Finding Your Way in the Dark: The Retrosplenial Cortex Contributes to Spatial Memory and Navigation Without Visual Cues

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Path integration is presumed to rely on self-motion cues to identify locations in space and is subject to cumulative error. The authors tested the hypothesis that rats use memory to reduce such errors and that the retrosplenial cortex contributes to this process. Rats were trained for 1 week to hoard food in an arena after beginning a trial from a fixed starting location; probe trials were then conducted in which they began a trial from a novel place in light or darkness. After control injections, rats searched around the training location, showing normal spatial memory. Inactivation of the retrosplenial cortex disrupted this search preference. To assess accuracy during navigation, rats were then trained to perform multiple trials daily, with a fixed or a different starting location in light or darkness. Retrosplenial cortex inactivation impaired accuracy in darkness. The retrosplenial cortex may provide mnemonic information, which decreases errors when navigating in the dark.

Path integration is the ability to keep track of movement through space on the basis of internally generated cues (Etienne, 1992; Etienne et al., 1998). Self-motion cues may include proprioceptive feedback, vestibular activation during movement, optic flow, and perhaps efference copy. Behavioral studies have demonstrated that animals can effectively navigate by means of path integration (Etienne et al., 1998), but an inherent problem with self-localization is that errors are cumulative (Etienne, 1992; Gallistel, 1990). It is generally assumed that stable and reliable visual features of the environment serve to update and correct for "drift" that may occur during path integration (e.g., Etienne, 1992; Gallistel, 1990). It is important to note that knowledge of landmark stability can only be determined by reference to previous experience. Therefore, long-term spatial memory is an integral component of navigation, and this may be true of path integration as well.

We have previously hypothesized that the retrosplenial cortex provides spatial memory for use in updating and correcting for errors that occur during path integration (Cooper & Mizumori, 1999; Mizumori, Cooper, Leutgeb, & Pratt, 2001). Therefore, while animals are using self-motion cues to identify their location in space, they are also using memory for reliable features of the

environment to enhance their accuracy in identifying their homeward directional vector. Our hypothesis was an extension of computational models of navigation, anatomical observations, and lesion and electrophysiological data (Chen, Lin, Barnes, & McNaughton, 1994; Chen, Lin, Green, Barnes, & McNaughton, 1994; Guazzelli, Bota, & Arbib, 1999; McNaughton et al., 1996; Samsonovich & McNaughton, 1997; Sutherland & Hoesing, 1993; Sutherland, Whishaw, & Kolb, 1988; van Groen, Vogt, & Wyss, 1993). Anatomical findings show that visual information may arrive in the retrosplenial cortex via the geniculostriate and tectocortical visual systems (for reviews, see Wyss & van Groen, 1992; Zilles & Wree, 1995). Self-motion cues may arrive in the retrosplenial cortex via connections with the anterior thalamic nuclei or posterior parietal cortex (Zilles & Wree, 1995). Information from the retrosplenial cortex may arrive in the hippocampal formation via efferents projecting to the entorhinal cortex and the pre-, para-, and postsubiculum (van Groen et al., 1993). Permanent lesion studies have demonstrated that the retrosplenial cortex is required for water maze acquisition and retention of a hidden, but not a visible, platform location (Sutherland et al., 1988; Sutherland & Hoesing, 1993; but see Warburton, Aggleton, & Muir, 1998). Electrophysiological studies in cats have demonstrated that single cells in the retrosplenial cortex are controlled by multimodal sensory information (Musil & Olson, 1993). In rodents, a subpopulation of cells in the retrosplenial cortex fires maximally when subjects face a particular direction in space; these are called *head direction* cells (Chen, Lin, Barnes, & McNaughton, 1994; Chen, Lin, Green, et al., 1994). Taken together, the anatomical, lesion, and electrophysiological data suggest that the retrosplenial cortex may contribute multimodal sensory information for use during navigation.

A number of researchers have used a simple hoarding paradigm to evaluate both spatial memory and path integration (Barnes, 1979; Etienne et al., 1998; Etienne, Maurer, Saucy, & Teroni, 1986; Etienne, Teroni, Hurni, & Portenier, 1990; Maaswinkle, Jarrard, & Whishaw, 1999; Whishaw & Gorny, 1999; Whishaw, McKenna, & Maaswinkel, 1997). In these studies, a large circular

arena with a discrete starting location, or "nest site," is used. Subjects leave the nest site, search for food, and upon locating the food, hold it in their mouth and return directly to their starting point. After returning to the nest site, subjects consume the food. There are several advantages in using this hoarding paradigm for studying navigation. First, the behavioral repertoire of the animals during performance of this task is clearly defined and tractable. Second, hoarding is an innate behavior of rodents and does not require special training procedures; subjects will perform this task with or without visual information. Last, the role of local arena cues is easily manipulated by rotating the testing apparatus (independent of the animal).

Of particular importance for the current work, this task has been used to dissociate spatial memory and path integration strategies for navigation (Whishaw & Maaswinkel, 1998). In this study, rats were trained with a consistent starting location and then, on probe test days, were started from a novel location. When the rats were started from the novel place with visual cues available, they returned directly to the familiar starting location. This indicated the use of spatial memory to return home. However, if the rats were started in darkness, they returned directly to the location from where they started the probe trial and not to the familiar training site. The latter solution was consistent with the use of path integration, not spatial memory, to guide navigation. Whishaw and colleagues have used this task to provide evidence that the hippocampus is critical for path integration (Maaswinkel et al., 1999; Whishaw et al., 1997, but see Alyan & McNaughton, 1999).

Our previous work demonstrating that retrosplenial cortex inactivation resulted in dark-selective impairments during radial maze performance suggests that this cortical area may also contribute to path integration (Cooper & Mizumori, 1999). Our hypothesis suggested that path integration could operate by using spatial memory to increase the subject's accuracy in identifying locations in space. Therefore, we used a hoarding paradigm on a circular arena to evaluate our hypothesis that (a) the retrosplenial cortex does indeed contribute to spatial memory and (b) accurate navigation in darkness (i.e., path integration) may be facilitated by intact spatial memory. To test the spatial memory component of our hypothesis, we trained rats with a consistent starting location and then conducted probe trials that required them to begin the trial from a novel starting location. Each rat was tested with two probe trials, one after inactivation of the retrosplenial cortex and another after a control injection. Separate groups started the probe trial with visual cues available or in darkness. If the retrosplenial cortex contributes to spatial memory or path integration selectively, then the use of this task should enable the dissociation of these strategies. However, if the retrosplenial cortex contributes to both processes, then its inactivation should impair probe trial performance in both light and darkness (spatial memory and path integration).

The probe trials evaluate the rats' behavior in a novel testing situation and do not necessarily test how the retrosplenial cortex may contribute to how accurately they can find their way home. To examine accuracy during navigation (the second component of our hypothesis), we conducted two additional experiments (Experiments 2 and 3). In Experiment 2, a reference memory task was used that required the rats to perform the task with a fixed starting location across days. The rats were tested in the presence or absence of visual cues, and navigational accuracy was measured after control injections or inactivation of the retrosplenial cortex.

In Experiment 3, a task that could be solved with working memory was used. In this experiment, the starting location varied across days and therefore placed a demand on working memory. Accuracy was measured after control injections and when the retrosplenial cortex was temporarily inactivated. During dark trials in both the reference and working memory versions of the tasks, rats could rely on path integration to navigate. We predicted that the rats would show dark-selective behavioral impairments, which would be consistent with our previous work on the radial maze (Cooper & Mizumori, 1999). Rats that were tested in Experiments 2 and 3 also took part in Experiment 1. This within-subject experimental design was used to facilitate comparisons across the different testing conditions. The combined results were expected to shed light on the role of the retrosplenial cortex in spatial memory and path integration. These data could also be used to address the issue of whether normal spatial memory increases accuracy during navigation without visual cues.

General Method

Subjects

Eleven male Long–Evans rats were purchased from Simonsen Laboratories (Gilroy, CA). The rats were housed in a temperature- and humidity-controlled environment and kept on a constant 12-hr light–dark cycle with lights on at 0700. Rats were housed individually in rectangular clear plastic bins (47 cm long × 22 cm wide × 26 cm high). The bottom of the cage was covered with wood shavings about 2.5 cm deep. The bedding was changed twice a week on a regular schedule, after completion of that day's experimental procedures.

Apparatus

The circular platform measured 137 cm in diameter and was elevated 78 cm above the floor of the testing room. The testing apparatus was on a "lazy susan" turntable that enabled rotation of the entire circular arena. Three boxes were placed at the edge, but separate from, the circular table. One "home box" and two "distractor boxes" were located adjacent to the arena, sitting approximately 5 cm below the circular table. The home box corresponded to the rat's starting location for that day. The distractor boxes were always located on each side of the home box, at 45° angles with respect to the circular arena. The home and distractor boxes were smaller versions of the rat's home cage, measuring 29 cm × 18 cm × 13 cm. The home box was filled with shavings from the rat's cage, and the distractor boxes were filled with clean shavings. The two types of shavings were used to evaluate whether local cues might guide behavior during control and inactivation trials. In our pilot experiments, we determined that the rats did not use olfactory cues from the home box to control navigation, but it was possible that such cues may control behavior during inactivation trials. Therefore, we evaluated the contribution of these cues as part of the experimental procedure (Experiments 1 and 3). Furthermore, the design was intended to be similar to the procedures used by Etienne and colleagues (for review, see Etienne, 1992), who made the starting location for hoarding trials the subject's home during the experimental procedures. Thus, we expected that subjects would be more likely to hoard food if they perceived their "home" in the test situation as similar to their home during the experimental period.

The boxes were placed so that the experimenter could not see the home cage when looking across the table from the "rat's-eye level." Therefore, it is unlikely that rats used the home cage as a visual cue for navigating home. The room was dimly lit by a 15-W light bulb projecting from the ceiling over the center of the arena. The testing apparatus was placed in an open room with numerous potential distal cues. An infrared camera was

mounted on the ceiling of the testing room, enabling the experimenter to monitor the rats from an adjacent room. The experimenter entered the room to replenish the food on the table only when the rat was eating the food pellets.

