Changes in Fos expression in the rat brain after unilateral lesions of the anterior thalamic nuclei

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Abstract

Activity of the immediate early gene c-fos was compared across hemispheres in rats with unilateral anterior thalamic lesions. Fos protein was quantified after rats performed a spatial working memory test in the radial-arm maze, a task that is sensitive to bilateral lesions of the anterior thalamic nuclei. Unilateral anterior thalamic lesions produced evidence of a widespread hippocampal hypoactivity, as there were significant reductions in Fos counts in a range of regions within the ipsilateral hippocampal formation (rostral CA1, rostral dentate gyrus, ‘dorsal’ hippocampus, presubiculum and postsubiculum). A decrease in Fos levels was also found in the rostral and caudal retrosplenial cortex but not in the parahippocampal cortices or anterior cingulate cortices. The Fos changes seem most closely linked to sites that are also required for successful task performance, supporting the notion that the anterior thalamus, retrosplenial cortex and hippocampus form key components of an interdependent neuronal network involved in spatial mnemonic processing.

Introduction

The normal functioning of the hippocampus is critically dependent on other structures, one of which appears to be the anterior thalamic nuclei (ATN). Behavioural evidence for this comes from a disconnection study that investigated crossed, unilateral lesions between the ATN and hippocampus (Warburton et al., 2001). Rats with crossed, unilateral lesions were impaired on various spatial tasks, including the radial-arm maze and Morris water maze (Warburton et al., 2001), unlike animals with lesions in both sites in the same hemisphere. This result indicates that these structures operate within an integrated system to support spatial memory. As these regions are reciprocally connected (Shibata, 1993; van Groen & Wyss, 1995), disconnection studies cannot isolate the contribution of the efferents from the ATN. Evidence for their role has largely come from electrophysiological studies; these have shown that the anterior dorsal nucleus of the ATN contains head direction cells (Blair & Sharp, 1995; Taube et al., 1996), and that the anterior dorsal nucleus is a critical relay for head direction information reaching the postsubiculum (Taube, 1995; Blair et al., 1997; Goodridge & Taube, 1997; Taube & Muller, 1998). This leaves unresolved the contribution of the remaining ATN nuclei and the extent to which ATN lesions disrupt brain regions other than the postsubiculum. The present study therefore sought to map the effects of ATN lesions across multiple brain regions, including the various subfields of the hippocampus.

The immediate early gene, c-fos, is widespread throughout the brain and has been used as a marker of neuronal activation (Dragunow & Faull, 1989). There is, in addition, evidence that the c-fos gene may have a more specific contribution to learning processes (Herdegen & Leah, 1998; Tischmeyer & Grimm, 1999).

Consistent with this, it has been found that blocking c-fos expression in the dorsal hippocampus impairs spatial memory formation in the radial-arm maze (He et al., 2002). In addition, performing the radial-arm maze task leads to increased Fos, the protein product of c-fos, in a network of interlinked sites including the ATN, hippocampus, subicular complex and retrosplenial cortices (Vann et al., 2000a, b; He et al., 2002). Thus, by measuring the disruptive effects of anterior thalamic lesions upon the c-fos activation associated with spatial learning it is possible to identify those loci where the ATN are most likely to influence spatial memory.

In the present study, rats with unilateral ATN lesions were trained on a working memory task in the radial-arm maze and Fos levels compared across hemispheres. Unilateral lesions were selected as a previous disconnection study (Warburton et al., 2001) showed that they are sufficient to disrupt hippocampal function. Furthermore, within subject comparisons provide the closest behavioural control. This is important as rats with bilateral ATN lesions are consistently impaired on the radial-arm maze task (Aggleton et al., 1996; Byatt & Dalrymple-Alford, 1996), and so it would not be possible to match their abnormal arm choices with those of a control group also performing the radial arm maze task. It is for this reason that a related study into the effects of bilateral ATN lesions on Fos activity (Jenkins et al., 2002) could not use an explicit spatial memory test.

