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Research report

Dentate gyrus-selective colchicine lesion and performance in temporal and spatial tasks

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Abstract

The effects of multiple-site, intradentate, colchicine injections on the performance of a temporal, 'differential reinforcement of low rates of responding' (DRL-20 s) task and a spatial, 'delayed non-matching-to-place' (DNMTP) task in a plus-maze were investigated in rats trained in both tasks prior to the lesion. Quantitative analysis revealed a greater than 86% reduction in the dentate gyrus (DG) of the colchicine-injected rats compared to the sham-operated controls. Dentate gyrus damage rendered rats less efficient than sham-operated controls in the performance of the DRL-20 s task. The DRL inter-response time (IRT) distribution for the DG-lesioned rats and the sham-operated controls was similar; however, while the distribution peak for the control rats was 20 s, it was 16 s for the DG-lesioned rats, indicating that the latter rats underestimated time. Performance of the DG-lesioned rats was also disrupted in the DNMTP task. However, DG-lesioned rats recovered control levels of performance during repeated training with an intertrial interval equal to 3 s. An increase in intertrial interval in lesioned and sham-operated controls disrupted performance in both groups; however, while DG-lesioned rats performed at chance levels when the intertrial interval was increased to 4 min or longer, the sham-operated controls performed at chance levels only when the intertrial interval was increased to 16 min. These results seem most parsimoniously interpreted following the cognitive map theory of hippocampal function.

Keywords: Hippocampus; Dentate gyrus; Cognitive map theory; Working memory; Timing process; Spatial discrimination; NMTP task; DRL task

1. Introduction

The hippocampal formation plays a critical role in brain function by regulating behavior and experience. While several different models of the hippocampal function coincide in ascribing memory functions to this brain structure, they disagree strongly with regard to the nature of the memory involved.

O'Keefe and Nadel [52] distinguished among alternative strategies used by animals to navigate through the environment, and suggested that more than one strategy 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 to indicate the goal location; egocentric orientation strategies are based on the rotation of the body axis relative to other axes. These strategies may be sustained by different neural systems; the hippocampal formation may be necessary for place learning. In addition, O'Keefe [53] 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, when this is possible. Normal rats apparently use these strategies simultaneously to solve spatial navigation challenges in the Morris' water maze task [81]. Further, Eichenbaum et al. [18] suggest that the integrity of the hip-

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pocampal formation is necessary for creating a cognitive map that supports flexible, spatial navigation in the environment. Their results show that rats with hippocampal damage manage to adopt taxon and egocentric orientation strategies to deal with the requirements of the task to be solved.

Honig [30] and Olton [55,57] distinguished between reference memory and working memory; working memory contains information relevant to a given trial, and is context specific, while reference memory contains information relevant to several trials, and is context independent (see [34,54,56,57]). Olton et al. [54] proposed that the hippocampal formation is involved in working memory but not in reference memory (see [37,57,62,78]). However, Meck et al. [45] noted that most demonstrations that the hippocampal system is involved in working memory, but is not required by reference memory, are based largely on tests providing spatial information. This begs the question of whether the hippocampal lesion effect results from interference with working memory for spatial but not for non-spatial information.

Different laboratories have reported that damage to the hippocampus impairs performance in a non-spatial, 'differential reinforcement of low rates of responding' (DRL) task [1,3,7,8,10,33,64,66,71]. In a DRL task, reinforcement is contingent on responses occurring a pre-defined time interval after the preceding response; that is, the rats must suppress response until a minimum time interval has elapsed since the last response, which is considered to require working memory and thus to be dependent on the hippocampal formation [42,46,50]. Further to this discussion, Meck et al. [45] emphasized that transection of the fimbria-fornix does not affect the rats' sensitivity to time, but does affect temporal working memory as revealed in a peak procedure situation with gaps.

Olton [56] argued that memory for a fixed interval schedule of reinforcement is processed by the reference memory; this may explain why performance by lesioned animals is not affected, rendering them able to complete the task. Meck et al. [45] suggested that the performance deficit exhibited by hippocampal rats in DRL tasks results from disruption of working memory rather than a deficit in estimating time.

Rawlins [65] proposed that hippocampectomy would restrict memory storage capacity, thus generating impairment to "bridging gaps" between stimuli in order to associate them, which is necessary to build up an overall map of the environment. Consistent with this view, Bannerman et al. [3] showed that rats with cytotoxic-induced hippocampal damage exhibit impairments in spatial tasks, including the water maze and the elevated T-maze, and non-spatial tasks, including the DRL task.

Different sources of evidence suggest the involvement of the dentate granule cells in encoding mnemonic information [16,17,82]. The demonstration by Goldschmidt and Steward [26,27], that the topical application of colchicine into the hippocampus produces the selective loss of dentate granule cells and mossy fibers, while leaving other hippocampal subfields reasonably intact, provided a model for studying the behavioral effects of selective neuronal loss. Using the so-called conventional lesion techniques (e.g., aspiration, and 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 [31,32]. Thus, many of the behavioral changes observed after lesions targeting the hippocampal formation may result from extra-hippocampal damage or a combination of hippocampal and extra-hippocampal damage. Since the use of colchicine minimizes some of this problem, several laboratories have used this alkaloid to investigate the effects of damage to the dentate gyrus (DG) granule cells and mossy fibers on memory function [16,17,43,44,73,79,82,83].

Although colchicine exhibits preferential toxicity for granule cells, some damage to hilar and pyramidal cells also has been reported [15,40,75,76,83]; for instance, the length of the pyramidal cell layer of Ammon's horn is significantly reduced following intradentate colchicine injection [20,75,76,79].

The aim of the present study was to investigate learning and memory changes induced by the selective loss of DG granule cells in parallel tasks involving spatial and temporal processes in the same animal. The effect of selective, colchicine-induced, DG granule cell loss was investigated on performance of both (1) a temporal task (DRL-20 s), including a detailed analysis of the time-course of bar press responses to evaluate an animal's ability to estimate time intervals precisely, and (2) a delayed non-matching-to-place (DNMTP) task in a plus-maze.

Thus, the experimental procedure allows analysis of the participation of DG granule cells in different cognitive processes. Additionally, the study provides a discussion of the theoretical underpinnings of both theories as to how information is processed in temporal and spatial tasks.

2. Experiment IA—Effect of DG-selective lesion on performance of a DRL-20 s task

Sinden et al. [71] showed that complete, ibotenate-induced, hippocampal pyramidal cell loss disrupts efficient performance of a DRL task. In the present experiment, we evaluated the effect of colchicine-induced, DG granule cell loss on performance of a pre-lesion acquired, DRL-20 s task. This experiment was run in parallel with the delayed non-matching-to-place task (see Experiment IB, below).

2.1. Materials and methods

2.1.1. Animals

Twenty naive, male Wistar rats, bred at the Central Colony Facility of the University of São Paulo at Ribeirão Preto were used. The rats were 90 days old at the beginning of the experiments, weighing from 220 to 280 g. Throughout all experiments, the animals were housed singly in steel cages in the laboratory colony room, on a 12 h light:12 h dark cycle (lights on from 8:00 to 20:00 h). The rats were kept on a food deprivation schedule at 80% of their ad libitum body weight by limiting access to food. On the 3 days preceding the

start of the behavioral pre-lesion training, the animals were individually handled for 1 min. They were also manipulated daily during weighing.

2.1.2. Apparatus

Three, identical, operant test chambers (Lafayette model 80201) were used, each measuring 20 cm × 20 cm × 23 cm. Each chamber possessed a response lever 7 cm above the floor, in the center of one of the walls. Below and to the left of the lever, was a circular opening through which food pellets (45 mg) were released as reinforcement by a dispenser. A 5 W lamp located in the center of the ceiling constantly illuminated the chamber. An interface (MRA—Electronic Equipment, Ribeirão Preto, Brazil) connected the conditioning boxes to a PC computer, which controlled the experiment and registered the data. Each experimental chamber was held within a sound proof wooden box (55 cm × 55 cm × 55 cm) provided with a 20 cm × 15 cm, transparent, acrylic window. These sets were located in a 6.0 m × 1.6 m × 3.0 m room; the interface and the computer were located in an adjacent room.

2.1.3. Pre-lesion training in the DRL-20 s task

In the first session, each rat was placed into the experimental chamber and trained to bar press for food. In the second session, which lasted 30 min, the animals were submitted to a continuous reinforcement frequency (CRF) schedule in which bar pressing, at any moment, was always followed by reinforcement. Subsequently, the rats were trained in the DRL-20 s task; bar presses were reinforced only if a minimum time of 20 s had elapsed from the previous response. Any responses exhibited less than 20 s since the last response were not rewarded and their occurrence reset the system and re-established the 20 s requirement of no response for release of the reward. A limited-hold contingency was not used. Approximately 20 min before the DRL sessions each animal received 4 g of food. Each training session lasted for 30 min. All rats received a single training session per day for 24 days. These training sessions in the DRL-20 s task were performed approximately 30 min after training in the delayed NMTP task (see Experiment IB, below).

At the end of each training session, the rats were returned to their home cages and given access to food sufficient to maintain them within the planned body weight schedule.

2.1.4. Surgery

Fifteen days after the training phase, the animals were submitted to surgery following the guidelines described by Xavier et al. [83].

Rats anesthetized with equitesin i.p. were positioned in a Kopf stereotaxic device, and the incisor bar adjusted 3.3 mm below the inter-aural 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 with a drawn glass pipette adapted to the end of the needle, mounted on a stereotaxic frame and held by a microinjector. Nine different sites in each hemisphere of 10 rats were injected with 0.06 μl colchicine (7 mg/ml) dissolved in phosphate-buffered saline (pH 7.4) to destroy the DG granule cells (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 then washed thoroughly with saline. After colchicine infusion (at 0.25 μl/min), the pipette was held in position for more 60 s to avoid colchicine back-flux up the needle tract; during this period, the dura mater was kept wet with saline to avoid cortical damage (lesioned group).