Procedure

The procedures used in the present study were designed to synthesize the methods used by Etienne and colleagues (for review, see Etienne, 1992) and Whishaw and colleagues (for review, see Whishaw, 1998). The food pellets were 300 mg (Bio-Serve, Frenchtown, NJ), similar to those used in similar hoarding experiments (cf. Maaswinkel et al., 1999; Whishaw & Gorny, 1999). To reduce neophobic responses to the food, the rats were given pellets in their home cage while their weights were being reduced. Rats were food restricted to 80% of their free-feeding weight. After the rats were consistently hoarding food, their weights were gradually raised to and maintained at 90% of their free-feeding weight.

Training was conducted in two phases: habituation and hoarding. The habituation phase consisted of two 20-min sessions of exposure to the room, circular arena, home box, and distractor boxes. During these 20-min habituation sessions, the rats were allowed to freely explore the arena and investigate the home box and distractor boxes. Food was scattered on the entire table, and observation of the rats showed that they frequently ate the pellets on the circular table, in their home box, and in the distractor boxes. After the 2 days of habituation, the hoarding phase of training began. From this time forward only 2 pellets were located on the table and the rats performed 10 hoarding trials per day (consuming about 20 pellets per day). The reduction in the quantity of food from the habituation phase to the hoarding phase appeared to encourage the natural hoarding response of rodents; almost all the rats immediately began to hoard food when this procedure was implemented. Food was always placed on the half of the circular arena that was opposite to the location of the rat's home box (see additional description below). The placement of the food varied pseudo-randomly across trials. When subjects began to hoard food, they made direct return paths back to the home box or one of the distractor boxes to consume the food. A more detailed description of the experimental procedures for the individual experiments is provided below.

Description of the hoarding behavior.   Most rats did not require special training procedures for hoarding food, but 2 were excluded from the study because they failed to reliably hoard food. The remaining rats all performed similar hoarding behaviors; when they encountered the food, they placed both pieces of food in their mouths and returned back to their home box or one of the distractor boxes before consuming the food. When rats approached either their home box or a distractor box, they would pause and investigate before jumping down into the box. The difference in bedding (clean vs. home cage bedding) may have encouraged rats to learn to avoid the distractor boxes (see Experiments 1 and 3 for an additional description of this issue). When the subjects entered the distractor boxes, they were gently picked up by an experimenter and placed in their home box before they started the next trial. After several days of training, they rarely entered the wrong boxes. Consumption of the food took approximately 15–25 s, which provided the self-paced intertrial interval for training. Trials began when the rat left the home box and ended when it returned to the home box or one of the distractor boxes. The subjects were trained to perform 10 trials per day with room lights on, which usually required 25 min during initial training and 10–15 min after they became proficient at the task. For Experiment 1 and 2, the presurgical criterion was 1 week of training for 10 trials per day. In Experiment 3, the criterion was the subjective judgment of the experimenters that the rat made a direct path back to the starting location after locating the food. This required up to 3 weeks of training.

Surgical procedure.   The rats were food and water deprived for 1 day before surgery. They were anesthetized with 35 mg/kg of pentobarbital (Abbott Laboratories, North Chicago, IL) to establish deep surgical anesthesia, after which they were given 0.20 ml of atropine (ip) to prevent

respiratory distress. The rat's head was shaved and cleaned with Betadine, and an incision was made exposing the skull. Guide cannulas, measuring 1.20 cm in length, were constructed from 25-gauge stainless steel tubing and were placed bilaterally 6.0 mm posterior to bregma, 1.0 mm lateral to midline, and 1.0 mm ventral to the surface of the brain. Vacuum grease was placed around the guide cannulas and the craniotomy to prevent dental cement from contacting the brain. Stylets remained in the guide cannulas to prevent occlusion of the tubing. Eight anchor screws were placed in the skull, and dental cement was used to hold the guide cannulas in place. A plug was attached to the top of the skull so that an infrared diode could be mounted onto the top of the rat's head during subsequent behavioral testing. After surgery, 0.1 ml (300,000 units/ml) of Bicillin was administered intramuscularly in each hind limb to guard against infection. The rats were monitored continuously after surgery until they showed coordinated movements in their home cage. At this time, they were given free access to food and water for 1 week. After the recovery period, experimental procedures resumed.

Postsurgical training.   After 2 days of food restriction, training continued, and rats were tested in both light and dark conditions. In darkness, the rats were monitored by observation of the infrared diode attached to the rat's head. The diode pack contained two 3-V batteries, which left the rat free from extraneous cables. Subjects performed 10 trials each day, 5 in light and 5 in darkness. The light and dark trials were intermixed in a consistent fashion, with 3 light trials followed by 3 dark trials, then 2 light trials followed by 2 more dark trials. During this training, the rats were accustomed to the injection regimen by removing and inserting stylets before testing.

Injection regimen.   Immediately before behavioral testing, the stylets were removed from the guide cannulas and replaced with injection needles, and tetracaine (2% wt/vol) or saline was injected (1.0 μl). The solution was infused through a 10-μl Hamilton syringe (Hamilton, Reno, NV) that was connected to the injection needles by neoprene tubing. The injection needles protruded approximately 0.5–1.0 mm beyond the tip of the guide cannulas. One minute was allowed for diffusion prior to removing the injection needles. After injection, the subjects were placed in the home box and immediately carried into the testing room, where they performed the task. The injection procedure is similar to those we have used in previous experiments (Cooper & Mizumori, 1999; Mizumori, McNaughton, Barnes, & Fox, 1989; Mizumori, Miya, & Ward, 1994).

Data Analysis

Position data were based on the location of the infrared diode and were obtained with a Dragon Tracker (Boulder, CO) system that provided the time and location (in a 256 × 256 pixel grid) of the diode in 50-ms intervals. During recording of the data set, experimenters used the Discovery program from DataWave (Longmont, CO) to enter "event flags" for behaviors of interest. The event flags were stored in the data file along with the time and location of the rat when the event flag was entered. Thus, while rats were performing the task, event flags were inserted into the data file "on-line," marking when the rat started the trial, when it located the food, and when the end of the trial occurred. Trials in which subjects left their home cage and returned without finding food were discarded. To correct for errors in marking event flags during the trials, a custom software program was used that replayed the position data "off-line," or after the data were collected. The program graphically displayed the position data for the trials in the identical 50-ms samples recorded during the experiment. The graphical display of the trials indicated the current location of the rat (in the 256 × 256 grid), the position data showing where the rat was in the 500 ms before reaching the current location, and 500 ms of the "future" position data. In addition, the location of the event flag was also graphically displayed at the position where it was originally entered. Event flags that were inserted incorrectly were deleted, and corrected event flags were then inserted into the data file and saved for the subsequent analyses.

Using the well-defined behavioral patterns of the rats performing this task, we were able to establish clear criteria for each of the behaviors of interest. The start of the trial was defined as the first position point from which the remaining position data moved consistently out of the home box and onto the arena. This corresponded to the rat jumping out of the home box and onto the circular platform to search for food. When rats located food, they would pause briefly and then make a direct path back toward the home box. The pause resulted in an accumulation of the position data in a similar location on the grid, and locating food was defined as the location from which the rat moved directly toward the home box. Locating food during hoarding trials was easily identified because rats quickly and immediately headed toward the trial's starting location. In contrast, if rats paused without locating food, they would continue to search at the opposite perimeter of the circular table instead of heading homeward. The end of the trial was defined as an accumulation of position data in a similar location at the edge of the arena near the home or distractor boxes. The ability to graphically display the coordinates of the subject's past, present, and future position data in the 50-ms intervals recorded during the experiment made identification of these behaviors very clear. We have used similar procedures to identify behaviors of interest on the radial maze (Cooper, Miya, & Mizumori, 1998; Pratt & Mizumori, 1998). Specific data analyses for the individual experiments are described below.

Histology

Before histological procedures, the rats were injected with permanent ink to mark the location of the injection site. They were then overdosed with pentobarbital and transcardially perfused with saline followed by phosphate-buffered Formalin. Brains were extracted and placed in a 30% (wt/vol) sucrose–Formalin solution. After the brains sank, they were frozen, and every third section was taken at 40-μm intervals with a cryostat. Brains were stained with cresyl violet, and guide cannula placements were verified with a light microscope. Sections were drawn and compared with the Paxinos and Watson (1986) rat atlas to identify the approximate location of the injection sites.

Experiment 1: Does the Retrosplenial Cortex Contribute to Spatial Reference Memory?

Rationale

Using a similar hoarding paradigm as described above, Maaswinkel and Whishaw (1999) have demonstrated that animals rely on spatial memory to identify their home location when visual cues are available and path integration when visual cues are obscured. We have previously speculated that the retrosplenial cortex provides mnemonic information for updating and correcting for errors that occur during path integration (Cooper & Mizumori, 1999). However, we have not directly tested whether spatial memory is a critical contribution of the retrosplenial cortex. Therefore, we trained the rats with a consistent starting location and then started a probe trial from a novel location. If rats searched around the original training location, it would be consistent with a spatial memory search strategy. However, if they returned directly home, it would be consistent with the use of path integration (which would be predicted in darkness). We compared the effects of inactivation of the retrosplenial cortex with control injections in rats that performed the probe trials in light or dark testing conditions.

Method

Subjects. Nine male Long–Evans rats were used in this experiment. All of the subjects from this experiment also contributed to Experiment 2 or 3 (see details below).

