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ORIGINAL ARTICLE

Impaired spatial working memory after anterior thalamic lesions: recovery with cerebrolysin and enrichment

Elena A. Loukavenko · Mathieu Wolff · Guillaume L. Poirier · John C. Dalrymple-Alford

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Abstract Lesions to the anterior thalamic nuclei (ATN) in rats produce robust spatial memory deficits that reflect their influence as part of an extended hippocampal system. Recovery of spatial working memory after ATN lesions was examined using a 30-day administration of the neurotrophin cerebrolysin and/or an enriched housing environment. As expected, ATN lesions in standard-housed rats given saline produced severely impaired reinforced spatial alternation when compared to standard-housed rats with sham lesions. Both cerebrolysin and enrichment substantially improved this working memory deficit, including accuracy on trials that required attention to distal cues for successful performance. The combination of cerebrolysin and enrichment was more effective than either treatment alone when the delay between successive runs in a trial was increased to 40 s. Compared to the intact rats, ATN lesions in standard-housed groups produced substantial reduction in c-Fos expression in the retrosplenial cortex, which remained low after cerebrolysin and enrichment treatments. Evidence that multiple treatment strategies restore some memory functions in the current lesion model reinforces the prospect for treatments in human diencephalic amnesia.

Keywords Diencephalic amnesia · Reinforced alternation · Hippocampal system · Retrosplenial cortex · c-Fos expression

E. A. Loukavenko and M. Wolff contributed equally.

E. A. Loukavenko (✉) · J. C. Dalrymple-Alford (✉)
Department of Psychology, New Zealand Brain Research Institute, University of Canterbury, Private Bag 4800, Christchurch 8140, New Zealand
e-mail: elena.moran111@gmail.com

J. C. Dalrymple-Alford
e-mail: jc.dalrymple.alford@gmail.com; john.dalrymple-alford@canterbury.ac.nz

M. Wolff (✉)
Univ.Bordeaux,INCIA, UMR 5287, 33400 Talence, France
e-mail: mathieu.wolff@u-bordeaux.fr

M. Wolff
CNRS, INCIA, UMR 5287, 33400 Talence, France

G. L. Poirier
Brain Mind Institute, École Polytechnique Fédérale de Lausanne, AAB201, Station 19, 1015 Lausanne, Switzerland
e-mail: guillaume.poirier@gmail.com

J. C. Dalrymple-Alford
Department of Medicine, University of Otago, Christchurch, New Zealand

Introduction

Clinical evidence shows that injury to the anterior thalamic nuclei (ATN) or its principal afferents makes a significant contribution to diencephalic amnesia (Carlesimo et al. 2011; Harding et al. 2000; Van der Werf et al. 2003a, 2003b). In rats, ATN lesions produce deficits in spatial memory, contextual fear memory and temporal memory for events, supporting the view that the ATN occupy a nodal point in an extended hippocampal system (Aggleton 2010; Aggleton et al. 2011; Henry et al. 2004; Marchand et al. 2013; Mitchell and Dalrymple-Alford 2006; Ward-Robinson et al. 2001; Wolff et al. 2006, 2008a). While spontaneous recovery of behavioral deficits after ATN lesions in rats does not occur, recovery of spatial working memory has been found when rats were placed in a complex enriched environment, even when this intervention was delayed 40 days post-surgery (Loukavenko et al. 2007).

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Restoration of the flexible use of spatial reference memory has also been shown (Wolff et al. 2008b).

The behavioral recovery from ATN lesions after enrichment has been encouraging, but pharmacological treatments may be more feasible in the clinical field. Cerebrolysin offers a unique pharmacotherapeutic option as it is a parenterally administered mixture of different active fragments of neurotrophic factors and peptides, including nerve growth factor and brain-derived neurotrophic factor (Sharma et al. 2012; Windisch 2000). Like enrichment (Will et al. 2004), various animal models have shown that cerebrolysin has pleiotropic actions that may facilitate recovery of function after brain injury. Cerebrolysin reduces apoptosis, promotes neurogenesis in the adult hippocampus and subventricular zone, improves glucose transport, increases dendritic complexity in hippocampus and cortex, and facilitates behavioral recovery in models of stroke, neurodegeneration and hippocampal system lesions (Francis-Turner and Valouskova 1996; Hartbauer et al. 2001; Juarez et al. 2011; Rockenstein et al. 2007; Ubhi et al. 2013; Vazquez-Roque et al. 2012). Cerebrolysin has also received favorable reports in Alzheimer's disease and vascular dementia clinical trials (Plosker and Gauthier 2009). The similarity of the effects following environmental enrichment and cerebrolysin suggests that cerebrolysin may also produce recovery of spatial memory after ATN lesions and that the combination of cerebrolysin and enrichment may have a stronger effect on recovery than enrichment alone.

The functional consequences of ATN damage on the neurobiology of the extended hippocampal system are most evident in the retrosplenial cortex (Aggleton 2008; Aggleton et al. 2011; Van Groen et al. 1993), where the expression of the immediate-early gene (IEG) products c-Fos and Zif268 is dramatically reduced in the granular superficial layers (Amin et al. 2010; Jenkins et al. 2002a, b, 2004; Poirier and Aggleton 2009; Albasser et al. 2007; Vann and Albasser 2009). In addition to spatial memory, therefore, we examined whether functional recovery of spatial working memory associated with cerebrolysin or enrichment after ATN lesions coincided with improved expression of c-Fos in the retrosplenial cortex. As in other studies (e.g., Jenkins et al. 2002a), we also examined the effects of ATN lesions on c-Fos in the hippocampus and other cortical regions.

Materials and methods

Animals, surgery, housing conditions and cerebrolysin administration

We used 49 female hooded rats (PVGc strain; 5–6 months old, between 149 and 210 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 surgery, recovery, and during subsequent 30-day treatment period. 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.

Comparisons across four groups with ATN lesions examined the effects of enriched housing (Enr) versus standard housing (Std) and cerebrolysin (Cere) versus saline (Sal) in the context of this thalamic injury (ATN-Enr-Cere; ATN-Enr-Sal; ATN-Std-Cere; ATN-Std-Sal). A reference control group, akin to a healthy control group in the clinical field, was provided by rats that were given sham lesions, standard housing and saline treatment (Sham-Std-Sal). Sham groups with enrichment and cerebrolysin were not included because (a) the primary outcome was behavioral recovery after ATN lesions, based on working memory for reinforced spatial alternation and (b) in intact rats there is little or no benefit on spatial memory of enrichment (Loukavenko et al. 2007) or cerebrolysin (Valouskova and Gschanes 1999). The second focus was to examine c-Fos activity in the granular superficial layers of the retrosplenial cortex in the ATN groups by comparison to the Sham-Std-Sal group.

The rats were anesthetized with ketamine (75 mg/kg i.p.) followed by domitor (medetomidine; 1 mg/kg i.p.), supplemented by subcutaneous mepivacaine (3 mg/kg) applied under the shaved scalp and ketofen (0.50 mg/kg) applied under the nape of the neck. After surgery, antisedan was administered (5 mg/kg i.p.) to reverse the effects of the domitor. Once anesthetized, the rats were placed in a stereotaxic frame with atraumatic ear bars (Kopf, Tujunga, CA) and the incisor bar at −7.5 mm below the interaural line. Two ATN lesion sites were used: AP −2.5 mm from bregma, laterality ±1.65 mm, ventrality −5.55 mm from dura; and AP −2.3 mm, laterality ±1.10 mm and ventrality −5.85 mm. A microinfusion pump (Stoelting, Reno, NV) delivered 0.12 µl (dorsal site) and 0.10 µl (ventral site) of 0.12 M *N*-methyl-D-aspartic acid (NMDA; Sigma, Castle Hills, NSW, Australia) in phosphate buffer (pH 7.2) at 0.04 µl/min using a 1-µl Hamilton syringe. The needle was left in situ at each site for 3 min post-infusion. The needle was lowered to 1.50 mm above lesion coordinates without infusion for the Sham-Std-Sal group.