Table 1
Stereotaxic coordinates for colchicine injections [59]

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 dorso-ventral coordinate corresponds to the dura mater level.

After the injections, the wound was sutured and the animals transferred to their cages for recovery.

Ten control rats received the same treatment using phosphate-buffered saline alone (sham-operated control group).

One rat in the sham-operated group, and two rats in the lesioned group died from the anesthesia and/or surgery. Another animal from the sham-operated group was unable to perform the spatial task after surgery, and was excluded from the experiments. Thus, the data derive from eight rats in the lesioned group and eight rats in the sham-operated group.

Behavioral post-lesion testing started 20 days after surgery (recovery period).

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

2.1.5. Post-lesion testing in the DRL-20 s task

After the post-surgery recovery period, which lasted for 20 days, the animals were tested in the DRL-20 s task and, concomitantly, were tested in the delayed NMTP task (see Experiment IB, below); the procedures were identical to those used during the pre-lesion training. Twenty-four testing sessions in the DRL-20 s were run.

2.1.6. Histology

At the end of all behavioral testing in both the DRL-20 s and the delayed NMTP tasks, the animals were deeply anesthetized with ether and perfused intracardiacally with 400 ml sulphide solution. After perfusion, the brains were removed, fixed in Carnoy solution, and processed until their final embedding in paraffin. Ten-micrometer-thick coronal sections taken every 150 μm along the hippocampus were stained with cresyl-violet for anatomical analysis. Area estimates of the DG granule-cell and CA1 pyramidal-cell layers were performed using an image analysis program (Kon Tron Bildanalyse Image Analyser) coupled to a light microscope (Zeiss, Germany); all sections for each rat were included in the area estimates.

2.1.7. Data analysis

The temporal efficiency index (TEI) (see [66]) was calculated as follows: $\mathrm{TEI}\,(\%) = \{[N' + \sum_{\mathrm{Xi}<T}(\mathrm{Xi}/T)]/N\} \times 100$, N' being the number of reinforced responses, N the total number of responses, T the critical time, i.e., $T = 20$ s, and Xi the duration of the inter-response times (IRT) less than T.

TEIs were calculated daily for each rat and averaged for the statistical analysis. Data were analyzed using a repeated measures analysis of variance (ANOVA); the post hoc Newman–Keuls comparisons were conducted to establish where overall and session dif-

ferences existed among groups. Only differences with significance levels equal to or less than 0.05 were considered.

The areas of the DG in both lesioned and sham-operated control rats were compared using a *T*-test.

2.2. Histology—results and discussion

Light-microscopic evaluation of Nissl-stained sections from the brains of the lesioned rats revealed (1) extensive, bilateral, DG cell loss along the septotemporal axis of the hippocampus associated with (2) a small loss of dorsal, pyramidal CA1 cells. In addition, (3) there was no apparent cell loss in the overlying cortex (Fig. 1).

While the mean area of the DG granule-cell layer for the sham-operated control rats was $1.80 \pm 0.3\,mm^2$, the corresponding parameter in DG-lesioned rats was $0.25 \pm 0.1\,mm^2$; therefore, there was an 86% reduction in the DG after the lesion. The *T*-test showed these figures differ significantly ($T = 11.41$, $P = 0.00001$). Histological analysis also showed that the mean area of CA1 pyramidal-cell layer for sham-operated control rats was $0.92 \pm 0.3\,mm^2$, and that the corresponding parameter for the colchicine-injected rats was $0.72 \pm 0.1\,mm^2$. Even though these figures correspond to a 22% reduction in the CA1 pyramidal-cell layer associated with the colchicine-induced granule cell loss, statistics (*T*-test) revealed lack of significant difference ($T = 1.58$, $P = 0.15$). These findings replicate those described by Xavier et al. [83].

Xavier et al. [83] selectively destroyed about 90% of the DG granule cells, with diminished cell loss within the CA4 (33%) and CA1 (23%), and lack of damage to the CA3 hippocampal subfields (as revealed by quantitative stereological estimates), employing multiple-site colchicine injections throughout the DG (nine sites in each hemisphere, 60 nanoliters at each site). Spatial reference and working memory assessed in the Morris' water maze revealed that lesioned rats were significantly disrupted in place learning; however, the data showed 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 were disrupted 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, lesioned rats showed no improvement in the match-to-place procedure, with either 0 or 5-min inter-trial intervals (ITI), suggesting that their working memory for places was disrupted. Therefore, although capable of acquiring relevant information about the task, DG-lesioned rats exhibit dramatic difficulty with place strategies. Thus, interruption of the trisynaptic circuit at the DG level produces a substantial performance deficit in spatial memory tasks in the water maze task; presumably, the disynaptic and monosynaptic circuits are maintained almost completely intact since very minor damage was observed in the CA1 pyramidal subfield, and no damage was noted in the CA3 pyramidal subfield.

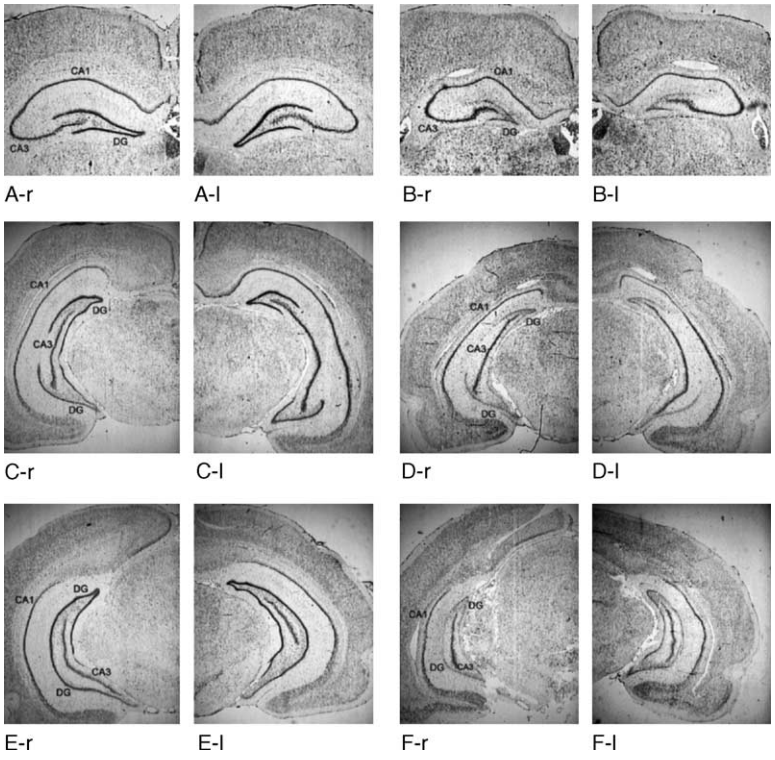

Fig. 1. Photomicrographs of cresyl-violet stained coronal sections of the hippocampus. A, C and E: septal, intermediate and temporal poles of a control rat hippocampus and dentate gyrus, respectively. B, D and F: typical multiple-site, colchicine-induced dentate gyrus at corresponding levels. r, Right hemisphere; l, left hemisphere. DG, dentate gyrus; CA1, CA1 pyramidal cell subfield; CA3, CA3 pyramidal cell subfield.

Moser et al. [49] reported that a small transverse block of the hippocampus (down to 26% of the total, including all hippocampal subfields) can support spatial learning in the water maze as long as it is located in the septal pole. In the present experiment, quantitative analysis revealed that colchicine-injected rats, relative to controls, exhibit a 22% reduction in the CA1 pyramidal cell layer. Since this CA1 area reduction was distributed throughout the septo-temporal axis of the hippocampus, it seems unlikely that it is responsible for the observed behavioral changes.

2.3. Behavior—results

Fig. 2 shows the mean TEIs for six blocks of four sessions each in the pre-lesion training and post-lesion testing in the DRL-20 s task.

A retrospective analysis of pre-lesion data, including only data from the eight rats later included in the lesioned group and the eight rats later included in the sham-operated control group, showed no statistical 'group' differences ($F_{1,14} = 0.33$, $P = 0.57$) or 'group × block' interaction effects ($F_{5,70} = 1.79$, $P = 0.12$); in addition, the ANOVA revealed a significant 'block' effect over pre-lesion training ($F_{5,70} = 131.84$, $P < 0.0001$). Together, these results show that both the 'to-be-lesioned' and the 'to-be-sham-operated' rats learned the DRL-20 s task at the same rate (Fig. 2A). It is thus assured that the results obtained in the post-lesion tests are due to the lesions and not to prior inter-group differences.

In the early stages of post-lesion testing, the performance of both lesioned and sham-operated control rats did not differ, as revealed by the TEIs, which were around 65% for both groups (Fig. 2B). Thus, the small decrement in performance during this phase relative to the late pre-lesion training may be related to the interruption of the DRL-20 s task training for the surgical procedures. Apparently, the DG lesion itself did not interfere with retention of the DRL-20 s temporal task.