Procedure. Before starting the probe trial sequence, the rats were trained for 6 days with the home box in the same spatial location (i.e., fixed location training). On the 7th day of training, rats ran a probe trial. This was followed by 2 days of regular fixed location training, after which they performed one more probe trial (Figure 1A displays the probe trial sequence across days). For the probe days (7 and 10), rats were started 135° to 180° away from their normal home location. Immediately before onset of the probe trial, the rats received a vehicle control or tetracaine injection into the retrosplenial cortex. Rats were then carried into the testing room with the room lights on or in darkness, and they performed one trial (which consisted of leaving home, locating food, and returning home). If a rat did not locate the food after it left the home box, and instead returned back to the starting location, it was given one more trial that day. All rats located the food within one or two departures from the home box. No time limits

A. Probe Sequence
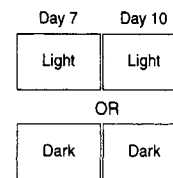
B. Injection Sequence
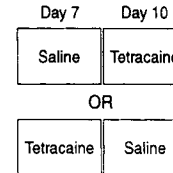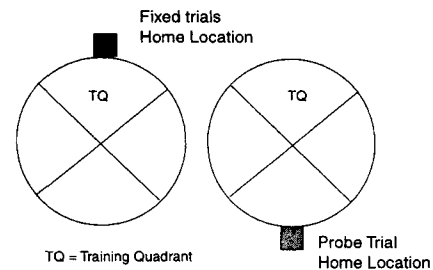
C. Probe Trial Procedure

TQ = Training Quadrant

Figure 1. Probe trial sequence, injection procedure, and quadrant analysis. A: Rats were tested with two probe trials, either in light or in darkness. The probe trials occurred on Day 7 and Day 10 of training. B: Control and tetracaine injections were counterbalanced, and rats were randomly assigned to receive tetracaine or saline on the first probe trial and vice versa for the subsequent probe trial. C: For the spatial memory probe trials, rats were started 135° to 180° opposite from their fixed location training. The quadrant of the arena next to the fixed training location was defined as the "training quadrant," and the percentage of time spent in the training quadrant after saline or tetracaine injections was used to measure spatial memory.

were imposed for the probe trials. Although the data were not measured, there were no noticeable differences in the amount of time rats remained in the home box before starting the probe trial after saline or tetracaine infusion.

To encourage homogeneous behavioral patterns for the probe trials, the food was always placed at locations between the border of the training quadrant and one of the adjacent quadrants. The location of the food varied between these two locations across days for each rat. Subjects were randomly assigned to receive a saline or tetracaine injection and to light or dark testing for the first probe trial (Figure 1B). On test days between probe trials, rats were tested daily with the original fixed location of the home box. These daily sessions required the rats to run 10 trials, 5 in light and 5 in darkness, using the interleaved light and dark testing schedule described in the General Method section.

Data analysis. The center of the circular arena was estimated, and the platform was divided into four equal pie-shaped quadrants. The training quadrant was defined as the quadrant of the arena around the fixed location training site (Figure 1C). On the basis of the quantity and location of position data (sampled every 50 ms), the time in each quadrant of the circular arena and total time for the probe trial was calculated. Data points that were on the dividing lines between quadrants were omitted. Time per quadrant was normalized as a percentage of the total time required for the rat to complete the probe trial. Three comparisons were made for the within-subjects statistical analyses. First, the amount of time spent in the training quadrant during the control probe trials was compared with the amount of time spent in the training quadrant during the inactivation probe trial. Second, to determine whether rats spent more time searching for the food, but not preferentially searching in the training quadrant, an average proportion of time spent in the three nontraining quadrants was computed. The three quadrants were averaged because rats started and finished the trial in one quadrant of the arena. The averaging was designed to reduce the bias this might introduce into the data. It is important to note that, given the search patterns of the rats after control injections, it was clear that the critical analysis was the time spent in the training quadrant. Third, the total amount of time required to complete each probe trial (control and inactivation) was calculated. A within-subjects t test, with significance defined with an a priori criterion of $\alpha = .05$, was used to evaluate the effects of retrosplenial cortex inactivation compared with the effects of vehicle control injections.

Results

Histology. Figure 2 displays the location of the guide cannula tips for the 9 rats in this experiment. The guide cannulas were centered on the posterior areas of the retrosplenial cortex, and the spread of ink into the tissue usually diffused in an approximate 1-mm circumference around the injection site. Injections most likely affected the retrosplenial granular and agranular regions, medial areas of posterior parietal cortex and the adjacent cingulum bundle. These injection sites are consistent with previous work from our laboratory (cf. Cooper & Mizumori, 1999).

Light probe trials. Five rats were randomly assigned to perform the probe trials with visual information available. Of these, 3 were first trained in Experiment 3, and 2 were subsequently tested in Experiment 2. One rat in this group was excluded from the probe trial data analyses because he did not show a preferred search pattern in his first probe trial. The first probe trial for this rat occurred after a vehicle control injection, with visual cues available. It is important to note that this subject was initially trained to perform the working memory version of the task (Experiment 3). This suggested that this rat, unlike the remaining 4, did not show normal reference memory for the probe location. Therefore, his data were excluded. The behavioral pattern for the
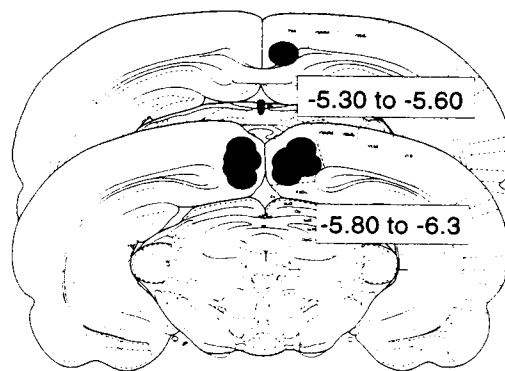

Figure 2. Tetracaine injection sites. Rats received bilateral cannula implants into the posterior retrosplenial cortex (aimed at -6.0 mm posterior to bregma, ± 1.0 mm lateral to midline); the spread of tetracaine likely affected the cingulum bundle and medial areas of the posterior parietal cortex. From *The Rat Brain in Stereotaxic Coordinates* (2nd ed., Figures 39–43), by G. Paxinos and C. Watson. 1986, Sydney, Australia: Academic Press. Copyright 1986 by Academic Press. Adapted with permission.

remaining 4 rats was very consistent. Figure 3A displays these rats' search path for the probe trial after a saline or a tetracaine injection into the retrosplenial cortex. After the control injection, the rats searched around the previous training location, presumably trying to locate their home box. After inactivation of the retrosplenial cortex, the very same rats did not show a preferential search for the home location (Figure 3A).

Given this search behavior, we quantified the amount of time rats spent in the training quadrant and compared this between the control and inactivation conditions. Figure 4A displays the average proportion of the probe trial that subjects spent in the training quadrant during control trials and inactivation trials. After control injections in the light, rats spent an average ($\pm$ *SEM*) of 61% ($\pm$ 9%) of the total probe trial time searching in the familiar training quadrant. After inactivation of the retrosplenial cortex, rats only spent an average of 17% ($\pm$ 6%) of the probe trial in the training quadrant. This difference was significantly lower than that of the control probe trial, $t(3) = 3.28, p < .05$. To determine whether there were substantive differences in the time that rats spent in the remaining areas of the arena, we calculated the average proportion time spent searching in the other three quadrants of the arena. Figure 4B shows that rats spent slightly more time in the remaining quadrants, but that this increase was not statistically significant. After control injections, rats only spent 12% ($\pm$ 7%) of the trial time in the nontraining quadrants, and after inactivation of the retrosplenial cortex, they spent 28% ($\pm$ 2%) of the trial time in the nontraining quadrants, $t(3) = -1.39$, *ns*. During the control and inactivation trials, there was no difference in the amount of time required to complete the probe trials. During control trials, the average time required to complete the probe trial was 26.53 s ($\pm$ 4.58), and rats spent an average of 18.93 s ($\pm$ 1.74) searching on the arena during the inactivation trial, $t(3) = 1.37$, *ns*.

Dark probe trials. Four subjects were randomly assigned to perform the probe trials in darkness. Of these, 3 were subsequently trained in Experiment 2, and 1 was first trained in Experiment 3. During the dark probe trials, a pattern of data was observed that
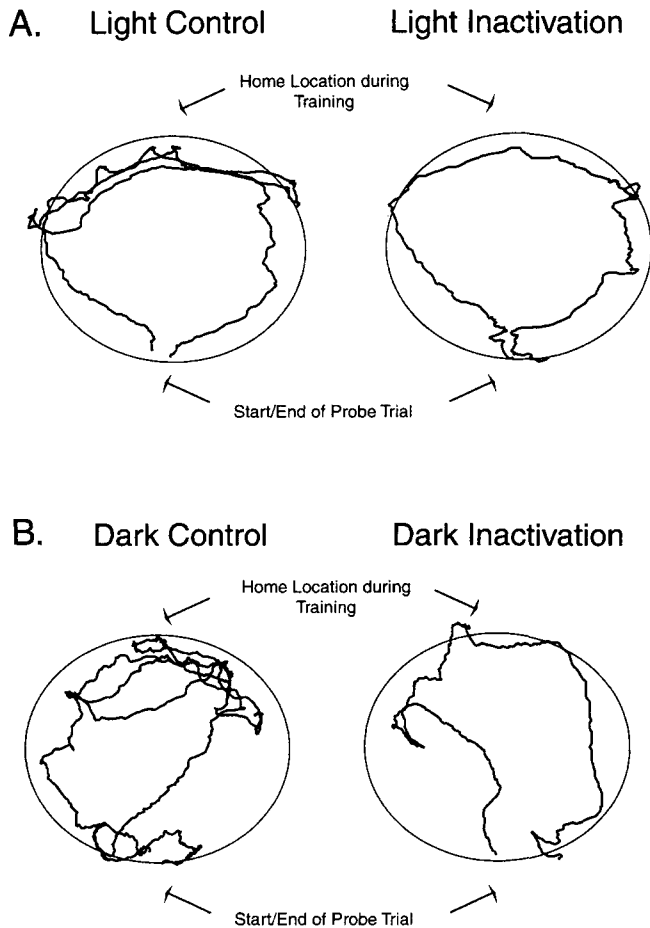
A. Light Control   Light Inactivation

B. Dark Control   Dark Inactivation

Figure 3. Inactivation of the retrosplenial cortex impaired spatial memory in light and dark testing conditions. A: The search pattern of a rat after a control injection and after inactivation of the retrosplenial cortex. Rats were trained with a familiar start location, and then a probe trial was conducted in which they started a trial from a novel place on the maze. Rats searched around the training quadrant after control injections (northern portion of circular arena) but not when the retrosplenial cortex was inactivated. The searching around the northern portion of the arena is not present in this rat after inactivation of the retrosplenial cortex. B: The search pattern of a rat that was tested in darkness with the probe trials is displayed. Similar to their performance on light trials, the rats searched around the familiar training location (northern portion of arena) after control injections but did not show the same search preference after inactivation of the retrosplenial cortex. Injection order and training locations were randomly assigned but for clarity in presentation are displayed with a consistent training location and injection order.

was very similar to the light probe trials. Figure 3B displays the search pattern of a rat that received a vehicle control injection and began to search around the familiar training location. In Figure 3B, the position data acquired from the same rat after inactivation of the retrosplenial cortex is displayed. In this case, the rat did not show a preferential search pattern for the familiar training quadrant. It is important to note that, although the data are presented graphically, with the control injection occurring before the tetracaine injection, the actual order during testing was randomly

varied for each subject (see *Method* section above). Thus, order effects are unlikely to have influenced these data.

