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Cognitive Performances and Locomotor Activity Following Dentate Granule Cell Damage in Rats: Role of Lesion Extent and Type of Memory Tested

Hélène Jeltsch, Fabrice Bertrand, Christine Lazarus, and Jean-Christophe Cassel

Laboratoire de Neurosciences Comportementales et Cognitives, Université Louis Pasteur/CNRS, UMR 7521, 12 rue Goethe, F-67000 Strasbourg, France

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Intradentate injection of colchicine is one of the techniques used to destroy granule cells. This study compared the behavioral effects of various amounts of colchicine (1.0, 3.0, and 6.0 μg; Col 1, Col 3, and Col 6, respectively) injected into the dentate gyrus of adult Long–Evans male rats. Starting 10 days after lesion surgery, behavioral testing assessed home-cage and open-field locomotion, alternation in a T-maze, water-maze, and radial-maze learning according to protocols placing emphasis on reference, and working memory. All of these tasks are sensitive to hippocampal disruption. Histological verifications showed that the extent of the lesions depends on the dose of colchicine (index of dentate gyrus shrinkage: −33% in Col 1, −54% in Col 3, and −67% in Col 6 rats). Colchicine dose-dependently increased nocturnal home cage activity (an effect found 10 days but not 5 months after surgery), but had no significant effect on open-field locomotion or T-maze alternation. A dose-dependent reference memory impairment was found during the acquisition of spatial navigation in the water maze; Col 3 and Col 6 rats were more impaired than Col 1 rats. During the probe trial (platform removed), control rats spent a longer distance swimming over the platform area than all rats with colchicine lesions. In the working memory version of the test, all rats with colchicine lesions showed significant deficits. The deficits were larger in Col 3 and Col 6 rats compared to Col 1 rats. The lesions had no effect on swimming speed. In the radial-maze test, there was also a dose-dependent working memory impairment. However, reference memory was disrupted in a manner that did not differ among the three groups of lesioned rats. Our data are in line with the view that the dentate gyrus plays an important role in the acquisition of new information and is an integral neural substrate for spatial reference and spatial working memory. They also suggest that damage to granule cells might have more pronounced effects on reference than

Address correspondence and reprint requests to Hélène Jeltsch, LN2C, Université Louis Pasteur/CNRS, UMR 7521, 12 rue Goethe, F-67000 Strasbourg, France. Fax: 33 388 358 442. E-mail: helene.jeltsch@psycho-ulp.u-strasbg.fr.

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on working memory in the radial maze. Finally, they demonstrate that part of the variability in the conclusions from previous experiments concerning the role of granule cells in cognitive processes, particularly in spatial learning and memory, may be due to the type of tests used and/or the extent of the damage produced.

Key Words: colchicine; granule cells; hippocampus; open field; radial maze; reference memory; T-maze; water maze; working memory.

INTRODUCTION

Even though there is converging evidence for hippocampal participation in learning and memory (e.g., Moser & Moser, 1998), less is known about the intrinsic processes allowing the hippocampus to elaborate, store, and use memory traces. In that regard, several questions remain open. For instance, does the hippocampal formation operate as a unitary structure, or are different regions involved in different functions? Is there more than one type of hippocampus-dependent memory? If so, are specific mnemonic functions restricted to specific regions of the hippocampal formation? Are specific subpopulations of hippocampal neurons, or rather, networks interconnecting various types of neurons dedicated to particular functions? Over the past, many studies have addressed such questions. Some of them more precisely focused on the possible role which certain structural (sub)components of the hippocampus may play in mediating cognitive processes such as learning and memory.

Evidence suggests that the hippocampus is functionally heterogeneous, with different portions along its longitudinal axis having different functional roles. The hippocampus is described as a largely unidirectional transverse loop of excitatory pathways through the dentate gyrus, CA3, CA1, and subiculum. Although the intrinsic pattern of connectivity basically repeats itself along the longitudinal axis of the hippocampus (Andersen, Bliss, & Skrede, 1971), the morphological organization of afferent and efferent connections changes from the septal to the temporal pole, an observation suggesting that the dorsal (septal) and ventral (temporal) regions of the hippocampus might be responsible for different functions (Moser & Moser, 1998). This is in line with early work showing that behavior can be affected differentially by dorsal and ventral lesions of the hippocampus (Hughes, 1965; Nadel, 1968; Stevens & Cowey, 1973). For instance, maze learning can be disrupted by dorsal and not by ventral hippocampal damage. Later work with more selective lesions confirmed that lesions of the dorsal hippocampus resulted in severe impairment of spatial memory, whereas lesions of the ventral hippocampus produced only modest effects (Moser, Moser, & Andersen, 1993).

Other results suggest that, among all hippocampal cells (e.g., granule cells, pyramidal neurons), several subpopulations seem crucial for the expression of normal memory functions. For instance, lesions of CA3 pyramidal neurons by injections of kainic acid (Handelmann, Olton, O'Donohue, Beinfeld, Jacobowitz, & Cummins, 1981; Sutherland, Whishaw, & Kolb, 1983) or ischaemic lesions of CA1 pyramidal neurons (Olsen, Scheel-Krüger, Møller, & Jensen, 1994; Volpe, Pulsinelli, Tribuna, & Davis, 1984; Volpe, Davis, Towle, & Dunlap, 1992) impair spatial learning in rats. Similar deficits are also observed after damage to granule cells in the dentate gyrus (Sutherland et al., 1983).

Concerning the role of the dentate gyrus in the mediation of memory processes, research has been facilitated by the fact that intragyral injections of colchicine, a neurotoxicant binding to tubulin and blocking mitosis and axoplasmic transport (Hanson & Estrom, 1978), preferentially destroy granule cells and mossy fibers within the dentate gyrus (Goldschmidt & Stewart, 1982). Other lesion methods consist of subjecting neonates to X-irradiation (e.g., Bayer, Brunner, Hine, & Altman, 1973) or adults to adrenalectomy (e.g., Conrad & Roy, 1993). Granule cells are the target of several afferents (e.g., from the septal region, the entorhinal cortex) and the first link of a loop from where mossy fibers project to CA3 pyramidal neurons which, over the Schaffer collaterals, contact CA1 pyramidal cells (Andersen et al., 1971). Concerning the role played by the granule cells in memory, studies reported that intrahippocampal injections of colchicine disrupted reference memory performances in the Morris water maze (Nanry, Mundy, & Tilson, 1989; Sutherland et al., 1983) and impaired acquisition and retention of a working-memory task in the eight radial-arm maze (Walsh, Schulz, Tilson, & Schmechel, 1986). In order to test working memory and reference memory in the same session, Jarrard, Okaichi, Stewart, and Goldschmidt (1984) used a procedure in which only four of eight arms of the maze were consistently baited. These authors showed that intradentate colchicine had no effect on the working memory component of the task, whereas the reference memory component was transiently affected. These results are not only at variance with the aforementioned ones, but also with those reported by McLamb, Mundy, and Tilson (1988) who used the same task as Jarrard et al. (1984) and showed that intradentate colchicine injections impaired the acquisition of working memory, but did not affect reference memory. Such different outcomes might be due to differences in the tasks (radial maze vs water maze) or the testing protocols used in a given task (working memory vs reference memory), as well as in the lesion procedures (amounts injected, number of injection sites, injection coordinates) which may have produced differences in the extent of the colchicine-induced damage. For example, McNaughton, Barnes, Meltzer, and Sutherland (1989) injected a total amount of 2.4 μg / hippocampus using six sites / hemisphere, McLamb et al. (1988) used a total amount of 5 μg / hippocampus with two sites / hemisphere, Sutherland et al. (1984) used 6 μg / hippocampus with three sites / hemisphere, Jarrard et al. (1984) injected 7 μg / hippocampus using two sites / hemisphere, and Emerich and Walsh (1990) injected up to 14 μg / hippocampus using two sites / hemisphere.

The possibility that different testing procedures or variable lesion extents might account for the aforementioned discrepancies was addressed in the present experiment. This experiment used adult Long–Evans male rats to investigate and compare the behavioral consequences of intragyral injections of various amounts of colchicine (1.0, 3.0, and 6.0 μg of colchicine). Particular emphasis was placed on the comparison of reference and working memory performances in two test situations used previously by other authors, a water-maze and a radial-maze test. Working memory was also assessed in a T-maze alternation task. Finally, as colchicine has been reported to induce hyperactivity (e.g., Tilson, Rogers, Grimes, Harry, Peterson, Hong, & Dyer, 1987; Walsh et al., 1986), the rats were also tested for locomotor activity in their home cage and in an open field. After completion of behavioral testing, all rats were sacrificed and the brains were examined to determine the extent of the lesions in the hippocampus.

