# Dentate Gyrus-Selective Colchicine Lesion and Disruption of Performance in Spatial Tasks: Difficulties in "Place Strategy" Because of a Lack of Flexibility in the Use of Environmental Cues?

Gilberto F. Xavier,* Francisco J.B. Oliveira-Filho, and Andréa M.G. Santos

*Departamento de Fisiologia, Instituto de Biociências, Universidade de São Paulo, São Paulo, São Paulo, Brazil*

**ABSTRACT:** The effects of intradentate colchicine injections on the performance of tasks requiring spatial working and reference memory are controversial. Multiple-site colchicine injections (7 µg/µl; via a drawn micropipette) throughout the dentate gyrus (DG) of rats (nine sites in each hemisphere, 0.06 µl at each site) selectively destroy about 90% of the DG granule cells, as revealed by quantitative stereological estimates; stereology also revealed minor neuronal losses in the CA4 (33%) and CA1 (23%) subfields, but lack of damage to the CA3 hippocampal subfield. Spatial reference and working memory were assessed in Morris' water maze; in the reference memory task, the rats were required to learn a single, fixed location for the platform over several days of training; in the working memory task, animals were required to learn a new platform location every day, in a matching-to-place procedure. Compared to sham-operated controls, lesioned rats showed significant disruption in acquisition of the reference memory water maze task; however, the data reveal that these rats did acquire relevant information about the task, probably based on guidance and orientation strategies. In a subsequent probe test, with the platform removed, lesioned rats showed disruption in precise indexes of spatial memory (e.g., driving search towards the surroundings of the former platform location), but not in less precise indexes of spatial location. Finally, the lesioned rats showed no improvement in the match-to-place procedure, suggesting that their working memory for places was disrupted. Thus, although capable of acquiring relevant information about the task, possibly through guidance and/or orientation strategies, DG-lesioned rats exhibit a marked difficulty in place strategies. This is particularly evident when these rats are required to deal with one-trial place learning in a familiar environment, such as in the working memory version of the water maze task, which requires flexibility in the use of previously acquired information. *Hippocampus 1999;9:668–681.*
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**KEY WORDS:** dentate gyrus; hippocampus; entorhinal cortex; CA3; CA1; learning

## INTRODUCTION

Both the anatomical and functional integrity of the components of the rodent hippocampal formation (the dentate gyrus, Ammon's horn, the-subicular complex, and the entorhinal area) seem to be necessary for the successful performance of spatial working (e.g., Aggleton et al., 1992; Sziklas et al., 1996, 1998) and reference (e.g., Morris et al., 1982; Sutherland and Rodrigues, 1989; Whishaw and Jarrard, 1995; Good and Honey, 1997; Arolfo et al., 1998; Cassel et al., 1998) memory tasks.

Dentate gyrus granule cells are of special interest, as they receive excitatory input from the entorhinal cortex via the perforant path into the molecular layer, and activate pyramidal cells in CA4 and CA3, being in a position therefore to control the flow of information within the hippocampus. Stimuli presented during discrimination learning can evoke electrical potentials in the molecular layer of the dentate gyrus, an effect that disappears with the extinction of the learned behavior (Deadwyler et al., 1979a; 1979b). Winson and Abzug (1977) showed that neural transmission in dentate granule cells changes during conditioning, suggesting their participation in encoding information into some kind of memory. Later, Jung and McNaughton (1993) demonstrated that the dentate gyrus granule cells exhibit a clear, spatially, and directionally selective discharge during performance of a spatial working memory task on the eight-arm maze, which is at least as selective as that exhibited by CA3 pyramidal cells recorded under the same conditions; after comparing the discharge patterns that dentate granule cells and CA3 pyramidal cells exhibited under different testing conditions, these authors concluded that granule cells provide their CA3 targets with a high degree of spatial information. However, information may also be transmitted down to the hippocampus through mono- (entorhinal cortex-CA1) and disynaptic (entorhinal cortex-CA3-CA1) circuits (Yeckel and Berger, 1990). Jones (1993) advocated that the direct perforant path projections to CA3 and CA1 are "more important than thought previously," emphasizing that neuroanatomical studies have strongly influenced the development of the idea that the perfo-

*Correspondence to: Dr. Gilberto F. Xavier, Department of Physiology, Biosciences Institute, University of São Paulo, Rua do Matão, Travessa 14, 321, 05508–900, São Paulo, São Paulo, Brazil. E-mail: gfxavier@usp.br

rant path input to the dentate gyrus was the principal pathway by which information was transferred.

Since the discovery by Goldschmidt and Stewart (1980, 1982) that the topical injection of colchicine into the hippocampus caused preferential damage to the dentate gyrus granule cells, this approach has been used to investigate the contribution of these cells to hippocampal function. Using the so-called conventional lesion techniques (e.g., aspiration, electrolytic, and thermocoagulation), damage is not restricted to the target area: passage fibers may be destroyed, and the amount of damage to the vasculature is unknown (Jarrard, 1983). Thus, many of the behavioral changes observed after lesions targeting the hippocampal formation may result from extrahippocampal damage or a combination of hippocampal and extrahippocampal damage. Since the use of colchicine minimizes this type of problem, several laboratories have been using this alkaloid to investigate the effects of damage to the dentate gyrus granule cells and mossy fibers on memory function. For instance, intradentate colchicine injections impair the acquisition (Walsh et al., 1986; McLamb et al., 1988) and retention (McLamb et al., 1988) of the eight-arm radial maze task. However, Jarrard et al. (1984) described a rather different pattern of results following intradentate colchicine lesions. Rats were preoperatively trained in a modified version of the eight-arm radial maze that allows concurrent testing of working and reference memory for places and cues in the same animal. The animals then received bilateral, intradentate colchicine injections. Relative to controls, performance in the reference memory task for places was disrupted early during postoperative testing; this effect, however, disappeared over the test period such that dentate-lesioned rats reached the same level of performance as the controls. No disruption of the reference memory task for cues or the working memory task for both cues and places was observed following dentate lesion. Sutherland et al. (1983), however, reported that intradentate colchicine injection strongly interfered with the acquisition of a version of the water maze task that is usually considered to measure spatial reference memory; in addition to dentate granule cell loss, there was also damage to the overlying parietal cortex, associated with the injection needle track, and related damage to the lateral posterior nucleus of the thalamus. This extradentate damage is important since, as acknowledged by the authors, "there is a possibility that the impairment of spatial localization reflects disruption of these different outlying structures." Intradentate colchicine injection has also been used to investigate the contribution of dentate granule cells to the place-related activity of hippocampal pyramidal cells. For example, McNaughton et al. (1989) claimed that destruction of more than 75% of the dentate gyrus granule cells severely disrupted performance in three different spatial tasks; however, despite this lesion, there was little effect on CA3 and CA1 spatial selectivity of place cell discharge in an eight-arm radial maze. The authors' conclusion was that "most of the spatial information exhibited by hippocampal pyramidal cells is likely to be transmitted from the cortex by routes other than the traditional 'trisynaptic circuit.'"

There seems to be controversy about which processes intradentate colchicine injections affect. Considering that the volume and concentration of colchicine injected, as well as the number of injection sites, varied considerably in these studies, part of the controversy may reside in the quantity of cells spared both in the granule cell layer and in the hilus. In fact, the extent and selectivity of granule-cell loss does appear to depend on the local concentration of colchicine (Dasheiff and Ramirez, 1985), and extradentate damage may interfere with behavioral performance. In addition, as granule cells of the fascia dentata send mossy fibers to the CA3 in a parallel but divergent projection (Blackstad et al., 1970; Gaarskjaer, 1978; Claiborne et al., 1986; Amaral and Witter, 1989), and since there are extensive lateral interactions among cells in the fascia dentata (Hjorth-Simonsen and Laurberg, 1977; Laurberg, 1979; Laurberg and Sørensen, 1981; Swanson et al., 1981) through excitatory interneurons located in the hilus, the degree of dentate lesion seems to interfere crucially with the flow of information along the trisynaptic circuit. In other words, even a small number of spared granule cells seems to interfere with the flow of information down through the hippocampal circuit. However, recurrent feedback loops among dentate gyrus (DG) granule cells, mossy cells, and hilar pyramidal neurons seem to perform a critical role in hippocampal information processing (Amaral and Witter, 1989); as a matter of fact, damage to this feedback system interferes with the performance of tasks that require normal hippocampal function (Samuel et al., 1997).

