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Retrosplenial cortex inactivation selectively impairs navigation in darkness

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THERE is an emerging consensus that retrosplenial and posterior parietal cortex importantly contribute to navigation. Several theories of navigation have argued that these cortical areas, particularly retrosplenial cortex, are involved in path integration. In an effort to characterize the role of retrosplenial cortex in active navigation, the effects of temporary inactivation of retrosplenial cortex on spatial memory performance were evaluated in light and dark testing conditions. Inactivation of retrosplenial cortex selectively resulted in behavioral impairments when animals were tested in darkness. These data support the hypothesis that retrosplenial cortex contributes to navigation in darkness, perhaps by providing mnemonic associations of the visual and nonvisual environment that can be used to correct for cumulative errors that occur during path integration. *NeuroReport* 10:625–630 © 1999 Lippincott Williams & Wilkins.

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Introduction

Accurate navigation typically requires integration of visual and self-motion cues. The use of self-motion cues during navigation, referred to as path integration, includes the use proprioceptive feedback, vestibular information, and perhaps efference copy. Recent theories of navigation have argued that retrosplenial cortex and posterior parietal cortex may provide self-motion cues to hippocampus to enable accurate navigation [1–3]. More specifically, we hypothesize that retrosplenial cortex may contribute to path integration by providing mnemonic associations of the visual and nonvisual environment which can be used correct for cumulative errors that occur when animals rely on self-motion cues to navigate.

A variety of spatial correlates are observed in retrosplenial cortex, many of which are modulated by an animal's movement [4–6]. For example, individual cells in retrosplenial cortex fire when an animal is facing a particular direction in space, regardless of current location. Such 'head direction' coding probably reflects a function of the path integration system since the preferred direction can persist in darkness [5]. Further support of this path integration hypothesis comes from the finding that the firing rate of the head directaion cells is modulated by the movement state of the animal. This movement-related modulation of the head direction signal in retrosplenial cortex may be derived from anterior thalamic nuclei input; a structure with head direction cells showing similar firing rate modulation during turning behaviors [7]. Visual spatial information may arrive in retrosplenial cortex via direct connections from the tectocortical (lateral dorsal and lateral posterior thalamic nuclei) and geniculostriate (primary and secondary visual cortices) visual pathways [8]. The electrophysiological findings, together with anatomical data, support the current hypothesis that retrosplenial cortex can function to update the path integration system based on mnemonic representations of the visual surroundings.

Lesion studies have both observed and failed to detect spatial learning impairments following damage to retrosplenial cortex [9,10]. One solution to the disrepancy in spatial learning impairments is that the different tasks used in the different studies may cause animals to rely more heavily, or less heavily, on path integration. Based on behavioral experiments, Brown and colleagues have argued that when animals are tested on the 8-arm radial maze under normal lighting conditions they rely on successive 'go/no go' decisions based on the constellation of visual cues [11,12]. However, when animals are tested in darkness on the radial maze they rely on a combination of local, distal and self-motion cues to solve the task [11,12]. Therefore, if our current hypothesis is correct, retrosplenial cortex may contribute to spatial memory performance in dark but not light testing conditions.

To evaluate the hypothesis that retrosplenial cortex encodes mnemonic representations of visual and non-visual cues for use in path integration, we tested

animals on a radial maze in light and dark testing conditions following reversible inactivation of retrosplenial cortex. In the light, which requires minimal use of path integration, we expected retrosplenial cortex inactivation to have minimal effects on behavioral performance. Our hypothesis assumes that in darkness animals depend on mnemonic associations of visual (and non-visual) cues to correct for cumulative errors that occur duing path integration [11–13]. Therefore, we expected to observe behavioral impairments when animals performed in darkness after retrosplenial cortex inactivation. Temporary lesions of retrosplenial cortex indeed resulted in behavioral impairments on the radial maze only when animals were tested in darkness.