MATERIAL AND METHODS

Subjects and design

Forty-four young adult Long–Evans male rats (R. Janvier, France) weighing 250–275 g at the time of surgery were used. They were housed individually in transparent Makrolon cages (42 × 26 × 15 cm). The colony and testing rooms were maintained on a 12:12 h dark–light cycle (lights on at 7:00) under controlled temperature (23° ± 1°C). Animals were housed with *ad libitum* access to food and water throughout the experiment except for training and testing in the radial maze for which they were kept under a food-restricted diet in order to maintain their body weight at approximately 80% of its initial value. The behavioral performances of the rats were assessed in several tests known for their sensitivity to hippocampal lesions or denervations. Upon completion of behavioral testing, at about 5 months postlesion, all rats were sacrificed and the brains processed for histological examination.

All procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national (council directive 87848, October 19, 1987, Ministère de l'Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animales; authorization 67-14 bis to H.J. and 6212 to J-C.C.), and international (NIH Publication 86-23, revised 1985) laws and policies.

Colchicine lesions

At approximately 60 days of age, the rats were allocated into four groups. Three groups received bilateral infusions of colchicine (Sigma, St. Louis, MO) administered at different doses while the control group received an equal amount of vehicle alone. All surgical procedures were conducted under aseptic conditions, using equithesin anesthesia (3.2 ml/kg; ip). Rats were lesioned using 1.0 μg (Group Col 1, $n = 11$), 3.0 μg (Group Col 3, $n = 11$), or 6.0 μg (Group Col 6, $n = 11$) of colchicine (Sigma). Colchicine (1.0, 3.0, or 6.0 mg/ml) in sterile 0.1 M phosphate buffer (pH 7.4) was injected into five sites (0.1 μl was delivered at each site over 1 min) along the dorsal extent of the dentate gyrus in each hemisphere. Anteroposterior (AP), lateral (L), and vertical (V) coordinates (in millimeters) were taken relative to bregma (Paxinos & Watson, 1986): (1) AP = −2.2, L = ±1.0, V = −5.0; (2) AP = −3.0, L = ±1.4, V = −4.9; (3) AP = −4.0, L = ±2.0, V = −4.8; (4) AP = −4.7, L = ±2.6, V = −5.1; (5) AP = −5.3, L = ±3.5, V = −5.2. The incisor bar was placed at the level of the interaural line. Injections were made through a 2.0-μl Hamilton syringe. After each injection, the needle was left *in situ* for 2 min to allow diffusion before being retracted.

Behavioral studies

Behavioral studies were begun approximately 10 days after lesion surgeries. Five tests were run in the following order: a locomotor activity test, first in the home cage and then in an open field, an alternation test in a T-maze, and two tests assessing spatial learning and memory capabilities in a Morris water maze and a radial-arm maze.

Home-Cage Activity

The spontaneous activity of the rats was recorded in their home cages for 23 h, starting at 11:00. This test was performed at two postoperative delays (10 days and 5 months after lesion surgery). The rats were brought to the experimental room 15 min before recording of the activity was started. A first period of observation (habituation to experimental conditions) lasting for 3 h was distinguished from a second period that lasted for 20 h and comprised the nocturnal period (19:00–07:00). Each cage was traversed by two infrared light beams targeted on two photocells 4.5 cm above floor level and 28 cm apart. The number of crossings of the cage (successive interruptions of the beams) was monitored continuously by a microcomputer. Data collected were the number of cage crossings during the habituation period and the diurnal and the nocturnal periods.

Open-Field Locomotion

The open field was an unpainted wooden square enclosure with 43.5-cm-high walls and a 65 $\times$ 65 cm floor divided into 25 equal squares. The illumination was provided by a 45-W white light placed centrally, 1.80 m above the field. Each rat was placed in a corner square, facing the corner. The number of squares crossed and the number of rearings were counted by the experimenter for a period of 10 min divided into five blocks of 2 min. This test was run 13 days after the surgery.

T-Maze Alternation with a Forced Choice

The apparatus was a grey Perspex T-maze (10 cm high $\times$ 10 cm wide) with a transparent Perspex roof and a 40-W white lamp located 105 cm above the choice point. The 45-cm-long stem and 21-cm-long side arms ended in 20-cm-long interchangeable start / goal boxes. Guillotine doors were located at the entrance to the stem and on each side arm; they allowed the rat to be confined in the start box or in the chosen arm. For a trial, the rat was first subjected to a forced run for which it was placed in the start box for 10 s before the guillotine door was opened. Once the rat had reached the end of the forced arm (the other arm being closed), it remained in this first arm for 20 s before another run was allowed (with both arms open). Two trials (one trial consisting in a forced run followed by a test run) were given each day during 5 days, so that 10 possible alternations were tested for each rat over the 5-day period. Start and goal boxes were interchanged with the rat inside and the intertrial interval on each day lasted for about 2 h. The test was run from the 17th to the 21st days after the surgery.

Morris Water Maze

Apparatus. The Morris water maze consisted of a circular pool (diameter 160 cm; height 60 cm) filled with water to a height of 30 cm. The water (21°C) was made opaque with powdered milk. The pool was located in an experimental room surrounded by cues external to the maze (e.g., chair, computer, desk, animal cages) which could be used by the rat to guide its navigation. The pool was virtually divided into four quadrants of equal surface and different starting points were identified. A circular platform (diameter 11 cm) was placed in the pool, 1 cm underneath the water surface. For each trial, the rat was

placed in the pool, facing the wall at a randomly designed starting point from where it was released and given a maximum of 60 s to reach the submerged platform. When the rat had climbed onto the platform, it was allowed to remain there for 10 s before being removed and placed on the next (in the reference memory procedure) or the same (in the working memory procedure) starting point. If the rat failed to find the platform within 60 s, it was placed on it for 10 s by the experimenter. Using a video-tracking system (Noldus, The Netherlands), the latency to reach the platform and the distance swum by the rat were recorded for each trial. At the end of the trials, the rat was removed from the pool, dried, and returned to its home cage. This test was performed with two procedures, one sensitive to disruption of reference memory and the other to disruption of working memory.

Reference memory procedure. During 5 consecutive days, the platform was placed in the NW quadrant (Q3). Each day, each rat was given four trials, with the starting point differing for each trial. When the last trial of the last day was completed, the platform was removed and the rat was given a probe trial for 60 s. The testing procedure used before the probe trial is generally considered to provide a measure of spatial reference memory, while the probe trial is considered to measure the strength of spatial learning. This test was performed during the 4th week after lesion surgery.

Working memory procedure. The testing protocol was similar to that used for testing reference memory except that the platform was placed in a new location each day and the rat was released from a single starting point for the four consecutive daily trials separated by 10 s. The rat was tested for 5 consecutive days. This test was performed during the 5th week after lesion surgery.

Radial-Arm Maze (RAM)

Apparatus. Starting 8 weeks after the surgery, RAM training and testing were run using two identical gray polyvinylchloride RAMs placed in an experimental room with several different visual cues around the mazes. The radial maze, elevated 68 cm above floor level, had eight arms (56 cm long and 10 cm wide) radiating from a central octagonal platform (diameter 40 cm), with a concave food well located 3 cm from the end of each arm. A 3-cm-high border was fixed to the arms and 30 x 20 cm walls were fixed to each arm entrance. Access to each arm was controlled by guillotine doors. In each maze, 16 infrared photocells (2 per arm, 1 at 12 cm from the entrance, and the other 10 cm from the end, with the infrared beam 4 cm above floor level) enabled the entries and movements of rats to be followed. Sequences of photocell beam interruptions were monitored with a microcomputer. The mazes remained in the same location with respect to extramaze cues.

Training procedure. The body weight of all rats was reduced progressively (over 10 days) and subsequently maintained at about 80% of the free-feeding value throughout testing. Water was available *ad libitum.* All rats were habituated to eat food pellets (45 mg, Noyes, distributed by Sandow Scientific, UK) in the maze on 5 days prior to testing. On the first day, only one arm was accessible and its food well was filled with eight calibrated food pellets. On the second day, two adjacent arms were accessible with four pellets placed in each food well. For the 3 following days, three adjacent arms were accessible with two pellets placed in each food well.

