

Short communication

Effects of thalamic lesions on repeated relearning of a spatial working memory task

K. Ulrich^a, P.N. Aitken^a, W.C. Abraham^a, J.C. Dalrymple-Alford^b, N. McNaughton^{a,*}^a Department of Psychology and Brain Health Research Centre, University of Otago, POB56, Dunedin 9054, New Zealand^b Department of Psychology, University of Canterbury, POB4800, Ilam, Christchurch, New Zealand

HIGHLIGHTS

- Control rats show repeated forgetting and relearning with long re-test intervals.
- Anterior thalamic lesions repeatedly block relearning except with a short interval.
- Spatial working memory allows repeated within-subject testing of pro-cognitive agents.

ARTICLE INFO

Article history:

Received 16 September 2013

Received in revised form

14 November 2013

Accepted 2 December 2013

Available online 12 December 2013

Keywords:

Thalamus

Learning

Animal model

Diencephalic amnesia

ABSTRACT

Anterior thalamic (ATN) dysfunction produces memory deficits in rats and humans. The current study shows that, with a substantial delay between post-surgery tests, controls show repeated relearning on a spatial working memory task whereas rats with neurotoxic ATN lesions showed repeated relearning deficits. Rats were pre-trained to criterion, but not over trained, on the spatial task. ATN lesions produced the expected spatial memory and relearning deficits about two weeks post-surgery and again either one or 15 weeks later. Control rats also showed forgetting post-surgery and after a 15 week break, relearning the task on each occasion. Controls with only a 1 week break before their final re-test showed negligible forgetting. Thus, a short break between re-tests replicated previous findings with ATN lesions, but a long break allows repeated comparison of rates of learning from a common starting point in sham and ATN-lesioned animals, providing a useful paradigm for future testing of pro-cognitive treatments.

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A wide range of brain disorders and injuries lead to memory deficits and amnesia. Studies in both rats and humans have suggested that the thalamus, and especially the anterior thalamic nuclei (ATN), are involved in normal memory functioning and in “diencephalic” amnesias [1,2]. Typically, damage to the ATN or one of its major afferents, the mammillo-thalamic tract, results in learning and memory deficits in monkeys, rats [3–6] and human Korsakoff’s syndrome [7,8], where ATN damage appears critical [9]. Of particular note, experimental disruption of ATN produces severe deficits in spatial and temporal working memory [3,10,11]. Here we report that the effects of ATN damage on spatial learning can be repeatedly tested with control and ATN rats starting at similar level, providing a useful platform for testing treatments that could ameliorate these learning and memory deficits.

While repeated testing of effects of ATN damage on anterograde amnesia is straightforward, repeated testing of learning deficits

is usually complicated by higher (often close to asymptotic) initial performance of shams when retested. We, therefore, tested whether a long break between re-tests would allow comparison of rates of learning in sham and ATN-lesioned rats starting from a similar performance baseline. We trained rats to criterion, without overtraining, before surgery and then re-tested them both shortly after surgery (4 weeks after their first test) and a second time with either a short (one week) or a long (15 weeks) break between the two post-surgery re-tests.

All experiments were approved by the University of Otago Animal Ethics Committee (101/2008), and conducted in accordance with New Zealand animal welfare legislation. Thirty-nine adult male Long–Evans rats (19 for the long-break experiment, 20 for the short-break experiment) were obtained from the University of Otago animal breeding unit. The long-break rats were 22 weeks old (550–650 g) and the short-break rats were 9–10 weeks old (283–359 g) on their arrival in the laboratory and were kept in groups of 3–4 rats (except immediately after surgery) on a 12 h/12 h light–dark cycle (lights on at 6 am) with food and water provided *ad libitum*. They had at least 10 days after arrival before the start of testing. For testing, they were gradually adapted to, and then

* Corresponding author at: Department of Psychology, University of Otago, POB56, Dunedin 9016, New Zealand. Tel.: +64 3 479 7634; fax: +64 3 4798335.

E-mail address: nmcn@psy.otago.ac.nz (N. McNaughton).

Table 1

Table of A–P coordinates, which depended on the rat's measured bregma–lambda difference (B–L) for AM and AV Lesion Sites (in mm).

B–L	6.0–6.5	6.6–6.9	7.0–7.5	7.6–8.0	8.1–8.5	8.6–9.0
AV site	–2.55	–2.60	–2.65	–2.70	–2.75	–2.80
AM site	–2.45	–2.50	–2.55	–2.65	–2.65	–2.70

maintained on, one hour per day access to food, with weights checked frequently and maintained above 85% of free feeding weight.

