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Publication Date

2018-11-01

DOI

10.1016/j.nlm.2018.07.013

Peer reviewed

HHS Public Access

Author manuscript

Neurobiol Learn Mem. Author manuscript; available in PMC 2019 May 31.

Published in final edited form as:

Neurobiol Learn Mem. 2018 November ; 155: 157–163. doi:10.1016/j.nlm.2018.07.013.

Recent and remote retrograde memory deficit in rats with medial entorhinal cortex lesions

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Abstract

The hippocampus is critically involved in the acquisition and retrieval of spatial memories. Even though some memories become independent of the hippocampus over time, expression of spatial memories have consistently been found to permanently depend on the hippocampus. Recent studies have focused on the adjacent medial entorhinal cortex (MEC), as it provides major projections to the hippocampus. These studies have shown that lesions of the MEC disrupt spatial processing in the hippocampus and impair spatial memory acquisition on the watermaze task. MEC lesions acquired after learning the watermaze task also disrupt recently acquired spatial memories. However, the effect of MEC lesions on remotely acquired memories is unknown. The current study examined the effect of MEC lesions on recent and remote memory retrieval using three hippocampus-dependent tasks: the watermaze, trace fear conditioning, and novel object recognition. MEC lesions caused impaired retrieval of recently and remotely acquired memory for the watermaze. Rats with MEC lesions also showed impaired fear memory when exposed to the previously conditioned context or the associated tone, and this reduction was seen both when the lesion occurred soon after trace fear condition and when it occurred a month after conditioning. In contrast, MEC lesions did not disrupt novel object recognition. These findings indicate that even with an intact hippocampus, rats with MEC lesions cannot retrieve recent or remote spatial memories. In addition, the involvement of the MEC in memory extends beyond its role in navigation and place memory.

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INTRODUCTION

A central question in behavioral neuroscience concerns how long-term memory is organized and stored in the brain. It is generally accepted that new memories are gradually transformed from a labile state to a more permanent state as a result of time-dependent modifications in circuits that support memory storage and retrieval – a process that is known as systems consolidation. A key feature of systems consolidation is that memories that were once hippocampus-dependent, gradually become hippocampus-independent. Studies of humans with damage that includes the hippocampus have reported such a temporal gradient within the memory impairment, in which memories acquired long before the lesion are spared relative to those acquired closer to the time of damage (Kapur and Brooks, 1999; Manns et al., 2003; Squire and Bayley, 2007). This phenomenon of temporally graded retrograde amnesia has been demonstrated in animal models (for review, see Squire et al., 2001, 2004; Frankland and Bontempi, 2005), with the consistent exception of rats tested in the Morris watermaze (Bolhuis et al., 1994; Mumby et al., 1999; Sutherland et al., 2001; Clark et al., 2005; Martin et al., 2005). Hippocampal lesions in rats, even 14 weeks after watermaze training, impairs memory retrieval (Clark et al., 2005). A theory for explaining this flat temporal gradient in the memory impairment is that hippocampal lesions produce an impairment in performance or navigation in the watermaze task, independent of memory (Clark et al., 2007).

More recent work has begun to focus on structures outside the hippocampus in adjacent brain regions, such as entorhinal cortex. One such study found that inducibly disrupting CaMKII activity in the entorhinal cortex in mice immediately after learning the watermaze task disrupted memory (Yasuda and Mayford, 2006). However, memory was intact when the transgene induction happened three weeks after training. These findings support temporally graded retrograde amnesia resulting from cellular processing disruptions in the entorhinal cortex in mice. However, given that cellular processes are disrupted in only a subset of cells, it is impossible to determine if memory has been reorganized to an extent to become independent of that structure. Accordingly, permanent lesions of the structure are critical.

Recent studies using permanent lesions have substantiated the involvement of the entorhinal cortex in spatial memory. Complete lesions of the medial aspect of the entorhinal cortex (MEC) in rats disrupt acquisition of the Morris watermaze task, and the deficits reported were comparable to those seen with hippocampal lesions (Hales et al., 2014). These results, therefore, show that MEC lesions cause anterograde spatial memory deficits similar to the effects of hippocampal lesions. In an earlier study, rats that received lesions of the dorsolateral band of the entorhinal cortex within 36 hours of watermaze training showed impaired memory retention for the previously learned platform location (Steffenach et al., 2005), which suggests that MEC lesions also cause retrograde memory impairments for recently acquired spatial memories. However, remote spatial memories were not examined.