Similar to the light trials, an average proportion of the total probe trial time was computed for time spent searching in the training quadrant. Figure 4C displays the proportion of the time rats spent in the training quadrant after control injection or inactivation of the retrosplenial cortex. After control injections, an average of 47% ($\pm$ 5%) of the total probe trial was spent in the familiar training quadrant. Inactivation of the retrosplenial cortex significantly reduced this search bias, and rats spent only 13% ($\pm$ 1%) of the trial searching in the training quadrant of the arena, $t(3) = 4.24, p < .05$. In darkness, retrosplenial cortex inactivation significantly increased the amount of time rats spent in the remaining three quadrants of the arena. After control injections, rats spent 18% ($\pm$ 2%) of the probe trial time in the nontraining quadrants of the arena, and this increased to 29% ($\pm$ 2%) of the probe trial time in the nontraining quadrants when the retrosplenial cortex was not active, $t(3) = -4.23, p < .05$ (Figure 4D). Inactivation of the retrosplenial cortex did not change the total amount of time required to complete the probe trial. After control injections, rats completed the probe trial in 33.04 s; they required 24.46 s to complete the probe trial after inactivation of retrosplenial cortex, $t(3) = 1.01, ns$.

The preferential searching around the familiar training location suggests that the rats were able to use nonvisual cues (and perhaps memory of the visual cues in relation to these cues) to identify the home training location. However, after inactivation of the retrosplenial cortex, they did not show the same preferential searching pattern. Thus, the rats did not use path integration in darkness to solve this task. Potential reasons for this are discussed in the General Discussion section. It is important to note that the searching behavior of the rats, particularly in darkness, made several types of data analyses difficult. For example, the first location where rats stopped at the edge of the circular arena was difficult to unambiguously determine because the rats would frequently pause at the edge of the arena for varying amounts of time. In addition, the time when rats located the food was also difficult to determine because they did not always immediately begin the typical hoarding behavior of making a direct path back home. This made it difficult to identify when the rats were pausing during their search to investigate or when they were pausing to pick up the food. Given these issues, the time rats spent searching was the most clear and consistent method for measuring differences between treatment conditions.

Experiment 2: Navigational Accuracy and Spatial Reference Memory

Rationale

In Experiment 1, rats showed impaired spatial reference memory in light and darkness when the retrosplenial cortex was not active. However, the probe trials were not measuring how accurately rats navigate. To assess accuracy during navigation, we compared the effects of control and tetracaine injections into the retrosplenial cortex during performance of a reference memory task.

Rats were trained and tested in light and darkness; local cues were controlled for by conducting trials with the testing arena
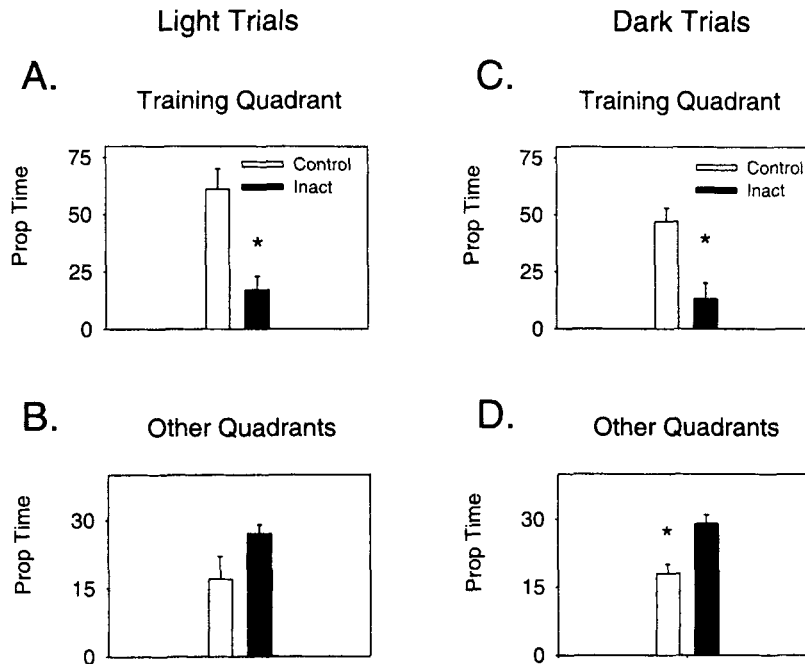
Light Trials

A.
Training Quadrant

B.
Other Quadrants

Dark Trials

C.
Training Quadrant

D.
Other Quadrants

Figure 4. Retrosplenial cortex inactivation disrupted spatial reference memory. A: Inactivation (Inact) of the retrosplenial cortex caused rats to spend a significantly smaller proportion of time (Prop Time) in the training quadrant than they did after control injections. B: Time spent in the remaining quadrants did not change significantly with retrosplenial cortex inactivation. C: In darkness, the rats spent significantly less time in the training quadrant when the retrosplenial cortex was temporarily inactivated. D: Time in the remaining quadrants increased significantly in darkness after inactivation of the retrosplenial cortex. Error bars indicate *SEM.* $*p < .05$.

rotated by 45°. We predicted that the behavioral effects of retrosplenial cortex inactivation would be comparable to our previous work on the radial maze, which demonstrated dark-selective navigation impairments (Cooper & Mizumori, 1999). Thus, when rats are navigating in darkness, spatial memory may serve to enhance how accurately they are able to locate their goal.

Method

Subjects, apparatus, and procedure. Five rats contributed to this experiment. To provide comparisons across experiments, they all participated in Experiment 1. The apparatus and testing room were identical to those used in Experiment 1. After rats completed the probe trials for Experiment 1, they were given 2 more days of training with the home in the original fixed location. The rats were then tested daily for 8–10 days with either a control or tetracaine injection occurring immediately before behavioral testing with the home box still at its original location (four to five injections were made for each injection condition). Rats ran five light and five dark trials in the same manner as described in the General Method section. Although tetracaine is active for about 20 min, the most pronounced effects are observed in the first 5–10 min (Cooper & Mizumori, 1999, 2001; Mizumori et al., 1989, 1994). The design of light and dark trials ensured that tetracaine was approximately equally active during both lighting conditions.

Data analysis. Four measures were used in the present experiment. The first was search distance, which was calculated as the distance traveled from start of the trial until the rat located the food. The second measure was accuracy, which was determined by calculating a difference score between the distance traveled from the food source to the home cage minus the

shortest possible distance between the food and home. Similar difference scores have been used to determine accuracy of navigation in the water maze (cf. Barnes, Suster, Shen, & McNaughton, 1997; Colombo, Wetsel, & Gallagher, 1997; Whishaw, Cassel, & Jarrad, 1995). The last measures were search time and return time, which were the times required to locate food and return home, respectively. Each rat contributed a single average difference score (search distance, accuracy, search time, and return time) for each trial during saline and tetracaine testing conditions. A one-way repeated measures analysis of variance (ANOVA) was used to determine whether there were significant differences between control and tetracaine injections, with $\alpha = .05$.

Local cue probe trials: Arena rotation. During the course of testing, bedding would frequently accumulate on the arena next to the home box. In addition, it is possible that rats would scent mark the circular arena to indicate the location of the home box. Therefore, we wanted to evaluate whether these local cues might control homing during navigation in this experiment. During the last 4 test days, local cue probe trials were conducted. After the rats performed six regular trials (three in light and three in darkness) and before they began the next trial, the testing arena was rotated 45°. Clockwise and counterclockwise rotations were used and were determined randomly for each probe trial (see Figure 5). The rotation of the arena will be referred to as the "rotated arena location," and the original spatial location of the arena (relative to the home box) will be referred to as the "normal arena location." During all local cue probe trials, food was located 180° opposite to the normal home box location. If rats follow local arena cues, then a 0° deviation relative to the rotated arena location would be expected. If local cues are not used, then it is expected that there would be a 45° deviation from the rotated arena location and that rats would return to the normal arena location.
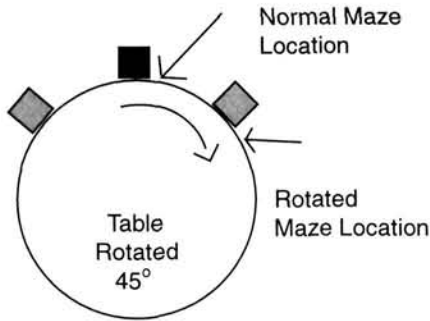
Local Cue Probe Trials

Figure 5. Procedure for local cue probe trials. Local cues on the arena were manipulated by rotating the circular platform immediately before a trial in light or darkness, during control or inactivation trials. Immediately before the sixth maze trial for the day, the platform was rotated by 45°; this is referred to as the rotated maze location. Normal maze location refers to the location of the home box relative to the arena. Angular deviation relative to the rotated arena location was calculated for each rat during each probe trial. An angular deviation of 0° would be expected if rats followed local cues; a deviation of 45° if they returned to the starting location, therefore relying on spatial cues.

Rats performed four local cue probe trials across 4 test days after saline or tetracaine injections. Two of the probes occurred in light, and two occurred in darkness. It is important to note that the local cue probe trials only rotated the arena and not the rat's home box. Therefore, this probe does not control for olfactory cues from the home box (but see Experiments 1 and 3). It could be argued that the rotation of the arena did not control for cues left at the start of each trial because the arena was rotated before the start of the probe trial. However, if rats were using cues left from each trial, the olfactory gradient would still be expected to be higher in the rotated arena location because rats had previously performed six trials with the arena in a fixed location.