As post-lesion testing proceeded, both groups improved their performance; however, while the level of performance achieved by the sham-operated control rats was about 80%, it reached only about 70% in the lesioned group (Fig. 2B). Repeated measures ANOVA revealed a lack of significant 'group' difference ($F_{1,14} = 3.44$, $P = 0.08$), and significant 'session' ($F_{5,70} = 26.01$, $P < 0.0001$) and 'group × session' interaction ($F_{5,70} = 2.64$, $P < 0.03$) effects. Post hoc Newman–Keuls comparisons showed that the groups differed among each other from the third block on. Fig. 2B shows that performance by the sham-operated control group was better than that of the lesioned group; the plateau of the TEIs is higher in sham-operated controls.

ANOVA also revealed that performance in pre-training and post-testing sessions differed between the sham-operated controls and lesioned animals. There were significant 'phase' ($F_{1,14} = 21.83$–41.20, $P < 0.0001$), 'session' ($F_{5,70} = 51.36$–83.34, $P < 0.0001$) and 'phase × session' interaction ($F_{5,70} = 8.28$–19.43, $P < 0.0001$) effects. Post hoc comparisons (Newman–Keuls Test) with data from the sham-operated controls showed that, except for data from the second block, all post-lesion TEIs differed significantly from those in block 6 of pre-lesion training (see Fig. 2B for relevant statistical comparisons). Differently, post hoc comparisons of data (Newman–Keuls Test) from the lesioned animals revealed that the TEIs in the first block of the post-lesion testing were smaller compared to the corresponding TEIs in block 6 of the pre-lesion sessions (see Fig. 2B for relevant statistical comparisons). Thus, both sham-operated controls and lesioned animals learned how to space the bar press response. However, during post-lesion testing, while the sham-operated controls exhibited an additional improvement in performance relative to pre-lesion training, this was not observed in the lesioned animals i.e., during post-lesion testing, lesioned animals did not surpass the level of performance achieved during the pre-lesion training.

The inter-response times for both the sham-operated and the lesioned groups during the pre-lesion training (i.e., retrospective analysis for the "to be" animals included in the lesioned and sham-operated control groups) (Fig. 3A) and post-lesion testing sessions (Fig. 3B) were calculated. The IRTs express the evolution of the DRL-20 s task acquisition

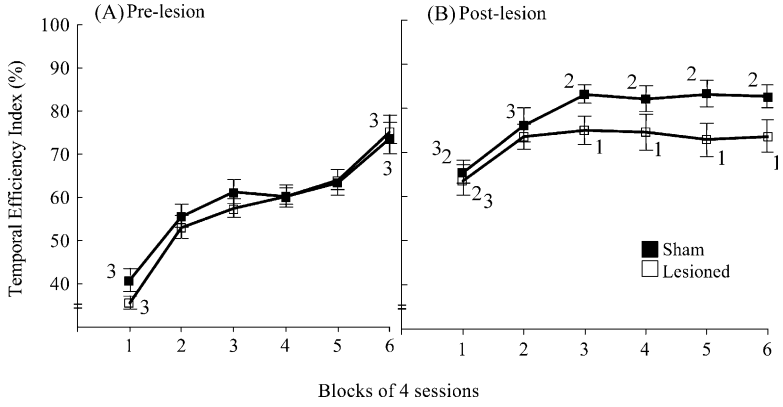

Fig. 2. Mean (±S.E.) temporal efficiency indexes (%) for lesioned and sham-operated control groups over six blocks of four sessions each during the (A) pre-lesion training, and (B) post-lesion testing of the DRL-20 s temporal task. (1) $P < 0.05$ relative to the sham-operated control group in the respective block; (2) $P < 0.05$ relative to pre-lesion block 6; (3) $P < 0.05$ relative to all other pre-lesion training and post-lesion testing blocks (Newman–Keuls test).

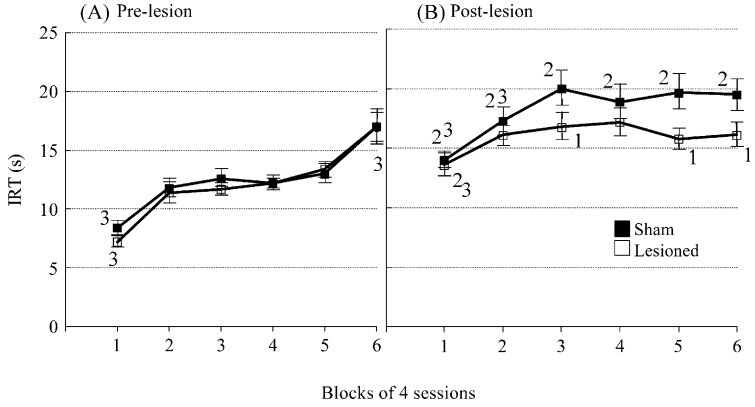

Fig. 3. Mean (±S.E.) inter-response times (IRT) for sham-operated controls and lesioned animals in both (A) the pre-lesion training, and (B) post-lesion testing of the DRL-20 s task. (1) $P < 0.05$ relative to the sham-operated control group in the respective block; (2) $P < 0.05$ relative to pre-lesion block 6; (3) $P < 0.05$ relative to all other pre-lesion training and post-lesion testing blocks (Newman–Keuls test).

and performance during both the pre-lesion training and post-lesion testing sessions; progress is revealed by an increase in the IRTs. As Fig. 3A shows, in the first block of pre-lesion training sessions, IRTs were around 7–8 s; however, as training proceeded, the IRTs increased to about 16 s for both groups in the last block of sessions (note that this is a retrospective analysis). ANOVA revealed a lack of significant IRT differences for 'group' ($F_{1,14} = 0.36$, $P = 0.56$) and 'group × session' interaction ($F_{5,70} = 0.96$, $P = 0.45$) effects, in the pre-lesion training; conversely, there was a significant 'session' effect ($F_{5,70} = 71.42$, $P < 0.0001$), indicating that "both groups" learned equally well how to increase IRT during the pre-lesion training (Fig. 3A).

In the first block of post-lesion testing, the IRTs of both lesioned and sham-operated controls did not differ (see Fig. 2B for relevant statistical comparisons) and were around 14 s; thus, selective damage to the DG does not disrupt the retention of the IRT acquired previously to the lesion. Repetitive, post-lesion testing lead sham-operated controls to reach a mean IRT of about 20 s, and the lesioned rats an IRT of about 16 s (Fig. 3B). ANOVA just failed to reach a significant 'group' effect ($F_{1,14} = 4.08$, $P < 0.06$). On the other hand, there were significant 'session' ($F_{5,70} = 17.78$, $P < 0.0001$) and 'group × session' interaction ($F_{5,70} = 2.96$, $P < 0.01$) effects, showing that there is a marked difference in the rate of increase in IRT both groups. Fig. 3B clearly shows this difference. Post hoc comparisons showed that the post-lesion IRT scores of the sham-operated controls were greater than those of corresponding pre-lesion session 6, indicating that these rats improved with the additional post-surgical testing in the DRL-20 s task; conversely, for the lesioned group, post-lesion IRT scores from blocks 2 to 6 did not differ from those of corresponding block 6 in the pre-lesion training (see Fig. 3B for relevant statistical differences), indicating that IRTs in the lesioned rats did not increase after damage to the DG.

To evaluate the animals' ability to discriminate among time intervals after training, an IRT distribution analysis was performed, including the four final pre-lesion training sessions (Fig. 4A) and the four final post-lesion testing sessions (Fig. 4B). While ANOVA, as expected, revealed a lack of significant 'group' effects for IRT scores in the four final sessions of the pre-lesion training ($F_{1,7798} = 0.04$, $P = 0.84$ (Fig. 4A), it revealed a significant 'group' difference for the IRT scores in the four final post-lesion testing sessions ($F_{1,6468} = 253.27$, $P < 0.0001$) (Fig. 4B). Inspection of Fig. 4B reveals that IRT

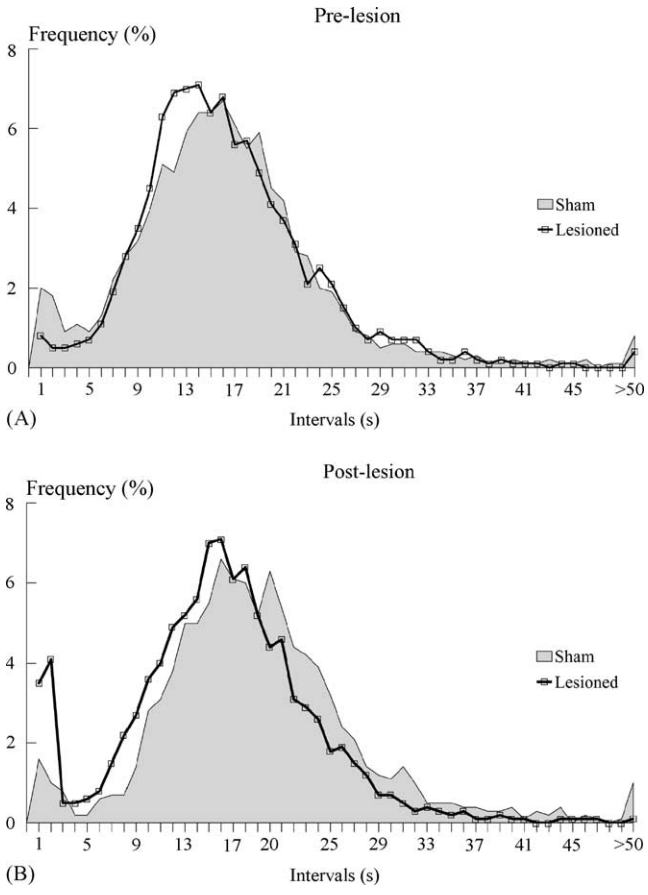

Fig. 4. Inter-response time distribution in the four final pre-lesion training sessions (A), and the four last post-lesion testing sessions (B) in the DRL-20 s task for both the sham-operated controls and the lesioned animals.

distribution of the lesioned rats parallels that seen in the sham-operated controls. Thus, like controls, lesioned rats are capable of discriminating among time intervals. However, the IRT distribution of lesioned rats is displaced 4 s to the left as if the lesioned rats had accelerated their clock.