The current study was designed to further probe the involvement of the MEC in memory retrieval. We probed three different hippocampus-dependent memory tasks: the Morris watermaze, trace fear conditioning, and novel object recognition. Rats received MEC lesions

1–3 days after or one month after learning in order to probe recently and remotely acquired memories, respectively.

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 80 experimentally naïve, 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. Sixty-four of the rats were trained in the Morris Watermaze (MWM) and Trace Fear Conditioning (TFC) tasks and were matched for performance on the final day of training. Rats were then assigned to receive NMDA lesions of the medial entorhinal cortex (MEC; $n = 32$) 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; $n = 32$). Some rats had surgery 1–3 days post watermaze and TFC training (MEC recent, $n = 24$; SHAM recent, $n = 24$, but two SHAM rats died after surgery), while the other rats had surgery 29–31 days post MWM and TFC training (MEC remote, $n = 8$; SHAM remote, $n = 8$). The other 16 rats were trained in the Novel Object Recognition (NOR) task, and they had surgery 1 day after the last day of training (MEC, $n = 8$, but one MEC rat died in surgery; SHAM remote, $n = 8$).

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 for MEC lesions. NMDA (Tocris) was dissolved in aCSF (Harvard Instruments) to provide a solution with a concentration of 10 mg/ml and was injected at a rate of 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 syringe needle was lowered to the target and left in place for 1 min before beginning the injection. After the injection, the syringe needle was left in place for 1 min to reduce the spread of drug up the needle tract. NMDA was injected into 8 sites (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. 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. From the brain surface, the needle was lowered to 8 different DV coordinates (−5.2, −4.7, −4.2, −3.7, −3.2, −2.7, −2.2, −1.7 mm).

Behavioral testing

Morris Watermaze (MWM)—The Morris watermaze is the benchmark test for hippocampus-dependent memory in rodents. Rats received MEC or sham lesions 1–3 days or 1 month after training in the MWM, and memory for the platform location was measured and compared between lesions groups.

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. An Atlantis platform (12.7-cm diameter) was used which could be raised or lowered remotely (Spooner et al., 1994). When the platform was in the lowered position, the rat could neither detect the platform nor escape from the water. When the platform was in the raised position (1.5 cm below the surface of the water), it remained invisible to the rat but provided a means to escape the water. The Atlantis platform provides the opportunity to present reinforced probe trials; that is, a probe trial can be presented (to assess retention) with the platform in the lowered position. When the probe trial ends, the platform can be raised so that the rat can escape and be rewarded for searching in the correct location.

Acquisition.: Rats began each of the 7 acquisition days with a reinforced probe trial followed by four standard training trials (with the same platform location for all trials). During the reinforced probe trial, rats were placed in the water facing the pool wall at one of four start points (counterbalanced across animals). The platform remained lowered for the first 60 s of the probe trial. The platform was then raised, and the rat had an additional 60 s to reach the platform before being guided to it by the experimenter. After escaping the water, the rat remained on the platform for 30 s. Performance on the probe trial was calculated by measuring, within the first 60 s, the percentage of time that a rat spent in the quadrant of the pool where the platform had been located during training (chance performance = 25%). In addition, we calculated the percentage of time that each rat spent in a circular zone (30 cm diameter) centered on the point where the platform had been located during training (Moser et al. 1993); chance performance = 4% (i.e., a 30-cm circle represents 4% of the total area of the pool). During the remaining four standard training trials, the platform remained in its raised position to permit escape from the water. Rats were given a maximum of 2 min to find the platform before being guided to the platform by the experimenter. After escaping, the rats remained on the platform for 30 s before they were returned to their home cage. Following training, rats were matched by performance on the last training probe and were divided into MEC and SHAM lesion groups. Rats underwent surgery 1–3 days post-training (recent) or 29–31 days post-training (remote). *Retention probe*. One 60-s nonreinforced probe trial, with the platform remaining inaccessible in the lowered position, was administered 12–14 days after surgery. Rats were placed in the water facing the pool wall at one of four start locations (counterbalanced across animals). Performance on the probe trial was calculated by measuring the percentage of time that each rat spent in the quadrant of the pool where the platform had been located during training (chance performance = 25%) and

the percentage of time that a rat spent in a circular zone directly above the platform location (chance performance = 4%).