Prior to surgery, all rats were housed in groups of 3 or 4 per standard plastic cage (50 × 30 × 23 cm high). Post-surgery all rats were housed individually for 5 days. Based on errors to criterion in preoperative performance, matched pairs of rats were randomly assigned postoperatively to either an enriched environment for 30 days or standard caging during which no behavioral testing occurred. Rats in

the standard-housed groups were placed with new cagemates in a plastic cage. Rats in the Enr groups were housed with 10 or 11 new cagemates in large wire-mesh cages (85 × 60 × 30 cm high) with all the enrichment objects changed daily and the position of food and water and cage placement in the colony room varied every 2–4 days (per Loukavenko et al. 2007). Rats with ATN lesions were randomly assigned to cerebrolysin (Cere, 2.5 ml/kg i.p., provided without restriction by Dr Moessler of EBEWE Arzneimittel, Austria) or saline groups (Sal, 2.5 ml/kg i.p.) starting 3 days post-surgery as in previous work with cerebrolysin (Francis-Turner and Valouskova 1996). Starting 5 days post-surgery, each enrichment or standard cage included rats from both conditions. Daily cerebrolysin or saline administration for all rats continued for 30 days and rats were weighed prior to injection. The Sham-Std-Sal group received saline injections in the same manner. After 30 days, the enriched rats were rehoused in groups of three in standard cages. Rats were retested for spatial working memory at 45 days post-surgery. After access to their food ration, rats from the enriched cages were returned to an enriched environment for about 12 h (lights on) at the end of each day's testing.

Apparatus and spatial working memory testing

We examined spatial working memory, that is spatial information encoded within a trial, because this measure provides a robust impairment in standard-housed rats with ATN lesions that is generally severe when an alternation test is used (Aggleton and Nelson 2014). The deficit is found even after preoperative training (Warburton and Aggleton 1999), which enables a familiar environment to be used and minimizes the confound of procedural learning differences after surgery. To increase the likelihood of severe deficits after ATN lesions, we employed a T-maze configuration within a cross-maze apparatus (Alcaraz et al. 2014; Loukavenko et al. 2007).

The gray wooden cross maze was elevated 75 cm above floor level. The two 72-cm-long stems, each with a door and an extra 28 cm length for the "North" and "South" start areas, were 10.5 cm wide with 2.5-cm-high edges. The goal arms were shorter (40 cm) and the end of each held a small block with inaccessible rewards below the recessed food well. Moveable wooden blocks (10.5 × 10 × 30 cm high) were used to restrict access to any stem or arm. The maze was located diagonally in a windowless room (334 by 322 cm) which contained a number of distal cues. Beyond the goal arms were a computer, poster, desk and chair on one side, and a curtain on the other; beyond the start areas was a closed door at one end, and hub to a radial arm maze at the other. The horizontal distance between the maze and the nearest surface varied from 25 to 70 cm. Diffuse lighting was provided by overhead fluorescent lights. The rats were familiarized individually to the maze over 3 days with 0.1 g chocolate pieces freely available on the arms and then food wells.

Prior to surgery, rats were trained to criterion (85 % accuracy on 2 consecutive sessions) for 10–14 sessions with a standard ~5-s intra-trial delay in the start area between sample and test runs of the trial, after a 10-s confinement in the sample arm while it ate a 0.1 g chocolate food reward. Six trials with an inter-trial interval of 3–4 min were conducted per session throughout testing, with half the rats tested on alternate days 6 days per week. Correct performance on the test run required the rat to choose the alternate arm from that previously "forced" during the sample run of that trial (reinforced spatial alternation). A pseudorandom half of the six trials each day used either the South or the North start area for the sample run. Half of the trials used the same start position, such as only North or only South, for both sample and test runs; the other half of the trials used the start area in the opposite start position for the test run (e.g., North for the sample run then South for the test run for a given trial). After the 10-s confinement in the sample arm, the rat was picked up and returned to the appropriate start area for the intra-trial delay (between sample and test run), before a free choice was allowed between two open maze arms (hind foot down an arm; no retracing) with the opposite stem blocked. If the rat chose the previously blocked arm (non-matching alternation), it was rewarded with two 0.1 g food rewards and returned to its home cage for the 3–4 min inter-trial interval. If the rat returned to the arm previously visited on the sample run, it was confined to that arm for 10 s without reward. Each rat experienced a pseudorandom sequence of correct forced arm choices (left or right), North or South start position for the sample run, and same *versus* opposite start position for the test run to define the two trial types, all of which varied across rats and sessions (Fellows 1967). Two trial types were used to restrict the number of strategies that might support spatial memory. Always starting the rat from the same start arm (either North or South) for both sample and test runs of a trial could reflect spatial behavior that depends on either the association of reward with an egocentric response (alternate body turns), intramaze cues such as olfaction, or distal visual cues, such as place or visually guided direction. The trial type with opposite start arms used across the sample (e.g., North) and test run (then South) emphasized the use of distal visual cues to locate the reward on the test run or intramaze cues, but egocentric cues would lead to an incorrect response. Evidence of differential performance across the two trial types makes it unlikely that a rat uses either intramaze cues to solve trials, a complex conditional strategy such as

perseveration of body turn for "opposite start position" trials but alternation of body turn for "same start position" trials, or simply the direction of the arm at the choice point.

Rats were retested in the same room post-surgery, starting 10 sessions at 45 days post-surgery. Repetition using the same room emphasized the working memory characteristics of the task rather than retrieval of spatial reference memory or the acquisition of new spatial cues. Two days later, they were given 1 day of familiarization and three test sessions using a longer intra-trial delay (40 s) between sample and test run. A 40-s delay is known to impair performance in reinforced spatial alternation in group-housed rats with ATN lesions in instances when they have shown above chance performance at a short delay (Aggleton et al. 1995). Three to five days after the end of behavioral testing using the 40-s delay between sample and test runs, the rats were divided into 4 squads (8–9 animals per squad) balanced across each experimental group as far as possible. At this point, the first squad of rats was retested on the cross maze for three consecutive days, but now with the standard (5 s) delay in the start area between the sample and test runs, and four (or five) rats were run together to maintain an inter-trial interval of 3–4 min. Following each rat's completion of the six test trials on each day, it was placed individually in a standard cage, in a dark quiet room for a period of 90 min to allow habituation to a post-testing procedure that maximizes test-related c-Fos activation when killed on the last day of testing (Zangenehpour and Chaudhuri 2002). After the 90-min period in the dark, rats were returned to standard (group) cages for their daily food allocation prior to being placed in the enriched environment cage overnight or returned to their standard group-housed cage, except on the last day of testing when they were euthanized with an overdose of sodium pentobarbital and the brain extracted for c-Fos processing. We did not introduce a new environment or a different behavioral task prior to euthanasia to minimize new changes or novelty that might confound the interpretation of c-Fos activity, and instead focus on the association between spatial working memory and c-Fos expression. Moreover, the large reduction in retrosplenial cortex c-Fos activity has been reported in rats with ATN lesion by comparison to intact controls even when the rats were taken from their home cages prior to euthanasia (Jenkins et al. 2004), which means that the fundamental differences of interest should only be increased by behavioral testing.

To summarize, rats received lesion or sham surgery after pretraining to criterion on spatial working memory in the cross maze (5-s delay in the start area between runs). Five days post-surgery, they were housed in an enriched environment or in standard-housed cages for 30 days, with rats given daily saline injections or daily cerebrolysin injections (for 30 days, starting 48 h post-surgery as in Frances-Turner and Valouskova (1996), before spatial working memory was retested (5-s delay) for 10 sessions. Three to five days later three sessions with a 40 s delay were conducted, followed by a return to the 5-s delay and rats euthanized after the last of these trials which included a 90-min period in the dark after testing.

Histology

Rats were perfused with cold saline followed by 4 % paraformaldehyde and brains post-fixed overnight before 50 μm coronal sections were collected using a vibratome. Lesion extent was assessed using standard Nissl-stained sections. EL and JDA agreed the lesion extent in each rat using the relevant plates of a rat brain atlas (Paxinos and Watson 1998) while blind to any individual behavioral data. The lesions were drawn on electronic copies of the atlas and automated pixel counts of the damaged regions were used to estimate lesion volumes by factoring in the distances provided by the atlas (Mitchell and Dalrymple-Alford 2006). Collapse of areas surrounding the ATN and adjacent thalamic region and variability in angle of sections required a conventional visual, rather than direct image, analysis.

For c-Fos staining, three sections from a series of one in five were used to assess the following regions: prefrontal cortex (prelimbic/infralimbic [ventromedial]/anterior cingulate cortex [dorsomedial]); somatosensory and motor cortex; dorsal hippocampal/anterior retrosplenial region and intermediate hippocampal/posterior retrosplenial region (Fig. 1). Free-floating sections were washed several times in 0.1 M PBS containing 0.3 % of Triton X-100 (PBST) and treated with 0.5 % hydrogen peroxide in PBST to quench endogenous peroxidase. The sections were incubated overnight at room temperature with a c-Fos rabbit polyclonal primary antibody (1:5000 in PBST, Santa Cruz) and the sections rinsed several times with PBST the next day before incubation for 2 h with a goat anti-rabbit secondary antibody (1:1000 in PBST; Jackson Immuno-Research) in 1 % normal goat serum. After further rinses, the sections were incubated for 2 h in 1:200 avidin–biotin–horseradish peroxidase complex (ABC elite, Vector Laboratories) and visualized using a 0.001 % diaminobenzidine in 0.0004 % hydrogen peroxide solution. The reaction was stopped by cold PB rinses and sections were mounted and dried on gelatine-coated slides.