For surgery, rats were anaesthetised with ketamine (0.75 ml/kg) and Domitor (0.5 ml/kg); and injected with atropine (0.2 ml/kg), Carprive (5 ml/kg) and Amphoprim (0.2 ml per rat); local anaesthetics (Marcaine: 0.4 ml/kg; and Lidocaine: 0.2 ml/kg) were infused into the scalp and the rat was placed in a stereotaxic frame using atraumatic ear bars (Kopf, Tujunga, USA) and the incisor bar set at 7.5 mm below the inter-aural line to minimise damage to the fornix from the injection needle. Three neurotoxin infusion sites in the anteroventral (AV) and the anteromedial (AM) thalamic nuclei in each hemisphere maximised ATN damage while minimising other damage. A–P coordinates varied with the distance between bregma and lambda as shown in Table 1.

The ML coordinate was ± 1.46 mm from the midline and depth from dura was -5.65 mm for the first AV lesion site and -5.80 mm for the second AV lesion site. AM coordinates were as for the AV site except that the A–P coordinate (see Table 1) was 0.1 mm more anterior than for AV and a single infusion was placed ± 1.16 mm lateral from the midline at -5.90 from dura. For the two AV lesion sites on each side, $0.16 \mu\text{l}$ N-methyl-D-aspartic acid (NMDA, Sigma–Aldrich Chemie GmbH, Steinheim, Germany) in phosphate buffer (pH 7.2) was infused, and for the AM lesion site on each side $0.18 \mu\text{l}$ NMDA was infused. Infusions were administered at $0.03 \mu\text{l}$ per minute using a motorised $1 \mu\text{l}$ Hamilton syringe and then the needle was left *in situ* for 3 min to allow diffusion before slow retraction. For sham rats, the needle was lowered to 1.5 mm above the AM and the dorsal AV lesion site and remained in place for the same time as normal but no liquid was infused. After surgeries, rats were held in individual cages and were closely monitored for at least 12 days before they were re-introduced to group housing conditions. In the older and heavier long-break rats 7 died during or shortly after surgery as a result of anaesthetic complications; in the short-break rats, 4 died.

Rats were trained prior to surgery on a spatial working memory task as described by Loukavenko et al. [12]. A T-maze embedded in a Plus-maze configuration was used.

The apparatus was raised 82 cm above the floor including two 1 m long start arms with guillotine doors ($38 \text{ cm} \times 11.4 \text{ cm} \times 0.3 \text{ cm}$) and two 0.4 m long bait arms with raised food wells at the end of the arms (2.5 cm diameter, 1 cm depth). The runways of all four arms had a width of 10.5 cm and were lined with metal edges (2.5 cm). Wooden blocks ($30 \text{ cm} \times 7 \text{ cm} \times 9.5 \text{ cm}$) with perspex wings (protruding 9.5 cm from the sides of the wooden blocks) were used to block the start/bait arms depending on the trial. Start and bait arms were labelled according to the cardinal directions (start arms: North (N) and South (S), bait arms: East (E) and West (W)) for trial sequencing. The maze was placed in a room with poster cues and a service duct and sockets on the walls, a chair at the south end of the South start arm, a black box ($35 \text{ cm} \times 35 \text{ cm} \times 35 \text{ cm}$) at the North end of the North start arm and two 22 cm diameter coloured bins, one on top of the other, containing Coco Pops. Two 15 W desk lamps mounted on the wall provided illumination.

Before the start of the first training sessions, rats were familiarised with the apparatus over the course of 5 days where they first were placed on the maze in groups (two days) and then individually, with Coco Pops scattered in the maze. For spatial working memory, subjects received six trials per day consisting of one forced

and one choice run per trial. All trials were non-matching to sample and sequences of start arms and of goal arms were counterbalanced. Half of the test runs used the opposite start arm to that used for the sample run, while the other half used the same start arm for both runs. There were 6 trials on each day of training. For each forced trial, a rat received four Coco Pops as food reward. For the choice runs, rats received four Coco Pops if they entered the correct arm. If they entered the wrong arm during training pre-surgery, they were given a correction trial; but no correction trials were given after surgery. Initial training continued for 14 days for long-break rats and for 10 days for short-break rats. The rats were then matched in pairs according to their percent correct scores over the last 4 days of pre-surgery training and one of each pair was assigned to lesion and the other to sham surgery.

