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Contributions of Cingulate Cortex to Two Forms of Spatial Learning and Memory

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The contribution of anterior and posterior cingulate cortical areas to spatial learning and memory was examined in 4 experiments using the place-navigation task. Rats with complete bilateral cingulate cortex aspiration or aspiration of posterior cingulate cortex (area 29) alone could not swim directly to a hidden platform located in a fixed place. When animals with these lesions were tested for 40 d in a place-alternation task in which they received 16 daily trials with the platform placed in a new location each day, they did not show reliable improvement in place navigation. The inability to swim to changing locations or to a single location was not overcome by preoperative training in these tasks. Rats with anterior cingulate cortex aspirations showed a less severe impairment in both tasks and, with more training than is necessary for control rats, they acquired near-normal place-navigation accuracy. Rats with complete cingulate cortex aspiration were almost as accurate as control rats in learning to swim to a visible platform.

The results imply that posterior cingulate areas play an essential role in the use of topographical information, probably by transmitting and elaborating information passing between the hippocampal system and neocortical association areas.

Cingulate cortical areas occupy a pivotal position in the mammalian forebrain, conveying information in both directions between neocortical and limbic structures. As a result of recent work using anatomical tracing techniques, we now know a great deal about the afferent and efferent connections of cingulate cortex in rodents and primates. In addition to having identified the origin and termination of cingulate connections, these studies have clearly shown that anterior (area 24) and posterior (area 29) cingulate cortex receive very different inputs and project to different target structures (Vogt, 1983). In the rat, anterior cingulate cortex receives thalamic input primarily from anteromedial and mediodorsal nuclei, but posterior cingulate cortex receives connections predominantly from anteroventral, anterodorsal, lateroposterior, and laterodorsal nuclei. These 2 cingulate regions differ in their neocortical inputs, with posterior areas receiving more extensive connections from visual areas 18b, 18a, and 17. There are also major differences between anterior and posterior cingulate areas in their connections with parahippocampal cortices. Cells throughout subicular cortices project to posterior, but not anterior, cingulate areas. Similarly, posterior cingulate cortex has a more extensive output to these areas than does anterior cingulate cortex (Domesick, 1972; Van Hoesen et al., 1975; Seltzer and Pandya, 1976; Meibach and Siegel, 1977; Rosene and Van Hoesen, 1977; Swanson and Cowan, 1977; Vogt et al., 1979; Vogt and Miller, 1983; Finch et al., 1984a, b).

The preponderance of work on the functional significance of cingulate areas has been concerned with their role in "emotional reactivity" or responses to noxious stimuli (Papez, 1937; Kaada, 1951; Pribram and Fulton, 1954; Peretz, 1960; Thomas and Slotnick, 1963; Lubar, 1964; Lubar and Perachio, 1965; McCleary, 1966; Trafton, 1967; Kimble and Gostnell, 1968; Thomas et al., 1968; White and Sweet, 1969; Talairach et al., 1973; Sutton et al., 1974; Woodruff et al., 1981; Buchanan and Powell, 1982; Jurgens, 1983). Deficits have also been described in food hoarding, nest building, sexual behavior, delayed response tasks, and spatial reversal tasks (Shipley and Kolb, 1977; Kolb, 1984). All of these relationships to behavior are seen more prominently after anterior cingulate manipulations than after manipulations involving posterior cingulate cortex.

Using a multiple-unit recording technique, neuronal activity has been recorded in anterior and posterior cingulate cortex during discriminative active avoidance learning in rabbits. Cells in both cingulate areas begin to preferentially discharge during the positive conditioned stimulus early in training, before acquisition of the behavioral avoidance response, and the discriminative neuronal activity develops earlier in anterior than in posterior cingulate cortex; see Gabriel et al., (1980) and Orona and Gabriel (1983a, b). These authors suggest that the activity of cingulate cortex is important in the associative process whereby the neuronal representation of environmental events acquires motivational significance. They further suggest that this process initially involves a flow of information from anterior to posterior cingulate areas.

A key feature of cingulate connectivity, particularly in posterior cingulate areas, is the extensive reciprocal connections with posterior association neocortex, on the one hand, and with parahippocampal cortices, on the other. It has been firmly established in rats and monkeys that the hippocampal system is critical for normal usage of topographical or spatial memories (O'Keefe and Nadel, 1978; Sutherland et al., 1980, 1982, 1983; Morris et al., 1982; Parkinson and Mishkin, 1982; Sutherland, 1985) and the evidence is clear, at least in primates, that those posterior neocortical association zones that are reciprocally connected with posterior cingulate cortex are also essential for normal usage of topographical representations and memories (Pohl,

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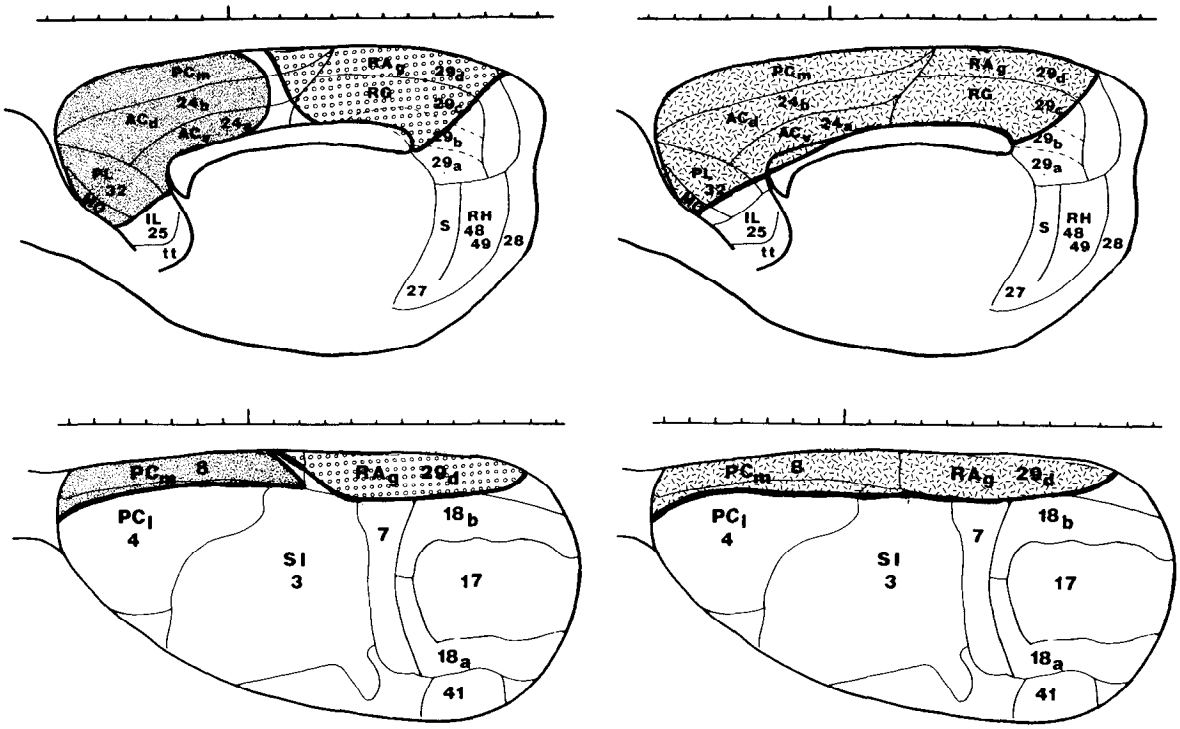

Figure 1. Dorsal and sagittal views of the extent of anterior or posterior (*on left side*) and complete (*on right side*) cingulate damage in representative rats.

