Comparing the effects of selective cingulate cortex lesions and cingulum bundle lesions on water maze performance by rats

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Abstract
The ability of rats to learn the location of a hidden platform in a swim maze was compared in animals with excitotoxic lesions of the anterior or posterior (retrosplenial) cingulate cortex or radiofrequency lesions of the cingulum bundle or fimbria-fornix. Performance of this allocentric spatial task was unaffected by the posterior cingulate cortex lesions, while anterior cingulate cortex damage produced only a mild acquisition deficit. Transection of the fornix and lesions of the cingulum bundle produced similar patterns of impairment on initial acquisition, but the cingulum bundle lesions had less effect on reversal of the task. The results from the water maze, and from a subsequent T-maze alternation task, indicate that cingulum bundle lesions can produce a spatial deficit that is similar, but milder, to that observed after fornix transection. The results of the excitotoxic lesions suggest that previous studies examining conventional cingulate lesions may have been influenced by damage to adjacent fibre tracts, such as the cingulum bundle.

Introduction
The cingulate cortex is not an anatomically homogeneous region but rather can be subdivided into anterior and posterior components (Vogt, 1985, 1993; Van Groen et al., 1993) that can be distinguished on the basis of their connectivity as well as their cytoarchitecture. For example, the projection areas of the anterior cingulate cortex (area 24) include mediodorsal thalamus, amygdala and prelimbic cortex, while connections of the posterior cingulate region (areas 23 and 29) include the anterior thalamic nuclei, hippocampal formation and posterior association cortices (Vogt, 1985, 1993; Lopez da Silva et al., 1990). In addition, rich reciprocal projections exist between these two regions.

It has also become increasingly apparent that the anterior and posterior cingulate cortices can be dissociated functionally as well as anatomically (Vogt et al., 1992). In a comprehensive series of unit recording studies, Gabriel and colleagues have shown that discriminative neuronal activity develops earlier in the anterior than in the posterior cingulate cortex during acquisition of an active avoidance task (Gabriel et al., 1991; Gabriel, 1993). This accords with lesion studies which have revealed that anterior cingulate lesions impair early acquisition of the conditioned avoidance response while posterior lesions impair late learning (Gabriel et al., 1991). In support of such findings, a behavioural dissociation of these two areas of cingulate cortex during learning has recently been demonstrated using a conditional visual discrimination task in which lesions of the anterior cingulate cortex affected early learning whereas lesions of the posterior cingulate were found to impair learning only during the late stages of acquisition (Bussey et al., 1996).

It has been suggested that the posterior cingulate cortex plays a specific part in spatial learning. For example, Pandya & Yeterian (1984) have proposed that as a result of the extensive reciprocal connections between posterior cingulate and parahippocampal cortices, the posterior cingulate has an important part in analysing the significance of objects within a topographical representation and in passing on this representation to the hippocampal system for memory formation. Evidence in support of a contribution to spatial processing comes from the finding that aspiration lesions of the combined anterior and posterior cingulate cortex markedly impair T-maze alternation (Markowska et al., 1989). More specifically, it has been demonstrated that aspirative lesions of the posterior cingulate cortex in rats impair acquisition of the Morris water maze while anterior cingulate lesions produce a less severe impairment (Sutherland et al., 1988; Sutherland & Hoesing, 1993).

These conclusions have, however, been called into question by the outcome of studies using bilateral excitotoxic lesions of the cingulate cortex. In one study (Neave et al., 1994), excitotoxic lesions of either the anterior or posterior cingulate cortex failed to affect three different tests of spatial memory (delayed non-matching-to-position, spatial reversal learning and forced alternation in a T-maze), all of which are sensitive to both fornical and hippocampal damage (Aggleton et al., 1986, 1991, 1995a,b). In a related study, extensive excitotoxic lesions of the combined anterior and posterior cingulate cortices had no apparent effect on T-maze alternation (Aggleton et al., 1995b). Further support for this distinction between conventional and cytotoxic lesions is provided by Meunier & Destrade (1988) who reported that
the effects of electrolytic cingulate lesions in mice on Hebb–Williams maze performance did not occur when fibres of passage, including the cingulum bundle, were spared by using an excitotoxin. All of these studies indicate a contribution from adjacent white matter, most notably the cingulum bundle. This view is supported by recent studies indicating that lesions of this tract are sufficient to impair T-maze alternation (Aggleton et al., 1995b; Neave et al., 1996, 1997). Mindful of these potential problems, Sutherland & Hoesing (1993) re-examined the effects of posterior cingulate lesions on the acquisition of the Morris water maze. This was achieved by injecting quisqualic acid into the posterior cingulate cortex in one hemisphere, while aspirating the corresponding cortex in the other hemisphere. These animals were found to perform as poorly in the Morris water maze as those that had received bilateral aspirative lesions. However, to date an investigation of the effect of bilateral excitotoxic lesions of the posterior cingulate cortex on spatial memory in the water maze has not been conducted, nor have comparisons been made with excitotoxic anterior cingulate cortex lesions.

