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# ANTERIOR THALAMIC NUCLEI LESIONS IN RATS DISRUPT MARKERS OF NEURAL PLASTICITY IN DISTAL LIMBIC BRAIN REGIONS

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**Abstract—In two related experiments, neurotoxic lesions were placed in the anterior thalamic nuclei of adult rats. The rats were then trained on behavioral tasks, immediately followed by the immunohistochemical measurement of molecules linked to neural plasticity. These measurements were made in limbic sites including the retrosplenial cortex, the hippocampal formation, and parahippocampal areas. In Experiment 1, rats with unilateral anterior thalamic lesions explored either novel or familiar objects prior to analysis of the immediate-early gene *zif268*. The lesions reduced *zif268* activity in the granular retrosplenial cortex and postsubiculum. Exploring novel objects resulted in local changes of hippocampal *zif268*, but this change was not moderated by anterior thalamic lesions. In Experiment 2, rats that had received either bilateral anterior thalamic lesions or control surgeries were exposed to novel room cues while running in the arms of a radial maze. In addition to *zif268*, measurements of c-AMP response element binding protein (CREB), phosphorylated CREB (pCREB), and growth associated protein43 (GAP-43) were made. As before, anterior thalamic lesions reduced *zif268* in retrosplenial cortex and postsubiculum, but there were also reductions of pCREB in granular retrosplenial cortex. Again, the hippocampus did not show lesion-induced changes in *zif268*, but there were differential effects on CREB and pCREB consistent with reduced levels of hippocampal CREB phosphorylation following anterior thalamic damage. No changes in GAP-43 were detected. The results not only point to changes in several limbic sites (retrosplenial cortex and hippocampus) following anterior thalamic damage, but also indicate that these changes include decreased levels of pCREB. As pCREB is required for neuronal plasticity, partly because of its regulation of immediate early-gene expression, the present findings reinforce the concept of an 'extended hippocampal system' in which hippocampal function is dependent on distal sites such as the anterior thalamic nuclei.** 

**Key words: CREB, GAP-43, hippocampus, phosphorylated CREB, retrosplenial cortex, *zif268*.**

## INTRODUCTION

Behavioral experiments have shown that anterior thalamic–hippocampal interactions are critical for spatial and contextual memory (Parker and Gaffan, 1997a,b; Aggleton and Brown, 1999; Warburton et al., 2000, 2001; Henry et al., 2004). Clinical studies add further weight to the notion that the anterior thalamic nuclei comprise part of an extended-hippocampal system that supports key elements of episodic memory (Van der Werf et al., 2003; Tsivilis et al., 2008; Aggleton et al., 2011; Carlesimo et al., 2011). The present study sought to understand why the anterior thalamic nuclei and hippocampus are often interdependent. For this reason, two related experiments examined the impact of anterior thalamic lesions on the activity of the hippocampus and related limbic regions by assaying levels of various molecules linked to neuronal plasticity.

Previous studies have shown how anterior thalamic lesions can reduce activity of the immediate early gene (IEG) *c-fos* within the hippocampus (Jenkins et al., 2002a,b; see also Vann and Albasser, 2009), potentially helping to explain why such lesions disrupt spatial memory tasks (see Liu et al., 2012). In addition, medial diencephalic pathology that includes the anterior thalamic nuclei disrupts cholinergic activity in the hippocampus (Savage et al., 2003; Roland and Savage, 2007). The present study pursued this inter-relationship by comparing the consequences of lesions in the anterior thalamic nuclei on activity levels of another IEG, *zif268* (also known as *egr-1* or *krox24*). The IEG *zif268* was studied as its expression is often closely associated with hippocampal plasticity and so it appears to be involved in spatial learning and memory (Richardson et al., 1992; Abraham et al., 1993, 1994; Herdegen and Leah, 1998; Tischmeyer and Grimm, 1999; Jones et al., 2001; Guzowski, 2002; Davis et al., 2003; Lindecke

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*Abbreviations:* ATN, anterior thalamic lesion; Audp, primary auditory cortex; BSA, bovine serum albumin; cRdg, caudal dysgranular retrosplenial cortex; CREB, c-AMP response element binding protein; cRga, caudal granular retrosplenial cortex, area a; cRgb, caudal granular retrosplenial cortex, area b; DAB, diaminobenzidine; dSub, dorsal subiculum; GAP-43, growth associated protein43; Hpc, hippocampus; iCA1, intermediate CA1; iCA3, intermediate CA3; iDG, intermediate dentate gyrus; IEG, immediate early gene; IL, infralimbic cortex; lEnto, lateral entorhinal cortex; mEnt, medial entorhinal cortex; NMDA, *N*-methyl-D-aspartic acid; PBS, phosphate buffer saline; PBST, PBS containing 0.2% Triton X-100; pCREB, phosphorylated CREB; PFA, 4% paraformaldehyde in 0.1 M PBS; PL, prelimbic cortex; Prh, perirhinal cortex; pSub, postsubiculum; rRdg, rostral dysgranular retrosplenial cortex; rRgb, rostral granular retrosplenial cortex, area b; tCA, temporal CA1; tCA3, temporal CA3.

