Behavioural Brain Research 208 (2010) 566–575

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

# Effects of selective granular retrosplenial cortex lesions on spatial working memory in rats

Helen H.J. Pothuizen[1], Moira Davies, John P. Aggleton, Seralynne D. Vann*

*School of Psychology, Cardiff University, Tower building, Park Place, Cardiff, CF10 3AT, UK*

ARTICLE INFO

*Article history:*
Received 13 November 2009
Received in revised form 3 January 2010
Accepted 4 January 2010
Available online 12 January 2010

*Keywords:*
Cingulate cortex
Radial-arm maze
Water-maze
Spatial memory
T-maze

ABSTRACT

The rat retrosplenial cortex comprises two major subregions (granular and dysgranular) that differ in morphology and connectivity. Although the effects of selective dysgranular retrosplenial cortex (area 30) lesions and the effects of selective lesions within separate sub-areas of the granular retrosplenial cortex have been described, the effects of complete granular lesions (area 29) remain unknown. The present study, therefore, contrasted excitotoxic lesions of the total granular retrosplenial cortex with complete retrosplenial cortex lesions (dysgranular plus granular) using two spatial working memory tasks variably sensitive to complete retrosplenial damage. The granular retrosplenial and complete retrosplenial lesion groups were comparably impaired throughout most of radial-arm maze acquisition, including when subsequently challenged by having the maze rotated mid-trial or being tested in the dark. The other test, reinforced spatial alternation in a T-maze, provided a slightly different result as it was the rats with selective granular cortex lesions that were most impaired when the rats were tested in two, parallel mazes (one for the sample run, the other for the test run). These findings reveal the importance of the granular retrosplenial cortex for learning across a variety of different spatial tasks. Combining these findings with the results of previous functional and anatomical studies suggests that the granular and dysgranular retrosplenial subregions function in close conjunction to support spatial learning.



## 1. Introduction

It is now evident that the retrosplenial cortex plays an important role in spatial learning and navigation, both in rats [1,13,14,19,25,28,34,35,37,41] and humans [11,15,20]. This conclusion is consistent with its dense anatomical connections with other structures known to support spatial memory, e.g. the hippocampal formation and the anterior thalamic nuclei [28,31–33,40,42]. However, the precise nature of the contribution of the retrosplenial cortex to these processes remains largely a matter of conjecture [37].

One factor hindering the development of a comprehensive model of retrosplenial function is that the region is not uniform. The retrosplenial cortex is composed of two distinct cytoarchitectonic subregions, areas 29 and 30 [18,39]. These two subregions correspond, respectively, to the granular and dysgranular areas within the retrosplenial cortex [31–33]. Alternative accounts exist of further subdivisions within the granular retrosplenial cortex of the rat, and we will adhere to the terminology of Van Groen and co-workers [31–33] as their nomenclature has been preferred in previous investigations into functional differences within the rat retrosplenial cortex [e.g. 26,30,36]. Using their terminology, the granular subregion has been further subdivided into an area a (Rga) and an area b (Rgb), each with their different patterns of connectivity [31–33]. Of the two major retrosplenial subregions, the dysgranular area (Rdg) has many more connections with visual regions, e.g. areas 17, 18b, while the granular subregion is more interconnected with sites containing head-direction cells, e.g. the postsubiculum and anterior dorsal thalamic nucleus [31–33]. These anatomical differences suggest that the granular and dysgranular subregions contribute to retrosplenial function in different ways.

Only two studies have investigated the behavioural effects of selective lesions within the retrosplenial subregions. One study [36] showed that selective dysgranular (Rdg) lesions impair the effective use of distal visual cues in a spatial working memory task in the radial-arm maze. The other study [30] found that lesions of Rgb, but not Rga, are sufficient to impair spatial learning in the Morris water-maze. Surprisingly, the effects of *total* granular (Rga plus Rgb, i.e. area 29) lesions have yet to be reported. The present study, therefore, examined the impact of granular cortex lesions intended to remove both Rga and Rgb, on two spatial working memory tests

* Corresponding author. Tel.: +44 29 2087 6253; fax: +44 29 2087 4858.
*E-mail address:* VannSD@Cardiff.ac.uk (S.D. Vann).
[1] Present address: Laboratory of Behavioural Neurobiology, Swiss Federal Institute of Technology (ETH) Zurich, Schorenstrasse 16, CH-8603 Schwerzenbach, Switzerland.

