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Nonredundant roles for hippocampal and entorhinal cortical plasticity in spatial memory storage

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Abstract

Recently, it has been demonstrated that targeted blockade of the extracellular signal-regulated kinase (ERK) cascade in either the entorhinal cortex (EC) or hippocampus (HIP) results in spatial memory deficits. However, it is unclear if ERK-mediated plasticity in these structures has redundant functions or unique roles. In this report, we contrast the role of long-term plasticity in these two structures with side-by-side comparisons of the effects of PD098059 infusion following water maze training. Analysis of performance during the long-term retention test indicates a role for plasticity in the EC in storing broad location information. In contrast, blocking plasticity in the HIP resulted in deficits in indices of precise location information and goal-directed navigational error. To distinguish between a navigational and location deficit, a "two-room" experimental design was employed. Training in the first room allowed animals to consolidate information regarding navigational strategies prior to training and drug infusion in the second room. Hippocampal-PD098059-infused animals demonstrated behavior suggestive of an expanded representation of the platform location and, thus, a loss of precise location information, suggesting that plasticity in these structures is involved in nonredundant, but complementary, processes necessary for spatial memory.

Keywords: MAPK; Medial entorhinal cortex; Spatial memory; Long-term memory

1. Introduction

It is well accepted that the entorhinal–hippocampal circuit is obligatory for performance in spatial memory tasks. However, it is unclear if these structures have redundant function(s) or unique roles in spatial memory storage. Although selective lesions of brain structures have been very informative in memory research, they often cause compensatory changes in remaining structures, making it difficult to assess a direct role of the structure. Posttraining treatment paradigms to block plasticity within a target area have been a highly effective technique for examining the role of a specific brain structure in memory storage (McGaugh, 1966; Schafe et al., 2000; Walz et al., 2000). In this paradigm, the function or development of plasticity is altered experimentally only after learning, and therefore, treatments do not alter memory acquisition or neuronal function within the target structure (Blum et al., 1999). Furthermore, because the treatment is temporary and localized, compensatory or generalized effects are less likely, as are direct alterations in performance during the retention testing. In this report, we combine these three approaches; blocking plasticity rather than activity, applying the treatment posttraining, and targeting infusions into specific structures to compare the roles of extracellular signal-regulated-kinase (ERK)-mediated plasticity in the entorhinal cortex (EC) and the hippocampus (HIP) in spatial memory storage.

The activation of ERK has been repeatedly shown to be a molecular correlate for long-term memory (Martin et al., 1997; Brambilla et al., 1997; Crow et al., 1998; Blum et al.,

Abbreviations: ERK, extracellular signal-regulated kinase; EC, entorhinal cortex; HIP, hippocampus; MEA, medial entorhinal area; LEA, lateral entorhinal area.

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1999; Kanterewicz et al., 2000; Roesler et al., 2000; Wu et al., 2001; Hebert et al., 2002). PD098059, a cell-permeable inhibitor of the kinase that activates ERK, mitogen-activated protein kinase kinase (MAPKK or MEK), specifically blocks the ERK cascade (Alessi et al., 1995; Blum et al., 1999; Davies et al., 2000) and has been extensively used to demonstrate a role for ERK activity in long-term memory storage (Berman et al., 1998; Schafe et al., 1999; Blum et al., 1999; Walz et al., 1999; Hebert et al., 2002; Runyan et al., 2004). For instance, posttraining intrahippocampal infusion of PD098059 impairs long-term spatial memory, but not short-term memory or acquisition (Blum et al., 1999). These and other studies indicate that blockade of the ERK cascade within a brain structure can be used to asses its role in long-term memory storage.

In this report, we contrasted the role of ERK-mediated long-term plasticity in the HIP versus the EC in spatial memory storage with detailed comparisons of the effects on a retention test of bilateral posttraining infusion of PD098059 into either of these two structures. Retraining to test for savings and the utilization of a two-room experiment were employed to help dissociate differences in strategy versus location deficits.

2. Materials and methods

2.1. Subjects

Male Long-Evans rats (220–250 g) were obtained from Charles River Laboratories (Wilmington, MA). Rats were housed individually on a 12-h light/dark cycle with ad libitum access to food and water.

2.2. Surgery

All protocols involving the use of animals were in compliance with the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee. Rats were initially anesthetized using 4% isoflurane with a 2:1 N_2O/O_2 mixture and were maintained via a facemask under a 2% isoflurane/2:1 N_2O/O_2 mixture. A small burr hole in the skull was prepared to allow the implantation of bilateral guide cannulae (22 gauge, stainless steel). The guide cannulae were aimed 1.5 mm above either the entorhinal area (AP −6.7, L ±5.5, and V 6.0) or the HIP (AP −3.3, L ±2.0, and V 1.5; Paxinos and Watson, 1997). Guides were secured to the skull with screws and dental cement. Animals were allowed to recover from the surgery for 10 days before the initiation of behavioral testing.

2.3. Drug preparation and infusion

PD098059 (Biomol, Plymouth Meeting, PA) was initially dissolved in DMSO and then diluted in sterile saline prior to use. Freely moving animals were bilaterally infused with 1 μl/side of 0.2 μg PD098059 or vehicle using a dual syringe infusion pump (Stoelting, Wood Dale, IL) at a rate of 0.25 μl/min. Following the infusions, the cannulae were left in place for an additional 2 min to allow for diffusion. The infusion cannulae extended 1.5 mm beyond the guide cannulae to give a depth of 7.5 mm for the EC and a depth of 3.0 mm for the HIP.

2.4. Drug effectiveness and diffusion

To confirm the inhibition of ERK in the target structures and to determine if surrounding structures, especially those known to be involved in spatial processing, were affected, the phosphorylation of ERK was examined using immunohistochemistry following unilateral infusion of 0.2 μg of PD098059 into either the HIP or EC with an equal volume of vehicle (1 μl) infused into the contralateral side of the same animal. Twenty minutes following the infusion, the animals were killed, and the brains processed with anti-phospho ERK1/2 (rabbit polyclonal antibody, 1 μg/ml; Cell Signaling Technologies, Beverly, MA) or anti-NeuN (mouse monoclonal antibody, 0.5 μg/ml; Chemicon, Temecula, CA), visualized using ABC and DAB kits (Vector Laboratories, Burlingame, CA) on free-floating 40-μm thick slices. The immunoreactivity of phospho-ERK and of NeuN was visually compared between the PD098059- and vehicle-infused sides, and areas lacking phospho-ERK-positive cells were measured.