Materials and Methods

Five male Long Evans rats (Simonson's Laboratories) were used in this study. Following a 1-week acclimation period to the laboratory, animals were food-restricted, and maintained at 80% of their free feeding weights. Animal testing was conducted in an AAALAC approved facility, within the guidelines of NIH animal care and use. Animals were trained to perform a spatial memory task on an 8-arm radial maze using procedures similar to those described elsewhere [14,15]. Briefly, the maze is elevated (77 cm) above the floor and consists of eight arms with 0.5 cm railings radiating from a center platform (19.5 cm diameter). Arm access is afforded or restricted via remote control by raising or lowering the proximal portion of the maze arm, respectively. A camera positioned above the maze allowed the experimenter to monitor the animals from an adjacent room. In darkness, animals were monitored by observing the movement of an infrared diode attached to the nine pin connecting socket on the rats head (see below) [14].

Following habituation to the maze, animals were trained to retrieve chocolate milk from the end of the maze arms. After animals readily pursued reward at the end of the arms, win-shift spatial memory training began. Win-shift testing consisted of two phases: in the first phase animals were presented with four randomly selected arms individually and sequentially. While animals were on the fourth arm the second phase began by raising all eight maze arms. Animals were allowed to chose freely among the eight arms with arm reentries considered errors. This partial forced choice procedure largely prevents animals from developing a response-based strategy for solving the task. After animals performed eight trials daily (2 min ITI) for seven consecutive days in < 1 h, they were given free access to food for 2–4 days prior to surgery.

Guide cannuli were cut from 25 G stainless steel tubing at a length of 1.1–1.3 cm. Removable stylets were constructed from 33 G stainless steel tubing and were placed inside the guide cannuli to prevent occlusion of the tubing. Using a stereotaxic drill assembly, holes were drilled in 10 mm thick nylon sheeting, and guide cannuli were glued in place 2 mm apart. Injection needles were made of 33 G stainless steel tubing glued inside 25 G stainless steel tubing, and protruded ~0.5 mm beyond the tip of the guide cannuli. Animals were deprived of food and water 24 h prior to surgery, then anesthetized with sodium pentobarbital (33 mg/kg). Under deep anesthesia, they were given 0.2 ml atropine to prevent respiratory distress. Eight burr holes were drilled and self-tapping anchor screws were inserted into the holes. Craniotomies were drilled at 6.0 mm posterior to bregma and 1.0 mm lateral to midline. Dura was cut and guide cannuli were implanted 1.0 mm ventral to the surface of the brain. Vacuum grease was packed around the guide cannuli to protect the surface of the brain from exposure to dental cement. Dental cement was then applied to the surface of the skull and a 9 pin plug was cemented directly in front of the guide cannuli. After surgery, animals were given one week of free feeding prior to resuming food restriction and behavioral testing.

Following recovery from surgery animals were retrained on the spatial memory task until they performed 15 trials for 7 consecutive days in < 1 h. After this retraining period animals underwent daily testing sessions with inactivation of retrosplenial cortex occurring during behavioral testing. Tetracaine (2% dissolved in bacteriostatic water) was loaded into the injection cannuli with an air bubble to allow for visual verification of infusion. Prior to placing animals on the maze, the stylets were removed from the guide cannuli, and in two of the animals, injection needles were placed into the guide cannuli for the duration of the behavioral testing. In these two animals the injections occurred while they remained on the center platform of the maze, thereby allowing for infusion of tetracaine without removing the animals from the testing room. For the remaining three animals, they were removed from the testing room and injection needles were always inserted imoediately prior to the injection. We have previously shown that removing the animals for 5 min and then resuming behavioral testing does not affect spatial memory performance [14]. A 5 µl Hamilton syringe was used to infuse 1.0 µl/hemisphere by hand over the course of about 2 min; at least 1 min was allowed for diffusion prior to removal of the injection needles. Previous work has shown that tetracaine is active for 20–25 min, which

is the equivalent of about five maze trials [15]. Therefore, all baseline and injection trials consisted of five spatial memory trials. For light spatial memory trials, animals performed five trials, and then tetracaine was injected. After the injection, animals performed five more light trials. For dark testing, animals performed five light trials and then 10 trials in darkness. Tetracaine injections occurred at one of two time points during dark testing; they were made either immediately prior to the onset of the dark trials (i.e. between trials five and six) or after animals had performed five dark trials (i.e. between trials 10 and 11).