0166-4328/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbr.2010.01.001

that are variably sensitive to the loss of the complete retrosplenial cortex or just its dysgranular subregion [25,34,36].

For both spatial tasks, training was conducted in both the light and the dark in response to evidence that the distinct demands imposed by these conditions can differentially affect the impact of retrosplenial cortex lesions [6,25]. The T-maze alternation study included testing with two adjacent T-mazes, making it possible to preclude the use of intra-maze cues and to examine strategies [9,12,25] such as direction alternation (alternation around a known heading or bearing) and egocentric alternation (alternation with respect to body turn). For comparison purposes, the present study sought to contrast groups of rats with either: (a) complete retrosplenial cortex lesions (i.e., granular plus dysgranular); (b) granular retrosplenial cortex lesions; (c) dysgranular retrosplenial cortex lesions; and d) surgical controls. If both retrosplenial subregions require each other for spatial learning then granular lesions should be as disruptive as complete retrosplenial lesions or dysgranular lesions, but if the dysgranular and granular cortices have some independent spatial functions then it might be expected that granular lesions would be less disruptive than complete retrosplenial cortex lesions.

## 2. Materials and methods

### 2.1. Experimental design

#### 2.1.1. Subjects

Subjects were 39 male, pigmented rats (Dark Agouti strain; Harlan Bicester, UK) weighing 223–248 g at the time of surgery. Animals were housed in pairs under diurnal light conditions (14 h light/10 h dark) and testing was carried out during the light phase at the same time each day. The rats were food deprived to 85% of their free-feeding body weight and maintained at this level, or above, throughout the experiment (unless stated otherwise). Water was available *ad libitum*. The animals were handled daily for at least one week before surgery, when they were randomly assigned to one of the four surgical groups: receiving 'complete' retrosplenial cortex lesions ('Compl rspl', $n = 9$), selective 'granular' retrosplenial cortex lesions ('Gran rspl', $n = 12$), selective 'dysgranular' retrosplenial cortex lesions ($n = 11$), or Sham operations ('Sham', $n = 8$). All experiments were carried out in accordance with UK Animals (Scientific Procedures) Act, 1986 and associated guidelines, and all efforts were made to minimise animal suffering.

#### 2.1.2. Surgery

Animals were deeply anaesthetised by an intraperitoneal (i.p.) injection (60 mg/kg) of 6% sodium pentobarbital (Sigma Chemical Company Ltd, Poole, UK, freshly dissolved in saline). The rats were each placed in a stereotaxic headholder (David Kopf Instruments, Tujunga, CA) with the nose bar at +5.0. The scalp was then cut and retracted to expose the skull. A craniotomy was performed to expose the cortex. After all surgeries, the skin was sutured and an antibiotic powder (Acramide; Dales Pharmaceuticals, Skipton, UK) applied topically. Animals also received subcutaneous injections of glucose saline (5 ml) and were given paracetamol and sucrose in their drinking water for at least four days post-surgery. All rats were allowed approximately four weeks to recover before the start of behavioural testing.

*2.1.2.1. Granular retrosplenial lesions.* The granular retrosplenial lesions were made by multiple bilateral infusions of 0.054 M *N*-methyl-D-aspartic acid (NMDA; Sigma) dissolved in 0.1 M phosphate buffer (PB, pH 7.2) using the stereotaxic coordinates and volumes indicated in Table 1. The infusions were made using a custom-made 34G needle attached to a 5 μl microsyringe (SGE Europe Ltd., Milton Keynes, UK) placed in a microinjection unit (David Kopf Instruments) at a rate of approximately 0.05 μl/min. The needle was left in place for 3 min after each infusion.

*2.1.2.2. Dysgranular retrosplenial lesions.* The dysgranular lesions were made by multiple bilateral injection of 0.09 NMDA (in PB) according to the coordinates indicated in Table 1 (adopted from Ref. [36]). The infusions were made using a standard 25G needle attached to a 1 μl Hamilton syringe (model 7001, Bonaduz, Switzerland) at a rate of 0.1 μl/min. The needle was left in place for an additional 3 min after each infusion.

*2.1.2.3. Complete retrosplenial lesions.* The complete retrosplenial lesions were made by injecting a solution of 0.09 M NMDA (in PB) using the coordinates and volumes as indicated in Table 1. The same needle, syringe and infusion rate was used as for the granular lesions. The needle was left in place for 3 min after each infusion.

