL (AM lesion) of 0.12 M *N*-methyl-D-aspartic acid (NMDA; Sigma, Castle Hills, NSW, Australia) in phosphate buffer (pH 7.2) was infused over 3 min at each site, using an automated Stoelting microinfusion pump and a 1-μL Hamilton syringe (Reno, NV, USA). The needle was left *in situ* for a further 3 min for diffusion at each site before slow retraction. ATN sham controls received the same surgical procedure, but the needle was lowered to 1.50 mm above the lesion coordinates and no material was infused.

Prior to surgery all rats were housed in standard (small group) housing conditions of three or four rats per opaque plastic cage (50 cm long by 30 cm wide by 23 cm high). Following surgery all rats were housed individually for a recovery period of 5 days and then, based on matched preoperative spatial working memory performance, pairs of rats were randomly assigned to either an enriched environment condition (EE) for 30 days or the standard housing conditions (SC). No behavioural testing occurred during the 30 days of enrichment. The rats in the EE groups were housed postoperatively with 11 or 12 rats in enrichment cages, made of wire mesh (85 cm long by 60 cm wide by 30 cm high) with a solid metal floor covered with sawdust. An array of objects, such as Perspex tunnels, PVC tubing, running wheel, metal chains, ladders, boxes, glass cups and plates, and plastic toys were placed in this cage and were changed on a daily basis, plus the position of food and water and the placement of the enrichment cages in the colony room were varied every 2–4 days. The rats in the EE group were then re-housed in standard conditions (i.e. three or four rats per cage) with their cage mates from the same EE cage. All rats (from SC and EE cages) were food deprived prior to retesting for spatial working memory, which started at 40 days post-surgery. After the end of continuous enrichment and thereafter in Experiment 1, the rats from enriched cages were returned to the EE for a period of 1.5–2 h at the end of each day, followed by their daily food ration on return to the standard cages. After confirmation of accurate lesions, the final group numbers were: SC-SHAM, $n = 11$; SC-ATN, $n = 8$; EE-SHAM, $n = 12$; and EE-ATN, $n = 7$ (in addition, four SC-ATN rats and four EE-ATN rats were excluded due to the ATN lesions being too small; see Histology and Lesion evaluation sections).

Apparatus and behavioural testing

Reinforced spatial alternation. Spatial working memory was tested using a T-maze configuration, embedded in a cross-maze raised 75 cm above the floor. The wooden runways were 10.5 cm wide and painted grey, with 2.5-cm-high metal edges. The two stems were 1 m long with a guillotine door located 28 cm from each end to create a North (N) and a South (S) start area. The two goal arms were 40 cm long, the end of which included a raised wooden food well (2.5 cm diameter, 1 cm deep). Wooden blocks (10.5 cm wide by 30 cm high by 10 cm deep) were used to restrict access to any stem or arm. The maze was located diagonally in a windowless room (334 cm by 322 cm), which contained a number of distal cues (to the side of the goal arms, computer, poster, desk and chair on one side, curtain on the other; beyond the start areas, closed door at one end, hub to a radial-arm maze at the other). The horizontal distance between an end of the maze and the nearest surface varied from 25 cm to 70 cm. Diffuse lighting was provided by overhead fluorescent lights.

All rats were trained to criterion on the spatial working memory task prior to surgery, and then retested for 10 sessions at 40 days post-surgery on the same task. Six trials were conducted per session and sessions were conducted on alternate days throughout, with half the rats tested per day for 6 days per week. The rats were familiarized individually to the maze over 3 days with chocolate pieces (0.1 g) freely available on the arms and then the food wells. The rats were initially trained for six or seven sessions (to 85% accuracy on two consecutive sessions) using the start area in the N or the S stem for half the six daily trials and with the same start area used for both the 'sample' and 'test' (choice) run for any given trial. Correct performance on the test run required the rat to choose the alternate arm from that previously visited during the sample run of that trial (reinforced spatial alternation). To ensure that the rats were not simply using an egocentric strategy (alternating body turn) from sample to test runs, a pseudorandom half of the trials on each of the subsequent 10–12 preoperative sessions (again to criterion of 85% accuracy for two sessions) used the opposite start area across the 'sample' and 'test' runs (e.g. S for the sample run and N for the test run for a given trial). The other half of the daily trials used the same start area, such as only S or only N, for both sample and test runs. On the sample run, the rat was placed in the start area, the door raised and the rat allowed to enter one open arm, due to the placement of wooden blocks on the alternate arm and stem area, where it was confined for approximately 10 s while it ate a 0.1 g chocolate food reward. It was then picked up and returned to the appropriate start area for a delay of 10 s while the arm barriers at the choice point were removed or repositioned as required. When the door was raised for the test run the rat was thus allowed a free choice between the two maze arms (hindfoot down that arm; no retracting). If the rat chose the previously blocked arm (non-matching alternation) it was rewarded with two food rewards, confined in that arm for about 10 s while it ate the reward and returned to the home cage. If the rat returned to the arm previously visited on the sample run it was confined to that arm for 10 s and returned to the home cage. The rats were tested in groups of three or four with each rat having one trial in

turn, so that the intertrial interval was approximately 3–4 min. Each rat experienced a pseudorandom sequence of correct arm choices (left or right), N or S start position for the sample run, and same vs opposite start position for the test run, which varied across rats and sessions (Fellows, 1967).

Spatial pattern separation problems. A 12-arm radial maze was used (85 cm above floor, 1.65 m diameter), which comprised a 35-cm-wide wooden hub (painted black) and 12 aluminium arms (65 cm long by 8.5 cm wide, 3-cm-high borders). Single clear Perspex barriers (20 cm long by 25 cm high) extended along each arm from the central hub to discourage rats from jumping across arms. A black wooden block (5 cm long by 8.5 cm wide by 3 cm high) containing a food well (2 cm diameter, 1 cm deep) was located at the end of each arm and housed inaccessible food (0.1 g chocolate pieces) to provide constant olfactory cues. Clear Perspex guillotine doors, each with a white horizontal label (4.5 cm wide by 2.5 cm high) centred 3 cm above the door's base, controlled access to the arms and could be raised singly or together by an overhead pulley system. The radial maze was located in the same room where the previous cross-maze testing had been conducted.