Although colchicine exhibits preferential toxicity for granule cells, some damage to hilar and pyramidal cells has also been reported (Lothman et al., 1982; Dashieff and Ramirez, 1985; Tilson et al., 1987, 1988). For instance, the length of the pyramidal cell layer of Ammon's horn is significantly reduced following intradentate injection (Walsh et al., 1986; Tilson et al., 1987, 1988; Emerich and Walsh, 1990). Unfortunately, the degree of neuron loss in the dentate and hippocampal subfields was not quantified in the above-mentioned studies, impeding comparison of the behavioral outcomes.

In the present study, quantitative stereological techniques (Gundersen et al., 1988; Gundersen and Jensen, 1987; West and Gundersen, 1990) were used to estimate the effects of the injection of minute amounts of colchicine at multiple sites on the number of intact-appearing neurons in the dentate gyrus granule cell layer, dentate hilus, and hippocampal subfields, to evaluate the degree of selectivity of the induced damage. This study was also designed to investigate the nature of the cognitive deficit following such a lesion.

O'Keefe and Nadel (1978) distinguished alternative strategies used by animals to navigate through the environment and suggested that more than one may be used simultaneously to solve spatial tasks. According to these authors, while place (or locale) strategies involve cognitive mapping, guidance (or taxon) strategies depend on a particular prominent object or stimulus indicating the location of the goal; egocentric orientation strategies are based on the rotation of the body axis relative to other axes. According to O'Keefe and Nadel (1978), these strategies would be subserved by different neural systems; the hippocampus would be necessary for place learning. In addition, O'Keefe (1983) suggested that when the use of one of these strategies is not possible, e.g., after lesions of the related system, the animal may

rely on the remaining systems to solve the task, if this is possible. Normal rats seem to be able to use these strategies simultaneously to solve spatial navigation in Morris' water maze task (Whishaw and Mittleman, 1986). In the variable-start-position version of this task, the rat is trained to reach the hidden platform, departing from different starting points at the pool edge; thus, optimal performance requires knowledge of the relative positions of the multiple extramaze cues and of the platform relative to these cues; this involves navigation based on place strategies. Although this procedure restricts acquisition based on guidance strategies, which is additionally restricted by the use of large-diameter pools, this latter type of acquisition is not completely precluded.

Reference memory is considered to contain general information applicable to many different instances of the same class of events (e.g., Olton, 1983). In this sense, it does not depend on the temporal/personal context in which the information is presented. Behavioral tasks requiring reference memory emphasize relevant information applicable to all trials. According to this view, the hidden platform version of the water maze task might be considered a spatial reference memory task if the platform position is kept constant throughout all training sessions. Working memory, however, is considered to contain information about specific personal and temporal contexts (Olton, 1983). Tasks requiring working memory emphasize relevant information applicable to a specific trial that does not apply to others. Thus, if a different position for the hidden platform is used on each day of training, the critical spatial information would apply only to that specific session; therefore, one should to consider it a working memory task (Xavier and Oliveira-Filho, 1995; Morris and Frey, 1997).

The present experiments associate 1) a surgical procedure to maximize damage to dentate gyrus granule cells and minimize damage to other hippocampal subfields and to the overlying parietal cortex, through the use of multiple-site injections of minute amounts of colchicine throughout the dentate gyrus via a glass micropipette glued onto a microsyringe needle with frequent washing of the exposed dura mater with saline; 2) stereological estimates of the amount of granule-cell loss in the dentate gyrus and of pyramidal-cell loss of the hilus, CA3, and CA1 subregions; and 3) cognitive evaluation of spatial reference memory acquisition and working memory, using different versions of Morris' water maze task.

## EXPERIMENTAL PROCEDURES

### Animals

Twenty-six naive, male Wistar rats weighing 270–330 g at the beginning of the experiments were used. Groups of 4–5 rats were maintained in the same cage. Light was provided from 07:00–19:00 h, and room temperature was maintained at 22 ± 3°C. These housing conditions lasted until the end of the experiments.

*TABLE 1.* _____

**Stereotaxic Coordinates for Colchicine Injections (Paxinos and Watson, 1986)***

| AP | ML | DV (dura = 0) |
|------|------|------|
| −2.3 | ±1.0 | −3.4 |
| −3.0 | ±1.4 | −3.4 |
| −4.0 | ±2.0 | −3.3 |
| −4.8 | ±3.1 | −3.5 |
|      | ±3.9 | −7.2 |
| −5.7 | ±4.1 | −3.8 |
|      | ±4.9 | −4.0 |
|      |      | −4.8 |
|      |      | −5.6 |

*Note that the zero dorsoventral coordinate corresponds to the dura mater level.
AP, anteroposterior; ML, mediolateral; DV, dorsoventral.

### Surgery

Rats anesthetized with equitesin intraperitoneal (i.p.) were positioned in a Kopf stereotaxic device (David Kopf Instruments), and the incisor bar was adjusted 3.3 mm below the interaural line. The cranium overlying the region to be lesioned was perforated; special care was taken to avoid damage to the cortex. Standard stereotaxic procedures were used. Injections were made using a 5-μl Hamilton microsyringe (Hamilton Company) with a drawn glass pipette adapted to the end of the needle, mounted on a stereotaxic frame and held with a microinjector. Nine different sites in each hemisphere of 13 rats were injected with 0.06 μl of colchicine (7 mg/ml) dissolved in phosphate-buffered saline (pH 7.4) to destroy the granule cells of the dentate gyrus (see coordinates in Table 1). The glass pipette was inserted slowly to penetrate the dura mater; its tip positioned at the injection site and the dura mater were then washed thoroughly with saline. After colchicine application, the pipette was kept in position for 60 s to avoid colchicine spread back up the needle tract. During this period, the dura mater was kept wet with saline to avoid cortical damage (DG group).

After the injections, the wounds were sutured and the animals were transferred to a heated room (33°C) until recovery from the anesthesia.

Thirteen control rats received the same treatment, using phosphate-buffered saline alone (control group).

Behavioral testing started 2–3 weeks after surgery.

Seizures were not observed in any animal throughout the experimental period.

### Apparatus

A round, black, fiberglass pool, 200 cm in diameter and 50 cm high, was filled to a depth of 25 cm with water (26 ± 1°C) rendered opaque by the addition of milk. A movable, transparent circular plastic platform 9 cm in diameter, mounted on a plastic column, was placed in the pool about 1–2 cm below the water surface. The platform location depended on the behavioral testing

procedure (see below). A video camera positioned approximately 290 cm over the center of the pool was connected to a video tracking digitizing device (VP112, HVS Image Ltd., Hampton, UK) and sampled, in units of 0.1 s, by a PC computer system programmed to collect and analyze the animals' swim-paths.

For descriptive data analysis, the pool was divided into four equal-area quadrants. The quadrants bordered each other along lines which intersected the edge of the pool at the arbitrary cardinal start locations called north, south, east, and west. The time spent within the training counter (an area three times the platform diameter, surrounding each platform position), as well as the percent time spent in that counter (in relation to three symmetrically located neutral areas of equal size), provided more specific indexes of spatial location.

The swimming pool was located in a 3.15 × 6 m room with several salient cues hanging on the walls.

## Behavioral Procedure

In the water-maze reference memory training task, the platform was located in a single, fixed position in the center of the northwest quadrant. Each animal received two trials per session, one session a day, during 12 days. In this phase, the starting locations varied between the cardinal points south, southeast, east, and northeast in random order and mixed combinations for each day. Rats were run in squads of 6–7 with a 4–7-min intertrial interval.

A 180-s probe test, with the platform removed, was conducted 24 h after the end of the training phase; during this test, the animals were allowed to swim freely in the pool. The percentage and total amount of time the animal spent within the training counter, the percent time in the training quadrant, and the number of times the animal swam across that counter (counter frequency), in three time bins of 60 s each, allowed 1) assessment of long-term memory for platform location through the extent of spatial bias in the first time bin, and 2) evaluation of the rate of extinction of this behavior by comparing the same parameter over the three consecutive time bins. The heading angle (a measure of initial divergence from the direct path to the platform) provided another measure of spatial bias. Note that percent time within the training counter reflects a more specific index of spatial location in relation to the percent time spent within the training quadrant (QD), given that the first parameter refers to a smaller pool area; further, the chance level of performance for these parameters is 25%, higher scores indicating spatial bias towards the former location of the platform.