The aim of the present study was therefore to examine the effects of bilateral excitotoxic lesions of the posterior (retrosplenial) cingulate cortex and of the anterior cingulate cortex on spatial memory using the Morris water maze task. (It should be noted that in the rat brain the posterior cingulate region is composed of retrosplenial cortex, there being no area 23. For this reason the term retrosplenial is used to describe the corresponding lesion group in the present study.)

Given the possibility that some of the behavioural effects obtained with conventional cingulate cortex lesions may be due to interrupting adjacent fibres of passage, a third group of animals received radiofrequency lesions centred in the cingulum bundle. A fourth group of animals received lesions of the fornix. This group was included to confirm the sensitivity of the spatial task to hippocampal system damage. A final group of sham surgical controls was also tested. Following completion of the Morris water maze task a subset of animals (from the sham, cingulum bundle and fornix lesion groups) were tested on a T-maze alternation task, in response to the effects of these lesions on the water maze task.

**Materials and methods**

**Subjects**

Sixty naive, male rats of the pigmented DA strain (Bantin and Kingman, Hull, UK) were used in this study. All subjects were housed in pairs under diurnal conditions (14 h light/10 h dark) and all testing occurred at a regular time during the light period. At the start of testing, the animals were aged 4 months and weighed between 215 and 230 g. All animals had free access to food diet (Harlan Teklad, Bicester, Oxfordshire, UK) and to water.

**Apparatus**

**Morris water maze**

The water maze used in this experiment was a 2-m-diameter white fibreglass pool, 60 cm high and mounted 58 cm above the floor. The pool was situated in a room which contained posters on the wall which served as distal cues, including a curtain used to conceal the experimenter. Lighting was provided by four floor mounted spotlights (500 W) placed in each corner of the room. The water in the maze was made opaque by the addition of three pints of milk. An escape platform was placed 2 cm beneath the water surface and kept in a constant position in the pool during the acquisition trials. Temperature of the water was 25 °C at the beginning of each testing period. The paths of the rats were tracked using a video camera suspended directly above the pool and all sessions were recorded on video tape. Data were collected and analysed on-line using an HVS image analyser connected to an Archimedes RISC computer using Watermaze software (Edinburgh, UK).

**T-maze**

All testing for the forced-alternation task was carried out in a modifiable T-maze. The floors of the maze were 10 cm wide and made of wood, and the walls were 17 cm high and made of clear Perspex. The stem was 70 cm long with a guillotine door located 25 cm from the end of the stem, so creating a start area. The cross-piece was 140 cm long, and at each end there was a food well 2 cm in diameter and 0.75 cm deep. The entire maze was supported by two stands 94 cm high. Lighting was provided by a fluorescent light suspended 164 cm above the apparatus.

**Surgical and histological procedures**

Each animal was deeply anaesthetized by intraperitoneal injection of pentobarbitone sodium (Sagatal) at a dose of 60 mg/kg. The animal was then placed in a stereotaxic headholder (David Kopf Instruments, Tujunga, CA, USA), and the scalp retracted to expose the skull. Lesions of the anterior cingulate cortex (n = 10) were made by infusing 0.3 µL of 0.09 M N-methyl-D-aspartate (NMDA) at each of the following sites (from Ear Bar zero, with the incisor bar set at +5.0): (i) AP + 7.6; L + 0.7; DV – 2.0 (from the top of the cortex); (ii) AP + 6.4; L + 0.8; DV – 2.0; (iii) AP + 5.2; L + 0.8; DV – 1.8. Lesions of the retrosplenial cortex (n = 10) were made by infusing 0.09 M NMDA at the following co-ordinates and using the volumes indicated: (i) 0.3 µL, NMDA at AP + 4.3; L + 0.8; DV – 1.7; (ii) 0.3 µL NMDA at AP + 2.6; L + 0.8; DV – 1.7; (iii) 0.1 µL NMDA at AP + 0.9; L + 0.8; DV – 2.2; (iv) 0.15 µL NMDA at AP – 0.1; L + 0.8; DV – 1.6. Sham control lesions of these cortical areas were carried out using the same procedure except that buffered saline was infused into the region rather than the excitotoxin.

Radiofrequency lesions of the cingulum bundle (n = 13) and of the fornix (n = 14) were made using an RFG4-A Lesion Maker (Radionics Inc., Burlington, USA). The electrode (0.3 mm tip length, 0.25 mm diameter) was lowered vertically and at each site the temperature of the tip was raised to 75 °C for 60 s. For lesions of the cingulum bundle the co-ordinates were (relative to Ear Bar zero, with the incisor bar at +5.0): (i) AP + 1.4; L + 0.9; DV – 1.8 (relative to the top of the cortex); (ii) AP + 5.0; L + 1.1; DV – 2.1. For lesions of the fornix the co-ordinates were: AP + 5.3; L + 0.7; DV – 3.7; (ii) AP + 5.3; L + 1.7; DV – 3.8. Sham control lesions of these structures were made using the identical procedure as described above except that the temperature of the tip of the electrode was not raised.