1973; De Renzi, 1982; Pandya and Yeterian, 1984). Pandya and Yeterian (1984) have proposed a central role for cingulate cortex in several aspects of spatial memory in primates. According to them, cingulate cortex, particularly the posterior part, is involved, albeit in concert with posterior neocortical association zones, in analyzing the significance of objects within a topographical representation, in passing on this topographical representation to the hippocampal system for memory formation, and in controlling the execution of movements in relation to topographical representations of the environment via projections to frontal cortex.

The hypothesis that cingulate cortical areas participate in an important way in the circuitry underlying spatial learning and memory is clearly consistent with our current understanding of their neuroanatomical connections, but comparable supporting evidence from behavioral studies is lacking. In the present study we specifically examine spatial learning and memory skills in rats with anterior cingulate (area 24), posterior cingulate (area 29), or complete cingulate lesions using several versions of the place-navigation task of Morris (1981). This task requires that rats swim from several directions to a small platform in a large, circular pool of cool water. In different conditions, the platform is clearly visible above the surface of the water or completely invisible (i.e., slightly submerged). Normal rats learn very rapidly to swim directly to the platform in both conditions from any direction. Thus, it is possible to determine how readily rats can learn to approach a visual cue in order to escape from cool water (visible platform condition) and how readily they can learn to swim to a goal using the topographical relationships among cues outside the pool (invisible platform condition). We also examined performance in a situation in which the invisible platform was moved to a new location each day (place alternation). After training in the latter condition, normal rats learn the new platform location on the first trial of each day, and in all subsequent trials that day they navigate almost directly to it (Whishaw, 1985a, b). In addition, we evaluated the effects of cingulate cortex damage in these tasks in rats that were trained preoperatively.

Materials and Methods

Subjects. The subjects were male hooded rats of the Long-Evans strain (300–350 gm at the start of the study) obtained from the University of Lethbridge vivarium. They were housed in pairs with continuous access to food and water in a room illuminated on a 12:12 hr light:dark cycle and were tested during the light phase of the cycle.

Surgical procedure. The midline cortex of the rat includes 10 distinct cytoarchitectonic zones, including the infralimbic, dorsal and ventral anterior cingulate, prelimbic, medial orbital, medial precentral (all defined by Krettek and Price, 1977), and areas 29a, 29b, 29c, and 29d (see Fig. 1). In the complete cingulate lesion group (combined lesions), the intended removal included all of the zones (Fig. 2). For the other 2 groups, the intended lesions either included all of area 29 (posterior cingulate) or included all midline cortex anterior to area 29 (anterior cingulate). The approximate line of demarcation between the anterior and posterior cingulate areas was taken to be 2 mm posterior to the bregmoidal intersection.

The rats were anesthetized with sodium pentobarbital (65 mg/kg). The cortex was exposed by removing a long piece of skull on either side of the midline, such that a strip of bone approximately 2 mm wide remained over the sagittal sinus. The dura was incised with a no. 11 scalpel blade and the cortex was aspirated using gentle suction with the aid of a surgical microscope. Following hemostasis, the skin was sutured closed. Control animals were anesthetized and the skin incised and sutured.

Histological procedure. At the completion of the behavioral experiments, the rats were anesthetized and perfused intracardially with 0.9% saline, followed by 10% formol saline. The brains were weighed and placed in 30% sucrose formalin for at least 48 hr, then cut frozen at 40 μm. Every tenth section was mounted and stained with cresyl violet.

Behavioral apparatus. The Morris water task was employed in all experiments. A circular pool (diameter, 1.7 m; height, 0.6 m) was used.

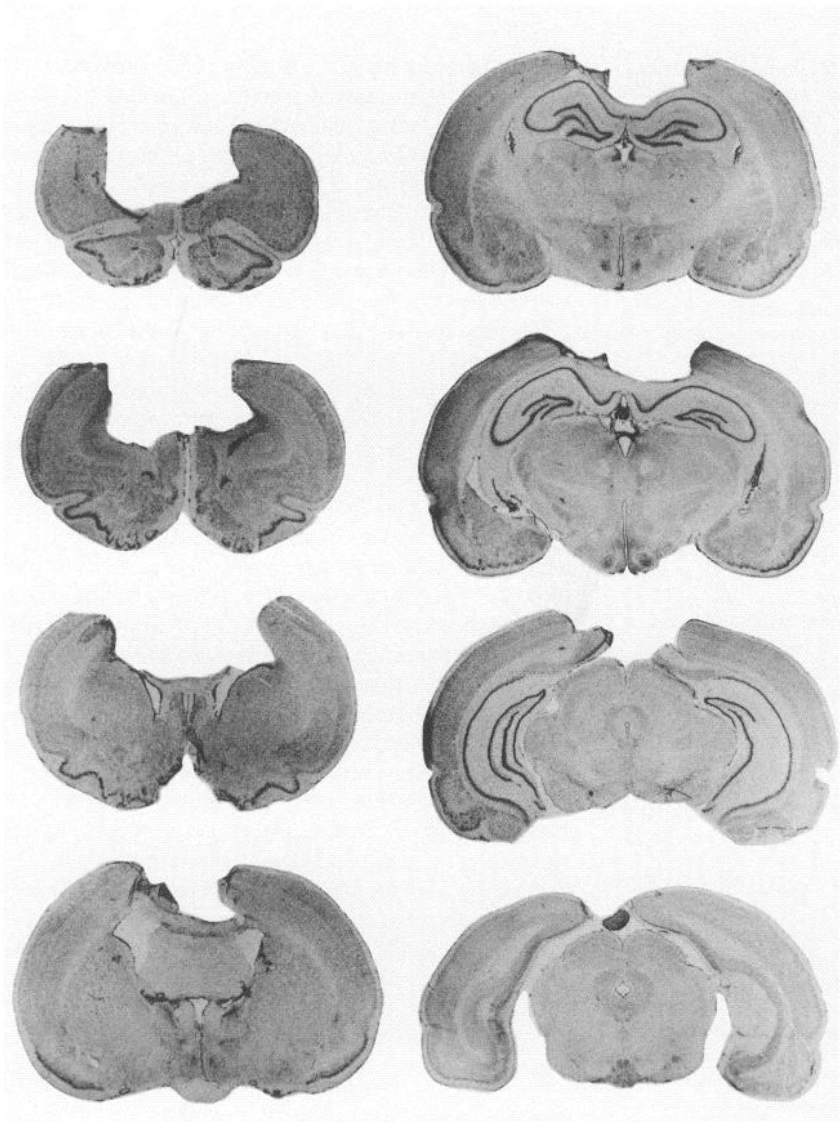

Figure 2. Coronal sections from a representative rat with complete cingulate cortex aspiration.