One week after the probe test, water-maze working memory task training started. The animals were trained using four trials/day with a different platform position on each of 17 days. For the first 11 days (phase 1), the intertrial interval (ITI) was 5 min, while on the last 6 days (phase 2) it was virtually zero (the rats were taken from the platform straight to the next trial). Four different remote starting locations were used in a different order each day. For instance, if the platform was located in the southwest quadrant closest to the south cardinal point, the starting locations were north, east, northeast, and northwest.

Each trial in both the reference and the working memory versions of the task constituted placing the rat near the side of the pool facing the wall at one of the starting locations and allowing it to swim until the platform was found. The collection of data by the computer system started as soon as the rat was placed in the pool. When the rat found the hidden platform, data collection was stopped, ending that trial. If the rat did not find the platform within 60 s, the animal was then manually guided to the platform, where it remained for a 10-s period. The rat was then dried thoroughly with a towel and placed into either the waiting box until the next trial or returned to its cage at the end of the trials.

Acquisition of the reference memory task was assessed by decreases in latency, path length, and heading angle, and by increases in the percent time spent in the quadrant where the platform was located.

In the first trial on each day of the working memory task, the rats reached the platform by chance and scanning, so latencies and path lengths would not be expected to reflect group differences. At the end of the first trial, however, the animals had obtained information about the platform location, and the second and subsequent trials required matching to the position for that day (Xavier and Oliveira-Filho, 1995; Morris and Frey, 1997). Thus, the percentage saving from the first to the second trial allowed assessment of working memory, while the slope over the four trials provided a measure of speed of acquisition (Morris et al., 1990). Unpublished observations from our laboratory (by Xavier) show that at the beginning of the first trial, normal rats search for the platform within the counter where it was located the day before, indicating that they remember its former position. As the platform location changes with training, the information about the new location is updated every day. The percent time spent within the critical day-before counter provided a 24-h delay test of memory for the precise former platform location.

Swim speed was obtained by dividing swim path length by latency, and provided an index of motivation.

## Histology

At the end of behavioral testing, the animals were deeply anesthetized with ether and perfused intracardiacally with 400 ml of sulphide solution. The brains were removed immediately and frozen. Two parallel, 30-μm-thick coronal sections were taken every 150 μm throughout the hippocampus. One set of sections was stained with cresyl-violet to estimate cell loss, while the adjacent set was processed with Timm stain for heavy metals to estimate the loss of mossy fibers.

## Stereology

The number of intact-appearing neurons was estimated using stereological principles and systematic sampling (Gundersen et al., 1988; Gundersen and Jensen, 1987; West and Gundersen, 1990) in four major subdivisions of the hippocampal formation, including the granule-cell layer, the dentate hilus (CA4), CA3, and the CA1 pyramidal-cell layers. The total volume of each hippocampal region, estimated by systematic point-counting techniques and the use of the principle of Cavalieri (see Gundersen and Jensen,

1987), and estimates of the numerical density of neurons, obtained with an optical disector (see West and Gundersen, 1990), in each subdivision in the same sections, allowed calculation of the total number of intact-appearing neurons in each hippocampal region (West and Gundersen, 1990). The volume of each hippocampal region and the relative numerical density of neurons in the different sectors of each section allowed evaluation of the septotemporal distribution of cell loss in the different hippocampal subfields. These estimates were performed bilaterally for 6 typical DG-lesioned rats and the controls; data from both sides were added.

The precision of estimates of both the total volume of each hippocampal region and the corresponding numerical densities of neurons in a set of sections for each animal was expressed using coefficients of error (CE) (Gundersen and Jensen, 1987). The stereological sampling scheme was considered adequate when the CE was less than 0.10 (see West and Gundersen, 1990).

## Data Analysis

Water-maze results in the reference memory task were analyzed by a repeated measures analysis of variance (ANOVA) with groups as the between, and sessions and trials as the within-subjects factors (SAS Institute, Inc., Cary, NC). Separate ANOVAs were used for each measure.

Probe-test latency scores were not analyzed, as all rats remained in the pool for 180 s. Probe-test quadrant and counter data were analyzed with group as the between- and time bin as the within-subjects factors. Heading angles were compared using group as the between-subjects factor.

Working memory scores of percentage saving from the first to the second trial were separately analyzed for 0-min and 5-min ITI schedules, with groups as the between- and sessions as the within-subjects factors. Further, means of latency, path length, and percent time within the critical day-before counter scores for the four trials across the last 6 days in the 5-min ITI schedule (days 6–11), and the 6 days in the 0-min ITI schedule (days 12–17), were individually compared, with group as the between, and phase and trials as the within-subject factors.

The estimates of the number of neurons in each subdivision of the hippocampal formation were compared by analysis of variance, with groups as the between-subjects factors; an individual analysis was run for each hippocampal region.

<div style="text-align:center">**RESULTS**</div>

## Histology

Light-microscopic evaluation of Nissl-stained sections from the brains of the DG rats indicated 1) shrinkage of the dentate granule-cell and molecular layers, and dentate hilus, with a small increase in the size of the lateral ventricles, and 2) marked granule-cell loss throughout the septotemporal axis of the hippocampus, with a small loss of other classes of hippocampal neurons

except in 2 rats which exhibited visible pyramidal CA3 and CA1 cell loss close to the dentate gyrus region; these animals were excluded from the behavioral analyses. Two other rats were excluded from the behavioral analysis as they exhibited much less granule-cell loss than the other animals. In the 9 DG animals included in the behavioral analysis, multiple-site colchicine injection destroyed most of the granule cells, while pyramidal cell loss was much less (see quantitative data below). Figure 1 shows Nissl-stained sections through the septal pole (Fig. 1A) and temporal pole (Fig. 1B) of a control dentate gyrus and hippocampus, while Figure 1C,D illustrates granule-cell loss in a typical, DG colchicine-lesioned rat at the respective corresponding levels.

The efficacy of multiple-site colchicine injection in inducing granule-cell death was confirmed in the Timm-stained sections (Fig. 2). While control rats (Fig. 2A) exhibited the typical laminar hippocampal organization, in the DG rats (Fig. 2B) the Timm-positive, mossy fiber terminals almost completely disappeared from the hippocampus; disorganization of the typical laminar molecular layer arrangement was evident. Finally, these rats apparently did not exhibit cell loss in the overlying cortex (Fig. 1C).

## Stereology

The appropriateness of the sampling scheme employed for the estimates of the number of intact-appearing cells in each brain region for each rat, as revealed by the CEs, was confirmed.

Figure 3 shows the estimated number of neurons for each subdivision of the hippocampus and dentate gyrus both for DG-lesioned and control animals. Note that the estimates of neuron number for the control hippocampal subfields are of the same order of magnitude seen in other studies involving estimates of hippocampal cell populations in the same rat strain (e.g., Bayer, 1982; Boss et al., 1987; West et al., 1991). Differences can be accounted for by factors such as age of the animals and the estimation methodology. Relative to controls, multiple-site colchicine-injected animals exhibited about 90% cell loss in the dentate gyrus granule cell layer (Fig. 3A) (ANOVA, $F_{1,15} = 42.90$, $P < 0.0001$), 33% neuronal loss in the CA4 layer (Fig. 3B) (ANOVA, $F_{1,15} = 27.25$, $P < 0.0001$) and 23% neuronal loss in the CA1 layer (Fig. 3D) (ANOVA, $F_{1,15} = 15.04$, $P < 0.0015$). These neuronal losses were equally and bilaterally distributed along the septotemporal axis of the hippocampal formation; there was no neuronal loss in the CA3 layer (Fig. 3C) (ANOVA, $F_{1,15} = 3.04$, $P > 0.10$).

## Behavior

Throughout all behavioral tests, the swim speeds of the DG and control groups did not differ significantly. Thus, differences in other scores are unlikely to be due to motor or motivational effects.