Acquisition of three successive spatial problems, using fixed rewarded locations in the maze, began about 100 days post-surgery. One of two non-rewarded arms was paired with the rewarded arm on any given trial, for each of the 12 daily (massed) trials. That is, access was always available to the designated rewarded arm and to one of two non-rewarded arms, with the left/right position of the non-reward arm determined using a pseudorandom sequence (Fellows, 1967). The second rat in each group used an arm allocation one arm clockwise from the first rat's arm allocation, the third rat two arms from the first rat's arm allocation, and so on. The two non-reward arms were: (i) for the first problem, five or six arms distant from the reward arm (wide separation, with four or five unused arms in between; for example, arm 12 was rewarded and arm 6 and arm 7 were the non-rewarded arms); (ii) for the second problem, four arms distant (intermediate separation, with three unused arms in between; for example, arm 9 was rewarded and arm 1 and arm 5 were the non-rewarded arms); and (iii) for the third and last problem, one arm distant (close separation, adjacent arms; for example, arm 3 was rewarded and arm 2 and arm 4 were the non-rewarded arms). For any given rat no arm that was used as a reward or non-reward arm in one problem was used for that rat in any subsequent problem. Each successive problem was run for a minimum of 6 days with a day's break between problems. The rats were initially familiarized to the radial maze for three trials per day for 3 days, with access to any two of the arms (baited with two 0.1 g chocolate pieces) that would be used in the first problem. For each problem, testing continued until a rat reached a criterion of 10 out of 12 correct choices on each of two consecutive days, or a maximum of 15 days testing for the first two problems and 18 days for the last problem. At the start of each session, the rat was placed on the centre platform and, after a 20–25-s delay, the doors to the rewarded arm and to one of the non-reward arms were opened simultaneously. The first arm entered by the rat's hind legs was recorded as the rat's choice and the door to the non-entered arm closed. Once the rat had returned to the centre platform the second door was closed. For the second and all subsequent trials for that session, the maze was rotated according to a randomly determined sequence to minimize the use of intramaze cues, while the rat was still present on the centre platform (20–25-s intertrial interval). The randomly determined sequence of maze rotations ensured that each specific arm on the maze was the reward arm once in the daily block of 12 trials, but the spatial orientation of the rewarded arm remained constant.

Histology

On completion of Experiment 1, all rats received an overdose of sodium pentobarbital and were transcardially perfused with cold saline followed by 4% formalin. The brains were postfixed for 2 days, cryoprotected in 30% sucrose, and every coronal 50-μm section throughout the thalamic region was collected for Cresyl violet staining of cell bodies. Lesion extent was replicated on electronic copies of the Paxinos & Watson (1998) atlas (see Mitchell & Dalrymple-Alford, 2005). 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, but not more than 40% damage to the corresponding adjacent lateral thalamic region (LT, which included the intralaminar and lateral mediodorsal nuclei) and posteromedial thalamic region (MT, which included the medial and central mediodorsal nuclei). The latter regions were of interest because they have also been suggested as possible causes of some aspects of diencephalic amnesia, but only ATN lesions are associated with spatial memory deficits (Bailey & Mair, 2005; Mitchell & Dalrymple-Alford, 2005, 2006; Gibb *et al.*, 2006).

Experiment 2 – enrichment delayed until 40 days post-surgery

Animals, surgery and housing conditions

Forty-five female PVGc hooded rats were used (6–7 months old, between 152 and 198 g at surgery). Body weights were restricted to 85–90% of free-feeding weight throughout Experiment 2, except free food access around the period of surgery, during immediate recovery and the period of differential housing.

The same surgery and histology procedures described for Experiment 1 were used, with minor adjustment to improve the reliability of the lesions (fewer rejections) in that the anterior–posterior coordinates moved 0.1 mm posterior for both AV and AM lesions sites. The AV lesion was now ±1.50 mm lateral from the midline and −5.55 mm ventral from dura, and the AM lesion was ±1.20 mm lateral from the midline and −5.80 mm ventral from dura.

Unless otherwise stated, all rats were housed in SC (group) of three or four rats per opaque plastic cage (per Experiment 1). After the initial post-surgery re-evaluation of spatial working memory performance, which started at 14 days post-surgery, pairs of rats with ATN lesions and pairs of rats with sham lesions were matched in terms of their postoperative performance and assigned to either an EE condition for 30 days or continued with SC (rats in both housing conditions were housed with new cage mates). Specifically, the introduction to enrichment was delayed until 40 days post-surgery and after initial confirmation of equal deficits in the two lesion groups. The rats in the EE group were housed in groups of 11 or 12 in an enrichment cage (per Experiment 1). Following the 30 days of continuous enrichment the rats in the EE group were re-housed in standard conditions of three or four rats per cage, with cage mates from the same enrichment cage, and all rats were placed under restricted food access for the second period of post-surgery testing (starting at 75 days post-surgery). Rats from the enriched cages were returned daily to the EE for a period of 1.5–2 h at the end of each session of this second post-surgery period of spatial working memory testing, followed by their daily food ration on return to the standard cages. After completion of this second period of post-surgery testing, however, all rats were returned to standard caging and no further enrichment was provided. Rats were retested for the third and final post-surgery reinforced spatial alternation test starting at 120 days post-surgery. After confirmation of accurate

lesions, the final group numbers were: SC-SHAM, $n = 11$; SC-ATN, $n = 8$; EE-SHAM, $n = 10$; and EE-ATN, $n = 11$ (four additional SC-ATN rats were excluded due to the ATN lesions being too small and one EE-ATN rat was excluded due to a misplaced lesion; see Histology and Lesion evaluation sections).

Apparatus and non-matching-to-sample spatial working memory testing

The same cross-maze apparatus and test room was used as described for Experiment 1, and sessions were again conducted on alternate days. All rats were trained to criterion on the spatial working memory task prior to surgery, and then re-tested on the same task for 10 sessions on each occasion at 14 days, 75 days and 120 days post-surgery. The only modification to the cross-maze procedure described in Experiment 1 was that preoperative training began from the start of testing with the 'opposite start position' for the sample and test runs for half of the six trials per session, which continued until the rats reach the criterion of 85% correct for each of two consecutive sessions (requiring between 10 and 14 sessions). The initial post-surgery testing provided a behavioural assay for the lesion deficit during a postoperative delay prior to enriched housing, and thus also enabled matched pairs of sham and ATN rats to be allocated to the subsequent housing environments. The third, and final, post-surgery test evaluated the durability of any enrichment and lesion effects, after which histology was conducted as per Experiment 1.