The c-Fos-stained cells were examined using a Nikon Eclipse E-800 microscope equipped with digital camera (Nikon DS-U2). Counts of c-Fos-positive cells were made using a ×10 objective. The regions of interest were manually drawn and an in-house computer program automatically labeled and counted the number of c-Fos-positive cells that met the threshold criterion of three SD greater

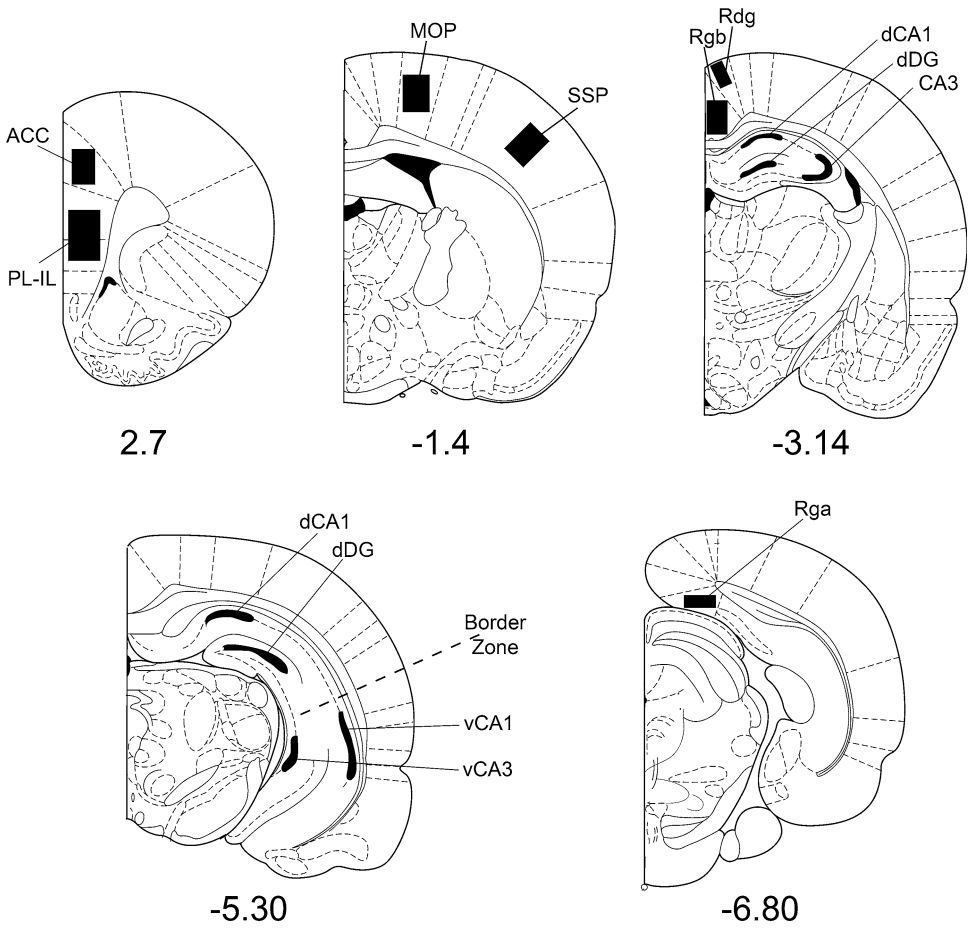

Fig. 1 Regions of interest for c-Fos analyses. *Numbers* refer to distance (mm) from bregma (Paxinos and Watson 1998). *ACC* anterior cingulate cortex, *dCA1* dorsal CA1, *dCA3* dorsal CA3, *dDG* dorsal dentate gyrus, *vCA1* ventral CA1, *vCA3* ventral CA3, *MOP* primary motor cortex, *PL-IL* prelimbic–infralimbic ventromedial prefrontal cortex, *Rdg* dysgranular retrosplenial cortex, *Rga* caudal retrosplenial granular cortex, *Rgb* rostral retrosplenial granular cortex, *SSP* primary somatosensory cortex. The border zone between dorsal/ventral hippocampus is shown by the *dotted line*

than the mean gray-scaled value within any region. Counts were taken from at least three sections from both hemispheres and the mean across sections counted in each region of interest was expressed as the number of c-Fos-positive cells/mm^2. Separate counts were made for three retrosplenial subregions (rostral granular cortex, Rgb; caudal granular cortex, Rga; and dysgranular cortex, Rdg). As in previous studies, Fos-positive cells were counted separately in the superficial (layer II and upper III) and deeper laminae (lower III to VI) of the Rgb, Rga, and Rgd regions (Dupire et al. 2013; Jenkins et al. 2004; Poirier and Aggleton 2009).

Statistical analyses

The focus on recovery of spatial working memory in rats with ATN lesions was examined across the four lesion groups using a balanced 2 (Enr, Std) × 2 (Cere, Sal) ANOVA with the repeated-measures factor of Trial type (same start position across sample and choice runs, opposite start position). As in our previous study, we examined the mean percent correct score for first three post-surgery sessions (at 5-s intra-trial delay) and the last three of these sessions (Loukavenko et al. 2007). A similar analysis was conducted for the three sessions with a 40-s intra-trial delay. Each lesion group was also compared with the intact reference group (Sham-Std-Sal) using independent *t* tests and the significance level increased to $p < 0.0125$ by Bonferroni correction to compensate for the multiple comparisons with the sham group.

The primary analyses for c-Fos examined counts in each region for the lesion groups expressed as a percent score relative to the mean count for the Sham-Std-Sal control group. That is, the count in the sham groups was converted to a transformed mean score of 100 % (±SD) in any given region (Fig. 5; mean raw counts are shown in Table 1). One-sample *t* tests then compared each lesion group's c-Fos data against that of the sham group (using the Bonferroni-adjusted $p < 0.0125$ for statistical significance). The effects of housing and drug treatment across the four lesion groups were assessed for c-Fos expression using a 2 × 2 ANOVA. Each region was analyzed separately, rather than using an omnibus ANOVA across all regions, to achieve a suitable balance with respect to Type I (false positive) and Type II (false negative) errors; a balance that reduces false negatives is likely to encourage similar studies in the future (Fiedler et al. 2012).

Table 1 Mean (±SEM) c-Fos-positive cells per mm^2 in each region of interest (absolute counts)

Regions	Sham-Sal	ATN-Std-Sal	ATN-Std-Cere	ATN-Enr-Sal	ATN-Enr-Cere
Retrosplenial					
Rgb sup	788 (136)	397 (100)	366 (94)	329 (96)	310 (140)
Rgb deep	667 (115)	447 (138)	462 (91)	350 (68)	358 (85)
Rga sup	522 (64)	348 (269)	201 (101)	214 (36)	203 (74)
Rga deep	217 (68)	195 (85)	240 (94)	222 (38)	227 (61)
Rdg sup	271 (63)	207 (35)	244 (53)	200 (79)	210 (60)
Rdg deep	325 (41)	314 (46)	304 (31)	307 (61)	338 (112)
Prefrontal					
Pl-IL	550 (58)	568 (109)	478 (30)	451 (122)	484 (59)
ACC	502 (85)	542 (102)	342 (76)	556 (104)	339 (84)
Cortical					
Sensory	619 (144)	532 (110)	594 (189)	451 (122)	411 (60)
Motor	691 (152)	598 (63)	583 (120)	556 (104)	459 (108)
Hippocampus					
Anterior dorsal					
CA1	251 (74)	302 (74)	273 (37)	275 (47)	275 (47)
DG	228 (77)	207 (66)	203 (52)	222 (63)	177 (39)
CA3	166 (47)	164 (31)	155 (20)	157 (16)	137 (19)
Posterior ventral					
CA3	213 (19)	214 (36)	220 (46)	206 (29)	226 (29)
CA1	321 (41)	357 (50)	344 (74)	356 (85)	315 (42)
Posterior dorsal					
CA1	283 (65)	307 (32)	309 (69)	299 (49)	304 (32)
DG	201 (31)	214 (41)	223 (51)	243 (50)	203 (18)