After surgery rats were given a recovery period of at least twelve days before being placed back into group housing and on food deprivation. Surgeries took 2 weeks (with timing counterbalanced across groups) and two weeks after the last surgery (i.e. 4 weeks after their initial training) rats were tested for spatial working memory again for 5 days and then received either a short-break of 1 week or a long-break of 15 weeks before testing was repeated for another 5 days. After completion of experiments, rats were deeply anaesthetised with sodium pentobarbitone intraperitoneally (300 mg/ml) and perfused transcardially with physiological saline and 10% formalin in physiological saline. Frozen coronal sections were taken at $40\text{--}150 \mu\text{m}$ through the areas of interest and stained with thionin. Sections were mapped to Paxinos & Watson [13] and lesion area identification was checked by a second person independently.

All rats that were infused with NMDA displayed large ATN lesions characterised by loss of neural cells as well as significant gliosis in the target regions. The smallest and biggest lesions of rats that were infused with NMDA are shown in Fig. 1. Lesion extent varied according to the distance from bregma and percentage damage was assessed from A–P -1.18 to -2.28 in 0.12 mm steps separately for each hemisphere. The mean extent of damage across these A–P steps and pooling the two hemispheres were 91% for AV and 89% for AM for the long-break rats, and 81% for AV and 70% for AM for the short-break rats. No rats were excluded from final analysis, with the long break having 6 lesioned and 6 sham rats and the short break having 6 lesioned and 10 sham rats.

Most of the lesioned animals showed some additional damage in adjacent structures. These included a range of thalamic nuclei (anterodorsal, reticular, reticulostriatal, ventral anterior, mediodorsal, paratenial, ventromedial, laterodorsal, central medial, reuniens and paracentral) and the sublenticular extended amygdala (medial part), the caudate/putamen, and the globus pallidus. The damage to these various areas was generally marginal; and very few of them were damaged in multiple rats.

All rats acquired the spatial working memory task prior to surgery within 14 days for long-break rats and 10 days for short-break rats (data not shown). In the first post-operative re-test (Fig. 2A), all rats started with a relatively low level of performance ($\sim 60\%$ correct; chance = 50%). A test of the last day of pre-surgery training against the first day of post-surgery training (data not shown) showed significant forgetting (day $F_{(1,20)} = 12.9$, $p = 0.002$) that was similar in lesioned and matched sham rats (day \times surgery $F_{(1,20)} = 0.16$, $p > 0.6$; day \times surgery \times break $F_{(1,24)} = 0.017$, $p = 0.90$).