Following the completion of the experiment, animals were anaesthetized with Euthatal and transcardially perfused with saline followed by 10% formol-saline. The brain was removed and postfixed in formol-saline for a minimum of 2 h before being transferred into 20% sucrose in 0.2 M phosphate buffer and left overnight. Coronal sections were cut at 60 µm on a freezing microtome and stained for Nissl using cresyl violet to allow examination of the extent of lesion-induced neuronal loss. Nomenclature and cytoarchitectonic borders are taken from Swanson (1992).

**Behavioural procedures**

**Water maze**

Four start positions, equally spaced around the edge of the pool, were designated as N, S, E and W, relative to the experimenter. The rats...
were divided into two groups and were trained to locate the underwater escape platform, which was positioned in the same quadrant throughout the acquisition period. For half of the animals the escape platform was located in the SW quadrant, for the other half in the NE quadrant. At the beginning of a trial the rat was placed in the pool facing the wall at one of the four start locations. The order in which the four start positions were used was kept constant for all the rats in one group, although the order varied randomly across sessions. As these two groups were required to locate the platform in different locations, the start positions for the two groups of animals were balanced accordingly, i.e. if the trial order for the rats which had to locate the platform in the SW quadrant was as follows N, S, W and E, the trial order for the group of animals which had to locate the platform in the NE quadrant would be S, N, E and W.

Owing to the large number of animals to be tested, the experiment was run in two halves. Sham control animals, anterior (ANT) cingulate and retrosplenial (RET) lesioned animals were run in a first cohort, followed by a second cohort of animals which consisted of further Sham controls, plus cingulum bundle (CB) and fornix (FX) lesioned animals. The testing regimen for all groups was as follows. Animals were initially tested with four trials per session for 6 consecutive days. Each acquisition trial was terminated either when the animal located the hidden escape platform or after 120 s had elapsed. If the rat located the hidden platform, it was allowed to remain on the platform for 30 s. The rat was then placed in the pool at the second start location, and so on for four trials. If the rat failed to find the platform after 120 s it was placed on the platform and allowed to remain on it for 60 s.

Following these six sessions, animals received a spatial probe trial (day 7) in which the platform was removed from the maze, and the swim path and distance swim in each of the four quadrants was recorded over 60 s. For this probe trial, each rat was placed at a start position directly opposite to where the platform had been located. For example, if the platform had been located in the SW quadrant, the rat was placed in the water, facing the wall, at the NE position.

In response to the poor performance of all animals, including the Sham group on this first probe trial, animals were given a further four training sessions on the task (days 8–11) commencing the following day. As before, each rat received four trials per session making a total by day 11 of 40 acquisition trials across 10 sessions. This was followed by another 60 s probe trial (day 12) with the platform removed from the tank. The final stage of testing in the water maze examined the ability of animals now to acquire a new location for the hidden platform. This was assessed by relocating the platform to the opposite quadrant of the tank from that used during the initial task acquisition. Animals then received 10 consecutive sessions of four trials per day with the platform in this new location followed by a 60 s probe trial on day 11 of the reversal.

### T-maze alternation

Animals from the three groups (sham surgery, fornix lesion, cingulum bundle lesion) that formed the second cohort were trained on the acquisition of a spatial forced alternation task. Each animal was food deprived to 85% of body weight and given several days of pretraining so that they would run reliably down the stem of the maze to find food pellets in the food wells in both arms. This was immediately followed by a series of six acquisition sessions, each of six trials. Two of the 13 rats in the FX group could not be trained on this task despite extensive habituation to the apparatus.

Each trial was divided into two stages, a ‘sample run’ followed by a ‘choice run’. At the start of each trial, three food pellets (45 mg; Sandown Instruments, UK) were placed in each food well and a metal barrier was placed at the neck of the T-maze, so closing one arm. On the sample run the animal was placed in the start area and the guillotine door raised. A metal barrier prevented the animal from entering one of the arms and thus forced the rat to enter the open arm of the maze, where it was confined for 10 s while it ate the food. The rat was then picked up and confined to the start area for a delay of 15 s while the barrier at the choice point was removed. The door to the start area was then raised and the animal allowed a free choice between the two arms of the T-maze.

On this choice run the animal was deemed to have selected an arm when it had placed a hind foot down that arm; no retraction was allowed. If the rat had alternated, i.e. had entered the arm not previously visited on the sample run, it was allowed to eat the food reward and was then returned to the home cage. If the other arm was chosen, i.e. the same arm as visited on the sample run, the animal was confined to that arm for 10 s, and then returned to the home cage. Animals were tested in groups of four with each rat having one trial in turn, so that the intertrial interval was 3–4 min. Each animal received six trials a day, and each day contained a pseudorandom sequence of correct choices between the two arms.