Materials and methods

Animals

Nine male pigmented rats (Dark Agouti strain; Harlan, UK) weighing from 220 g to 250 g were used. Ten days after surgery animals were food deprived to 85% of their free-feeding body weight and maintained at this level throughout the experiment. Water was available ad libitum. Animals were housed in pairs under diurnal
conditions (14 h light and 10 h dark) and all testing occurred at a regular time during the light period. Animals were thoroughly habituated to handling before the study began. All experiments were carried out in accordance with the UK Animals (Scientific Procedures) Act (1986) and associated guidelines.

**Apparatus**

Testing was carried out in an eight-arm radial maze which consisted of an octagonal central platform (34 cm diameter) and eight equally spaced radial arms (87 cm long, 10 cm wide). The base of the central platform and the arms were made of wood, whereas panels of clear Perspex (24 cm high) formed the walls of the arms. At the end of each arm was a food well (2 cm in diameter and 0.5 cm deep). At the start of each arm was a clear Perspex guillotine door (12 cm high), which controlled access in and out of the centre area. Each door was attached to a pulley system enabling the experimenter to control access to the arms. All animals were tested in the same rectangular room (295 × 295 × 260 cm) which contained salient visual cues such as high contrast stimuli and geometric shapes on the walls.

**Surgery**

Rats were anaesthetized by intraperitoneal injection of pentobarbitone sodium (Sagatal) at a dose of 60 mg/kg. Animals were then placed in a stereotaxic frame (David Kopf Instruments, Tujunga, USA) and the scalp cut and retracted to expose the skull. A tone sodium (Sagatal) at a dose of 60 mg/kg. Animals were then

Animals were trained to run in the maze using a standard working memory procedure as described by Olton et al. (1978). Thus, at the start of a ‘run’ all eight arms were baited with a single food pellet (45 mg; Noyes Puriﬁed Rodent Diet, Lancaster, NH, USA). When the rat returned to the central platform, all doors were closed for approximately 5 s before they were again opened, permitting the animal to make a choice. This continued until all eight arms had been visited. Retrieving all eight pellets constituted a single ‘run’, composed of a minimum of eight arm choices. Training consisted of four sessions. The only unusual aspect of the training was that each session consisted of multiple runs in the radial-arm maze, one after the other, so that each session lasted for 30 min. Therefore, after entering all eight arms the animal was removed from the maze while it was rebaited and then returned to the maze to perform a new trial. This was repeated for 30 min. The delay between each run (2 min) was the time it took to rebait all of the arms, and during this period the animals were placed in a travelling box which had an aluminium top, base and sides (10 × 10 × 26 cm).

**Final session**

The final (fifth) session was the same as those in training, i.e. 30 min of radial-arm maze testing (approximately six radial-arm maze runs). Immediately before every session, including the final session, each animal was placed in a soundproof box in a dark, quiet room for 30 min. At the completion of every session, including the final session, each animal was returned to this box for 90 min before perfusion. This quiet period was to minimize c-fos activation in the periods before and after the radial-arm maze task.

**Immunohistochemistry**

Ninety minutes after completing the final radial-arm maze session, rats were anaesthetized deeply with pentobarbitone sodium (60 mg/kg) and perfused transcardially with 0.1 M phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in 0.1 M PBS. The brains were removed and postﬁxed in 4% paraformaldehyde for 4 h and then transferred to 30% sucrose overnight. Coronal sections were cut at 30 µm on a freezing microtome and a 1 in 2 series was collected in 0.1 M PBS containing 0.2% Triton X-100 (PBST). To inhibit endogenous peroxidase the sections were transferred to 0.3% hydrogen peroxide in PBST for 10 min and then washed several times with PBST. Sections were incubated in PBST containing Fos rabbit polyclonal antibody (1:5000; Ab-5, Oncogene Science, UK) for 48 h at 4°C with periodic rotation. Sections were then washed with PBST and incubated in biotinylated goat anti-rabbit secondary antibody (diluted 1:200 in PBST; Vectastain, Vector Laboratories, Burlingame, CA, USA) and 1.5% normal goat serum for 2 h at room temperature on a rotator. Sections were again washed and processed with avidin-biotinylated horseradish peroxidase complex in PBST (Elite Kit; Vector Laboratories) for 1 h at room temperature, also with constant rotation. Sections were washed again in PBST and then in 0.05 M Tris buffer. The reaction was then visualized using diaminobenzidine (DAB Substrate Kit, Vector Laboratories). The reaction was stopped by washing in cold PBS and then sections were mounted on gelatin-coated slides, dehydrated through a graded series of alcohols and coverslipped. A separate 1 in 4 series of sections was made in a standard frame sample area (0.84

