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Reversible Inactivation of the Hippocampal Mossy Fiber Synapses in Mice Impairs Spatial Learning, but neither Consolidation nor Memory Retrieval, in the Morris Navigation Task

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The role played by hippocampal mossy fibers in the learning and memory processes implemented in the Morris swimming navigation task has been studied in C57BL/6 mice by selective and reversible inactivation of mossy fiber synaptic fields by diethyldithiocarbamate. The functional integrity of the mossy fibers proved essential for the storage of the spatial representation on the modifiable synapses of the recurrent collaterals of the CA3 pyramidal cells, whereas it is not necessary for the consolidation and recall of spatial memories. The results suggest that mossy fibers are preferentially involved in new learning. They are consistent with the hypothesis that the hippocampal CA3 region might act as an autoassociation memory.

INTRODUCTION

One of the most crucial problems that animals have to solve when they live in the wild is that of spatial orientation. They have to acquire and memorize information from spatial cues and beacons to be able to orient themselves toward an invisible goal or to make a shortcut retreat toward a shelter.

Lesion studies have emphasized the prominent role played by the hippocampus in the processing of spatial information (Sutherland & Rudy, 1988; O'Keefe, 1991; Morris, Garrud, Rawlins, & O'Keefe, 1992). Unfortunately, permanent lesions do not allow conclusions to be drawn as to the specificity of the role played by the hippocampus because all the various stages of the learning and memory processes are affected. Reversible lesions, on the other hand, allow a refined interpretation of the brain mechanisms involved in

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behavior, as underlined by Bures and Buresova (1990). For instance, they make possible the dissociation of the effects of the structural lesion on the performance vs. the learning process. With reversible lesions, the subject in its normal state can be tested again after the effect of the temporary deafferentation has disappeared. It can thus be used as its own control so that acquisition, consolidation, or memory recall can be evaluated independently in the absence of the target structure. Thus, Gallo and Candida (1995) showed that the reversible inactivation of the dorsal hippocampus by tetrodotoxin selectively impairs acquisition but not retrieval of the conditional blocking of taste aversion in rats. Electrophysiology studies realized in the early seventies by O'Keefe and Dostrovsky (1971), then more recently by O'Keefe and Burgess (1996), Burgess and O'Keefe (1996), and Cressant, Muller, and Poucet (1997), have shown that, in the CA1 and CA3 regions of the rodent hippocampus, there are place cells which respond to the spatial location of the subject. Cells which respond to the orientation of the head have also been discovered in the postsubiculum (Taube, Muller, & Rank, 1990) and in various other brain structures (Blair & Sharp, 1996). They probably make up the basic elements of a neural net that provides the animal with a spatial representation of its vital domain (Dudchenko & Taube, 1997).

Behavioral neurogenetics has shown that the variation of structures in the brain is controlled by genetic factors (see Lassalle, 1996, for a review) and led us to question their internal functioning, namely, to try to understand how the genetic variation of the hippocampal circuitry can control cognitive abilities. Different results have shown, sometimes with conflicting evidence, that the intraspecific variations in the size of the different hippocampal mossy fiber synaptic fields present genetic correlations with the variation of novelty responses (Crusio, Schwegler, & Van Abeelen, 1989; Roullet & Lassalle, 1990), open-field activity (Hausheer-Zarmakupi et al., 1996), intermale aggression (Guillot et al., 1994), and with various forms of associative (Lipp, Schwegler, Crusio, Wolfer, Leisinger-Trigona, Heimrich, & Driscoll, 1989) and spatial learning (Schwegler, Crusio, Lipp, Brust, & Mueller, 1991; Schwegler & Crusio, 1995). Our aim was to analyze the role played by mossy fibers in hippocampal functioning and, if possible, to find clues that would allow to understand through what kind of mechanism the variation in size of the mossy fiber synaptic fields could influence behavioral differences between strains of mice.

Treves and Rolls (1992, 1994) and Rolls (1994) proposed a functional hypothesis of the role played by mossy fibers. Their model assigns the hippocampal circuitry of the CA3 region (see Fig. 1) the role of an autoassociation network that would allow the storage of neural representations of episodic memories or spatial representations, the recall of which can be triggered by a fragmental input (see also McNaughton & Smolensky, 1991). This model predicts that mossy fiber synapses are essential to drive information storage, which corresponds to the process of learning. On the other hand, they are not necessary for memory retrieval, which is initiated by the synapses of the alvear pathway. The aim of the present work is to test this model. In order to dissociate the role played by the mossy fibers in the learning and memory processes of a spatial location in the Morris navigation task, we used selective and reversible inactivation of hippocampal mossy fiber synapses. This was obtained by diethyldithiocarbamate (DDC) infusions, a powerful technique the interest in which in behavioral studies remains nevertheless unexplored. DDC chelates the zinc contained in the giant mossy fiber synapses (Haug, 1967;