### Statistical analysis

Escape latencies were used as a measure of acquisition of the spatial water maze task. These were compared in a repeated measures analysis of variance (ANOVA) using the means of each set of four trials from the 10 acquisition sessions and the means of the 10 reversal sessions. In addition, the swim speeds of the animals (m/s) during the initial acquisition trials were examined. For the transfer probe test, ANOVA was used to analyse: (i) the percentage of time animals spent in the quadrant in which the escape platform had previously been located; (ii) the swim speed of the animals during this trial; (iii) the number of crossings of the platform area (annulus crossings) providing a measure of the accuracy with which the animals were crossing the exact location of the target platform; (iv) mean directionality, a measure of the extent to which the animal’s swim path deviates from a direct path to the hidden platform during the period of the probe session. The smaller the directionality score the more direct the path, while a completely random path gives a mean directionality of 90; (v) the percentage of swim time spent within 15 cm of the side wall (a measure of the animals’ persistence for swimming close to the edge of the pool).

Acquisition of the T-maze task was analysed using a repeated measures ANOVA conducted on the total number of correct responses made on each session (maximum of six correct responses per session).

### Results

#### Histology

One of the 10 ANT cases and one of the 10 RET animals had lesions that were substantially smaller than the remainder of the animals in each group and were excluded from further analyses. Figure 1 shows the extent of the lesions in the remaining nine anterior cingulate and nine retrosplenial animals. All of the animals in the ANT group (n = 9) had extensive bilateral lesions of the ACD and ACd regions caudal to the genu of the corpus callosum, with some additional damage to the secondary motor region (Fig. 1). These lesions extended rostrocaudally to the level of the fornix, and within the boundaries of the lesion the loss of neurones appeared complete (Fig. 2). Animals in the RET group (n = 9) had largely complete lesions of the RSPd and RSPv regions dorsal to the corpus callosum (Figs 1 and 2). In all of the RET animals the bilateral lesion extended caudally from...
Fig. 1. Series of standard coronal sections from Swanson (1992) showing the extent of the largest (grey) and the smallest (black) of the lesions in the anterior cingulate cortex (ANT CING) and retrosplenial cortex groups. The numbers refer to the corresponding sections in the atlas of Swanson (1992).
Fig. 2. Photomicrographs of Nissl-stained coronal sections showing the appearance of the anterior cingulate (1, 2) and the posterior cingulate (3, 4) excitotoxic lesions. Sections 2 and 4 are twice the magnification of sections 1 and 3, and show the details of the cingulate region. Note the lack of neurones in the cingulate cortex, contrasting with the normal appearance of the cingulum bundle. The vertical dark lines in the cingulate cortex are injection tracts. The scale bars represent 1 mm in sections 1 and 3, and 500 µm in sections 2 and 4.
the level of the fornix to the level of the splenium. In three cases the lesion extended for another 0.5 mm caudal to the splenium, while in the remaining six cases the lesions continued for 1.0–2.5 mm beyond the level of the splenium, often destroying the retrosplenial neurons to the subposticular border. There was, however, bilateral sparing of the most caudal portions of RSPd and RSPv. Five of the RET animals also showed very limited cell loss in the dentate gyrus and CA1 field of the hippocampus and in two of these cases the damage was bilateral (Fig. 1). This damage was restricted to that part of the dorsal hippocampus closest to the cingulate cortex (Fig. 2).

All but one of the animals in the FX group had complete bilateral transections of the fornix. In this one case, which was rejected, the lateral third of the fimbria-fornix was spared bilaterally. This left a total of 13 FX animals (Fig. 3). In addition to lesions of the fornix, there was also minor damage to the dorsal septum, the corpus callosum, and the dorsal edge of the anterior dorsal and anterior ventral thalamic nuclei. In one case the thalamic damage extended into the medial dorsal nucleus. The 13 animals which received radiofrequency lesions of the cingulum bundle (CB) showed complete or near complete bilateral lesions of the bundle both rostrally, at the level of the septum/fornix, and caudally, just in front of the splenium. In some cases, however, the lesions extended ventrally to involve restricted portions of the dorsal hippocampus or fornix. In three cases this damage was bilateral and appreciable, and these cases were excluded from all further analysis. A fourth animal was excluded as histological analysis revealed evidence of an infection. Of the remaining nine animals (Figs 4 and 5), three had lesions that did not invade any of the hippocampal system, while six had extremely limited hippocampal system damage that was often unilateral (Fig. 5). Initial analyses were conducted on these nine CB animals.

**Behavioural results**

**Water maze**

**Acquisition.** As the experiment was run in two series the first analysis compared the acquisition data for the two cohorts of Sham control animals (NS of 8 and 11). There was no evidence of a difference in the latencies of these two groups across the 40 acquisition trials. F < 1. Consequently, for the subsequent analyses the two Sham groups were combined and the two series treated as one.

