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Research report

Reduced cytochrome oxidase activity in the retrosplenial cortex after lesions to the anterior thalamic nuclei

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HIGHLIGHTS

- ATN damage reduced cytochrome oxidase activity in the granular retrosplenial cortex.
- A modest reduction was also apparent in the anterior cingulate cortex.
- No other change was observed in the limbic circuitry.
- The same lesions severely impaired spatial discrimination.
- CO activity measures enable to examine cortical diaschisis following thalamic damage.

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ABSTRACT

The anterior thalamic nuclei (ATN) make a critical contribution to hippocampal system functions. Growing experimental work shows that the effects of ATN lesions often resemble those of hippocampal lesions and both markedly reduce the expression of immediate-early gene markers in the retrosplenial cortex, which still appears normal by standard histological means. This study shows that moderate ATN damage was sufficient to produce severe spatial memory impairment as measured in a radial-arm maze. Furthermore, ATN rats exhibited reduced cytochrome oxidase activity in the most superficial cortical layers of the granular retrosplenial cortex, and, to a lesser extent, in the anterior cingulate cortex. By contrast, no change in cytochrome oxidase activity was observed in other limbic cortical regions or in the hippocampal formation. Altogether our results indicate that endogenous long-term brain metabolic capacity within the granular retrosplenial cortex is compromised by even limited ATN damage.

1. Introduction

Injury to the anterior thalamic nuclei (ATN) impairs learning and memory in many behavioural tasks that are also disrupted by hippocampal dysfunction [1–8]. Given the strong neural connections between these structures [9], this behavioural evidence gave rise to the influential proposal that the ATN and hippocampus constitute an interdependent functional system in support of episodic memory [10,11]. Moreover, both ATN and hippocampal lesions trigger a dramatic hypoexpression of immediate early gene (IEG) products in the retrosplenial cortex (RSG), particularly evident in the superficial layers of the granular RSG, a region that itself interconnects with both structures [12–19]. Importantly, the retrosplenial cortex still shows normal appearance by standard histological means so that IEG hypoexpression in this region has been classified as a "covert pathology" [1].

In view of these findings, the aim of the current study was to further investigate an early observation that large unilateral ATN lesions reduced cytochrome oxidase (CO) activity in the retrosplenial cortex [20]. The measure of CO activity reflects metabolic capacity, which may be more directly associated with intrinsic functionality than IEG expression because it determines the

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amount of ATP available, therefore constraining the amount of activity that a neuron can sustain [21]. Unfortunately, this early observation was not supported by quantitative data as a single case study was the only material provided [20]. A more serious concern arose from the use of electrolytic lesions which were unusually large. Several additional thalamic loci such as the midline, intralaminar or mediodorsal nuclei were thus damaged [22], which may obscur the interpretation of the results. We therefore made highly specific, fibre-sparing, bilateral ATN lesions in rats to examine CO activity in the limbic circuitry, with a particular emphasis on regions that receive strong ATN afferents such as the granular RSG and the anterior cingulate cortex. We hypothesized that CO activity may represent a more stable indicator of cortical diaschisis following focal thalamic damage than IEG measures because it is not influenced by short-term environmental stimulations. Spatial memory performance of the same ATN rats was also examined in the radial-arm maze to confirm the effectiveness of the lesions at the behavioural level.

2. Methods

2.1. Animals

14 male Long Evans rats obtained from the Centre d'Elevage Janvier (France) were used (weight 275–325 g at surgery). They were housed in pairs throughout the experiment in standard rat cages (polycarbonate 49 × 26 × 20 cm) with ad libitum food and water and a 12/12 h light cycle (lights on at 7.00 a.m.). All experiments were conducted in agreement with the French (council directive 87848, October 19, 1987) and international (directive 86-609, November 24, 1986, European Community) legislation regarding animal experiments, and care was taken at all stages to minimize stress and discomfort to the animals.

2.2. Surgery

Rats were anaesthetized with 4% Isoflurane and placed in a stereotaxic frame with atraumatic ear bars (Kopf, Tujunga, CA) in a flatskull position. Anaesthesia was maintained with 1.5–2% Isoflurane complemented by subcutaneous administration of diazepam (0.2 ml of Valium© per rat) and Carprophen (Norocarp©, 5 mg/kg). Eight rats received neurotoxic ATN lesions using multiple NMDA micro-injections. Twenty μg/μL NMDA (Sigma-Aldrich) in artificial cerebrospinal fluid (CMA Microdialysis AB, Solna, Sweden) was pressure-injected into the brain through a glass micropipette (outside diameter: around 100 μm) connected to polyethylene tubing (Picospritzer, General Valve Corporation, Fairfield, NJ). Two lesion sites per side were used: AP − 1.3 mm from bregma, laterality ± 1.3 mm, ventrality − 5.4 mm from dura; and AP − 1.5 mm, laterality ± 1.5 mm and ventrality − 5.5 mm. Each site was injected with 0.12 μL of NMDA and the pipette was left in place 3 min after injection before slow retraction. The Sham rats ($n = 6$) received similar surgery except that the micropipette was lowered only in the cortex and no injection was made (ventrality −2.5 mm). Rats were given at least 10 days of recovery before behavioural testing began.