## Reference Memory Task Acquisition

Multiple-site colchicine injection into the dentate gyrus disrupted the acquisition of a spatial navigation reference memory task in the water maze, as revealed by the significant group effect for latency, path length, percent time in training quadrant ($F_{1,20} = $
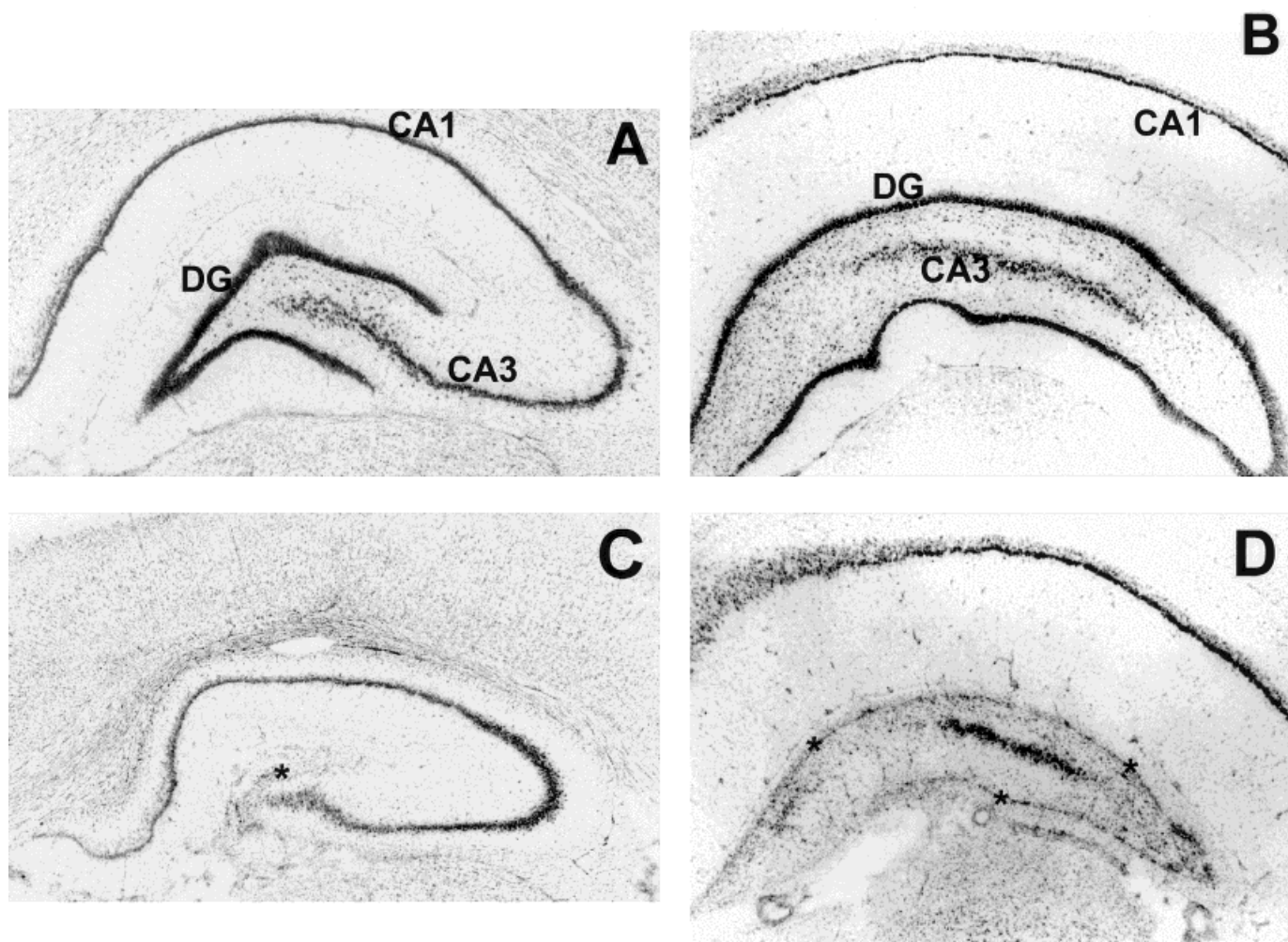
**FIGURE 1.** Photomicrographs of cresyl-violet stained sections of the hippocampus. A, B: Septal and temporal poles of a control rat hippocampus and dentate gyrus (DG), respectively. C, D: A typical multiple-site, colchicine-injected dentate gyrus at corresponding levels. Asterisks indicate previous locations of granule cells and glial scarring.

16.75–17.77, $P < 0.005$), and heading angle ($F_{1,20} = 7.40$, $P < 0.0132$) (Fig. 4).

Repeated-measures ANOVA also revealed a significant session effect for latency, path length, percent time in the training quadrant, and heading angle ($F_{11,220} = 4.81$–10.44, $P < 0.0002$), but no significant group $\times$ session interaction for latency and percent time in the training quadrant ($F_{11,220} = 0.66$–1.58, $P > 0.10$). Therefore, the rate at which latency and percent time in the training quadrant changed did not differ between the two groups, indicating that DG animals also exhibit improvements with repeated training.

Conversely, for path length and heading angle parameters, group $\times$ session interaction did differ significantly ($F_{11,220} = 1.85$–1.92, $P < 0.0477$), revealing that DG animals did not improve like the controls in the water maze task acquisition: i.e., DG rats exhibited improvements in latency and percent time in the training quadrant, but not in path length and heading angle parameters.

In summary, these results show that DG-lesioned rats took longer to find the platform than did controls (Fig. 4A,B), and also exhibited a highly impaired, precise spatial search strategy, as shown by a decreased percentage of time in the training quadrant (Fig. 4C) and an increased heading angle (Fig. 4D). Additionally, DG-lesioned rats showed less improvement than the controls during training in relation to their initial swimming towards the specific location which contained the platform, as expressed by the lack of decrease in heading angle scores. While DG-lesioned animals were clearly impaired in the acquisition of information about the precise spatial location of the platform, their improvements in latency and percent time in the training quadrant suggest that they have acquired some information about the presence of the hidden platform in the pool, and have also acquired a general idea about the area in which it is located. However, once within that area, DG rats do not know where to search with precision.
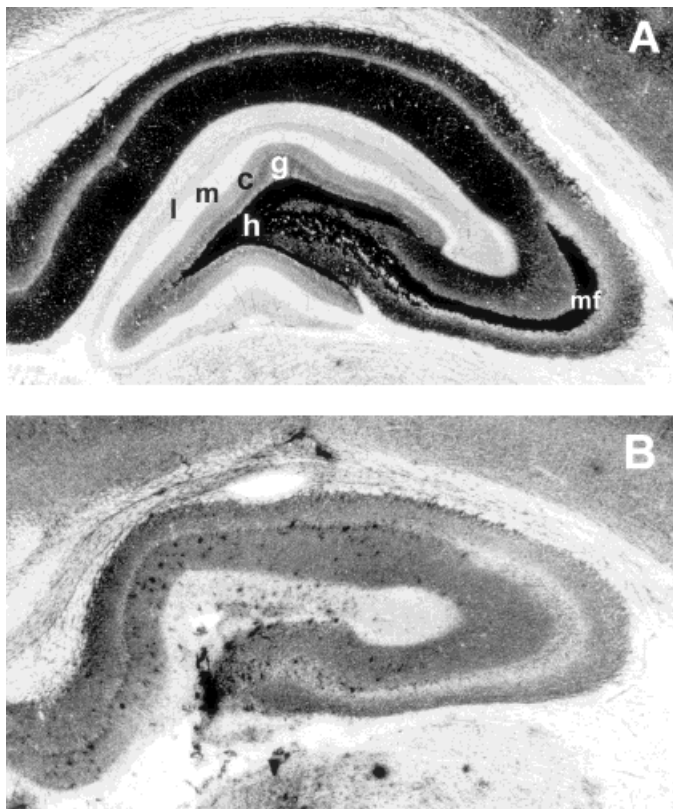
## Probe Test

