Testing the importance of the retrosplenial guidance system: effects of different sized retrosplenial cortex lesions on heading direction and spatial working memory

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Abstract

The present study: (1) tested the importance of the retrosplenial cortex for learning a specific heading direction and distance and, (2) determined if lesion size could explain apparent inconsistencies in the results of different research groups. Dark agouti rats received either ‘complete’ cytotoxic retrosplenial cortex lesions or ‘standard’ lesions, the latter sparing the caudal retrosplenial cortex. Animals were first tested on two versions of a ‘landmark’ task in a water maze. In condition 1 animals could use both heading direction and allocentric position, while in condition 2 only heading direction was effective. In condition 1, animals with complete retrosplenial lesions were impaired by the end of training, their profile of performance being consistent with a failure to use allocentric position information. When the water maze task changed (condition 2) so that allocentric cues became redundant, the animals with complete retrosplenial lesions were able to head in the appropriate direction although they showed longer swim paths. Subsequent testing in the radial-arm maze provided more evidence that retrosplenial lesions can disrupt the use of distal (allocentric) room cues. The impairments seen with retrosplenial lesions were often mild but throughout the study performance of rats with ‘complete’ lesions was more disrupted than those with ‘standard’ lesions, who often did not differ from the controls. These findings show that lesion size is a critical factor and may explain why some studies have failed to find comparable deficits after retrosplenial cortex lesions.

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1. Introduction

The rodent retrosplenial cortex (area 29) is thought to be important for spatial learning and memory. Evidence comes from the lesion induced deficits found for both reference and working memory tests in the water maze [10,24,27,29], and for tests of spatial working memory in the radial-arm maze [6,27,29]. The present study had two interlinked goals. The first was to identify more precisely the nature of these spatial impairments. For this, attention focused on the hypothesis that the retrosplenial cortex provides directional guidance information [12]. The second goal arises from the large variations found between studies in the severity of spatial impairment associated with retrosplenial lesions. By comparing lesions of different sizes in the same study we provide the first direct test of the importance of this factor.

The retrosplenial cortex is densely connected with the anterior thalamus and laterodorsal thalamus [26], regions thought to be important for directional learning [25,32]. Furthermore, like the anterodorsal and laterodorsal thalamic nuclei, the retrosplenial cortex contains head direction cells [4,5]. For these reasons the first experiment tested the ability to head in a fixed direction and distance from a cue (landmark) placed within a water maze [21]. Two task variants were used. In the first condition rats could use not only the direction of the platform from the landmark but also its absolute position [21]. In the second condition the landmark and, hence the platform, changed position on every trial, so providing a purer test of the ability to learn a specific trajectory and distance. In further tests we also examined the nature of the retrosplenial lesion deficit found in the radial-arm maze. Previous studies have indicated that this deficit may reflect a reduced
reliance on extramaze cues [27,29], but this account remains to be tested systematically.

The second goal addressed apparent inconsistencies between the findings of different research groups concerning the effects of retrosplenial lesions in rats. Two different explanations have been provided. First, that these inconsistencies reflect strain differences as the Dark Agouti strain, used by some groups, is claimed to be relatively insensitive to retrosplenial lesions [10]. Second, that these inconsistencies reflect variations in lesion extent and nature, with key predictors of lesion effects being the amount of area 29 damage and the involvement of the underlying cingulum bundle [2,27]. To examine these accounts we compared two groups of Dark Agouti strain rats with different lesion sizes. One group (complete) received surgeries intended to remove the full extent of area 29. In the second group (standard) the lesions spared caudal area 29, so corresponding to the lesion placement most typically reported in studies on this region (e.g., [16,24]).

2. General methods

2.1. Subjects

Subjects were 32 male, pigmented rats (Dark Agouti strain) weighing between 226 and 268 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. Animals were given free access to water throughout. All experiments were carried out in accordance with UK Animals (Scientific Procedures) Act, 1986 and associated guidelines.

2.2. Surgery and histology

Animals were deeply anaesthetised by intraperitoneal injection (60 mg/kg) of sodium pentobarbital. The 12 rats receiving ‘complete’ retrosplenial cortex lesions (CMPrspl) and the 10 rats receiving ‘standard’ lesions (STDrspl) were then 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. The lesions were made by injecting a solution of 0.09 M N-methyl-D-aspartic acid (NMDA; Sigma Chemical Company Ltd., UK) dissolved in phosphate buffer (pH 7.2). Injections for the CMPrspl animals were made in six sites, 0.15 µl was injected in the third site and 0.25 µl was injected in the most caudal site. Animals receiving more confined lesions (STDrspl) received bilateral injections of NMDA in the four rostral injection sites used for the complete lesions. These injections were of the same volume and in the same locations as those in the CMPrspl group.