ATN neurotoxic lesion of the anterior thalamic nuclei, *Enr* enriched environment, *Cere* cerebrolysin, *Std* housed in standard group conditions, *Sal* saline, ACC, anterior cingulate cortex, *dCA1* dorsal CA1, *dCA3* dorsal CA3, *dDG* dorsal dentate gyrus, *vCA1* ventral CA1, *vCA3* ventral CA3, *MOP* primary motor cortex, *PL-IL* prelimbic–infralimbic ventromedial prefrontal cortex, *Rdg* dysgranular cortex, *Rga* caudal granular cortex, *Rgb* rostral granular cortex, *sup* superficial layers, *deep* deep layers

Results

Lesion evaluation

The largest and smallest lesions are shown in Fig. 2. As in previous studies, only rats with lesions that encompassed 50 % or more of the ATN and less than 40 % damage to the adjacent dorsal medial (MT) and lateral (LT) thalamic nuclei were included for analysis (see Mitchell and Dalrymple-Alford 2006). Lesion failures occurred in 15 rats, which were not processed further (7 small unilateral; 4 too anterior; 4 too posterior). The final sample sizes were: Sham-Std-Sal, $n = 8$; ATN-Std-Sal, $n = 7$; ATN-Enr-Sal, $n = 7$; ATN-Std-Cere, $n = 6$; and ATN-Enr-Cere, $n = 6$. There were no effects on lesion volume for either Housing or Cerebrolysin factors for either the ATN, MT or LT regions (all $F < 1$). For the ATN-Std-Sal group, there was 74 % (range 60–95 %) damage to the ATN (and MT region, 2 %; LT region, 7 %). In the ATN-Enr-Sal group, suitable lesions produced a median of 83 % (range 61–92 %) damage to the ATN (and MT, 6 %; LT, 15 %). For the ATN-Std-Cere group, there was 79 % (range 73–92 %) damage to the ATN (and MT, 4 %; LT, 14 %). In the ATN-Enr-Cere group, there was 89 % (range 57–92 %) damage to the ATN (and MT, 4 %; LT, 16 %). There were no differences across groups in terms of damage to other adjacent structures (all $F < 1$), which was minimal apart from the interanteromedial nucleus: ATN-Std-Sal, 58 % (1–69 %); ATN-Enr-Sal, 48.2 % (11–91 %); ATN-Std-Cere, 40 % (16–92 %); ATN-Enr-Cere, 61 % (29–71 %). There was no significant association between ATN lesion size and memory performance. After lesions, all other brain regions, including the retrosplenial cortex and its laminar pattern, appeared normal from visual inspection of cresyl violet staining, as in previous studies (Dupire et al. 2013; Jenkins et al. 2004; Mendez-Lopez et al. 2013; Poirier and Aggleton 2009).

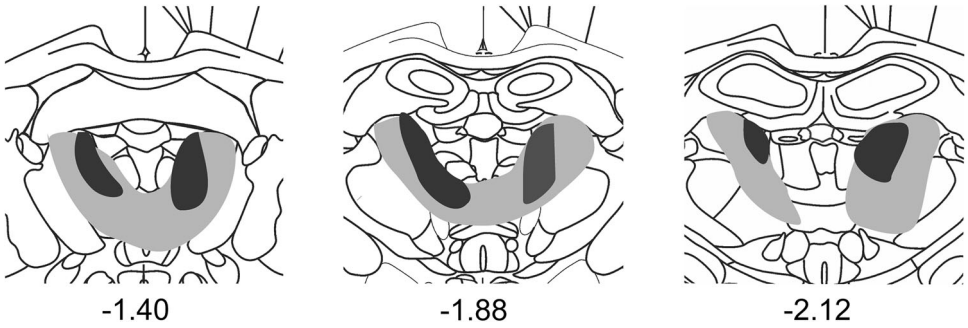

Fig. 2 Coronal schematics through the medial thalamus showing smallest (*black*) and largest (*gray*) lesion, across all ATN groups. *ATN* neurotoxic lesion of the anterior thalamic nuclei. *Numbers* refer to the distance from bregma (Paxinos and Watson 1998)

Non-matching to sample spatial working memory (standard 5-s intra-trial delay)

The rats retained after lesion verification revealed no significant group differences in spatial working memory at the end of training prior to surgery (all $p > 0.3$; data not shown). Figure 3a describes the post-surgery spatial working memory performance combined across both trial types (i.e., irrespective of "same start position" and "opposite start position" trials) for all groups during the postoperative sessions with the standard (5 s) intra-trial delay between the two runs. The Sham-Std-Sal (control) group rapidly reacquired the task and achieved near perfect performance by the third postoperative session, whereas significantly poorer overall performance was evident in all four ATN lesion groups (all $p < 0.001$). The ATN-Std-Sal group displayed close to chance levels of spatial working memory throughout the 10 postoperative sessions, whereas the other three lesion groups attained an intermediate level of performance.

Performance as a function of trial type for the first three and last three sessions of testing with the 5-s intra-trial delay is shown in Fig. 3b. For the first three sessions, the $2 \times 2 \times 2$ ANOVA for the four ATN lesion groups (Housing: enriched *versus* standard; Drug: cerebrolysin *versus* saline; repeated measure of Trial type: same start position *versus* opposite start position) produced a highly significant Trial type effect ($F_{(1,22)} = 22.79$; $p < 0.0001$), with better performance for the same start position trials, but no other significant effects or interactions were detected (all $p > 0.05$). By contrast, the ANOVA for the last three sessions (Fig. 3c) confirmed that the enriched ATN groups performed better than the standard-housed ATN groups (Housing, $F_{(1,22)} = 23.01$; $p < 0.0001$) on both types of trial (Trial Type, $F_{(1,22)} = 35.92$; $p < 0.0001$; Housing $\times$ Trial Type, $F < 1$). As suggested by Fig. 3c, there was also a significant Cerebrolysin effect ($F_{(1,22)} = 4.70$; $p = 0.04$) and a Housing by Cerebrolysin interaction ($F_{(1,22)} = 5.40$; $p = 0.03$). The ATN-Std-Cere group showed improved performance on both trial types compared to the ATN-Std-Sal group ($p < 0.01$), but cerebrolysin did not improve performance of the ATN rats housed in enrichment. No other significant interactions were evident.

Comparison of the lesion groups with the Sham-Std-Sal group, for the same start position trials in the first three sessions (Fig. 3b), confirmed significantly worse performance in the ATN-Std-Sal and ATN-Std-Cere groups (both $p < 0.01$), but this comparison did not reach the $p < 0.0125$ adjusted significance level for the ATN-Enr-Sal ($p = 0.04$) and ATN-Enr-Cere groups ($p = 0.1$). For the opposite start position trials in the first three sessions, the Sham-Std-Sal group was again significantly superior to the ATN-Std-Sal and ATN-Std-Cere groups ($p < 0.01$) and the comparison failed to reach significance relative to the ATN-Enr-Sal ($p = 0.12$) and the ATN-Enr-Cere groups ($p = 0.02$). All groups bar the ATN-Std-Sal group ($p > 0.3$) performed above chance level (all $p < 0.01$) for the same start position trials in the first three sessions; none of the lesion groups were significantly better than chance for the more difficult opposite start position trials ($p > 0.3$), unlike the Sham-Std-Sal group (Fig. 3b).

Comparison with the Sham-Std-Sal group on the same start position trials for the last three sessions (Fig. 3c) showed that the ATN-Std-Sal group remained significantly worse ($p = 0.002$). All three treatment groups did not significantly differ from the intact control group for these trial types ($p > 0.3$). For the opposite start position trials in the last three sessions, the Sham-Std-Sal group outperformed both the ATN-Std-Sal ($p < 0.0001$) and the ATN-Std-Cere ($p = 0.0003$) groups, whereas the difference did not reach the adjusted significance level for either the ATN-Enr-Sal group ($p = 0.02$) or the ATN-Enr-Cere group ($p = 0.07$). All ATN groups now performed significantly above chance levels on the same start position trials (all $p < 0.01$). An important finding, however, was that the three treatment ATN groups were now also significantly above chance level for the opposite start position trials in the last three sessions (all $p < 0.01$), whereas the ATN-Std-Sal group remained at chance ($p > 0.3$) on this measure.