The inner surfaces were smooth and uniformly white. The pool was filled to within 20 cm of the top with cool water (18°C) that was rendered opaque by the addition of 1500 cm^3 of instant powdered skim milk. An escape platform (13 × 13 cm) was positioned at various locations in the pool. The platform was constructed of clear plastic and its top surface was 1.5 cm below the surface of the water. Thus, there were no intramaze cues that could be used to locate the platform.

Experiment 1: postlesion acquisition of place navigation. Thirty rats were randomly assigned to the following 4 groups: Control ($n = 6$); anterior cingulate aspiration ($n = 9$); posterior cingulate aspiration ($n = 9$); and hippocampal lesions ($n = 6$). The latter lesions were made using multiple microinjections of colchicine (3 sites in each hippocampus, 2 $\mu g/0.5\ \mu l$ physiological saline per site), which cause widespread degeneration of granule cells throughout the dentate gyrus, according to procedures previously described (Sutherland et al., 1983; Sutherland, 1985; Whishaw, 1987). Two weeks after surgery, training began in the invisible platform condition of the Morris water task. Briefly, the hidden platform was always located in the center of the southeast quadrant of the pool. On a particular trial, a rat was released facing the wall of the pool from one of 4 starting locations (north, south, east, or west), according to a pseudorandom sequence. Within each block of 4 trials, each rat was exposed to each of the starting locations once; there were 8 trials/d. On every trial in which the platform was successfully located, the rat was allowed to remain on the platform for 10 sec; if the platform was not located after 90 sec, the trial was terminated and the rat was lifted out of the water by an experimenter. Testing continued for 10 d, and on the last block of 4 trials, the platform was repositioned in the center of the northwest quadrant. On every trial the latency to find the platform and the distance traveled were measured and, in addition, during the block of trials when the platform was repositioned, the percentage of the distance traveled within each of the 4 quadrants of the pool was measured.

Experiment 2: place-alternation task. The place-alternation task was similar to that described previously (Whishaw, 1985a, b, 1987). Four different platform locations were used, and the platform was moved each day to one of these locations according to a designated sequence. Location 1 was in the center of the southeast quadrant of the pool; location 2 was in the center of the southwest quadrant; location 3 was in the center of the pool; and location 4 was about 8 cm away from the wall between the northeast and northwest quadrants of the pool (it was slightly closer to the wall of the pool than were locations 1 and 2).

These platform positions were chosen to frustrate a number of nonplace learning strategies that normal rats may adopt. A rat may attempt to locate the platform by swimming in a circular path around the pool: if this strategy is adopted, a platform located at position 3, in the pool's center, will not be found. A rat may turn away from the wall and swim at a given angle: this strategy will not help it reach platform location 4, which is immediately adjacent to a start position and located slightly closer to the wall than are locations 1 and 2, and which requires that the rat swim toward the center of the pool to locate it. A rat may concentrate swimming in one quadrant or half of the pool: the asymmetric locations of the platforms will limit the utility of this strategy. At the beginning of a trial, a rat is gently placed into the water facing and touching the wall of the pool at one of the 4 cardinal compass points.

Testing was conducted on consecutive days, with each rat receiving

16 trials on each day. If, on a particular trial, a rat found the platform, it was permitted to remain there for 10 sec. A trial was terminated after 120 sec if a rat failed to find the platform; the rat was removed from the water by hand and returned to its cage. Trials were given in pairs. The second trial of each pair was given immediately after the 10 sec stay on the platform, and the same starting location was used. At the end of the second of each pair of trials, the rat was returned to a holding cage and approximately 5–8 min elapsed (during this interval, the remaining rats were tested) before the next pair of trials from a new starting location was initiated. Trial pairs were given so that the rats started from each of the 4 locations on each of the first and second 4 pairs of trials. The sequence in which starting positions were used was randomly generated.

The swim path was drawn on a map of the pool as the rat completed each trial. The latency to find the platform was recorded for every trial. An additional error measure was used to evaluate navigational accuracy. For this error measure, an 18-cm-wide path from the start point to the platform was designated the correct route, so that if a rat deviated from this route at any point, it received an error on that trial.

Experiment 2a: postlesion acquisition of place alternation. Twenty-three rats were randomly divided into the following 4 groups: Control ($n = 6$); anterior cingulate aspiration ($n = 6$); posterior cingulate aspiration ($n = 5$); and complete cingulate cortex aspiration ($n = 6$). Two weeks following cortical aspirations, testing began in the place-alteration task and continued for 40 consecutive d.

Experiment 2b: transfer to single-place navigation. After the fortieth day of testing on the place-alternation task, the same rats were given 11 additional d of training on a place task (similar to Experiment 1) in which the platform was always located in the center of the southwest quadrant of the pool. Each rat received 8 training trials (2 blocks of 4 trials) each day for 10 d, with 5–8 min between trials. On day 11, the platform remained in the southwest quadrant of the pool for the first 4 trials (trial block 21), but for the next 4 trials, the platform was moved to the center of the northeast quadrant of the pool.

Experiment 3: postlesion retention of place alternation. Thirteen rats were used. All received prelesion training in the place-alternation task for 10 d. The rats were then randomly assigned to 3 groups. One group ($n = 4$) received anterior cingulate aspiration, one ($n = 4$) received posterior cingulate aspiration, and the third group ($n = 5$) received anesthesia only. Seven days following surgery, the rats were returned to the place-alternation task and received a further 48 consecutive d of testing.

Experiment 4: postlesion acquisition of navigation to a visible cue. Twelve rats were trained in the single-place navigation task described above for 6 d (8 trials/d). They were then randomly assigned to one of 2 groups: Control ($n = 6$) and complete cingulate aspiration ($n = 6$). The rats of the control group were anesthetized and the skin on their heads was incised and sutured. Seven days after surgery, testing began again and continued for 6 consecutive d. On the first 2 d (8 trials/d), the rats were tested for retention of single-place navigation. On the next 4 d, the procedures were identical, except that a solid black platform that protruded 5 cm above the surface of the water was used in place of the hidden platform.