At the completion of all surgeries, the skin was sutured and an antibiotic powder (Acramid; Dales Pharmaceuticals, Skipton, UK) applied topically. Animals also received subcutaneous injections of 5 ml glucose saline and were given paracetamol in their drinking water for 3 days post-surgery. The 10 animals acting as surgical controls (Sham) received the same procedure and drugs as the animals receiving lesions. This involved the removal of a bone flap and the needle being lowered but without the injection of NMDA. For five of the animals this corresponded with the sites for the complete lesion and for the other five with the sites for the standard lesion.

On completion of the experiments, the animals were irreversibly anaesthetised with sodium pentobarbital (140 mg/kg) and perfused transcardially with 0.1 M phosphate-buffered saline followed by 10% formol-saline. The brains were removed and post-fixed in 10% formol-saline and then transferred to 25% sucrose overnight. Sections were cut at 40 µm on a freezing microtome in the coronal plane, and a one-in-three series of sections was mounted onto gelatine-coated slides and stained with cresyl violet, a Nissl stain.

2.3. Water maze “landmark” task

2.3.1. Apparatus

The maze (2 m in diameter, 60 cm deep) was made of white fibreglass and mounted 58 cm above the floor. The pool was filled with water (24 ± 1 °C) made opaque by the addition of non-toxic emulsion (Opacifier, Chesham Chemicals, Harrow, UK). An escape platform (10 cm diameter, 2 cm below water surface) could be placed in the pool. The visible landmark was a spherical object 13 cm in diameter, positioned on a stick so that it appeared to rest on the surface of the water. While the bottom half of the sphere was painted black, the top half was painted white so that the automatic tracker would not fix on its position. The distance from the edge of the landmark to the centre of the platform was 20 cm. The position of this landmark was always 20 cm north of the platform for half of the rats and 20 cm south for the other half. The pool was in a room (440 cm × 400 cm) lit by four 500 W floodlights placed on the floor in the corners of the room. Salient visual cues were placed on the walls. A plain white curtain that could be drawn closed to encircle the water maze was hung from the ceiling in a corner of the test room. The paths of the rats were tracked with a video camera suspended directly above the pool and recorded on videotape. Data were collected and analysed on-line with an HVS image analyser connected to a computer that used Watermaze Software (Edinburgh).
that were measured corresponded to circles of 20 cm radius, where the platform had been relative to the landmark, in allowed to swim for 120 s and the amount of time spent in the pool and the platform was removed. The animals were placed in eight locations in the pool, approximately 50 cm from the side wall. While the platform always remained in the same relative position to the landmark, and the location of the platform and landmark remained constant across the four trials of a given session but these locations varied between sessions. The start position (E, N, NE, NW, S, SE, SW, W) changed between every trial in a pseudo-random order. This arrangement means that animals can use the platform’s constant heading direction and distance from the landmark equally for trials 1–4, but can also incrementally use the constant allocentric position of the platform for trials 2–4. Thus trial 1 provides the most selective measure of heading vectors. Each trial terminated when the animal had either located the platform or 120 s had elapsed. The animals were then left on the platform for 30 s. The next trial began almost immediately afterwards, giving an inter-trial interval of about 15 s. All rats were transported to and from the test room in aluminium boxes that ensured that the rats could not see their surroundings until they were removed for testing.

After completion of training, rats received three probe sessions. Probe 1 consisted of four trials, the first 3 of which were identical to those of the previous 12 sessions. On the fourth trial the curtain was drawn closed round the pool to cover the distal cues. During probe 2 the curtain was left open and the first 3 trials were again identical to those in the 12 sessions of training. On the fourth trial the platform remained in the same position as it had been for the previous three trials but the landmark was removed from the pool. For probe 3 the first three trials involved the platform and landmark being moved to different locations on each trial. On the fourth trial the landmark was placed in the centre of the pool and the platform was removed. The animals were allowed to swim for 120 s and the amount of time spent in the areas north and south of the landmark were measured. Thereby, the amount of time spent searching in the area where the platform had been relative to the landmark, in training, could be measured. The areas around the landmark that were measured corresponded to circles of 20 cm radius, the area of each was 4% of the total area of the pool. This is, therefore, the percentage of time the animals would spend in that area if swimming randomly.