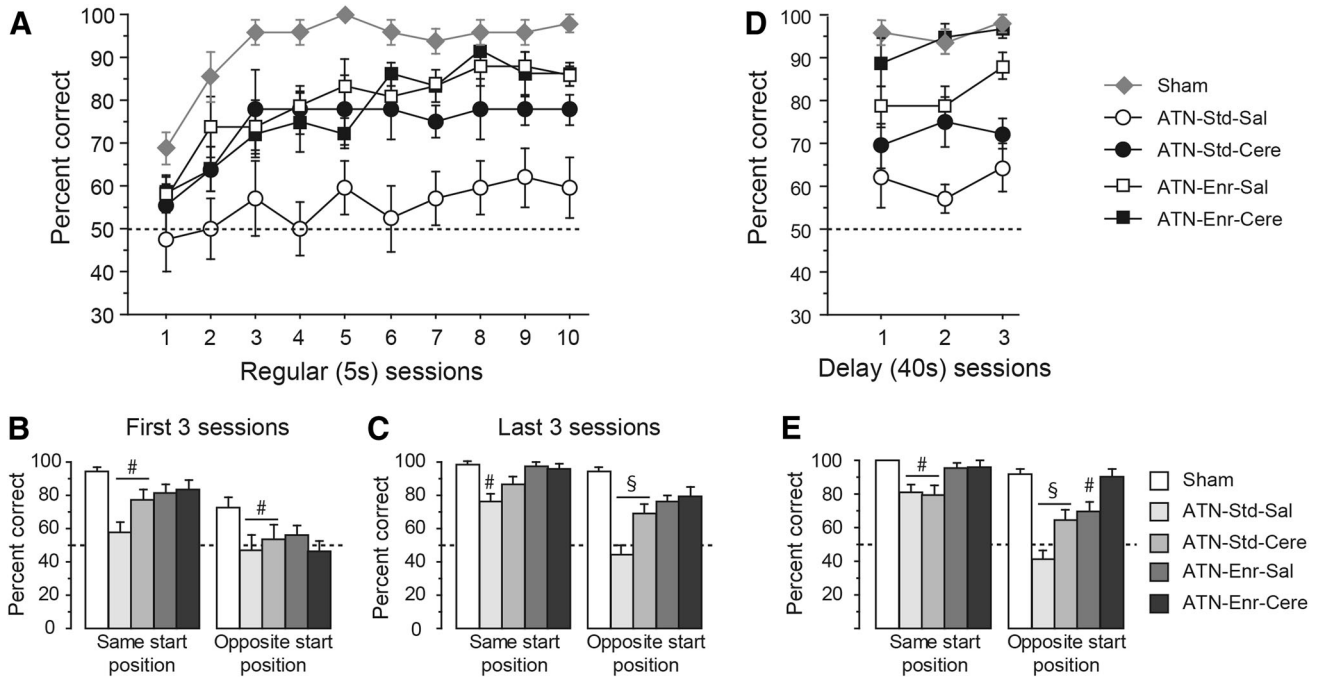

Fig. 3 Spatial working memory in the cross maze. Performance is expressed as mean (±SEM) percent correct. The *symbols* indicate the level of significance of the independent *t* test ran between ATN groups and the Sham control group. $^{\#}p < 0.01$, $^{\S}p < 0.001$. **a** Performance in the five groups across ten sessions with a standard (5 s) intra-trial interval between the sample and test runs, collapsed across the two types of trial. **b** Performance for the first three sessions for the same start position trials (same start position used for both sample and test run) and opposite start position trials (opposite start position used for the test run). **c** Same as **b**, but for the last three sessions. **d** Performance across three sessions with a 40-s intra-trial delay between the sample and test runs, collapsed across trial types. **e** Performance across the 40-s intra-trial delay sessions for the same start position and opposite start position trials. *ATN* neurotoxic lesion of the anterior thalamic nuclei, *Cere* cerebrolysin, *Enr* enriched environment, *Std* housed in standard group conditions, *Sal* saline, *Sham* Sham-Std-Sal. *Dashed lines* chance performance

Non-matching to sample spatial working memory (40-s intra-trial delay)

Performance of the 5 groups when the delay between sample and test run was 40 s is shown in Fig. 3d, e. This phase of testing suggested that the combination of enrichment and cerebrolysin had an additive beneficial effect relative to each treatment in isolation. The enriched groups (ATN-Enr-Sal and ATN-Enr-Cere) outperformed their corresponding standard-housed groups (Housing, $F_{(1,22)} = 28.52$; $p < 0.0001$), but cerebrolysin was now associated with more accurate performance in both housing conditions (Cerebrolysin, $F_{(1,22)} = 7.62$; $p = 0.01$; Housing × Cerebrolysin interaction, $F < 1$). As before, there was a Trial type effect ($F_{(1,22)} = 42.05$; $p < 0.0001$). In this case, the Trial type by Cerebrolysin interaction was also significant ($F_{(1,22)} = 11.51$; $p = 0.003$), which reflected the influence of cerebrolysin in improving performance on the opposite start position trials without affecting performance in the same start position trials. All three ATN treatment groups continued to perform above chance level for both same start position and opposite start position trials ($p < 0.01$). As before, the ATN-Std-Sal group performed above chance levels on the same start position trials ($p < 0.01$), but close to chance on the opposite start position trials ($p = 0.11$). Both standard-housed ATN groups continued to perform worse than the Sham-Std-Sal group on both trial types (Same start trials, both $p = 0.002$; Opposite start trials, both $p < 0.001$). The ATN-EE-Sal group performed as well as the sham group on the same start trial only ($p > 0.1$) but was impaired on opposite start conditions ($p = 0.008$). Only the ATN-Enr-Cere group's performance matched that of the sham group on both trial types ($p > 0.2$), suggesting the added benefit of the combination of both treatments.

Spatial working memory related to c-Fos expression

Performance on the last day of behavioral testing (5-s delay) when rats were euthanized 90 min later to collect the brain for c-Fos immunostaining is shown in Fig. 4. The 2 × 2 ANOVA across the four groups with ATN lesions produced significant effects of Housing ($F_{(1,22)} = 17.45$; $p = 0.0004$), Cerebrolysin ($F_{(1,22)} = 5.09$; $p = 0.034$) and a Housing × Cerebrolysin interaction ($F_{(1,22)} = 4.67$; $p = 0.042$), with no difference of drug vs saline in the enriched ATN groups ($F_{(1,11)} < 1.0$; $p > 0.50$), but a

significant difference between drug vs saline in the standard-housed ATN groups ($F_{(1,11)} = 6.05$; $p = 0.032$). This last day of testing revealed significant differences between both the ATN-Std-Sal group ($p = 0.0002$) and the ATN-Std-Cere group ($p = 0.005$) when each was compared with the Sham-Std-Sal group. By contrast, the ATN-EE-Cere group ($p = 0.099$) and the ATN-EE-Sal group ($p = 0.063$) did not differ significantly from the Sham-Std-Sal group. Of the 30 possible bivariate correlations between memory performance on the last trial and c-Fos expression in the six subregions of the retrosplenial cortex for the 4 ATN groups and all ATN rats combined as a single group (Sham group excluded as it had little variance and only high values), the only significant association was for the Rdg deep layers in the ATN-Std-Sal group ($r = -0.77$, $p = 0.039$; all other $p > 0.07$, all other r −0.32 to 0.78).

c-Fos expression

Retrosplenial cortex

Compared to the Sham-Std-Sal group (Fig. 5a), both standard-housed groups with ATN lesions showed substantially reduced c-Fos expression in the superficial and deep laminae of the rostral granular retrosplenial cortex (Rgb): ATN-Std-Sal (both regions $p < 0.01$); ATN-Std-Cere ($p < 0.001$ and $p = 0.004$, for the two regions, respectively). Lower c-Fos expression in the Rgb was also found in the ATN-Enr-Sal and ATN-Enr-Cere groups ($p < 0.001$) when compared to the Sham-Std-Sal group (Fig. 5a). The influence of treatments on c-Fos expression in the superficial lamina of the Rgb is of particular interest, because these layers have shown the most consistent decreases after ATN lesions in previous reports. The 2 × 2 ANOVA across the four lesion groups revealed no significant effects on c-Fos expression in the superficial lamina of the Rgb for Housing, Cerebrolysin or Housing × Cerebrolysin interaction ($F < 1$). In the deep laminae of the Rgb, cerebrolysin did not change the reduced c-Fos expression and enrichment was associated with a reduction of c-Fos activation (Housing effect, $F_{(1,22)} = 6.54$; $p = 0.02$; Cerebrolysin, and Cerebrolysin × Housing, $F < 1$). Examples of c-Fos staining in the Rgb region in the five groups are shown in Fig. 6a.