The escape latencies of the five groups are shown in Fig. 6. There was a significant main effect of group (F(4,54) = 5.40, P < 0.01) as well as a group by session interaction F(36,486) = 1.59, P < 0.02. Newman–Keuls post hoc comparisons (P < 0.05) of the significant group effect revealed that the escape latencies of animals of the ANT (mean 46.84 s), CB (mean 50.56 s) and FX (mean 57.44 s) groups were significantly longer than those of the Sham control animals (mean 31.14 s). The escape latencies of the animals in the retrosplenial group (RET; mean 41.91 s) did not differ significantly from those of the Sham group, but they were significantly quicker than the FX group (P < 0.05). In light of the minor hippocampal system damage in some of the CB animals a final analysis compared the Sham escape latencies with those of the four CB animals with the most discrete lesions (three suffered no hippocampal system damage, while the fourth had very minor, unilateral damage in a restricted portion of the hippocampus—see Fig. 5). This subgroup of CB animals still showed a clear impairment, F(1,20) = 6.64, P = 0.018. Analysis of the swim speeds of these animals across the 10 session acquisition period revealed that there was no significant group difference with respect to swim speed, F(4,54) = 1.08, P > 0.05. The groups did, however, differ in the number of trials in which the rat completely failed to locate the platform in the 120-s test period (Kruskall–Wallis H = 17.7, P = 0.0014). Subsequent Mann–Whitney tests showed that the fornix, cingulum bundle and anterior cingulate groups all had significantly more such trials than the Sham group.

The final analyses assessed whether any of the lesion effects on escape latency were due to an increase in the persistence of swimming around the edge of the pool. Comparisons of the percentage of time spent within 15 cm of the side walls revealed a clear group difference (Fig. 7). While the groups with cingulate or cingulum bundle damage performed normally on this measure, the fornix lesions resulted in a marked decrease in the proportion of time spent close to the side wall. Although there was no overall group effect, F(4,54) = 1.54, there was a significant group by session interaction, F(36,486) = 1.68, P < 0.01. This interaction arose from the increased tendency of the FX animals to swim into the centre of this pool, and this was confirmed by an analysis of the simple main effects. Inspection of the data revealed that one FX animal displayed the opposite pattern of behaviour (i.e. it persisted in swimming around the perimeter of the pool throughout the acquisition period). This animal proved to have by far the largest extent of thalamic damage and removal of this one animal from the analyses produce a highly significant group effect, F(4,53) = 13.3, P < 0.0001, as well as a significant group by session interaction, F(36,477) = 1.79, P = 0.004.

**Acquisition probes.** Examination of the 60 s probe trial following the initial 6 days of acquisition revealed that the percentage of time animals spent swimming in the quadrant in which the platform had been located during the acquisition trials was little better than chance (25%) for all groups (Sham = 29.1%; ANT = 31.0%; RET = 29.3%; CB = 28.8%; FX = 31.3%) and that there was no evidence of a group difference on this measure, F(4,54) = 0.072, P > 0.05. As a result of the poor performance of all groups during this first probe trial (day 7), animals received a further 4 days of acquisition training on the task and then received a second probe trial (day 12). During this second probe trial (Fig. 8), animals spent a substantial amount of their time in the quadrant in which the platform had been located (Sham = 40.9%; ANT = 39.4%; RET = 50.5%; CB = 34.7%; FX = 48.1%). However, analysis of variance of this measure showed that there was no significant difference between groups, F(4,54) = 1.54, P > 0.05. Comparisons based on the number of annulus crossings on day 12 indicated that there was a lesion effect, but this just failed to reach the criterion significance level F(4,54) = 2.48, P = 0.055. Inspection of the data showed that the CB group made the fewest number of annulus crossings (means CB = 0.67, Sham = 2.0). Analysis of swim speeds on probe day 12 revealed a significant effect of lesion group, F(4,54) = 14.81, P < 0.001 with animals of the CB and FX groups swimming significantly slower than animals of the other groups (P < 0.05) (Sham = 0.33; ANT = 0.36; RET = 0.38; CB = 0.28; FX = 0.25 m/s). Analysis of the mean directionality of the animals during probe day 12 also revealed a significant effect of group, F(4,54) = 19.24, P < 0.001, which post hoc Newman–Keuls analyses revealed as due to the animals of the CB and FX groups swimming in a more random pattern than animals of the other three groups (P < 0.05). In contrast, the swim paths of the other groups indicated that during the probe trial the animals headed more directly towards the location of the (absent) platform.