2.3.2. Condition 2. Immediately after probe 3 the animals were given three further acquisition sessions (each of four trials) where the landmark and platform again kept the same relationship to each other but now they were also in different locations for each trial of each session. Again, new start positions were used for every trial. This meant that animals were no longer able to navigate using absolute position but would be solely reliant on heading direction. On both the fourth and sixth session the animals received three trials where the platform and landmark were again in different locations for each trial, but on the fourth trial the landmark was placed in the centre of the pool and the platform removed (probes 4 and 6; same as probe 3, condition 1). Again the animals were allowed to swim for 120 s and the times spent in the different regions around the landmark were measured. The fifth session was similar to sessions four and six in that the platform and landmark were in different positions for the first three trials and on the fourth trial the landmark was placed in the centre of the pool and the platform was removed (probe 5). For this probe trial the curtains were drawn closed around the pool and the animals were allowed to swim for 120 s.

2.4. Radial-arm maze task

2.4.1. Apparatus

Testing was carried out in an eight-arm radial maze. The maze consisted of an octagonal central platform (34 cm diameter) with eight equally spaced radial-arms (87 cm long, 10 cm wide). The floor of the central platform and the floors of the eight arms were made of wood, while clear Perspex (24 cm high) formed the walls of the arms. Close to the furthest 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) 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. The maze was in a rectangular room (255 cm × 330 cm × 260 cm) that contained salient visual cues such as geometric shapes and high contrast stimuli on the walls. A plain white curtain that could be drawn closed to encircle the radial-arm maze was hung from the ceiling in a corner of the test room.

2.4.2. Behavioural training

Prior to training the animals were placed on a restricted diet but their weight remained at or above 85% of their free-feeding body weight. Water remained available ad libitum. Testing began 8 months after surgery. Pretraining 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
food pellets (45 mg; Noyes Purified Rodent Diet, UK) were scattered down the arms. This was followed by formal training which lasted for 31 sessions and consisted of three stages.

Stage 1 (sessions 1–15) was the standard working memory version of the radial-arm maze task [18,19] where the animals’ optimal strategy was to retrieve the reward pellets from all eight arms without re-entering any previously entered arms. At the start of a trial all eight arms were baited with a single food pellet. The animal would make an arm choice and then return to the central platform and all the doors were closed for about 10 s before they were opened again, permitting the animal to make another choice. This continued until all eight arms had been visited. On completion of a trial the number of sequential choice responses was calculated. This assessed whether the animals selected immediately adjacent arms in a constant direction (an egocentric strategy). Sequential choices were measured by giving the animal a score of +1 (clockwise) or −1 (anticlockwise) if the arm is immediately adjacent to the previous choice and 0 for any other arm choice. A higher absolute score would therefore reflect the use of a sequential response strategy [18].

Stage 2 (sessions 16–27) was to determine the nature of the disruption caused in previous studies using maze rotation in a middle of a trial. Different forms of disruption, all of which occur in maze rotation were separately assessed. These conditions were counterbalanced across animals and sessions so that at the end of stage 2 all animals had four sessions of each of the three conditions. For all conditions the first half of the session was the same, the animals selecting the first four arms. After the first four choices animals were either: (1) removed from the maze and the rat rotated, then immediately replaced in the centre of the maze in order to complete the trial; (2) confined in the centre of the maze for 60 s before being allowed to continue the session; (3) confined in the centre of the maze while the maze was rotated and the arms rebaited so that the spatial locations of the remaining pellets were unchanged with respect to the room cues.

Stage 3 (sessions 28–31) involved the rats performing two sessions of the standard version of the radial-arm maze task followed by two sessions when a curtain was drawn closed around the radial-arm maze.

2.5. Spontaneous locomotor activity

2.5.1. Apparatus and procedure

After completion of the above tests, while still food deprived to 85% of their free-feeding body weight, all rats were placed in novel test cages (56 cm × 39 cm × 19 cm) in a novel room. Activity was measured using pairs of photo-beams situated 20 cm apart and 18 cm from the end of the cage (Paul Fray Limited, Cambridge, UK). The total number of beam breaks were recorded. Data were gathered in 12 intervals of 10 min each.

3. Results

3.1. Histological analysis

All the lesion surgeries, except one, resulted in extensive cell loss to area 29. As shown in Figs. 1 and 2 the CMPrspl lesions involved much of the entire anterior–posterior extent of area 29.
of area 29, while the STDrspl surgeries spared the caudal 2.2–2.6 mm of this region. Within the lesion boundaries much of the tissue had completely collapsed, but in other areas where tissue was still intact the remaining cells looked abnormal and there was evident gliosis. In all cases there was retrograde degeneration in a limited part of the anterior ventral thalamic nucleus. The single case with unusually restricted damage (STDrspl) was removed from further analyses. In addition, one of the CMPrspl animals had extensive bilateral hippocampal and subicular damage and so was also removed. Finally, an additional CMPrspl animal only had retrosplenial cell loss to a level just caudal of the splenium and was therefore included in the STDrspl group. The final group numbers were, therefore, 10 complete retrosplenial cortex lesion animals (CMPrspl), 10 standard retrosplenial cortex lesions (STDrspl) and 10 surgical controls (Sham).