2.5. Behavioral training

All behavioral trainings were performed by an experimenter blind to the treatment groups.

2.5.1. Experiment 1

To directly compare the effects of inhibition of ERK, hippocampal- and entorhinal-cannulated animals were trained together in pairs. Spatial memory was examined using protocols similar to the hidden platform version of the Morris water maze task, with all trials on a single day and a 4-min intertrial interval as described previously (Blum et al., 1999; Hebert et al., 2002). The animals were given a minimum of eight training trials and were trained until they found the hidden platform on three consecutive trials with an average latency of 10 s or less. Animals that failed to reach criterion after 16 trials were eliminated from the study (two animals). Immediately following training (within 5 min), the animals were bilaterally infused with either 0.2 μg PD098059 or vehicle (1 μl/side at 0.25 μl/min). Spatial memory was assessed 48 h later with a 60-s retention test, in which the platform was removed. Movement within the maze was recorded using a digital camera and Chromotrack tracking software (San Diego Instruments, San Diego, CA). For retraining, after the completion of the retention test, the platform was replaced in the same location as during

training. Animals were exposed to the platform for 30 s and then immediately given retraining trials (minimum of three), followed by a second retention test 48 h later.

2.5.2. Experiment 2

To test for a deficit for the location of the platform versus a navigational strategy deficit, a two-room training protocol similar to that outlined by Bannerman et al. (1995) was employed. Training and testing protocols were the same as previously reported (Hebert et al., 2002), unless otherwise stated. Rats were trained and given a long-term retention test (48 h) in the first room. The next day, animals were trained in a second room, infused with 0.2 μg PD098059 or vehicle into the HIP (1 μl/side at 0.25 μl/min), and given a second retention test 48 h later. The platform placement, extra-maze cues, the color of the tank and water, and the room lighting used during training in the second room were arranged to be distinctly different from those used during training in the first room. During training in the second room, animals were given a minimum of 7 and a maximum of 12 training trials to meet the criterion. One animal did not satisfy the criterion and was eliminated from the study. Following the second retention test, animals were retrained and given a third retention test 48 h later.

2.6. Retention test analysis

The tracking software automatically calculates time and distances traveled. Swimming speed was calculated as the total distance traveled divided by the total time in the tank. Initial heading error was measured as the angle away from the target after the animal had traveled 36 cm. Computer traces of the animal's movements were printed with the following areas marked: concentric circles representing the platform location and the gray annulus (an annulus twice the diameter of the platform), the perimeter, and the quadrants. The printouts were coded and analyzed by an experimenter blind to the treatment groups. The printouts were used to determine the number of approaches to the platform (attempts). An attempt was defined as any time that the path of the animal (1) entered or touched the gray annulus; (2) demonstrated a sharp turn (>30°) away from the tank wall, followed by a straight swim of at least 9 cm, which, if continued, would have entered the previous platform location; and (3) demonstrated a longer straight swim (at least 18 cm), crossed into the training quadrant, and came within 20 cm of the edge of the platform. A category of behaviors resulting in unsuccessful attempts that included altered approaches and premature turns were called "turns too soon" and were quantified using

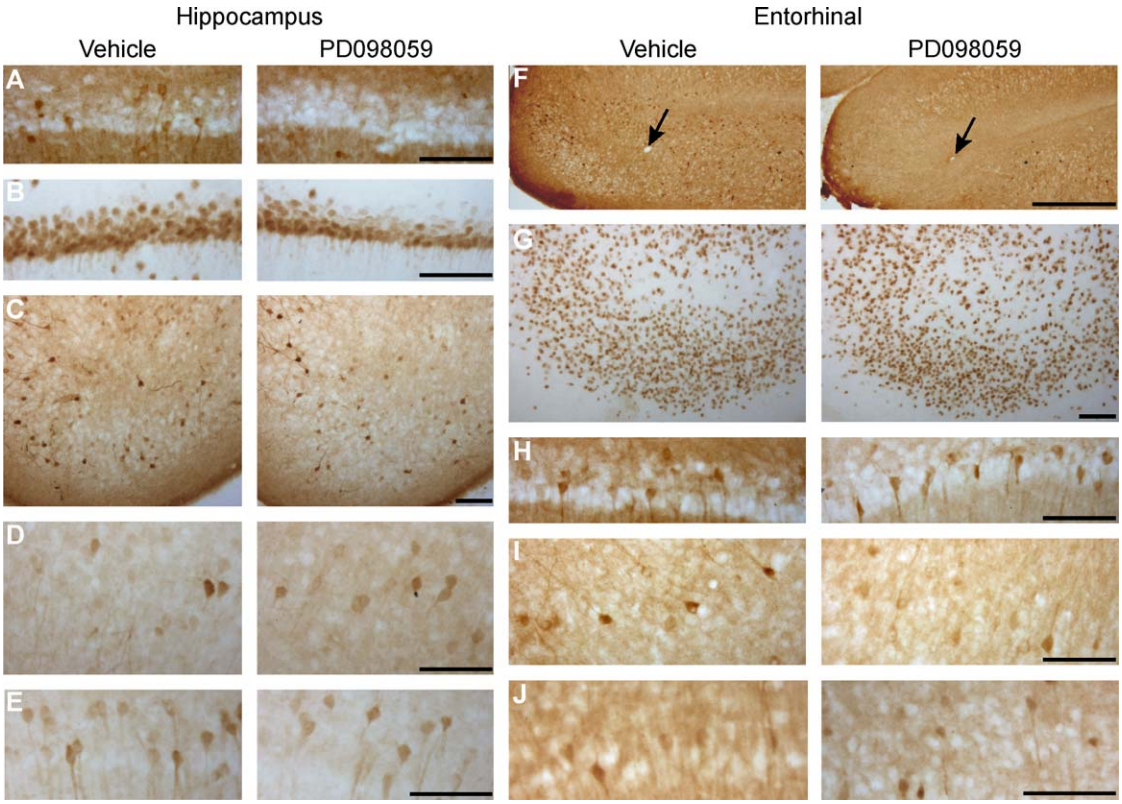

Fig. 1. Intrahippocampal PD098059 infusion decreases phospho-ERK (A) but not NeuN immunoreactivity (B) in the dorsal HIP and does not affect phospho-ERK immunoreactivity in the EC (C), dorsal subiculum (D), or ventral HIP (E). Intraentorhinal infusion of PD098059 decreases phospho-ERK immunoreactivity in the EC that extends approximately 0.5 mm from the infusion site (F). Intraentorhinal infusion of PD098059 did not affect NeuN immunoreactivity in the EC (G) and does not affect phospho-ERK immunoreactivity in the dorsal HIP (H), ventral subiculum (I), or ventral HIP (J). All compared with vehicle-infused contralateral side. Scale bar in Panels A–E and G–J represents 50 μm; scale bar in Panel F represents 500 μm. Arrows indicate the terminus of the infusion tract.