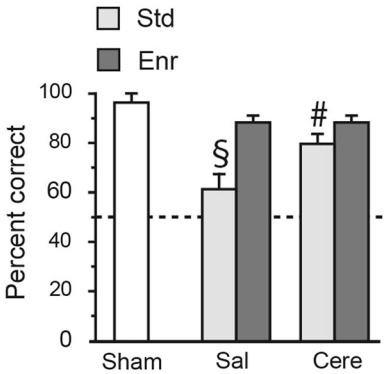

Fig. 4 Spatial working memory in the cross maze in the last session before rats were euthanized for c-Fos assessment. Performance is expressed as mean (±SEM) percent correct and is collapsed across the two types of trial. The *symbols* indicate the level of significance of the independent *t* test ran between ATN groups and the Sham control group. $^{\#}p < 0.01$, $^{\S}p < 0.001$. *ATN* neurotoxic lesion of the anterior thalamic nuclei, *Cere* cerebrolysin, *Enr* enriched environment, *Std* housed in standard group conditions, *Sal* saline, *Sham* Sham-Std-Sal. *Dashed lines* chance performance

In the superficial layers of the caudal granular retrosplenial cortex (Rga; Fig. 5a), c-Fos in the ATN-Std-Sal group was non-significantly lower than the mean value of the Sham-Std-Sal group ($p = 0.10$), and these counts were significantly reduced in the ATN-Std-Cere group ($p < 0.0001$). Significant reductions on this measure, compared to the Sham-Std-Sal group, were also evident in the ATN-Enr-Sal and ATN-Enr-Cere groups ($p < 0.0001$). The 2 × 2 ANOVA of c-Fos in the superficial layer of Rga in the four ATN groups, however, revealed no significant differences (all $F < 1$). In the deep layers of the Rga, no differences were evident between the Sham-Std-Sal group and any of the ATN lesion groups (all $p > 0.1$) or across the four ATN groups (all $F < 1$).

When compared to the sham group, only the ATN-Std-Sal group exhibited significantly reduced c-Fos activation in the superficial layer of the Rdg ($p = 0.003$, Fig. 5a). However, the 2 × 2 ANOVA across the four lesion groups did not show any significant main effects or Housing × interaction (Fs < 1) in either the superficial or the deep layers for the Rdg.

Medial prefrontal cortex

Compared to the Sham-Std-Sal group, c-Fos counts in the prefrontal cortex were not reduced by ATN lesions in either of the standard-housed groups (ATN-Std-Sal; ATN-Std-Cere; $p > 0.3$ for both regions, Fig. 5b). For the prelimbic/infralimbic cortex, a lower value relative to the Sham-Std-Sal group was significant in the ATN-Enr-Sal group ($p < 0.001$) but the difference was non-significant in the ATN-Enr-Cere group ($p < 0.05$). Lower values for both enriched ATN groups relative to the Sham-Std-Sal group were evident for the anterior cingulate cortex ($p < 0.01$). The 2 × 2 ANOVA across the four lesion groups showed significant Housing effects for both the prelimbic/infralimbic prefrontal cortex ($F_{(1,22)} = 11.14$; $p = 0.003$) and the anterior cingulate ($F_{(1,22)} = 20.28$; $p = 0.0002$), but there was no Cerebrolysin effect or Cerebrolysin by Housing interaction (all $F < 1$).

Primary motor and sensory cortex

There was no difference in c-Fos for either of these regions in the two standard-housed groups with ATN lesions

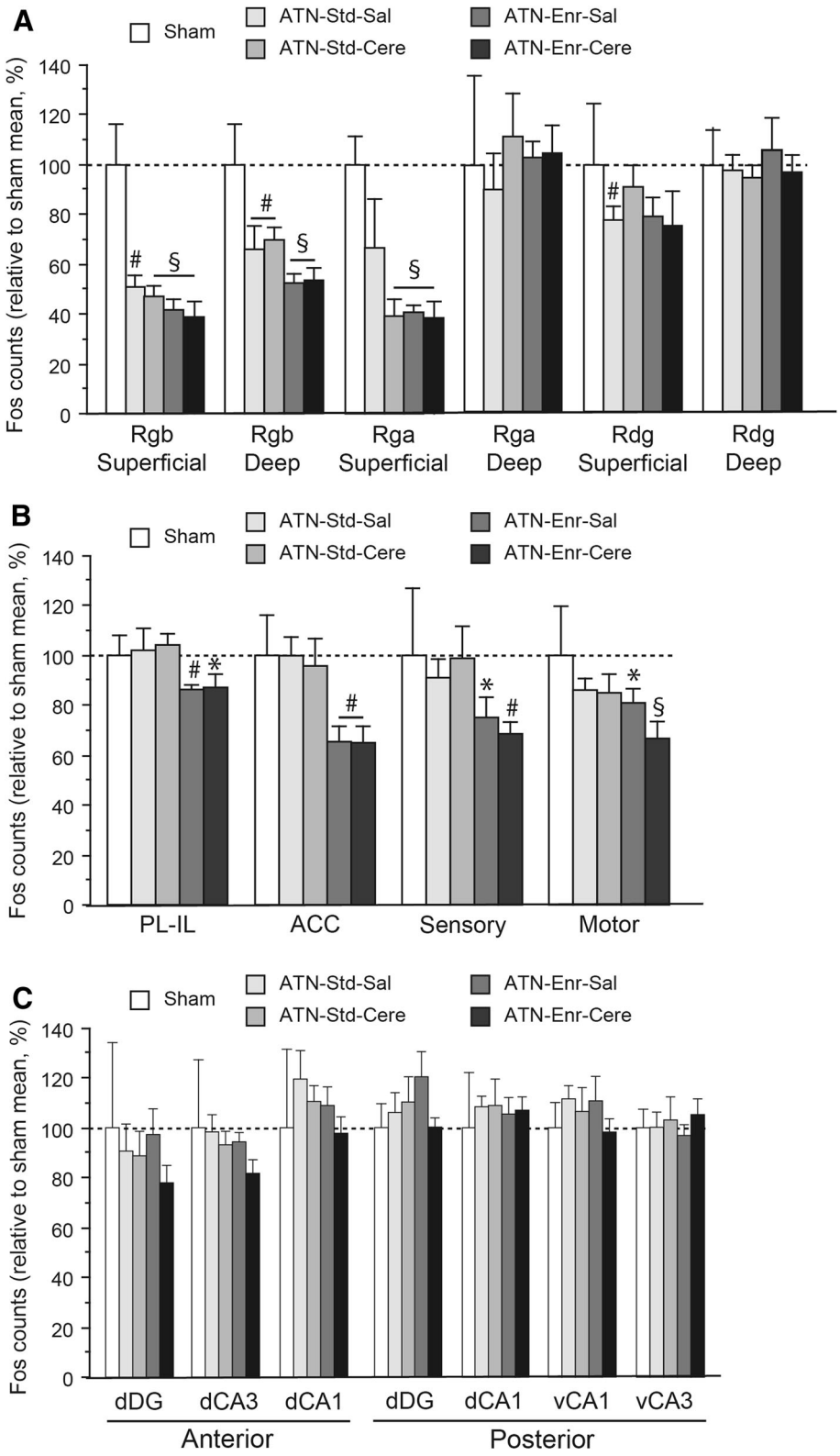

Fig. 5 **a** Mean (±SEM) c-Fos counts in the retrosplenial region expressed relative to the mean obtained in the Sham-Std-Sal group (i.e., Sham = 100 % activation, dashed line). **b** Same as **a**, but for other cortical regions. **c** Same as **a**, but for the hippocampal regions. The *symbols* indicate the level of significance of the independent *t* test ran between ATN groups and the Sham control group. $^{*}p < 0.05$, $^{\#}p < 0.01$, $^{\S}p < 0.001$. *ATN* neurotoxic lesion of the anterior thalamic nuclei, *Enr* enriched environment, *Cere* cerebrolysin, *Std* housed in standard group conditions, *Sal* saline, *ACC* anterior cingulate cortex, *dCA1* dorsal CA1, *dCA3 dorsal CA3*, *dDG* dorsal dentate gyrus, *vCA1* ventral CA1, *vCA3* ventral CA3, *MOP* primary motor cortex, *PL-IL* prelimbic–infralimbic ventromedial prefrontal cortex, *Rdg* dysgranular cortex, *Rga* caudal granular cortex, *Rgb* rostral granular cortex, *SSP* primary somatosensory cortex

compared to the Sham-Std-Sal group (ATN-Std-Sal, ATN-Std-Cere, $p > 0.3$; Fig. 5b). Compared to the sham group, there were lower c-Fos counts in the primary motor cortex (MOp) and primary somatosensory area (Ssp) in the ATN-Enr-Cere group ($p = 0.008$ and $p = 0.006$, respectively). Weaker effects were evident in the ATN-Enr-Sal group