2.4. Behavior—discussion

The present experiment shows that rats with selective, DG granule cell loss are less efficient than controls in a DRL-20 s task acquired prior to the lesion (Fig. 2).

Previous studies have shown that damage to the hippocampus disrupts performance in DRL tasks. For instance, rats whose hippocampus has been removed by aspiration experience difficulty in acquiring DRL-12 s [10] and DRL-20 s [64,68] tasks. In addition, electrolytic hippocampal lesions caused acquisition impairment in both the DRL-20 s and DRL-40 s tasks [22]. However, the impairment observed in these studies is much greater than that seen in the present experiment. While the animals in the present experiment may show less disruption in performance than those of previous studies because they received pre-lesion training in the DRL-20 s task, this interpretation is not supported by data from Tonkiss et al. [77], showing that training in a DRL-18 s task before hippocampal aspiration does not prevent profound and enduring loss of efficiency when the lesioned rats are subsequently tested in the DRL-18 s task. Another plausible explanation is that the damage induced by the aspiration and electrolytic lesion procedures is greater than that induced by the use of neurotoxins. That is, using conventional aspiration and electrolytic lesion procedures, damage is not restricted to the target area; passage fibers may be destroyed, and the degree of damage to the vasculature is unknown. Since colchicine minimizes some of these effects, its use may lead to smaller disruptions. Contrary to this notion, however, Sinden et al. [71] showed that cytotoxic, ibotenate-induced lesions of the hippocampus, CA3 subfield and subiculum caused marked acquisition impairment in a DRL-18 s task, while Bannerman et al. [3] showed that NMDA-induced damage in the hippocampus strongly disrupts acquisition of a DRL-18 s task. Since the topical administration of colchicine induces DG granule cell loss, and topical administration of ibotenate or NMDA leads to hippocampal pyramidal cell loss, it seems more likely that functional pyramidal cells are more critical for the performance of DRL tasks than are DG granule cells.

There have been several proposals that the hippocampal system underlies the performance of tasks requiring both temporal and spatial working memory, but is not required for the performance of tasks requiring reference memory (e.g. [45,46,56]).

Even while suffering massive, DG granule cell loss, the animals in the present experiment were able to postpone their bar presses (Figs. 3B and 4B); the distribution of IRTs from lesioned rats closely parallel that seen for sham-operated controls, except for a 4-s displacement of the distribution curve to the left (Fig. 4B). Apparently, DG damage leads the lesioned animals (trained in a DRL-20 s task) to under-estimate time intervals; on average, this under-estimation was 4 s. That is, while the IRT distribution peak for control animals was 20 s, as expected, it was 16 s for lesioned rats (Fig. 4B). This "acceleration of the clock" may account for the less efficient performance by the lesioned rats since an accurate estimation of a 20-s interval is required by the task.

Similar results were reported by Olton [56], who trained rats with fimbria-fornix lesions and sham-operated controls in a 20-s, fixed-interval (FI-20 s) schedule of reinforcement. The moment at which the maximum rate of responses occurred was defined as the peak time. Peak time was 20 s for control rats and 16 s for fimbria-fornix lesioned rats. Olton [56] argued that reference memory processes information in a fixed interval schedule of reinforcement; according to this author, this would explain why the performance of lesioned animals was not completely disrupted, and why they were able to complete the task. Meck et al. [45] proposed that the performance deficit in DRL tasks that follows hippocampal damage results from the disruption of working memory; in a DRL task, the absence of an external stimulus to indicate that an interval should be timed adds a component of working memory to the task since the animals must store the time elapsed from the last bar press in their working memory to complete the task. Thus, Meck et al. [46] accept the interpretation that the fixed interval value is obtained from reference memory. According to these authors, if lesioned rats showed a change in clock speed, they should be able to learn how to re-scale stimulus duration; thus, their deficit would not be permanent. On the contrary, assuming that the speed of memory storage increased would lead to underestimation of the reinforcement intervals and the IRT peak would be permanently displaced leftwards. Thus, Meck et al. [46] favor the interpretation that a change in the speed of storage of information occurs, rather than a change in internal clock speed.

Together, these results show that hippocampus dysfunction caused either by DG granule cell loss or by transection of the fimbria-fornix cause animals to under-estimate time intervals in tasks that require temporal estimation.

According to Richelle and Lejeune [66], collateral behaviors mediate temporal control. Killeen and Fetterman [35] have accepted the notion that adjunctive behaviors provide the basis for conditional discriminations of the passage of time, formalizing this view in a behavioral theory of timing to account for phenomena such as temporal generalization and discrimination, subjective shortening and paired comparisons of intervals. Later, Lejeune et al. [38] showed that the effects of drugs on temporal regulation in FI and DRL tasks are secondary to the non-specific activation of motor activity. It is widely known that damage to the hippocampal formation induces hyperactivity in rats (see [28] for review), an effect that has been ascribed to their difficulty in constructing cognitive maps of the environment [52]. Since the destruction of DG granule cells following colchicine also results in hyperactivity [4,20] and impairment of tasks that

require the construction of cognitive maps (e.g. [73,83]), it is tempting to propose that the underestimation of time seen in the DG-lesioned rats is related to this spatial difficulty.

3. Experiment IB—Effect of DG-selective lesion on performance of an NMTP task

Extensive damage to the DG granule cells strongly disrupts acquisition of working and reference memory tasks in the water maze [83]. In the present experiment, we evaluated the effect colchicine-induced, DG granule cell loss on performance of a pre-lesion acquired, NMTP task. Note that this experiment was run in parallel with the DRL-20 s task reported above.

3.1. Materials and methods

3.1.1. Animals

Since the same rats used in the DRL-20 s task were trained and tested in parallel in the NMTP task, the extent of DG granule cell damage and time-course of pre-lesion training and post-lesion testing were exactly the same, making inter-task comparisons possible.

3.1.2. Apparatus

A plus-maze made of transparent acrylic plastic (Fig. 5) was used. Each arm of the plus-maze measured 50 cm × 10 cm × 10 cm and was connected by a guillotine door to a box measuring 30 cm × 30 cm × 10 cm (box doors). Four guillotine doors connected the central square (10 cm × 10 cm × 10 cm) of the maze to the arms (arm doors). Thus, any arm and its corresponding box could be isolated from the rest of the maze by closing the arm door. Two boxes connected to opposite arms were used as starting boxes (Fig. 5, boxes S′ and S); the remaining two arms were used as reward boxes (Fig. 5, boxes A and B). In each of the boxes, 4 cm from the wall opposite the door, was an 8-mm deep, circular hole in the floor, into which food pellets could be placed. The maze was laid on a wooden structure, elevated 100 cm from the floor. This set was placed in a 1.85 m × 2.30 m room with a 100 W lamp located in the ceiling above the maze center, with visible objects on the walls, including a door, a light switch, an electrical outlet, horizontal iron bars hanging from one of the walls, a window, and a window curtain of the same color as the room walls. During training and testing, the experimenter was positioned beside the starting box (S).

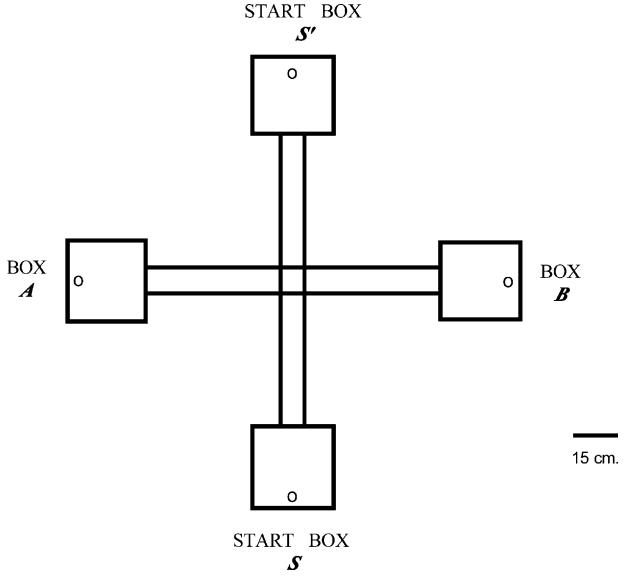

Fig. 5. Schematic representation of the plus-maze.

3.1.3. Pre-lesion training in the NMTP task

The animals were submitted to two 5-min sessions of pre-exposure to the maze, one session per day. During pre-exposure, the arm doors were open, except that of the arm giving access to the starting box S′. The rats were individually placed in the starting box S and allowed to explore the available arms and boxes freely. Three food pellets were placed in the floor holes of both boxes A and B.

The animals were then submitted to five pre-training sessions of eight trials each. In each trial, the arm door giving access to the S′ box and the arm door leading to one of the rewarding boxes (A or B) were closed, thus impeding the animal to get into those arms; the other arm and box remained opened. The rat was placed in the starting box S and was allowed to move towards the available arm and its corresponding box rewarded with two food pellets. After the food was consumed, the rat was returned to the starting box S for another trial; after the eighth trial, the animal was returned to its home cage. The rewarding box available varied from trial to trial, in a balanced fashion. In addition, with respect to the time taken to train the animals in this task, the rats were divided in two groups, each trained separately every other day.