In the probe test, when the platform was removed, while control animals spent a substantial amount of time searching for the platform at its previous location during the reference memory
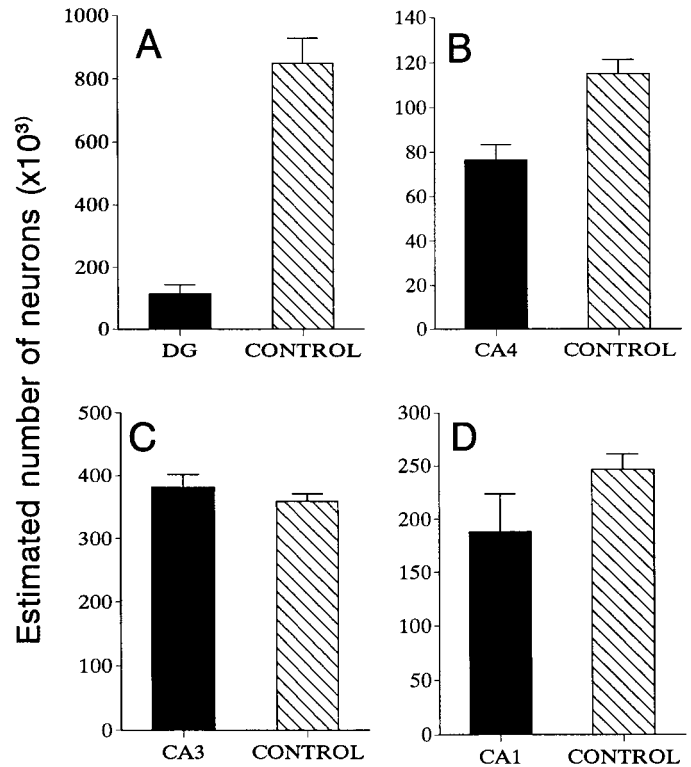
training, such behavior was disrupted in DG rats (Fig. 5). There were significant group effects for percent time within the training counter (Fig. 5A), total amount of time within the training counter (not shown), and counter frequency (Fig. 5B) ($F_{1,20} = 5.67–14.01$, $P < 0.0273$); note that the percent time within the training counter, total amount of time within the training counter, and the counter frequency represent precise indexes of spatial location memory.

Thus, DG rats failed to remember the precise location of the platform. This was confirmed by the heading angle scores, which were significantly lower in the control animals compared to DG rats ($F_{1,20} = 7.98$, $P < 0.01$) (Fig. 5C). Interestingly, however, DG animals did not differ from controls, at the 5% level, in relation to the percent time spent in the training quadrant ($F_{1,20} = 3.40$, $P > 0.08$), in agreement with the assumption that they do remember the general area of the pool in which the platform was located (Fig. 5D).

The extinction of this bias towards the original location of the platform was evaluated by comparing the scores in three consecutive 60-s time bins. A repeated-measures ANOVA revealed

**FIGURE 3.** **Mean estimated number of neurons in multiple-site, intradentate colchicine (DG)- or phosphate buffer (CONTROL)-injected animals in the dentate gyrus granule cell layer (A), the CA4 (B), and the CA3 (C) and CA1 (D) pyramidal layers of the hippocampal formation. Vertical bars indicate SEM.**
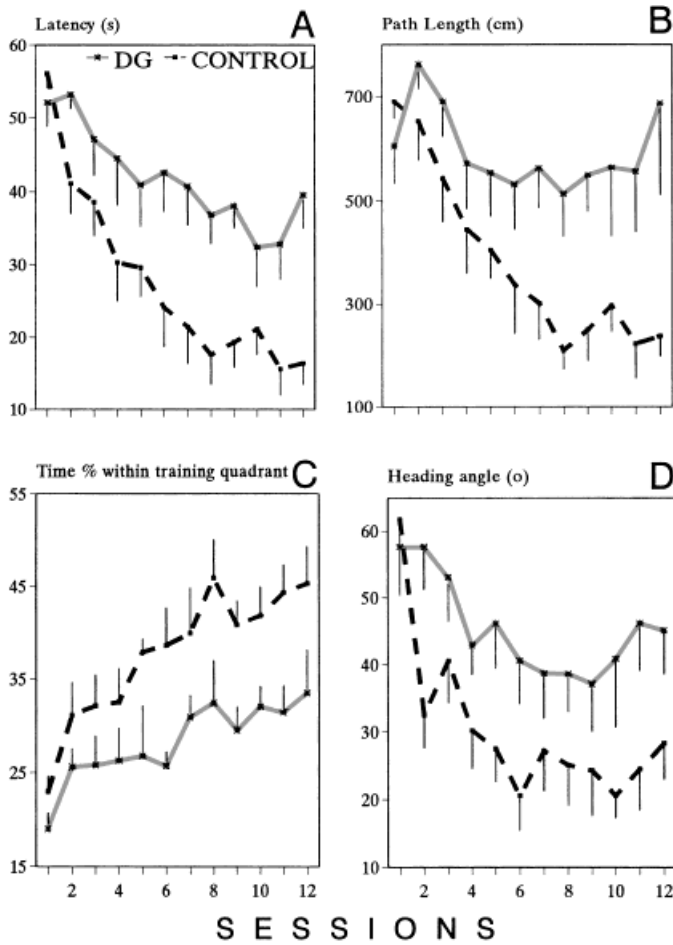
significant time bin effects for the total amount of time within the training counter, and counter frequency ($F_{2,40} = 3.64–4.42$, $P < 0.035$), indicating extinction. Furthermore, the group × time bin interactions were not significant ($F_{2,40} = 0.60–0.82$, $P > 0.55$), suggesting that the rate of extinction, at least for these parameters, was similar in both groups. In relation to the scores for percent time spent within training quadrant, there was no significant time bin effect ($F_{2,40} = 0.59$, $P > 0.56$); the group × time bin interaction just failed to reach significance ($F_{2,40} = 2.85$, $P < 0.07$). Inspection of Figure 5D shows that control rats present a typical extinction curve for this parameter, while DG animals apparently do not extinguish this search for the platform, persisting in that area of the pool.

## Working Memory Task

Working memory was assessed by requiring the rats to learn a new platform position every day, in a matching-to-place procedure with four trials/day. In phase 1 (11 days), the ITI was 5 min. As DG rats were strongly disrupted, the ITI was reduced to 0 min (phase 2, 6 additional days) to evaluate the possible 5-min-delay disruptive effect.

As Figure 6 shows, the percentage saving from trial 1 to 2 quickly improves for control rats in both phases 1 and 2, while DG animals do not show improvement, independent of ITI. There were significant differences between groups for both the 5-min ITI ($F_{1,20} =$

**FIGURE 2.** **Photomicrographs of Timm-stained sections through the septal pole of the hippocampus. A: Normal laminar terminal fields of the lateral (l) and medial (m) perforant pathways and the commissural-associational projections (c) in the molecular layer, the dentate gyrus granule cells (g), and the dentate mossy fiber projection to CA3 (mf) and dentate hilus (h). B: A typical multiple-site, colchicine-injected dentate gyrus. Note the disorganization of the laminar terminal fields and the almost complete disappearance of the dentate mossy fiber projections.**
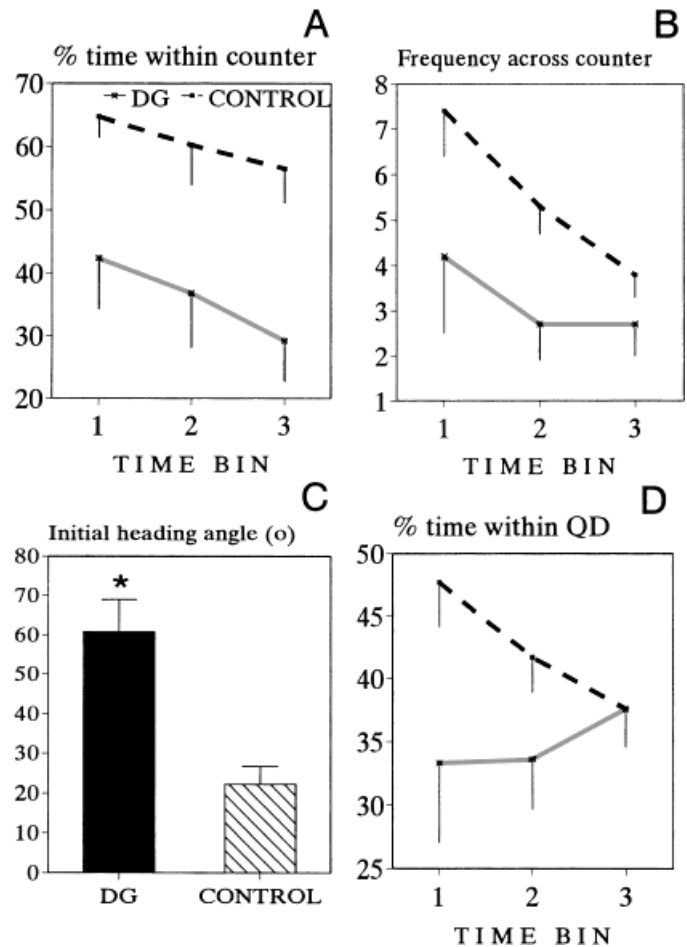
**FIGURE 4.** Effect of multiple-site, intradentate colchicine (DG), or phosphate buffer (control) injections on the acquisition of a spatial navigation reference memory task in the water maze task over 12 training sessions, as revealed by latency (A), path length (B), percent time within the training quadrant (C), and heading angle (D). Data are mean ± SEM. For representational purposes, mean values of two trials per session were used.

