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A role for medial entorhinal cortex in spatial and nonspatial forms of memory in rats

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Abstract

Many studies have focused on the role of the medial entorhinal cortex (MEC) in spatial memory and spatial processing. However, more recently, studies have suggested that the functions of the MEC may extend beyond the spatial domain and into the temporal aspects of memory processing. The current study examined the effect of MEC lesions on spatial and nonspatial tasks that require rats to learn and remember information about location or stimulus-stimulus associations across short temporal gaps. MEC- and sham-lesioned male rats were tested on a watermaze delayed match to position (DMP) task and trace fear conditioning (TFC). Rats with MEC lesions were impaired at remembering the platform location after both the shortest (1 min) and the longest (6 hr) delays on the DMP task, never performing as precisely as sham rats under the easiest condition and performing poorly at the longest delay. On the TFC task, although MEC-lesioned rats were not impaired at remembering the conditioning context, they showed reduced freezing in response to the previously associated tone. These findings suggest that the MEC plays a role in bridging

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temporal delays during learning and memory that extend beyond its established role in spatial memory processing.

Keywords

medial entorhinal cortex; memory; rat; temporal; spatial; trace fear conditioning

INTRODUCTION

The formation and retrieval of episodic memories, which contain both spatial and temporal components, is dependent on the medial temporal lobes. The hippocampus has a well-established role in spatial processing (i.e., place cells) and spatial memory [1,2]. In addition, the contribution of the medial entorhinal cortex (MEC) to spatial processing (i.e., grid cells) and to spatial memory has also been shown [3,4].

To better understand the relationship between spatial processing and memory in the hippocampus and MEC, a recent study used precise excitotoxic lesions of the entire MEC to examine hippocampus-dependent memory ability and place cell firing [5]. Following almost complete MEC lesions, rats displayed similar spatial memory impairments as seen following excitotoxic hippocampal lesions; however, MEC-lesioned rats did not show impairments on hippocampus-dependent non-spatial memory tasks. Consistent with the spatial impairments, MEC lesions also impaired the stability and precision of hippocampal place cell firing [5].

Besides the finding of anterograde deficits in spatial memory acquisition, MEC lesions also caused retrograde memory deficits, as rats were impaired at locating a previously learned reference platform location following MEC lesion surgery [6]. Additionally, following fear conditioning with a temporally discontiguous tone and foot shock, rats with MEC lesions showed impaired fear memory retrieval when exposed to either a previously conditioned context or an associated tone. These findings suggest that the role of the MEC in memory retrieval is not limited to tasks involving navigation or place memory, but instead may include temporal aspects of memory. Further evidence of MEC involvement in temporal aspects of memory is that MEC lesions disrupt theta phase precession in the hippocampus, suggesting involvement of the MEC in the temporal organization of activity in the hippocampus [7]. The hippocampus plays a known role in both temporal processing, containing “time cells” [8], and temporal aspects of memory formation [9,10]. This recent finding of MEC lesions disrupting temporal firing patterns in the hippocampus further supports the possibility that the MEC may play a larger role in memory, extending into the temporal processing domain. Despite the attention paid to the role of the MEC in spatial processing and memory, researchers have only begun to examine the temporal functions of the MEC.

Despite the recent evidence of MEC lesions disrupting the retrieval of fear memory for a tone previously associated with a temporally discontiguous foot-shock, the precise role of the MEC in temporal aspects of memory formation is still unclear. Studies have shown that retrograde memory tests are far more sensitive to hippocampal lesions than anterograde tests [11]; with anterograde designs, secondary brain areas can compensate when a primary

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memory structure, such as the hippocampus, is damaged. However, retrograde designs are unable to assess whether or not a brain area is *essential* because damage to nonessential brain areas that are merely involved in encoding will also result in retrieval deficits, due to portions of the memory representations being encoded by these structures. Therefore, only anterograde designs are able to assess whether a brain area is *essential* for memory formation.

The current studies were designed to directly examine the involvement of the MEC in bridging temporal delays in memory encoding in both spatial and non-spatial tasks. Rats with complete bilateral excitotoxic lesions of the MEC or sham lesions were tested on two different tasks: the delayed matching to position (DMP) task in the watermaze and trace fear conditioning (TFC).

MATERIALS AND METHODS

Subjects

All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of California, San Diego. The subjects were 78 male Long–Evans rats weighing between 300 and 400 g at the beginning of the experiment. Rats were housed individually on a 12-h light/dark cycle with continuous access to food and water. Testing was performed in the light phase. Rats were randomly assigned to receive NMDA lesions of the medial entorhinal cortex (MEC; $n = 39$) or sham lesions to serve as the control group, in which rats underwent the same initial surgical procedures as the lesion groups, but the dura was not punctured (SHAM; $n = 39$). After they recovered, thirty-one of the rats were trained on the Delayed Matching-to-Position (DMP) task (MEC, $n = 16$; SHAM, $n = 15$), and forty-seven were trained on Trace Fear Conditioning (TFC; MEC, $n = 23$; SHAM, $n = 24$).