Results

Experiment 1 – enrichment starting 5 days after ATN surgery

Lesion evaluation

The largest and smallest acceptable lesions in the two lesion groups are shown in Fig. 1 (top panels). Only rats with ATN lesions meeting the histology criteria (eight SC and seven EE rats) were included in the behavioural analyses. In the EE-ATN group, there was a median (and range) of 79.4% ATN damage (54.2–87.4%), and only 0.8% damage to the LT (0.1–4.7%) and 2.9% damage to the MT region (1.8–9.4%). In the SC-ATN group, there was a median (and range) of 73.3% ATN damage (64.3–90.9%), 7.9% damage to the LT (1.5–21.2%) and 2.2% damage to the MT (0.6–19.9%). The volume of ATN and MT damage was similar in the EE-ATN and SC-ATN groups ($t_{13} < 1$), but the damage to the LT region, although still relatively minor, was slightly greater in the SC-ATN group than the EE-ATN group ($t_{13} = 2.8$; $P < 0.05$). The minor difference in LT damage in Experiment 1 is unlikely to have affected the results, particularly as both housing groups with ATN lesions in Experiment 2 experienced about 24% damage to the LT region. The median damage (and range) to the other adjacent structures was generally minimal in both groups. For the EE-ATN group, these were: interanteromedial nucleus, 1.8% (0.0–28.9%); laterodorsal nucleus, 1.0% (0.4–5.0%); and parataenial nucleus, 2.9% (2.4–17.7%). For the SC-ATN group, these were: interanteromedial nucleus, 17.65% (1.0–96.5%); laterodorsal nucleus, 3.8% (0.4–43.2%); and parataenial nucleus, 9.6% (2.4–43.9%). There was no damage to the paraventricular and posterior paraventricular nuclei, rhomboid nuclei or reuniens. All of the rejected lesion cases had ATN lesions that were too small, and the median damage (and range) sustained was: for the four EE-ATN cases, 14.7% ATN (4.0–24.6%), 1.1% LT (0.1–18.1%) and 0.1% MT (0.1–2.4%); for the four SC-ATN cases, 18.5% ATN (5.3–25.5%), 2.6% LT (0.8–3.1%) and 0% MT (0.0–0.2%).

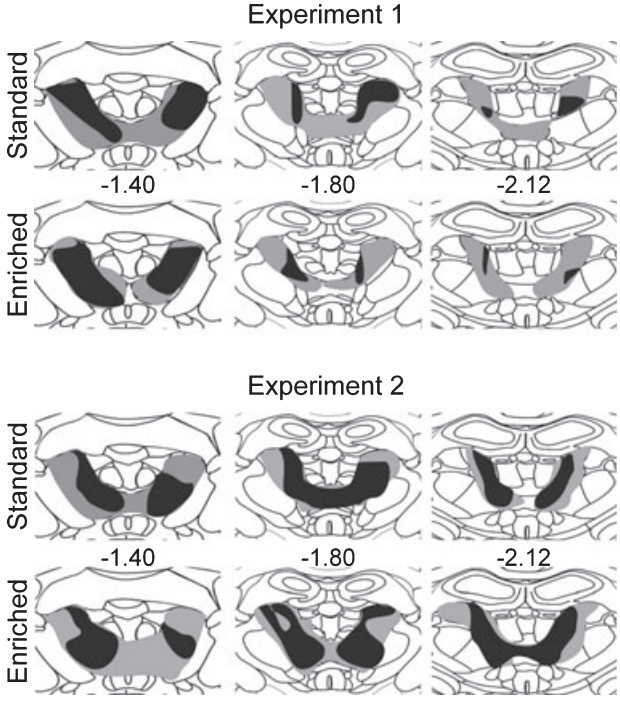

FIG. 1. Coronal schematics through the medial thalamus showing the area of cell loss in the smallest (black) and largest (grey) ATN lesions in SC and EE groups in Experiment 1 (top panels) and Experiment 2 (lower panels).

Non-matching to sample spatial working memory

Figure 2A shows spatial working memory performance in terms of overall percent correct, combined across both trial types (i.e. irrespective of 'same start position' and 'opposite start position' trials). Performance in all four groups of rats was equivalent at the end of training prior to surgery ($F < 1$). The sham groups, especially the SC-SHAM group, showed an initial reduction in performance at 40 days post-surgery, after the postoperative differential housing period, but they rapidly reacquired the task and achieved 80–90% correct performance. As expected the SC-ATN group displayed poor spatial working memory performance throughout the 10 postoperative test sessions, which was consistently around chance levels. The important finding, however, was that the EE-ATN group clearly displayed an overall performance that was superior to that of the SC-ATN group, although less accurate than that of the two sham groups. This improvement was particularly clear towards the latter part of testing, when the performance of the EE-ATN group more consistently approached that of the SC-SHAM group.

A four-way ANOVA (group by housing by session by trial type) revealed highly significant effects for lesion ($F_{1,34} = 194.9$; $P < 0.0001$), reflecting the severe ATN lesion deficit, and for housing ($F_{1,34} = 41.73$; $P < 0.0001$), confirming that rats housed in EE performed better than the SC rats. However, the marked overall beneficial effects of postoperative enrichment on the ATN lesion deficit was confirmed by a highly significant lesion by housing interaction ($F_{1,34} = 23.01$; $P < 0.0001$). *Post hoc* Newman–Keuls tests verified that, collapsed across the 10 sessions and across trial types, the EE-ATN group reliably performed far better than the SC-ATN group ($P < 0.001$). But the EE-ATN group still achieved lower percent correct scores than both sham groups ($P < 0.001$), which did not differ ($P > 0.2$). All groups performed better when 'same start position' trials were used than when 'opposite start position' trials were used, resulting in a highly significant effect for

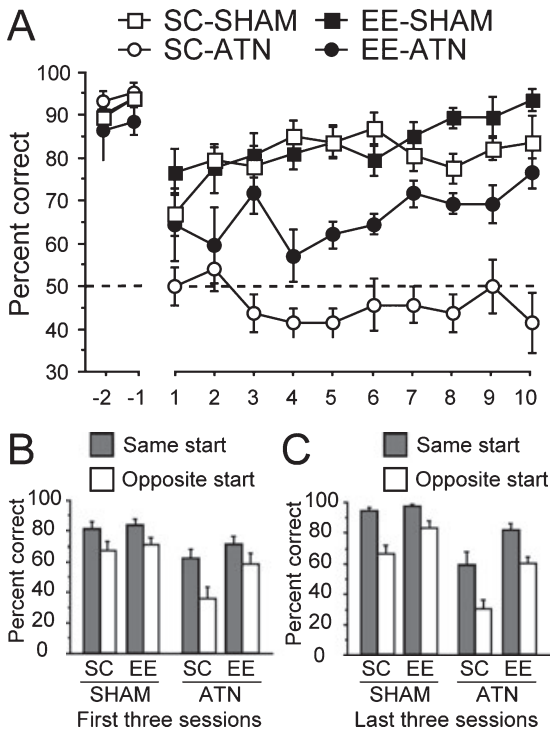

FIG. 2. Spatial working memory in the cross-maze (Experiment 1). (A) Mean (± SEM) performance for the last two sessions of presurgery testing (−1, −2) and across 10 sessions of post-surgery testing after the 30-day enrichment period. (B) Performance for the first three sessions expressed separately for the 'same start position' trials (same start position used for both sample and test run per trial) and 'opposite start position' trials (opposite start position used for the test run). (C) Same as (B), but for the last three sessions. ATN, neurotoxic lesion of the anterior thalamic nuclei; EE, enriched environment; SC, housed in standard group conditions.