the following criterion. Altered approaches were incidences of the animal initiating a swim, with a target trajectory that was altered by making a sharp turn (>30°), such that the animal did not cross the previous platform location. Premature turns were defined as an attempt where the animal initiated the attempt turn too early; if it had turned slightly later (9 cm), the attempt would have been successful. The printouts were also used to determine the distance from which animals were able to make an attempt that reached the proximity of the platform location. Concentric circles were placed over the platform, and the circles of initiation and of termination of each attempt were recorded. Any attempt that terminated in the circle most proximal to the platform was considered for further analysis. For these attempts, the number of circles crossed (distance from platform) was averaged for each animal.

These data were analyzed using analysis of variance followed by post hoc analysis for Experiment 1, and using a Student's *t* test for unpaired variables for Experiment 2.

2.7. Verification of cannulae placement

Infusion sites were confirmed by histological analysis. Animals were killed and bilaterally infused with 1.5 μl Coomassie Blue dye to mark the infusion sites. The brains were then removed and processed, and 40-μm cryosections were prepared. Sections were stained with cresyl violet and the infusion sites recorded. Only animals with infusion sites within the target structures were included in the analysis.

3. Results

3.1. PD098059 infusion results in an inhibition of ERK activity surrounding the infusion site

The MEK inhibitor PD098059 has been previously shown to be specific for the ERK cascade (Alessi et al., 1995; Davies et al., 2000). Consistent with this, we have reported that intrahippocampal infusion of 2.0 μg PD098059 (1 μl/side) does not inhibit the activities of calcium/calmodulin-dependent protein kinase, protein kinase A, stress-activated protein kinase, or protein kinase C (Blum et al., 1999). This dosage and a 10-fold lower concentration (0.2 μg/ μl) of PD098059 were found to be effective in decreasing ERK phosphorylation in the HIP and caused an impairment in long-term spatial memory, but not short-term memory or acquisition (Blum et al., 1999). To confirm the inhibition of ERK in the HIP and EC for the present study and to determine if surrounding structures were affected, the basal level of

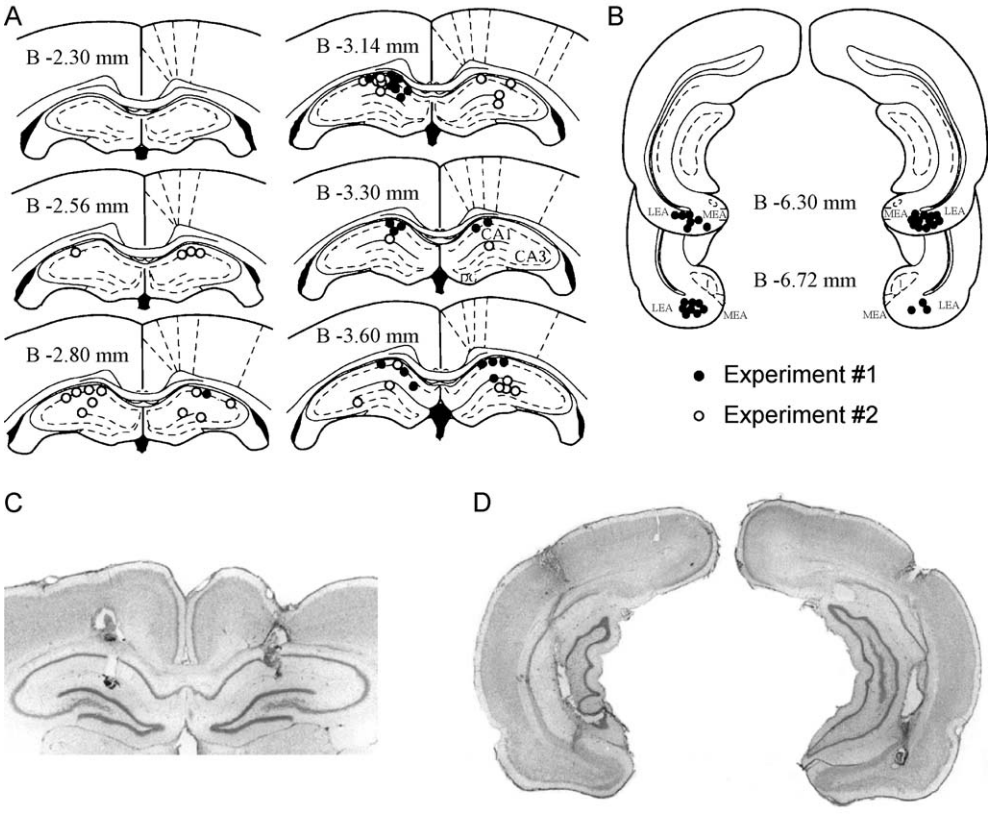

Fig. 2. Unique infusion sites for (A) hippocampal-cannulated (Experiment 1 n=21, Experiment 2 n=16) and (B) entorhinal-cannulated (n=15) animals as shown on coronal atlas plates from Paxinos and Watson (1997). Cresyl-violet-stained sections from representative animals for the (C) hippocampal- and (D) entorhinal-cannulated groups. Abbreviations: bregma (B), medial entorhinal area (MEA), and lateral entorhinal area (LEA).

phosphorylation of ERK was examined using immunohistochemistry following unilateral infusion of 0.2 μg of PD098059 into the EC or HIP with an equal volume of vehicle (1 μl) infused into the contralateral side of the same animal.