In half of the STDrspl and CMPrspl animals there was a very localised, bilateral cell loss in the dorsal hippocampus. This was typically at the very rostral level of CA1 or in the dorsal subiculum at the level of the splenium. The remaining STDrspl and CMPrspl animals either had no hippocampal damage or slight unilateral damage to the same restricted parts of the hippocampus or subiculum. When it occurred, the extent of this hippocampal damage was plotted and measured by someone who was ‘blind’ to the experimental groups. This hippocampal damage was found to be equivalent in both the STDrspl and CMPrspl lesion groups ($t < 1$). The mean percentage ($\pm$ S.E.) of hippocampal damage in the STDrspl and CMPrspl lesion groups was 2.25 ($\pm$0.59) and 2.87% ($\pm$0.60), respectively. The percentage of damage ranged from 0 to 5.41% in the CMPrspl group and 0–5.73% in the CMPrspl group.

Prior to proceeding to the full data analyses we first compared the results of those animals with some bilateral hippocampal damage to those with unilateral or no hippocampal damage to help determine if it had an additional impact on task performance. Thus for each of the two surgeries the five cases with bilateral hippocampal damage were compared to the five cases with unilateral/no additional damage. In addition, the five cases of standard shams were compared to the five cases of complete shams. For all analyses (latency and path length for acquisition of the “landmark” task in conditions 1 and 2, task entries on RAM, scores on rotation trials) $F < 1$ and, accordingly, the subgroups were combined.

### 3.2. Water maze “landmark” task

#### 3.2.1. Condition 1

**3.2.1.1. Task acquisition.** During condition 1 the platform and landmark remained in the same location for each session, but changed after each session. This means that trial 1 can only be solved effectively by using heading information, while trials 2–4 can be solved using both heading direction and the absolute position of the platform as specified by extramaze cues.

**Fig. 3** illustrates the mean path lengths and escape latencies of the groups during acquisition of the water maze “landmark” task. Analyses using path length with factors group, session and trial revealed a borderline group difference ($F_{(2,22)} = 3.35, P = 0.05$). There were significant effects of session ($F_{(3,3)} = 16.64, P < 0.001$) and trial ($F_{(1,3,3,11)} = 42.24, P < 0.001$), and a borderline group x session interaction ($F_{(2,2,2)} = 1.54, P = 0.06$). All individual groups showed an effect of trial (all $P < 0.005$) but there was no group x trial interaction ($F < 1$).

Analyses using escape latency revealed a similar pattern of results. There was a borderline group difference ($F_{(2,2,2)} = 2.96, P = 0.07$), with significant effects of session ($F_{(1,3,3,11)} = 10.87, P < 0.001$) and trial ($F_{(1,3,3,11)} = 41.12, P < 0.001$). All groups showed significant effects of session (CMPrspl $P < 0.05$; STDrspl $P < 0.001$; Sham $P < 0.005$) and trial (all $P < 0.005$). There was no group x trial interaction ($F_{(6,3,3,11)} = 1.36, P > 0.2$) but there was a group x session interaction ($F_{(2,2,2)} = 1.71, P < 0.05$) reflecting the different acquisition patterns for the CMPrspl...
and STDrspl groups. While the STDrspl group were impaired in the initial stages, the CMPspl group were impaired in the later stages of acquisition. The initial STDrspl deficit was reflected in the simple effects as group differences were found for sessions 2–4 (all $P < 0.05$).

Trials 1 and 2 were also considered separately as trial 1 provides the most selective measure of heading vectors while trial 2 is the first trial that can be solved using memory for the absolute location (Fig. 4). For this reason, trial 2 most taxes working memory. Trial 1 data revealed no overall group difference using either path length ($F_{(2, 27)} = 1.13, P > 0.3$) or escape latency ($F < 1$). However, for trial 2 there were group differences using both path length ($F_{(2, 27)} = 3.41, P < 0.05$) and escape latency ($F_{(2, 27)} = 4.14, P < 0.05$). Subsequent post-hoc analyses using path length and latency (Newman–Keuls) revealed that the Sham group outperformed both lesion groups (both $P < 0.05$). Analyses with trials 1 and 2 also revealed significant group $\times$ day $\times$ trial interactions using both path length ($F_{(22, 207)} = 1.74, P < 0.05$) and escape latency ($F_{(22, 207)} = 1.71, P < 0.05$), reflecting the differences in acquisition across groups (Fig. 4).