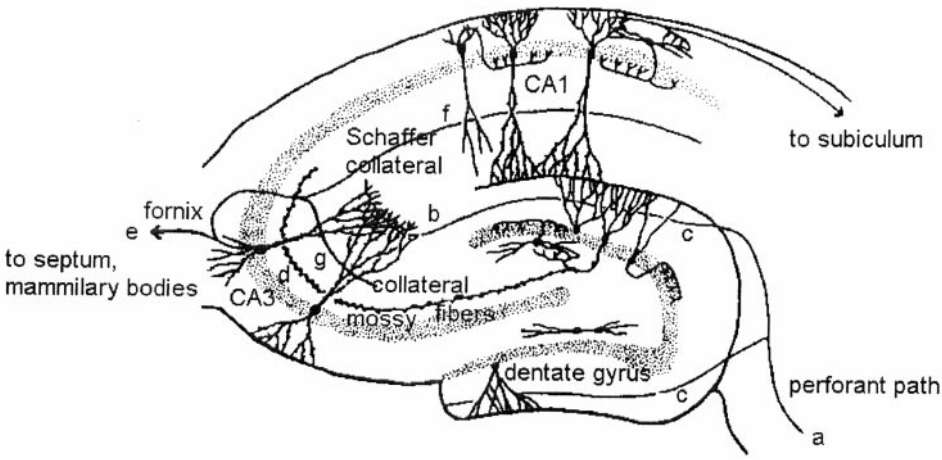

FIG. 1. Schema of the intrahippocampal connections (adapted from Rolls, 1994). The inputs from the entorhinal cortex reach the hippocampus through the perforant path (a). Some of these afferences synapse directly on the distal part of the apical dendrites of the CA3 cells (b) and constitute the alvear path, whereas the other perforant axons relay on the granular cells of the dentate gyrus (c). The axons of the granular cells contact "en passant" the proximal part of the apical dendrites and the basal dendrites of the CA3 cells, close to the cellular body, through the giant mossy fiber synapses (d). Three collaterals issue from the CA3 axon. One projects to the lateral septum and mammilary bodies through the fornix (e). The Schaffer collateral innervates the apical dendrites of the CA1 pyramidal neurons (f). The associative recurrent collateral contacts the median part of the apical dendrites of neighboring CA3 neurons by modifiable synapses (g).

Perez-Clausell & Danscher, 1985) which are inactivated for a 30- to 45-min duration, resulting in reversible working memory disruption (Frederickson, Frederickson, & Danscher, 1990). This time interval is long enough to allow a three-trial learning session in the Morris navigation task to be performed. Under the same conditions, control mice received an infusion of Ca-ethylenediamine tetraacetic acid (Ca-EDTA), which is also a zinc chelator that cannot penetrate the synaptic membrane and does not chelate the zinc within axonal boutons (Fredens & Danscher, 1973). This constitutes the right control for such an experiment. Infusions were made in the dorsal hippocampus which appeared the most appropriate location for that. Indeed, an accumulating body of evidence suggests that the hippocampus is a functionally and genetically heterogeneous structure along its rostrocaudal axis. For instance, Wimer and Wimer (1985) claimed that the hippocampus is a highly differentiated structure dependent upon four different genetic systems. More recently, Moser et al. (1993) have shown, in female rats, that the ventral and dorsal parts of the hippocampus may process qualitatively different kinds of information, the dorsal part being more important for spatial learning than its ventral counterpart. They showed that a 20% lesion volume of the dorsal hippocampus was sufficient to produce a long-lasting deficit in spatial learning in the Morris navigation task, whereas to be effective, a ventral lesion had to be large enough to have lesioned some cells of the dorsal hippocampus. Consequently, it appeared more relevant to study the effects of focal lesions of the mossy fiber pathway in the dorsal hippocampus.

Two experiments were carried out. The first was designed to dissociate the effects of the inactivation of the mossy fibers by DDC in the acquisition and memory processes of the spatial task and to assert the reversibility of the effects of DDC. The second was

planned to analyze the effects of DDC on the processes of memory consolidation and recall and to replicate the results of the first experiment.

GENERAL METHODS

Subjects

Ninety- to 120-day-old male mice from the C57BL/6 inbred strain were used. All mice came from the IFFA-CREDO breeding center and were 6–7 weeks old at their arrival at the laboratory. They were housed by groups of five to six in 30 × 20 × 14 polycarbonate cages placed in a rearing room at constant temperature (23 ± 1 °C) with a reversed 12–12 LD schedule, the onset of the dark phase being at 8:30 AM. Food and water were given ad libitum. Sawdust bedding was changed only once a week, at the end of each series of experiments. Experiments were always run in the afternoon between 1:30 and 6:00 PM.