Animals underwent at least three light injections, four dark injections, and two control vehicle injections across a minimum of 9 test days. If unilateral injections occurred, animals received extra injections to ensure equal number of bilateral injections across animals. Therefore, some animals experienced more injections (depending on the number of unilateral injections), but all animals had an equal number of bilateral injections. The order light and dark testing with inactivation was pseudorandom and counterbalanced with the exception that control injections occurred during the middle of the injection schedule and at the conclusion of the injection regimen. In order to reduce the number of injections each animal incurred, control injections were only conducted during dark testing because pilot data suggested that inactivation of retrosplenial cortex during light testing did not effect spatial memory performance. The statistical analyses, described below, compared the baseline (no injection) trials to the inactivation or control injection trials.

The experimenter recorded the number of errors (i.e. repeat arm entries) and time taken to complete each trial during the course of the behavioral testing. The mean number of errors was calculated for each trial (five baseline and five inactivation trials) for each animal during light and dark testing. Time per choice (TPC) was calculated by dividing the amount of time taken to complete the trial by the number of arms visited within the trial. A mean TPC for each trial was then calculated. Therefore, each animal contributed a single (average) score (error and TPC) for each of the five baseline and injection trials during light, dark and vehicle injections. A two factor (uninjected baseline or injection condition) repeated measures (five spatial memory trials) within-subjects analysis of variance (ANOVA) was used to determine statistical significance with $\alpha = 0.05$.

Following completion of behavioral testing, animals were overdosed with sodium pentobarbital and 1.0 µl permanent ink was infused into retrosplenial cortex. Animals were perfused with saline followed by 10% phosphate buffered formalin; brains were extracted and placed in 30% sucrose formalin. After the brains sunk, they were frozen and cut at 40 µm, taking every third slice around the injection site. Sections were stained with cresyl violet and viewed under a light microscope to identify injection site and spread of ink.

Results

Figure 1 displays the central location of the injection sites in the current study. The injection of permanent ink spread into the retrosplenial granular and agranular cortex [16]. In addition, limited areas of posterior parietal cortex (OC2M) and the cingulum bundle were marked with ink. One animal was excluded from the data analyses because the tip of the injection site was located in the superficial layers of superior colliculus.

Because the injection cannuli sometimes became clogged, there were several cases of unilateral injections (three of the four animals, range 1–3 unilateral injections) in both light and dark spatial memory testing. Because the pattern of data was the same for the unilateral and bilateral injections, they were combined for all statistical analyses.

Figure 2 shows that the mean number of errors

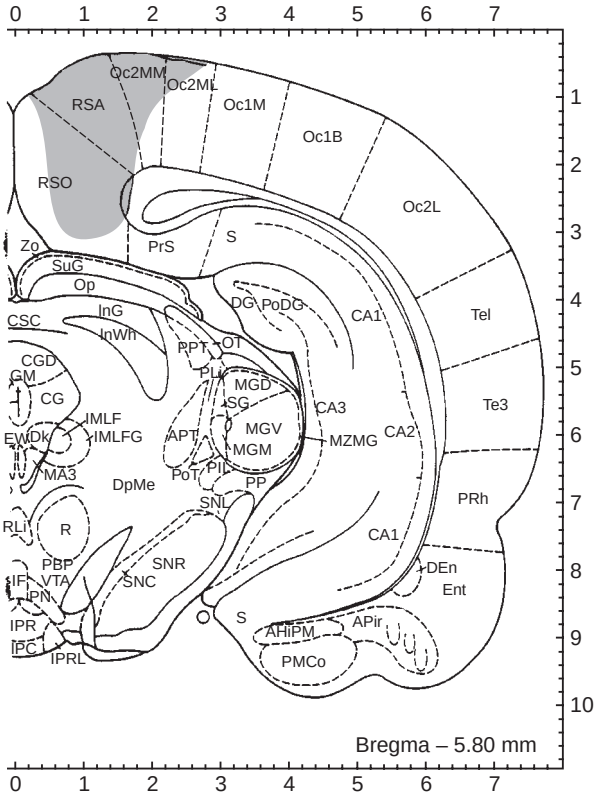

FIG. 1. The site of tetracaine injections was marked with the infusion of permanent ink. A representative section of the injection site, and spread of ink in that area, is depicted here. Retrosplenial granular and dysgranular areas were stained with the ink. The ink also spread into the adjacent cingulum bundle and posterior parietal cortex (OC2m).