The animals were then subjected to six training sessions in an NMTP task, each session with eight pairs of trials, with a 10-min inter-pair interval. In some trials, the arm door leading to the box S′ was kept closed, and the S box was used as the starting box; in other trials the reverse was done, i.e., the arm door leading to the S box was kept closed, and the S′ box was used as the starting box (see below). In the first trial of each pair (always run with the arm door leading to S′ closed), the arm door leading to one of the rewarding boxes (A or B) was closed, and the arm door leading to the other rewarding box was opened; two food pellets were placed in the floor hole of this latter arm. The animal was placed in the starting box S and was allowed to seek out the baited box (information trial). Three seconds after the rat had consumed the food pellets, it was replaced either into the starting box S or the starting box S′ (the arm door of the remaining starting box remained closed) and allowed to complete a second trial; in this trial, the doors of the rewarding boxes A and B were open, so that the animal had a choice of where to go (choice trial). However, only the arm not visited in the previous information trial was baited with two food pellets; therefore, independently of the starting box, the best strategy for the rat was to avoid entering the arm visited in the information trial, and to choose the arm not visited in that trial. If the rat made the wrong choice, i.e., entered the arm visited during the information trial, it was held within that box for 30 s and then returned to its home cage where it remained until the next pair of trials. This quasi-random variation of the starting box was used to minimize the adoption, by the rats, of a behavioral strategy relying on egocentric body axis orientation. Subjects were trained up to a minimum of 75% correct choices, which was achieved in two consecutive sessions.

3.1.4. Surgery

Surgical procedures were as described in Experiment IA; the same rats were used both experiments.

3.1.5. Post-lesion testing in the NMTP task

After the post-surgical recovery period, which lasted for 20 days, the animals were tested in the DRL-20 s task (see Experiment IA) and, in parallel, were tested in the NMTP task. Initially, the rats were given two pre-test sessions, a procedure identical to the pre-training phase described in Section 3.1.3. The animals were then tested six sessions using the procedures described in the training sessions of Section 3.1.3.

3.1.6. Data analysis

A spatial efficiency index (SEI) was calculated using the formula: SEI (%) = (8 − number the incorrect choices) × 12.5.

Data were analyzed using ANOVA and post hoc Newman–Keuls comparisons were conducted to establish where overall and sessions differences existed among groups. Only differences with significance levels equal to or less than 0.05 were considered relevant.

3.2. Results

Fig. 6 shows the SEIs achieved by the animals during the pre-lesion training sessions (Fig. 6A) (a retrospective analysis for rats to be included in the lesioned and sham-operated control groups is shown) and post-lesion testing sessions (Fig. 6B).

During pre-lesion training, the rats readily learned how to perform the NMTP task, quickly achieving about 85% correct responses (Fig. 6A). ANOVA revealed a lack of significant 'group' ($F_{1,14} = 0.23$, $P = 0.60$), 'session' ($F_{5,70} = 0.76$, $P = 0.60$) and 'group × session' interaction ($F_{5,70} = 0.23$, $P = 0.95$) effects, indicating that (1) performance of the groups to be subjected to lesioning and to the sham-operation was similar prior to neuro-surgery, and (2) acquisition of the task occurred early during the initial trials of pre-lesion session 1, a rate of about 85% correct responses being maintained thereafter (Fig. 6A).

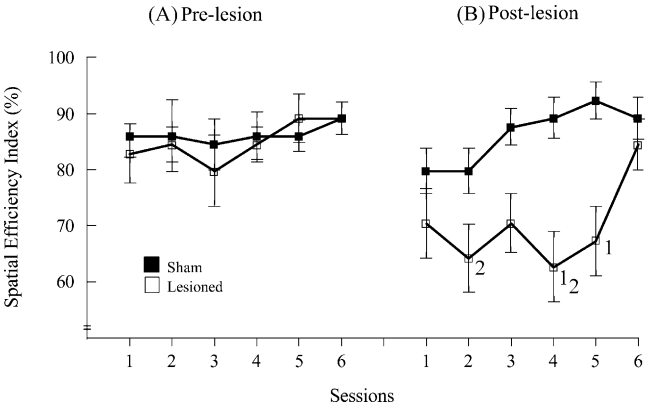

Fig. 6. Mean (±S.E.) spatial efficiency indexes (%) for sham-operated controls and lesioned groups during both (A) pre-lesion training sessions, and (B) post-lesion testing sessions in the NMTP task. (1) $P < 0.05$ relative to the corresponding sham-operated control session; and (2) $P < 0.05$ relative to the sixth pre-lesion training session (Newman–Keuls test).

Relative to post-lesion testing data, ANOVA revealed significant 'group' ($F_{1,14} = 24.47$, $P < 0.0001$) and 'session' ($F_{5,70} = 2.41$, $P < 0.04$) effects, but the lack of a significant 'group × session' interaction effect ($F_{5,70} = 1.68$, $P < 0.15$) (Fig. 6B). Thus, rats with DG granule cell loss were disrupted, relative to controls, when performing the NMTP task acquired prior to the lesion. In addition, the data show that these rats improved their performance over repetitive, post-lesion testing sessions. Post hoc Newman–Keuls comparisons revealed that lesioned animals differed significantly from sham-operated controls at post-lesion sessions 2, 4 and 5, but not at post-lesion session 6, suggesting that repeated testing helped these rats to achieve performance levels equivalent to those of the controls (see Fig. 6B for relevant statistical comparisons). Congruently, while post hoc tests did not reveal significant differences between the scores of the post-lesion testing sessions of the control group, significant differences were present between post-lesion sessions 2 and 4 compared to session 6 (see Fig. 6B for relevant statistical comparisons).

The comparisons of the pre-lesion training and post-lesion testing scores for lesioned and sham-operated controls reveal even more interesting results; while sham-operated controls do not show any statistical differences between pre-lesion training and post-lesion testing scores ('phase' effect: $F_{1,14} = 0.00$, $P = 0.99$; 'session' effect: $F_{5,70} = 1.19$, $P = 0.32$; 'phase' × 'session' interaction effect: $F_{5,70} = 0.99$, $P = 0.43$), the scores of lesioned rats did differ significantly ('phase' effect: $F_{1,14} = 25.48$, $P < 0.0001$; 'session' effect: $F_{5,70} = 2.011$, $P = 0.09$; 'phase' × 'session' interaction effect: $F_{5,70} = 1.12$, $P = 0.36$). The SEIs of lesioned animals during post-lesion testing sessions 1–5 were less than those seen over the six pre-lesion training sessions; only post-lesion testing session 6 showed scores that did not differ from the pre-lesion scores.

Therefore, while sham-operated controls preserved the performance levels achieved before the neuro-surgery, lesioned rats exhibited impairment of performance over sessions 1–5 of post-lesion testing, recovering pre-lesion performance levels at post-lesion session 6.

3.3. Discussion

These results clearly show that colchicine-induced, DG granule cell loss disrupts performance of an NMTP task acquired prior to the lesion, and that repetitive, post-lesion testing leads to the improvement of task performance (Fig. 6). Note that the quasi-random variation of the starting boxes used throughout both training and testing was performed to preclude adoption, by the rats, of ego-centric orientation strategies; performance thus relied either on place or guidance strategies (see [52]). In addition, independently of the strategy used, performance in the NMTP task requires the maintenance, in working memory, of critical information, acquired in the information trial, about the last arm visited, to

allow the choice of a different arm in the choice trial (see [54]).

Congruent with the present results are reports that hippocampal dysfunction interferes with performance of non-matching-to-sample (NMTS) and spontaneous alternation tasks [23]. Markowska et al. [42] showed that damage to the fornix substantially disrupts performance in an NMTS task in a T-maze during postoperative testing; when later tested in conditional object discrimination tasks in which the conditional stimuli changed in each discrimination and included (1) the location of the maze in the room, (2) the direction that the rat took to approach objects, and (3) the side (left or right) to which the rat turned, fornix-lesioned rats were not impaired in choice accuracy. These findings lead Markowska et al. [42] to additionally test the animals in the NMTS task to evaluate the possible occurrence of post-lesion recovery or performance. Differently from the results of the present experiment, Markowska et al. [42] showed that there was no functional post-lesion recovery since the rats were still substantially disrupted in the NMTS task, leading the authors to propose the involvement of the hippocampus in spatial working memory.

Similarly to the results of the present experiment, Emerich and Walsh [21] showed that rats with colchicine-induced DG damage exhibit a transient deficit of performance in an NMTS T-maze task, and that with time, all rats were able to reacquire the task to preoperative performance levels. The authors ascribed this recovery to the incomplete lesion of the DG, associated with extensive pre- and post-operative training. Giving the connectivity of the hippocampus, it is plausible to propose that an intact portion of the DG might support reacquisition of the task since mossy fibers project to the CA3 subfield in parallel but divergent manners [6,9,24], and since there are extensive lateral interactions among the DG cells [29,74] through excitatory interneurons located in the hilus. Favoring this proposal, Moser et al. [49] showed that even a transverse block of the hippocampus down to 26% of the total in the septal portion can support spatial learning in the water maze. Nevertheless, it is unlikely that this interpretation applies to the present results since histological analysis revealed a reduction of 86% in the area of the DG of the lesioned animals compared to sham-operated controls. In addition, such reduction in the area of the DG possibly underestimates the real granule cell loss because in association with the reduction in DG area there seems to have been a reduction in granule cell numerical density. Using quantitative stereological estimates for analyzing neuronal loss in rats subjected to the same surgical procedure as that used in this experiment, Xavier et al. [83] showed that there was about 90% DG granule cell loss, associated with 33% CA4 and 23% CA1 neuronal loss, but lack of damage to the CA3 hippocampal subfield. As a matter of fact, the neurosurgery used in this study differed substantially from that used by Emerich and Walsh [21] which injected colchicine at two sites per hemisphere; otherwise, in the present study, nine sites per hemisphere were injected throughout the septo-temporal axis of the DG, leading to greater granule cell loss. In addition, rats in the present experiment were not subjected to extensive pre- and post-lesion testing; recovery of performance was observed after only 48 post-lesion testing trials, i.e., much less than the 120 postoperative testing trials in Emerich and Walsh's [21] study.