In addition to recording the latency to find the platform on every trial, we calculated the percentage of the swimming distance in each of the 4 quadrants of the pool, as well as a measure of accuracy of the initial segment of the swim trajectory (heading deviation). This latter measure has previously been described (Sutherland et al., 1983; Sutherland and Dyck, 1984). Briefly, an imaginary line is drawn from the starting location on each trial through the rat's position after it has traveled 16 cm, and the deviation of this line from a second imaginary line from the starting location through the center of the platform is measured in degrees.

Results

Experiment 1: postlesion acquisition of place navigation

The control rats showed a rapid decrease in latency to find the platform, such that by the fourth block of trials, they were performing at an asymptotic level of accuracy (Fig. 3). When the platform was repositioned on the last block of trials, the control rats showed marked increases in latency to find the platform and in distance traveled, and concentrated most of their swimming within the quadrant of the pool that had previously contained the hidden platform. Relative to the control rats, the rats of all 3 lesion groups were impaired. Throughout all of the blocks of trials of training, each of the lesion groups was slower than the controls in finding the platform. Despite their impairment relative to controls, all 3 lesion groups showed a decline in latency across training. The posterior cingulate- and hippocampal-damaged groups were very similar to each other during training, and both of these groups had longer overall latencies to find the platform than the anterior cingulate group. It is important to note that swimming speeds were similar for all groups and that the longer latencies shown by the lesion groups cannot be attributed to a difference in swimming speed, since the trend was for lesion rats to swim more quickly. When the platform was repositioned, both cingulate-damaged groups showed increased latencies to find the platform, whereas the hippocampal-damaged group was unaffected.

Statistical analyses confirm these observations. In the overall analysis of variance, there were significant Group, Trial, and Group × Trial interaction effects (all p's < 0.01). Followup tests using Fisher's LSD method (Loftus and Loftus, 1982) ($p < 0.05$) showed that throughout training each of the 3 lesion groups had longer latencies than that of the control group, that the posterior cingulate and hippocampal groups did not differ from each other, and that by the completion of training the anterior cingulate group had significantly shorter latencies than either of the other 2 lesion groups. The control, anterior cingulate, posterior cingulate, but not the hippocampal, groups all showed statistically significant increases in latency between the last block of trials of training and the block of trials for which the platform had been repositioned.

In order to rule out the possibility that the longer latencies might reflect a simple motor deficit affecting swimming, we compared the average swimming speed (swim distance/latency) for each group of rats. There was no significant difference among the groups ($F < 1.0$).

Experiment 2a: place-alternation task

All 3 cingulate lesion groups were impaired with respect to the control group on both the latency and error measures. A summary of mean daily latency and mean daily errors is presented in Figure 4. As illustrated, the control group reached asymptotic performance on both measures after 5 d of training. The cingulate lesion groups had longer latencies and made more errors on initial acquisition. They required more trials to reach asymptotic performance, and their asymptotic performance was inferior to that of control rats. The impairments produced by the posterior cingulate lesions were greater than those produced by the anterior cingulate lesions, but both of these groups showed superior performance compared with the group with complete cingulate lesions. In fact, whereas the former groups eventually learned to swim to the platform relatively quickly, the group with complete cingulate lesions seemed to reach the platform only by chance. A final feature of the performance of the rats with cingulate lesions, particularly the groups with anterior or posterior cingulate lesions alone, was the variability that occurred on some tests. Most sharp increases in latency and error scores, illustrated in Figure 4, occurred on days when the platform was in the center of the pool. Typically, the rats adopted a circling strategy of swimming around the pool in a wide circle away from the wall of the pool. By adopting this strategy rats could relatively efficiently reach the platform when it was located in the other 3 positions, but it did not help them reach the

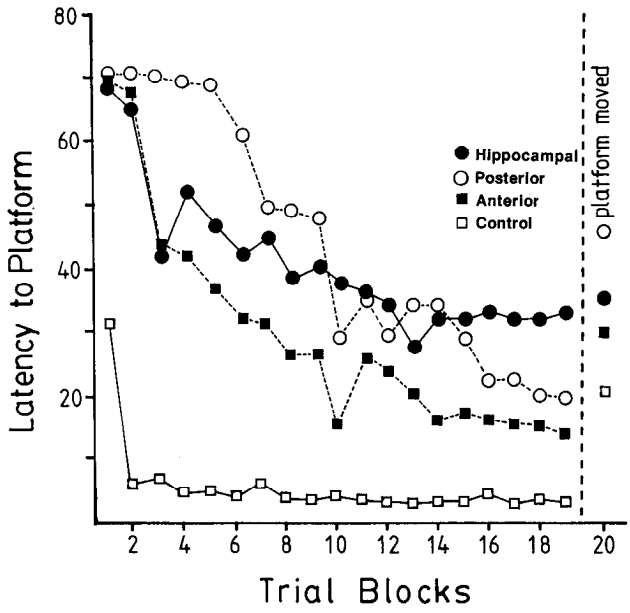

Figure 3. Mean latency (sec) per trial to find the hidden platform on each block of 4 trials for each group in Experiment 1. For trial block 20, the hidden platform was repositioned in the center of the diagonally opposite quadrant.

platform when it was located in the center of the pool. Figure 5 depicts examples of swim paths of a rat from each group during the last test day, with the platform in the center of the pool.

Analysis of variance and Neuman-Keuls followup tests supported these conclusions. Analysis of latency showed that there was a significant Group effect ($F(3,19) = 9.74$, $p < 0.001$), a significant effect of Training ($F(39,741) = 2.95$, $p < 0.001$), as well as a significant Group × Training interaction ($F(117,741) = 2.95$, $p < 0.001$). The followup tests showed that the control group was superior to the other groups, and also showed that the differences between the other groups were statistically significant. Analysis of error scores revealed that there was a significant Group effect ($F(3,19) = 38.92$, $p < 0.001$), a significant effect of Training ($F(39,741) = 2.27$, $p < 0.001$), and a significant Group × Training interaction ($F(117,741) = 2.27$, $p < 0.001$). The followup tests showed that the control group was superior to other groups, and also showed that the anterior cingulate group was superior to the posterior cingulate and complete cingulate groups, which did not differ from each other.