All rats made stereotypical return trajectories. After leaving the food location, they would frequently make initial heading corrections (during the first 500 ms), then begin a direct line toward the edge of the arena. After reaching the edge of the circular arena, they would pause, presumably to look for their home. When rats paused at the edge of the arena, the position data would accumulate in an analogous location. This first stopping point was estimated by identifying accumulated position data in the same location for at least five consecutive data points (250 ms). This provided a conservative estimate of the first place the rat stopped at the edge of the arena, and this location was used to calculate the angular deviation of the homeward trajectory relative to the rotated arena location. The average angular deviation was calculated for the four local arena cue probe trial conditions: light–control, light–inactivation, dark–control, and dark–inactivation. Ninety-five percent confidence intervals were used to evaluate whether rats followed local arena cues (Etienne et al., 1990; Batschelet, 1981).

Results

Accuracy and search distance with visual cues. Figure 6A shows that, during light testing, inactivation of the retrosplenial cortex did not change the rat's accuracy scores, $F(1, 4) = 2.47$, *ns*, and that there was no improvement across trials, $F(1, 4) = 1.21$, *ns*. The lack of improvement across trials suggests that rats remembered the location of the home across test days, because they
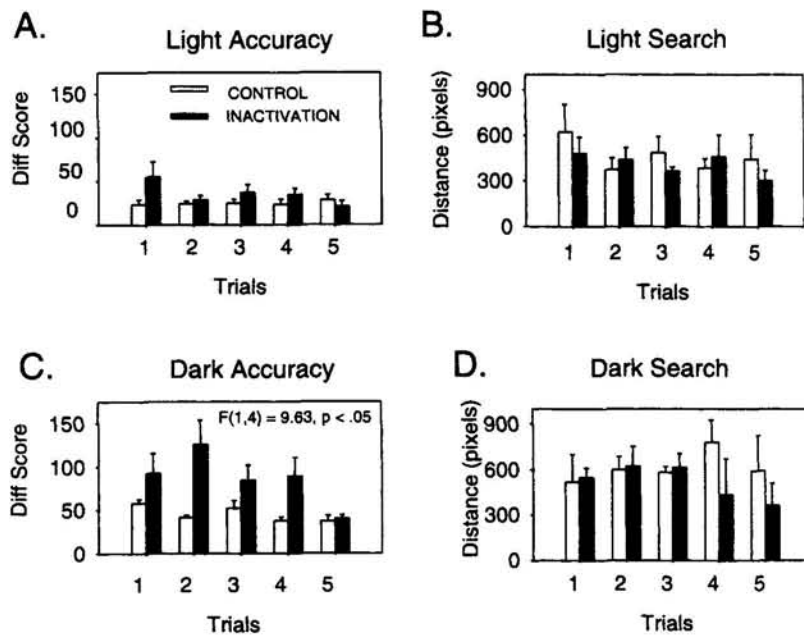

Figure 6. Inactivation of the retrosplenial cortex impaired navigation in darkness during performance of a reference memory navigation task. A: Difference (Diff) scores in light did not change as a function of injection. B: Search distance to locate the food was not affected by the injection condition. C: Difference scores increased significantly (impaired accuracy) when rats were tested in darkness after inactivation of the retrosplenial cortex. D: Search distance in darkness was not affected by retrosplenial cortex inactivation, suggesting that nonspecific sensory deficits are not a likely cause of the behavioral effects. Error bars indicate *SEM*.

were just as accurate on the first trial as they were on the subsequent trials. Figure 6B displays the distance required to locate the food with the room illuminated. Search distance did not change as a function of injection condition, $F(1, 4) = 0.26$, ns, and there was no improvement across trials, $F(1, 4) = 1.06$, ns.

Return and search time with visual cues. Figures 7A and 7B display the time required to return home and to locate food during the light trials, respectively. The time required to return home was increased significantly after inactivation of the retrosplenial cortex, $F(1, 4) = 8.65$, $p < .05$. There was no significant change in the return times across trials, $F(1, 4) = 2.24$, ns, but there was a significant interaction between the injection condition and trials, $F(1, 4) = 4.47$, $p < .05$. A Newman–Keuls post hoc analysis demonstrated that the first trial after retrosplenial cortex inactivation required significantly more time for the rats to return home ($p < .05$). The amount of time rats spent searching for food did not change after inactivation of the retrosplenial cortex, $F(1, 4) = 0.14$, ns, and did not change across trials, $F(1, 4) = 1.30$, ns.

Accuracy and search distance without visual cues. In Figure 6C, the dark-specific impairment in accuracy after retrosplenial cortex inactivation is displayed, $F(1, 4) = 9.63$, $p < .05$. Performance in darkness improved across trials, $F(1, 4) = 3.48$, $p < .05$, and there was no interaction between injection condition and trials, $F(1, 4) = 2.56$, ns. Inactivation of the retrosplenial cortex did not affect search distance in darkness, $F(1, 4) = 3.31$, ns, and search distance did not change across trials, $F(1, 4) = 0.22$, ns.

Return and search time without visual cues. Figures 7C and 7D display the time required to return home and to locate food. The amount of time to return home in darkness was increased significantly after inactivation of the retrosplenial cortex, $F(1, 4) = 8.45$, $p < .05$. Return times did not change across trials, $F(1, 4) = 0.18$. Retrosplenial cortex inactivation did not change the amount of time required to locate food on the arena, $F(1, 4) = 1.00$, ns. In addition, there was no change in time spent searching for food across trials, $F(1, 4) = 2.20$, ns.

Velocity during light and dark trials. During the light trials, there was no significant difference in accuracy, but there was a significant increase in the amount of time required to complete the trials. To determine whether the increase in the amount of time during inactivation was due to movement differences, we estimated the rats' velocity when they were returning home during hoarding trials. We used the distance traveled (in pixels) divided by the time required to return home to compute the velocity of each trial for each rat. Inactivation of the retrosplenial cortex did not affect velocity of movement in light or in darkness: light trials, $F(1, 4) = 1.42$; dark trials, $F(1, 4) = 3.59$; and velocity did not change across trials in either lighting condition: light trials, $F(1, 4) = 0.56$; dark trials, $F(1, 4) = 0.93$. Thus, the speed with which the rats performed the task was not solely responsible for the increased time to complete the light trials after retrosplenial cortex inactivation.
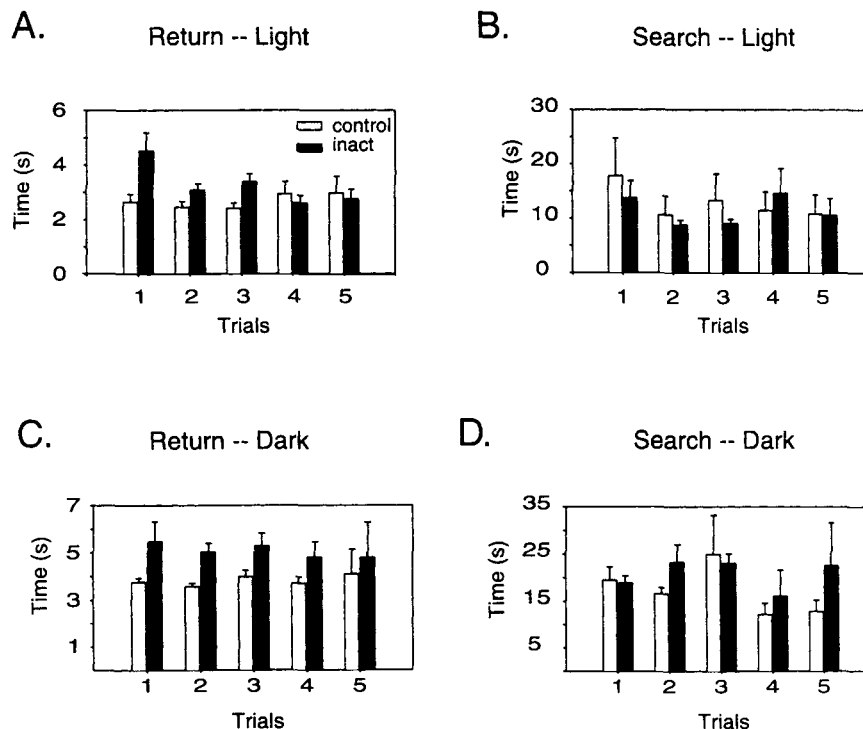

Figure 7. Inactivation (inact) of the retrosplenial cortex increased the amount of time required to return home, but not to locate the food, in the reference memory task. A: Return times with visual cues available were increased significantly after inactivation of the retrosplenial cortex. B: Search time to locate the food on the arena was not affected by inactivation of the retrosplenial cortex. C: In darkness, rats required more time to return home after locating the food if the retrosplenial cortex was inactivated. D: Similar to the light trials, search time in darkness was not influenced by the injection condition. Error bars indicate *SEM*.

Performance in light versus darkness. To assess the role of visual information in performance of the task, we compared the light–control data to the dark–control data for several of the measures (accuracy, return time, and velocity). To determine whether inactivation changed the pattern of data, we also compared the light–inactivation data to the dark–inactivation data for each of these measures.

After control injections, rats were significantly less accurate in darkness than they were with visual cues available, $F(1, 4) = 53.91, p < .002$, and there was no improvement across trials, $F(1, 4) = 0.19, ns$. Thus, performance of this task is facilitated by the availability of visual information. After inactivation of the retrosplenial cortex, visual information did not dramatically facilitate performance. There was only a marginally significant decrease in accuracy when rats were tested in darkness, $F(1, 1) = 6.86, p = .06$. There was a significant improvement across trials, $F(1, 4) = 4.70, p < .01$, but no interaction between lighting condition and trials, $F(1, 4) = 1.88, ns$.

After control injections, rats required more time to return home in darkness than they did during light trials, $F(1, 4) = 10.58, p < .05$. Return times did not change across trials, $F(1, 4) = 0.90, ns$, and there was no interaction between injection condition and trials, $F(1, 4) = 0.31, ns$. After retrosplenial cortex inactivation, rats tended to require more time to return home in darkness, but this difference was not statistically significant, $F(1, 4) = 7.00, p = .06$. Similar to the control trials, there was no change across trials in the amount of time required to return home, $F(1, 4) = 1.82, ns$, and no interaction between trials and lighting condition, $F(1, 4) = 0.54, ns$.