Testing procedure. Following training, all rats were tested once a day for 32 trials. The procedure used allowed to determine two types of memory failures (working and reference memory; Jarrard, 1983) within the same session. On a single trial, the rat was placed on the central platform with all guillotine doors open, but only four arms were baited. Two patterns of baited arms were used: 1, 3, 4, 6 and 2, 5, 7, 8. The baited arms were always the same for one given rat. The rats remained on the maze until all four reinforcements had been eaten or until 5 min had elapsed, whichever occured first. Reference memory supposes information that remains constant over time to be stored and used appropriately (i.e., the never-baited arms) and difficulties in reference memory are thus reflected by choices of arms that were never baited; working memory supposes information that is pertinent only within a short period of time to be stored and used appropriately, and impairments in working memory are indicated by repeated entries into arms that have already been visited within the trial. Working memory errors can be divided further into repeated entries into baited arms that have been already visited (working memory correct: WM-C) and reentries into arms that are never baited (working memory incorrect: WM-I). The testing procedure is that described by Jarrard et al. (1984). After 32 trials, testing was interrupted for 10 days. Retention performances were then measured over 8 trials.

Histological verifications

After completion of all behavioral testing, each rat was given an overdose of sodium pentobarbital (100 mg / kg) and perfused transcardially with 50 ml of 0.9% saline, followed by 60 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4, 4°C). After extraction, the brain was postfixed for about 4 h and transferred into a 0.1 M phosphate-buffered 20% sucrose solution for about 36–40 h. Coronal sections (30 μm) were cut on a freezing microtome and were collected onto gelatin-coated slides. The sections were dried at room temperature and stained with cresyl violet (Sirkin, 1983). An evaluation of the extent of the lesions was performed using a morphometric approach. The areas of the remaining parts of the dentate gyrus and Ammon's horn were measured in both hemispheres at -1.8, -2.8, -3.8, -4.8, and -5.8 mm anterior to bregma (Paxinos & Watson, 1986). At -4.8 and -5.8 mm from bregma, the area of Ammon's horn was measured only up to a depth of 5.5 mm from the dura. The comparisons considered the surface of each remaining area at each level of anteriority with the data from the right and the left hemisphere added according to the hippocampal region considered and the level of anteriority.

Statistical analysis

All data were analyzed using an analysis of variance (ANOVA) followed, when appropriate, by 2×2 comparisons based on the Neuman–Keuls test (Winer, 1971).

RESULTS

Behavioral data

Home Cage Activity

Data are shown in Fig. 1.

Habituation period (Fig. 1A). ANOVA of the average hourly activity scores recorded

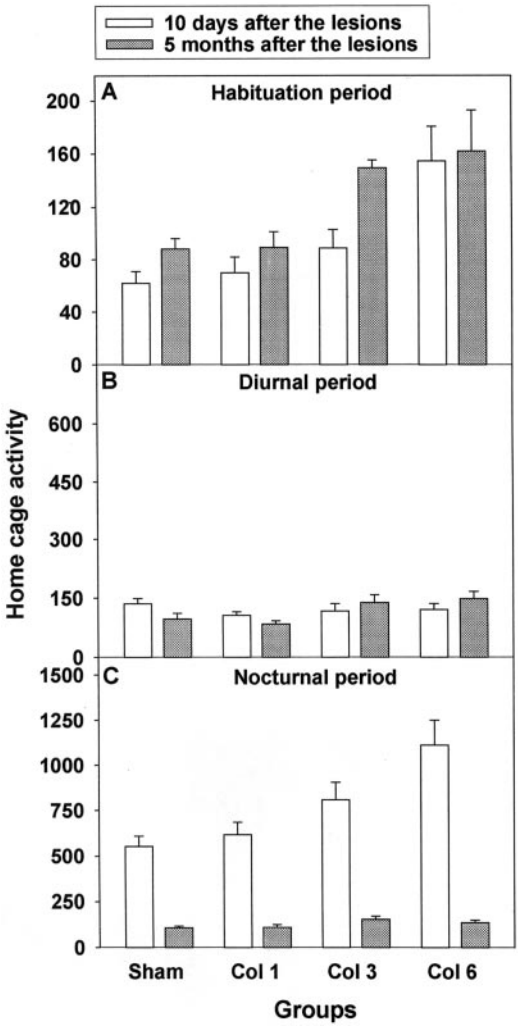

FIG. 1. Mean (±SEM) home cage activity scores recorded during the 3 h of habituation (A) and the subsequent diurnal (B) and nocturnal (C) period of the cycle in rats subjected to the injection of vehicle (Sham) or to that of 1.0 (Col 1), 3.0 (Col 3), or 6.0 μg (Col 6) of colchicine.

during the habituation period 10 days and 5 months after the lesion surgeries showed a significant "Group" effect, $F(3, 40) = 11.38$, $p < .001$, which was due to increased locomotor activity in Col 6 rats compared to Sham, Col 1, or Col 3 rats ($p < .01$ in each case) and in Col 3 rats compared to Sham and Col 1 rats ($p < .01$ in each case). No significant difference was found between Sham and Col 1 rats. There was also a significant "Delay" (time between surgery and each test) effect, $F(1, 40) = 4.38$, $p = .04$, but no significant "Group × Delay" interaction. The Delay effect was due to an overall increase of locomotion at the second delay compared to the first one ($p < .05$).

Day period (Fig. 1B). Scores recorded during the diurnal phase did not differ significantly among the four groups, $F(3, 40) = 2.49$, $p = .07$, or between both postsurgical delays, $F(1, 40) = .06$, $p = .81$.

Night period (Fig. 1C). Concerning the nocturnal phase, ANOVA showed significant Group and Delay effects, $F(3, 40) = 6.83$, $p = .0008$, and $F(1, 40) = 196.24$, $p < .001$, respectively, as well as a significant Group × Delay interaction, $F(3, 40) = 6.56$, $p = .001$. The Group effect was due to overall activity scores which were significantly increased in Col 6 rats compared to Sham and Col 1 rats ($p < .01$). There was no significant difference among Sham, Col 1, and Col 3 rats or between Col 3 and Col 6 rats. The significant Delay effect was due to an overall level of locomotion which was smaller on the second as compared to the first delay ($p < .001$), while the interaction is due to the disappearance of the lesion-induced effects over time.

Open-field locomotion. Data are shown in Fig. 2. ANOVA of the locomotion scores only showed a significant "Block" effect, $F(4, 160) = 27.45$, $p < .001$, which was due to an overall decline of locomotion over time, the activity scores decreasing significantly from Block 1 to Block 5 (e.g., Blocks 2, 3, 4, and 5 vs Block 1; Blocks 3, 4, and 5 vs Block 2, and Block 5 vs Blocks 3 and 4, $p < .05$, at least, in each case).

T-maze alternation with forced choice. Data are shown in Fig. 3. ANOVA showed no Group effect, $F(3, 40) = .27$, $p = .84$.

Morris Water Maze

Reference memory. Data are shown in Fig. 4. The ANOVA of the mean escape distances (Fig. 4A) showed significant effects of factors Group, $F(3, 40) = 17.70$, $p < .001$, and Day, $F(4, 160) = 32.3$, $p < .001$, as well as a significant interaction between both, $F(12, 160) = 4.6$, $p < .001$. Similarly, the ANOVA of the latencies (Fig. 4B) to reach the platform showed significant effects of factors Group, $F(3, 40) = 22.8$, $p < .001$, and Day, $F(4, 160) = 46.1$, $p < .001$, as well as a significant interaction between both, $F(12, 160) = 3.1$, $p < .001$. The Group effect was due to significantly impaired

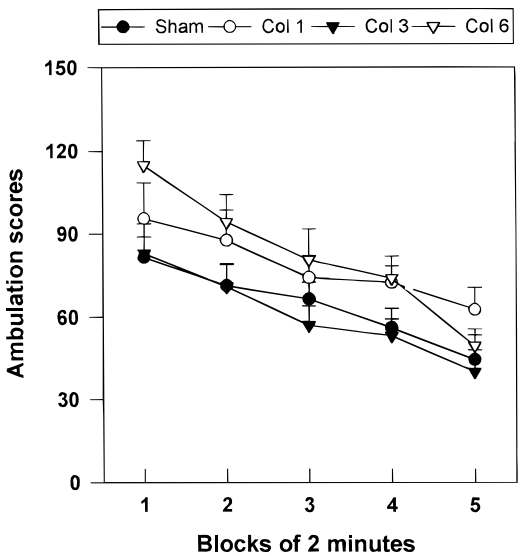

FIG. 2. Mean (±SEM) open-field ambulation scores found 13 days after surgery in rats subjected to the injection of vehicle (Sham) or to that of 1.0 (Col 1), 3.0 (Col 3), or 6.0 μg (Col 6) of colchicine.