Surgery

All surgery was performed using aseptic procedures. Anesthesia was maintained throughout surgery with isoflurane gas (0.8%-2.0% isoflurane delivered in O_2 at 1 L/min). The animal was positioned in a Kopf stereotaxic instrument, and the incisor bar was adjusted until *Bregma* was level with *Lambda*. The bone overlying the target site was removed using a high-speed drill. After completion of each lesion, the wounds were closed, and the animal was allowed to recover from anesthesia on a water-circulating heating pad. Behavioral testing began ~two weeks after surgery.

Excitotoxic lesions were produced by NMDA. NMDA (Tocris) was dissolved in aCSF to provide a solution with a concentration of 10 mg/ml and was injected at a rate of approximately 0.1 μl/min using a 10 μl Hamilton (Reno, NV) syringe mounted on a stereotaxic frame and held with a Kopf model 5000 microinjector. The needle was lowered at ML ± 4.6 mm at an angle of 22° (in the posterior to anterior direction) with the needle tip placed immediately anterior to the transverse sinus. Once the syringe needle was lowered to the target, it was left in place for 1 min to let the brain tissue settle before beginning the injection. NMDA was injected into eight different DV coordinates (−5.2, −4.7, −4.2, −3.7,

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−3.2, −2.7, −2.2, −1.7 mm; total volume 1.04 μl) within each hemisphere of the brain to lesion the areas with grid cells along the entire dorsoventral axis of the medial entorhinal cortex and in the parasubiculum. After each injection, the syringe needle was left in place for 1 min to reduce the spread of drug up the needle tract.

Behavioral testing

Delayed Matching-to-Position (DMP)

Apparatus.: Testing was conducted in a pool of water (1.8 m diameter at the water level) that was rendered opaque by the addition of powdered milk. The testing room contained a number of constant, salient visual cues (posters, objects, and equipment). A video camera mounted on the ceiling directly above the pool was used in conjunction with a video tracking system (San Diego Instruments) to record the swim path of each rat. A platform (12.7-cm diameter) was used, which remained 1.5 cm below the surface of the water, and provided a means to escape the water.

Testing.: All rats were trained on the standard watermaze prior to receiving sham or MEC-lesion surgeries (data reported in [6]). Following recovery from surgery, rats were pretrained on the DMP task for five days. Each day, rats received four trials with the platform remaining in the same location for each trial and a 15 s intertrial interval (ITI). At the start of each trial, rats were placed in the water facing the pool wall at one of four start points (counterbalanced across animals and across trials). Once the rat found the platform, it remained on the platform for 30 s before returning to the home cage for 15 s. The platform location in the pool changed each day, but remained constant for all rats within a day. For each trial, rats were given a maximum of 2 min to find the platform before being guided to the platform by the experimenter. By the end of the fifth pretraining day, all rats exhibited an efficient search strategy and showed an improvement in locating the platform across Trials 1–4 each day.

Rats received 16 days of training with the platform location changing each day. During the four daily trials, the rat starting positions changed each trial and the ITIs between Trial 2 and Trial 3 (T2-T3) and between Trial 3 and Trial 4 (T3-T4) were 15 s each. There were four different possible delay times between Trial 1 and Trial 2 (T1-T2): 1 min, 20 min, 90 min, or 6 hr. Rats received each T1-T2 delay four times over the 16 testing days, receiving each delay type once before receiving them a second time. T1-T2 delay lengths were counterbalanced between rats and across days. Rats were given one final day of testing where the location was different on T2–4 compared to T1 to ensure that the rat was unable to detect the location of the platform on T1 and was actually making the decision on T2 based on the location learned on T1. Performance on Trial 1 and Trial 2 were calculated by measuring the swim path distance (in cm). After escaping the water, the rats remained on the platform for 30 s before they were returned to their home cage for all ITIs. Because the location of the platform on Trial 1 was always unknown to the rat, distance traveled on Trial 1 is an index of performance without memory. The distance to find the platform on Trial 2 can be used as an index of memory for the platform location on Trial 1. Performance on Trials 3 and 4 were not analyzed as these trials were used to reinforce learning that the platform remained in the same location for each trial within a day.

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Trace Fear Conditioning (TFC)—Trace fear conditioning is a hippocampus-dependent task, in which a rat learns to fear a tone that had been previously paired with a temporally discontiguous foot shock. We measured the amount of freezing displayed by rats when they were returned to the same environment (Context A) in which they had previously been shocked (context test) and the amount of freezing resulting when the same tone was presented in a novel environment (Context B; tone test). We compared these measures between rats that received MEC versus SHAM lesions prior to conditioning.