**Image analysis**

Sections were scanned using a Leitz Dialux 20 microscope equipped with a Dage MTI CCD72S camera interfaced to a PC computer. After image processing, counts of the stained nuclei were carried out using the public domain Scion Image 4.0 program. Cortical areas were assessed using counts of nuclei labelled above threshold, and were taken across all layers of cortical regions. The threshold was set at the same level for both hemispheres of each section sampled. Counts were made in a standard frame sample area (0.84 × 0.63 mm) using a 10 × objective and the camera was positioned so that the counts were taken across all cortical layers. For dorsal and ventral hippocampal counts and hippocampal subﬁeld (dentate gyrus, CA3 and CA1) counts the entire extent of the target region within the selected coronal sections was assessed by creating a montage of the target area on a Power Macintosh computer (8500/150) using the public domain National Institutes of Health (NIH) Image program, and subsequently counted.
For all brain areas analysed (Fig. 1), counts were taken from four consecutive sections from each hemisphere (lesioned and intact side). The counts were then normalized across the two hemispheres by dividing the scores from one hemisphere by the sum from both hemispheres, and the result expressed as a percentage. Thus, all pairs of normalized scores add up to 100. By normalizing the data in this way it was possible to reduce the variance between animals that is a consequence of carrying out the immunohistological procedures in separate batches. The normalization procedure also equates the cell counts from different regions, which would otherwise vary considerably so ensuring individual areas do not have a disruptive effect on any overall analysis. These normalized data were then used for the statistical analyses, unless otherwise stated. The separate groups of regions were analysed in overall analyses of variance with two factors: hemisphere and brain region. When appropriate, the simple effects for each brain region were analysed as recommended by Winer (1971).

**Regions of interest**

Cytoarchitectonic subfields were identified from coronal sections, using the nomenclature of Swanson (1992). Regions analysed are depicted in Fig. 1. Sites were selected either because they had previously been implicated in memory processes or because they served as ‘control’ cortical regions. All of the sites from which it was decided *a priori* to count Fos-positive cells are presented.

Cytoarchitectonic subfields within the hippocampal formation included the rostral dentate gyrus (DG), rostral CA3, rostral CA1, and subicular region (Fig. 1). The subicular region involved separate counts for the dorsal and ventral subiculum, as well as the presubiculum, parasubiculum, and postsubiculum. More caudal ‘dorsal’ and ‘ventral’ hippocampal counts were taken from the same coronal slices at the middle section of the hippocampus, corresponding to AP level ±5.0 mm relative to bregma in Swanson (1992). The border between these two regions (Fig. 1) corresponded to height ±5.0 mm from bregma (Moser *et al*., 1995). The ‘dorsal’ and ‘ventral’ hippocampal counts involved just the DG and fields CA1 and CA3, i.e. not the subiculum complex. At this level the

**TABLE 1. Abbreviations of brain regions used in figures and tables**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Brain region</th>
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<tr>
<td><strong>Control regions</strong></td>
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<tr>
<td>AUDp</td>
<td>Primary auditory area</td>
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<tr>
<td>MOp</td>
<td>Primary motor area</td>
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<tr>
<td>SSP</td>
<td>Primary somatosensory area</td>
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<td>VISp</td>
<td>Primary visual area</td>
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<td>CA1</td>
<td>Rostral CA1</td>
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<td>CA3</td>
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<td>DG</td>
<td>Rostral dentate gyrus</td>
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<td>dHPC</td>
<td>Dorsal hippocampus</td>
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<td>vHPC</td>
<td>Ventral hippocampus</td>
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<td>Parasubiculum</td>
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<td>Pre</td>
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<td>Postsubiculum</td>
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<td>Subd</td>
<td>Dorsal subiculum</td>
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<td>Subv</td>
<td>Ventral subiculum</td>
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<td><strong>Cingulate cortices</strong></td>
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<td>cACA</td>
<td>Caudal anterior cingulate cortex</td>
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<td>rACA</td>
<td>Rostral anterior cingulate cortex</td>
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<tr>
<td>PL</td>
<td>Prelimbic cortex</td>
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<tr>
<td>cRSP</td>
<td>Caudal retrosplenial cortex</td>
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<tr>
<td>rRSP</td>
<td>Rostral retrosplenial cortex</td>
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<td>Porh</td>
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<td><strong>Thalamic nuclei</strong></td>
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<td>AD</td>
<td>Anterodorsal thalamic nucleus</td>
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<td>AM</td>
<td>Antero medial thalamic nucleus</td>
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<td>AV</td>
<td>Anteroventral thalamic nucleus</td>
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Fig. 1. Diagrams of coronal sections indicating the areas investigated. The numbers indicate the distance (in millimeters) of the section from bregma (Swanson, 1992). See Table 1 for list of abbreviations.
dentate gyrus is present in both the ‘dorsal’ and ‘ventral’ hippocampus.