Fig. 1 shows representative photomicrographs of slices taken from HIP- or EC-infused animals. Following the infusion of 0.2 μg PD098059 into the HIP, a reduction in phospho-ERK immunoreactivity in the dorsal HIP can be seen compared with the vehicle-infused contralateral side, with a lack of phospho-ERK-positive cells for approximately 0.5 mm from the infusion site (Fig. 1A). Fig. 1B shows adjacent slices processed with antibodies against NeuN (neuron-specific nuclear protein), indicating the presence of intact cells and that the infusion of 0.2 μg PD098059 does not affect the general immunoreactivity of the tissue. Intrahippocampal PD098059 infusion does not affect phospho-ERK immunoreactivity in the EC (Fig. 1C), the dorsal subiculum (Fig. 1D), or in the ventral HIP (Fig. 1E). Following intra-EC infusion of 0.2 μg PD098059, there was a lack of phospho-ERK-positive cells within approximately 0.5 mm radius of the infusion site (Fig. 1F), including a reduction in phospho-ERK immunoreactivity in both the lateral entorhinal area (LEA) and in the medial

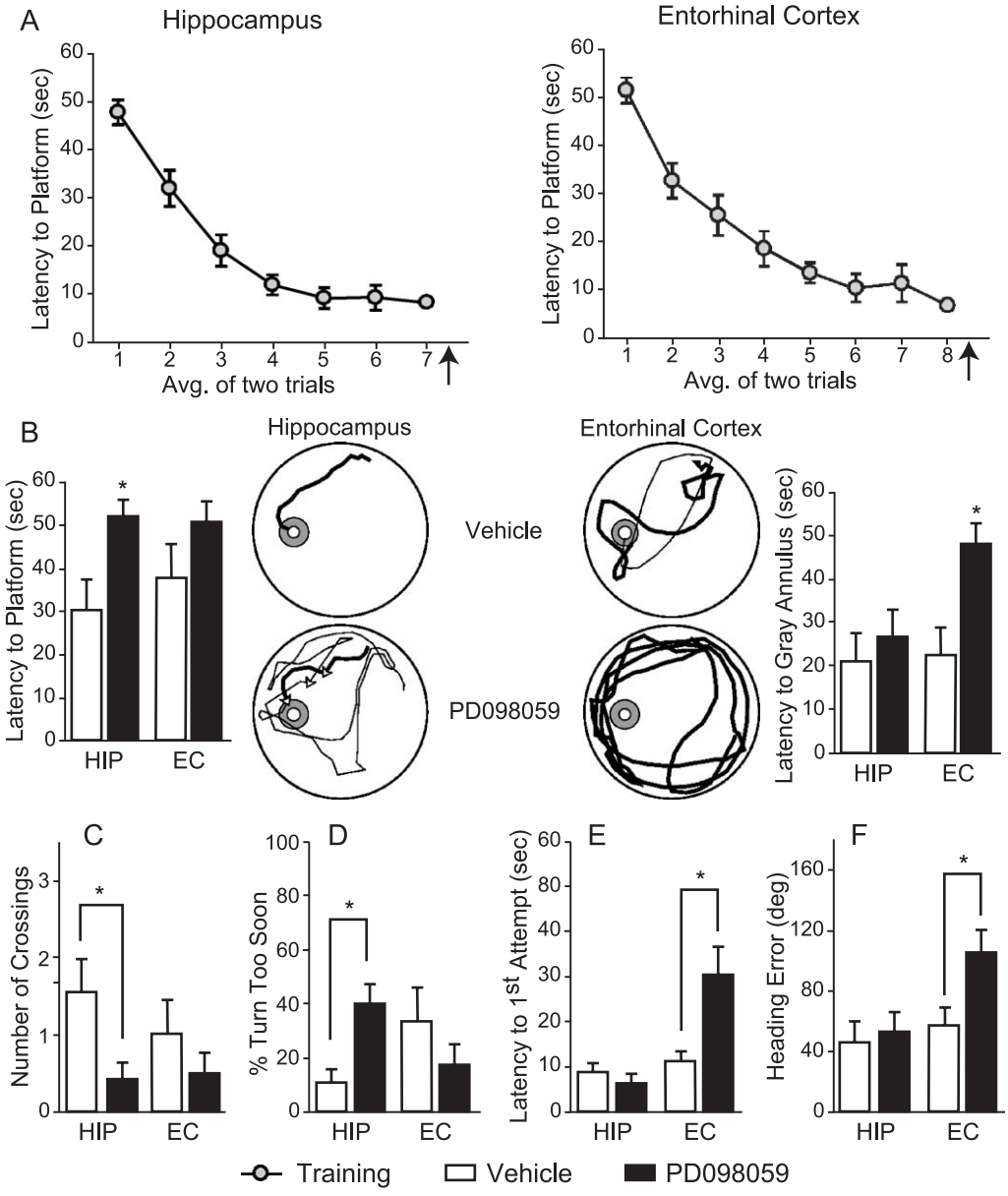

Fig. 3. Infusion of PD098059 into either the HIP or into the EC following training blocks long-term memory. (A) Latency to platform during training. Data were binned into blocks of two trials starting with the first trial. Arrows indicate infusion. (B–F) Performance during the retention test. Representative retention test traces show the platform location (white circle) and the gray annulus (gray circle). Thick lines: traces up to first gray annulus crossing. Thin lines: traces after the first gray annulus crossing up to the first platform location crossing. Arrowheads indicate examples of turning too soon. HIP: PD098059 (n=10) and vehicle (n=9); EC: PD098059 (n=8) and vehicle (n=7). Data are expressed as mean±S.E.M., *P<05.

entorhinal area (MEA) of the EC, which are thought to process different types of information (Witter et al., 2000). No difference in NeuN immunoreactivity in the EC was observed between the drug- and vehicle-infused sides (Fig. 1G). Intra-EC PD098059 infusion did not affect phospho-ERK immunoreactivity in the dorsal HIP (Fig. 1H), ventral subiculum (Fig. 1I), or in the ventral HIP (Fig. 1J). Although the duration of inhibition following PD098059 infusion has not been determined for this study, previously, we have shown that unilateral PD098059 administration is effective for up to one hour (Blum et al., 1999).