Examination of latencies and swim patterns of the rats indicated that the control and anterior cingulate groups showed marked improvements in latency and error scores between the first and second trials of each trial pair, particularly the first trial pair. The percentage improvement in latency and error score between the first and second trials of the first trial pair of each day across the 40 test days was calculated. This percentage improvement is illustrated in Figure 6. Overall analysis of variance on latency and error measures indicated significant ($p < 0.001$) Group, Trials, and Group × Trials effects. Followup analyses of the individual groups were therefore performed. For the control group there was a significant Trials effect ($F(1,10) = 104.4$, $p < 0.001$), and a significant Training effect ($F(39,390) = 14.4$, $p < 0.001$), but no significant Trials × Training interaction. Similarly, for the anterior cingulate group there was as significant Trials effect ($F(1,10) = 15.52$, $p < 0.001$), a significant Training effect ($F(39,390) = 9.6$, $p < 0.001$), and no significant Trials ×

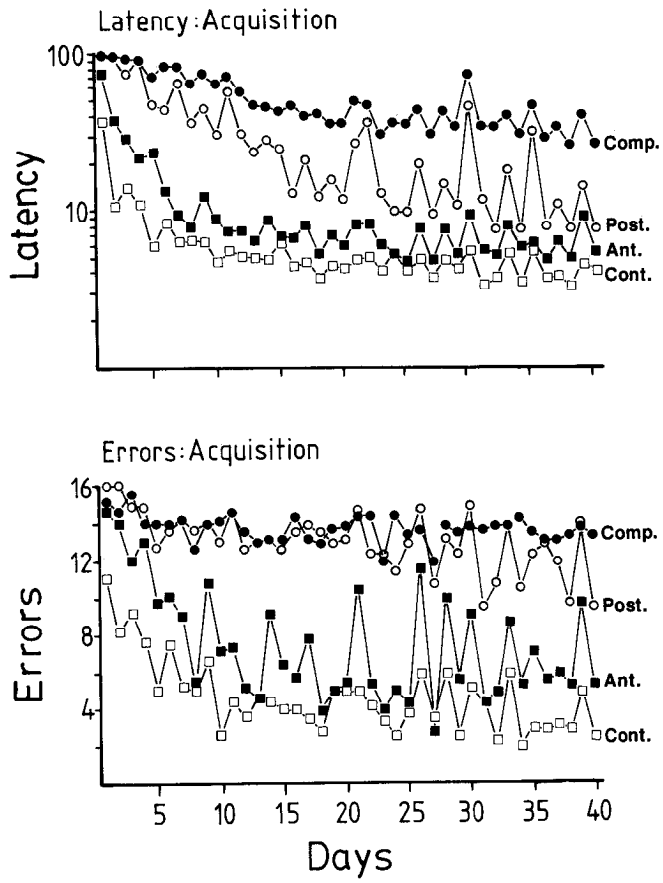

Figure 4. Mean latency (*upper panel*) per trial and errors (*lower panel*) per day for the anterior cingulate (*Ant.*), posterior cingulate (*Post.*), complete cingulate (*Comp.*), and control (*Cont.*) groups in Experiment 2a.

Training interaction. Thus, for both groups the second trial improvement in performance was present throughout testing, even as performance improved. For the posterior cingulate and complete cingulate groups, there were no significant differences between first and second trial performances. Analysis of errors by the rats of the control group revealed a significant Trials effect ($F(1,10) = 25$, $p < 0.001$), but no significant Training effect or Trials × Training interaction. Analysis of the errors made by rats of the anterior cingulate group revealed a significant Trials effect ($F(1,10) = 17.8$, $p < 0.001$), a significant Training effect ($F(39,390) = 2.97$, $p < 0.001$), as well as a significant Trials × Training interaction ($F(39,390) = 1.74$, $p < 0.01$). Thus, for the control group, the first to second trial improvement was present throughout testing, whereas inspection of the performance of the anterior cingulate group shows that the first to second trial improvement did not begin to emerge until after about 16 d of training. No significant first to second trial improvements in error scores were obtained from the posterior cingulate or complete cingulate lesion groups.

The trial-to-trial improvement across the 16 daily trials was analyzed over the last 8 d of testing, at which time the rats in all groups had reached asymptotic performance. The mean trial × trial latency and error scores for these last 8 d are illustrated in Figure 7. The overall analysis of variance of latency showed that there was a significant Group effect ($F(3,19) = 4.44$, $p < 0.001$), a significant Trials effect ($F(15,285) = 7.42$, $p < 0.001$), and a significant Group × Trials interaction ($F(45,285) = 1.46$,

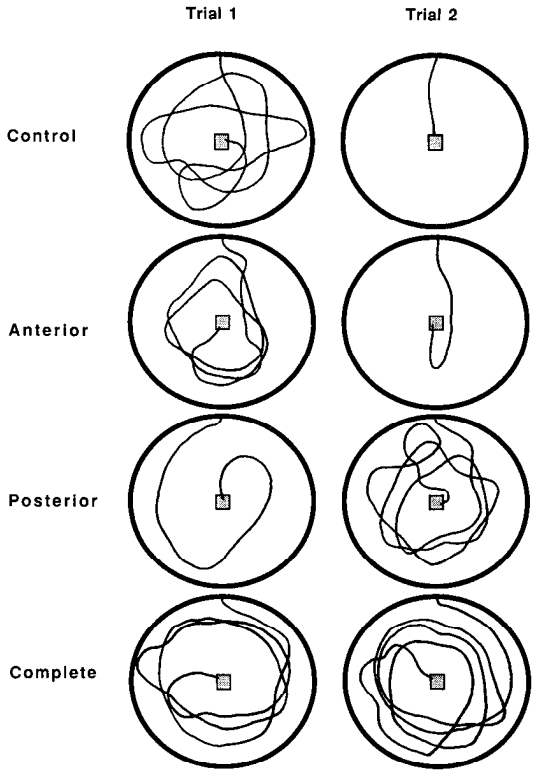

Figure 5. Swim paths from the last day of testing with the hidden platform in the center location for the median rat of each group.

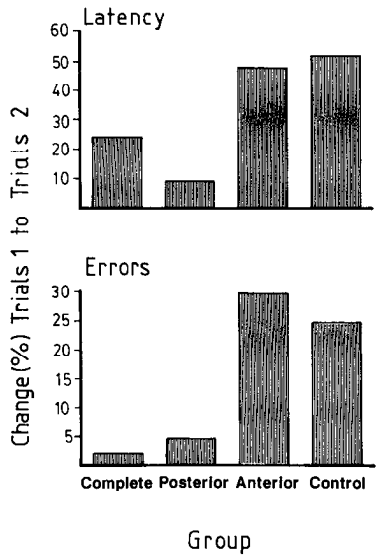

Figure 6. Percentage improvement in mean latency to find the platform (*top*) and in mean number of errors (*bottom*) from trial 1 to trial 2, averaged across each day of testing in Experiment 2a.

$p < 0.05$). The analysis of errors showed a significant Group effect ($F(3,19) = 18.73$, $p < 0.001$), a significant Trials effect ($F(15,285) = 9.72$, $p < 0.001$), and a significant Group × Trials interaction ($F(45,270) = 7.42$, $p < 0.001$). The most interesting feature of the latency measures was the conspicuous decrease in latency between the first and second trials displayed by the control and anterior cingulate groups. This decrease was statistically significant (t tests; $p < 0.05$) and demonstrates that rats in both groups reached asymptotic latency levels in one trial. A similar rapid improvement was not seen in the posterior cingulate group. The changes in latency displayed by the rats in the complete cingulate group were interesting because the rats did not show an improvement in performance across trial pairs even though there was a tendency for their latency to decrease on the second trial of each trial pair. However, this decrease was not statistically significant.