$P < 0.0001$), and trial × group ($F_{3,60} = 21.01$–$22.03$, $P < 0.0001$) effects, but a lack of significant differences for phase, phase × trial, and phase × trial × group ($P > 0.09$) effects, both for mean latency and path length. Thus, DG rats are as capable as controls of finding the hidden platform in the first trial each day, suggesting that the general information about the procedures necessary to perform the water maze task is kept in memory. Differently from controls, however, the DG rats do not benefit from the specific information about the platform location. These findings confirm that the present results do not involve differences in motor or motivational states.

The water-maze working memory task also provided a 24-h delay test of memory for the precise, former platform location. Given that the platform position changed every day, the spatial bias towards the day-before platform location in the first trial could be considered a delayed-matching-to-position working memory test, while its decrease over the remaining trials provided a measure of extinction associated with the acquisition of information on the new platform location. The mean percent time spent within (Fig. 8A) and the mean frequency across (Fig.



**FIGURE 5.** Effect of multiple-site, intradentate colchicine (DG), or phosphate buffer (CONTROL) injections on the platform removal 3-min probe test, represented as the percent time within the training counter (A), frequency within the training counter (B), initial heading angle (C), and percent time within the training quadrant (D) in each of three 1-min consecutive time bins (except for heading angle). Data are mean ± SEM.

49.35, $P < 0.0001$) and the 0-min ITI ($F_{1,20} = 15.49$, $P < 0.0008$); nevertheless, the lack of a significant session and session × group interaction effect observed in both phases ($P > 0.45$) indicates that in the critical second trial, DG rats do not benefit from their experience regarding platform location in trial 1.

This result cannot be ascribed to differences between the groups' knowledge of the general aspects of the water maze task (e.g., knowledge of the presence of the hidden platform and how to get on to it once it is found), since in the first trial each day, when the critical information about platform location was unknown for both the DG and control rats, the latency and path length scores for both groups did not differ (Fig. 7, trial 1). However, in trial 2, when the rats could use the information about the platform location obtained in trial 1, only control animals exhibited latency (Fig. 7A) and path length (Fig. 7B) decrements, while DG rats did not improve, independent of ITI schedule. Repeated-measures ANOVA revealed highly significant group ($F_{1,20} = 30.99$–$52.65$, $P < 0.0001$), trial ($F_{3,60} = 24.88$–$44.52$,
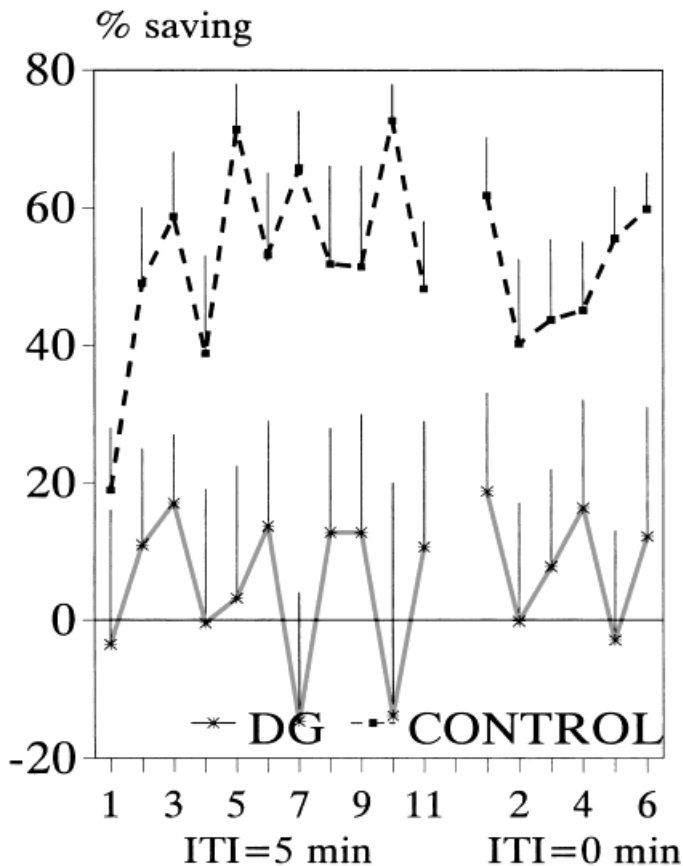
**FIGURE 6.** Effect of multiple-site, intradentate colchicine (DG), or phosphate buffer (CONTROL) injections on the percent saving from trial 1 to 2 over 11 sessions in phase 1 (5-min ITI) and 6 subsequent sessions in phase 2 (0-min ITI) of the working memory task. Data are mean ± SEM.



**FIGURE 7.** Effect of multiple-site, intradentate colchicine (DG), or phosphate buffer (control) injections on latency (A) and path length (B) in the working memory task. Individual scores for latency and path length in trials 1–4 were averaged over the last 6 days of testing in phase 1 (5-min ITI) and over the 6 days of testing in phase 2 (0-min ITI). Note that a novel platform location was used each day. Data are mean ± SEM.

dentata in both the dorsal and ventral hippocampus (Figs. 1, 3A). These observations agree with previous studies on the effects of colchicine injection into the DG (Goldshmidt and Stewart, 1980, 1982; Walsh et al., 1986; Tilson et al., 1988). The association between colchicine and multiple-site injection strategies allowed destruction of about 90% of the DG granule cells, as revealed by stereological estimates (Fig. 3). There was also almost complete mossy-fiber Timm-positive terminal loss (Fig. 2) throughout the septotemporal hippocampus, with no visible damage to the overlying parietal cortex (Fig. 1). Quantitative stereological estimates also revealed some neuronal loss in the CA4 (33%) (Fig.

8B) the day-before critical counter for the four trials assessed memory of the former platform location. It is evident that the performances by DG and control rats are different.

Control rats showed a strong spatial bias towards the previous-day platform location in the first trial, particularly in phase 1, and a sharp decrease in this behavior over trials, while DG rats performed around the chance level (ANOVA significant trial effect, $F_{3,60} = 14.94$, $P < 0.0001$, and trial × group effect, $F_{3,60} = 4.15$, $P < 0.009$). Furthermore, performance by control rats over trials was different in both phases (ANOVA phase × trial significant effect, $F_{3,60} = 3.41$, $P < 0.023$). As seen in Figure 8, the spatial bias of controls in trial 1 was greater in phase 1 than in phase 2; given that phase 1 corresponds to the mean scores for sessions 6–11 and phase 2 to those for sessions 12–17, as training proceeds, extinction of this searching behavior may occur as well.