Apparatus.: Rats were tested in a sound attenuating fear-conditioning chamber (MED-Associates, Burlington, VT). Each chamber had a mounted infrared digital video camera connected to a PC computer with software that computes a frame-by-frame comparison to determine the amount of freezing (Med-Associates). Foot shock (1.0 mA; 2 s) was delivered through the floor's steel rods. A 20-sec pure tone (90 dB) was delivered through a speaker placed within each of the conditioning chambers. All rats were conditioned in Context A, which consisted of uncovered transportation of animals to the testing room, low room lighting, total interior chamber darkness, and metal shock grid floors cleaned with 95% ethanol.

The context test was also conducted in Context A. The tone test was conducted in Context B, which consisted of covered transportation of animals to the testing room, high ambient room lighting, high interior chamber lighting, and white plastic floor boards and black triangular enclosures within the chambers which were all cleaned with bleach. Rats were tested in different operant conditioning chambers for Contexts A and B.

Conditioning in Context A.: Rats were placed into the fear conditioning chambers for a 25-min, 20-s conditioning session during which there were 4 min of baseline followed by five tone–shock pairs (20-s auditory tone, 30-s stimulus-free trace interval, 2-s (1.0 mA) shock, and 240-s inter-trial interval). The conditioning session ended 60 s after the last trial. *Context A Test.* Twenty-four hours after conditioning, rats were placed for 8 min into the same chamber used for fear conditioning, and freezing was measured to assess retention of context fear memory. *Context B Habituation.* The next day, rats were habituated to Context B for 8 min. This new context included a different conditioning chamber with a triangular façade and a plastic floor to cover the steel shock rods. *Tone Test in Context B.* Retention of the conditioned fear response to the tone was assessed 24 hr later. Rats were placed back into Context B, which they were habituated to the day before. After a 4-min baseline period, the rats received one 10-s tone and remained in the chamber for the remainder of the 8-min trial while freezing was measured. Cumulative freezing after the onset of the tone was calculated at the end of the trial.

Statistical analysis

For the DMP analyses, the distance traveled to locate the platform on Trial 2 was compared between MEC ($n = 16$) and SHAM ($n = 15$) rats for each delay length using an unpaired t-test. Effects were considered significant at $p < 0.05$. A repeated measures ANOVA was also used to examine group differences across delays. In order to calculate the percent time freezing for each rat, the cumulative time each rat spent freezing was divided by the testing

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period (x 100). For the context test, the 420 s testing period was the entire length of the test; for the tone test, the 420 s testing period was the length of the test that followed the 10 s tone (the 240 s that preceded the tone was used to calculate baseline freezing). Freezing was measured using Video Freeze software (sampling rate = 30 frames/sec). For the context and tone tests, difference in percent time freezing between MEC ($n = 23$) and SHAM ($n = 24$) rats was calculated using an unpaired t-test. Effects were considered significant at $p < 0.05$. Effect sizes were calculated for each analysis using Cohen's *d*.

Neurohistological Methods

At the completion of testing, rats were administered an overdose of sodium pentobarbital and perfused transcardially with buffered 0.9% NaCl solution followed by either 4% or 10% formaldehyde solution (in 0.01 M phosphate buffer). Brains were then removed from the skull and cryoprotected in a solution of 20% glycerol and 10% formaldehyde or kept in a solution of 4% formaldehyde followed by 30% sucrose. Sagittal sections (40 or 50 μm) were cut with a freezing microtome beginning just lateral to the hippocampus and continuing medially through the hippocampal region for each hemisphere. Every fourth section was mounted and stained with cresyl violet to assess the extent of the lesions. An additional series of sagittal sectioned brains was prepared for immunolocalization of neuron-specific nuclear protein (NeuN) by using an anti-NeuN (1:15000, Chemicon) monoclonal mouse antibody. A biotinylated anti-mouse IgG (H+L) (1:1000, Vector BA-2000) was used as the secondary antibody. Quantification of the MEC lesion was obtained by calculating the percent damage in 0.35mm increments through the lateral-medial extent of the MEC (3 sections from each hemisphere, from ± 4.6 – 3.9 mm from midline) [12].

RESULTS

Neurohistological Findings

Figure 1 shows photographs of sagittal sections through the MEC moving lateral to medial in an MEC- and sham-lesioned rat. The MEC-lesioned rats had damage to 93.6% of the total MEC volume, with damage to 97.3% of layer II, 94.0% of layer III, and 89.4% of deep layers (supplemental table 1 provides the percent damage for each cell layer assessed at lateral, intermediate, and medial sections for each rat). Only five of the 39 MEC-lesioned rats had damage that extended beyond the MEC cell layers: three rats had slight damage that spread anterior to the presubiculum/subiculum, five had moderate damage to the parasubiculum, three had damage that extended superior to the MEC into the postrhinal cortex, and only one had very slight damage to the lateral entorhinal cortex. There was no evidence of damage to the amygdala or thalamus in any animal.