To examine how quickly rats ran the task in light and darkness, we compared the velocity during light–control trials to velocity during dark–control trials. Rats moved significantly slower in darkness than they did with visual cues available, $F(1, 4) = 46.68, p < .002$, but there was no significant change in running speed across trials, $F(1, 4) = 1.26, ns$. After retrosplenial cortex inactivation, rats ran slower in darkness than they did with visual cues available, $F(1, 4) = 24.13, p < .01$. Similar to light trials, there was no change in velocity across trials, $F(1, 4) = 1.06, ns$.

Local cue probe trials. Figure 8A displays the individual rats' angular deviation relative to the rotated arena location during light–control and light–inactivation trials. If rats were following local cues, a deviation of 0° would be expected. However, if they were returning back to the location from which they started the trial, the angular deviation would be 45°. During light–control local cue probe trials, the 95% confidence interval was 37.65° ± 12.00°. During inactivation trials with room lights available, the 95% confidence interval for the average return trajectories was 67.07° ± 32.00°. The higher mean was due to 1 rat that approached the distractor box opposite to the rotation of the arena. Thus, in both conditions, the rotated arena location (0°) does not fall within the 95% confidence interval, but the normal arena location does. This suggests that local arena cues did not control the rat's return path with room lights available and that rats were accurately returning to the location from which they started the trial.

Figure 8A also displays the individual angular deviation for each of the rats during dark–control and dark–inactivation trials. During dark–control local cue probe trials, the 95% confidence interval was 35.19° ± 17.00°. During inactivation of the retrosple-

A. Experiment 2 -- Reference Memory Task
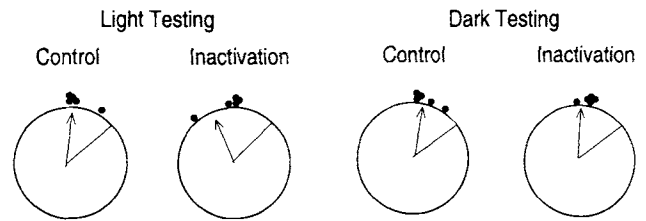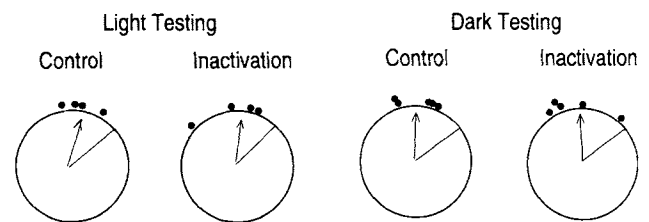

B. Experiment 3 -- Working Memory Task

Figure 8. Local arena cues did not control homing in the circular arena task. To control for local arena cues, the circular arena was rotated immediately before the start of the sixth trial. The circular arena is depicted in this figure, with the solid line indicating the "rotated arena location," the arrow indicating the mean angle (θ) and the length of the vector corresponding to the mean vector length. The dots at the perimeter of the arena correspond to the first location where rats stopped at the edge of the arena during the control trials. If rats followed local cues, they would be expected to stop near the solid line. If spatial cues were used, then a 45° deviation would be predicted. A: During light testing, rats did not follow local cues and instead were aligned with spatial cues. The rotated arena location is outside of a 95% confidence interval for the mean angle (see *Results* section). A similar pattern of data was also observed in dark testing, regardless of injection condition. After control injections or inactivation of retrosplenial cortex, rats returned toward the spatial location of the home box and did not follow arena cues in darkness. B: Similar to the reference memory task, rats did not rely on local cues to guide their return trajectories in the working memory task. In all testing conditions, the rotated arena location was outside of a 95% confidence interval for the return trajectories.

nial cortex in darkness, the 95% confidence interval was 41.71° ± 10.00°. In control and inactivation trials, the rotated arena location does not fall within the 95% confidence interval.

Experiment 3: Does Retrosplenial Cortex Inactivation Impair Navigation in Darkness in a Working Memory Task?

Rationale

In Experiment 2, rats were impaired in both light and darkness in a reference memory task. We therefore sought to extend those results by examining performance in a working memory task. Furthermore, in Experiment 1 it was demonstrated that path inte-

gration was not the dominant strategy used to solve the task. To encourage the use of strategies that do not rely on reference memory and to encourage the use of path integration in darkness, we trained rats to navigate from different starting locations each day. Six subjects (4 participated in Experiment 1, but not Experiment 2) were trained to perform this task.

Method

Subjects, apparatus, and procedure.   The injection regimen started after rats began to make direct paths back to their home starting location for at least 5 consecutive days in both light and dark conditions (i.e., experimenter's subjective judgment that the rats were not following the perimeter of the arena or entering distractor boxes). Subjects were tested daily after injection of tetracaine or vehicle control. After the injection, rats were brought into the testing room and tested for 10 trials, 5 with room lights on and 5 with room lights off. The light and dark trials were intermixed in the same order as the original training (3 light, 3 dark, 2 light, 2 dark). A maximum of four injections of tetracaine and four injections of saline were given to each rat. The variables of interest and data analyses were identical to those described in Experiment 2.

Local cue probe trials.   To control for local arena cues, we used two types of probe trials. The first involved rotating the arena after rats performed six trials (Figure 4 and see Experiment 2); the second involved switching the rat's home box with one of the distractor boxes. The first type of probe trial controlled for local cues on the arena (bedding at the edge of the arena from the home box, scent marks on the arena, etc.). If rats used local arena cues, then they would be expected to deviate their homeward trajectory with the rotation of the arena. The second type of probe trial assessed the role of olfactory cues from the home box. Switching the home box with a distractor box required two experimenters; one remained in the testing room, and one observed the movement of the rat from an adjacent room. When the rat was at or near the opposite side of the table, the

experimenter in the adjacent room knocked quietly on the wall. The knock provided the signal to the experimenter in the testing room to switch the home boxes. In two cases, the knocking apparently frightened the rats, and they immediately returned to the home box and would not continue to perform the task for the remainder of the day. Data were obtained from 2 rats that did not show disrupted behavior during dark–inactivation trials.

Results

Accuracy and search distance with visual cues.   Inactivation of the retrosplenial cortex did not result in a significant change in homing accuracy with room lights available, $F(1, 5) = 0.19$, ns (see Figure 9A). In light, there was a significant improvement across trials, $F(1, 4) = 5.61, p < .01$, but no interaction between trials and injection condition, $F(1, 4) = 0.97$. Thus, after control injections or inactivation of the retrosplenial cortex, rats performed similarly, and both groups showed pronounced improvement across trials. Figure 9B displays equivalent search distances to locate the food in light as a function of the injection condition, $F(1, 5) = 0.28$, ns, and shows that there was no improvement across trials in finding the food, $F(1, 4) = 0.55$, ns.

Return and search time with visual cues.   Figures 10A and 10B display the return and search times during the light–control and light–inactivation trials. The amount of time required to return home did not change after inactivation, $F(1, 5) = 1.87$, ns. The time required to return home decreased significantly across trials, $F(1, 4) = 3.80, p < .02$, but there was no interaction between injection condition and trials, $F(1, 4) = 1.27$, ns. Retrosplenial cortex inactivation did not change the amount of time required to search for and locate the food, $F(1, 5) = 0.41$, ns, and there was no significant change in search time across trials, $F(1, 4) = 0.91$.
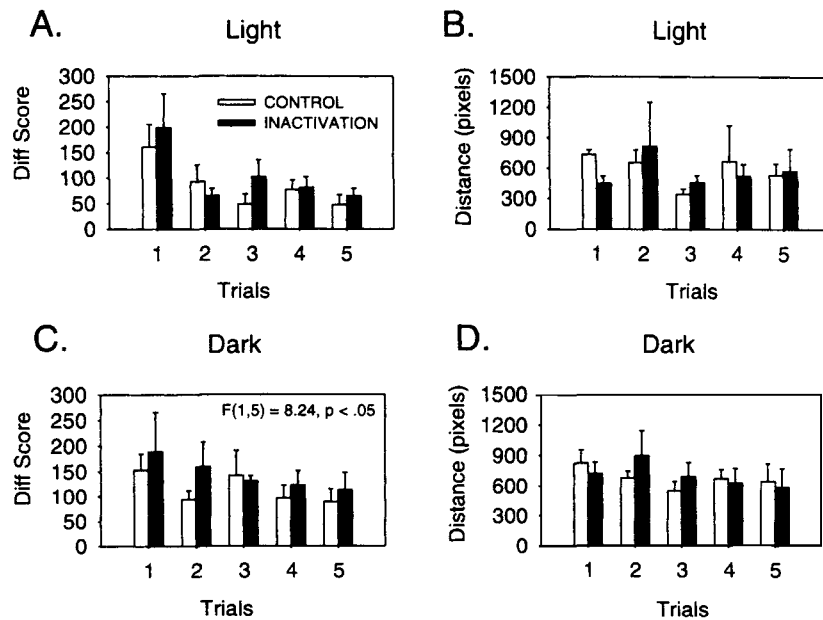