The control and anterior cingulate groups displayed a decline in error scores across trial pairs, but this decline was more gradual than that obtained for latency. The posterior cingulate and complete cingulate groups showed no statistically significant changes in errors across trials.

Experiment 2b: single-place location task

After the fortieth day of testing in the place-alternation task, the rats were trained with the platform in only one location for all trials. Training continued for 21 blocks of 4 trials, for 11 d, with 2 blocks of trials each day. On the twenty-second trial block, the platform was moved to a new location. The mean latency and error scores for all 22 trial blocks are shown in Figure 8. It is noteworthy that even though the place task was simplified in this way, there were still marked differences in performance. In terms of mean latency and errors, the groups differed as follows: control < anterior cingulate < posterior cingulate < complete cingulate. Notwithstanding these mean performance differences, all groups except the complete cingulate cortex group showed increases in latency and errors during the block of trials for which the platform was repositioned. Thus, except for the complete cingulate group, all groups had acquired information about the location of the platform relative to the distal cues by the end of training.

The statistical analysis of latency and errors during training and when the platform was repositioned supports these conclusions. For the latency to find the platform, there was a significant Group effect ($F(3,19) = 6.99$, $p < 0.001$), a significant Trials effect ($F(20,380) = 3.77$, $p < 0.001$), and a significant Group × Trial interaction ($F(20,380) = 2.0$, $p < 0.001$). Followup Neuman-Keuls tests showed that the overall differences among all groups were significant. Student's t tests for correlated samples indicated that the latency increases that occurred when the platform was repositioned were statistically significant ($p < 0.05$) for the control, anterior cingulate, and posterior cingulate groups, but not for the complete cingulate group. For the error measure, there was a significant Group effect ($F(1,19) = 16.86$, $p < 0.001$), a significant Trials effect ($F(10,190) = 4.84$, $p < 0.001$), but no significant Group × Trials interaction. The followup tests showed that the differences among all groups were statistically significant, and, with the exception of the complete cingulate group, the increases in error scores when the platform was relocated were statistically significant ($p < 0.05$).

Experiment 3: place-alternation retention task

A summary of the latency to find the platform in the place-alternation retention task is shown in Figure 9. The control rats maintained asymptotic performance when returned to the task, whereas the anterior cingulate group displayed a slight increase in latency. Nevertheless, the accuracy of the anterior cingulate group, although poorer than that of the control group, was maintained at a high level and reached asymptote as early as the first 2 d of retention testing. On the other hand, the posterior cin-

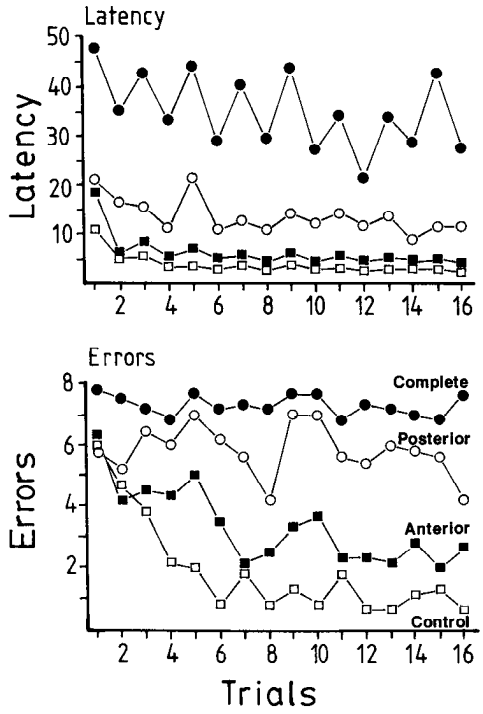

Figure 7. Mean latency to find the platform (*top*) and mean number of errors (*bottom*) on each of the 16 daily trials, averaged across the last 8 d of testing in Experiment 2a.

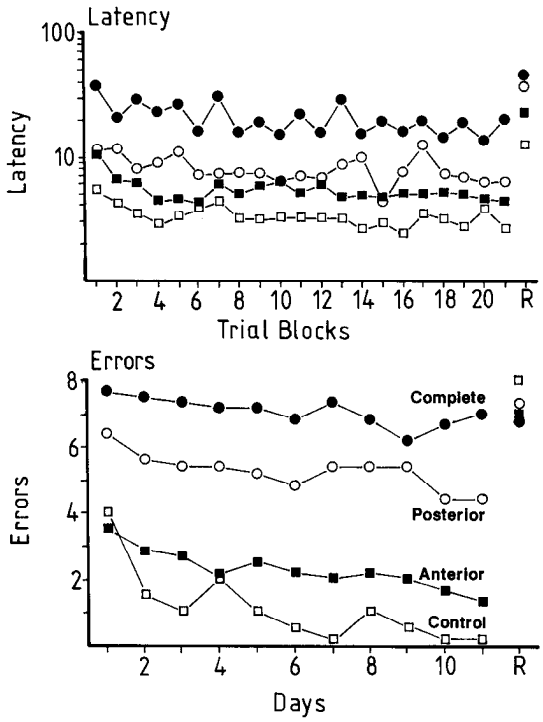

Figure 8. Mean latency to find the hidden platform in a fixed location (*top*) and mean number of errors (*bottom*) per trial for the groups in Experiment 2b.

gulate group was profoundly impaired relative to the anterior cingulate and control groups when returned to the task, and it required about 7 d of training to reach asymptotic performance. The analysis of variance for the latency results showed that there was a significant Group effect ($F(2,10) = 53.20$, $p < 0.001$), a significant Training effect ($F(47,470) = 15.73$, $p < 0.001$), and a significant Group × Training interaction ($F(94,470) = 13.53$, $p < 0.001$). The analysis of the error measure also gave a significant Group effect ($F(2,10) = 65.61$, $p < 0.001$), a significant Training effect ($F(47,470) = 6.88$, $p < 0.001$), and a significant Group × Training interaction ($F(94,470) = 2.63$, $p < 0.001$). The mean number of errors on each trial was 2.9 for controls, 4.5 for anterior cingulate-damaged rats, and 10.0 for posterior cingulate-damaged rats. Thus, the experiment showed that the control and anterior cingulate groups displayed marked savings on the retention test, with anterior cingulate cortex rats displaying a performance deficit about equal to that observed after asymptotic performance on the acquisition test (see Fig. 4). Despite their poor retention, the posterior cingulate cortex rats did appear to show some savings because they reached asymptotic performance slightly more quickly than did the posterior cingulate cortex rats previously tested on acquisition (see Fig. 4).