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et al., 2006; Kubik et al., 2007; Poirier et al., 2008a). Previous studies have shown that anterior thalamic lesions lower levels of both c-*fos* and *zif268* in the retrosplenial cortex (Jenkins et al., 2004b; see also Albasser et al., 2007), suggesting that anterior thalamic lesions will also cause *zif268* hypoactivity in the hippocampus, i.e., *zif268* will again follow the pattern seen with c-*fos*. Reflecting this focus on the hippocampus, other closely related sites, e.g., the retrosplenial and parahippocampal cortices (Diana et al., 2007; Vann et al., 2009) were also examined.

In Experiment 1, all rats received unilateral anterior thalamic lesions to allow inter-hemispheric comparisons of *zif268* activity levels. These rats were divided into two groups (Group Novel and Group Familiar). One group was given novel objects to explore on the final test session before IEG analysis while the other group received just familiar objects to explore. Based on previous measures of c-*fos* expression using this protocol (Albasser et al., 2010b) it was expected that the Group Novel rats would show higher perirhinal and hippocampal IEG expression in the intact hemisphere than Group Familiar, potentially making it easier to detect any impact of anterior thalamic damage on limbic activation.

The lack of hippocampal *zif268* changes after unilateral anterior thalamic damage in Experiment 1 prompted a second experiment. In Experiment 2, the rats received bilateral anterior thalamic lesions, so that any null result would not be due to cross-hemispheric connections. This change meant that a separate surgical control group was required. The animals were also given a different behavioral task immediately prior to IEG analysis, the task more explicitly involving spatial learning. Experiment 2 also broadened the search for hippocampal-related activity changes after anterior thalamic lesions by looking at three additional molecules, as well as *zif268*. These additional targets were: (1) c-AMP response element binding protein (CREB), (2) phosphorylated CREB (pCREB), and (3) growth associated protein43 (GAP-43). The first two molecular targets were selected as there is a wealth of evidence highlighting the importance of the conversion of CREB to pCREB for the consolidation of new learning, including hippocampal-dependent learning (Silva et al., 1998; Guzowski et al., 2001; Guzowski, 2002; Mizuno et al., 2002; Winograd and Viola, 2004; Countryman et al., 2005; Warburton et al., 2005). As part of its functions, pCREB is a transcription factor for the induction of *c-fos* and *zif268* (Herdegen and Leah, 1998; Silva et al., 1998; Davis et al., 2003). For these reasons both CREB and pCREB were examined.

The final target, GAP-43, was selected as it is a regulator of growth cone motility that has been repeatedly associated with neuronal plasticity, e.g., its expression often changes in neurons undergoing neural growth (Gionotti et al., 1992; Benowitz and Routtenberg, 1997; Carmichael et al., 2005). Furthermore, GAP-43 is upregulated following axonal injury and is regarded as a key component of the systems controlling axonal regeneration (Plunet et al., 2002; Snider et al., 2002). Previous studies have established that lesions in the anterior thalamic nuclei can have chronic distal effects on c-*fos* and *zif268* levels in the retrosplenial cortex, yet not cause overt structural changes in the same region (Jenkins et al., 2004b; Poirier et al., 2008b; Poirier and Aggleton, 2009). For this reason, GAP-43 is of particular interest as any changes in this protein might reflect chronic responses to deafferentation that are not revealed by standard histological methods. Indeed, previous studies have found that hippocampal changes in GAP-43 can be observed for at least 70 days after entorhinal cortex lesions (Steward, 1995; Hardman et al., 1997).

## EXPERIMENT 1

Levels of *zif268* expression were analyzed immunohistologically in rats with unilateral anterior thalamic lesions. The rats were divided into two groups (Novel, Familiar) according to the nature of their behavioral training. The principal difference between Group Novel and Group Familiar was whether the rats had explored novel or familiar objects during the final session prior to immunohistochemical analysis.

### Materials and methods

*Subjects.* The subjects were 25 male Lister Hooded rats (*Rattus norvegicus*) housed in a 12-h light/dark cycle and weighing 270–320 g at the beginning of the experiment (Harlan, Bicester, UK). Water was provided *ad libitum* throughout, but the rats were maintained at 85% of their free-feeding weight for the duration of the experiment. The rats were divided into two groups: Novel ($n = 13$) and Familiar ($n = 12$), and where possible housed in pairs of one Group Novel rat and one Group Familiar rat. Both Experiments 1 and 2 were performed in accordance with the UK Animals (Scientific Procedures) Act (1986) and associated guidelines, thereby complying with APA ethical standards for the treatment and care of animals.

*Surgery – anterior thalamic lesions.* Surgery was performed under pentobarbitone sodium anesthesia (60 mg/kg i.p., Sigma–Aldrich Company Ltd., Dorset, UK). Once anesthetized, the animal was placed in the head-holder of the stereotaxic apparatus (Kopf Instruments, CA, USA) with the incisor bar adjusted to +5.0 relative to the horizontal plane. Following an incision, the scalp was retracted to expose the scull. A craniotomy was made and the dura cut to expose the cortex above the target location. Unilateral lesions were made by injecting 0.12 M *N*-methyl-D-aspartic acid (NMDA; Sigma Chemicals, UK) dissolved in sterile phosphate buffer (pH 7.4) into two separate sites within one hemisphere with the use of a 1-μl Hamilton syringe (Hamilton, Switzerland) that was attached to a moveable arm mounted on the stereotaxic frame. The other hemisphere was left intact. At each site, 0.22 μl of NMDA was injected over a period of 5 min, and the