Gilbert et al. [25] also described similar results. These authors trained rats in a delay matching-to-sample task on a circular platform. During the information trial the animals received food reward associated with an object located in one specific place on the platform. During the choice trial two identical objects, different from that seen in the information trial, were presented, one in the same location as that of the information trial and the other 15–105 cm apart, depending on the trial; the animals were rewarded if they searched for food in the same location. Then, the rats were subjected to bilateral colchicine injections (two sites per hemisphere) into the dorsal dentate gyrus and, after recovery, were re-trained in the delay matching-to-sample task. The results showed that the greater was the distance between the objects, the smaller was the disruption of performance seen in dentate gyrus lesioned rats. That is, once the lesioned rats did not have difficulties to perform the task when the two objects were 105 cm apart but did have difficulties when the objects were closer from each other, the authors proposed that the dentate gyrus supports spatial pattern separation. The spatial separation between the arms in the present experiment was 100 cm; therefore, according to Gilbert et al.'s proposal [25], there should be no disruption of performance in the dentate gyrus lesioned rats. Contrary to this prediction, however, the dentate gyrus lesioned rats of the present study showed substantial disruption of performance early in the post-lesion training sessions. It is difficult to conciliate this result with Gilbert et al.'s interpretation [25].

According to O'Keefe and Nadel [52], animals navigate through the environment using place, guidance and egocentric strategies; more than one of these strategies may be used simultaneously to solve spatial tasks. The hippocampus would be required for the use of place strategies but not for the use of guidance and egocentric orientation strategies. O'Keefe [53] proposed that when the use of one of these strategies is not possible, e.g., after lesion of the related system, the animal may rely on the remaining systems to solve the task. Normal rats seem to be able to use these strategies simultaneously to solve spatial navigation in the Morris' water maze task [81]. Even though the emphasis in the strategy preferentially adopted by the animals depends on the previous schedule of training, normal rats would give priority to the use of place strategy [47,52]. Xavier et al. [83] tested rats with extensive, colchicine-induced granule cell loss in both reference and working memory versions of the water maze task. In the reference memory version of the water maze task, the hidden platform location is kept constant throughout all training sessions; thus, the information acquired in 1 day is useful for other sessions. Differently, in the working memory version of the water maze task, a different location for

the hidden platform is used on each day of training; therefore, the information acquired in 1 day is useful only for the trials run during that day, and is not applicable to other sessions. It is important to mention that during training in both the reference and working memory versions of the water maze task, rats departed from different starting points at the pool's edge; therefore, optimal performance required knowledge of the relative positions of the multiple extra-maze cues and of the platform relative to these cues, involving navigation based on place strategies (see [81]). The results show that DG-lesioned rats were disrupted in the reference memory version of the water maze task, indicating their inability to use place strategies; however, the data suggest that some relevant information about the requirements of the task were acquired by the lesioned rats, implying that this improvement relied on guidance and orientation strategies obtained throughout repeated training [83]. In addition, lesioned rats showed no improvement in the working memory version of the water maze task, indicating that not only their working memory for places was disrupted but also that guidance and orientation strategies were not efficient in supporting performance in the water maze task.

The animals in the present experiment were trained in the NMTP task prior to surgery; thus, considering the usual priority given by normal rats to rely on place strategy to perform this type of task [47,52], it is reasonable to suggest that they learned the task based primarily on place strategy. After surgery, however, DG-lesioned rats could no longer rely on place strategy to perform the task (see [83]), differing from the sham-operated controls that could. This would explain why lesioned rats, but not sham-operated controls, were impaired in the early, post-operatory testing sessions (Fig. 6B). In addition, as post-lesion testing proceeded, lesioned rats re-acquired the pre-lesion performance, but now relying on guidance strategy (see [63]); that is, even though lesioned rats reached a level of performance equivalent to control rats at the sixth post-lesion session (Fig. 6B), this performance would be based on a different strategy. It is not clear why the post-lesion acquisition rate for DG-lesioned rats was less than that seen in pre-lesion acquisition training. One possible hypothesis is that acquisition rates for this NMTP task, using either place or guidance strategies, are intrinsically different even for normal rats. Alternatively, damage to the DG may slow down acquisition of this task relative to controls. The present results do not allow deciding among these interpretations.

Performance in NMTP tasks like that used in the present study is believed to depend on a functional working memory; i.e., the critical information collected during the information trial (about the box visited, including related intra- and/or extra-maze cues) must be maintained as long as required for a correct decision of where to go in the choice trial. Since the initial proposal by Olton et al. [54], that the hippocampal formation is necessary for working memory, there have been many reports showing that damage or disconnection of this brain structure disrupts performance in tasks that require this function [37,42,45,46,50,57,62,78]. Similar results were observed when damage was restricted to the DG granule cells [43,79,83]. The data gathered in the present experiment, however, do not support this view since, after a transient, post-operatory disruption of performance, rats exhibiting DG granule cell loss achieved control performance level (Fig. 6). Whatever the strategy used by the rats to perform the NMTP task (place, guidance or ego-centric), this strategy involves the temporary maintenance of information in working memory. Note, however, that the time interval between the information and the choice trials in the present experiment was only 3 s. It is possible that an increase in this time interval might reveal a time-dependent, working memory deficit. Experiment II addressed this possibility by testing the rats' performance with ITIs varying from 0 to 16 min.

The experimental design used in this study attempted to preclude the use of an egocentric orientation strategy; the starting box in the choice trials varied quasi-randomly from trial to trial. Experiment III tested whether this behavioral manipulation was effective in preventing the animals from adopting an egocentric orientation strategy.

4. Experiment II—Effect of increasing the interval between information and choice trials on performance of the NMTP task

Previous studies have produced controversial results regarding the effect of hippocampal damage on rats' ability to perform delayed matching-to-sample and delayed non-matching-to-sample tasks (e.g. [2,48,60,80]). For instance, rats with hippocampal lesions exhibit delay-dependent impairments in a delayed non-matching-to-sample task involving two arms selected at random during every trial in an eight-arm radial maze [60]. Similarly, Morris et al. [48] showed that an ibotenate acid-induced lesion of the hippocampus impairs performance in a delayed matching-to-place task in the water maze. Interestingly, Aggleton et al. [2] provided evidence suggesting that this impairment seems to be associated with tasks that involve spatial information; i.e., the same rats that were disrupted in a spatial, forced-choice, alternation task were not disrupted in an object, delayed non-matching-to-sample task in a Y-maze, even with retention delays of as long as 60 s. This result suggests that the origin of the controversy may be related to the nature of the information the animals must maintain in working memory during the delay to perform the task. However, Prusky et al. [61] developed a non-spatial, picture-based, trial-unique, delayed matching-to-sample task for rats analogous to that often used for testing working memory in primates, showing that selective lesions of the rat hippocampus impaired performance in this delay-dependent visual (non-spatial) working memory task. Long and Kesner [39] tested rats with hippocampal lesion in working memory tasks for egocentric distance and place information, showing that the animals

were disrupted in both types of task; however, impairment for the egocentric distance information was mild.

In the present experiment, a delayed non-matching-to-place test allowed evaluation of to what extent animals with colchicine-induced, granule cell loss are capable of maintaining the level of performance achieved by the end of the post-lesion testing in Experiment IB, when the interval between the information and the choice trials was 3 s or 1, 2, 4, 8, or 16 min.

4.1. Materials and methods

The subjects and apparatus were the same as used in Experiment IB. Thirty days after the end of the Experiment I, seven DG-lesioned rats and another seven sham-operated controls were retested during nine sessions, employing a behavioral procedure identical to that used in the pre-lesion training and post-lesion testing of Experiment IB (Retest); the animals were returned to their home cages for approximately 3 s between the information and choice trials. By the ninth session, the rats had achieved the minimum criterion of six correct responses per session, over two consecutive sessions; this criterion was defined based on a binomial distribution, with P less than 0.05 (two animals in the lesioned group did not reach the limiting criterion and were not tested in the DNMTP; thus, the data refer to five lesioned animals and seven sham-operated controls). The DNMTP procedure was then initiated and consisted of maintaining the animals in their home cages for an interval of 1, 2, 4, 8 or 16 min between the information and choice trials. Each interval was tested in a different session every other day; the each-day intervals were randomly distributed among the sessions and animals, in a counter balanced design. There were five sessions where delays varied (1, 2, 4, 8 or 16 min), one session for each interval. Thus, rats were tested once at each of five different delays. One day before each delay session, i.e., interspersed with the DNMTP sessions, there were sessions with a 3-s (0.05 min) delay, similar to the Retest sessions, to aid the animals in maintaining performance over the DNMTP sessions, and to allow comparison between them. Thus, there were five sessions with a 3-s (0.05 min) delay. Each session consisted of eight pairs of trials.