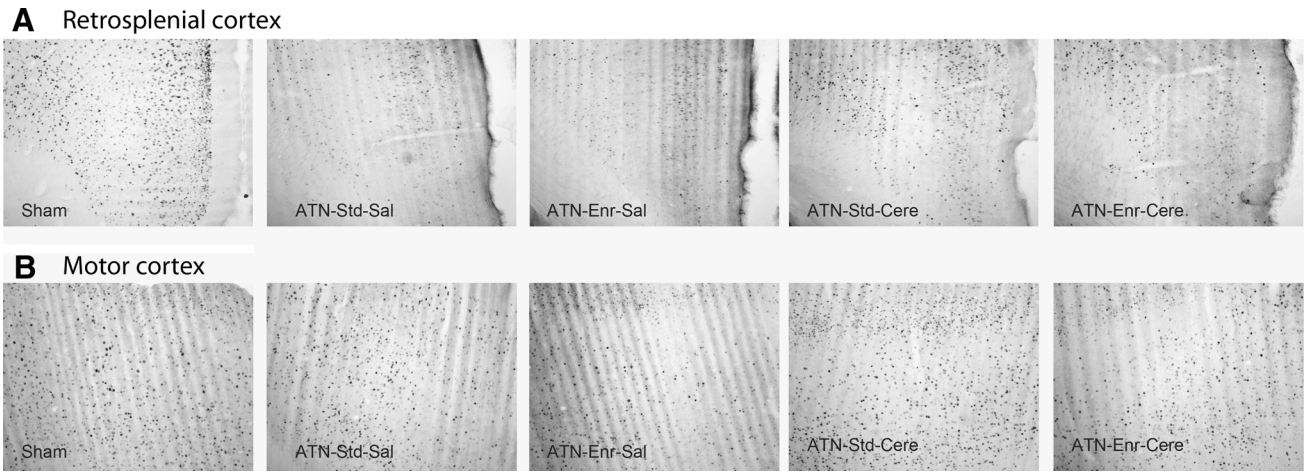

Fig. 6 Photomicrographs showing c-Fos immunoreactivity at the level of the retrosplenial cortex (**a**) and the motor cortex (**b**). While c-Fos levels are markedly reduced in the retrosplenial group for all ATN groups (**a**), the lesion did not affect c-Fos expression in the motor cortex (**b**)

compared to the sham group for these two cortical regions ($p < 0.05$). The 2 × 2 ANOVA across the four lesion groups showed significant Housing effects in both cortical regions (MOp, $F_{(1,22)} = 4.42$; $p = 0.05$; Ssp, $F_{(1,22)} = 6.89$; $p = 0.02$), but no cerebrolysin effects or interactions (all $F < 1$). Examples of the c-Fos staining in the motor cortex in the five groups are shown in Fig. 6b.

Hippocampus

No significant differences were evident in any c-Fos measure in the hippocampus (all t and $F < 1$; Fig. 5c).

Discussion

The current study has provided novel evidence that the parenterally administered neurotrophic drug cerebrolysin reduced spatial working memory deficits in ATN rats. We also replicated the beneficial effects of enrichment on spatial memory after ATN lesions (Loukavenko et al. 2007; Wolff et al. 2008b). Enrichment tended to produce better recovery than cerebrolysin, but the combination of these two treatments produced an additive improvement in spatial working memory when a longer (40 s) delay was used between the sample and test runs. Our study replicated reports that ATN lesions in rats produce a dramatic reduction in IEG expression in the retrosplenial granular cortex (Amin et al. 2010; Dumont et al. 2012; Dupire et al. 2013; Jenkins et al. 2002a, b, 2004; Poirier and Aggleton 2009). Cerebrolysin in standard-housed rats with ATN lesions did not, however, restore c-Fos expression in the retrosplenial cortex when compared with standard-housed ATN rats given saline. Enrichment, either alone or in combination with cerebrolysin, was also associated with low c-Fos activity in the retrosplenial cortex.

These findings extend previous evidence that cerebrolysin promotes recovery from impairments associated with neurodegeneration and hippocampal lesions (Francis-Turner and Valouskova 1996; Ubhi et al. 2013). Hence, there may be therapeutic benefits of cerebrolysin in human diencephalic amnesia, in addition to Alzheimer's and vascular dementia (Plosker and Gauthier 2009). The animal model used in the current study is based on the importance of the ATN but other thalamic regions are also implicated in diencephalic amnesia, so it would be interesting to determine the effects of both cerebrolysin and enrichment in other lesion or thiamine-deficiency animal models of diencephalic amnesia (Carlesimo et al. 2011; Gold and Squire 2006; Mair et al. 2011; Pergola and Suchan 2013; Savage et al. 2012; Vann 2010).

The beneficial effect of enrichment and cerebrolysin in the reinforced spatial alternation task was evident both when the opposite start position was used on the sample and test runs and when the same start position was used. The ATN-Std-Sal group showed mildly improved performance for the same start position trials as testing progressed over time, but remained at chance level on the opposite start position trials. The interpretation of performance in this apparently simple working memory task is not straightforward. One conclusion is that the task is particularly sensitive to ATN lesions precisely because it engages an array of spatial cues and strategies subserved by ATN subregions and by other brain regions that are functionally impaired as a consequence of this brain injury (Aggleton and Nelson 2014). Rats generally fail to show strong evidence of egocentric responding in this alternation task, instead showing evidence of place alternation, alternation of intramaze cues

and alternation of direction of the two arms (Baird et al. 2004; Dudchenko 2001; Pothuizen et al. 2008). Rats with ATN lesions are, however, unimpaired in egocentric discrimination or its reversal, at least when trained in a reference memory task (Warburton et al. 1997; Wolff et al. 2008a). The alternation task tested working memory, not reference memory, but it is possible that non-treated rats with ATN lesions developed an unusual reliance on egocentric strategies, such as alternating body turn at the choice point from the sample run to the test run, which would facilitate performance on the same start position trials (Aggleton and Nelson 2014). On the opposite start position trials, this egocentric strategy would be unhelpful and could even lead to increased errors because the rat would now alternate to the previously visited arm. Reliance on intramaze cues such as olfaction at the choice point was unlikely, because this strategy in our task should produce roughly equal performance for trials using the same start position and trials using the opposite start position. Another possibility is that the rats with ATN lesions, in particular, may behave as if they were being run in a different maze or on a new trial when the opposite start position was used (Aggleton and Nelson 2014), but this is also unlikely because they experienced lengthy testing and a 3–4-min interval between trials whereas the interval between runs was brief. A basic failure to process direction cues can also be ruled out, because the rats with ATN lesions would then also have been equally impaired on both types of trial (the direction of the arms remain unaltered, irrespective of the type of trial). Thus, there must be something important about traversing the same or different stem during the choice run that differentially influenced performance. The directional cues while the rat traversed the stem of the maze and cues in the start area changed within the trial for the opposite position trials. Hence, one reason for poor performance on these trials in rats with ATN lesions that did not show recovery was that these rats were susceptible to the increase in retroactive interference. While many cues and strategies may have guided performance, it therefore seems more parsimonious to propose that improved processing of allocentric spatial cues and increased resistance to interference were responsible for better performance in sham rats and ATN rats that showed recovery of spatial working memory. Recovery of allocentric memory and resistance to interference to explain improved performance on the opposite start position trials in the treatment groups is consistent with evidence that enriched ATN rats showed relatively little disruption when required to use allocentric cues in a flexible manner in the water maze (Wolff et al. 2008b). Nonetheless, it would be interesting to know whether the benefit of a combination treatment continues with longer periods of training and under different test conditions.