**Water maze reversal—acquisition of a new location.** Following the second probe trial, animals were required to locate the hidden platform which was now located in the quadrant opposite to the previous location. Analysis of the escape latencies across the 10 acquisition sessions given to each animal using this new location (Fig. 6) once again revealed a significant lesion group by session interaction, F(36,486) = 2.70, P < 0.001. Newman–Keuls post hoc comparisons
Fig. 3. Series of standard coronal sections from Swanson (1992) showing the extent of the largest (grey) and the smallest (black) of the lesions in the fornix. The numbers refer to the corresponding sections in the atlas of Swanson (1992).
Fig. 4. Series of standard coronal sections from Swanson (1992) showing the extent of the largest (grey) and the smallest (black) of the lesions in the cingulum bundle (CB) region. The sections on the left depict the rostral cingulum bundle lesions, those on the right depict the caudal cingulum bundle lesions. The numbers refer to the corresponding sections in the atlas of Swanson (1992).
Fig. 5. Photomicrographs of Nissl-stained coronal sections showing the appearance of the rostral cingulum bundle lesions (1) and the caudal cingulum bundle lesions (2). The rostral lesions are at the level of the caudal septum while the posterior lesions are just rostral to the splenium. Note the very minor, unilateral hippocampal involvement in section 2. The scale bar represents 1 mm.
carried out on the main effect of group, $F(4,54) = 13.17, P < 0.001$, showed that animals of the FX lesion group had significantly longer escape latencies during reversal learning than animals of the other four groups (all $P < 0.01$, Sham = 14.9; ANT = 26.2; RET = 25.7; FX = 51.1 s). Analysis of the probe trial conducted at the end of the acquisition trials for this new location (Fig. 8) revealed that there was no significant difference between the groups in terms of the percentage of time spent in the quadrant in which the platform had been previously located, $F(4,54) = 0.31, P > 0.05$. There was, however, an effect of lesion group on swim speed, $F(4,54) = 3.67, P = 0.01$, with animals of the FX group swimming significantly slower ($P < 0.01$) than animals of all four other groups (Sham = 0.31; ANT = 0.28; RET = 0.29; CB = 0.28; FX = 0.25 m/s). There was no effect of the lesions on annulus crossings, $F(4,54) = 1.41, P > 0.05$, but there was an effect of the FX lesion on mean directionality, $F(4,54) = 6.63, P < 0.01$, with this group showing a more random path pattern than animals of the other four groups ($P < 0.01$).

**Spatial forced alternation.** Following completion of the water maze testing, animals of the second cohort (Sham controls, $n = 11$; CB, $n = 9$; and FX, $n = 11$) were also tested on forced alternation in the T-maze (Fig. 9). Analysis of variance revealed a significant main effect of lesion group, $F(2,28) = 8.15, P < 0.001$, with animals of the FX group performing close to chance (mean correct responses per session of a possible six Sham = 5.4; CB = 4.8; FX = 2.9). Post hoc analyses using the Newman–Keuls test showed that the scores of the FX group were significantly lower than those of the other two groups (both $P < 0.01$). The CB group was also impaired as their scores again differed from those of the Sham controls ($P < 0.01$). It should be noted that the subgroup of four CB animals with the most discrete lesions still differed from the Sham group, $F(1,13) = 6.48, P = 0.024$.

**Discussion**

The present study investigated the contributions of the anterior and posterior (retrosplenial) cingulate cortical areas to spatial learning and memory. Previous studies using aspirative lesions have suggested that lesions of both regions can impair acquisition of the Morris water maze, and there is evidence that posterior cingulate lesions can result in the greater deficits (Sutherland et al., 1988). The present study re-examined these effects but, for the first time, used bilateral excitotoxic lesions of the anterior and posterior cingulate cortices. The need to spare fibres of passage arose from evidence that damage to the adjacent cingulum bundle might be sufficient to induce the spatial deficits associated with conventional cingulate lesions (Aggleton et al., 1995b; Neave et al., 1997). For this reason we also examined the effects of cingulum bundle lesions on water maze performance.

In contrast to previous studies (Sutherland et al., 1988; Sutherland & Hoesing, 1993), loss of the retrosplenial cortex did not significantly disrupt spatially guided behaviour in this task. Thus rats with bilateral excitotoxic lesions of this cingulate region were as efficient as sham control animals in learning the location of the hidden platform as measured by latencies during acquisition and by performance during probe trials. Rats with excitotoxic lesions of the anterior cingulate cortex were, however, significantly impaired in learning to locate the hidden platform, as were those animals with radiofrequency lesions of the fornix or of the cingulum bundle. As the swim speeds for all five groups were equivalent during the acquisition trials, the latency deficits observed for the anterior cingulate, cingulum bundle and fornix lesion groups were not simply due to a motoric impairment. Although analysis of the probe data following task acquisition failed to reveal any clear group differences relating to the time spent in the correct quadrant, both the cingulum bundle and the fornix lesion animals swam in a more random manner when attempting to locate the region of the platform and swam significantly slower than animals of the other three groups.

Following the first acquisition series the location of the hidden platform was changed to the opposite quadrant. In this reversal condition the performance of all of the experimental groups appeared normal, with the exception of the animals in the fornix group. The animals in the FX group maintained escape latencies that were significantly higher than those of the other four groups. The animals...
with fornix lesions also demonstrated significantly slower swim speeds during the probe trial and showed more random swim paths than animals of the other four groups. In contrast, none of the cingulate groups nor the cingulum bundle group appeared to behave abnormally in this regard. As in the initial acquisition task, all groups spent an equivalent proportion of their time in the correct quadrant during the probe trial, a finding that also applied to the animals with fornix lesions.