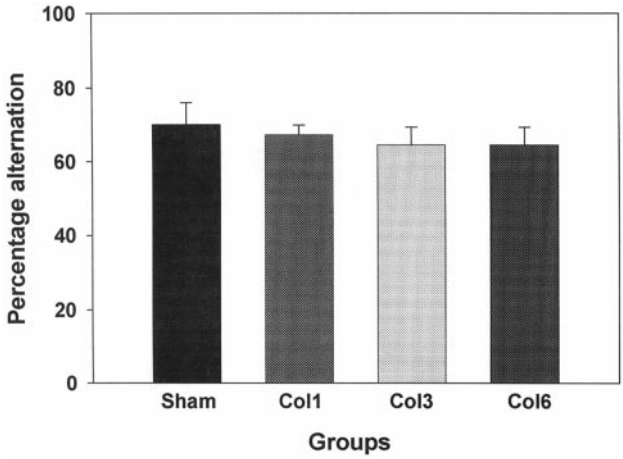

FIG. 3. Mean (±SEM) percentage of alternation in the forced T-maze test in rats subjected to the injection of vehicle (Sham) or to that of 1.0 (Col 1), 3.0 (Col 3), or 6.0 μg (Col 6) of colchicine.

performances in all lesioned rats (particularly groups Col 3 and Col 6) in which distances and latencies were increased compared to Sham rats ($p < .001$ in each case). While there was no significant difference between groups Col 3 and Col 6, Col 1 rats showed an overall level of performances which was significantly better than in Col 6 and Col 3 rats ($p < .05$ at least, in each case), but was still significantly worse than that of Sham rats ($p < .05$). The Day effect was due to a significant improvement of the overall level of performance over time, the overall latencies becoming significantly lower. All possible differences among days 1 to 5 were significant ($p < .001$ in all cases), except that the average values on days 4 and 5 did not differ significantly from each other. Also, the overall distances decreased significantly over time, except that the distances swum on day 2 were significantly longer than on day 1 in the four groups of rats ($p < .05$ in all cases). Finally, the interaction between both factors can be interpreted as reflecting a marked decrease of distances and latencies in the group of Sham rats compared to a weaker decrease, particularly in Col 3 and Col 6 rats. This was confirmed by a linear trend analysis: for distance and latency, the linear trends of Sham and Col 1 rats did not differ significantly from each other, but they were significantly different from those of Col 3 and Col 6 rats ($p < .01$ for each variable, in each case). The average swimming speed was also analyzed (Fig. 4C). There was no significant Group effect, but significant effects of the Day, $F(4, 160) = 39.6$, $p < .001$, and of the interaction between both factors, $F(12, 160) = 2.4$, $p = .006$. The Day effect was due to an overall swimming speed which was significantly larger on days 2 to 5 compared to the first day ($p < .01$ in all cases) and significantly smaller on days 3 to 5 compared to day 2 ($p < .01$ in all cases). The interaction between both factors was due to a marked decrease of the swimming speed of Sham rats from day 2 to 3 as opposed to more stable (Col 1, Col 3) or even increasing (Col 6) values noted in all lesioned rats ($p < .05$ in all cases). Mean group values (over all days) were, in centimeters per second, 24.01 ± 1.0 in Sham rats, 23.66 ± 1.2 in Col 1 rats, 22.11 ± 1.1 in Col 3 rats, and 23.58 ± 1.3 in Col 6 rats.

Probe trial. Data are shown in Table 1 and Fig. 5. During the probe trial, the rats showed average swimming speeds which did not differ significantly across the four groups.

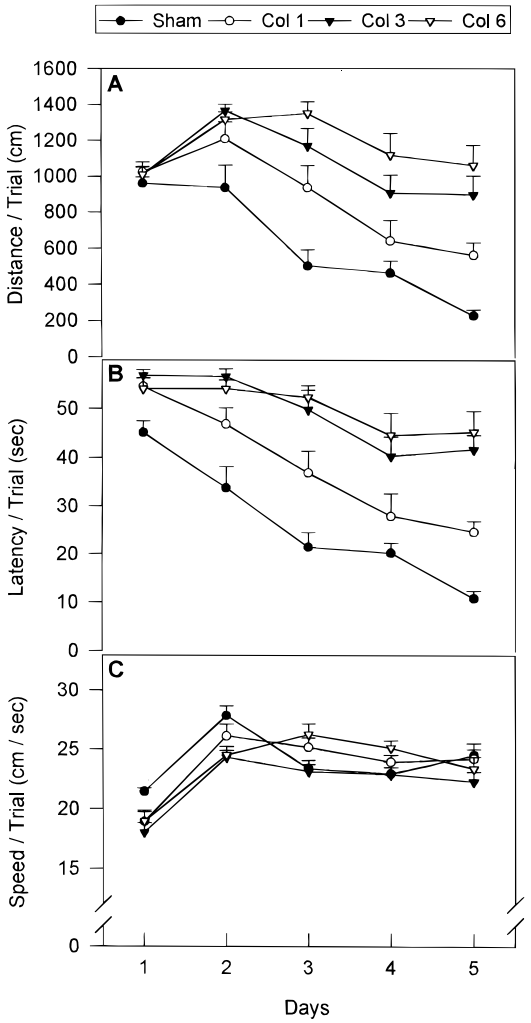

FIG. 4. Mean (±SEM) distances (A), latencies (B), and swimming speeds (C) to reach the platform in the water-maze test made according to a protocol placing emphasis on reference memory. The rats were given four trials each day with different release positions. Group abbreviations refer to rats subjected to the injection of vehicle (Sham) or to that of 1.0 (Col 1), 3.0 (Col 3), or 6.0 μg (Col 6) of colchicine.

Mean group values were, in centimeters per second, 25.03 ± 1.0 in Sham rats, 26.68 ± 1.0 in Col 1 rats, 24.87 ± 1.1 in Col 3 rats, and 24.68 ± 1.1 in Col 6 rats. ANOVA of the average time spent in the platform quadrant (Q3) failed to show a significant Group effect, $F(3, 40) = 1.6$. ANOVA of the average distance swum in the platform quadrant showed a tendency toward a significant Group effect, $F(3, 40) = 2.6$, $p < .10$, which was due to average values, which in Col 3 and Col 6 rats tended to be lower than in Sham rats ($p < .10$ in each case). Concerning the distances swum over the area where the platform was located during the acquisition phase of the test, the ANOVA showed a significant Group effect, $F(3, 40) = 1.1$, $p = .01$. This effect was due to Col 3 and Col 6 rats searching with significantly shorter distances over this location than Sham or Col 1 rats (p .01 in each case). The Col 1 group performed at a level which was intermediate

TABLE 1

Performances and Variables Recorded during the 1-min Probe Trial in the Morris Water Maze

Behavioral variable	Sham				Col 1				Col 3				Col 6			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Swimming speed (cm/s)	27.7 (±0.8)	28.9* (±1.8)	24.5 (±0.9)	25.0 (±0.6)	28.9 (±1.0)	28.4 (±0.8)	26.5 (±0.9)	26.3 (±0.9)	25.3 (±1.0)	26.3 (±0.9)	25.4 (±0.9)	24.7 (±0.9)	24.8 (±1.7)	25.4 (±1.3)	24.8 (±1.1)	25.5 (±1.1)
Time (s)	9.3* (±0.9)	10.7* (±1.2)	23.1 (±1.5)	14.7* (±1.4)	9.7* (±1.4)	15.3 (±1.2)	19.8 (±1.8)	14.3 (±1.1)	11.2* (±2.3)	14.4 (±1.5)	19.5 (±2.3)	14.2 (±1.4)	11.0 (±0.9)	16.5 (±1.6)	19.0 (±1.5)	12.9 (±1.1)
Distance (cm)	254.7* (±27.7)	294.4* (±34.0)	546.6 (±5.0)	370.2* (±41.0)	278.1* (±43.0)	419.2 (±33.0)	506.3 (±38.0)	366.7 (±25.0)	271.3 (±53.0)	367.1 (±43.0)	473.2 (±49.0)	345.0 (±33.0)	264.0* (±27.0)	403.6 (±39.0)	461.3 (±43.0)	321.7 (±31.0)
Distance (cm) on platform area			46.4 (±8.0)				28.9 (±7.5)				15.0** (±3.3)				19.8** (±6.3)	

All data are means (±SEM). Sham: sham-operated rats; Col 1: 1.0 μg of colchicine; Col 3: 3.0 μg of colchicine; Col 6: 6.0 μg of colchicine.