3.2. Cannulae site verification

Fig. 2A and B is a compilation of the bilateral infusion sites for animals included in the behavioral analysis, with only nonredundant infusion sites shown. Cresyl-violet-stained histological sections from representative animals are shown for the hippocampal- (Fig. 2C) and entorhinal-cannulated (Fig. 2D) groups.

3.3. ERK-mediated plasticity stores nonredundant, but complementary, aspects of spatial memory in the HIP versus the EC

Experiment 1 was designed to test for differences in the effects of blocking plasticity in the EC or the HIP on the retention of spatial memory. Hippocampal- and entorhinal-cannulated animals showed comparable memory acquisition (Fig. 3A). The infusion of PD098059 into either structure resulted in deficits in performance during the retention test. A comparison of latencies to the previous platform location showed significantly longer latencies for hippocampal- ($P<0.05$) (corroborating previous findings) but not entorhinal-drug-infused animals (Fig. 3B). The latency to gray annulus (an area with twice the diameter of the platform) analysis demonstrated significantly longer latencies in the entorhinal-drug-infused group ($P<05$) (corroborating previous findings), with no deficit in the hippocampal-drug-infused group (Fig. 3B). Analysis of swim speed did not reveal any significant differences (HIP: PD098059 26.52±1.09 cm/s, vehicle 25.63±1.80 cm/s, n.s.; EC: PD098059 26.96±0.91 cm/s, vehicle 28.35±1.30 cm/s, n.s.).

Retention test traces for hippocampal- and entorhinal-cannulated animals were further analyzed to examine differences in performance using measures of localization (number of platform crossings), directionality (heading error), and goal-related navigation (attempts) (Table 1). The number of platform crossings was significantly decreased for hippocampal-drug-infused animals ($P<05$) but not for entorhinal-drug-infused animals (Fig. 3C). To explain the short latency to the gray annulus, the longer latency to the platform, and the low number of platform crossings seen in hippocampal-drug-infused animals, an analysis of unsuccessful attempts due to altered approaches and premature turns ("turns too soon") was performed. The percent incidence of "turns too soon" was significantly increased for hippocampal-drug-infused animals ($P<05$), but not for entorhinal-drug-infused animals (Fig. 3D). To test the reliability of this measure, previously published data showing a long-term memory deficit following intrahippocampal-PD098059 infusion was analyzed for "turns too soon" to determine if this parameter is affected by differences in handling or subtle differences in protocol (Blum et al., 1999). The results for these previously published data were found to be consistent with what was observed in the present study (PD098059 35.12±7.36%, vehicle 6.25±4.09%, $P<05$).

Initial heading error, a measure of directionality, was significantly increased for entorhinal-drug-infused animals ($P<05$), but not for hippocampal-drug-infused animals (Fig. 3E). Entorhinal-drug-infused animals also demonstrated a significant increase in the latency to first attempt ($P<05$), whereas the hippocampal-drug-infused animals did not (Fig. 3F). However, there were no differences in the total number of attempts for either group compared with its control (HIP: PD098059 4.30±0.42 attempts, vehicle 3.89±0.45 attempts; entorhinal: PD098059 3.75±0.88 attempts, vehicle 4.71±1.06 attempts; n.s.).

Deficits in the measures of directionality (initial heading error) and in initiation of goal-directed navigation (latency to first attempt) in the entorhinal-drug-infused animals suggest either an initial disorientation effect, as both are measured early in the probe trial, or a deficit in the ability to navigate from a distance. To discriminate between these possibilities, an analysis of how often animals successfully navigated from a distance to the proximity of the platform throughout the whole retention test was examined. There was no difference in the total number of attempts that reached the proximity of the platform from anywhere in

Table 1
Summary of Experiment 1 retention test results

	PD098059 infusion location	
	Hippocampus	Entorhinal cortex
Location information		
Precise		
Latency to previous platform location	Impaired	n.s.
Number of platform crossings	Impaired	n.s.
Broad		
Latency to gray annulus	n.s.	Impaired
Goal-directed navigation		
Number of attempts	n.s.	n.s.
Turns too soon (aborted and premature attempts)	Impaired	n.s.
Latency to first attempt	n.s.	Impaired
From a distance		
Initiation distance of proximal attempts	n.s.	Impaired
Initial heading error	n.s.	Impaired

Impaired=significant difference ($P<05$), n.s.=nonsignificant difference compared with vehicle-infused control animals.

the tank (HIP: PD098059 1.82±0.40 attempts, vehicle 2.30±0.30 attempts; entorhinal: PD098059 1.37±0.37 attempts, vehicle 2.43±0.84 attempts, n.s.); however, there was a difference in the start location of these attempts, with a significant decrease in the distance from which animals could reach the proximity of the platform in the entorhinal-drug-infused group, but not in the hippocampal-drug-infused group (HIP: PD098059 6.03±1.50 circles, vehicle 8.77±0.61 circles, n.s.; entorhinal: PD098059 4.75±1.08 circles, vehicle 10.53±1.02 circles, $P<.05$). There were also no differences in measures of thigmotaxic behavior (data not shown).

The term "savings" is often used to refer to faster relearning following extinction of a learned behavior. In this case, we tested savings following the retention test using the same protocol and criterion as for original training, with a second retention test given 48 h later. The number of trials to retrain for both hippocampal-cannulated groups is significantly decreased as compared with the original training, demonstrating savings ($P<.05$); however, the drug-infused animals required more trials to reach criterion, on average, than the vehicle-infused animals do ($P<.05$), demonstrating a loss of information that the animals had to relearn (Fig. 4A and B). The performance of hippocampal-drug-infused animals was equivalent to the control animals during the second retention test (Fig. 4C), indicating that hippocampal function was not permanently impaired.

Fig. 4D shows the training and retraining curves for the entorhinal-cannulated animals demonstrating savings in both the drug- and vehicle-infused groups. Fig. 4E shows the average number of trials to reach criterion for training and retraining. The entorhinal-drug- and vehicle-infused groups both took significantly fewer trials to retrain when compared with original training ($P<.05$), but not when compared with each other; however, the retraining curve for drug-infused animals demonstrated higher latencies to platform over the retraining period when compared with the vehicle-infused group. Drug-infused animals were not statistically different from vehicle-infused animals in latency to platform location during the second retention test (Fig. 4F).