Before considering the effects of the various cingulate lesions, the performance of the animals with fornix lesions will be briefly discussed. As expected, these animals required longer latencies to locate the escape platform (Cassel & Kelche, 1989; Sutherland & Rodriguez, 1989; Whishaw et al., 1995). In fact, the fornix lesions also led to the animals spending proportionately less time close to the side walls in the initial stages of the swim maze task. This shows that their increased escape latency was not caused by perseverative swimming around the pool perimeter, and indicates that they were unusually efficient at learning to swim away from the side walls. This measure also underlines the poor navigation of the fornix animals as they still took longer to find the hidden platform even though they started searching more rapidly. On the probe trials the animals with fornix lesions were, however, able to identify the correct quadrant, even though they also showed a significantly more random combination of swim paths as determined by the directionality measure. The apparent sparing on the former measure was not due to incomplete lesions as the histological reconstructions confirmed that the tract was consistently severed bilaterally. Furthermore, these same animals displayed profound deficits on the T-maze alternation task indicative of severe hippocampal system dysfunction. The relatively large numbers of animals in the FX and Sham groups suggest that this pattern of water maze performance is a robust finding and, indeed, this same pattern of probe performance has since been observed in our laboratory in other rats of this strain with complete sections of the fornix (unpublished findings).

The finding that rats with fornix lesions could identify the correct quadrant yet show poor directionality and longer escape latencies suggests that these animals have difficulty in effectively reaching the correct location but are nevertheless able to identify the location once they have arrived. Precisely the same distinction was made in a recent set of studies in which it was shown that rats with fornix lesions could eventually learn the location of a hidden platform but their swim paths were less direct (Whishaw et al., 1995). In both this (Whishaw et al., 1995) and the present study, an array of different start points had been used in the training procedure and, hence, the rats could not simply learn to head off in a constant angle from the side of the pool. The apparent distinction between 'getting there' and 'knowing where' (Whishaw et al., 1995) led to the suggestion that fornix transection results in an impairment in some process of motor control such as path integration, rather than in learning the location of the platform in relation to room cues. This impairment might, for example reflect a failure to use distal cues to update a current position and so fail to provide the most direct route to the platform location. Another possibility is that rats with fornix lesions can learn a limited number of room views to guide their swim paths, but this would leave them inflexible and prone to less accurate swim paths (Eichenbaum et al., 1990). While these issues are not yet resolved these studies do indicate that transection of the fornix does not abolish the ability to learn a place location and that with appropriate training or appropriate cues (Eichenbaum et al., 1990; Whishaw et al., 1995) the animals can identify the correct quadrant in a swim pool. In this regard the effects of this surgery differ from complete hippocampectomy (Morris et al., 1982), indicating the importance of non-fornical hippocampal connections.
The failure of excitotoxic lesions of the posterior cingulate cortex to impair acquisition of the water maze is perhaps surprising in the light of previous reports of substantial impairments on this task following lesions of this area (Sutherland et al., 1988; Sutherland & Hoesing, 1993). However, the present study is the first time animals have been tested on the swim pool task following bilateral excitotoxic-induced lesions of this cortical region. Previous studies have examined either bilateral aspirative lesions of this area (Sutherland et al., 1988) or unilateral aspirative lesions combined with a contralateral excitotoxic lesion (Sutherland & Hoesing, 1993). Although there was sparing of the most caudal portions of the retrosplenial cortex in the present study, the extent of the cortical damage is comparable with that reported in previous studies which have reported severe deficits (e.g. Sutherland et al., 1988). As the NMDA appeared to destroy all neurones within the confines of the lesion, the principal difference appears to be the bilateral sparing of adjacent white matter.

The potential importance of the sparing of white matter is highlighted by a number of other studies that have also failed to find evidence of spatial deficits following excitotoxic lesions involving the posterior cingulate cortex (Neave et al., 1994; Aggleton et al., 1995b). These studies used a variety of spatial tasks, including T-maze alternation which, like the swim pool, taxes allocentric spatial processes (Aggleton et al., 1996; Neave et al., 1997). Other relevant evidence comes the finding that the effects of aspirative lesions of the cingulate cortex on Hebbl–Williams maze performance in mice were no longer apparent when fibres of passage, including the cingulum bundle, were spared by using an excitotoxin (Meunier & Destrade, 1988). While these results show that the retrosplenial cortex is not necessary for navigation tasks they do not preclude a contribution to other spatial functions, such as relating object with location (Pandya & Yeterian, 1984; Ennaceur et al. 1997). These results do, however, suggest that the effects of conventional posterior cingulate cortex are often confounded by tract damage.

It was for these reasons that the present study included a group with cingulum bundle lesions. The results of this group support the view that damage to this tract can influence the outcome of a cingulate lesion. It was found that radiofrequency lesions of the cingulum bundle significantly impair acquisition of the water maze task, as reflected by a significant increase in escape latencies. The behaviour of this group was similar to that of the FX group as the CB animals also showed more random swim patterns and slower swim speeds during the post-acquisition probe trial. They also showed the fewest annulus crossings during this probe trial. The acquisition impairments were not, however, due to impaired swim speed as the cingulum bundle lesions had no effect on this measure. While the performance of the rats with cingulum bundle lesions appeared to be poor on the reversal phase, their escape latencies did not differ from those of the Sham groups as measured in the overall ANOVA. The similarities of the CB group with the fornix transection group, along with the finding that both groups were impaired on T-maze alternation, suggests that the cingulum bundle lesion deficit is one of spatial processing. Although some of the CB animals revealed slight damage to the hippocampal system this does not account for the behaviour of this group, as shown by those comparisons involving a subset of the animals with the most selective cingulum bundle damage.