Fos-reactive cells were also counted in the parahippocampal region (Witter et al., 1989; Burwell et al., 1995). The perirhinal counts involved both areas 35 and 36 (Burwell et al., 1995), whereas the postrhinal cortex only involved cortex posterior to the perirhinal cortex and dorsal to the rhinal sulcus (Burwell & Amaral, 1998; corresponding to the ectorhinal area in Swanson, 1992). The lateral and medial entorhinal cortices were considered separately in light of their different connection patterns (Witter et al., 1989; Naber et al., 1997). Other cortical counts were taken from the prelimbic area, the rostral and caudal levels of the anterior cingulate cortex, and rostral and caudal levels of the retrosplenial cortex (Fig. 1). The retrosplenial counts involved both the granular and dysgranular portions of the cortex. For all cortical regions, Fos counts were made across all lamina.

Counts were also taken from a number of ‘control’ cortical regions for purposes of comparison in order to determine the selectivity of any findings. These regions were selected on the criteria that they were not directly connected with the anterior thalamus and were not known to have a specific role in spatial memory. The cortical areas were the visual cortex (primary visual area; VISp), the somatosensory cortex (primary somatosensory area; SSP), the auditory cortex (primary auditory area; AUDp) and the motor cortex (primary motor area; MOp).

Results

Anterior thalamic lesions

Two cases with lesions involving <50% of the anterior thalamic nuclei were removed from all analyses. In the remaining seven cases with larger unilateral thalamic lesions, the cell loss was centred in the anterior thalamic nuclei. As a consequence, the three principal anterior thalamic nuclei were the sole common lesion site across all cases. The extent of the largest and smallest of these seven lesions is shown in Fig. 2. The NMDA injections created discrete regions of cell loss (Fig. 3) within the anterior thalamic nuclei that did not cross over into the opposite hemisphere. All three anterior thalamic nuclei showed extensive cell loss, though in six cases there was sparing at the more caudal limits of the complex. Whereas the anterior dorsal nucleus displayed the most complete cell loss, the caudal and the most medial portions of the anterior medial nucleus showed some sparing in most cases. In two cases there was cell loss restricted to the rostral portion of the lateral dorsal nucleus. Although the hippocampus was intact in all cases, there was very minor involvement of the parataenial and reticular nuclei in the cases with the largest lesions. The remaining midline nuclei were intact.

Behavioural results

On the final test day, the animals performed the standard version of the eight-arm radial maze. Testing took place over a 30 min session, and animals typically ran a total of six runs (each run comprises the retrieval of all eight pellets) in this session. The mean number of

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FIG. 2. Series of five coronal sections showing the area of cell loss in the largest (left) and smallest (right) anterior thalamic lesions. Numbers refer to the distance in millimetres from bregma (Paxinos & Watson, 1997).

FIG. 3. Photomicrographs of a Nissl-stained coronal section contrast the anterior thalamic nuclei in a normal hemisphere (left) and a lesioned hemisphere (right). (A) intact; (B) lesioned. AD, anterior dorsal thalamic nucleus; AM, anterior medial thalamic nucleus; AV anterior ventral thalamic nucleus.
errors per run across all runs within this session (± SEM in parenthesis) was 1.6 (0.3). The mean number of correct responses in the first eight choices, across all trials in the final session, was 7.2 (0.1). These performance levels are similar (1.1 and 7.2, respectively) to those of normal rats that were trained and tested in exactly the same way (Vann et al., 2000a, b).