Experiment 4: postlesion acquisition of navigation to a visible cue

Figure 10 depicts the average latency for both groups on the last 2 d of training, on the 2 d of single-place navigation retention testing, and on the 4 d of acquisition of navigation to the visible cue. Before surgery, both groups had attained a similar level of performance in single-place navigation. After surgery, however, the control group showed good retention of single-place navigation, but the complete cingulate lesion group was markedly impaired—they appeared to swim more or less randomly around the pool during the first 2 d of postsurgical testing. After the introduction of the visible platform, both groups were navigating at a similar level of accuracy. The overall analysis of variance on latency confirmed these impressions. There was a significant main effect of Group ($F(1,10) = 10.7$, $p < 0.008$), Trial Block ($F(23,230) = 30.2$, $p < 0.001$), and a significant interaction between Group and Trial Block ($F(23,230) = 4.3$, $p < 0.001$. Followup tests using Fisher's LSD method, with all p's < 0.05, revealed that the 2 groups did not differ from each other before surgery or during acquisition of navigation to the visible cue, but that the complete cingulate group was significantly slower across the 4 blocks of trials of single-place navigation retention testing.

The analysis of variance on heading deviation, averaged across all 8 trials of each day, revealed a significant Group effect ($F(1,10) = 6.4$, $p < 0.03$), and Day effect ($F(11,110) = 6.7$, $p < 0.001$). Fisher's LSD tests ($p < 0.05$) indicated that only on the days of single-place navigation retention testing and on the first day of acquisition of swimming to the visible cue were the complete cingulate rats significantly worse than the controls (42.3° vs 21.8° heading deviation, on average, across the 2 d of single-place navigation retention testing).

The analysis of variance on percentage of swimming distance in the quadrant that contained the platform indicated a significant Group effect ($F(1,10) = 7.6$, $p < 0.02$), a significant Day effect ($F(11,110) = 5.2$, $p < 0.001$), and a significant interaction between Group and Days ($F(11,110) = 2.7$, $p < 0.004$). The followup tests, using Fisher's LSD method, revealed that only on the 2 d of testing for retention of single-place navigation was the percentage of swimming in the correct quadrant significantly

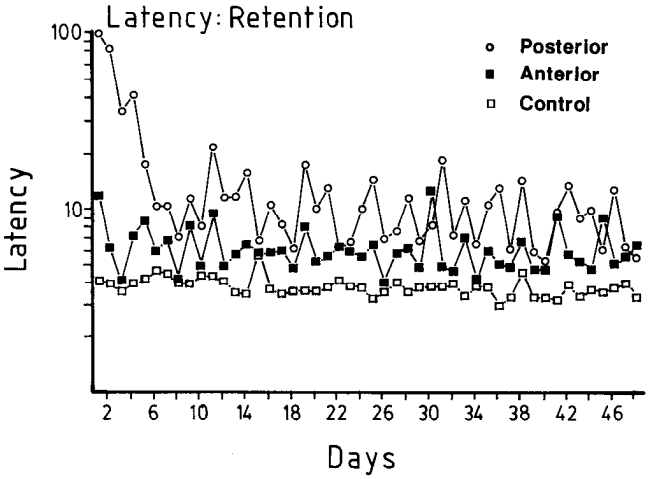

Figure 9. Mean latency to find the hidden platform per trial by rats preoperatively trained in place-alternation task (Experiment 3).

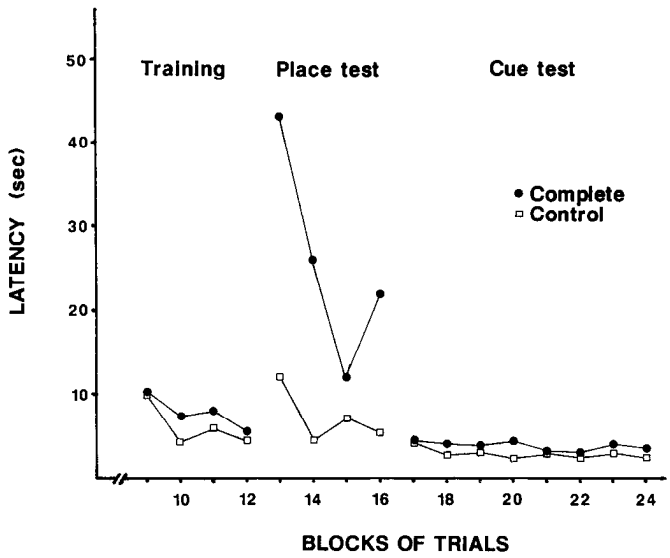

Figure 10. Mean latency to find the hidden platform per trial before (blocks 9–12) and after (blocks 13–16) surgery in Experiment 4. A black, visible platform was used for blocks 17–24.

lower for the complete cingulate rats than for the control rats (31 vs 48%).

Anatomical results

Analysis of the brain weights showed that the lesions had a rather small effect on overall brain weight, producing a loss of about 5% relative to control values (mean weights: control, 2.2481 gm; anterior cingulate, 2.1308 gm; posterior cingulate, 2.1525 gm; complete cingulate, 2.1245 gm). There were no statistically significant differences among the lesion groups.

Each brain was inspected for damage to each of the 10 cytoarchitectonic divisions of the midline cortex (see Figs. 1, 2). On the whole, the lesions were slightly smaller than intended. All rats in the anterior cingulate groups had complete removal of the dorsal anterior cingulate and medial precentral cortex, and all but one had the prelimbic zone removed. The infralimbic and medial orbital zones were largely intact in all rats, and only 2 rats had significant removal of the ventral anterior cingulate area. Rats with posterior cingulate lesions all had complete removal of areas 29a, 29b, and 29c, but the posterior part of area 29d was spared in all rats. There was slight damage to the very posterior part of the anterior cingulate cortex in all rats. Three rats had damage to the posterior corpus callosum. The complete lesions were effectively identical to the 2 partial groups combined, the one difference being that the ventral anterior cingulate zone was removed in all rats. There was virtually no retrograde degeneration in the anterior cingulate group. The rats with posterior or complete cingulate lesions all had degeneration in the dorsal and ventral anterior nuclei, as well as a restricted area of degeneration in the dorsal and posterior lateral nuclei, largely along the border with the posterior nucleus. No rat had damage to either the striatum, subiculum, hippocampal formation, or visual cortex.

Discussion

These results provide the first clear behavioral evidence that cingulate cortical areas make an important contribution to the control of spatially guided behavior. Rats with bilateral damage to cingulate cortex could not readily learn to swim to a place in space using distal cues; yet, in the same situation, rats with complete cingulate cortex removal had virtually no difficulty in learning to swim directly to a visual landmark. The latter finding indicates that the impairment in place navigation produced by cingulate damage is probably attributable to a difficulty in making appropriate use of the topographical relationship among distal cues, rather than to changes in aversive motivation, locomotor control, or other behavioral capacities necessary for normal performance in our tasks.