Behavioral Analysis

The circular swimming pool (70 cm in diameter and 30 cm in height) was made of ivory-colored PVC, filled with water (25 ± 0.5 °C) made opaque with the Opacifier 631 to 12 cm below the edge of the wall. A circular goal platform (5 cm in diameter) laid 0.5 cm under the surface of the water and 7 cm from the wall. The device was placed in a regular room. Dropped into the water from a different quadrant on each trial, mice had to learn to navigate to the invisible platform using the spatial cues available in the room. After a three trial pretraining session to find out the procedural components of the task, the mice were given three consecutive trials a day for 4 days, according to the procedure described by Chapillon and Roullet (1996). After the third trial of the last session, the mice were submitted to a probe test for spatial bias. The platform was removed and the mouse, starting from the opposite quadrant, was allowed a 1-min search for the platform. The path was recorded on videotape and a spatial bias index was computed as the difference between the number of times an 8-cm-diameter annulus surrounding the former location of the platform was crossed and the mean number of crossings of three annuli, symmetrically laid out in the quadrants where the platform had never been, divided by the total number of annulus crossings.

Surgery, Drug Administration, and Histology

Selective and reversible inactivation of mossy fibers was obtained through direct infusion of DDC in the dorsal hippocampus. Under the same conditions, control mice received an infusion of Ca-EDTA. Mice were operated under deep chloral hydrate anesthesia (500 mg/kg). A holder made of methacrylate resin with two guide tubes spaced 4 mm apart and protruding 2 mm out of the base was fastened to the skull. The guide tubes were positioned according to stereotaxic coordinates from the atlas of Slotnick and Leonard (1975) (AP: 1.6 mm posterior to bregma; Lat: 2 mm; Vert: 1.6 below dura) so that the tip of the guide tubes was close to the dorsal part of the hippocampus, near the CA1 field. The mice were given a 5- to 7-day recovery period after surgery.

Just before the injections, two beveled injection tubes were introduced into the guides and their lengths adjusted so that the tip of the injection tube reached the CA3 pyramidal

layer. DDC and EDTA were administered as aqueous solutions (200 mM). Injections were monitored by a Bioblock infusion pump which infused 0.25 μl in 2 min simultaneously in both hippocampi. The injection tubes were maintained in place for 2 min after the end of the injection, so that the solution could not escape through the guide tube. Adjusted lengths of steel entomology pins smeared with paraffin oil were placed in the tubes to seal them between injections. After the injection, mice were replaced in their home cages for a 15-min period before testing.

Three days after the end of the experiments, mice were given a new injection of DDC; then, after a lapse of 20 min, they were perfused intracardially under lethal anesthesia with (a) 0.9% NaCl, (b) 0.1% sodium sulfide in phosphate buffer, (c) 3% glutaraldehyde fixative, and (d) 0.1% sodium sulfide. Their brains were then processed for Timm staining of the mossy fibers (Danscher & Zimmer, 1978) and counterstained with thionin for cellular bodies. The position of the cannulae was verified on 25-μm coronal sections. In all cases, damage to the dorsal cortex, close to the CA1 field, indicated that the guide tubes had been set at the right locations. Figure 2 presents the location of the tips of 10 guide tubes in each hemisphere that cover the entire area where injections were made. Bleaching of Timm stain of the mossy fibers (Frederickson et al., 1990) covered a large but not complete part of the mossy fiber synaptic field in the CA3 region. This suggests that the observed behavioral effects of reversible lesions of mossy fibers by the DDC result from moderate size focal lesions. Bleaching was not apparent in three mice. This could be due to an infusion problem liable to occur when many injections have already been made. Anyway, these animals did not appear to perform as outliers in their proper experimental group and consequently, it was more conservative not to discard them from the analysis.

Statistics

Box plots were used to look for outliers. A repeated measures ANOVA design was performed to analyze the effects of the treatment, of the session, and of their interaction on swimming latencies with the Multiple General Linear Model (Wilkinson, 1987). The influence of the treatments on the spatial bias index was analyzed by the nonparametric Mann–Whitney *U* test.

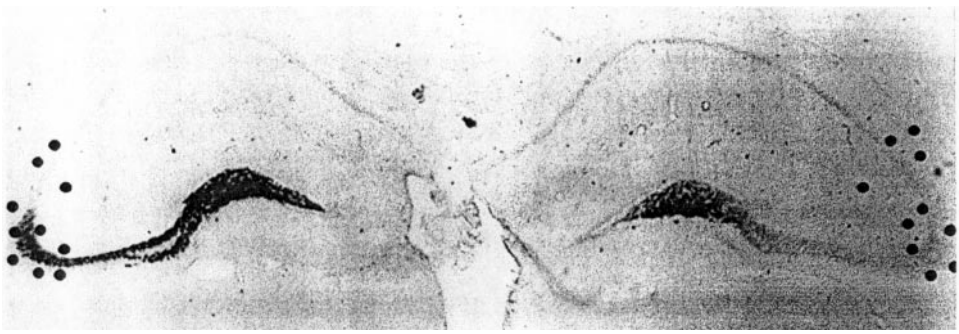

FIG. 2. Digitalized image of a coronal brain section showing (i) Timm bleaching of mossy fibers after DDC infusion on the right side compared to normal Timm staining on the left side and (ii) histological verification of cannulae placement in the dorsal hippocampus. Black dots indicate the location of 10 pairs of cannulae that cover the entire region where injections were made.