Statistics: *significantly different from value found in Q3, $p < .05$; **significantly different from sham-operated rats, $p < .05$.

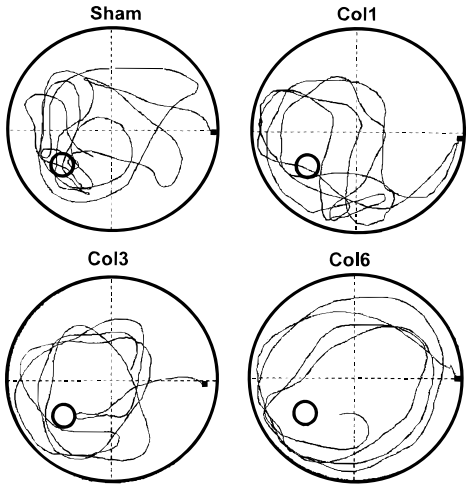

FIG. 5. Swimming patterns during the probe trial in rats subjected to the injection of vehicle (Sham) or to that of 1.0 (Col 1), 3.0 (Col 3), or 6.0 μg (Col 6) of colchicine. The start point is indicated by the filled square and the location of the platform during the acquisition trials by the open circle.

between the control and the two other lesion groups, but their performances failed to differ significantly from those of any of these groups. Representative swimming patterns are shown in Fig. 5.

From the data of the acquisition trials, one should expect that the rats given colchicine perform poorly in the probe trial, especially in groups Col 3 and Col 6 in which the rats failed to show good learning performances. The aforementioned statistical analysis indicates that this was not the case. Our explanation is that the rats knew approximately where to look for the platform, but they were unable to locate it precisely. Therefore, they did not find the platform with much success during the acquisition trials, although they were searching for it in the appropriate area, and this failure was subsequently reflected in the probe trial: Col 1, Col 3, and Col 6 rats were more often in the area of the platform compared to the other three (see Table 1), but within this area, they did not swim as often as Sham rats over the correct location of the platform (see Fig. 5).

Working memory. Data are shown in Fig. 6. The ANOVA (Group $\times$ Trial) of the mean escape distances (Fig. 6A) showed significant effects of factors Group, $F(3, 40) = 10.7$, $p < .001$, and Trial, $F(3, 120) = 2.6$, $p = .05$, as well as a significant interaction between both, $F(9, 120) = 3.5$, $p < .001$. The Group effect was due to overall distances which were significantly higher in the three groups of lesioned rats compared to their sham-operated counterparts ($p < .001$ in all cases). There was no significant difference among the three lesion groups. The Trial effect was due to overall distances which were significantly higher on trials 1, 2, and 3 than on trial 4 ($p < .001$ in all cases), the average values on trials 1, 2, and 3 differing not significantly from each other. Finally, the interaction between both factors can be interpreted as reflecting the important decrease of distances swum by Sham rats as opposed to the more stable distances in all other rats (linear trend analysis showed a significant decrease over trials only in Sham rats, $p < .001$). ANOVA of the latencies to reach the platform (Fig. 6B) showed significant effects of factors Group, $F(3, 40) = 13.1$, $p < .001$, and Trial, $F(3, 120) = 33.3$, $p < .001$, but not of the

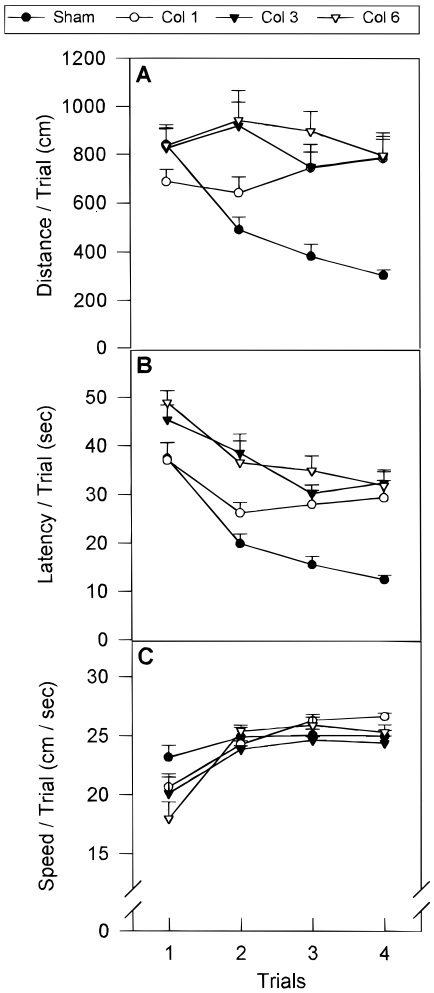

FIG. 6. Mean (±SEM) distances (A), latencies (B), and swimming speeds (C) to reach the platform in the water-maze test made according to a protocol placing emphasis on working memory. The rats were given four trials each day with an identical release position. Group abbreviations refer to rats subjected to the injection of vehicle (Sham) or to that of 1.0 (Col 1), 3.0 (Col 3), or 6.0 μg (Col 6) of colchicine.

interaction between both factors. The Group effect was due to overall latencies which were significantly increased in groups Col 3 and Col 6, compared to Sham or Col 1 rats ($p < .001$ in each case). There was no significant difference between groups Col 3 and Col 6. Col 1 rats performed significantly better than the rats from both other lesion groups, but their performance did not reach the level observed in Sham rats ($p < .05$ in all cases). The Trial effect was due to an overall improvement of the performance over time, the latencies on trials 2, 3, and 4 being significantly lower than latencies on trial 1 ($p < .01$ in all cases). Globally, this was observed in the Sham group but also in all other groups, an observation which may explain the lack of a significant Group × Trial interaction. Concerning the swimming speed (Fig. 6C), data are similar those ones noted in the reference memory task. ANOVA showed no significant Group effect, but significant effects of factor Trial, $F(3, 120) = 37.3$, $p < .001$, and of the interaction between both factors,

$F(9, 120) = 2.7$, $p = .006$. The Trial effect was due to an overall swimming speed which was significantly larger on trials 2 to 4 compared to the first trial ($p < .01$ in all cases). All other differences were not significant. The interaction between both factors was due to a marked increase of the swimming speed of all lesioned rats on trial 2 as opposed to more stable values noted in Sham rats ($p < .05$ in all cases). Mean group values were, in centimeters per second, 24.54 ± 0.4 in Sham rats, 24.48 ± 1.3 in Col 1 rats, 23.25 ± 1.0 in Col 3 rats, and 23.66 ± 1.8 in Col 6 rats.

Radial-Arm Maze

Analyses were run on maze performances (errors) averaged over four-trial blocks. Data are shown in Fig. 7.