Figure 9.   Dark-selective navigation impairments were observed during performance of a navigation task that required working memory (A) or path integration (B). Inactivation of the retrosplenial cortex did not affect performance in the light or change the distance traveled to locate the food. C: Difference (Diff) scores increased significantly when rats were tested in darkness without a normally functioning retrosplenial cortex. D: Search distance in darkness was not affected by inactivation of the retrosplenial cortex.
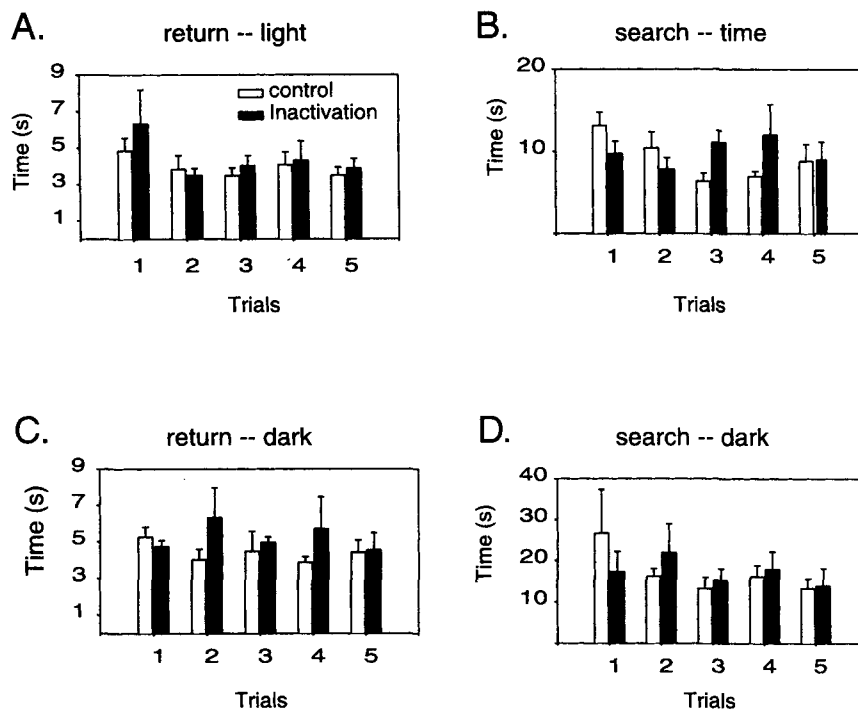
Figure 10. Inactivation of the retrosplenial cortex did not increase the amount of time required to return home or to locate the food in the working memory task. A: Return times were not increased significantly after inactivation of the retrosplenial cortex with visual cues available. B: Search time was not affected by inactivation of the retrosplenial cortex. C: In darkness, inactivation of the retrosplenial cortex did not change the amount of time required to return home after locating the food. D: Similar to the light trials, search time in darkness was not influenced by the injection condition.

Accuracy and search distance without visual cues. In darkness, retrosplenial cortex inactivation resulted in a significant increase in the average difference scores, $F(1, 5) = 8.24, p < .05$ (Figure 9C). There was no significant improvement across trials in darkness, $F(1, 4) = 1.63, ns$, and no interaction between injection condition and trials, $F(1, 4) = 0.33, ns$. Within-subjects analyses are extremely sensitive to consistent patterns of data across subjects, rather than the overlap in variance between groups. Although the graphical representation of the effect is modest, the significant ANOVA likely reflects the within-subject consistency of the behavioral impairment observed after inactivation. Figure 9D displays the absence of a change in search distance to locate the food in darkness as a function of injection condition, $F(1, 5) = 0.35, ns$, and shows that there is no improvement across trials, $F(1, 4) = 0.41, ns$.

Return and search time without visual cues. Figures 10C and 10D display the return and search times for the dark–control and dark–inactivation trials. The time required to return home after locating the food was not significantly longer during inactivation trials than it was during control trials, $F(1, 5) = 3.52, ns$. Return times also did not change significantly across trials, $F(1, 4) = 0.87, ns$. The time rats spent searching for food did not change during retrosplenial cortex inactivation, $F(1, 5) = 0.01, ns$, and there was no change across trials, $F(1, 4) = 1.04$.

Velocity in light and darkness. During light trials, retrosplenial cortex inactivation significantly decreased the running speed of the

rodents, $F(1, 5) = 8.64, p < .05$. There was no change in velocity across light trials, $F(1, 4) = 0.59, ns$. In darkness, there was no significant increase in running speed after inactivation of the retrosplenial cortex, $F(1, 5) = 1.40, ns$, and no change across trials, $F(1, 4) = 1.03, ns$.

Performance in light versus darkness. To evaluate the contribution of visual cues during performance of the task, we compared light–control trials to dark–control trials for several of the measures (accuracy, return time, velocity). To determine whether inactivation of the retrosplenial cortex changed this pattern of data, we also compared the light–inactivation trials to the dark–inactivation trials for each of these measures.

The control injection trials were compared between light and dark to determine whether there was a significant difference between illumination conditions. No significant difference in behavioral performance between lighting states was observed, $F(1, 5) = 3.28, ns$, but there was a significant improvement across trials, $F(1, 4) = 8.06, p < .01$. Thus, visual information did not facilitate performance in this task.

Return times after control injections were significantly faster during light trials than they were during dark trials, $F(1, 5) = 14.93, p < .02$, and there was a significant decrease in the amount of time required to return home across trials, $F(1, 4) = 27.89, p < .005$. This pattern of data was preserved during inactivation of the retrosplenial cortex. Rats returned home more quickly during light–inactivation trials than they did in darkness,

$F(1, 5) = 9.51, p < .05$. Return times did not decrease significantly across trials, $F(1, 4) = 1.96, ns$.

During control trials, rats ran faster with visual cues available than they did in darkness, $F(1, 5) = 13.83, p < .02$. There was no significant change in running speed across trials, $F(1, 4) = 0.81, ns$. During dark–inactivation trials, rats ran significantly faster with visual cues available, $F(1, 5) = 23.88, p < .01$, and there was no change across trials, $F(1, 4) = 0.50$.

Local cue probe trials. The circular arena was rotated by 45° before the start of the probe trial. The angular difference between the first place where the rat stopped at the edge of the arena and the rotated arena location was determined. Most rats experienced each probe trial condition, with the exception of 2 rats that either did not find the food or did not leave the starting location during the local cue probe trial. The data from these subjects were excluded from the analysis. To determine whether their return trajectories deviated in agreement with the local cues, a 95% confidence interval was determined on the basis of the average return trajectories for light and dark control and inactivation trials (Batschelet, 1981). As with Experiment 2, an angular deviation of 0° would be expected if rats followed local arena cues, and a deviation of 45° would be expected if they followed distal spatial cues. Figure 8B displays the data for the light and dark probe trials after control injections and retrosplenial cortex inactivation.

After control injections with room lights available, the average angular difference between the first stopping place and the rotated arena location was 34.80° ± 13.00°; after inactivation of the retrosplenial cortex, the angular difference was 43.50° ± 32.00°. In darkness, the angular deviation was 50.98° ± 19.00° after control injections and 47.16° ± 25.00° after inactivation of the retrosplenial cortex. In all of these cases, the rotated arena location (0°) is outside a 95% confidence interval for the average homeward journey.

Figure 11 displays an example return path of a rat during performance of a dark–inactivation probe trial with the home cage moved 45° counterclockwise. The search for the food source is displayed in Panel A, the return from the food source to the edge of the arena is shown in Panel B, and the ensuing search for the home box around the arena is depicted in Panel C. Although this rat showed the most pronounced effect, 1 other rat tested with this manipulation showed a similar pattern of data. Rats made an initial homeward trajectory independent of the new location of the home, paused at the edge of the arena, and then began to search for the home location. Even when the rat passed the location of the box, it would frequently hesitate before entering. This can be observed in Figure 11C by the position data showing repeated entries into the goal box. In these cases, the rat investigated the box but did not leave the arena and enter the box.

General Discussion

The results of these experiments demonstrated that the retrosplenial cortex is important for spatial memory (extending beyond 1 day) and for accurate navigation in darkness. We suggest that the findings are interrelated such that, when visual information is not available to reduce error, memory for visual and nonvisual features of the environment may serve this function. In the first experiment, rats were trained to retrieve food from a consistent starting location. After training, they were given a single probe

A. Search for Food
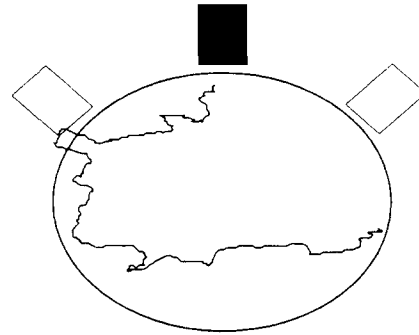
B. Return Home $\phi = 65°$
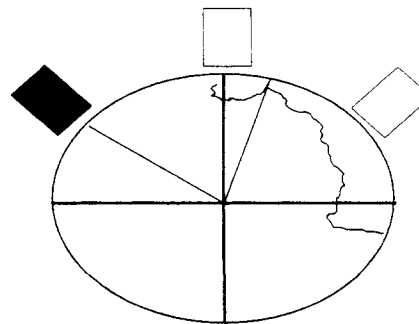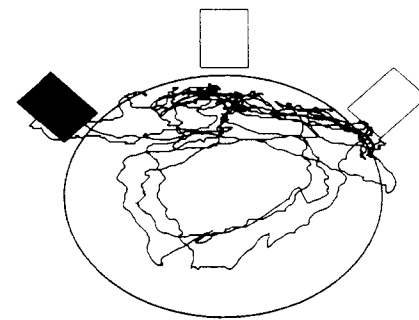
C. Search for Home

Figure 11. Rats did not follow cues from the home box to find their way home but instead used local cues. A: The path taken by the rat from the home to the food source, after the experimenter switched the home box with the northwest distractor box. B: After locating the food, the rat returned home despite the change in the location of the home box. C: When the rat realized that the home box was not in its anticipated location, it began to search the arena for the home box. The rat investigated the new home box location several times before entering the home box. Thus, a combination of local cues and memory for spatial location defined the home box location, but only absolute spatial location guided the rat's homeward journey.

trial in which they began a hoarding excursion from a novel location in the arena. After control injections, rats searched extensively around the location in which they were trained. This suggested that they were searching for the remembered home location. However, inactivation of the retrosplenial cortex disrupted this

search pattern, and rats did not show preferential searching in any particular area of the arena. Thus, without the normal activity of the retrosplenial cortex, rats showed impaired spatial memory. It is important to note that rats searched around the training location in darkness after control injections, suggesting that the rats in this study were not relying only on movement-related cues to identify their homeward trajectory.