EXPERIMENT 1

Mice were first given a pretraining session with a visible platform (without drug injection) and then two series of four daily sessions with the submerged platform with a 72-h rest in between. The session started 15 min after the end of the injection. After the last trial of the last session, on day 4 of the first and second week, mice were submitted to the spatial probe test.

Thirty male mice were randomly assigned to three different groups: two groups of 12 mice each were equipped with guide tubes. These mice served alternatively as experimental and injected control groups according to a cross procedure. During the first week, group 1 mice were infused with EDTA. They were able to learn the task normally and were used as a control group to test the effects on learning of the DDC injected into mice of group 2. During the second week, group 2 mice received in turn the infusion of EDTA and served as controls of group 1 mice, which were then infused with DDC. The study of the performance of group 1 mice during the second week allowed analysis of the effects of DDC on long-term memory retrieval, whereas the study of the performance of group 2 mice checked for the reversibility of the inactivation of mossy fibers by the DDC and for the absence of residual effects according to the following predictions: (i) if mossy fiber synapses are not necessary to memory recall, group 1 mice should show normal performances under DDC during the second week of training; (ii) if DDC effects during week 1 are fully reversible, group 2 animals should show normal learning during week 2. One group of 6 nonimplanted and noninfused mice served as controls for the side effects of surgery and injections during the first week.

The escape performance of a session was the sum of the latencies of the three trials for a mouse within that session. Escape latencies were submitted to a $\log_{10}$ scale change in order to normalize the shape of the distributions and to homogenize the variances of the different experimental groups.

Results

Figure 3A shows that during the first week of training, mice displayed a significant linear improvement of their escape performance across sessions [$F(3,84) = 3.55$, $p = .018$] without significant treatment $\times$ session interaction [$F(6,84) = 0.783$, NS].

The ANOVA showed that there was no overall significant treatment effect either [$F(2,28) = 1.817$, NS]. Although it fluctuated greatly from the first to the second session, the performance of EDTA mice did not differ from that of controls [$F(1,16) = 0.005$, NS], which indicates that neither the implantation of the cannulae nor the infusion of EDTA significantly modified their learning performance. Mice infused with DDC during the first week of the experiment improved their performance more slowly than EDTA mice, but reached the same level of performance on the fourth day of training. Over the four sessions, the effect was nonsignificant [$F(1,23) = 3.324$, $p = .081$] but marginal. The probe test (Fig. 3B) demonstrated that the spatial performance of mice infused with EDTA during the first week of training did not differ from that of control mice [Mann–Whitney $U = 29.5$, $\chi^2 = 0.124$, $df = 1$, NS] and that they searched the platform at the right place. On the other hand, mice infused with DDC during the first week do not learn the location of the platform and searched for it everywhere in the water maze (DDC vs. EDTA: $U = 130.5$, $\chi^2 = 11.715$, $df = 1$, $p = .001$).