Reference memory. Reference memory was impaired in all colchicine-lesioned rats. ANOVA of the number of reference memory errors (Group × Block) showed significant Group, $F(3, 40) = 18.04$, $p < .001$, and Block, $F(7, 280) = 19.31$, $p < .001$, effects, as well as a significant interaction between both factors, $F(21, 280) = 5.08$, $p < .001$. The Group effect was due to the poor performance of all lesioned rats which showed a significantly increased number of errors compared to their sham-operated counterparts ($p < .01$ in each case). There was no significant difference among Col 1, Col 3, and Col 6 groups. Regardless of the dose of colchicine, the Block effect was mainly due to a significant decline of the overall number of reference memory errors over time and more precisely during the three last blocks compared to all previous ones ($p < .001$ in each case). However, and this observation might partly account for the interaction, the improvement of the scores over the last trial blocks was significant only in Sham and Col 1 rats. In Sham rats, the performances in Blocks 6, 7, and 8 were significantly better than in all previous blocks, $p < .001$ in each case, and in Col 1 rats, the performances in Block 8 were significantly better than in all previous ones, $p < .05$ in each case. The scores of the

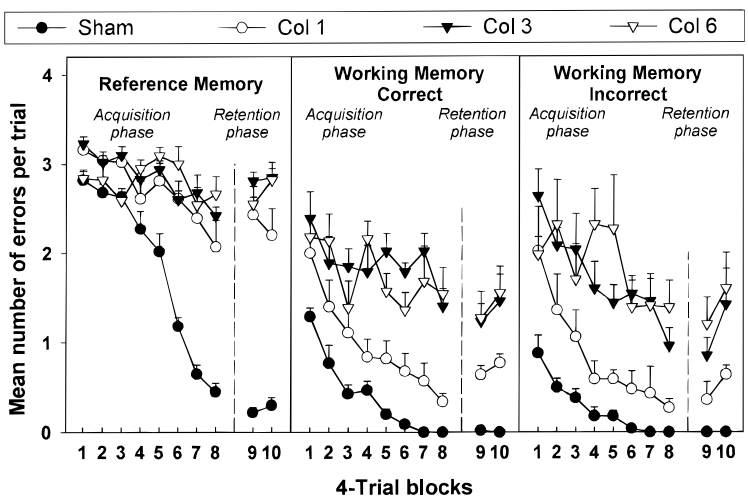

FIG. 7. Mean (±SEM) number of reference (A), working memory correct (B), and working memory incorrect (C) errors in the radial-maze test. Group abbreviations refer to rats subjected to the injection of vehicle (Sham) or to that of 1.0 (Col 1), 3.0 (Col 3), or 6.0 μg (Col 6) of colchicine.

other lesioned rats did not fluctuate significantly over trial blocks. A comparison of the linear trends in the different groups showed the scores of Sham rats to decrease significantly faster than in the three other lesion groups ($p < .001$); the only other significant difference was between Col 1 and Col 6 rats.

Working memory. The ANOVA of the number of working memory correct as well as that of the working memory incorrect errors (Group × Block) showed significant effects of factors Group [WM-C: $F(3, 40) = 11.71$, $p < .001$; WM-I: $F(3, 40) = 8.48$, $p < .001$] and Block [WM-C: $F(7, 280) = 8.96$, $p < .001$; WM-I: $F(7, 280) = 8.08$, $p < .001$], but no significant interaction between the two factors.

For the WM-C type of errors, the Group effect was due to significantly impaired performances in all colchicine-lesioned rats compared to the sham-operated ones ($p < .01$ in all cases). Also, while there was no significant difference between the performances of Col 3 and Col 6 rats, the rats from both groups performed significantly more poorly than those from the Col 1 group ($p < .01$ in all cases). For the WM-I type of errors, the Group effect was due to significantly impaired performances in Col 3 and Col 6 rats compared to either Sham or Col 1 rats ($p < .001$ in all cases). The difference between Col 3 and Col 6 rats, as well as that between Col 1 and Sham rats, was not significant.

As concerns the Block effect, it was due to an overall improvement of the performances over time. For the WM-C errors, this improvement was quite fast, the overall number of errors on Block 2 and on all other following ones being significantly lower than on Block 1 ($p < .05$ in all cases). In the same way, the overall number of errors observed on Block 2 was significantly higher than on all other following blocks ($p < .05$ in all cases). For the WM-I type of errors, the improvement was apparently slower than for WM-C errors, the overall number of errors on Blocks 1 and 2 differing not significantly from each other but being significantly higher than on all subsequent blocks ($p < .05$ in all cases).

In comparing the scores observed in the last block (Block 8) of the acquisition phase and the ones observed in the first block (Block 9) of the retention-testing phase, it becomes apparent that the interruption of testing did not result in any significant increase of the overall number of errors [reference memory: $F(1, 40) = 0.11$, $p = 0.73$; WM-C: $F(1, 40) = 0.46$, $p = 0.50$; WM-I: $F(1, 40) = 0.84$, $p = 0.36$].

As concerns the performances after the 10-day interruption of testing, ANOVA of the number of reference memory errors, as well as that of the number of WM-C errors, showed a significant impairment in all colchicine-lesioned rats [Group effect for reference memory: $F(3, 40) = 39.93$, $p < .001$; for WM-C: $F(3, 40) = 11.32$, $p < .001$] in comparison with their sham-operated counterparts ($p < .01$ in all cases). The difference between reference memory performances of Col 1, Col 3, and Col 6 rats was not significant. As to WM-C errors, Col 6 rats performed significantly more poorly than Col 1 rats ($p < .05$). Regarding WM-I errors, there was an overall Group effect, $F(3, 40) = 8.50$, $p < .001$, which was due to a larger number of errors in Col 3 and Col 6 rats compared to Sham rats ($p < .001$). In addition, Col 6 rats performed more poorly than Col 1 rats ($p < .05$).

Histological verifications

Typical examples of granule cell lesions in the dentate gyrus and, where observed, of pyramidal cells in Ammon's horn in each group are shown in Fig. 8. Examination of the

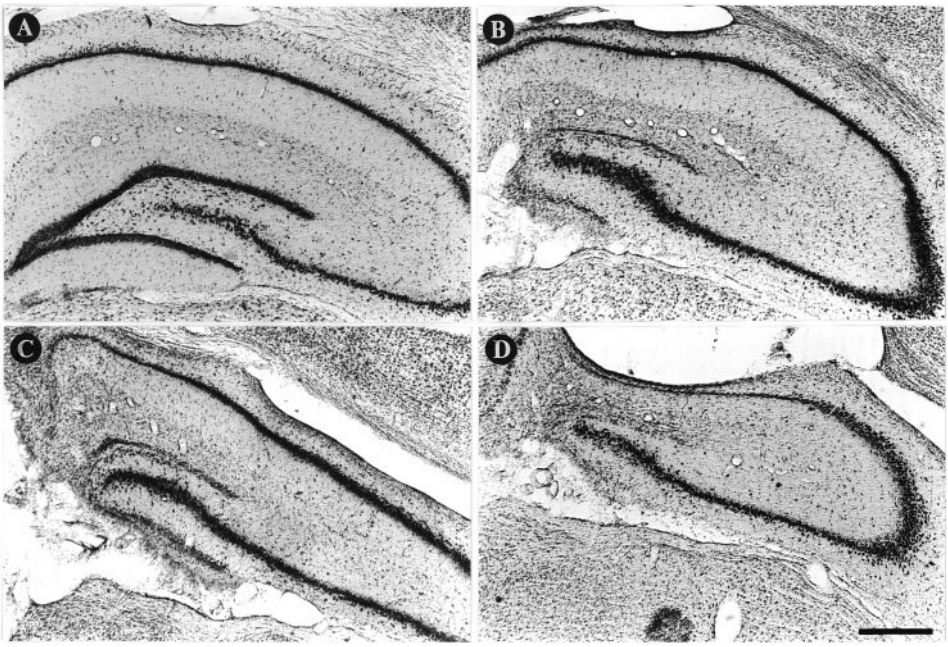

FIG. 8. Illustrations of typical colchicine-induced lesions on coronal sections through the dorsal hippocampus stained with cresyl violet. (A) Injection of vehicle; (B) injection of 1.0 μg colchicine; (C) injection of 3.0 μg colchicine; (D) injection of 6.0 μg colchicine. Scale bar = 500 μm.

sections stained with cresyl violet showed extensive loss of granule cells in the dentate gyrus of all rats, although the lesion extent clearly appeared to depend upon the amount of colchicine injected. In Col 1 rats, granule cells were damaged from about −2.8 to −5.8 mm from bregma (Paxinos & Watson, 1986), but the most lateral parts of the granule cell layer were preserved in almost all sections (damage complete at about −2.8 mm from bregma). Pyramidal neurons in regions CA1 and CA2 were not affected. In the most medial part of region CA3, there was a slight loss of neurons at about −3.8 mm from bregma. In Col 3 rats, the granule cell damage started already at about −1.8 mm from bregma and extended up to −5.8 mm. The lateral parts of the granule cell layer found to be preserved in Col 1 rats were completely gone bilaterally. There was a clear-cut disruption (no cell visible, or cells visible but layer thinner than in sham-operated rats) of one-half to two thirds of the pyramidal cell layer in region CA1. The damage in region CA3 was more extensive than in Col 1 rats, but was not complete. In Col 6 rats, the lesions were comparable to those found in Col 3 rats except that they were larger as to their anterior, posterior, and lateral extents.