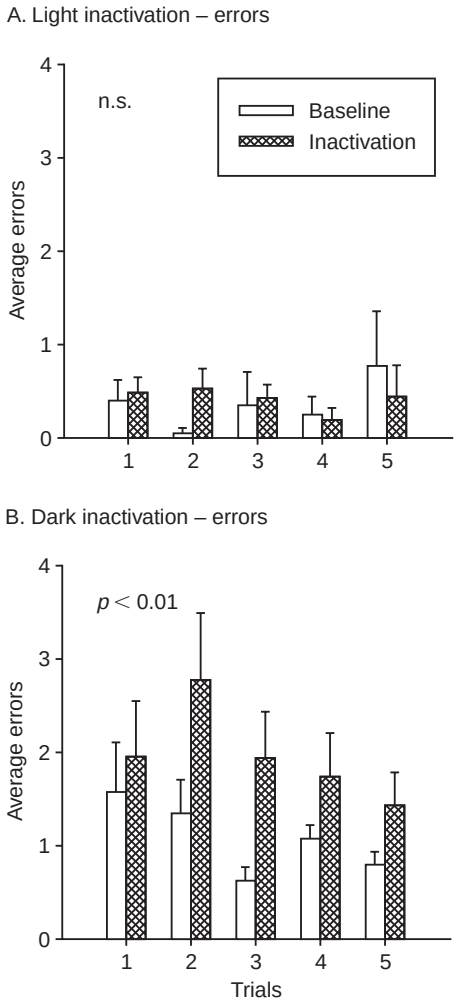

FIG. 2. Temporary inactivation of retrosplenial cortex impairs navigation during dark testing, but not in light. (**A**) The average number of errors (± s.e.m.) across trials following inactivation did not change when animals were tested with room lights on. (**B**) When animals were tested without explicitly available visual cues, temporary inactivation of retrosplenial cortex caused animals to make significantly more errors compared to baseline dark trials. These data suggest that retrosplenial cortex importantly contributes to non-visual navigation; perhaps by providing mnemonic associations of the visual environment for use in correcting cumulative errors that occur during path integration. Animals performing the task by randomly selecting maze arms would make an average of 9 errors per trial [12].

committed following inactivation of retrosplenial cortex during light and dark testing. A within-subjects repeated measures ANOVA revealed that the mean (± s.e.m.) number of errors did not change significantly following inactivation of retrosplenial cortex in light testing ($F(1,3) = 0.08$, $p > 0.05$). Figure 2A shows the number of errors made across trials during baseline and inactivation light trials. The mean number of errors during baseline light trials was 0.36 ± 0.14, following inactivation of retrosplenial cortex the mean was 0.42 ± 0.09. The interaction of condition by trials was also not significantly different following retrosplenial cortex inactivation ($F(1,4) = 0.55$, $p > 0.05$).

Regardless of when inactivation occurred during dark testing, the same pattern of data was observed; therefore the data were combined for statistical analyses. Figure 2B displays the mean number of errors in the baseline and inactivation dark trials. The overall baseline dark average number of errors was 1.10 ± 0.14, following inactivation of retrosplenial cortex the mean number of errors increased significantly to 2.00 ± 0.24 ($F(1,3) = 57.01$, $p < 0.01$). There was no significant interaction of condition × trials during dark testing ($F(1,4) = 0.48$, $p > 0.05$). If the behavioral impairment observed in darkness was influenced by task difficulty, we predicted that as the task became easier, the inactivation would not be expected to cause a behavioral impairment. To evaluate this, we compared the last day of dark injection trials for each animal to determine if the behavioral impairment was present when the task was presumably easier for the animals. The mean number of errors in the baseline condition of the final day of dark testing was 0.80 ± 0.25, during inactivation the mean increased significantly to 1.4 ± 0.33 ($F(1,3) = 10.8$, $p < 0.05$). Thus, even with only 3–5 days of dark testing, the error rates were approaching those observed in light, yet the behavioral impairment remained suggesting that task difficulty was not the primary reason for the behavioral impairment. Across all of the dark inactivation trials each animal showed the same pattern of data, errors reliably increased only when they were tested with retrosplenial cortex inactivated (individual data not shown).