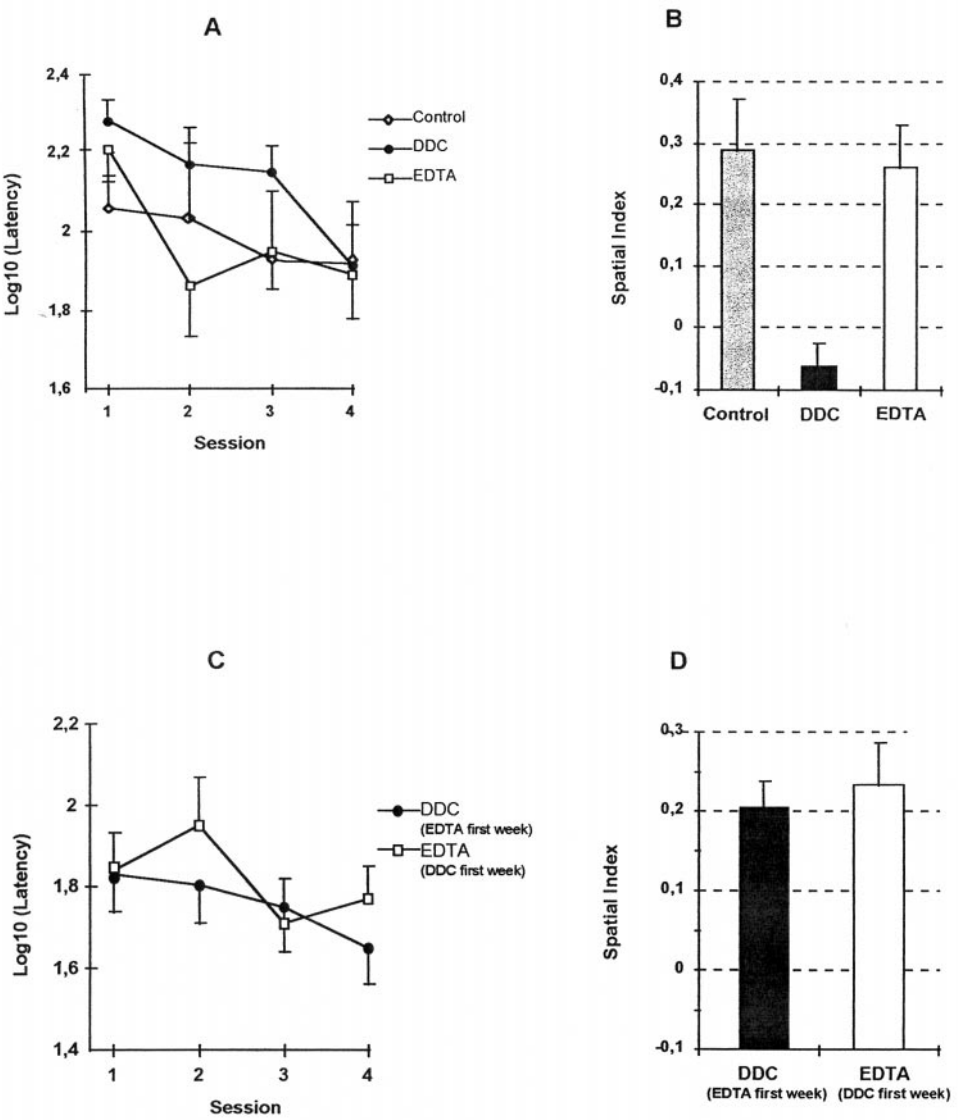

FIG. 3. (A) First week training. Log_{10} escape latencies (mean ± SEM) to find the platform in the Morris navigation task across sessions. Each latency is the sum of the three trial latencies within a session. (B) Spatial index values (mean ± SEM) during the probe test in the Morris navigation task at the end of the first week for control, EDTA, and DDC mice. (C) Second week training. Log_{10} escape latencies (mean ± SEM) to find the platform in the Morris navigation task after treatments have been crossed. (D) Spatial index values (mean ± SEM) during the probe test in the Morris navigation task at the end of the second week of training.

During the second week of the experiment (Fig. 3C), when treatments were rotated, mice continued to improve their escape latencies [$F(3,66) = 3.008$, $p = .036$] but, although latencies in EDTA mice fluctuated more than those of mice infused with DDC, both groups improved their performance at the same speed [$F(1,22) = 0.315$, NS]. Over the 2 weeks, mice infused with EDTA appeared, unexpectedly, more variable than their DDC counterparts.

The spatial probe test (Fig. 3D) showed that, whereas they were unable to learn the

spatial component of the task when they were infused with DDC during the first week of training, these mice can learn normally under EDTA during the second week. On the other hand, those which learned the spatial task under EDTA during the first week nevertheless displayed normal performances under DDC during the second week.

Discussion

The comparison of escape latencies and spatial index scores of EDTA and nonoperated, noninjected control mice proves that surgery and injection have no side effects and that EDTA has no effect on mossy fiber synapses. Consequently, EDTA mice are the right control for DDC mice. These results establish also that the effect of DDC is fully reversible, without any aftereffects, since DDC mice which were unable to learn the location of the platform during the first week of training learned normally during the next week under EDTA.

The analysis of escape latencies shows that the inactivation of the mossy fibers by DDC might slow the improvement of latencies, at least during the previous stages, but was unable to prevent it. Nevertheless the improvement of escape performance across sessions does not imply that mice learned to locate the platform. They might also have learned only that somewhere under the water, at a certain distance from the wall, there is a platform they can climb for a rest, so that they searched actively and efficiently for it, without being able to navigate there directly.

Actually, analysis of the spatial index shows that the DDC, by selectively blocking the synapses of the mossy fibers on the CA3 neurons, prevents learning the spatial component of the task, i.e., learning the location of the platform, whereas it does not impede learning the sensorimotor and procedural components of the task (hunting actively everywhere for a platform), which results in the improvement of escape latencies over sessions. These results support the involvement of the hippocampal structure, specially the CA3 region, in the specific learning of the spatial component of the task. This corresponds to a first dissociation.

Inactivation of mossy fibers by the DDC also reveals a second dissociation. According to the model presented by Treves and Rolls (1992, 1994), these results show that, whereas the activity of the mossy fibers is essential to the learning process, it is not necessarily involved in memory recall, since blocking mossy fiber activity during the second week of training does not prevent the recall of spatial information stored previously during the first week. Nevertheless, this last point holds only if it is considered that the information is still stored in the modifiable synapses about 72 h after the learning session. An alternative hypothesis to account for these results could be that instead of preventing the acquisition of spatial information, mossy fiber transient inactivation could interfere with the early processes of memory consolidation and thus impair memory storage. Posttrial DDC injections, which leave mossy fibers functional during acquisition but block their activity during the first 45 min of memory consolidation, would help answer this question.