These observations were confirmed by statistical analysis of the morphometrical data (Fig. 9). When the areas of the dentate gyrus and Ammon's horn were compared at each level of anteriority, we found an overall Group effect in each of these two subregions of the hippocampus [from −1.8 to −5.8 mm posterior to bregma, $F(3, 40) < 30.0$, $p > 0.001$, at all levels and in each hippocampal subregion]. When the total areas were analyzed (addition through all five levels from bregma; data not illustrated), we found a significant Group effect in the dentate gyrus, $F(3, 40) = 389.60$, $p < .001$, and Ammon's horn, $F(3, 40) = 161.30$, $p < .001$. In the dentate gyrus, this effect was due to a significant reduction

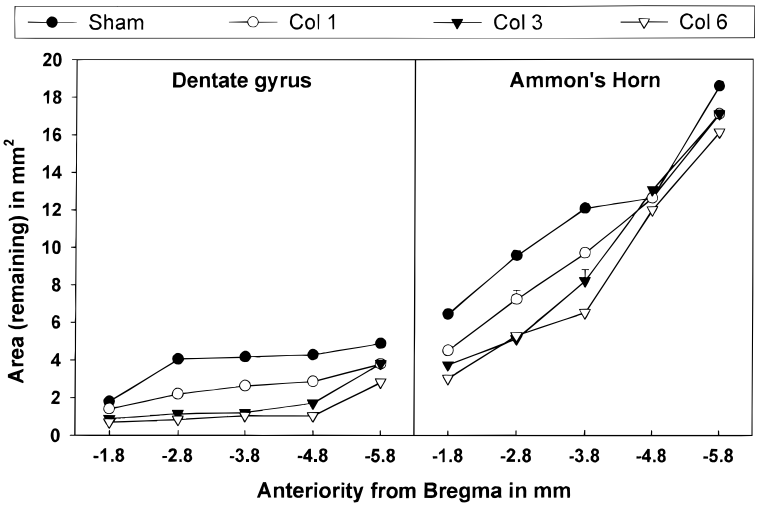

FIG. 9. Mean (±SEM) areas of the dentate gyrus (left) and Ammon's horn (right) at various levels posterior to bregma in rats subjected to the injection of vehicle (Sham) or to that of 1.0 (Col 1), 3.0 (Col 3), or 6.0 μg (Col 6) of colchicine. All values correspond to the surface of the remaining area of each hippocampal subregion (left and right hemisphere added). Nonvisible SEMs are masked by the symbols.

of the global area in Col 1 (−33 %, $p < .001$), Col 3 (−54 %, $p < .001$), and Col 6 (−67 %, $p < .001$) rats compared to their sham-operated counterparts, but also in Col 6 compared to Col 1 and Col 3 rats ($p < .001$), as well as in Col 6 as compared to Col 3 rats ($p < .001$). The percentages were computed as [(total area in lesioned/total area in intact) × 100] − 100. In Ammon's horn, we found exactly the same significant differences, although the percentage of reduction was lower than in the dentate gyrus (−19 % in Col 1, −25 % in Col 3, and −32 % in Col 6 rats).

DISCUSSION

The behavioral results first confirm that the locomotor activity increases dose-dependently. This dose-dependent effect only appeared in the home cage and was not persistent. In a study by Walsh et al. (1986), the hyperlocomotion was found to last for about 1 month. When open-field locomotion scores were considered, there was no significant effect of the lesions. Also, T-maze alternation scores were not affected significantly by colchicine. All of these observations are in line with previous reports (Tilson et al., 1987; Walsh et al., 1986). Colchicine produced a dose-dependent impairment of reference and working memory in the water-maze test. A similar observation was made in the radial-maze test, except that maximal impairment of reference memory was found already at the lowest dose. These observations all confirm a crucial involvement of the hippocampus and, within the hippocampus, of the dentate gyrus granule cells in spatial memory processes (e.g., Jarrard, 1993; O'Keefe & Nadel, 1978).

The morphometrical measurements show that the bilateral intradentate administration of 1.0 μg of colchicine caused a 33% decrease of the total volume index of the dentate

gyrus, whereas the volume index of Ammon's horn was decreased by only 19%. A bilateral administration of 6.0 μg of colchicine caused a 67% decrease of the volume index of the dentate gyrus, whereas that of Ammon's horn was decreased by 32%. The difference between the extent of the damage in the dentate gyrus and Ammon's horn confirms a higher sensitivity of colchicine toward granule cells compared to pyramidal cells and even, at a low dose, a certain selectivity of the toxin (Goldschmidt & Stewart, 1982; Jarrard et al., 1984; Lothman, Stein, Wooten, & Zucker, 1982; Tilson et al., 1987; Walsh et al., 1986). Although it may give an idea of the lesion extent, it is important to note that a reduction of the area of a given hippocampal region does not indicate that there is a proportional loss of neurons. In Col 1 and Col 3 rats, the volume index of Ammon's horn is reduced by 19 and 25%, respectively, but there is no morphological evidence of significant pyramidal cell loss in Col 1 rats. In Col 3 rats, the loss of pyramidal neurons is clear cut, but it is restricted to a medial portion of CA1. The partial destruction of pyramidal cells in Col 3 and Col 6 rats is in line with reports indicating that the selectivity of colchicine decreases at high doses (e.g., Tilson & Peterson, 1987).

The major goal of the present experiment was to investigate whether the cognitive impairments produced by intradentate administration of colchicine may be specific to a type of task (e.g., radial maze vs water maze) or a type of memory (reference memory vs working memory), and to what extent the severity or type of this impairment could depend on the extent of the lesion. Our results enable us to discuss each issue.

Colchicine-Induced Effects and Difficulty of the Test?

Regardless of the extent of the lesion, we provide evidence that the effects of colchicine may depend on the test used. T-maze alternation, radial-maze, and water-maze performances are considered sensitive to disruption of the hippocampus (e.g., Jarrard, 1993; O'Keefe & Nadel, 1978; Olton, 1983; Olton, Becker, & Handelmann, 1979). In contrast to the clear-cut effects in the water-maze and the radial-maze tests, intradentate administration of colchicine had no effect on T-maze alternation, even at the highest dose injected. Regardless of the intragroup variabilities, the mean alternation scores of the three lesion groups and those of the control group were extremely close to each other (within a 5% range). This absence of effect, as opposed to straightforward effects in both other tests, might be related to the fact that the spontaneous-alternation task can be solved easily on the basis of a single praxis strategy (e.g., turn left or turn right) and does not necessarily suppose high-level processings of spatial data (e.g., make a choice according to a cognitive map). Such a view is in line with studies suggesting that brain regions other than the hippocampus, e.g., the caudate nucleus, may contribute to cognitive performances by processing egocentric items (e.g., Cook & Kesner, 1988; Kesner, Bolland, & Dakis, 1993). It is also in line with a view considering that some of the learning which is spared after hippocampal damage might be mediated by the caudate nucleus (e.g., Packard, Hirsh, & White, 1989). Conversely, in the radial maze and the water maze, spatial information must be used and memorized for successful completion of the task. A dose-dependent impairment was found in each of these two tasks, suggesting that as long as the task places a high demand on spatial processes, a deficit can be expected after damage of granule cells. Such a view is compatible with the consideration that granule cells may operate with place-cell properties (McNaughton, Barnes, Mizumori, Green, & Sharp,

1991) and thus with neurophysiological characteristics thought to be linked with encoding of spatial information (e.g., Poucet, 1997). It is also possible that these data reflect various difficulty levels of the different tasks, a difficult task requiring probably more intact hippocampal tissue than an easier one. As suggested by an anonymous reviewer, in the working memory version of the water maze, rats had to learn a new position and to forget that of the previous day. This task is presumably more difficult than always remembering the same position from day to day (reference memory version). In the radial maze, rats may also use nonspatial cues to solve the working memory component of the task, possibly placing a lower demand on the hippocampal network than our reference memory version of the test. Based on recent experiments, it has been suggested that the dentate gyrus is involved in performing a spatial separation analysis (Gilbert, Kesner, & DeCoteau, 1998). Thus, it is possible that depending on the task (working vs reference memory) and the test (T-maze, water maze, radial maze), the demands in terms of spatial separation are different: for some tests, even the smallest lesion may have been sufficient to induce nearly maximal effects, whereas for others, a dose-dependency could be observed (see also McDonald & White, 1995, as to ambiguous vs unambiguous discriminations).