Control vehicle injections did not result in a significant change in errors. The mean number of errors was 0.50 ± 0.17 during baseline conditions and 0.77 ± 0.26 following vehicle infusion ($F(1,3) = 1.6$, $p > 0.05$). The interaction of condition × trials was also not significant ($F(1,4) = 0.86$, $p > 0.05$).

Figure 3 shows that the average TPC was not affected by inactivation of retrosplenial cortex. The mean TPC did not change significantly following inactivation; during baseline light trials the mean TPC was 13.84 ± 1.00 s and 14.86 ± 1.24 s following inactivation ($F(1,3) = 0.18$, $p > 0.05$; Fig. 3A). The interaction of condition × trials was also not significant ($F(1,4) = 0.44$, $p > 0.05$). Figure 3B displays the average TPC during dark testing for baseline and inactivation trials. During dark testing, mean TPC was 15.18 ± 0.90 s during baseline trials and 15.82 ± 0.79 s following infusion of tetracaine ($F(1,3) = 0.28$, $p > 0.05$). The interaction of condition by trials was also not significant ($F(1,4) = 0.95$, $p > 0.05$). Similarly, dark control injections resulted in equivalent mean TPC of 15.23 ± 0.96 s during baseline trials and 15.23 ± 0.97 s following vehicle injection ($F(1,3) = 0.00$, $p > 0.05$). Again, the inter-

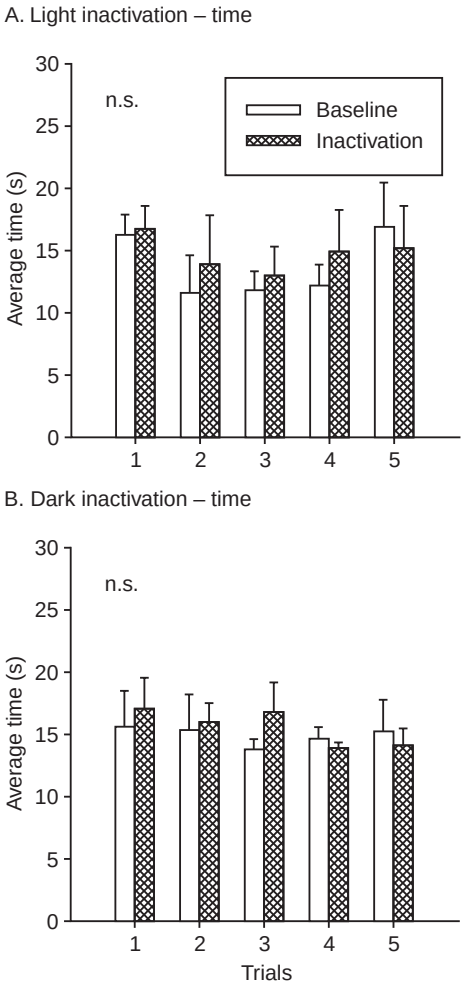

FIG. 3. Average time per choice (TPC) was not affected by inactivation of retrosplenial cortex. (**A**) In light testing the amount of time taken for each trial (± s.e.m.) was not changed following inactivation. (**B**) Similarly, inactivation in darkness did not change the TPC. Therefore, gross motor impairments are an unlikely explanation of the behavioral impairment observed during dark testing.

action of condition by trials was not significant ($F(1,4) = 0.95$, $p > 0.05$).