Even after extensive training (640 trials) in the place-alternation condition, when the rats were switched to the single-place navigation condition (Experiment 2b), the rats with anterior, posterior, or complete cingulate damage took longer to find the hidden platform and made more errors in trajectory than did control rats throughout testing. The cingulate deficit in place navigation is not due simply to a deficit in *learning* the appropriate spatial relationships in the task, since rats that were well trained preoperatively did not show accurate place navigation when retested in the same task 1 week after complete cingulate aspiration (Experiment 4). Nor can the deficit be attributed to a general defect in associative learning or in the ability to use exteroceptive cues to guide swimming, since rats with complete cingulate aspiration had little difficulty in learning to swim directly to a visible cue. Rather, the results, in the case of rats with complete cingulate removal and probably in those with posterior cingulate removal alone, are consistent with the hypothesis that these areas play an essential role in those situations when the generation of appropriate behaviors depends upon the relationships among stimuli, cues, or events.

It is important to note that in the 2 experiments that examined swimming to a single place by rats with posterior cingulate damage, these animals showed reliable increases in latency and trajectory errors when the hidden platform was repositioned. The increases are consistent with the possibility that these rats had learned to swim normally to the platform location. However, the fact that even by the end of extensive training, their latency to find the platform was much longer than that of normal animals, and that they made more errors, strongly suggests that their place navigation was not the same as normal rats'. Unlike normal rats, the posterior cingulate rats were not actually swim-

ming to the specific location of the hidden platform, but rather, with the extended training that they had received, they learned an alternate strategy. This alternate strategy may have involved learning to approach the correct side or area of the pool, defined by its proximity to a conspicuous extramaze cue or cue constellation. The latter hypothesis can account for both the observed increase in latency and in errors when the platform was repositioned and for the persisting inaccuracy in swimming to the original platform location by the posterior cingulate rats. The analysis of the performance of these rats in the place-alternation tasks provides further support for this conclusion.

Each of the cingulate lesion groups was clearly impaired relative to the control group in its acquisition of place navigation when the platform was in a new location each day (Experiment 2a). However, the rats with anterior cingulate damage were quite similar in performance to the control rats. Although a relatively small inaccuracy in swimming persisted, the anterior cingulate rats, like the control rats, showed (1) a reliable decline in latency to find the hidden platform with training, (2) a reliable decline in errors with training, (3) significant improvement in latency and errors between the first and second trials of each day, and (4) by the last 8 d of training, ability to attain asymptotic accuracy after the first time they found the platform in its new location each day. Thus, although the anterior cingulate-damaged rats were initially less accurate, they showed acquisition of place navigation to novel locations comparable to that of control rats. In contrast, the posterior- and complete cingulate-damaged rats never acquired normal place-alternation performance; any improvement in performance with training that did occur was attributable to their having learned to circle around the pool more efficiently. Even by the end of the fortieth day of training, these 2 groups did not reliably improve accuracy across the trials of each day. The complete cingulate lesion group did show some improvement between the first and second trial of each trial pair (when the intertrial interval was close to 0 sec), but inspection of their swim paths indicates that this could be attributed to an improvement in the efficiency of their search strategy (e.g., staying away from the wall of the pool), rather than to their having used specific positional information obtained on the first trial. Thus, complete cingulate removal, or removal of posterior cingulate cortex alone resulted in a loss of the ability to learn to swim to specific new locations.

The place-alternation task is procedurally more difficult than single-place navigation, since the rats must learn the rule that for all trials of each day the location of the hidden platform is fixed, but that from one day to the next the location changes. The failure of posterior cingulate-damaged rats to acquire normal performance in this task cannot be attributed to their never having acquired this rule, because posterior cingulate cortex damage disrupted performance even in rats that had preoperatively mastered the task (Experiment 3). In contrast, preoperatively trained control and anterior cingulate cortex-damaged rats displayed near-perfect retention of accurate swimming to new locations. The preoperatively trained posterior cingulate cortex-damaged rats showed faster acquisition than that of naive rats with the same damage, but their final levels of performance were equivalent.

We have emphasized the functions of the posterior cingulate region and its dissociation from the anterior cortex. Finally, we turn to the functions of the anterior cingulate region. Anatomically, this zone shares similarities with both the dorsolateral frontal and anterior cingulate regions of the primate brain. Indeed, this region is frequently referred to as "medial frontal" cortex, and the parallels with dorsolateral prefrontal cortex of the primate have been emphasized (e.g., Leonard, 1969; Kolb, 1984). More important here is the possible function of this region relative to the posterior cingulate cortex. It has been argued that the anterior cingulate (medial frontal) cortex has a special role in the temporal ordering of movements required for the execution of relatively complex chains of behaviors, especially if they require moving from one place to another (Kolb, 1984). Thus, anterior cingulate lesions in rats disrupt behaviors that require a series of movements in extrapersonal space—movements that are under the guidance of sensory information, especially visual, auditory, tactile, and kinesthetic (e.g., Eichenbaum et al., 1983; Kolb, 1984). Rodents with lesions of the anterior midline cortex therefore show impairments in food hoarding and nest building, whereas those with posterior cingulate lesions do not (Shipley and Kolb, 1977). The mild deficit of rats with anterior cingulate lesions on tests of avoidance learning or spatial orientation (e.g., Becker et al., 1980; Sutherland et al., 1982; Kolb et al., 1983) therefore results from a deficit in the temporal ordering of movements guided by distal sensory information, rather than from a specific difficulty in forming topographical representations, this latter problem being the basis of the more severe posterior cingulate deficit observed on tests of spatial learning and memory.

Two conclusions suggest themselves from these behavioral results: (1) Posterior cingulate areas are essential to the ability to move accurately to points in space using the relationships among distal cues, and (2) anterior cingulate areas make some, albeit nonessential, contribution to place-navigational accuracy. Given the importance of the hippocampal system for some forms of learning and memory, particularly those involving behaviors based on topographical representations of the environment, and given the anatomical relationships of posterior cingulate areas, it is obvious that the place-navigation impairment that we describe could be due to a partial disconnection of the hippocampal system from its normal cingulate and neocortical inputs and/or to a partial disconnection of neocortical zones from their normal inputs from parahippocampal and cingulate cortices. With regard to the latter possibility, Gabriel and Sparenborg (1986) have recently shown that the discriminative multiunit activity seen in posterior cingulate cortex during acquisition of active avoidance learning in rabbits does not occur after damage to the subiculum. Similarly, removal of posterior cingulate cortex blocks the discriminative multiunit activity in anterior cingulate cortex. Thus, the conduction of information from the hippocampal system via subicular cortices to posterior cingulate cortex and from there to anterior cingulate cortex and association zones in neocortex may be pivotal to the generation, elaboration, and utilization of topographical (and possibly other) representations.

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