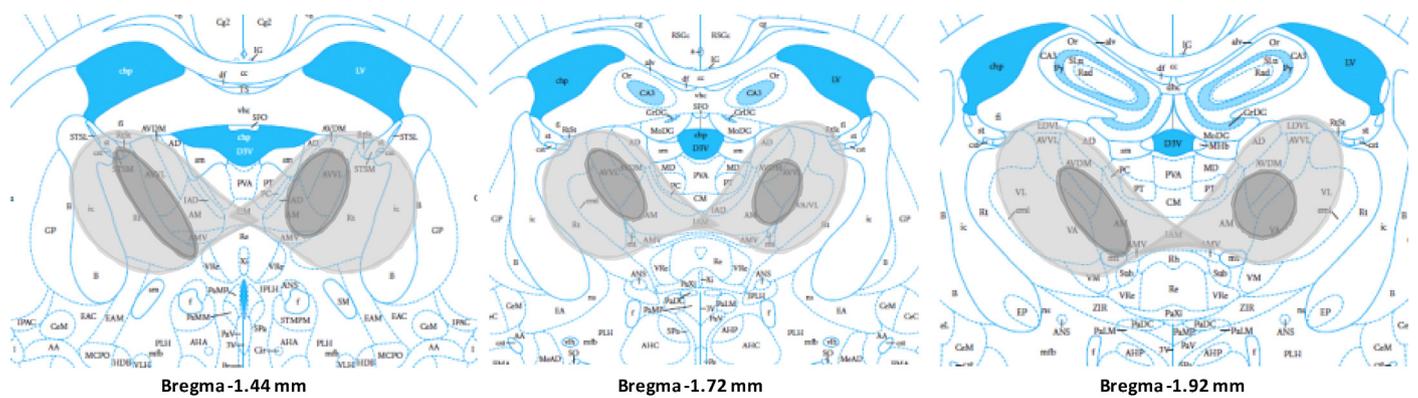


Fig. 1. Coronal schematics through the area of the anterior thalamus showing the extent of cellular loss and gliosis in the smallest (dark grey) and largest (light grey) lesions to the anterior thalamic nuclei. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Re-learning in sham condition rats on this first postoperative test stabilised at a high level (~80% correct); but lesioned rats did not show any improvement over the five days of training (final performance 55–65% correct). There were no apparent differences between the rats having a short break and those having a long break (except, perhaps, a drop in performance on the last day for the sham-short rats). Interestingly, after the long break (15 weeks, Fig. 2B), both sham and lesion groups again started at the same low level as at the start of the first post-operative test (~60%), indicating that even the sham rats forget the task after this interval. The sham group then relearned the task, although the terminal performance was not as good as in the first re-test. The lesion group showed no relearning effect, mirroring the first re-test. After the short break (1 week, Fig. 2C), the sham rats showed negligible forgetting, while the lesion rats also showed little change from the last day of the first re-test but this could be a floor effect rather than a lack of forgetting. Both groups showed signs of further learning.

Statistically, lesioned rats (collapsed across both breaks) performed significantly more poorly than the sham rats (surgery: $F_{(1,20)} = 14.97$, $p < 0.001$) and the extent to which the lesion affected the rate of relearning after the break depended on the length of the break (surgery \times break \times training day: deviation \times deviation \times linear: $F_{(1,20)} = 5.22$, $p = 0.002$). Post-hoc testing showed that the long-break rats showed a significant lesion effect that was particularly evident in the last few training days in both tests (days \times surgery, cubic \times deviation $F_{(1,10)} = 7.09$, $p = 0.024$); and there was no difference in the size of the lesion effect between the two re-tests (days \times test \times surgery, cubic \times deviation \times deviation $F_{(1,10)} = 0.22$, $p > 0.6$).

The most important result in the current study is that sham rats at the beginning of re-tests showed near chance performance both shortly after surgery and on a second re-test after a long break. In both cases, they then relearned the task to at least 75% correct within five days. This behaviour of our sham groups contrasts with the sham rats in Loukavenko et al. [12], where the same task was used but there was more extensive training and the rats reached higher performance levels in each re-test. Critically, their lesion rats started close to chance but their sham rats started well above chance at each re-test. With only a short break before the second re-test, the sham rats in the current study showed very little forgetting, and then showed some signs of further learning. Their failure in this second re-test (as with the sham long-break rats) to regain their level of performance in the first re-test is similar to the poorer performance observed for later re-tests by Loukavenko et al. [12]. While the short-break rats were younger than the long-break rats, and took fewer days to reach initial pre-surgery criterion, the duration of the break seems the most likely explanation for their lack

of forgetting in the second re-test. In their first re-test (i.e. after a 4 week break including surgery), short-break rats showed similar forgetting to long-break rats.

Pretraining before surgery was also used previously by Warburton et al. [6], using rats tested in both the water maze (a reference memory task) and a standard T-maze forced alternation task that were sensitive to ATN lesions for both tasks pre-surgery training was given for over two weeks with the water maze acquisition involving extensive overtraining such that performance was close to asymptote by the end of the first week (acquisition for the T-maze was not reported). Re-testing was carried out 2 weeks after surgery. In both tasks, there was very little sign of forgetting in the sham rats after surgery and any post-surgery learning by the shams was substantially less than for ATN lesion rats. As with the Loukavenko results, this suggests that for sham relearning to be clearly observed overtraining and short test-retest intervals should be avoided.

In the current study, rats with ATN lesions did not relearn the task either post-surgery or after the long break and their performance was only slightly above chance throughout each re-test. This demonstrates a persisting ATN-induced dysfunction, consistent with the standard-housed rats in the report by Loukavenko et al. [12]. The novel result in our experiment is that this learning deficit can be displayed relative to control rats starting with similar, poor, spatial working memory on multiple occasions after ATN lesions, provided that a long enough interval occurs between the tests. With only a 1 week break between re-tests, sham rats did not show substantial forgetting and both they and their matched lesion group showed similar rates of additional learning. In Loukavenko et al. [12], the rats were trained to a high level pre-surgery (reaching 95% correct) and neither post-surgery nor on later 10-day-long re-tests did sham performance on the first day of testing drop to the same level as lesioned rats. These data suggest that for repeated testing not only should the separation between the two tests be longer than 1 week but the initial test should not allow the control rats to become over-trained. While our data do not show where the critical limit for sham rats is between 1 and 15 weeks, it should be noted that the first re-test was after a break of 4 weeks, although, the highly significant forgetting in this case could have been produced or amplified by surgery.