## DISCUSSION

Intradentate colchicine injections produced massive destruction of granule cells in the superior and inferior blades of the fascia
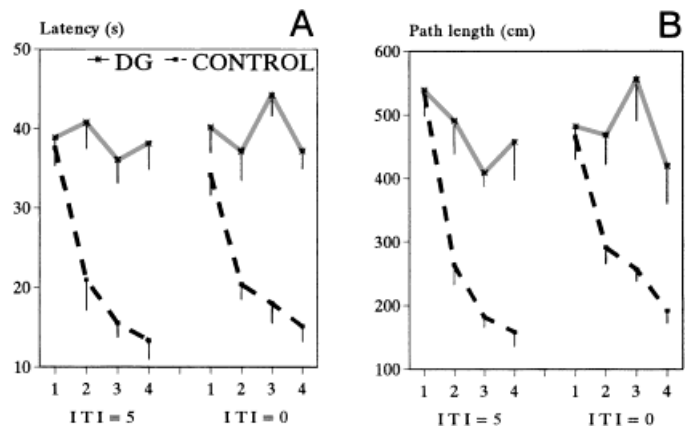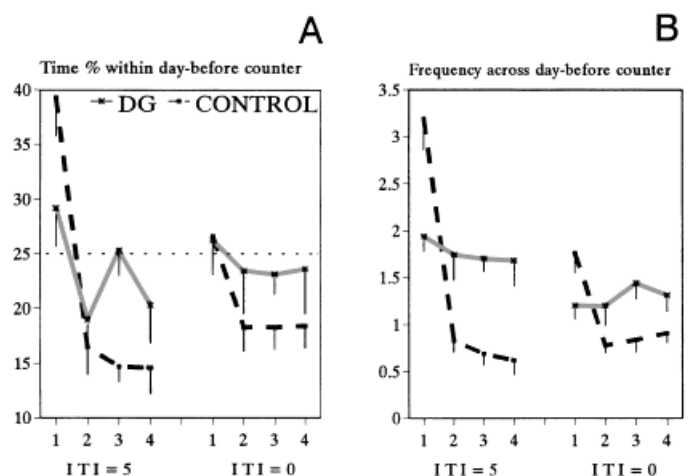


**FIGURE 8.** Effect of multiple-site, intradentate colchicine (DG), or phosphate buffer (control) injections on percent time within the day-before critical counter (A) and frequency across the day-before critical counter (B), in the working memory task. Individual scores in trials 1–4 were averaged over the last 6 days of testing in phase 1 (5-min ITI) and over the 6 days of testing in phase 2 (0-min ITI). Note that percent time within the day-before critical counter (A) chance level corresponds to 25%. Data are mean ± SEM.

3B) and CA1 (23%) (Fig. 3D) subfields, mainly surrounding the colchicine injection sites, confirming previous qualitative observations by Lothman et al. (1982), Dasheiff and Ramirez (1985), and Tilson et al. (1988). Conversely, the CA3 subfield did not exhibit neuronal loss (Fig. 3C).

It is difficult to estimate the contribution of these neuronal losses to the behavioral outcomes. For instance, recurrent feedback loops between the dentate gyrus granule cells, and mossy cells and pyramidal cells in the hilus, are thought to be critical for the control of information processing in the hippocampal circuit (e.g., Amaral and Witter, 1989). In fact, Samuel et al. (1997) showed greater performance impairment in a complex cued discrimination operant conditioning task in rats with ibotenate damage to the dentate hilum (without significant damage to the granule cells) compared to rats with CA2/CA3 damage. These results suggest that damage to the recurrent circuitry between granule cells and hilar neurons (via damage to the latter) strongly disrupts memory processes. In the present study, colchicine injected rats presented 90% dentate granule cell loss and 33% hilar neuronal loss; this massive damage to the recurrent feedback loops certainly strongly affected the animals' performance. The rats also showed 23% CA1 pyramidal cell loss, confirming previous descriptions (e.g., Robain et al., 1989) that intradentate colchicine produces damage to some pyramidal neurons in the CA1 pyramidal layer. In addition, CA1 cell loss was equally distributed along the septotemporal axis of the hippocampi. Moser et al. (1995) showed that spatial learning in the water maze following hippocampal damage was still possible as long as the rats maintained transverse tissue remnants greater than 26% of the total hippocampus; however, to be effective, such remnants must be located within the septal two thirds of the hippocampus (see Moser and Moser, 1998). Note that about 77% of the CA1 pyramidal cells, equally distributed along the septotemporal axis of the hippocampi, were preserved in the intradentate colchicine-injected rats in the present study. Also, there have been reports that spatial learning is inversely correlated to the loss of dorsal CA1 neurons following ischaemia (e.g., Rod et al., 1990; Kiyota et al., 1991; Volpe et al., 1992; Olsen et al., 1994; Milani et al., 1998). For instance, Olsen et al. (1994) showed that while total neuronal cell loss in the anterior-dorsal CA1 region of rats produces memory performance impairments in the water-maze task, rats with only partial damage to the dorsal CA1 region do not exhibit significant acquisition impairments. Such results do not favor the view that the 23% CA1 pyramidal-cell loss distributed along the septotemporal hippocampi has a significant impact on behavioral outcome; however, that such cell loss contributed to behavioral impairment in association with both the more significant dentate and hilar cell losses cannot be excluded.

It is well-known that the subcomponents of the hippocampal formation are linked by well-defined trisynaptic (entorhinal cortex → dentate gyrus → CA3 → CA1), disynaptic (entorhinal cortex → CA3 → CA1), and monosynaptic (entorhinal cortex → CA1) circuits. The interruption of these circuits can be achieved by damage either to their constituent neurons or to their projecting pathways. Intradentate colchicine injections produced massive damage to the dentate gyrus granule cells, interrupting the trisynaptic circuit. However, the effects on the disynaptic and monosynaptic circuits are not clear, i.e., since the CA3 pyramidal cells were intact and CA1 pyramidal cell loss was fairly small, it is unlikely that the disynaptic and monosynaptic circuits were greatly disrupted by damage to the neurons. However, the effect of intradentate colchicine on the projecting pathways was not directly determined in the present study. The occurrence of CA1 cell loss suggests that some colchicine diffused towards the hippocampal lacunosum moleculare layer, where entorhinal projections reach both the CA1 and CA3 subfields. It is well-established that colchicine exerts its primary actions by binding to and hence preventing the polymerization of tubulin, thereby blocking intracellular transport (Fink et al., 1973; Steward et al., 1984; Alonso, 1988). In fact, Contestabile and Villani (1984) have shown that colchicine injections in the septohabenulointerpeduncular system produce irreversible, although limited axonal damage to fibers crossing the injection area. Furthermore, Fonnum and Contestabile (1984) showed that colchicine injections in the supracommissural septum of rats caused choline acetyltransferase decrease in the dorsal hippocampus, suggesting damage to the fimbria-fornix fibers crossing the injected septal area. However, Morelli et al. (1980) emphasized that colchicine apparently does not damage myelinated axons of passage. Further, McNaughton et al. (1989) showed that even though colchicine-induced destruction of the dentate gyrus granule cells severely disrupts performance in three different spatial tasks, there is little effect on CA3 and CA1 spatial selectivity of place cell discharge in an eight-arm radial maze. These data suggest that spatial information obtained by the hippocampal pyramidal cells is transmitted from the cortex by routes that do not include the dentate gyrus granule cells and are not damaged by colchicine. Accordingly, McNaughton et al. (1989) concluded that "these routes may include the direct projections from entorhinal layers II and III to CA3 and CA1, respectively." Also, coronal, Timm-stained sections of dorsal to temporal levels of the CA1 and dentate gyrus of adult rats exhibit a septotemporal gradient of decreasing stainability in the lacunosum moleculare layer of CA1, which is believed to be related to the septotemporal distribution of lateral-medial perforant path terminals (Zimmer and Haug, 1978). This gradient of decreasing stainability in the lacunosum moleculare layer of CA1 was maintained in the colchicine-injected rats in the present study, suggesting that colchicine did not substantially interfere with these terminals.

Relative to controls, colchicine-lesioned animals were significantly disrupted in the reference memory water maze task acquisition (Fig. 4). In addition, the rates of latency decrease and the increase in percent time within the training quadrant in DG rats did not differ significantly over repeated training; conversely, the path length and heading angle showed a slower rate of decrement. This slower improvement in parameters that require a precise spatial search strategy (heading angle and path length) associated with the normal rate of improvement in less precise indexes of spatial location (latency and percent time within the training quadrant) suggests that DG lesions disrupt place strategy, confirming O'Keefe and Nadel (1978) in their proposal for the hippocampal formation. Further, these data show that lesioned

rats did acquire relevant information about the task, probably based on guidance and orientation strategies (see below).