4.2. Results

Fig. 7A shows that the level of performance of both lesioned and sham-operated control animals at the early stages of Retest was reduced compared to that seen by the end of the post-lesion testing in Experiment IB (Fig. 6B); since the behavioral procedure was exactly the same in both the testing phase of Experiment IB and the Retest in this experiment, we conclude that, as expected, the 30-day interval without testing lead to a decrement in performance. However, repeated testing lead to an improvement in performance by both groups (Fig. 7A); repeated measures ANOVA including Retest data revealed a significant 'session' effect ($F_{2,20} = 10.92$, $P < 0.0001$) associated with non-significant 'group' ($F_{1,10} = 1.78$, $P = 0.20$) and 'group × session' interaction ($F_{2,20} = 1.35$, $P = 0.27$) effects; post hoc Newman–Keuls comparisons revealed that the spatial efficiency indexes of the lesioned rats in the first block of

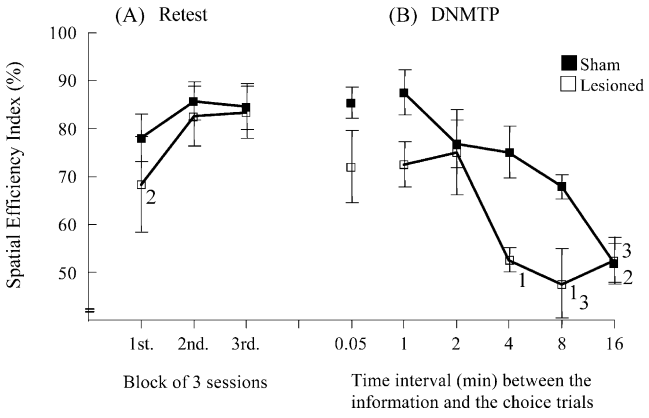

Fig. 7. Mean (±S.E.) efficiency indexes (%) for sham-operated controls and lesioned animals in the Retest (blocks of three sessions) and DNMTP sessions using time intervals of 0.05 (mean scores of five sessions), 1, 2, 4, 8 or 16 min. (1) $P < 0.05$ relative to sham-operated controls in the corresponding block (Retest) or delays (DNMTP); (2) $P < 0.05$ relative to other blocks (Retest) or delays (DNMTP) within the same group; (3) $P < 0.05$ relative to the remaining delays (DNMTP) (Newman–Keuls test).

the Retest were less than those for the second and third blocks (see Fig. 7A for relevant statistical comparisons). This was not seen for the sham-operated controls, which showed no statistical differences in their scores.

Fig. 7B shows the effect of increasing the time interval between the information and choice trials on animals performance; both lesioned animals and sham-operated controls decreased their spatial efficiency indexes as the interval between the information and choice trials increased (ANOVA 'time interval' effect: $F_{5,50} = 11.35$, $P < 0.0001$); even though this effect was significantly greater for the lesioned rats (ANOVA 'group' effect: $F_{1,10} = 10.93$, $P < 0.0001$), the rate at which it occurs is similar for both groups (ANOVA 'group × session' interaction effect: $F_{5,50} = 1.86$, $P = 0.10$). Inspection of Fig. 7B reveals that the lesioned animals reached chance levels of performance when the time interval between the information and choice trials was 4 min or more; for the sham-operated controls, the same effect was seen only for the 16-min time interval (see Fig. 7B for relevant post hoc statistical comparisons). For the interspersed sessions, in which the interval between the information and choice trials was 0.05 min (3 s), the efficiency index was 85% for the sham-operated animals and 72% for DG-lesioned rats (Fig. 7B); these scores were not significantly different.

During the early Retest sessions, performed 30 days (without behavioral training) after the end of the post-lesion testing in Experiment IB, both lesioned and sham-operated rats showed a small decrement in performance relative to their performance in the late post-lesion testing (compare Figs. 6B and 7A); this decline in performance was slightly greater in the lesioned rats compared to sham-operated controls (Fig. 7A). However, with repeated training, both groups improved their efficiency indexes, achieving levels of performance equivalent to those seen previously, which confirms the results of Experiment IB; note that over the nine Retest

sessions, the interval between the information and choice trials was only 3 s. In the DNMTP sessions, the increase in the interval between the information and choice trials resulted in a decrease of performance by both control and lesioned rats (Fig. 7B); lesioned animals, however, showed greater impairment at longer intervals, performing at chance levels when the interval was 4 min or greater, thus differing from the sham-operated controls, which performed at chance levels only when the time interval was increased to 16 min (Fig. 7B).

4.3. Discussion

Previous studies have shown that rats with hippocampal damage are particularly susceptible to the delay increments in spatial NMTS tasks [2,39,48,60]; however, there have been demonstrations that hippocampal damage also disrupts performance in non-spatial matching-to-sample tasks (e.g. [61]). Shapiro and Olton [70] noted that the magnitude of deficit in a DNMTS or a DMTS task is related to the interval between the information and choice trials, and that performance becomes closer to chance levels as the interval increases; damage to the hippocampal system seems to produce little – if any – impairment at shorter time intervals, and substantial impairment at longer time intervals. For Cohen and Eichenbaum [11], these results suggest that hippocampal-lesioned rats are able to acquire the critical information necessary for performance of the task; their difficulty seems to be related to maintaining this information in working memory.

Contrary to this notion, however, Xavier et al. [83] showed that DG-lesioned rats did not show any improvement in performance in a working memory version of the spatial navigation water maze task. Note that while the surgical procedure used by these authors and the resulting damage were identical to those of the present experiment, the behavioral procedures differed substantially. The variable-start-position version of the water maze task used by Xavier et al. [83] required the rats to reach the hidden platform departing from different starting points at the pool edge and, thus, knowledge of the relative positions of the multiple extra-maze cues and of the platform relative to these cues was required, limiting the use of guidance strategies to solve the task; therefore, impairment in the ability to use place strategies in this behavioral task could not be substituted by the adoption of guidance strategies. Congruently, DG-lesioned rats showed no improvement in the working memory version of the water maze task even when the intertrial interval was zero.

Differently, the NMTP task used in this experiment may be solved by using either place strategies or by remembering a prominent cue (or set of cues) signaling the side of the room on which boxes A and B were located; thus, guidance strategies would be also effective. In Experiment IB, rats with DG lesion were impaired in the NMTP task acquired prior to the lesion, but recovered control levels of performance with repeated training; differently, sham-operated controls maintained pre-operatory levels of performance throughout post-operatory testing (Fig. 6B). These results suggest the hypothesis that, while lesioned rats re-acquired the task using guidance strategies (see [63]), sham-operated controls simply maintained performance using the place strategy acquired prior to surgery (see Experiment IB). In this context, the slower rate of acquisition by lesioned rats in the post-lesion testing (Fig. 6B), relative to pre-lesion training (Fig. 6A), can be interpreted as revealing that acquisition of the NMTP task is more difficult when guidance strategies are required, as compared to when place strategies can be used. This may explain why (1) the lesioned rats show a greater decrement in performance induced by the 30-day interval without training between Experiments I and II (Fig. 7A), and (2) the lesioned rats exhibit a greater disruption of performance as the time interval between the information and choice trials increases (Fig. 7B). However, these differences also may be related to the direct effects of the damage on the rats' ability to maintain the critical information temporarily. Further studies are necessary to decide among these possibilities.

5. Experiment III—Do DG-lesioned rats rely on an egocentric strategy or a guidance strategy?

Although special care was taken to preclude adoption, by the rats, of egocentric orientation strategies, this experiment was performed to evaluate whether the lesioned animals might be using an egocentric orientation strategy to perform the NMTP task. A variation of the NMTP procedure was used. During the information trial, performed exactly as reported in Experiment IB, animals departed from the starting box S and were allowed to move towards one of the rewarding boxes (either A or B); the arm door leading to the remaining rewarding box was closed. During the choice trial, instead of departing from one of the starting boxes (either S or S′) as in Experiment IB, the animals departed from the rewarding box they had visited in the information trial; all arm doors of the maze were open giving access to the remaining rewarding box and both starting boxes. Thus, if an egocentric strategy were being used by the lesioned rats, they should make a body turn the opposite of that executed during the information trial; this would take them to one of the starting boxes. Differently, however, if lesioned rats were relying on a guidance strategy, they should move straight towards the opposite rewarding box. Similarly, since control rats are considered to adopt a place strategy to perform the NMTP task, they would be expected to move straight towards the remaining rewarding box.

5.1. Materials and methods

The subjects and apparatus were the same as those used in Experiments IB and II. The experiment started on the day following the end of Experiment II. The experiment involved three sessions in 1 day, with four pairs of trials (including the information and choice trials) per session. In the information trial of sessions 1 and 2, the rats were placed in the starting box S and allowed to move

towards one of the rewarding boxes (A or B) where they were rewarded; the arm door leading towards the other box was closed. The rats were then transferred to their home cages where they stayed for 3 s. They were then replaced in the box from which they had just been removed (A or B) and allowed to perform their choice trial, with all arm doors of the maze open; thus, the animals could move towards any box of the maze. In the information trial of session 3, the animals departed from the starting box S, with the arm doors leading to the starting box S′ and one of the arm doors leading to the rewarding boxes A or B open; thus, in this case, the rats could choose where to go in the information trial. Since the animals had not been rewarded in the starting box S′, this choice was considered an error; differently, the choice to move towards the rewarding box was considered a correct response. Three seconds after the information trial, the animals were subjected to the choice trial, which was identical to the previous procedures.

5.2. Results and discussion

The spatial efficiency indexes revealed that both groups exhibit levels of performance similar to those seen in the previous experiments (data not shown); in addition, the lesioned rats did not differ significantly from the sham-operated controls. On the third session of this experiment, when the animals could choose where to go in the information trial, the average number of correct responses was 77.5% for the sham-operated controls and 75.0% for the lesioned rats; similar levels of performance were seen in the choice trials. ANOVA revealed a lack of significant 'group' ($F_{2,16} = 0.00$, $P = 0.99$) and 'trial' ($F_{3,48} = 0.04$, $P = 0.99$) significant effects.