**Fos counts**

**Hippocampus proper**

The initial analysis involved counts taken across all subfields of the hippocampus proper (‘dorsal’ hippocampus, ‘ventral’ hippocampus, and rostral CA1, CA3, and dentate gyrus). There were higher Fos counts in the intact hemisphere as compared to the lesioned hemisphere (Fig. 4), and this was confirmed by the main effect of hemisphere ($F_{1,12} = 16.0$, $P < 0.01$). These hemispheric differences were found to be significant for the rostral CA1 region ($F_{1,60} = 8.8; P < 0.01$), rostral dentate gyrus ($F_{1,60} = 11.1; P < 0.01$) and ‘dorsal’ hippocampus ($F_{1,60} = 5.1; P < 0.05$). No hemispheric differences were found, however, when counts were made in the rostral CA3 ($F_{1,60} = 1.7; P > 0.1$) or ‘ventral’ hippocampus ($F_{1,60} = 1.4; P > 0.1$) (Fig. 4).

**Subicular region**

Comparisons involving the various subicular cortices (Fig. 5) revealed a significant hemispheric difference for the subicular region ($F_{1,12} = 8.1; P < 0.05$). Within the various subregions Fos counts were found to be significantly lower in the lesioned hemisphere in both the postsubiculum ($F_{1,60} = 9.0; P < 0.01$) and presubiculum ($F_{1,60} = 20.1; P < 0.0001$). No clear group differences were found in the remaining regions: dorsal subiculum ($F < 1$), ventral subiculum ($F < 1$) and parasubiculum ($F_{1,60} = 1.1; P > 0.1$).

**Cingulate cortices**

Counts were made in the prelimbic cortex, rostral and caudal levels of the anterior cingulate area and rostral and caudal levels of the retrosplenial cortex (Fig. 6). Overall, a significant hemispheric effect was observed ($F_{1,12} = 8.0; P < 0.05$), with significant reductions in Fos levels on the lesioned side compared to the intact side in the rostral retrosplenial cortex ($F_{1,60} = 13.8; P < 0.0001$) and caudal retrosplenial cortex ($F_{1,60} = 38.6; P < 0.0001$). No hemispheric differences were found, however, when counts were made in the prelimbic cortex ($F_{1,60} = 1.9; P > 0.1$), rostral anterior cingulate ($F < 1$), and caudal anterior cingulate area ($F_{1,60} = 2.8; P = 0.1$; Fig. 6).
was observed (Fet al (Aggleton radial-arm maze. This task was chosen because lesion studies have measured 90 min after rats completed a working memory task in the spatial memory impairments associated with pathology in this thalamic damage, and so identify those sites that could contribute to the The goal was to map out areas that are disrupted after anterior compared across hemispheres in animals with unilateral ATN lesions. Using Fos as a marker of neuronal activation, activity levels were compared across hemispheres in animals with unilateral ATN lesions. There was no evidence of a hemispheric effect (F < 1) or an area–hemisphere interaction (F3,36 = 2.9; P = 0.05) within this region (Fig. 7).

Control regions
The cortical control regions examined were the MOp, SSp, VISp, and AUDp (Fig. 8). Across the four cortical areas there was no significant hemispheric effect (F < 1). A hemisphere–area interaction, however, was observed (F1,36 = 6.9, P < 0.01), which upon further analysis reflected a significant increase in Fos levels on the lesioned side in the MOp (F1,4h = 6.0, P < 0.05) and a significant decrease in the VISp (F1,4h = 8.0, P < 0.01).

Discussion
Using Fos as a marker of neuronal activation, activity levels were compared across hemispheres in animals with unilateral ATN lesions. The goal was to map out areas that are disrupted after anterior thalamic damage, and so identify those sites that could contribute to the spatial memory impairments associated with pathology in this region (Aggleton & Brown, 1999). For this reason, Fos levels were measured 90 min after rats completed a working memory task in the radial-arm maze. This task was chosen because lesion studies have shown that it requires the integrity of the anterior thalamic nuclei (Aggleton et al., 1996; Byatt & Dalrymple-Alford, 1996). The present study found that unilateral ATN lesions lead to significant Fos decreases in five regions within the hippocampal formation, as well as the retrosplenial cortex. It is noteworthy that all of these same regions normally show an increase in Fos levels in the intact brain following radial-arm maze performance (Vann et al., 2000a, b).