Discussion

Reversible inactivation of retrosplenial cortex resulted in a dramatic increase in errors committed during spatial memory testing when animals were tested in darkness. In contrast, inactivation had no effect on choice accuracy when animals were tested in light conditions. Importantly, the time taken to complete each trial was not changed as a function of inactivation in light or dark conditions, and control vehicle injections did not influence dark spatial memory performance. The lack of a memory impairment in light, in the same animals showing consistent impairments in darkness, and no change in running time argues strongly against nonspecific motoric, attentional, task difficulty or perceptual deficits. Therefore, the behavioral effect of retrosplenial cortex inactivation is specific to spatial memory performance when well-learned visual features of the environment are removed.

Although the current data, and work from other laboratories, may suggest that the navigation impairment in darkness is due to a disruption of the path integration system, another possible interpretation of the current data is that this structure enables animals to switch strategies between visually and nonvisually based navigation. We do not believe that a failure to switch strategies can adequately explain the current data because behavioral impairments were observed in darkness regardless of whether the inactivation preceded dark trials or occurred during the middle of dark trials.

One could argue that inactivation of adjacent tissue caused the behavioral impairments. Tests of the spread of dye confirmed that some fibers from the cingulum bundle were likely influenced by the injection of tetracaine. Cingulum bundle inactivation is an unlikely explanation of the current data because permanent lesions of the cingulum bundle impair 8-arm radial maze performance in light conditions [10], and temporary inactivation in this study did not affect performance when animals were tested in light conditions. Spread of tetracaine into posterior parietal cortex may also have influenced the performance of the animals in when tested in darkness. The vast majority of posterior parietal cortical projections go through retrosplenial cortex, and then into the hippocampal formation [17]. Therefore, our manipulation could have been caused by a disruption of medial areas of posterior parietal cortex or of retrosplenial cortex. It is worth noting that head direction cells are observed in both of these structures [4,5]. Therefore, they may function as an integrated system relaying related head direction information to hippocampus. Future work can explore the unique contributions of posterior parietal cortex to navigation without explicitly available visual cues.

The dark impairments may arise from a failure to utilize local maze cues, or from an impairment in utilizing path integration for facilitating spatial memory performance. We believe the latter, rather than the former, is the more likely interpretation for two reasons. First, the local cue hypothesis seems unlikely because retrosplenial cortex lesions impair water maze performance, a task explicitly designed to have local cues provide little information about current location in an environment [9,18]. Furthermore, retrosplenial head direction cells are modulated by the movement state of the animal, or in some cases visual cues [5]. Thus far head direction coding based on local maze cues has not been identified. Therefore, it appears more likely that retrosplenial cortex may

be involved in memory-guided visual and self-motion integration necessary for accurate navigation when path integration strategies dominate.

Recent work has argued that hippocampus functions as a path integrator [1,3,19]. Animals with lesions of the fornix are unable to make direct return paths home after venturing out to a food source [20]. To effectively path integrate, one must keep track of directional heading (and changes in heading) along with the amount of time traveled after leaving a discrete starting point [21]. Input from retrosplenial cortex may provide experience-dependent head direction information to hippocampus. Temporal information may be derived from hippocampal theta, allowing for computation of current location in the absence of visual input. Indeed, a computational model of hippocampus argues that the architecture of CA3 may allow for such forms of path integration [3]. Importantly, the locations of the retrosplenial cortex injection sites are the same as the site of origin of direct projections to the septal pole of the CA3 subregion of hippocampus proper [22]. In addition to these hippocampal projections, there are indirect projections from retrosplenial cortex to the hippocampal formation via pre-, para- and postsubicular cortices [8]. Therefore, retrosplenial cortex may provide hippocampus (directly or indirectly) with directional information for use in determining current location based on time spent travelling from a discrete starting location. Supporting this hypothesis is the recent finding of head direction cells in the septal pole of hippocampus [23].

Conclusions

The current data argue strongly for a selective role of retrosplenial cortex (and possibly the adjoining posterior parietal cortex) in path integration. Specifically, we hypothesize that retrosplenial cortex provides mnemonic information about visual and nonvisual associations for use in correcting for cumulative errors that occur during path integration. The current data support this hypothesis. The location of the injection sites corresponds with known direct projections from retrosplenial cortex to CA3 of hippocampus. Therefore, it is possible that path integration, as involved in spatial memory performance, is supported in part by retrosplenial cortex input to hippocampus.

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