3.4. Experiment 2

The results from Experiment 1 suggest that hippocampal-drug-infused animals have a deficit for the precise, but not the broad, location of the platform. However, it is possible that the location information is intact and that the deficit is due to an inability to maintain a target trajectory or to perform path integration. To distinguish between these two

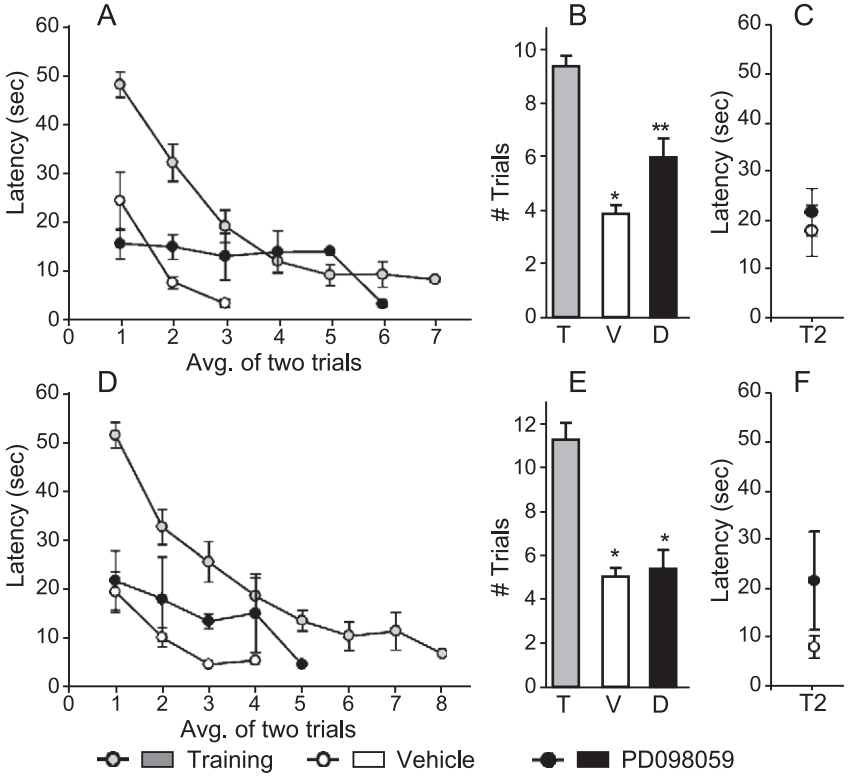

Fig. 4. Savings in hippocampal-cannulated animals as shown by training and retraining curves (A) and average number of trials to reach criterion (B) for vehicle- (n=8) and PD098059-infused (n=10) animals. Latency to platform location during second retention test (C). Savings in entorhinal-cannulated animals as shown by training and retraining curves (D) and average number of trials to reach criterion (E) for vehicle- (n=7) and PD098059-infused (n=8) animals. Latency to platform location during second retention test (F). Abbreviations: training (T), vehicle infused (V), PD098059 infused (D), and second retention test (T2). Training and retraining curves were plotted by binning data into blocks of two trials starting with the first trial. Data are presented as mean±S.E.M., *$P<.05$.

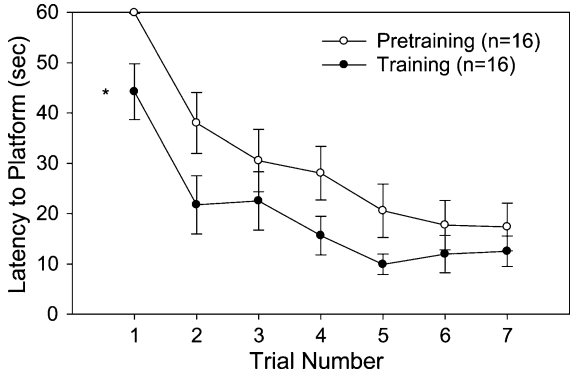

Fig. 5. Prior training in a separate water maze facilitates training. Latency to platform location for the first seven trials during training in the first (○) and second rooms (●). Data are presented as mean±S.E.M., *P<05.

alternatives, a two-room protocol was employed with a new group of hippocampal-cannulated animals.

3.4.1. Prior training attenuates the deficit following ERK inhibition in the HIP

Experiment 2 was designed with the idea that training in the first room will allow the animals to consolidate information regarding navigational strategies prior to training and drug infusion in the second room. To confirm the consolidation of information from training in the first room, a probe trial was performed. All animals demonstrated long-term memory for the training in the first room (Fig. 6A). If training in the first room allowed the animals to also consolidate information regarding the navigational strategies, training in the first room should facilitate training in the second room. A comparison of the initial portion of the training curves (broken down into individual trials) shows consistently shorter latencies to the platform during training in the second room (P<05; Fig. 5), demonstrating facilitation. In addition, the average number of trials during which animals failed to recognize the hidden platform as a means of escape (leaving the platform once found to continue swimming) was significantly decreased (first room 1.50±0.61 trials, second room 0.06±0.06 trials; P<05).

During the second retention test (following training and drug or vehicle infusion), drug-infused animals were not significantly different than vehicle-infused animals in the latency to cross the previous platform location (Fig. 6A). The vehicle-infused animals did demonstrate slightly longer