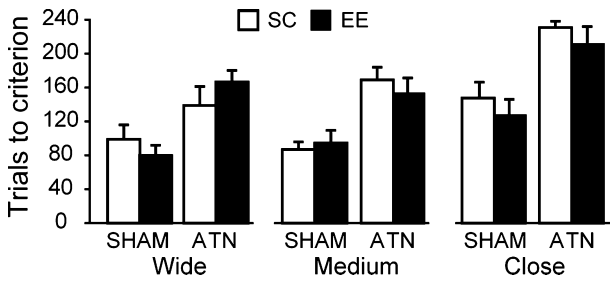

FIG. 3. Acquisition of fixed locations in the radial-arm maze (Experiment 1). Mean trials to criterion (± SEM) on each of the three spatial pattern separation problems (wide, intermediate and close). ATN, neurotoxic lesion of the anterior thalamic nuclei; EE, enriched environment; SC, housed in standard group conditions.

trial type ($F_{1,34} = 137.48$; $P < 0.0001$). There was also a significant session by trial type interaction ($F_{1,306} = 2.83$; $P < 0.01$), because overall performance on the 'same start position' trials generally improved across sessions, whereas that for the 'opposite start position' trials did not change markedly when the mean performance was assessed across all rats combined.

To provide a clearer perspective on the effects of trial type, ATN lesions and enrichment, performance for the two trial types was assessed for the first three sessions and then for the last three sessions (see Fig. 2B and C, respectively). For the first three sessions the EE-ATN group achieved better performance when compared with the SC-ATN group on both trial types (housing main effect, $F_{1,34} = 5.09$; $P < 0.05$; housing by lesion interaction, $F_{1,34} = 2.33$; $P > 0.10$; Fig. 2B, left panel). On these sessions, the EE-ATN group performed above chance (50%) for the 'same start position' trials ($t_6 = 4.49$; $P < 0.01$), but no other differences relative to chance were significant for the lesion groups. The more pertinent finding, however, was that for the last three sessions there was even clearer evidence of improved performance in the EE-ATN group compared with the SC-ATN group, resulting in a significant lesion by housing interaction ($F_{1,34} = 8.13$; $P < 0.01$). On these last sessions, the EE-ATN group was significantly above chance for both the 'same start position' trials ($t_6 = 9.84$; $P < 0.01$) and the 'opposite start position' trials ($t_6 = 2.51$; $P < 0.05$). By contrast, the SC-ATN group was at chance levels for the 'same start position' trials ($t_7 = 1.24$; $P > 0.25$) and below chance for the 'opposite start position' trials ($t_7 = -3.32$; $P < 0.02$). In summary, the main finding was that enrichment markedly improved performance after ATN lesions, and a mean improvement to above chance levels was evident on both types of spatial working memory trial.

Spatial pattern separation problems

This task commenced 100 days post-surgery. Performance was expressed as the trials to criterion for the four groups in each of the three spatial discrimination problems (Fig. 3). Rats that failed to achieve criterion within 15 days for the wide and medium spatial separation problems, and 18 days for the close separation problem, were given a 'trials to criterion' score equal to the maximum number of trials used for that task plus 2 days (204 trials for the wide and medium separation problems; 240 trials for the close separation problem). This addition of 2 days extended the trials to criterion score for a rat by the minimum number of trials (24) that would be theoretically needed to attain criterion. The number of rats per group that failed to reach criterion on each problem was as follows: the wide separation, three (of eight) SC-ATN rats, two (of seven) EE-ATN rats, one (of 11) SC-SHAM rats; the medium separation, one (of 12) EE-SHAM rats, four SC-ATN rats, three EE-ATN rats; close separation, three SC-SHAM rats, seven SC-ATN rats, three EE-SHAM rats and five EE-ATN rats.

Overall, the rats made more errors when learning the third problem that used adjacent arm locations than during acquisition of the first two problems in the radial-arm maze (problem main effect, $F_{2,68} = 19.28$; $P < 0.001$). The rate of acquisition did not differ between the wide and intermediate spatial separations (Newman–Keuls, $P > 0.5$), but the difference between these tasks and the third problem was highly significant ($P < 0.001$). There was also a highly significant effect for lesion ($F_{1,34} = 45.35$; $P < 0.0001$), with ATN rats being clearly impaired relative to sham rats. There was, however, no evidence that the acquisition of the fixed rewarded locations by ATN rats was minimized by the use of a 'wide' (easier) spatial pattern separation (lesion by problem interaction, $F_{2,68} < 1$). In addition, prior history of postoperative enrichment did not change the rate of acquisition on these spatial discrimination problems (housing, $F_{1,34} < 1$; housing by lesion interaction, $F_{1,34} < 1$; housing by lesion by problem interaction, $F_{2,68} = 1.54$; $P > 0.2$). The same conclusions were evident when the groups were evaluated using non-parametric analyses. For each problem, Kruskal–Wallis tests confirmed group differences (all $H_{3,38} > 13.00$; $P < 0.01$), and rats with ATN lesions performed worse than sham rats (Mann–Whitney U-test, $P < 0.002$), but there were no enrichment effects (Mann–Whitney U-test, $P > 0.50$). The same conclusions were also evident when using errors across sessions or the proportion of rats achieving criterion on each problem.