Altogether, if one disregards the type of memory tested in each task (see below), and also the fact that the testing procedures of each task are not necessarily sensitive to identical functional aspects of learning (Hodges, 1996), there does not seem to be a difference between the effects of granule cell lesions on radial-maze and water-maze performances. Therefore, one may consider that, between the radial maze and the water maze, it is not the type of test used that mainly accounts for the discrepant findings reported in the literature and summarized in the Introduction.

Specificity of Colchicine-Induced Effects to a Type of Memory?

From previous studies, it is known that granule cell lesions impair reference memory in a water maze (Nanry et al., 1989; Sutherland et al., 1983). We do confirm these findings and now show also that working memory is impaired in this particular test. Neither of these deficits can be ascribed to problems that rats with hippocampal damage might have had with their ability to swim correctly. Actually, in the three groups of colchicine-injected rats, the swimming speed was not different from that of controls, and the deficits were found on latencies and on distances. Distances, unlike latencies, are less sensitive to sensorimotor biases (e.g., Lindner, 1997). These observations are also in line with those of Vanderwolf, Kolb, and Cooley (1978), who have shown that, in rats, the ability to swim is not affected by hippocampal damage (see also Cassel, Cassel, Galani, Kelche, Will, & Jarrard, 1998; Morris, Garrud, Rawlins, & O'Keefe, 1982). Whereas Jarrard et al. (1984) found reference but not working memory to be impaired in the radial maze, McLamb et al. (1988) used the same task and reported working memory, not reference memory, to be impaired. When compared to our present findings, several remarks can be made. First, we found working memory to be impaired in both spatial tests but not in the T-maze, suggesting that the lesion-induced effects are certainly not an exclusive matter of working memory. Indeed, whatever the theoretical explanation for the motivation to alternate in a T-maze test may be, alternating requires an operational working memory, and alternation was not affected by the lesions. Second, we found both types of memory

to be impaired not only in one or the other test, but in both, and this is at variance with the data obtained by Jarrard et al. (1984) or McLamb et al. (1988).

Several maze procedures have been used to measure memory performances, including within-trial reentries in the radial-arm maze, alternation in a T-maze, learning-set or matching to position tasks in the water maze. Because an impairment in spatial working memory tasks is often found after damage to the hippocampus, these tasks have played a substantial role in the development of theories of hippocampal function. Eichenbaum, Otto, and Cohen (1994) have argued that the hippocampus processes relational information while the parahippocampal region stores information in working memory. As a corollary, damage of or within the hippocampus would be predicted to impair working memory in spatial, but not in nonspatial tasks (a view compatible with our present observation; e.g., T-maze vs radial maze), whereas lesions affecting the perirhinal and entorhinal cortices would impair working memory in any procedure, whether spatial or not. This issue has not been satisfactorily resolved because so many different methods have been used to lesion the hippocampus, and so many different tasks have been used to assess the lesion-induced effects. Our lesions have affected mainly granule cells, at least at the lowest dose. It is considered that the majority of information inputs being of spatial relevance to the hippocampus must transit through the granule cell layer of the dentate gyrus. These cells may receive them from the entorhinal cortex via the perforant path. In that way, studies reported that bilateral entorhinal cortex lesions impair spatial performances in the eight-arm radial-maze test (e.g., Hunt, Kesner, & Evans, 1994; Olton, Walker, & Gage, 1978; Otto, Wolf, & Walsh, 1997) and that the characteristics of the environmental control over hippocampal single unit activity are abolished after entorhinal cortex lesions (Miller & Best, 1980). In other words, granule cells seem to have a central position in the treatment of spatial informations within this multicellular circuitry, and this may be an explanation for the fact that, regardless of the type of memory considered, it could be the spatial load of a task that determines whether a deficit appears after granule cell damage. The picture, however, is not as simplistic as just mentioned. First, there are studies showing that lesions of the entorhinal cortex have no effect on spatial learning and memory (e.g., Jarrard & Hyko, 1994; Galani, Weiss, Cassel, & Kelche, 1998). Second, while the impairment of reference memory (but not that of working memory) was gradual in the water maze (roughly: Col 1 $<$ Col 3 $<$ Col 6), the impairment was not different among the three doses of colchicine in the radial maze, but this could be linked to variable difficulty levels of the task (see above).

Extent of the Lesions?

From the aforementioned discussion, it can be retained that the effects of granule cell lesions on spatial memory depend on the type of memory assessed but also on the type of test used to assess memory performances. In the water maze, reference and working memory are altered. The dose-dependency of this alteration is best illustrated in reference memory performances. In the radial maze, reference and working memory are also altered, but the dose-dependency now is best illustrated in working memory performances. Taken together, these observations clearly suggest that it is not possible, from a given task requiring spatial items to be processed and memorized, to make general conclusions as to the involvement of hippocampal granule cells in a given type of memory.

Interestingly, as to radial maze performances, a significant correlation was found between the volume index of each hippocampal subregion and the number of working memory errors, as well as between the volume index of only the dentate gyrus and the number of reference memory errors. Such a picture was, however, not observed when the water maze data were considered. There, the correlation between morphometrical and behavioral data was significant only for reference memory performances during the acquisition phase of testing. The lack of any apparent relation between the remaining area of the dentate gyrus and the spatial working memory or probe trial performances in the water maze may indicate that alternative searching strategies, which are perhaps independent (or less dependent) on hippocampal processes, may have compensated for the lost function.

Concerning the relation between the lesion extent (and specificity) and the behavioral deficits, it seems that further experimental studies are required in order to compare water-maze performances of rats with pyramidal cell lesions (e.g., by ischemia) with those of rats subjected to granule cell lesions (e.g., by intradentate colchicine injections). From previous studies, for instance, those based on ischemia-induced damage of pyramidal cells in region CA1 of Ammon's horn, it is known that these cells do also play a crucial part in spatial working memory (e.g., Hodges, Sowinski, Fleming, Kershaw, Sinden, Meldrum, & Gray, 1996; Nelson, Lebessi, Sowinski, & Hodges, 1997; Netto, Hodges, Sinden, Le Peillet, Kershaw, Sowinski, Meldrum, & Gray, 1993).

If one looks at the figures corresponding to spatial learning performances (Figs. 4, 5, 6, and 7), there seems to be a relationship between the dose of colchicine and either the amplitude of the working memory deficits in the radial maze or that of the reference memory in the water maze. As concerns working memory and probe trial performances in the water maze, such a relationship could not been evidenced graphically. This remark might indicate that for the memory processes just mentioned, our lesions have produced maximal functional disruptions even at the lowest dose of colchicine and, thus, that the functions involved might be more sensitive to granule cell lesions than those operating in the other testing variants.

CONCLUSIONS

Rats with colchicine-induced lesions exhibited clear-cut deficits in reference and working memory, whether tested in the radial maze or the water maze. When the spatial load of the task was weak (e.g., the T-maze alternation test), there was no deficit, suggesting that the lesions which we performed have altered mechanisms preferentially involved in processing of spatial information, rather than mechanisms involved in a type of memory process. This account is in line with the literature. With our present results, the question of whether granule cell damage alters spatial reference more than spatial working memory could not be broken up. It seems that both types of memory are sensitive to granule cell damage. Regarding the relationship discussed above between the extent of the damage and the level of impairment of each category of memory in each test, it could be that part of the discrepancy noticed in the literature is due to a multifactorial determination of the deficit: there may be a concomitant dependence on the type of task, the type of memory, and the extent of the lesion (in terms of completeness and selectivity). Our present data nevertheless suggest that for a working memory task in the water maze,

granule cells do seem to play a more important role, as for reference memory or probe trial performances in the same maze. Conversely, in the radial maze, the involvement of these cells could be of more comparable significance for both types of memory processes.

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