Delayed Matching-to-Position

Comparing Trial 2 distances after each of the four delay lengths for each rat, a repeated measures ANOVA (Group × Delay) revealed main effects of Lesion and Delay (lesion: $F_{(1,29)} = 7.37$, $p < 0.05$; delay: $F_{(3,29)} = 5.13$, $p < 0.05$). Although the interaction did not reach significance, unpaired t-tests revealed delay-dependent effects. After the shortest time delay of 1 min between Trial 1 and Trial 2, the MEC lesion rats traveled a longer distance to find the platform on Trial 2 than the SHAM rats (MEC mean ± SEM: 377.80 ± 62.66%;

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SHAM mean ± SEM: 198.34 ± 21.99%; $t_{(29)} = 2.63$, $p < 0.05$; Cohen's $d = 0.96$; Figure 2). MEC lesion rats also traveled a longer distance than SHAM rats to find the platform on Trial 2 after the longest time delay of 6 hr between Trial 1 and Trial 2 (MEC mean ± SEM: 583.58 ± 73.51%; SHAM mean ± SEM: 365.07 ± 45.08%; $t_{(29)} = 2.49$, $p < 0.05$; Cohen's $d = 0.90$). Although the MEC rats traveled numerically longer distances than the SHAM rats on Trial 2 after the intermediate delays of 20 min (MEC mean ± SEM: 379.93 ± 63.92%; SHAM mean ± SEM: 300.50 ± 62.60%) and 90 min (MEC mean ± SEM: 391.13 ± 50.29%; SHAM mean ± SEM: 284.53 ± 60.52%), the MEC lesion rat performance was not significantly different than that of the SHAM rats (20 min: $t_{(29)} = 0.89$, $p > 0.1$; Cohen's $d = 0.32$; 90 min: $t_{(29)} = 1.36$, $p > 0.1$; Cohen's $d = 0.49$). There were no differences between groups for swim path distances on Trial 1 (all $t < 1.20$, $p > 0.1$).

Trace Fear Conditioning

When rats were placed into the same context in which they were previously shocked, the cumulative amount of freezing at the end of the trial did not differ between MEC and SHAM groups (MEC mean ± SEM: 56.5 ± 5.8%; SHAM mean ± SEM: 59.4 ± 5.7%; $t_{(45)} = 0.36$, $p > 0.1$; Cohen's $d = 0.10$; Figure 3A). Rats were then habituated to a new context, and MEC rats showed less freezing in response to the tone (MEC mean ± SEM: 28.6 ± 4.9%; SHAM mean ± SEM: 48.0 ± 5.8%; $t_{(45)} = 2.56$, $p < 0.05$; Cohen's $d = 0.75$; Figure 3B), even though there was no difference between groups in the amount of freezing in the new context prior to the tone (MEC mean ± SEM: 5.5 ± 1.5%; SHAM mean ± SEM: 5.4 ± 1.3%; $t_{(45)} = 0.05$, $p > 0.1$).

DISCUSSION

Previous research has supported the involvement of the MEC in spatial memory acquisition [5] and retrieval [4,6] and recollection-based, non-spatial recognition memory [13]. However, the role of the MEC in temporal aspects of memory encoding had not been thoroughly explored. The current study examined whether lesions of the MEC disrupt rats' ability to learn and remember information about locations or stimulus-stimulus associations across short time delays. In the DMP task, rats were tested on their ability to learn and remember new platform locations across various time delays: 1 min, 20 min, 90 min, and 6 hr. Rats with MEC lesions were impaired at the shortest (1 min) and longest (6 hr) time delays, never performing as precisely as sham rats under the easiest condition and performing poorly at the longest delay (Figure 2). In the TFC task, rats were conditioned to associate a tone with a foot shock when a 30 sec stimulus-free "trace" period was separating the offset of the tone and onset of the shock. Rats with MEC lesions showed intact memory for the context in which they had been previously shocked, suggesting that MEC lesions did not interfere with acquisition of freezing or shock-reactivity. However, MEC-lesioned rats showed reduced freezing relative to sham rats when exposed to the associated tone in a novel context, suggesting that MEC lesions impaired their ability to associate a shock with a temporally discontiguous tone (Figure 3).

We have targeted the MEC because this area of the brain contains grid cells [3]. Grid cell activity emerges as hexagonally arranged firing fields that tile entire two-dimensional

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surfaces as an animal explores an open environment. Grid cell activity has an inherent connection to spatial processing because of its firing properties, but it is likely additionally important for spatial processing because the MEC receives input from place cells, border cells, head direction cells [14] and because the MEC is critical for normal place field size and stability [5]. Thus, the MEC is thought to be a critical component of the brain's navigation system that works closely with the hippocampus and place cells, specifically [15].