Experiment 2 – enrichment delayed until 40 days post-surgery

Lesion evaluation

The largest and smallest acceptable ATN lesions are shown in Fig. 1 (lower panels). Eight SC-ATN and 11 EE-ATN rats met the histology inclusion criteria. The ATN lesions were similar to Experiment 1, except that there was now slightly more damage to the LT region in both standard-housed and enriched groups, and fewer rats were excluded due to having small lesions. In the EE-ATN group there was a median (and range) of 78.7% damage to the ATN (53.7–97.9%), 4.3% damage to the MT region (0.7–13.9%) and 25.0% damage to the LT region (8.5–36.6%). The damage was almost identical in the SC-ATN group (all EE-ATN vs SC-ATN comparisons, $t_{17} < 1.01$), with a median (and range) of 78.6% damage to the ATN (59.0–93.7%), 3.1% to the MT region (0.2%-8.2%) and 23.3% to the LT region (12.4–36.4%). With the exception of the interanteromedial nucleus, the median damage (and range) to the other adjacent structures was again generally minimal in both groups. For the EE-ATN group these were: interanteromedial nucleus, 65.3% (3.6–86.3%); laterodorsal nucleus, 14.9% (1.8–30.1%); parataenial, 5.5% (1.2–18.5%); paraventricular and posterior paraventricular nuclei, 0%; rhomboid nuclei, 9.2% (0.6–18.1%) and reuniens nuclei, 0.2% (0.1–10.5%). For the SC-ATN group these were: the interanteromedial nucleus, 58.6% (35.1–91.9%); laterodorsal nucleus, 10.1% (7.3–23.3%); parataenial, 9.8% (4.1–28.2%); paraventricular and posterior paraventricular nuclei, 0%; rhomboid nuclei, 7.2% (1.2–20.6%); and reuniens nuclei, 1.6% (0.1–5%). The EE-ATN rat that was excluded had a posterior lesion (17.5% ATN, 48.9% LT, 28.4% MT), and the four excluded SC-ATN rats had limited ATN damage (median, 9.7%; range 9.4–23.9%) with 0.4% LT damage (0.2–9.2%) and 0.3% MT damage (0.2–5.9%).

Non-matching to sample spatial working memory

Initial post-surgery test. The initial spatial working memory test, conducted 14 days post-surgery and prior to enrichment, revealed the expected severe deficits after ATN lesions ($F_{1,36} = 605.01$; $P < 0.0001$; Fig. 4A, left panel). The matching procedure produced two groups of rats with ATN lesions that showed equally severe impairments throughout this first test. The only other significant finding for this pre-enrichment test was that all groups showed some improvements across the 10 sessions ($F_{9,324} = 9.82$; $P < 0.001$). As for Experiment 1, there was a main effect for trial type, with rats experiencing greater difficulty in solving the working memory task when the 'opposite start position' was used on the test run ($F_{1,36} = 132.25$; $P < 0.0001$).

The last three sessions were examined more closely to assess the effects of ATN lesions with respect to trial type when overall performance was relatively stable (Fig. 4B, left panel). For these sessions, the severely impaired overall performance of the ATN rats (lesion effect, $F_{1,36} = 255.81$; $P < 0.0001$) was relatively worse in terms of the 'opposite start position' trials (lesion by trial type, $F_{1,36} = 25.20$; $P < 0.001$), in both the SC-ATN group and the

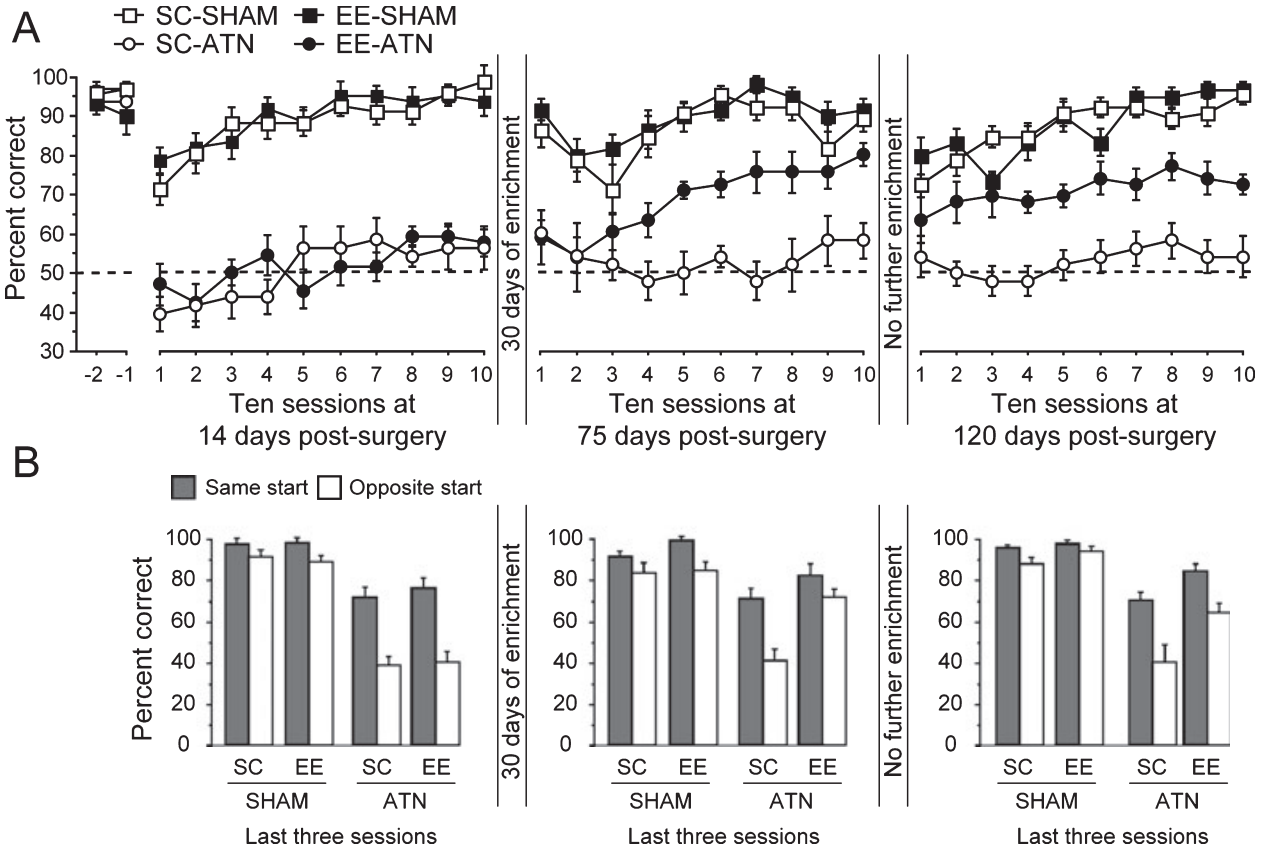

FIG. 4. Spatial working memory in the cross-maze (Experiment 2). (A) Mean (± SEM) performance for the last two sessions of presurgery testing (−1, −2) and 10 sessions of post-surgery testing prior to the enrichment period (left panel), after the 30-day period of enrichment (middle panel) and at 120 days post-surgery (right panel). (B) Performance for the last three sessions of each test expressed separately for the 'same start position' trials (same start position used for both sample and test run per trial) and 'opposite start position' trials (opposite start position used for the test run), prior to enrichment (left panel), after enrichment (middle panel) and at 120 days post-surgery (right panel). ATN, neurotoxic lesion of the anterior thalamic nuclei; EE, enriched environment; SC, housed in standard group conditions.