To relate these findings to our previous work showing that the retrosplenial cortex contributes to nonvisual navigation (Cooper & Mizumori, 1999), we examined accuracy during navigation in two versions of the hoarding task. In Experiment 2, rats were just as accurate on the first trial of a given day as they were on subsequent trials. This is consistent with the use of reference memory because they were able to accurately locate the home across test days. Inactivation of the retrosplenial cortex impaired accuracy during dark trials, but not light trials, in this task. On the first trial in the reference memory task, however, there was a slight impairment that was observed when we examined the time required for rats to return home during inactivation of the retrosplenial cortex. The difference in time to return home was not due to velocity of movement, as rats moved at similar speeds during control and inactivation trials. The increase in time, however, may reflect an interaction between two results that were statistically nonsignificant. Rats may have run slightly slower (but not significantly) and were less accurate (again, not significantly) when visual cues were available. This combination may have led to decreased time to return home after locating food and a significant difference between control and inactivation trials.

In Experiment 3, rats began hoarding excursions from different locations each day. Therefore, working memory for visual or nonvisual features of the environment, movement-generated cues, or a combination of these possibilities would enable successful performance of the task. Inactivation of the retrosplenial cortex resulted in dark-specific navigation impairments in accuracy, but did not increase the amount of time required to return home after finding the food in darkness. In contrast to the reference memory task, the availability of visual information did not enhance performance; control rats performed similarly in both lighting conditions. Thus, although visual information was not critical for this working memory task, spatial memory likely reflected the incorporation of visual and nonvisual information. The retrosplenial cortex may have used such a memory to enable accurate performance when rats located their home in darkness.

The combination of experiments showed that the retrosplenial cortex is critical for accurate navigation when rats are placed in novel testing situations (Experiment 1) or tested in a familiar task but without the aid of visual information. Our interpretation is that memory is critical for performance of a task when rats are placed in a novel testing situation or when major features of the environment are removed (e.g., testing without visual cues). The results suggest that the retrosplenial cortex likely processes mnemonic spatial information and that this is used for ensuring accurate navigation.

Sensory/Motor Controls

Nonspecific sensory or motor deficits are not likely causes for the observed effects. Experiment 1 demonstrated that if rats were using local cues from the home box, then they would have been expected to return directly home and not search for where the home box was no longer located. Furthermore, this experiment illustrates that local cues on the arena were not likely to guide navigation in this task. If local arena cues were guiding behavior, then rats would be expected to return toward the novel starting location and not to a location that they had not yet visited during the test day. Nonspecific effects of the injection are unlikely sources of the observed effects because behavioral performance was normal in Experiments 2 and 3 when room lights were available (except Trial 1 of Experiment 2). The velocity analysis provides further evidence that motor impairments are not the likely contributors to the pattern of data. Inactivation of the retrosplenial cortex (in most cases) did not change running speed in this task. Finally, the dark-specific impairments are not due to differences in task demands between the lighting conditions. In Experiment 3, accuracy was comparable with room lights on or off for control-injected rats, yet behavior after retrosplenial cortex inactivation was impaired only when visual cues were removed. Thus, the retrosplenial cortex may enhance the accuracy of navigation in darkness by providing mnemonic information about spatial features of the environment.

Neural Structures Contributing to the Pattern of Data

Inactivation of the retrosplenial cortex also includes inactivation of the adjacent cingulum bundle and medial areas of the posterior parietal cortex. Both of these areas play important roles in spatial processing and navigation (Aggleton, Neave, Nagle, & Sahgal, 1995; DeCoteau & Kesner, 1998; Kolb, Buhrmann, McDonald, & Sutherland, 1994; Long, Mellem, & Kesner, 1998; Neave, Nagle, & Aggleton, 1997; Neave, Nagle, Sahgal & Aggleton, 1996; Warburton, et al., 1998). On the basis of the data obtained from our radial maze experiments, we have argued that our effects are most likely due to inactivation of the retrosplenial cortex rather than the cingulum bundle (Cooper & Mizumori, 1999). Damage to the cingulum bundle, but not damage to the retrosplenial cortex, results in radial maze spatial memory impairments when room lights are available (Neave et al., 1997). We have previously shown that inactivation of the retrosplenial cortex does not disrupt radial maze performance when subjects have access to visual cues (Cooper & Mizumori, 1999). Furthermore, the preserved performance in light trials during Experiments 2 and 3 of the present study further suggests that inactivation of the cingulum bundle is not likely to result in this pattern of behavioral effects.

It has been suggested that a body-centered coordinate representation of the environment provided by the posterior parietal cortex is transformed into an environment-centered representation in the retrosplenial cortex (Chen, Lin, Barnes, & McNaughton, 1994). Thus, the posterior parietal cortex and the retrosplenial cortex may interact in mediating navigation. These findings are supported by anatomical data, which show that the most direct route for information from the posterior parietal cortex to reach the hippocampus is through the retrosplenial cortex (Zilles & Wree, 1995). The posterior parietal cortex may provide the retrosplenial cortex with proprioceptive feedback that can be used in the neural computation of directional heading within the context of remembered features of the spatial environment. This issue will be further explored below.

*Does the Retrosplenial Cortex Contribute to Path
Integration?*

Alyan and McNaughton (1999) have distinguished between
different forms of path integration that are commonly used in the
literature. They provide the distinction between "coordinate up-
dating," which uses spatial memory to guide navigation, and
"homing vector updating," which uses path integration based only
on self-motion cues. Homing vector updating is independent of
memory for spatial features of the environment. The data from the
current study are consistent with the idea that rodents can use
coordinate mapping to navigate; they provide little evidence for
homing vector updating.

Whishaw and Maaswinkel (1998) have shown that, in a similar
task, subjects rely on visual information when it is available, use
homing vector updating when visual information is removed, and
do not reliably use local cues to guide navigation. This was
demonstrated by training rats with a fixed location, then testing
them with visual cues or in darkness from a novel location. With
visual cues, the rats returned to the previously trained location, and
in darkness they returned directly to the novel starting location. In
the present study, rats did not return to the novel start location in
darkness as would be predicted from homing a vector updating
strategy. Instead, they searched for the original training location,
which is consistent with the use of coordinate updating. The reason
for this discrepancy in the use of behavioral strategies is currently
unclear.

Training procedures in the present study were designed to be
similar to those used by Whishaw and colleagues (Maaswinkel et
al., 1999; Whishaw & Maaswinkel, 1998). Therefore, it may be
that the different testing environments or subtle differences in
behavioral testing procedures encouraged the use of different nav-
igational solutions to the task. For example, in the current study,
rats were brought into the testing room by the same path each day.
This information is sufficient to establish location coding in the
hippocampus (Sharp, Kubie, & Muller, 1990). Memory for direc-
tion of entry into the testing room may account for the differences
in the current study and may have provided the rats with the
information that they were beginning hoarding trials from a novel
location. In the Whishaw and Maaswinkel (1998) experiment, it is
possible that such directional information was less prevalent and
did not provide sufficient information for rats to determine that
they were starting from a novel location.

We find the pattern of data across experiments to be supportive
of our hypothesis that the retrosplenial cortex may provide spatial
memory (reference and working) for identifying locations in space
when rats are relying heavily on movement-related information.
Indeed, navigation requires the coordinated representation of
movement and spatial knowledge. There is strong evidence from
monkeys that this integrative process is a critical function that is
mediated in part by the posterior parietal cortex (Andersen, 1997;
Colby & Goldberg, 1999). Our theoretical framework suggests that
such spatial processing requires a distributed neural system. The
unique functional contribution of this neural system will be ad-
dressed below.

Neural System Contributing to Navigation

On the basis of anatomical, electrophysiological, and lesion
data, we suggest that posterior parietal and anterior thalamic nuclei

may provide self-motion information to the retrosplenial cortex
(Chen, Lin, Barnes, & McNaughton, 1994; Chen, Lin, Green, et
al., 1994; Colby & Goldberg, 1999; Mizumori & Williams, 1993;
Taube, 1995; van Groen & Wyss, 1992). A unique function of the
retrosplenial cortex may be to integrate self-motion cues with
mnemonic spatial features of the environment. We suggest that
such an integrative process may enable rats to more accurately
identify locations in space when visual cues are reduced or ob-
scured. Save (1997) demonstrated that the longer rats are exposed
to visual information before performing a water maze task in
darkness, the better the performance. Such mnemonic processing
of visual information may be critical for self-localization.

The retrosplenial cortex may provide mnemonic spatial infor-
mation to the hippocampus for use in computing current location.
Indeed, temporary inactivation of the retrosplenial cortex changes
the normal location coding of place cells in the hippocampus
(Cooper & Mizumori, 2001). The hippocampal–retrosplenial in-
teractions may enable effective navigation by means of a compar-
ison between the currently experienced environment relative to
past experiences (Mizumori et al., 2001). Such interactions would
enable coordinate updating, thereby preventing cumulative errors
that occur when rats navigate in the absence of visual cues.

Processing of multiple types of sensory information provides an
effective means of identifying locations, particularly in ever-
changing environmental conditions. By methods that may be com-
parable to pattern completion, rats can identify a location on the
basis of partial information when one type of sensory information
is removed or reduced (e.g., in darkness). Indeed, hippocampal
cells represent both distal spatial features of the environment and
local cues when they are salient and reliable features of the
environment (Shapiro, Tanila, & Eichenbaum, 1997; Young, Fox,
& Eichenbaum, 1994, for review, see Eichenbaum, Dudchenko,
Wood, Shapiro, & Tanila, 1999). Furthermore, in darkness, local
cues can exert significant control over place coding by hippocam-
pal neurons (Poucet, Save, Lenck-Santini, 2000). Thus, nonvisual
navigation likely utilizes spatial memory, which, broadly defined,
includes knowledge of consistent and reliable relationships be-
tween visual cues, somatosensory cues, and perhaps auditory cues
in the environment. Therefore, knowledge about past experiences
with the locations of stimuli, which may be provided by the
retrosplenial cortex, could be used in coordinate updating. Previ-
ous lesion experiments have demonstrated that the cingulate cor-
tices (anterior and posterior) are involved in memory for the
locations of objects in the environment (Ennaceur, Neave, &
Aggleton, 1996). The current work extends these mnemonic func-
tions of the retrosplenial cortex and suggests movement-related
processing as an additional critical function. Interactions between
the retrosplenial cortex and hippocampus may contribute to iden-
tifying locations in the environment and ultimately mediate accu-
rate navigation.

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