The results of the present experiment unequivocally confirm that the lesioned rats did not use egocentric orientation strategies to achieve control levels of performance in the NMTP task; had the lesioned rats been using egocentric strategies, they would not move straight towards the opposite arm of the maze in the choice trial because this response was not rewarded during previous testing in the task.

It could be argued that the lesioned rats move directly through the adjoining arms because they exhibit a persistent running response of a hyperactivity instead of aiming at the opposite maze arm. In fact, hippocampal damage [14,84] and colchicine-induced, DG-granule cell loss [83] do induce persistent responses in rats. This question was specifically addressed in the third session of the present experiment by offering the animals the opportunity of either (1) moving straight through the arm towards an unbaited box, or turning to the right or to the left towards a previously baited box, in the information trial, or (2) moving straight through the arm towards a baited box, or turning to the right or to the left towards an unbaited box, in the choice trial. The results are straightforward: the rats only move straight through the arm when this response takes them to the rewarding box. Therefore, one can discard the interpretation that perseverance or hyperactivity was driving the animals' response can be discarded.

Taken together, these results indicate that the lesioned rats do not rely on egocentric orientation; the data suggest that lesioned rats use intra- and/or extra-maze cues to perform this task.

6. General discussion

The results constitute evidence for multiple functions involving the DG granule cells, including time control modulation, and spatial and working memories, congruent with previous proposals for the hippocampal formation (e.g. [3]). However, the data can be also interpreted following O'Keefe and Nadel's [52] line of reasoning (see below).

6.1. DG lesion and performance in a DRL-20 s task

Olton [56] proposed that reference memory processed information in a fixed interval schedule of reinforcement, providing an explanation of why the performance of lesioned animals was not completely disrupted, and why they were able to complete the FI-20 s task. In addition, Meck et al. [45] proposed that the less efficient performance in DRL tasks following hippocampal damage results from disruption of working memory. Note that the DRL task differs from the FI task in other respects. In the FI task, a bar press is associated with both reinforcement after a time interval has elapsed and lack of reinforcement during the interval; however, the animal is not punished, including when the bar is pressed before the correct time. Differently, in the DRL task, a bar press may be rewarded or punished, depending on when it occurs; thus, rats must solve the conflict of approaching the lever at certain times and avoiding it at other times. Despite these differences, the time-course for fimbria-fornix lesioned rats in the FI-20 s task [56] is very similar to that seen for DG-lesioned rats in a DRL-20 s task (Experiment IA). This suggests that a similar strategy is being used to solve both tasks, which does not involve working memory.

Rawlins [65] proposed that hippocampal damage interferes with the ability to deal with temporal discontinuity between events to be associated. Congruently, Bannerman et al. [3] showed that complete cytotoxic damage to the hippocampus disrupts performance in a DRL-18 s task and Sinden et al. [71] showed that complete hippocampal ibotenate damage markedly affected performance in a DRL-18 s task, while leaving performance in a DRL-12 s task almost intact. Differently, the present experiments show that rats with colchicine-induced, DG granule cell loss, even though slightly less efficient than controls when performing a DRL-20 task, were able to (under-) estimate time intervals. Together, these results suggest that the contribution of the hippocampus proper and the DG for performance of DRL tasks may be different.

When discussing performance of animals in DRL tasks, O'Keefe and Nadel [52] proposed that "... rats cannot 'count time', but rather bridge temporal intervals by engaging in any of a variety of behaviors which fill the required interval. The normal rat can call on behaviors based on place (go to the other side of the box), guidance (do not press unless

light is on), or orientation (engage in a sequence of collateral displacement behaviors) hypotheses." (p. 325). In agreement with this notion, Richelle and Lejeune [66] proposed a synthesis as to how collateral behaviors mediate temporal control, and Killeen and Fetterman [35] defended the notion that adjunctive behaviors serve as the basis for conditional discriminations of the passage of time. Further, Costa et al. [13] showed that there is a defined sequence of adjunctive behaviors during performance of a DRL task. Congruently, it has been shown that the presence of objects in the conditioning chamber facilitates performance in DRL tasks [1,36,72], possibly because such objects contribute to the appearance of adjunctive behaviors on which temporal discrimination would be based.

O'Keefe and Nadel [52] hypothesized that animals with hippocampal damage, even though unable to use a place strategy, taking them away from the lever, should be able to generate some form of collateral behaviors, relying on guidance or orientation strategies, to perform DRL tasks. In this context, the results of the present study suggest that these strategies are not as efficient as the place strategy, leading to time underestimation. In addition, these authors emphasized that since most DRL experiments are preceded by training on a continuous reinforcement schedule, and hippocampal rats acquire the CRF schedule more rapidly than do controls, this superior acquisition in the CRF schedule would lead to difficulty in finding collateral behaviors in DRL tasks to compete with the strategy developed during CRF training; congruently, while extensive CRF pre-training results in persistent deficit in DRL tasks [19], minimal CRF pre-training results in normal DRL performance by hippocampal rats [67]. In the present experiments, rats acquired the DRL task prior to the lesion; in addition, the degree of CRF training was very small. Therefore, it is likely that the prior CRF training had little, if any, effect on performance of the DRL. This helps explain why performance of these rats was only slightly less efficient than that of the controls after surgery. However, if the hypothesis that DG-lesioned rats perform the DRL task by relying on guidance and orientation strategies is correct, this would explain why their inter-response times were shorter than those of the controls.

Lejeune et al. [38] showed that the administration of amineptine (a tricyclic antidepressant whose major effect is to inhibit dopamine uptake and, at higher doses, to enhance dopamine release) decreases performance in a DRL task, while leaving performance in a time duration discrimination task intact. Thus, the hypothesis that this drug interferes with the timing mechanism was discarded; on the contrary, the authors suggested that the effects of amineptine on temporal regulation in the DRL task are secondary to non-specific activation of motor activity. In favor of this interpretation, retraction of the bars during time estimation was shown to prevent expression of the activated motor behavior to the benefit of the expression of unimpaired time estimation. Thus, similarly to other hyperactivity-inducing dopaminergic drugs (e.g., nomifensin [51] and buproprione [69]), amineptive increased the spontaneous motor activity in rats, decreasing performance in DRL tasks. Hippocampal damage in rats is widely known to induce hyperactivity (see [28] for review) associated with difficulty in constructing cognitive maps of the environment [52]. Similarly, colchicine-induced, selective DG granule cell loss in rats increases motor activity [4,20] and decreases performance in tasks that require the adoption of the place strategy (e.g. [73,83]).

Together, these findings suggest that DG-lesioned rats adopt guidance and/or orientation strategies, engaging themselves in a sequence of behaviors to measure time intervals (see [35,52,66]). However, since they also exhibit activation of motor behaviors (see [4,20]), it would take them less time to conclude such sequence. Consequently, these animals show smaller inter-response times and, therefore, a less efficient performance in the DRL task (Experiment IA). Furthermore, Costa et al. [12] showed that the disruption of performance in the DRL-20 s seen after colchicine-induced damage to the dentate gyrus depends upon the size of the experimental chamber; that is, the larger is the experimental chamber the smaller is the behavioral disruption.

6.2. DG lesion and performance in an NMTP task

Performance of rats in both delayed matching-to-sample and delayed non-matching-to-sample tasks is believed to depend on the integrity of working memory for the temporary maintenance of critical information for later use [54]. While former studies on the involvement of the hippocampal formation in working memory are based largely on spatial tasks [45], there have been proposals that this brain structure is also required for the maintenance of non-spatial information in working memory (e.g. [46]). The present study shows that damage to the DG disrupts performance of an NMTP task acquired prior to the lesion; however, repeated post-lesion testing lead to recovery of performance (Experiment IB). Note that the time interval between the information and choice trials throughout all phases of Experiment IB was 3 s. It is difficult to conciliate these results with the notion that working memory is disrupted since the recovered performance requires a preserved working memory, independently of the nature of the information maintained temporarily for performance of the task.

The demonstration that DG damage disrupts the ability to navigate relying on place strategies, preserving the ability to use guidance and orientation strategies [83], corroborates another more parsimonious interpretation of the NMTP task results. That is, place strategies supported performance in the NMTP task prior to the lesion, but were no longer available after damage; thus, performance was disturbed soon after the lesion. As training proceeded, the rats relearned the task, now relying on the available guidance strategies (Experiments IB and II). The training schedule was designed such as to prevent the use of orientation strategies (see above), and the results of Experiment III indicate that our design was effective in avoiding the adoption of orientation strategies.

The increase in time interval between the information and choice trials produced interesting results (Experiment II); both lesioned rats and sham-operated controls exhibited greater impairment at longer intervals; however, while lesioned rats reached chance levels of performance when the time interval was 4 s, the controls only reached chance levels of performance when the time interval was increased to 16 s.

These specific results may favor the notion that damage to the DG disrupts working memory, in contrast to the hypothesis advanced above to explain data from Experiment IB. However, congruent with this latter hypothesis, the temporary maintenance of information in memory when using place strategies may be longer relative to the temporary maintenance of information when using guidance strategies. In fact, Beatty and Shavalia [5] and Maki et al. [41] showed that spatial working memory has a long persistence, and Panakhova et al. [58] showed that spatial memory exhibits a slow decay. Thus, the time-interval effect would be stronger for the lesioned rats because, differently from controls, they did not use a long-lasting place strategy to perform the NMTP task. More studies are necessary to discern among these possibilities.

Taken together, the present results seem most parsimoniously interpreted following the cognitive map theory of hippocampal function [52].

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