Additionally, it is becoming clear that one of the functions of the grid cell area of the brain relates to the temporal organization of hippocampal firing patterns, which suggests that the mnemonic functions that depend on precise neuronal sequences in the hippocampal theta cycle are critically dependent on the MEC [7]. We reasoned that if MEC function includes timing functions, then MEC activity might be important for associating discontinuous temporal events like those required in trace classical conditioning (see [16] for review). Indeed, we found that the retrograde memory for trace fear conditioning was robustly impaired by lesions of the MEC [6], just as it is with lesions of the hippocampus [17]. However, it is clear that retrograde designs are more sensitive to disruptions caused by lesions than are anterograde designs (e.g., [11,18,19]). Presumably, the greater sensitivity of retrograde designs, compared to anterograde designs, is that during acquisition the memory representation becomes distributed across multiple brain structures that are not necessarily essential for the memory acquisition. Accordingly, only anterograde designs can identify what structures are essential for the basic memory association. The finding that MEC lesions impair the acquisition of trace fear conditioning but not delay fear conditioning [5], indicates that the MEC is not required for context-cued or tone-cued fear conditioning in general, but instead is essential for associating *discontinuous* nonspatial stimuli. These findings are consistent with early work that showed lesions of the entorhinal cortex impaired the acquisition of trace fear conditioning in rats [20], although the lesion in this case did not include the most dorsocaudal extent of the entorhinal cortex, where the highest proportion of grid cells are located. We note here that trace eyeblink classical conditioning was disrupted with reversible lesions of the lateral entorhinal cortex, but not the medial entorhinal cortex [21]. The discrepancy between those findings and the present findings could be due to the substantial differences between the brain circuits that are essential for fear conditioning and those that are essential for eyeblink conditioning [16]. Alternatively, the 250–500 ms trace interval commonly used in eyeblink conditioning may not be long enough to require the functions of the MEC, as opposed to the 20–30 s trace interval commonly used in trace fear conditioning studies.

The findings from the DMP experiment are particularly informative. One difficulty in interpreting ostensible memory impairments following lesions is that the lesions may cause nonspecific impairments that can be misinterpreted as primary memory impairments (e.g. impairments in perception, motivation, motor functions, or in other cognitive functions). Accordingly, the clearest examples of pure memory impairments in work with experimental animals are delay-dependent impairments (e.g., [22,23]. Thus, a memory impairment can be isolated if performance is possible when the demand on memory is minimal (i.e. shorter delays) and impaired when the demand on memory is greater by increasing the delay between encoding and the retention test. Prior work using the DMP task reported delay-

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independent impairments in rats with hippocampal lesions [24,25]. Findings like these make it impossible to disambiguate memory impairments from spatial or navigational impairments. By contrast, the present findings using the DMP task show that animals with MEC lesions performed well on the shorter delays of 1 min, 20 min, and 90 min, but were profoundly impaired on the longest delay of 6 hrs. This finding indicates that MEC function is critical for maintaining a spatial memory representation across delays of multiple hours. The impairment on the shortest delay is more difficult to interpret, but we suggest that perhaps on the shortest delay the sham rats' strong memory allowed them to employ an almost direct path to the platform location, whereas the MEC lesion group was unable to form such a precise memory. Alternatively, we cannot rule out that a subtle navigational impairment prevented the lesion group from matching the efficient performance of the sham group at the 1 min delay. It is possible that MEC lesions could influence movements by causing hyperactivity; however, such an effect could not explain our results. In the DMP task, hyperactivity in MEC lesion rats would be expected to cause similar impairments across all delays. Even though the distances were numerically longer for MEC lesion rats for all delays, the difference in performance between groups was not even marginally significant at the 20-min delay ($p = 0.38$) or 90-min delay ($p = 0.18$). In TFC, hyperactivity in MEC lesion rats would be expected to cause reduced freezing both to the context and to the tone. However, both sham and MEC lesion rats showed similar amounts of freezing to context and MEC lesion rats only showed a significant reduction in freezing to the tone. Therefore, any hyperactivity in the MEC lesion rats could not explain these results.

Suh et al. [26] found that in transgenic mice, inhibition of the layer III input from MEC to the hippocampus resulted in impaired encoding of trace fear conditioning and produced a mild impairment in the DMP task using a 30-s and 2-hr delay interval, but only once the platform size was reduced. The authors suggest that these results demonstrate a critical role of the MEC layer III inputs to the hippocampus in spatial working memory and temporal association memory, and such results are consistent with the present findings using rats and MEC lesions. Accordingly, it is possible that the memory impairments following MEC lesions observed in the present study might be due to the disruption of normal hippocampal function. For example, our finding that MEC lesions impair the acquisition of trace fear conditioning is consistent with several prior studies in rats with hippocampal lesions [27,28]. Thus, our observed impairment could reflect the disruption of hippocampal function that results from the loss of MEC input. However, this possibility would not account for our DMP findings. Prior work with hippocampal lesions and the DMP task revealed a complete inability of lesioned rats to perform this task irrespective of delay interval [24, see Figure 7A,B,C], whereas our MEC lesions produced delay-dependent impairments (Figure 2).