EXPERIMENT 2

This second experiment aimed at dissociating the effects of mossy fiber synapse inactivation by DDC on the learning, memory consolidation, and recall processes.

Thirty-two mice, equipped with injection tubes, were allotted to four groups receiving DDC or EDTA infusions either 15 min before the first trial of a session, in order to be active during the learning phase, or immediately after the last trial, to affect the initial phase of the memory consolidation process.

At the end of the learning session on the fourth day, after a 15-min pause, the mice were given a spatial probe test. The two postsession injection groups received a last DDC or EDTA infusion immediately after the last learning trial so that the effect of mossy fibers inactivation on memory recall could be checked 15 min later during the probe test. During this second experiment, the water temperature was lowered from 25 to 23°C in order to improve the motivation of animals to escape.

Results

Figure 4A showed that presession injected mice presented a significant global improvement of their latencies to find the platform from the first to the fourth sessions whatever treatment they received [$F(3,33) = 9.477$, $p < .001$]. Comparison of the performances between groups which received either DDC or EDTA before the training sessions supported the results of the first experiment; DDC mice showed longer latencies than EDTA mice before finding the platform, although the difference was again nonsignificant [$F(1,11) = 3.697$, $p = .081$] but marginal. As previously, they reached similar performance on the fourth session [$F(1,11) = 0.578$, NS]. Results of the spatial probe test (Fig. 4B) showed that mice which received a DDC infusion before the learning sessions did not learn the spatial location of the platform, whereas in the same conditions, EDTA mice could learn [$U = 41.5$, $\chi^2 = 8.6$, $df = 1$, $p = .003$].

On the other hand, when the infusions were given immediately after each learning session, DDC and EDTA did not affect consolidation in a different manner. The latencies of mice which received the DDC (Fig. 4C) did not differ globally from those which received EDTA [$F(1,14) = 0.619$, NS] and both improved significantly their performance across sessions [$F(3,42) = 8.887$, $p < .001$]. The difference observed on the first trial between the posttrial DDC and EDTA mice cannot be attributed to the effect of the molecules, since they had not received any infusion at that time. The results of the spatial probe test (Fig. 4D) showed that the spatial index of DDC mice did not differ from that of EDTA controls [$U = 34.5$, $\chi^2 = 0.069$, $df = 1$, NS].

Discussion

These results show that the inactivation of mossy fibers during the early stages of memory consolidation disrupted neither the storage of information acquired during the session immediately prior to the injection nor the recall of this information. They also confirm the findings of the first experiment; whereas mossy fiber inactivation during learning impaired the initial performance but did not prevent mice from improving their escape latencies during the next sessions, it nevertheless made spatial learning impossible.

GENERAL DISCUSSION

The results of these two experiments first confirm already known phenomena: (i) they corroborate the involvement of the hippocampus and particularly of the CA3 region in