2.3. Apparatus and behavioural procedures

The automated eight-arm radial maze used for spatial discrimination testing was made of grey plexiglas and consisted of a circular central platform (30 cm diameter) from which radiated in a symmetrical fashion eight arms (60 cm long and 12 cm wide). A food-pellet tray was situated at the end of each arm. Animals were food deprived to maintain their body weight at 90% of their ad libitum weight during testing, and were initially allowed free exploration sessions on two successive days with all arms baited with food to familiarize them with the maze and its environment.

For spatial discrimination testing, each subject was assigned a different set of three constantly baited arms (arms 1, 4, 6) with the specification that the sequence of angles between the baited arms was 135°–90°–135° as previously described [24]. Briefly, rats were tested for acquisition of this reference memory task over ten daily sessions composed of six trials. At the beginning of a trial, all eight doors were open. After each visit, the door giving access to that arm was automatically closed, preventing the subject from making working memory errors (re-entries into an already visited arm). Each trial was terminated after the animal retrieved the third food pellet and had returned to the central platform. Discrimination performance was assessed by the percentage of correct responses recorded for the first three arm choices (3 choices per trial times 6 trials per session = 18 choices) and by the number of runs necessary to obtain the three rewards.

2.4. Histology and cytochrome oxydase activity

Ninety minutes after the end of the behavioural procedures (between day 37 and day 39 post-surgery), the rats were decapitated, their brain dissected and frozen in isopentane. Coronal brain sections (30 μm) were processed for quantitative CO histochemistry according to the method described by Gonzalez-Lima and Jones [25]. The CO activity was quantified as previously described [26]. Briefly, quantification of CO staining intensity was measured densitometrically and converted to CO units using a computer-assisted image analysis workstation composed of a high precision illuminator, a digital camera and a computer with specific image analysis software (MCID, InterFocus Imaging Ltd., Linton, England). Each selected region was measured bilaterally using three consecutive sections in each rat. In each section, readings were taken as shown in Fig. 1. The measurements taken per region were averaged to obtain one mean per region for each subject.

Additional brain sections throughout the ATN region were collected for lesions verification. After coloration with thionin, Mathieu Wolff, while blind to behavioural and CO data, performed conventional observation under a microscope and used a computer-assisted method to provide an estimation of the volume of the lesions as extensively described elsewhere [2,6–8,27,28].

3. Results

3.1. Histology

Fig. 2 depicts the largest and smallest ATN lesions that met an *a priori* criterion of at least 50% injury to the ATN region together with minimal damage to adjacent thalamic regions. Two rats were excluded from subsequent analysis, due to insufficient damage to the ATN (i.e. <50%). The six rats with acceptable ATN lesions sustained moderate ATN injury, with a mean of 59.7% (range: 53.7–75.0%). The anterodorsal nucleus was more severely affected (mean: 69.7%, range: 56.9–94.9%) than the anteroventral (mean: 51.1%, range: 45.0–58.0%) and the anteromedial (mean: 47.3%, range: 40.0–64.9%) nuclei (see Tables 1 and 2 for individual values). This moderate damage was highly specific as it only marginally encompassed the adjacent lateral thalamic nuclei (comprising the rostral intralaminar nuclei and lateral mediodorsal thalamic nuclei, mean: 2.1%, range: 0.6–6.6%) and the more medial thalamic nuclei (comprising the central and medial mediodorsal thalamic nuclei and the intermediodorsal nucleus, mean: 0.3%, range: 0.0–1.1%). No significant damage was observed in the interanteromedial, laterodorsal, rhomboid, reuniens and paraventricular nuclei. The paratenial nucleus shows minimal damage (3.2%, range: 0.7–5.9%). No damage was apparent in the hippocampal formation or in the fornix.

3.2. Radial-arm maze testing

Fig. 3 shows the performance of rats submitted to a spatial discrimination task in the radial-arm maze over ten consecutive daily sessions (depicted as blocks of 2 days). While all rats improved their performance overall (Day, $F(4,40) = 6.95$, $P = 0.0002$; Day × Lesion, $F < 1$), ATN rats were considerably impaired relative to Sham rats, which was evident throughout the entire testing period (Lesion, $F(1,10) = 38.12$, $P < 0.0001$; Fig. 3A). The same findings arose from the analysis of the number of runs necessary to find the three rewards (Fig. 3B). All rats progressively improved over time (Day, $F(4,40) = 11.96$, $P < 0.0001$; Day × Lesion, $F(4,40) = 1.76$, $P = 0.16$) but ATN rats were less efficient (Lesion, $F(1,10) = 26.64$, $P = 0.0004$).

We also examined the time necessary to complete a trial (Fig. 3C). The analysis revealed a progressive reduction of this parameter over time (Day $F(4,40) = 7.43$, $P < 0.0001$) but, contrary to the two primary measures of performance, no effect of the Lesion (Lesion, Lesion × Day, $F_s < 1$). This final result is noteworthy because it confirms that both Sham and ATN rats had the opportunity to sample similar environmental stimulations to build up spatial representation. Hence, any difference in subsequent CO measures cannot be simply explained by differential experiences.