Probe 1. During probe 1 the curtain was drawn closed around the pool on trial 4 to exclude extramaze cues (Fig. 5). The Sham group showed a significant increase in path length between the trial preceding the probe (trial 3) and the probe trial itself ($F_{(1, 27)} = 4.47, P < 0.05$) indicating that the removal of the extramaze cues disrupted performance. In contrast, there was no significant difference in performance between the normal and probe trials for either the CMPspl or STDrspl lesion animals (both $F < 1$). Escape latencies revealed a similar pattern although it was less clear cut as there was no significant difference between trial 3 and the probe trial for any group (CMPspl and STDrspl both $F < 1$, Sham $P = 0.07$).

Probe 2. This probe was used to determine whether the animals were actually using the intramaze landmark, or whether they were solely reliant on extramaze cues. For this reason the landmark was removed on trial 4 (Fig. 6). When a comparison was made between the path lengths of the trial preceding the probe (trial 3) with the probe trial itself, both Sham ($F_{(1, 27)} = 13.33, P < 0.005$) and CMPspl groups ($F_{(1, 27)} = 6.21, P < 0.05$) were affected by the removal of the landmark. There was a similar (borderline) effect for the STDrspl group ($F_{(1, 27)} = 3.62, P = 0.068$). Consistent with these results there was no group $\times$ trial interaction nor effect of group (both $F < 1$), although there was a large effect of trial ($F_{(1, 27)} = 21.57, P < 0.001$). Once again, escape latencies produced the same pattern of results.

Probe 3. This probe investigated the search strategies used by the rats to locate the platform. After three trials with the
platform and landmark moving for each trial (curtain open), the landmark was placed in the centre of the pool and the hidden platform was removed. The rats were then allowed to swim for 120 s. As the landmark was in a novel position, the use of extramaze cues to determine the absolute position of the platform would no longer be beneficial (and might hinder performance) while directional cues would remain constant. Performance on the probe trial was measured as the time spent swimming in two areas adjacent to the landmark; one which would have included the platform position, e.g. north and the other which was directly opposite, i.e. south (Fig. 7). An ANOVA conducted on the time spent in the training area and the time in the opposite area revealed a significant effect of search area ($F_{(1,27)} = 12.78, P < 0.005$), but no group difference or group x area interaction (both $F < 1$). Examination of the simple effects revealed that both the CMPrspl and Sham groups had a significant preference for the appropriate training area (both $P < 0.05$).

### 3.2.2. Condition 2

The previous version of the “landmark” water maze task enables animals to use both directional and allocentric cues find the platform. In order to compare more specifically the use of directional cues, the next condition involved the landmark and platform moving every trial. By this means, extramaze cues for allocentric processing should become redundant. Due to a number of probes being carried out on trial 4, acquisition analyses were carried out using the first three trials of each session for six sessions (Fig. 8).

The first thing to note is that unlike condition 1 there was no overall effect of trial for path length or escape latency (both $F < 1$). This is consistent with a reliance on heading...
direction to solve the task. For path length there was, however, a significant effect of session \( (F(5, 135) = 3.73, P < 0.005) \) and of group \( (F(2, 27) = 3.97, P < 0.05) \). Subsequent post-hoc analyses (Newman–Keuls) revealed significantly longer swim paths for the CMPrspl lesion group than both the Sham and STDrspl group (both \( P < 0.05 \)). Although the CMPrspl group were also the poorest as measured by escape latency, this difference was not significant \( (F(2, 27) = 1.60, P > 0.2) \). Probes 4 and 6. These probes were identical and so were combined (Fig. 9a). They looked at the effect of removing the platform and placing the landmark in the centre of the pool for trial 4. The curtains were open so that extramaze cues were always visible. Analyses compared the time spent in two opposite areas over the 120 s of the probe trial (see probe 3, condition 1). There was no effect of group \( (F < 1) \) but a significant effect of training area \( (F(1, 27) = 23.89, P < 0.001) \).

Probe 5. On trial 4 the platform was removed and the landmark placed in the centre of the pool but with the curtains now drawn closed. Again the measures were the amount of time spent in the correct area, or in the opposite area, over the 120 s of the probe trial (Fig. 9b). There was no effect of group \( (F < 1) \) or training area \( (F(1, 27) = 2.36, P > 0.1) \). Analysis of the simple effects revealed that only the Sham group showed a preference for the training area \( (F(1, 27) = 6.18, P < 0.05) \).