In contrast to the behavioral improvements evident after cerebrolysin and enrichment, the c-Fos counts in the retrosplenial cortex remained low by comparison to the sham reference group. The marked disruption of this functional marker in the retrosplenial cortex is thus a robust effect of ATN lesions (Aggleton 2008, 2010). Variation in rat strain, age, sex, and other behavioral and procedural details may influence the precise c-Fos pattern in the retrosplenial cortex after ATN lesions, but the current study replicated the general pattern of c-Fos hypoactivation despite using female rats and different rat strains. As in previous studies, the loss tended to be greater in the superficial layers ($\sim$40–50 %) than the deeper cell layers ($\sim$30–40 %) of the rostral granular retrosplenial cortex (Rgb). Our Rgb changes were less severe than some, but not all, previous studies (Dupire et al. 2013; Jenkins et al. 2002a, b, 2004; Poirier and Aggleton 2009; Poirier et al. 2008). For the caudal granular retrosplenial cortex (Rga), the c-Fos reduction in the ATN-Std-Sal group did not reach significance in the superficial layers and there was no reduction in the deep layers. Reduced c-Fos expression in the dysgranular retrosplenial cortex (Rdg) may need 9–12 months before becoming evident after bilateral ATN lesions (Poirier and Aggleton 2009). At 4 months postsurgery, we found mildly reduced c-Fos in Rdg which was significant in only the ATN-Std-Sal group. There have been less consistent reports that ATN lesions also reduce IEG markers in the limbic prefrontal cortex and hippocampus (Dumont et al. 2012; Dupire et al. 2013; Jenkins et al. 2002b) and no change in these structures was evident in the ATN-Std-Sal group in our study.

It is plausible that IEG hypoactivation in the retrosplenial cortex contributes to the cognitive deficits associated with ATN lesions. For example, an interesting study by Czajkowski and colleagues used a transgenic mouse with a FosGFP reporter to show that the retrosplenial cortex processes both the encoding and the storage of spatial information in the Morris water maze (Czajkowski et al. 2014). Furthermore, spatial memory was enhanced in their study by overexpression of the phosphorylated cAMP response element-binding protein (pCREB), indicating that spatial processing within the retrosplenial cortex also relies on the activity of this molecule. Unlike c-Fos after ATN lesions, cerebrolysin and enrichment may influence many molecules associated with plasticity, energy metabolism and chromatin remodeling that are dysregulated in the retrosplenial cortex after ATN damage, including pCREB (Dumont et al. 2012; Dupire et al. 2013), and GABA-A *receptor delta subunit*, *serotonin receptor 2c, zif268*, *fra-2*, *kcnab2*, *cox6b, mmp9*, *cghB*, or *ruvbl1* which reflect protracted lesion-induced transcriptome disturbances (Amin et al. 2010; Poirier et al. 2008). It would also be interesting to know whether beneficial treatments reverse the loss of

electrophysiological plasticity in the superficial layers of the Rgb that occur after ATN lesions (Garden et al. 2009). Nonetheless, the contrast between functional recovery and enduring low c-Fos expression in the retrosplenial cortex in the current study suggests that the behavioral deficits in our task may not primarily arise from this retrosplenial dysfunction. Several points, however, need further consideration before a conclusive statement can be made regarding retrosplenial c-Fos activity and recovery of spatial memory.

The first point is whether the low c-Fos levels in treated rats were due to only a partial behavioral recovery and non-specific differences during testing that precluded a direct link between spatial memory and this limbic molecular marker. Overall, ATN rats showing recovery often showed poorer spatial working memory compared to the sham group. Perhaps the most critical data, however, concern spatial working memory performance on the last day of testing, 90 min prior to euthanasia and subsequent processing for c-Fos immunoreactivity. While the ATN-Std-Cere group continued to show intermediate recovery of spatial memory, the two enriched groups with ATN lesions performed only marginally and non-significantly below the level of the Sham group. While ceiling levels of accuracy in the Sham group may have contributed to the dissociation between c-Fos activity in the retrosplenial cortex and spatial memory, the size of the disparity in c-Fos levels makes it difficult to believe that the two measures are closely linked when recovery of function is observed. In addition, there was little evidence of an association between c-Fos counts in the retrosplenial cortex and performance on the spatial working memory task. We should, however, bear in mind the caveat that the sham group was standard housed, whereas the two groups showing almost full recovery were enriched housed. That is, absence of sham enriched groups means that enrichment itself may reduce c-Fos activity so that the disparity would be less dramatic than we found when the comparison was made to a reference control group that had been standard housed. Some support for this possibility comes from the general reduction of c-Fos activity across cortical brain regions that were associated with enrichment in the ATN rats. Another study also reported reduced c-Fos activity in the frontal cortex after enrichment, which was also evident in sham-operated enriched (male) rats in that study (Dupire et al. 2013). Everyday exposure to novelty in enriched rats may reduce neuronal excitability, which may lead to reduced expression of molecular markers of neuronal activity (Struthers et al. 2005). However, Dupire et al. (2013) also found that enrichment did not rescue low c-Fos expression in the retrosplenial cortex and in this case c-Fos expression was equivalently high in both the enriched and standard-housed sham rats.

The second limitation concerns the behavioral test and methodology that was employed in the current study. The rats were familiar with the behavioral test procedures, whereas the use of a novel room to test spatial memory prior to euthanasia may have elicited stronger c-Fos activity (Sheth et al. 2008; Wirtshafter 2005). That is, using a novel room may have been informative if c-Fos had then been elevated in the recovered groups by comparison with the non-recovered rats with ATN lesions. Nonetheless, the complete failure to find any increased c-Fos expression in the recovered groups suggests that c-Fos as a molecular marker for spatial working memory may be limited or task dependent. It is also relevant that the dramatic loss of c-Fos in the retrosplenial cortex is evident even when rats are simply removed from their home cage (Jenkins et al. 2004), suggesting a pervasive deficit after ATN lesions that should show some change if beneficial treatments were able to influence this covert functional pathology to any substantial degree. The conclusion instead is that an enduring c-Fos dysfunction in the retrosplenial cortex remains which may simply reflect the loss of excitatory thalamic afferents that cannot be countered by behavioral or pharmacological treatments. This conclusion is supported by the fact that the dramatic effect of ATN damage on c-Fos expression and reduced metabolic capacity (Mendez-Lopez et al. 2013; Van Groen et al. 1993) is most evident in regions of the retrosplenial cortex that receive a particularly dense thalamic innervation (Odagiri et al. 2011; Shibata 1993a, b; Shibata and Honda 2012; Shibata and Kato 1993).

It is possible that c-Fos in the retrosplenial cortex is limited to the specific modes of spatial memory that are most strongly linked with this limbic cortex. Lesions of the retrosplenial cortex in rats generally have weak, if any, effects on standard spatial memory tasks. Instead, deficits emerge when alternative behavioral tasks are used, such as navigating during darkness or once internal and external cues are placed in conflict when maze rotation is introduced after the first four choices in a radial arm maze (Pothuizen et al. 2008, 2010; Vann and Aggleton 2005). The latter task has revealed an association between increased c-Fos levels in the retrosplenial cortex and reduced spatial memory errors (Aggleton 2010; Pothuizen et al. 2009). Hence, these alternative behavioral methods may be more sensitive to the association between c-Fos in the retrosplenial cortex and recovery of function.

Given that the ATN represent a nodal point in a widely distributed memory system, it is possible that the recovery observed after enrichment, and potentially cerebrolysin, involves components other than the retrosplenial cortex. One obvious candidate is the hippocampal system. In fact, ATN lesions have been shown to reduce synaptic density in both the superficial layers of the retrosplenial cortex and on hippocampal CA1 neurons, but only the latter effect was

reversed albeit partially by enrichment (Harland et al. 2014). The ATN can also affect neurogenesis in the adult dentate gyrus. High-frequency stimulation of the ATN increases neurogenesis, which can rescue memory performance (Encinas et al. 2011; Hamani et al. 2011; Toda et al. 2008). Enrichment and cerebrolysin also increase adult neurogenesis in the dentate gyrus, which suggests one mechanism whereby these treatments improve spatial memory after ATN lesions (Olson et al. 2006; Rockenstein et al. 2007). Indeed, both enrichment and cerebrolysin have multiple effects across many brain structures that support learning and memory (Hirase and Shinohara 2014; Juarez et al. 2011; Leger et al. 2012). Conversely, the beneficial effects of these treatments do not rely on an intact hippocampal system, because they have been found to improve performance after hippocampal lesions, although lesions of the entorhinal cortex appear resistant to enrichment effects, so this parahippocampal structure may influence recovery after ATN lesions (Francis-Turner and Valouskova 1996; Vazquez-Roque et al. 2012; Will et al. 2004).

In summary, the present study shows that recovery of spatial working memory can be elicited after experimental ATN lesions by different therapeutic strategies, including behavioral and pharmacological treatments. Reduced expression of c-Fos in the retrosplenial cortex is a hallmark of secondary, and apparently permanent, distal dysfunction after ATN lesions. This low c-Fos activity was not, however, increased by two therapeutic strategies, even when both approaches were combined, which may underlie the incomplete recovery of memory functions. Further work is required to determine the extent to which secondary metabolic dysfunctions contribute to the behavioral deficits elicited by focal thalamic damage.

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Conflict of interest None.

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