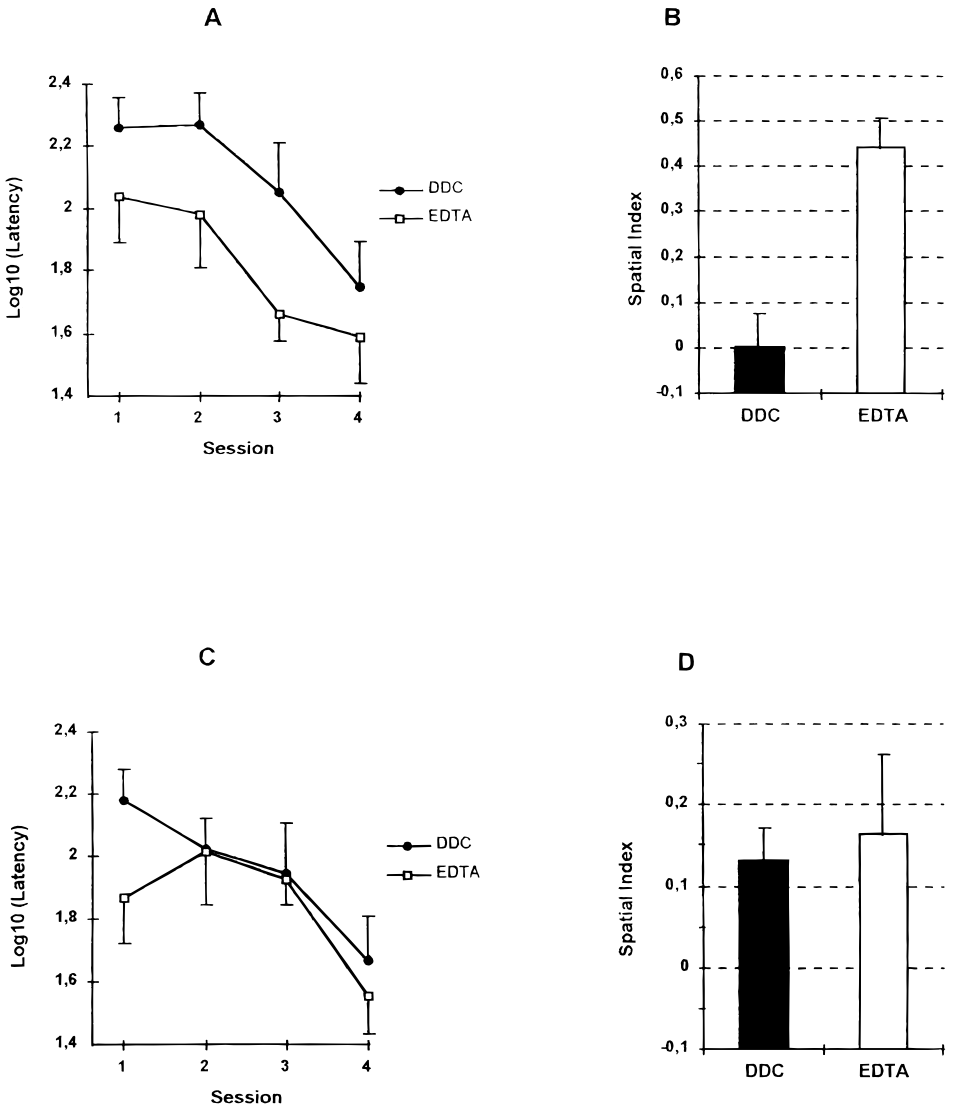

FIG. 4. (A) Presession injection. Log_{10} escape latencies (mean ± SEM) to find the platform in the Morris navigation task. (B) Spatial index values (mean ± SEM) during the probe test in the Morris navigation task. (C) Postsession injection. Log_{10} escape latencies (mean ± SEM) to find the platform in the Morris navigation task. (D) Spatial index values (mean ± SEM) during the probe test in the Morris navigation task.

the learning of a spatial location in the Morris navigation task, (ii) they validate the dissociation between procedural and sensorimotor learning on one side and spatial learning on the other side, as far as hippocampal functions are concerned. Above all, they bring new findings of potential importance to understanding the role played by mossy fibers in the learning and memory processes of a spatial task.

Since the first report on cognitive deficits due to bilateral hippocampal lesions by Brenda Milner in the fifties (Scoville & Milner, 1957), neuropsychologists have clearly demonstrated that whereas hippocampal subjects suffer anterograde amnesia and spatio-temporal desorientation, they are still capable of forms of procedural learning which

concern the acquisition rules or the implementation of sensorimotor abilities. On these lines, it is more and more widely acknowledged by specialists who study animal behavior that the escape latency in the spatial version of the Morris task is a rather complex behavioral performance involving various processes (attention, motivation, rule understanding, and specific and strain-specific strategies) so that it cannot represent a reliable assessment of the processes that control spatial orientation. Only the spatial probe test will give an appropriate measurement of the spatial learning ability of the subject [see Upchurch and Wehner (1989) for an earlier discussion of this point and Lipp and Wolfer (1998) for a more recent one]. Whishaw (1989) brought forward experimental arguments concerning the need for dissociating between performance and learning deficits in spatial navigation tasks in rats submitted to cholinergic blockade. Numerous experiments show that brain-lesioned rats (hippocampus, neocortex, subiculum, caudate putamen) can significantly improve their escape latencies toward an invisible platform in the Morris swimming pool, although they do not reach the same level of performance as controls. On the other hand, they display a significant deficit in the probe test (see for example Morris, 1990; Moser et al., 1993; Whishaw et al., 1987). Similarly, Frederickson et al. (1990) noticed that whereas the rat's ability to return directly to the specific platform position on the second trial of the delayed matching to sample task in the Morris swimming pool was severely impaired by hippocampal infusions of DDC, recall of the general procedure for solving the task, however, was relatively unaffected by the drug. Namely, no trained rat ever reverted to the naïve strategy of swimming around the tank in search of escape, for the duration of the trial. Nevertheless, it has been shown that overtraining hippocampal rats allows them to improve even more their escape latencies and finally their spatial performance in the probe test (Morris, 1990). In this experiment however, mice were given moderate training so that their performances ranged from a mean value of about 67 s on the first session to 15 s in the last session, which enabled few mice to have direct paths toward the platform. In this respect, our results match those of Mizumori, Perez, Alvaro, Barnes, and McNaughton (1990), who showed that in rats, reversible inactivation of the medial septum by tetracaïne impairs spatial learning on the radial maze, whereas it produces only a significant retardation of learning followed by a clear improvement over trials in the spatial reference memory task with the same experimental paradigm.