3.3. Radial-arm maze task

3.3.1. Stage 1

The 15 sessions of the standard radial-arm maze task were analysed in five blocks of three sessions. Analyses were performed using total number of errors and correct arm entries in the first eight choices (Fig. 10). Total error scores (Fig. 10a) showed that there was a significant main effect of group \( (F(2, 27) = 5.48, P < 0.02) \). Subsequent post-hoc analyses (Newman–Keuls) revealed error score differences between the CMPrspl and Sham groups \( (P < 0.01) \), and the CMPrspl and STDrspl groups \( (P < 0.05) \). There was a significant effect of block \( (F(4, 108) = 43.21, P < 0.001) \) but no group × block interaction \( (F < 1) \), as the groups showed comparable improvement across training.

Analyses using correct entries in first eight choices revealed the same pattern of results (Fig. 10b). There was a significant effect of group \( (F(2, 27) = 4.22, P < 0.05) \), and post-hoc analyses again revealed differences between the CMPrspl lesion group and both the Sham and STDrspl groups \( (P < 0.05) \). There was a significant effect of block \( (F(4, 108) = 51.09, P < 0.001) \) but no group × block interaction.
interaction ($F < 1$), again showing an improvement in all groups across training. Analyses of sequential choice responses revealed no group difference ($F_{(2,27)} = 1.74, P > 0.1$). The mean sequential choice scores were low (mean ± S.E.M.: CMPrspl = 1.09 ± 0.1, STDrspl = 1.37 ± 0.1, Sham = 1.28 ± 0.1), showing they were not using a simple response strategy.

To determine whether performance was equivalent at the end of acquisition, analyses on the last three sessions were carried out separately. There was no effect of group using either errors ($F_{(2,27)} = 1.45, P > 0.2$) or correct entries ($F_{(2,27)} = 1.37, P > 0.2$).

3.3.2. Stage 2
Following acquisition, rats were given four sessions with each of the three test conditions. Analyses also included the last four sessions of standard trials in order to enable baseline comparison (Fig. 11). Analyses using total errors (Fig. 11a) revealed significant main effects of group ($F_{(2,27)} = 3.91, P < 0.05$) and task condition ($F_{(3,81)} = 30.53, P < 0.001$), but no group by condition interaction ($F < 1$). Post-hoc analyses (Newman–Keuls) of the task condition revealed significant differences between the maze rotation and the three component conditions (standard, rat rotation, delay; all $P < 0.01$), but no other comparisons reached significance. Pairwise comparisons of the groups revealed that the CMPrspl lesion animals were significantly impaired compared to the Shams ($P < 0.05$) but no other comparisons were significant. Examination of the simple effects showed a significant group difference for the maze rotation condition ($P < 0.01$).

Analyses using correct entries in the first eight choices (Fig. 11b) revealed a very similar pattern of results. There was a significant effect of group ($F_{(2,27)} = 3.55, P < 0.05$) with post-hoc comparisons showing a significant difference between the CMPrspl and Sham groups ($P < 0.05$). As before, there was also a significant effect of task condition ($F_{(3,81)} = 24.67, P < 0.001$) with post-hoc analyses showing differences between the maze rotation condition and all three other conditions ($P < 0.01$). Once again there was no group × task condition interaction ($F < 1$), but an effect of group only on the maze rotation condition ($P < 0.05$).

3.3.3. Stage 3
The final stage involved the rats performing two sessions of the standard version of the radial-arm maze task followed by two sessions when a curtain was drawn closed around the radial-arm maze. Analyses using total errors showed no effect of group ($F_{(2,27)} = 1.17, P > 0.1$) but a significant effect of condition (curtains open versus curtains closed; $F_{(1,27)} = 6.32, P < 0.02$). There was no group × condition interaction ($F < 1$).

3.4. Spontaneous locomotor activity
There was no effect of group nor was there a group × block interaction (both $F < 1$) but there was a highly significant main effect of block ($F_{(1,20)} = 36.19, P < 0.0001$) showing the habituation of all groups to the novel environment.

4. General discussion
The present study had two goals: (1) to compare the importance of the retrosplenial cortex for spatial tasks that tax different modes of learning a target location; (2) to compare the effects of retrosplenial lesions that either remove essentially all of area 29 (CMPrspl) or spare the caudal portions of area 29 (STDrspl), the latter corresponding to lesions more typically reported. Retrosplenial lesions repeatedly disrupted the effective use of allocentric cues but appeared to have more subtle effects on learning a heading vector. The present study, which is the first to compare directly retrosplenial cortex lesions of different sizes, revealed that ‘complete’ lesions can produce impairments that are not observed in animals with more ‘standard’ retrosplenial lesions. These findings can explain apparent inconsistencies between previous studies.