In conclusion, if rats are trained only to criterion and given a 15 week break between tests, control and lesion performance drops to similar levels and the effects of ATN lesions on learning (and not just memory) can be repeatedly tested post-surgery. Our post-surgery results suggest that even a 4 week break may demonstrate this effect. This paradigm could be useful for within-subject pre-clinical testing of treatments for diencephalic amnesia.

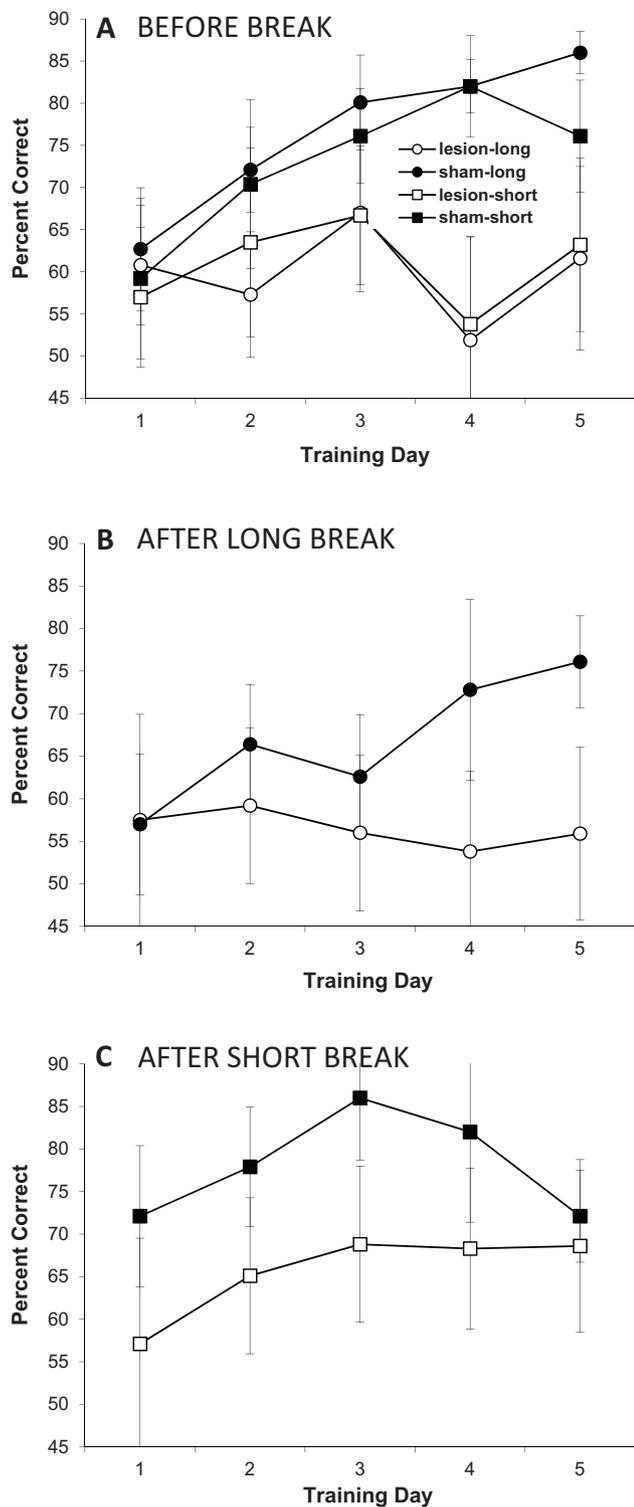


Fig. 2. Effects of thalamic lesions on working memory before and after a 1 week (short) or a 15 week (long) break from training. All rats were trained to criterion on the task pre-surgery (data not shown). (A) Data for the first re-test at least two weeks after surgery (BEFORE BREAK); “short” and “long” refer to the break the rats are about to experience. Both groups showed equivalent forgetting relative to pre-surgery, shams then show relearning and lesion rats do not; (B) Second post-surgery re-test, i.e. the third test overall, after the long-break. Forgetting is similar to the previous test, sham relearning does not reach the same high level of performance, and lesion rats (as before) show no relearning. (C) As for B but after the short break. Shams show little forgetting, both sham and lesion groups show signs of learning.

Acknowledgements

This work was supported by an International Investment Opportunities Fund grant from the Health Research Council of New Zealand to JD-A, WCA and NMcN. KU was supported by a University of Otago post-graduate scholarship.

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