EE-ATN group that would later be housed in enriched conditions. On this occasion, the ATN rats performed above chance for the 'same start position' trials ($t_{18} = 7.86$; $P < 0.0001$), and below chance for the 'opposite start position' trials ($t_{18} = -3.30$; $P < 0.01$).

Initial post-enrichment performance. For the test conducted at 75 days post-surgery, immediately after the 30-day enrichment period, the two sham groups continued to perform at a high level, while the SC-ATN group remained at its previous, near-chance level of performance (Fig. 4A, middle panel). The important finding, however, was that the EE-ATN group now demonstrated a reduced spatial working memory deficit. At the start of testing the EE-ATN group performed no differently to the SC-ATN group, but the performance of the EE-ATN group then improved towards that of the sham groups by the end of testing. These observations were supported by a highly significant lesion effect ($F_{1,36} = 108.92$; $P < 0.0001$), housing effect ($F_{1,36} = 13.28$; $P < 0.001$), and lesion by housing ($F_{1,36} = 5.55$; $P < 0.05$) and lesion by housing by session interactions ($F_{9,324} = 2.13$; $P < 0.05$). A highly significant main effect for trial type was also evident ($F_{1,36} = 90.01$; $P < 0.0001$), with 'opposite start position' trials again being more difficult to solve for all groups.

The effects of trial type for performance on the last three sessions (Fig. 4B, middle panel) confirmed the marked benefits of enrichment, with a significant housing effect ($F_{1,36} = 11.83$; $P < 0.02$), and significant interactions for housing by lesion ($F_{1,36} = 5.11$; $P < 0.03$) and housing by lesion by trial type ($F_{1,36} = 6.44$; $P < 0.02$). As in Experiment 1, the EE-ATN group displayed improved performance compared with the SC-ATN group for both types of trial. Specifically, the EE-ATN group achieved a significantly better than chance level of performance for both the 'same start position' trials ($t_{10} = 6.50$; $P < 0.0001$) and the 'opposite start position' trials ($t_{10} = 4.73$; $P < 0.001$). The SC-ATN group displayed above chance performance for the 'same start position' trials ($t_7 = 3.52$; $P < 0.01$) and only chance performance for the 'opposite start position' trials ($t_7 = -1.52$; $P > 0.15$).

Performance at 120 days (4 months) post-surgery. Overall performance on this final test replicated in large part the earlier findings (Fig. 4A, right panel). The SC-ATN group showed a sustained severe deficit in spatial working memory. The two sham groups maintained a high level of performance, although accuracy was lower at the start of testing and improved thereafter. Again, the important finding was that the EE-ATN group displayed an intermediate level of performance, which on this occasion did not approach that of the sham groups at the end of testing. These observations were supported by significant main effects for lesion ($F_{1,36} = 97.92$; $P < 0.0001$), housing ($F_{1,36} = 12.95$; $P < 0.001$) and session ($F_{9,324} = 9.01$; $P < 0.0001$), and a lesion by session interaction ($F_{9,324} = 2.19$; $P < 0.05$). As before, overall performance was better for the 'same start position' trials (trial type, $F_{1,36} = 81.16$; $P < 0.0001$). Most relevant, the lesion by housing interaction was again significant ($F_{1,36} = 11.87$; $P < 0.01$). Thus, the overall effect of enrichment on spatial working memory was resilient over time, at 4 months post-lesion, despite the enrichment period being introduced for a restricted period of 30 days that started at only 40 days post-surgery.

The effects of trial type for performance on the last three sessions (Fig. 4B, right panel) once again confirmed the beneficial effects of enrichment, with a significant housing effect ($F_{1,36} = 21.31$; $P < 0.001$) and housing by lesion interaction ($F_{1,36} = 8.77$; $P < 0.01$). The two sham groups showed improvements on the 'opposite start position' trials relative to the lesion groups at the end of testing, which produced a lesion by trial type interaction for the last three sessions ($F_{1,36} = 10.52$; $P < 0.01$). As before, the EE-ATN group displayed improved performance compared with the SC-ATN group on both trial types. Specifically, the EE-ATN group displayed above chance level of performance for both the 'same start position' trials ($t_{10} = 11.25$; $P < 0.0001$) and the 'opposite start position' trials ($t_{10} = 3.12$; $P < 0.02$). By contrast, the SC-ATN group displayed above chance performance for the 'same start position' trials ($t_7 = 5.78$; $P < 0.001$) and chance performance for the 'opposite start position' trials ($t_7 = -1.09$; $P > 0.30$).

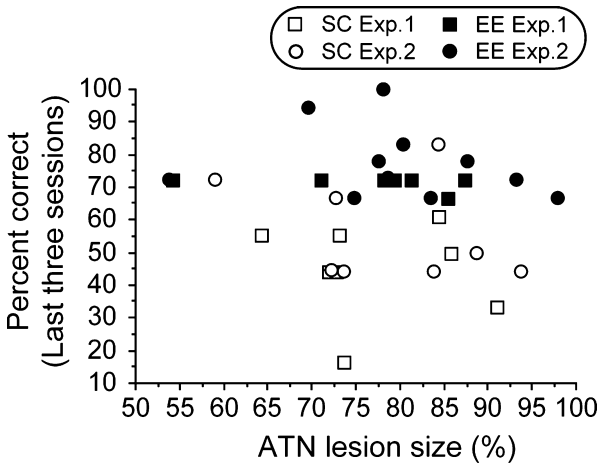

FIG. 5. Individual spatial working memory performance on the last three sessions on the test conducted immediately after the 30-day period of enrichment for all rats with ATN lesions in Experiment 1 and Experiment 2 (EE-ATN, $n = 18$; SC-ATN, $n = 16$). Overall percent correct is plotted as a function of lesion size. Some symbols overlap. ATN, neurotoxic lesion of the anterior thalamic nuclei; EE, enriched environment; SC, housed in standard group conditions.