First, we will consider the assessment of different modes of spatial learning. As navigation using allocentric cues and heading direction can be dissociated [21] they will be considered separately, although both will normally interact. Allocentric information is taxed both in condition 1 of the ‘landmark’ task and in the radial-arm maze task. As the platform position during condition 1 is fixed within each
session, allocentric cues can be used to find the platform on trials 2–4. Evidence for their use comes from the improved performance across trials 1–4, which was found for all three groups. Nevertheless, the rate of improvement differed between the groups, so that for the later sessions the CMPrspl group failed to show the normal, rapid improvement between trials 1 and 2. This test of working memory indicated that the animals with complete retrosplenial lesions were slower at using allocentric cues. Consistent with this, the CMPrspl group along with the STDrspl group was relatively insensitive to the removal of distal cues (probe 1).

The use of allocentric cues was also examined in experiment 2 in which animals were trained in a radial-arm maze to distinguish arms that had been visited from those that had not been visited in the current session [18]. This task is highly sensitive to lesions in the hippocampus and anterior thalamic nuclei (e.g. [1,3,17]), both of which have dense connections with area 29 [26]. Consistent with its connectivity, acquisition of this task was impaired in the CMPrspl group, although the animals were eventually able to achieve an accurate level of performance. Previous studies have found that the retrosplenial lesion deficit on this task is most evident when the rat is removed from the maze after making several choices, and is then returned after a delay during which the maze is turned and rebaited so that the food items are in the same positions relative to the distal room cues but in different positions with respect to intramaze cues [27,29]. In view of the complex nature of this manipulation rats in the present study were challenged with three separate components (rotation of the rat, rotation of the maze, and imposition of a delay). Maze rotation proved to be the most demanding manipulation, and was the only one to produce a group difference as the CMPrspl group made more errors and fewer correct choices before the first error than the Sham group. Maze rotation also reinstated a deficit as the CMPrspl group had only differed from the other two groups in the earlier stages of task acquisition. As maze rotation sets intramaze and extramaze cues in conflict, the results once again indicate that the CMPrspl animals less readily used extramaze cues.

The conclusion that retrosplenial lesions can bias the use of allocentric cues is supported by a number of other studies. Deficits on learning to swim to a submerged platform in a constant position in the water maze have been observed in a number of studies of retrosplenial lesions in rats [10,23,24,27,29,31]. While the magnitude of the deficit varies across studies, a common feature is that the animals require extended training but then show a significant preference for the target area in probe trials [10,27]. In other words, the allocentric deficit is manifested in a slower learning of the location rather than a complete failure to learn that location. Other evidence of slower allocentric learning after retrosplenial lesions is seen in the matching-to-place version of the water maze task, in which a new location is used for every session [10,29]. This deficit parallels that found for trial 2 of condition 1 of the “landmark” heading task, which can also be solved by matching-to-place. Despite this consistent effect of retrosplenial cortex lesions on allocentric memory tasks these impairments seen with this lesion are not as severe as those seen with either hippocampal or anterior thalamic lesions on tasks such as the radial-arm maze and water maze (e.g. [1,3,17]). While the retrosplenial lesion animals show an improvement across training on these tasks, this improvement is far less evident in animals with either hippocampal lesion or anterior thalamus lesions [1,3,7,14,15].

While condition 1 was designed to test the use of heading direction cues, the additional presence of allocentric information complicates interpretation. Nevertheless, probes 2 and 3 of condition 1 showed that all three groups used the landmark to guide their search and that all three groups had learnt to search in a particular direction from the landmark. These findings show that animals with retrosplenial lesions are able to learn a direction with respect to the landmark and some distal cues. Further support for their effective use of heading direction comes from the lack of a lesion effect on trial 1, when allocentric cues should be of little value.

Condition 2 was designed to provide a more stringent test of heading direction. Unlike condition 1, there was no within-session improvement as absolute location can no longer guide successive trials. Evidence that the CMPrspl group were impaired emerged from the path length data, although this deficit was not so evident for swim latency. This heading deficit only appears to be mild as the probe trials in condition 2 showed that the CMPrspl group were not only using the landmark to navigate from, but also had a clear preference for the correct pool region (probes 4 and 6). Part of the rationale for examining heading direction arose from the discovery of ‘head direction’ cells that can relay information to the hippocampal formation [7]. The retrosplenial cortex is not, however, alone in this function as the anterior thalamic nuclei, including the lateral dorsal nucleus, also contain ‘head direction’ cells that could relay information to the hippocampal formation [9,22]. The importance of these other regions is highlighted by the finding that discrete lesions centred in these thalamic head direction areas both disrupt hippocampal ‘head direction’ cell activity [8,13] and impair performance of the “landmark” heading task as used in condition 1 [32]. The existence of parallel, head direction inputs to the hippocampal formation may, therefore, explain the failure of the retrosplenial lesions to prove more disruptive. In view of the performance on the CMPrspl group on the various probes it should also be considered whether the impairment in the acquisition stage of condition 2 was not due to some other factor. One possible factor is the change in task requirements between the two conditions. Another is the possibility that accurate allocentric information might contribute to an effective search strategy, e.g. to not revisit a location in the same trial. Some additional support for the view that retrosplenial lesions can spare heading tasks comes from the finding that retrosplenial lesions (comparable to the present ‘standard’
some studies [10,11,24,27,29,31], but not in others [2,16,30]. Memory tests appear inconsistent with deficits present in effects of retrosplenial lesions on the performance of spatial retrosplenial lesions of differing degrees of completeness. The suggest that the retrosplenial cortex is not necessary. Preclude an involvement in direction heading, but strongly distance and angle [33]. Taken together, these data do not route in the water maze that involved swimming for a fixed lesions [10]. In particular, it is argued that the strain (Dark Agouti) used in those studies that found no apparent lesion effect [2,16,30] is innately poor at spatial memory tasks. This, in turn, is thought to mask some of the effects of retrosplenial lesions [10].