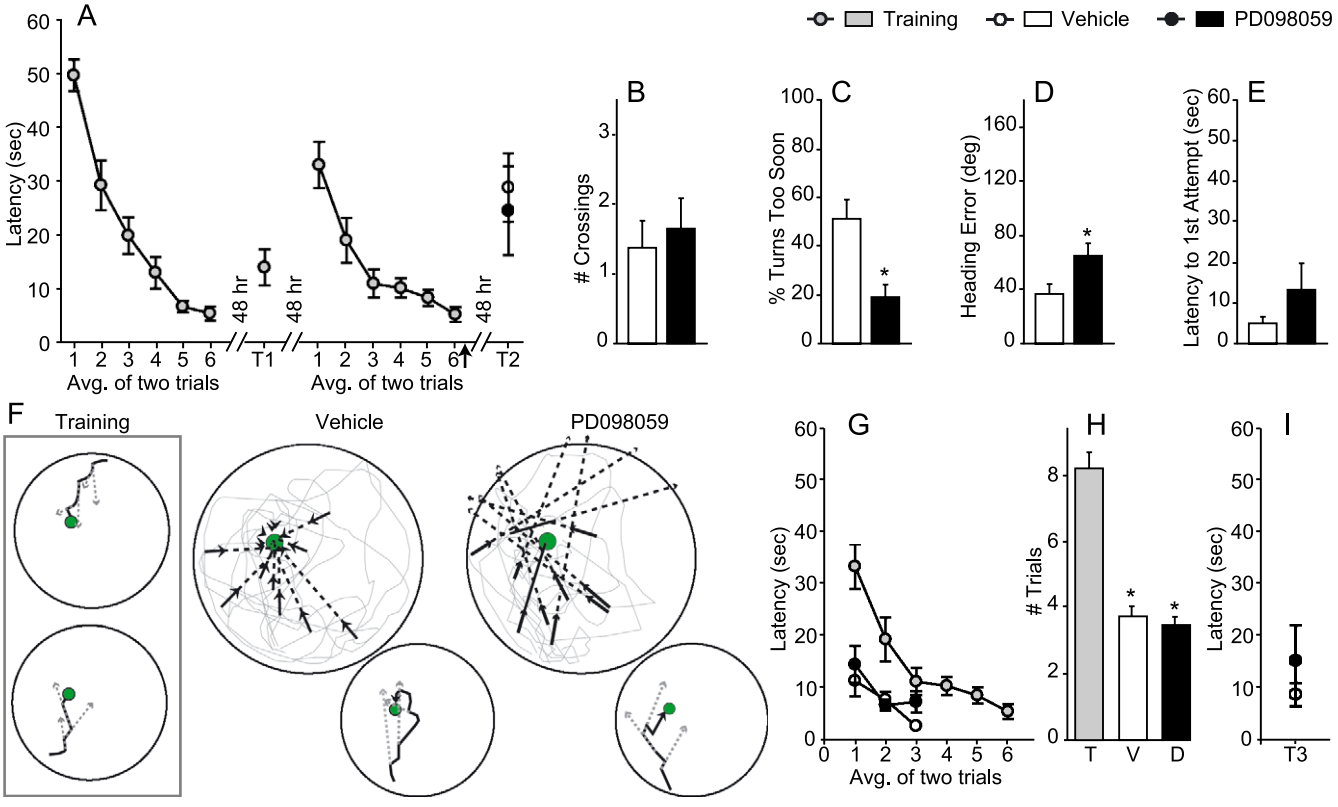

Fig. 6. Prior training attenuates, but does not prevent, the deficit induced by intrahippocampal PD098059 infusion. Latency to platform location during training in the first room, the first retention test (T1), training, and the second retention test (T2). Arrow indicates infusion (A). Retention test performance (B–E). Training and retention test traces (small circles) showing zigzag strategy (F). Dark line: animal's path. Gray dotted lines: trajectories. Large circles with superimposed traces for all animals (one drug-infused animal that circled the tank was left out for clarity) up to the first platform crossing with arrows and dotted lines showing trajectories aimed at (vehicle-infused animals) or near (PD098059-infused animals) the platform location. Savings are shown by the latency to platform location (G) and the average number of trials to reach criterion (H). Latency to platform location during the third retention test (I). PD098059 (n=8) and vehicle (n=8). Training and retraining curves were plotted by binning data into blocks of two trials starting with the first trial. Abbreviations: training (T), vehicle infused (V), PD098059 infused (D), third retention test (T3). Data are presented as mean±S.E.M., *P<05.

latencies to the platform location in the second retention test than in the first retention test; but because their latencies were similar to that of the vehicle-infused animals in Experiment 1, this is likely due to a general infusion effect. Consistent with the latency measure, drug- and vehicle-infused groups were also not different in the number of crossings of the previous platform location (Fig. 6B), latency to make a first attempt (Fig. 6E), total number of attempts (PD098059 3.75±0.86 attempts, vehicle 4.62±0.65 attempts; n.s.), or in swim speed (PD098059 24.28±1.51 cm/s, vehicle 22.46±2.00 cm/s; n.s.). However, the drug-infused animals did show significantly increased initial heading error ($P<.05$), which appears to be due to a decrease in this value in the control group relative to Experiment 1 (Fig. 6D), and a difference in the "turns too soon" measure. Interestingly, the values for the percent of "turns too soon" are opposite to that observed in Experiment 1. The drug-infused animals demonstrate significantly fewer "turns too soon" than did the vehicle-infused animals ($P<.05$; Fig. 6C). In addition, the vehicle-infused animals in Experiment 2 demonstrated more "turns too soon" compared with the vehicle-infused animals in Experiment 1 (Experiment 1 vehicle 11.33±4.94%, Experiment 2 vehicle 51.05±8.21%; $P<.05$). Individual training and retention test traces (Fig. 6F) demonstrate that due to the extra training provided by the two-room training protocol, both the vehicle- and drug-infused animals use a more advanced zigzag navigational strategy to find the hidden platform. The composite traces in Fig. 6F demonstrate the combined trajectories of all animals to visually show the "turns too soon" result, demonstrating that while the vehicle-infused animals aim directly at the platform location during their approaches, the drug-infused animals aim in the general vicinity of the platform, but not directly at it during their approaches.

Following the retention test, the animals were retrained to criterion. Drug-infused animals showed savings equivalent to control animals (Fig. 6G and H). Both hippocampal-drug- and vehicle-infused groups took significantly fewer trials to retrain when compared with the original training ($P<.05$). However, the drug- and vehicle-infused groups were not significantly different from each other. There was also no difference between vehicle- and drug-infused animals in latencies to platform location on the third retention test (Fig. 6I).

4. Discussion

Using a posttraining, targeted plasticity-blocking paradigm, the involvement of ERK-mediated long-term plasticity in two structures known to be involved in spatial memory has been contrasted. The key finding of this report is that ERK-mediated plasticity in the HIP (as compared with the EC) is involved in storing different aspects of spatial memory. Specifically, ERK inhibition in the HIP resulted in disruption in indices of spatial memory, suggestive of a loss of precise location information, while ERK inhibition in the EC resulted in a disruption in the indices of spatial memory, suggestive of a loss of broad location information. The deficits appeared to be attributable to the blockade of ERK activity in these structures, as immunohistochemical analysis did not reveal any influence of the infusion on phospho-ERK immunoreactivities in the surrounding structures. These results suggest that, together, information stored in these two structures may allow for navigation from a distance (entorhinal) to a precise (HIP) unmarked goal.