**Table 1**
Stereotaxic coordinates for the retrosplenial excitotoxic lesions. Anterior–posterior (AP) and midline (ML) coordinates are in millimeters from bregma; dorsoventral (DV) coordinates are in millimeters from the surface of the cortex. ML coordinates marked with an asterisks (*) were at 10° angle from the vertical.

| Lesion type/coordinates | | | |
|---|---|---|---|
| AP | ML | DV | Injected volume (μl) |
| Granular retrosplenial cortex: | | | |
| −2.3 | ±0.6* | −1.6 | 0.05 |
| −3.6 | ±0.6* | −1.6 | 0.05 |
| −4.7 | ±0.7* | −1.6 | 0.05 |
| −5.8 | ±0.8 | −2.0 | 0.10 |
| −6.5 | ±0.8 | −2.0 | 0.10 |
| −7.2 | ±1.0 | −1.6 | 0.10 |
| Dysgranular retrosplenial cortex: | | | |
| −1.9 | ±0.7 | −1.2 | 0.20 |
| −3.3 | ±0.7 | −1.2 | 0.30 |
| −4.9 | ±0.8 | −2.0 | 0.15 |
| −4.9 | ±1.0 | −1.2 | 0.25 |
| −6.0 | ±1.1 | −1.6 | 0.30 |
| −6.8 | ±1.3 | −1.2 | 0.30 |
| Complete retrosplenial cortex: | | | |
| −2.3 | ±0.6* | −1.6 | 0.05 |
| −3.6 | ±0.6* | −1.6 | 0.05 |
| −4.7 | ±0.5 | −1.6 | 0.05 |
| −5.8 | ±0.8 | −2.0 | 0.17 |
| −6.7 | ±0.8 | −2.0 | 0.17 |

*2.1.2.4. Sham operated controls.* The surgical operated controls received the same procedure and drugs as the lesioned animals, including the craniotomy, with the exception of lowering the needle into the brain and the injection of NMDA.

### 2.2. Experiment 1: Radial-arm maze

#### 2.2.1. Apparatus

Testing was carried out in an eight arm radial maze. The maze consisted of an octagonal central platform (diameter 34 cm) with eight equally spaced radial-arms (87 cm long, 10 cm wide) each with a recessed cylindrical food well (diameter 2 cm, 0.5 cm deep) at the end of each arm. The base of the central platform and the arms were made of wood, while clear Perspex formed the walls (24 cm high) of the arms. The central platform was placed on a clear, Perspex column (diameter 34.5 cm, 55 cm high). At the start of each arm was a clear Perspex guillotine door (12 cm high) that controlled access in and out of the central platform. Each door was attached to a pulley system enabling the experimenter to control access to the arms from a distance. The maze was in a rectangular room that contained salient visual cues such as geometric shapes and high contrast stimuli on the walls.

Illumination during initial habituation and training in the 'light' was provided by two standard ceiling lights giving a mean illumination of 671 lux measured in the middle of the central platform. During training in the 'dark', the illumination was provided by a standard lamp, fitted with a 60 W red light bulb placed inside the column of the radial-arm maze apparatus, immediately under the central platform. This arrangement for the 'dark' condition gave an average light intensity of 2.5 lux on the central platform. The room lights were switched off and a white cotton curtain was pulled around the outside of the radial-arm maze. The rats were transported in groups of four between the holding room and the radial-arm maze room in an opaque, aluminium travelling box.

#### 2.2.2. Procedure

One day before the start of the experiment, the rats were familiarized with the sucrose reward pellets (45 mg; Noyes Purified Rodent Diet, UK) in their home cage. Pre-training for the radial-arm maze involved three habituation sessions where the animals were allowed to explore the maze freely for 5 min. All the guillotine doors were raised and reward pellets were scattered down the arms. This was followed by formal training which lasted for 30 sessions and consisted of four stages.

*2.2.2.1. Stage 1: Acquisition (Light).* The acquisition phase (sessions 1–12) was the standard working memory version of the radial-arm maze task [23] where the animals' optimal strategy was to retrieve the reward pellets from all eight arms without re-entering any previously explored arms within that same trial. At the start of a trial all eight arms were baited with a single reward pellet. The animal would make an arm choice and then return to the central platform. All doors to the arms were closed for about 10 s before they were opened again, permitting the animal to make another choice. This procedure continued until all eight arms had been visited, i.e. a single 'trial' had been completed.