Kraus et al. [29] evaluated the extent to which grid cell activity in the MEC reflects elapsed time or distance traveled when isolated from external information that normally accompanies an animal at travel. They recorded MEC cells as animals ran on a treadmill. In this condition, activity was only weakly modulated by location with the most robust activity modulated by a combination of time and distance. These findings suggest that, in the absence of external dynamic cues, the MEC integrates self-generated time and distance information perhaps to help encode a representation of an episodic experience. Similarly, it has been found in mice that the MEC contains a representation of elapsed time when they

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were immobile with these time-encoding neurons active at specific moments during the immobile interval. Further they reported that time encoding neurons were preferentially active during periods of immobility compared to the space-encoding neurons which were preferentially active during periods of mobility [30]. These physiology results clearly suggest that the grid cell area of the MEC has both spatial and timing functions and could be used to encode spatial and temporal aspects of an experience.

Our finding that MEC lesions create a nonspatial impairment in associating two discontinuous events (in the case of trace fear conditioning) indicates that the MEC is additionally important for nonspatial processing. Our finding of a delay-dependent impairment on the DMP task demonstrates that the computations carried out by the MEC can be functionally linked to the normal maintenance of at least some forms of long-term memory. These findings are consistent with a recent study reporting that when animals are trained to find food in specific locations, rather than randomly foraging for food, the grid fields are attracted towards those rewarded locations [31]. Thus, the normally rigid grid fields seen in animals foraging for food can be distorted by adding a learning and memory component to the task. By adding a nonspatial component to these tasks, in this case time, impairments emerge in the anterograde direction that extend beyond the spatial domain and suggest a more general role of the MEC in learning and memory.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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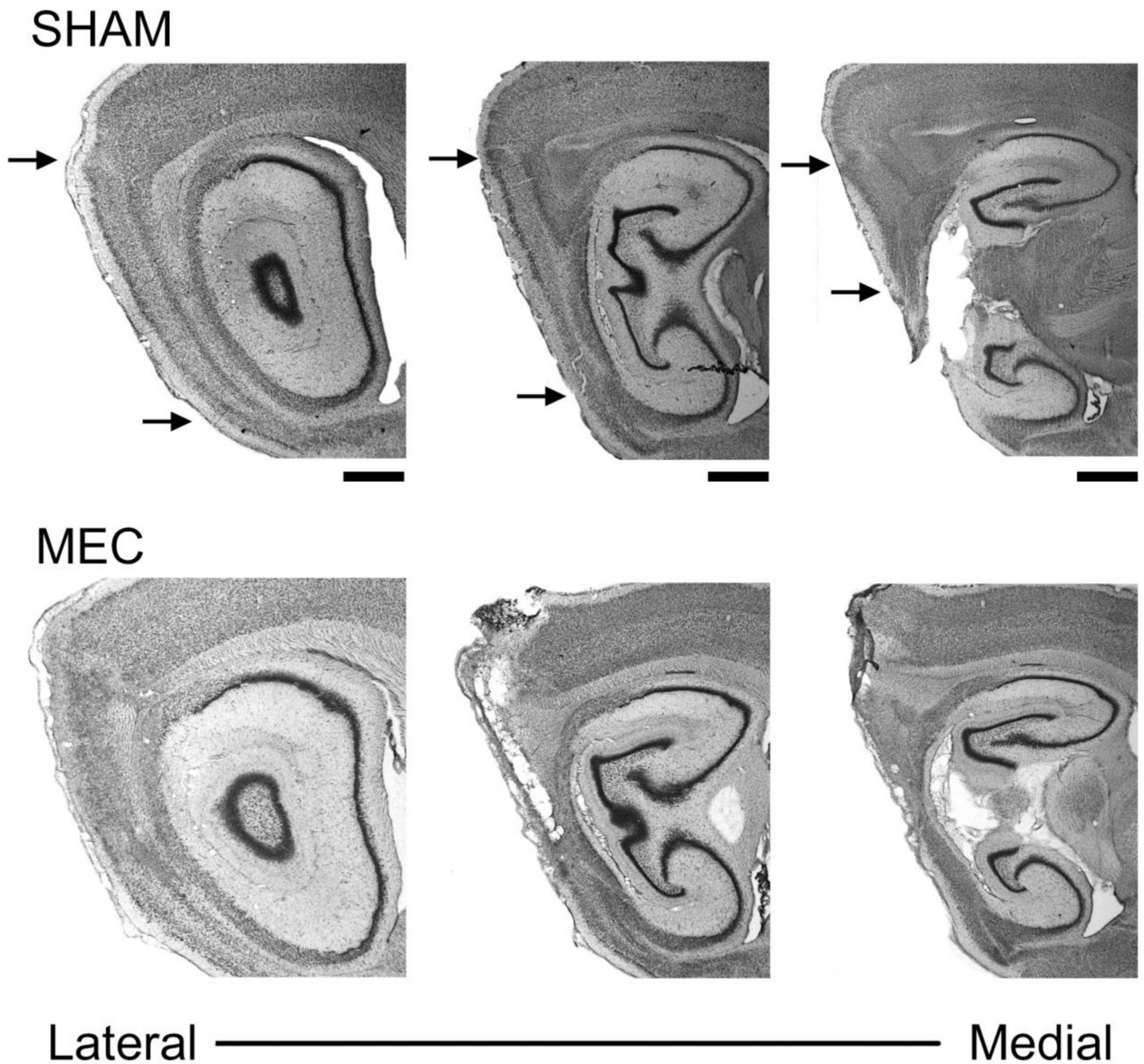