Individual performance, lesion size and spatial working memory

Figure 5 shows the spatial working memory performance in individual rats on the last three sessions on the test conducted immediately after the 30-day period of enrichment, combined across the two experiments for the EE-ATN ($n = 18$) and SC-ATN groups ($n = 16$). These data reflect the last 3 days shown in Fig. 2A and the middle panel of Fig. 4A. Clearly, there was little overlap in scores for the ATN rats that experienced postoperative enrichment and the rats that only experienced SC. There was no evidence that the benefits of postoperative enrichment varied as a function of lesion size. The performance–lesion size correlation was close to zero for both the combined SC-ATN group ($r = -0.17$, d.f. = 14, $P > 0.5$) and the combined EE-ATN group ($r = -0.16$, d.f. = 16, $P > 0.5$). The size of the lesion to adjacent thalamic regions also did not correlate significantly with performance in either group.

Discussion

This study provides the first evidence that some deficits associated with brain injury to the ATN, which are strongly implicated in diencephalic amnesia, may be amenable to therapeutic intervention. A deficit in non-matching-to-sample spatial working memory represents one of the core findings in the rat ATN lesion model (e.g. Aggleton *et al.*, 1995; Warburton & Aggleton, 1999; Warburton *et al.*, 1999, 2001; Ward-Robinson *et al.*, 2002). In the present study, near chance level of performance on this reinforced spatial alternation task was evident in the standard-housed rats in two experiments after highly localized ATN lesions. Such lesions are not subject to the interpretation confounds that arise when the ATN lesions extend to the

adjacent thalamic regions (Warburton *et al.*, 1999). As with previous reports, the severity of the deficit probably reflected at least in part the cross-maze procedure that was employed, which restricted the benefits of an egocentric strategy (Aggleton *et al.*, 1996; Warburton *et al.*, 1999). In addition, Experiment 2 provided new evidence of the long-term nature of this impairment when rats are housed in standard conditions, despite preoperative training and repeated postoperative testing on three occasions over a 4-month period. It is therefore of considerable interest that 30 days of enriched housing, irrespective of whether enrichment was introduced either at 5 days (Experiment 1) or delayed until 40 days post-surgery (Experiment 2), markedly ameliorated this severe and otherwise permanent spatial working memory impairment. It was especially remarkable that, on the spatial working memory tests conducted after the enrichment period, there was little overlap for the last three sessions between the performance of individual rats with ATN lesions that were housed in standard conditions and that of rats with ATN lesions that experienced an enriched environment. The EE-ATN group, however, remained impaired relative to the intact controls. It is also important that treatment gains for spatial working memory were maintained at 4 months post-surgery in Experiment 2, despite no further enrichment. These robust findings add significantly to previous literature that enrichment can promote recovery of function in some instances of acute brain injury (Dalrymple-Alford & Kelche, 1985; Will & Kelche, 1992; Johansson, 2003; Will *et al.*, 2004), as no previous evidence exists to our knowledge on the effects of enrichment after lesions to the limbic thalamus.

Experiment 1 also showed that ATN lesions produced clear deficits for the acquisition of spatial pattern separation problems based on fixed locations in a 12-arm maze, irrespective of the pattern separation between the correct and incorrect arms. The fact that enrichment did not reduce these deficits suggests caution in assuming that spatial memory in general may be ameliorated by enrichment of rats with ATN lesions. All groups found the adjacent arm discrimination, which provided overlapping spatial information, more difficult to solve than the wide or intermediate separation problems. There is little demand for pattern separation in the intermediate and wide problems because there would be little or no overlap of relevant spatial information in those tasks. Hence, the existence of a general impairment in ATN rats irrespective of the separation between arms suggests that the ATN are involved in more general aspects of spatial memory, such as consolidation, rather than spatial pattern separation. One important difference between the radial-arm maze tasks and the cross-maze task was that the latter examined working memory, which implies that the benefits of enrichment on spatial tasks after ATN lesions may be restricted to working memory processes. Alternatively, subtle procedural differences and variations in the cognitive and behavioural demands of the radial-arm and cross-maze tasks could explain the failure of enrichment to ameliorate acquisition of the simultaneous discrimination problems in the radial-arm maze. For example, the rats were already at the choice point (the central hub) for the discrimination problems, whereas the cross-maze procedure afforded rats the opportunity to approach a choice point by traversing a start stem. The latter procedure would more readily encourage path integration and the use of directional and allocentric cues to guide spatial behaviour (Whishaw *et al.*, 1995, 1997). Another difference was that the radial-arm maze was rotated between trials while the rat remained in the central hub, and this procedure would introduce additional distraction and vestibular stimulation, which is known to impair the ability to use spatial information (Kirwan *et al.*, 2005). Clearly, evidence from more tasks is required before concluding that postoperative enrichment in ATN rats does not improve spatial reference memory in general. The reason for doubting such a conclusion is that beneficial effects of enrichment have been found in the Morris water maze in other examples of brain injury, including hippocampal system lesions, transgenic Alzheimer models and neonatal hypoxia-ischaemia models (Galani *et al.*, 1998; Frick *et al.*, 2003; Jankowsky *et al.*, 2005; Pereira *et al.*, 2007). The water maze differs from the radial-arm maze spatial discrimination problems used here in that the former encourages continuous navigation in an 'open' arena and place acquisition that is based on many differing trajectories and viewpoints, the use of which may benefit from exposure to an enriched environment.

There is good evidence that the effects of ATN lesions often resemble those of hippocampal system lesions (e.g. Warburton *et al.*, 1999; Wolff *et al.*, 2006), which lends support to Aggleton & Brown's (1999) hypothesis that the ATN provide a critical nodal point in an extended hippocampal system. For example, hippocampal, fornix and restricted ATN lesions generally produce similar impairments in spatial working memory in a T-maze and spatial reference memory in a water maze (Aggleton & Brown, 1999; Warburton *et al.*, 1999). The view that the ATN and the hippocampal system function in an interdependent manner, at least for spatial memory tasks, finds support also from disconnection analyses that combined unilateral ATN lesions with either contralateral fornix or hippocampal lesions (Warburton *et al.*, 2000, 2001; Henry *et al.*, 2004). There is, however, some evidence that damage to these components of this extended system do not always produce the same impairments, at least in conditional associative tasks when rats are required to learn an arbitrary association. Specifically, impaired acquisition after both ATN and hippocampal lesions, but intact performance after fornix lesions, has been found when a rat must choose an object based on its location (Sziklas *et al.*, 1998; Sziklas & Petrides, 1999). By contrast, hippocampal lesions impair acquisition of a conditional task in which rats select a location, based on a visual cue, but in this instance both rats with ATN lesions and those with fornix lesions appear to be able to use either egocentric cues or place cues and achieve normal rates of acquisition (Sziklas *et al.*, 1998; Sziklas & Petrides, 1999, 2002, 2004, 2007). The deficit on the radial-arm maze discrimination problems after ATN lesions in the current study is thus interesting in providing a second example of a possible dissociation between the effects of fornix and ATN lesions, as fornix lesions produce a severe impairment when the arms are adjacent but have no effect when the arms are widely separated (McDonald & White, 1995). It is not known whether hippocampal lesions spare the acquisition of widely separated locations in a radial-arm maze; if so, the current findings would provide a unique effect of ATN lesions on spatial memory.