Although the effects of DDC observed in the present study are interpreted as a consequence of the binding of the zinc in hippocampal mossy fiber synapses, other possible effects of DDC have been investigated. DDC can also act as a dopamine-β-hydroxylase inhibitor which has been shown to reduce whole brain norepinephrine *in vivo* (Haycock et al., 1978; Frieder & Allweis, 1982). The effects of systemic injections of DDC have been investigated in one-trial avoidance tasks in rats. It has been shown that DDC injected prior to training does not impair learning and short-term memory performance (Hamburg & Cohen, 1973; Stein et al., 1975; Solanto & Hamburg, 1979; Frieder & Allweis, 1982). The effects of DDC observed in our experiments or those of Frederickson et al. (1990), however, cannot be explained by their effects through the noradrenergic system for various reasons. First, as underlined by Haycock et al. (1978), the lack of effect of other dopamine-β-hydroxylase inhibitors makes it difficult to attribute the amnestic effects of DDC solely to catecholaminergic effects. Second, the absence of effect of systemic or intracisternal injections of DDC on short-term memory, whereas they impair memory consolidation or recall processes, indicates clearly that the target is not the hippocampus. These effects

are in the opposite direction of those resulting from hippocampal insults. Third, the timing of the pharmacological interventions and their effects in these experiments are very different from those observed in our experiments. The delay (hours to days) between the injections and their effects as well as their duration are clearly incompatible with those of intrahippocampal infusions. Fourth, the cognitive processes involved in these experiments and in our learning task are also different and there is no evidence that the hippocampus is necessary to acquire a one-trial associative conditioning when there is no delay between the occurrence of the behavior to be suppressed (step down or step through) or the place to be passively avoided and the administration of the punishment. All these arguments and others developed by Danscher et al. (1973), namely, the fact that other zinc chelating agents, dithizone (Fleischhauer & Ohnesorge, 1958) and oxine (Danscher & Fredens, 1972), have the same effect on the Timm stain and have somewhat similar behavioral effects supports our claim that the effects of intrahippocampal infusions of DDC on spatial learning are mediated through the binding of zinc, which interferes with transmission in the mossy fiber synapses of the CA3–CA4 region.

On the whole, our results demonstrate that reversible inactivation of the mossy fibers by DDC disrupts the spatial learning process, whereas it has no effect on memory consolidation or memory recall in either working or reference memory. This new dissociation supports the selective involvement of the mossy fiber synapses in the learning process of a spatial location, whereas they are not necessary to memory recall, in accordance with the model developed by Rolls (1994) and Treves and Rolls (1992, 1994), in which the CA3 region of the hippocampus is supposed to act as an autoassociation memory matrix. The consequences of the inactivation of mossy fibers can be paralleled with those of reversible inactivation of the medial septal area (MSA), which sends cholinergic inputs either directly to the hippocampus via the fimbria fornix or indirectly through layer II of the entorhinal cortex. Our results are again consistent with those of Mizumori et al. (1990), since inactivation of MSA before learning increased error numbers during the test, whereas inactivation of the MSA after the initial learning phase had no effect on the test and thus can be said not to affect consolidation. On the other hand, they differ from those of Rashidy-Pour, Motghed-Larijani, and Bures (1996), which show that reversible inactivation of the MSA by tetrodotoxin impairs consolidation of a passive avoidance learning task in rats when administered 5 to 90 min after a single acquisition trial. Such inconsistencies stress either possible differences in the effects of the chemicals used in these studies or the diversity of the neural mechanisms underlying the two learning tasks, rather than a weakness of the reversible inactivation methodology, which proved extremely selective and efficient.

Genetic studies by Heimrich et al. (1985), Crusio et al. (1986), and Lassalle et al. (1999) indicate that size variations of the various hippocampal mossy fiber layers (suprapyramidal, intra-infrapyramidal, and CA4) are based on different genetic architectures. As already underlined, they also show genetic correlations with various behavioral processes, the physiological and cognitive bases of which are poorly understood. In most cases, behavioral variation correlates with the size of the intra-infrapyramidal mossy fiber projection rather than with the entire mossy fiber area. Therefore, it is important to know more about details of the role played by hippocampal mossy fibers in hippocampal functionning to decipher these correlations. As our results show that mossy fibers are involved in the storage of episodic memories, the focus should then be put on physiological mechanisms

that could explain that mice with a large intra-infrapyramidal projection are better performers. For instance, it would be relevant to know if mossy fibers that synapse on the basal dendrites of pyramidal cells are more efficient in teaching these cells or inducing mossy fiber potentiation, as has been shown in the CA1 field by Capocchi et al. (1992), Karbara and Leung (1993), and Arai et al. (1994). Also, as CA3, like CA1, pyramids are complex spike cells that have been considered basic elements of the spatial neural representations, research should be undertaken to discover whether they are involved only for the tuning of these place cells or whether they might as well be involved in nonspatial learning processes. Further studies, investigating the effects of reversible lesions in tasks involving spatial and nonspatial components, are currently under way in our laboratory that should help clarify this point.

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