The hippocampal formation was the principal region of interest. The ATN project directly to the retrohippocampal region, terminating in the presubiculum, parasubiculum, postsubiculum and dorsal subiculum (Shibata, 1993; van Groen & Wyss, 1995). Consistent with these direct projections, ATN lesions altered hippocampal Fos levels, although these changes were not restricted to the retro-hippocampal region. Of the ten subregions measured, the mean Fos count was always lower in the lesioned hemisphere. Of these ten subregions, significantly lower Fos levels were found in rostral CA1, rostral dentate gyrus, ‘dorsal’ hippocampus, postsubiculum and presubiculum. It therefore appears that the impact of unilateral anterior thalamic lesions extends beyond those hippocampal regions that receive direct thalamic inputs. The Fos changes in the ‘dorsal’ hippocampus are of special interest as blockade of c-fos expression in this region can disrupt both working and reference memory in the radial-arm maze (He et al., 2002). This evidence of a widespread hippocampal hypoactivity also helps to explain why crossed unilateral lesions of the anterior thalamic nuclei and the hippocampus can produce marked deficits on tests of spatial memory, including the radial-arm maze (Warburton et al., 2001).

The present evidence of a widespread hippocampal hypoactivity is all the more striking when it is considered that the hippocampus has very dense interhemispheric inputs via the hippocampal commissure (Blackstad, 1956; Swanson et al., 1987). As these afferents are likely to attenuate any lesion-induced effects, it is most likely that bilateral anterior thalamic pathology would have had an even more disruptive effect upon hippocampal Fos levels. It should also be noted that the lesions in the present study were carefully targeted in order to minimize the involvement of other thalamic nuclei. This sparing included the adjacent midline nuclei. This is potentially important as some of these nuclei (reuniens, parataenial, paraventricular) have dense projections to the hippocampus (Wyss et al., 1979) that could also alter levels of hippocampal activity.

Outside the hippocampal formation, the clearest lesion effect was observed in the retrosplenial cortex. These findings reinforce the very close functional relationship between the ATN and retrosplenial cortex (Gabriel, 1993; Gabriel et al., 1989). This cortical region has dense, reciprocal connections with all three anterior thalamic nuclei (van Groen et al., 1993), and consistent with this there was decreased c-fos activity in both the rostral and caudal retrosplenial regions. In agreement with the present findings, a study into the effects of much more extensive unilateral thalamic lesions reported decreased levels of cytochrome oxidase, a marker of metabolic activity, in the retrosplenial (Rbg) cortex of the lesioned hemisphere (van Groen et al., 1993). The circumscribed lesions used in the present study suggest that anterior thalamic pathology would be sufficient to induce these metabolic changes. The present lesion-induced abnormalities in the retrosplenial cortex also help to account for the findings of a disconnection study (Sutherland & Hoesing, 1993) that examined the behavioural effects of crossed unilateral lesions between the anterior thalamic nuclei and retrosplenial cortex. The resultant water maze deficit (Sutherland & Hoesing, 1993) indicates that unilateral anterior thalamic lesions are sufficient to render dysfunctional the ipsilateral retrosplenial cortex. Direct evidence for this assumption was found in the present study.

In contrast to the present study, which examined unilateral ATN lesions, a previous study mapped the effects of bilateral ATN lesions upon limbic Fos levels (Jenkins et al., 2002). The present study, however, offers a number of key advances. By using unilateral lesions, the present study avoided the problem posed by the abnormal arm choices associated with bilateral anterior thalamic lesions (Aggleton et al., 1996; Byatt & Dalrymple-Alford, 1996). Thus, unlike the present study, the earlier study (Jenkins et al., 2002) could not use an explicit memory task to recruit regions involved in spatial memory (Vann et al., 2000a, b). Instead, animals were placed in a novel room to induce c-fos activation, but their ability to use this spatial information was not taxed (Jenkins et al., 2002). The present study also made it possible to provide the closest control (interhemispheric) for potential differences in motor activity and sensory

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**Fig. 8 Normalized counts of Fos-positive nuclei in control cortical regions.** Data are shown as mean ± SEM. All normalized data sum to 100 (see Materials and methods). See Table 1 for abbreviations. Significance of hemispheric differences in normalized counts: *P < 0.05, **P < 0.01.