Figure 1. Extent of MEC lesions versus sham tissue

A. Photographs through the rat medial entorhinal cortex (MEC) at three sagittal levels (lateral to medial, centered around the target location of 4.6 mm lateral from the midline) for rats with sham or MEC lesions. The black arrows indicate the dorsal and ventral borders of the MEC. Scale bars below each sham tissue section indicate 1 mm.

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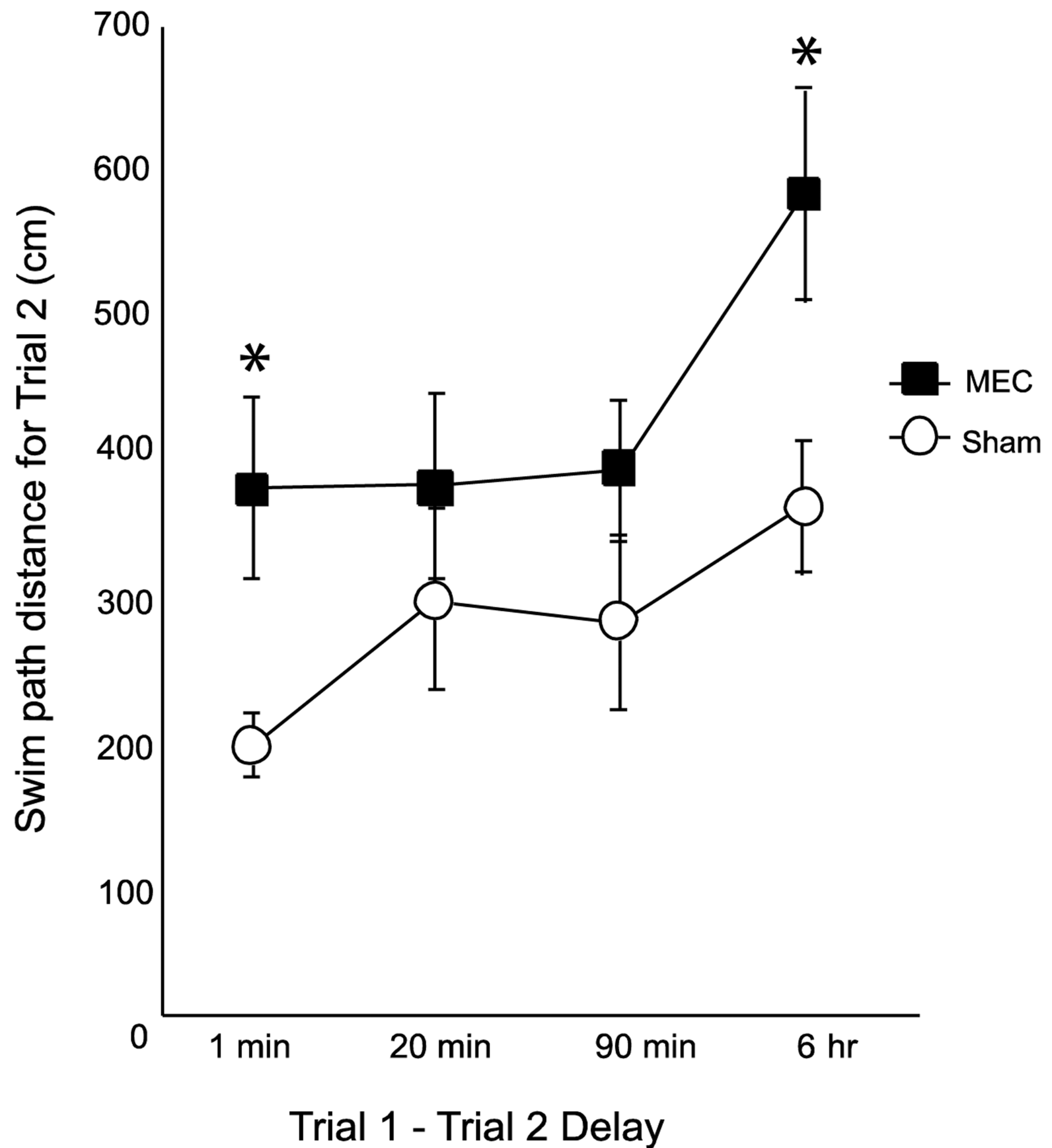

Figure 2. MEC lesioned rats show a delay-dependent impairment on the DMP task.
Swim path distances for Trial 2 across different delays between Trial 1 and Trial 2. MEC lesioned rats were impaired at finding the previously located platform when the delay between Trials 1 and 2 was long (6 hrs). MEC lesioned rats also failed to show superior memory at the shortest delay of 1 min between Trials 1 and 2, as shown by the SHAM rats. Error bars indicate SEM. Asterisks indicate difference from SHAM group (* $p < 0.05$).