Although the generality of the effects of enrichment as a potential therapy in animal models of diencephalic amnesia requires further study, this prospect receives strong support from several features of the spatial working memory improvements demonstrated in the current study. First, our findings provide a first step towards proof in principle that severe memory deficits after diencephalic injury can be modified to some extent. Second, behavioural therapy in clinical cases is likely to be delayed until after any initial deficits have stabilized. The current study demonstrated that severe ATN-induced deficits in spatial working memory can be ameliorated even when the treatment phase is introduced a long time post-injury, and despite the prior identification of severe memory impairments. This evidence is particularly encouraging, especially as surprisingly few studies have examined similar delayed-enrichment effects or the long-term effects of a brief period of prior enrichment. In ischaemic rats, enrichment introduced at 5 and 14 days produced benefits, but a delay of 30 days was too long to result in functional gains (Johansson, 1996; Biernaskie *et al.*, 2004). The only similar study to our knowledge of beneficial effects of

delayed enrichment was in the context of learning and memory deficits that gradually emerge after an earlier 192 IgG-saporin cholinergic forebrain lesion, when 3 months of enrichment starting 9 months after forebrain injury markedly compensated these deficits (Paban *et al.*, 2005). Our data show that a 40-day delay before enrichment still produced substantial functional gains in spatial working memory after neurotoxic ATN lesions. Moreover, these benefits remained at 4 months post-surgery, despite no further enrichment. While the ATN rats housed in standard conditions remained close to chance performance overall, the ATN group with prior enrichment continued to show impressive gains, although their spatial working memory performance was below that of the sham groups, which continued to improve towards optimal performance.

An important issue with respect to the therapeutic value of enrichment was whether it produced recovery or sparing of function. Also, the potential value of enrichment or similar therapy as a means to obtain lasting gains in diencephalic amnesia depends on the type of learning improvements achieved, so it is important to consider the nature of the spatial working memory improvements that were found. In Experiment 2, the initial postoperative non-matching-to-sample test clearly characterized the severe deficit produced by an ATN lesion, and the permanence of this deficit was evident in two subsequent tests. While recovery from a prior and otherwise sustained deficit was thus observed, as opposed to sparing of the emergence of a deficit, the enriched ATN rats were impaired at the start of testing. It is, then, clear that treatment and task-relevant training were required in combination for optimal functional gains. Rats appear to rely on more than one strategy during forced alternation working memory testing, including the use of spatial (allocentric; direction) and non-spatial cues (egocentric response; Dudchenko, 2001; Skinner *et al.*, 2003; Futter & Aggleton, 2006). As in previous reports (Aggleton *et al.*, 1996; Warburton *et al.*, 1999), even the intact rats in the current study found the 'opposite start position' trials more difficult than the 'same start position' trials, the latter of which could be solved by alternating body turns (egocentric cues). As this difference between the two types of trial was evident in both standard and enriched rats, it seems unlikely that the effect of enrichment was based primarily on the use of intramaze (e.g. olfactory) cues to alternate goal arms, because such cues would assist performance irrespective of the start position used on the test run. This observation makes it also unlikely that minimizing the availability of olfactory cues by maze rotation was responsible for the lack of enrichment effects in the radial-arm maze tasks. Standard-housed ATN rats were disproportionately impaired on the 'opposite start position' working memory trials, which replicates reports that ATN lesions produce a weakness in using spatial/non-egocentric cues and/or a preference in using egocentric cues (Aggleton *et al.*, 1996; Sziklas & Petrides, 1999; Warburton *et al.*, 2001). The enriched ATN group achieved significantly better than chance levels of performance on both types of trial, including the 'opposite start position' trials that specifically benefit from the utilization of spatial cues, so the benefit of enrichment on spatial working memory was not based simply on an improved use of an egocentric strategy or simply the inhibition of this non-spatial strategy. In addition to facilitating or improving the use of spatial cues, it is possible that enrichment in ATN rats improved spatial working memory in part through the early inhibition of non-optimal strategies, the use of multiple strategies, or flexibility in switching from one type of preferred strategy to another across training.

Enrichment has diverse effects on brain function, including increased angiogenesis, gliogenesis, neurogenesis, synaptogenesis, cortical dendritic morphology, and various biochemical and neurotrophic factors (van Praag *et al.*, 2000; Frick & Fernandez, 2003; Will *et al.*, 2004), any of which may influence behaviour. Thus, a number of potential neural mechanisms could explain the beneficial enrichment effects reported here, and an explanation of the current findings could be due to a number of such factors. The brain regions responsible for the current improvements are also uncertain, but may for example be due to changes in unrelated regions, such as the parietal cortex (Leggio *et al.*, 2005), or to the neurocircuitry directly influenced by ATN lesions. It is especially interesting that some of the effects of ATN damage may reflect changes in the functional status of the distributed neural systems of which the ATN is one component, so it is tempting to speculate that a reversal of some of these changes may at least in part explain the benefits observed in enriched ATN rats in the current study (Gabriel, 1993; Taube & Muller, 1998; Dalrymple-Alford *et al.*, 1999; Jenkins *et al.*, 2002; Savage *et al.*, 2003; Vann & Aggleton, 2004).

In conclusion, this study provides the first evidence that enrichment can ameliorate some of the severe and long-lasting deficits associated with ATN lesions, even when introduced a long time after the initial brain insult. The current findings on spatial working memory are most encouraging, but the lack of beneficial effects of enrichment in the fixed location problems in the radial-arm maze indicate that additional evidence from animal models is required before the prospect of therapeutic interventions for diencephalic amnesia. There is also a need to investigate the neuronal changes whereby enrichment may produce improvements after ATN damage, because these mechanisms may also suggest new therapeutic opportunities.

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Abbreviations

ATN, anterior thalamic nuclei; AM, anteromedial; AV, anteroventral; B–L, bregma to lambda; EE, enriched environment condition; LT, lateral thalamic region; MT, posteromedial thalamic region; SC, standard housing conditions.

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