**Parahippocampal cortices**
Within the parahippocampal cortices counts were taken from the lateral and medial entorhinal cortex, perirhinal and postrhinal cortex. The hippocampal formation was the principal region of interest. Of the ten subregions measured, the mean Fos count was always lower in the lesioned hemisphere. Of these ten subregions, significantly lower Fos levels were found in rostral CA1, rostral dentate gyrus, ‘dorsal’ hippocampus, postsubiculum and presubiculum. It therefore appears that the impact of unilateral anterior thalamic lesions extends beyond those hippocampal regions that receive direct thalamic inputs. The Fos changes in the ‘dorsal’ hippocampus are of special interest as blockade of c-fos expression in this region can disrupt both working and reference memory in the radial-arm maze (He et al., 2002). This evidence of a widespread hippocampal hypoactivity also helps to explain why crossed unilateral lesions of the anterior thalamic nuclei and the hippocampus can produce marked deficits on tests of spatial memory, including the radial-arm maze (Warburton et al., 2001).

The present evidence of a widespread hippocampal hypoactivity is all the more striking when it is considered that the hippocampus has very dense interhemispheric inputs via the hippocampal commissure (Blackstad, 1956; Swanson et al., 1987). As these afferents are likely to attenuate any lesion-induced effects, it is most likely that bilateral anterior thalamic pathology would have had an even more disruptive effect upon hippocampal Fos levels. It should also be noted that the lesions in the present study were carefully targeted in order to minimize the involvement of other thalamic nuclei. This sparing included the adjacent midline nuclei. This is potentially important as some of these nuclei (reuniens, parataenial, paraventricular) have dense projections to the hippocampus (Wyss et al., 1979) that could also alter levels of hippocampal activity.

Outside the hippocampal formation, the clearest lesion effect was observed in the retrosplenial cortex. These findings reinforce the very close functional relationship between the ATN and retrosplenial cortex (Gabriel, 1993; Gabriel et al., 1989). This cortical region has dense, reciprocal connections with all three anterior thalamic nuclei (van Groen et al., 1993), and consistent with this there was decreased c-fos activity in both the rostral and caudal retrosplenial regions. In agreement with the present findings, a study into the effects of much more extensive unilateral thalamic lesions reported decreased levels of cytochrome oxidase, a marker of metabolic activity, in the retrosplenial (Rbg) cortex of the lesioned hemisphere (van Groen et al., 1993). The circumscribed lesions used in the present study suggest that anterior thalamic pathology would be sufficient to induce these metabolic changes. The present lesion-induced abnormalities in the retrosplenial cortex also help to account for the findings of a disconnection study (Sutherland & Hoesing, 1993) that examined the behavioural effects of crossed unilateral lesions between the anterior thalamic nuclei and retrosplenial cortex. The resultant water maze deficit (Sutherland & Hoesing, 1993) indicates that unilateral anterior thalamic lesions are sufficient to render dysfunctional the ipsilateral retrosplenial cortex. Direct evidence for this assumption was found in the present study.

In contrast to the present study, which examined unilateral ATN lesions, a previous study mapped the effects of bilateral ATN lesions upon limbic Fos levels (Jenkins et al., 2002). The present study, however, offers a number of key advances. By using unilateral lesions, the present study avoided the problem posed by the abnormal arm choices associated with bilateral anterior thalamic lesions (Aggleton et al., 1996; Byatt & Dalrymple-Alford, 1996). Thus, unlike the present study, the earlier study (Jenkins et al., 2002) could not use an explicit memory task to recruit regions involved in spatial memory (Vann et al., 2000a, b). Instead, animals were placed in a novel room to induce c-fos activation, but their ability to use this spatial information was not taxed (Jenkins et al., 2002). The present study also made it possible to provide the closest control (interhemispheric) for potential differences in motor activity and sensory