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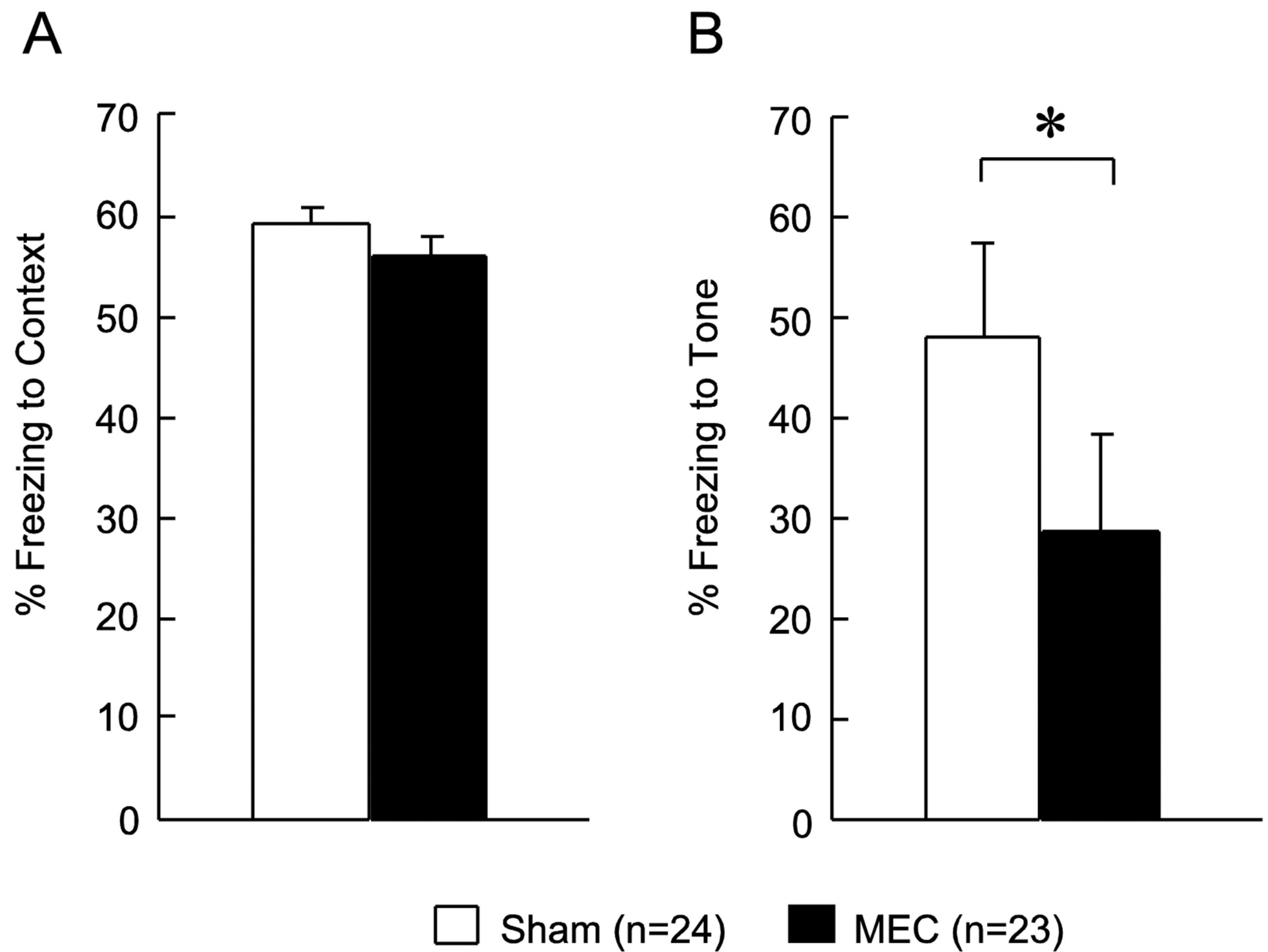

Figure 3. MEC lesions impair fear memory for temporally discontiguous tone, but not TFC context.

Mean percent freezing to the previously conditioned context (A) or to the associated tone (B) during 8-minute retention tests for 5 discontiguous tone-shock pairs. Although rats with MEC lesions showed intact fear memory when exposed to the previously conditioned context, they showed impaired fear memory when exposed to the associated tone in a new context. Error bars indicate SEM. Asterisks indicate difference from sham group (* $p < 0.05$).