Previous studies examined the contribution of DG granule cells to water-maze acquisition and retention (Sutherland et al., 1983; Mundy and Tilson, 1988; Nanry et al., 1989). For example, Sutherland et al. (1983) showed that bilaterally colchicine-damaged DG rats exhibit a great deficit in the acquisition of the water-maze task. Differently from the present study, however, these authors found no improvement by colchicine-lesioned rats during training, and concluded that rats with this type of lesion "did not show any evidence of having learned anything about the spatial aspects of the task." A possible explanation for this discrepancy may be related to the extradentate damage. While the colchicine-injected rats of Sutherland et al. (1983) did show damage to the overlying parietal cortex and to the lateral posterior nucleus of the thalamus (the latter presumably associated with retrograde degeneration because it was associated with parietal cortex lesion), no damage to these structures was observed in the colchicine-injected rats of the present study. Indeed, unpublished observations from our laboratory (Xavier and Oliveira-Filho) confirm that colchicine-induced thinning of the parietal cortex, by itself, interferes with acquisition of the water-maze task. Further, damage to the dentate gyrus associated with thinning of the parietal cortex induces response strategies that are incompatible with finding the safe platform, since these rats become strongly thigmotaxic and therefore tend to circle around the edge of the pool. This latter behavior was not observed in the DG rats in the present study. Another important difference concerns the diameter of the pool employed in these studies. Even though the diameter of the pool used by Sutherland et al. (1983) was much smaller than that used here (85 cm vs. 200 cm, respectively), and since pools of greater diameter supposedly make stronger demands on spatial learning, our intradentate colchicine-injected rats did show some improvement, while those of Sutherland et al. (1983) did not. Taken together, these data emphasize that lesion of the parietal cortex in association with dentate damage produces a greater behavioral disruption in the water-maze task, even in smaller pools, possibly by rendering the rats thigmotaxic, which is incompatible with finding the platform; damage restricted to the DG avoids this problem.

Nanry et al. (1989) also showed that bilateral, intradentate colchicine injections disrupted acquisition and retention in Morris' water maze. The magnitude of the deficit in the retention test was smaller than the deficit in the acquisition test, showing that the rats did benefit from the prelesion training. The authors concluded that the lesioned rats were still able to make use of guidance and orientation information acquired during the prelesion training to solve the task after the lesion. Note that their conclusion is similar to that of the present study, at least in terms of the nature of the processes considered to underlie the observed improvement in performance. However, while Nanry et al. (1989) stated that the relevant information was acquired during prelesion training, our results show that there is information acquisition after the lesion. In fact, the rate of latency decrease in the acquisition test of lesioned and control groups of Nanry et al. (1989) did differ significantly, contrasting with the findings of the

present experiment. A number of experimental differences between the present study and that of Nanry et al. (1989) may have led to unequal patterns of DG lesion and behavioral testing, leading to differences between results. These include 1) the number of sites of colchicine injection (4 vs. 18 sites), the volume injected at each site (0.5 vs. 0.06 μl), and the colchicine concentration (5 vs. 7 μg/μl), resulting in different total amounts of colchicine injected (10 vs. 7.56 μg); 2) the diameter of the swimming pool (148 vs. 200 cm); and 3) the training schedule (4 vs. 2 trials/day). Further, the present study did not address the effect of prelesion training; the animals were trained only after the lesion. Thus, DG colchicine-lesioned rats do make use of guidance and orientation strategies. In the present experiments, these strategies were acquired after the lesion.

This interpretation receives further support from the probe test results. DG rats were strongly disrupted in precise indexes of spatial memory (e.g., percent time within the training counter, counter frequency, and heading angle), but were not significantly disturbed in the percent time within the training quadrant, a less specific index of spatial memory (Fig. 5), in agreement with the assumption that they do remember some particular extramaze cue which guides them to the side of the pool where the platform is located. Furthermore, lesioned rats did not extinguish this search strategy, in agreement with the previous report by Dalland (1970) that hippocampally lesioned rats exhibit response perseverance.

The effects of intradentate colchicine have also been investigated through other behavioral tasks which are believed to evaluate the same cognitive processes. For instance, Jarrard et al. (1984) evaluated the effect of colchicine DG lesion on retention of a preoperatively learned, complex, place and cue task in the eight-arm radial maze, using a procedure in which (in both the place and the cue tasks) only four arms were baited. They showed that reference memory for places, but not for cues, was slightly impaired early in testing, while working memory for both places and cues was not affected by this lesion. These results contrast with those reported by McLamb et al. (1988) in a similar spatial version of the eight-arm radial maze with only four baited arms, showing that DG colchicine lesion impaired the acquisition of the working memory task, leaving the reference memory component of the task intact.

Data from the present study show that DG lesioned rats showed no improvement in the match-to-place procedure in the water maze, suggesting that their working memory for places was disrupted even when the ITI between the "information trial" (first trial in the water maze working memory task) and the "matching-to-place trial" (second trial) was virtually zero (phase 2) (Figs. 6, 7). Even though several differences between the water maze and the radial-arm maze tasks which affect the cognitive processes required for their acquisition and performance should be considered (see Hodges, 1996), the present study confirms, using the water-maze task, previous radial-maze task data showing working memory disruption after DG colchicine lesion (Walsh et al., 1986; McLamb et al., 1988).

Given that in the present experimental situation the platform is moved to a different site every day, the first trial data represent the level of performance achievable without knowledge of the plat-

form location. Interestingly, the performance of the DG and control rats in this (first) trial, both in terms of latency and path length, did not differ significantly (Fig. 7). This result has important implications. Firstly, it shows that when control rats do not know the platform location they perform like DG rats, emphasizing the view that the latter miss place information. Secondly, eventual group differences in relation to other requirements of the task, which may interfere in performance, including 1) lack of knowledge of the existence of an escape platform, 2) how to get on to it, 3) adequate motor control for swimming, and 4) motivation, can all be discarded. Thirdly, except for the initial search by controls within the day-before platform location area, which was incorporated into the scanning, the pattern of pool scan during the search for the platform exhibited by DG rats was surprisingly similar to that of the controls. Conceptually, an optimal search strategy of the pool on the first trial requires retention of information about places already visited during this trial and its integration with motor control, in order to select a path which distributes the search equally around the pool area. This operation requires a functional working memory for places, which was shown to be disrupted after DG lesion. The DG lesioned rats may be able to maintain some information for a short time, while performing the search, but the discontinuity represented by finding the platform and/or being removed from the pool for the second trial may cause them to lose critical information about platform location. Alternatively, the DG rats may have greater difficulty finding the platform in this trial, which is expressed in their latencies and path lengths. However, the initial bias towards the day-before critical location exhibited by the controls would also increase their latency and path length, masking eventual differences in relation to DG rats. In fact, Figures 7 and 8 reveal that decrements in the amount of time spent within the day-before critical counter in trial 1 from phase 1 (ITI = 5 min) to phase 2 (ITI = 0 min) by the control animals (Fig. 8) were accompanied by a corresponding decrease in latency and path length (Fig. 7); these decrements favor this latter interpretation.

Performance in the working memory task required the daily acquisition of new information. Supposedly, DG rats could make use of the same strategies that resulted in the improvement of their performance during the reference memory task and that allowed them to search for the platform within the correct quadrant, during the probe test. However, they did not exhibit any improvement during working memory training, showing that such strategies seem not to work in this latter task. Dentate gyrus-lesioned rats may require repeated exposure to the same information for acquisition to occur. Alternatively, these data may be interpreted according to the proposal by Eichenbaum et al. (1990), that the hippocampal formation is required for flexible transfer of relationships among cues within an environment from original learning patterns to novel situations. Consistent with this view, in the reference memory task, when a constant relationship between environmental cues and platform location was offered, the performance of DG rats showed some improvement (remember, however, that the starting points in each trial varied within a training session). Conversely, in the working memory task, when a new association between known environmental cues and platform location was required, the performance of DG rats completely deteriorated.

In conclusion, the present study shows that the primary loss in DG lesioned rats relates to the use of place information. However, repeated training in the reference memory task resulted in performance improvement, showing that these rats are able to acquire relevant information about the task, possibly through guidance and/or orientation strategies. Conversely, when DG rats were required to deal with one-trial place learning in a familiar environment, such as the working memory version of the water-maze task, which requires flexibility in the use of acquired information, disruption of performance was dramatic.

## Acknowledgment

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