Although the performance of the posterior cingulate group did not differ significantly from controls, it should be noted that the mean latencies of this group were greater than those of the control group during acquisition training. The marginal effect of the posterior cingulate lesion on performance in the water maze in the present study, could be accounted for by the sparing of some cortical cells by the excitotoxin. Thus, there may be a qualitative rather than a quantitative difference between posterior cingulate lesioned animals and the other lesioned groups. Conversely, the cingulum bundle lesion may have produced larger behavioural deficits because such a lesion would also have the additional effect of disrupting processing in downstream regions. This would result in a more effective overall disruption of cingulate cortical function compared with an excitotoxic lesion alone.

However, the contribution of the cingulum bundle in these spatial tasks may well reflect the fact that it is a major output route for the anterior thalamic nuclei. This is plausible as the anterior thalamic nuclei receive dense hippocampal projections via the fornix, and lesions in both regions impair swim maze acquisition (Sutherland & Rodriguez, 1989) and T-maze alternation (Aggleton et al., 1995a, 1996). If this is so, the contribution of the cingulum bundle is likely to involve the anterior thalamic projections to sites other than the retrosplenial cortex, although this does not preclude the additional impact of disconnecting cingulate regions.

While the posterior cingulate cortex lesions did not significantly affect swim maze performance, excitotoxic lesions of the anterior cingulate cortex significantly impaired acquisition of the initial spatial location. Unlike the fornix or cingulum bundle lesion groups these animals did not show abnormal swim speeds or path trajectories during the probe trial, nor were they impaired on the reversal task. This finding of a relatively mild impairment following anterior cingulate cortex lesions is consistent with a previous study using aspirative lesions (Sutherland et al., 1988). The pattern of deficits in the animals with anterior cingulate lesions was, however, different to that observed after hippocampal system damage, suggesting that it is not spatial in nature (Vogt et al., 1992). This accords with the failure of similar excitotoxic anterior cingulate lesions to disrupt T-maze alternation (Neave et al., 1994).

A number of studies have reported that anterior cingulate lesions can impair active shock avoidance learning (Thomas & Slotnick, 1963; Lubar, 1964; Lubar & Perachio, 1965; Gabriel et al., 1991), and the present acquisition deficit fits with this pattern. Explanations for these deficits include the possibility that the animals are less responsive to aversive stimuli (such as the cool water in the swim maze). This stems from evidence that damage to the anterior cingulate cortex can decrease emotional reactivity to aversive stimuli (Vogt et al., 1992; Devinsky et al., 1995). Such a change might affect initial acquisition, although it is the case that the animals with anterior cingulate lesions performed normally as regards swim speeds, reversal learning, and probe trial behaviour. With this in mind, it is relevant that excitotoxic anterior cingulate lesions can also disrupt appetitive learning tasks (Bussey et al., 1996), i.e. tasks that do not involve aversive stimuli.

The present study both supports and extends a series of experiments that have compared the contributions of the cingulate cortices and the cingulum bundle to various aspects of spatial learning and memory. A consistent pattern is now emerging where complete lesions of the cingulum bundle produce mild impairments on a variety of spatial tasks that are more severely impaired by lesions of the hippocampus, the fornix and the anterior thalamic nuclei (Aggleton et al., 1995a,b; Neave et al., 1996, 1997). In contrast, "Cingulate cortex and spatial memory" 633
selective lesions of the cingulate cortex have little or no apparent effect on the same tasks. Although the cingulum bundle contains fibres from many connections other than those with the anterior thalamic nuclei (Domesic, 1970), it would appear that the contribution of this tract in these spatial tasks is likely to involve these thalamic connections. Preliminary evidence for this conclusion comes from the finding that crossed conventional lesions of the anterior/medial thalamus and retrosplenial cortex (posterior cingulate cortex) produce a significantly greater deficit on water maze acquisition than ipsilateral lesions of the same sites (Sutherland & Hoesing, 1993). It now seems unlikely that this result can solely reflect the direct projections between the anterior thalamic nuclei and the retrosplenial cortex, suggesting that there are projections to other key sites that pass in the cingulum bundle. One possibility concerns the projections from the anterior thalamic nuclei to the hippocampus and to the retrohippocampal and subicular fields (Shibata, 1993; Van Groen & Wyss, 1995). The involvement of these projections may reflect the fact that the anterior thalamic nuclei project to the postsubiculum, and both contain ‘head direction’ cells, i.e. cells that encode directional heading independent of location (Taube, 1995; Taube et al., 1996). Of especial interest is evidence that the postsubicular signal follows that in the anterior thalamus, and that it is dependent on the integrity of the anterior thalamic nuclei (Taube et al., 1996). Such results further highlight the importance of these thalamo–retrohippocampal projections.

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Abbreviations

ACAd anterior cingulate area, dorsal part
ACAv anterior cingulate area, ventral part
RSPa retrosplenial area, dorsal part
RSPv retrosplenial area, ventral part

References