The present study, which used Dark Agouti rats, provides a direct test of whether differences in the extent of retrosplenial disruption could account for these inconsistencies. Complete retrosplenial lesions had significantly more disruptive effects than ‘standard’ lesions on a number of measures, including performance on the last four sessions of condition 1 of the “landmark” task, performance on probe 1, acquisition of condition 2 of the “landmark” task, and acquisition of the radial-arm maze task. In all these cases the rats with ‘standard’ lesions did not differ from the Sham animals. In addition, the CMPrspl group, unlike the STDrspl group, differed from the Sham animals when the radial-arm maze was rotated. The performance of the STDrspl lesion group on the radial-arm maze was, in fact, somewhere between these two other groups as they were not impaired relative to either. The inference, that the inclusion of causal retrosplenial damage can be critical in determining whether a deficit is observed, is supported by recent evidence that lesions confined to the causal retrosplenial cortex are sufficient to produce subtle deficits on the radial-arm maze and on matching-to-place in the water maze [29]. A corollary is that cingulum bundle damage is likely to be an important factor as inadvertent damage to this tract might disconnect the entire retrosplenial cortex, i.e. it will increase the impact of a subtotal retrosplenial lesion. This prediction is supported by studies into the effects of selective cingulum bundle damage [2,16, but see 10]. It cannot, however, be concluded from the present results that the causal retrosplenial cortex is more important than the rostral retrosplenial cortex in supporting forms of spatial memory. Previous gene imaging studies have shown that both rostral and causal retrosplenial cortex show the same increases in activation in rats performing a spatial working memory task [28], and it is more likely that the entire rostral-caudal extent of the retrosplenial cortex is involved in spatial memory but some level of spared tissue is sufficient to support some of these functions. It should lastly be noted that the significant performance differences between the CMPrspl and STDrspl groups show that the retrosplenial lesion effects were not merely a reflection of the limited hippocampal cell loss that was observed in half of the cases in each group. This possibility can be discounted as the extent of this additional cell loss was comparable in the two lesion groups, yet the CM-Prspl group was significantly more impaired on several key measures.

The present results not only support that view that the extent of retrosplenial damage can be a critical factor, but also appear to contradict the alternate hypothesis, i.e. that the use of the Dark Agouti strain can mask retrosplenial lesion effects [10]. This is because the present study used Dark Agouti rats and found clear spatial impairments following cytotoxic lesions. In fact, a number of other recent studies have also found spatial deficits following retrosplenial lesions in Dark Agouti rats [27,29], including studies examining matching-to-place (working memory) in the water maze [10,29]. Closer inspection reveals that the principal area of disagreement concerns learning a constant place in the water maze (reference memory). It has been reported that this task is impaired in Long-Evans [10] but not Dark Agouti [10,30] rats. In fact, the Dark Agouti rats with retrosplenial lesions reported by Harker and Whishaw [10] were significantly impaired on a quadrant preference probe. Furthermore, a study of complete retrosplenial lesions in Dark Agouti rats revealed a clear acquisition deficit for constant place learning [27]. Finally, in the study by Warburton et al. [30] the retrosplenial lesion group formed one of five different surgeries (fornix, anterior cingulate, cingulum bundle, retrosplenial cortex, sham control) that were compared in an overall analysis of variance. If these same data are re-analysed with only the retrosplenial and sham groups there is a highly significant acquisition deficit (see [29]). Taken together, it can be seen that Dark Agouti rats are sensitive to retrosplenial lesions but a critical factor is the extent of causal area 29 cell loss. This discovery helps to explain apparent inconsistencies in the effects of retrosplenial cortex lesions.

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References