The impairments seen following hippocampal-drug infusion as compared with entorhinal-drug infusion were readily distinguishable using standard measures of memory for this task, such as latency to platform (or gray annulus) and heading error, both of which are sensitive to the degree of learning (Morris, 1984; Guzowski et al., 1997; Blum et al., 1999; Teather et al., 2002; Hebert et al., 2002). The use of a 1-day training paradigm results in a lack of robust localized searching in control animals, as measured by dwell time in the training quadrant (Morris, 1984). However, measuring the number of platform crossings still allowed for distinguishing a difference between localized searching in control animals and a lack of localized searching in hippocampal-drug-infused animals. In addition, a 1-day training protocol has been previously shown to result in an increase in ERK activity in the dorsal HIP, which is necessary for the long-term memory for the task (Blum et al., 1999). This protocol also has the advantage of only requiring a single, posttraining infusion, removing the confound of an affect of ERK blockade on acquisition that is present when training over days. In addition to standard measures, novel behavioral measures were also used, allowing for a better description of the retention test performance. Several papers examining behavior in the water maze task have developed new measures to quantify a particular type of behavior (Whishaw et al., 1986; Cain, 1998).

Although not significant, during the long-term retentiontest, entorhinal-drug-infused animals demonstrated increased latencies to the platform. The lack of a statistical effect appears to be due to increased latencies in the vehicle-infused group, which were, on average, higher than in the hippocampal-vehicle-infused group, possibly the result of a general surgery effect (see Fig. 2). However, this did not result in significant differences between the hippocampal- and entorhinal-vehicle-infused animals in any of the parameters measured. Entorhinal-drug-infused animals displayed significant deficits in latency to the gray annulus and initial heading error, both indices of broad location information. In addition, over the entire retention test, the attempts made by drug-infused animals from far away did not reach the proximity of the platform location, and those attempts that did reach the platform location were initiated from a position closer than for the control

animals, demonstrating a deficit in navigation from a distance (Table 1). An inability to navigate from a distance may reflect a loss of broad location information because animals with memory for the position of the platform approach it from a distance (Morris, 1984). This raises the question of how these animals are able to cross the platform location as often as control animals do. As already mentioned, entorhinal-drug-infused animals were able to reach the proximity of the platform during attempts that were initiated from a position near the platform. It is possible that knowledge of the precise location of the platform, perhaps stored in the HIP, is still intact and is able to guide the animal if they are already close enough to the correct location as a result of random swimming.

In contrast, the hippocampal-drug-infused animals demonstrated increased latencies to the platform, but not to the gray annulus, indicating a deficit in precise location information, but not broad location information. The deficit in the measure "turns too soon" indicates that the drug-infused animals turned away from the platform during their attempts, which could be indicative of a navigational problem or consistent with the loss of precise location information (Table 1). Experiment 2 specifically tested whether PD098059 infusion into the HIP results in a navigational or location deficit, using a two-room training protocol. Both groups of animals employed a more advanced zigzag approach to the platform, presumably due to the extra training provided by the two-room design, suggesting the retention of navigational strategies in the drug-infused group. However, by comparison with the target-oriented, constrained zigzag employed by the vehicle-infused group, hippocampal-drug-infused animals displayed a broad, sweeping zigzag aimed in the general vicinity of the platform. This modification of the target-oriented approach suggests that the drug-infused animals have an expanded representation of the platform consistent with a loss of precise location information.

Savings is a term which often refers to faster relearning following normal forgetting or manipulations, such as pharmacological blockade or extinction. This faster relearning is thought to result from residual memory or plasticity. In Experiment 1, animals receiving either vehicle or drug infusion following original training needed fewer trails to retrain, indicating savings. Interestingly, the HIP-drug-infused animals demonstrated less savings than did the EC-drug-infused animals (see Fig. 4B and E), suggesting that the HIP plays a more prominent role in savings than the EC does. However, additional experiments would be needed to further examine the respective roles of these structures in savings.

These results are consistent with place-cell firing properties in the HIP and the EC. Electrophysiological studies suggest that, together, the HIP and the EC are important for forming a spatial representation of the environment. Both the HIP and the MEA contain spatially selective cells (O'Keefe and Dostrovsky, 1971; Quirk et al., 1992). The MEA cells fire at a higher background rate than hippocampal place cells do and have broader place fields (Quirk et al., 1992), and a higher number of MEA cells demonstrate prospective coding (firing patterns that correlate with where an animal will go next) following training in a goal-oriented task (Frank et al., 2000). By comparison, hippocampal place-cell firing can predict the behavior of an animal trained in a radial arm maze (O'Keefe and Speakman, 1987), and place fields show an increase in representation of the goal location following training in the Morris water maze, indicating goal-related firing (Hollup et al., 2001). In addition, altering hippocampal place cell firing disrupts map-based navigation (Lenck-Santini et al., 2001; Lenck-Santini et al., 2002; but see Jeffery et al., 2003).

Although we cannot rule out a contribution of ERK inhibition in cells without spatial selectivity, our results suggest that a loss of broad place field firing stability and prospective coding in the EC would lead to a lack of knowledge of the broad location of the platform and an inability to navigate to a goal from a distance, where a loss of constrained, goal-related place-cell firing in the HIP would lead to a lack of knowledge of the precise location of the platform. As NMDAR blockade following exposure to a novel environment disrupts place-cell stability (Kentros et al., 1998), and because the ERK cascade is known to be necessary for NMDAR-dependent LTP (English et al., 1997), the blockade of ERK may give rise to impaired long-term place cell stability in the targeted structure. However, it is possible that the attenuation of deficits seen following drug-infusion in the HIP in the two-room experiment could be due to a decreased necessity for NMDAR-dependent plasticity (Bannerman et al., 1995; Saucier et al., 1995; Morris et al., 2003). These results suggest that, together, information stored in these two structures may allow for navigation from a distance (entorhinal) to a precise (HIP) unmarked goal.

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