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**Fig. 8 Normalized counts of Fos-positive nuclei in control cortical regions.** Data are shown as mean ± SEM. All normalized data sum to 100 (see Materials and methods). See Table 1 for abbreviations. Significance of hemispheric differences in normalized counts: *P < 0.05, **P < 0.01.
anterior thalamic damage. A similar Fos increase in the motor cortex was found in the current study, but here it is most unlikely to be the result of increased motor activity (as this would presumably be the same for both hemispheres). It would therefore appear that the changes seen in the motor cortex are a secondary response to alterations elsewhere in the hemisphere. By the same logic, the unilateral Fos decrease in the visual cortex does not reflect a gross behavioural change. As neither the motor nor the visual cortical regions receive direct inputs from the anterior thalamic nuclei these effects must be mediated through other areas. The retrosplenic cortex provides a plausible candidate (van Groen et al., 1993; Vogt & Miller, 1988).

As might be expected, evidence of hippocampal hypoactivity was also seen after bilateral anterior thalamic lesions (Jenkins et al., 2002). In that study, significant Fos decreases were found in the ‘dorsal’ and ‘ventral’ hippocampus but not in those other subregions that were assessed. Thus in the present study, unilateral lesions resulted in additional, significant Fos decreases in CA1, dentate gyrus, postsubiculum and presubiculum. As the lesions in the present study were typically more restricted than those in the earlier study (Jenkins et al., 2002) it would seem that the abnormally low Fos levels in these additional regions reflects the use of an explicit memory task. This explanation is plausible as performance of the radial-arm maze task normally increases Fos levels in each of the ten hippocampal subfields (Vann et al., 2000a, b), and hippocampal lesions lead to very severe deficits in task performance. Thus, the recruitment of these areas in the normal brain appears to increase the likelihood of detecting a lesion induced change.

Comparisons can be made with the consequences of unilateral ATN and unilateral fornix lesions on Fos production (Vann et al., 2000c). This is of interest as both studies used unilateral lesions to examine c-fos activity after performance of the same radial-arm maze task (Vann et al., 2000c). Unilateral fornix lesions led to widespread Fos changes, as might be predicted given the large number of regions connected by this tract (Swanson et al., 1987). Of particular relevance is the finding that, with the exception of the visual cortex, all of the regions that showed a significant Fos decrease in the current study also showed a significant decrease after fornix lesions (Vann et al., 2000c). These common areas of hypoactivity in the hippocampal formation and retrosplenial cortex could help to explain why bilateral lesions in the fornix and ATN can produce deficits on tests of spatial memory that are often of similar magnitude (Aggleton et al., 1991, 1995; Warburton et al., 1999). In addition, these Fos imaging studies help to explain a disconnection study in which combined unilateral lesions in the fornix and the ATN induced deficits on the radial-arm maze task (Warburton et al., 2000). Both of these surgeries reduce hippocampal Fos levels, and there is evidence that the blockade of c-fos expression in the dorsal hippocampus impairs radial arm maze performance (He et al., 2002). Thus, these findings point to the common areas of dysfunction, e.g. in the dorsal hippocampus, that are most likely to be responsible for the lesion induced behavioural deficits.

By combining the present results with those of a previous study, which used bilateral anterior thalamic lesions but a passive memory task (Jenkins et al., 2002), a fuller picture emerges of the sites that could depend on the anterior thalamic nuclei for normal function. Hypoactivity can be found almost throughout the hippocampus proper, along with the presubiculum, postsubiculum, and parasubiculum. In addition, hypoactivity might also occur in the retrosplenial cortex, the anterior cingulate cortex and the prelimbic cortex (Jenkins et al., 2002). These sites include those regions, that like the anterior thalamus, contain head direction cells [i.e. retrosplenial cortex, postsubiculum and hippocampus (Taube, 1995; Blair et al., 1997; Goodridge & Taube, 1997; Taube & Muller, 1998; Leutgeb et al., 2000; Cho & Sharp, 2001)] but the consequence of this thalamic pathology is far more extensive. Although the motor cortex shows an upregulation of c-fos, it is clear that the overall effect of anterior thalamic damage is to produce hypoactivity throughout limbic brain regions. This, in turn, is likely to have negative consequences on learning and memory.

References