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 non-contiguous foot shock. We measured the amount of freezing displayed by rats when they were returned to the same environment in which they had previously been shocked (context test) and the amount of freezing resulting from the same tone played in a novel environment (tone test). We compared these measures between rats that received MEC versus SHAM lesions 1–3 days or 1 month after 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.

Conditioning.: On the last day of MWM training and following the final training probe, rats were placed into the fear conditioning chambers for a 25-min, 20-s conditioning session during which there were 4 min of quiet 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. Following conditioning, rats were divided into MEC and SHAM lesion groups. Rats underwent surgery 1–3 days post-training (recent) or 29–31 days post-training (remote). *Context Test*. Following the MWM probe test, 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. *New Context Habituation*. The next day, rats were habituated to a new context 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. The new testing environment also included a different experimenter, altered lighting, new olfactory cues, and a modified transportation experience. *Tone Test*. Retention of the conditioned fear response to the tone was assessed 24 hr later. Rats were placed back into the context 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.

Novel Object Recognition (NOR)—This task is a hippocampus-dependent test of recognition memory (see Clark and Martin, 2007 for review).

Apparatus.: The novel object recognition task was conducted in an opaque plastic box measuring 35 cm × 41.5 cm × 50 cm. Stimuli consisted of ceramic or plastic objects that varied in color and size (see Broadbent et al., 2010 for details).

Habituation and Familiarization.: Rats were acclimated to the testing room and habituated to the empty box for five min each day for two days. Rats then had 4 days of familiarization

during which they were placed in the box for 15 min per day and allowed to explore two identical objects. Each rat had the same objects during every familiarization day, and the specific object was counterbalanced across rats. Following familiarization, rats were divided into MEC and SHAM lesion groups. Rats underwent surgery 1 day post-training. *Test.* After a 14-day recovery period from surgery, rats were returned to the testing box and allowed to explore two objects (one novel object and a copy of the object from the familiarization phase) for 15 minutes. Using video recordings, object exploration was scored when a rat's nose was within 1 cm of the object and the vibrissae were moving. Object exploration was not scored when the rat reared upwards facing the ceiling or leaned on the object. Object recognition memory was inferred by a preference for the novel object compared to the familiar, and thus less interesting, object. The time spent exploring the novel object was divided by the time spent exploring the novel object + the time spent exploring the familiar object. This value was then multiplied by 100 (chance performance = 50%; see Broadbent et al., 2010 for details).

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.

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 89.6% of the total MEC volume (93.1% of layer II, 89.9% of layer III, and 86.8% of deep layers) with the majority of the sparing in the most lateral extent of the MEC. Cell loss in adjacent cortical areas was predominantly in the parasubiculum and postrhinal cortex and was minor in the ventral hippocampus and in the LEC. There was no evidence of damage to the amygdala or thalamus in any animal.

Morris Watermaze

Acquisition.—All rats learned the location of the platform, spending 61.43 ± 2.01% (mean ± SEM) of the time in the target quadrant and 20.74 ± 1.18% of the time in the platform location during the probe trial on the last day of training. These values were well above

chance values of 25% and 4%, respectively. Rats were divided into equivalent lesion and sham groups based on these performance scores.

Recent memory.—For the rats that had surgery 1–3 days after training, the MEC lesion group performed worse than the SHAM group, spending less time in the target quadrant (MEC mean ± SEM: 31.24 ± 2.61%; SHAM mean ± SEM: 49.76 ± 3.78%; $t_{(44)} = 4.09$, $p < 0.001$) and less time in the precise platform location (MEC mean ± SEM: 5.81 ± 1.40%; SHAM mean ± SEM: 15.55 ± 1.93%; $t_{(44)} = 4.14$, $p < 0.001$) during the retention probe trial (Figure 2). The SHAM group showed above chance-level performance in the time spent in the training quadrant (chance = 25%; $t_{(21)} = 6.55$, $p < 0.0001$) and in the precise platform location (chance = 4%; $t_{(21)} = 6.00$, $p < 0.0001$). The MEC group, however, only showed above chance-level performance in the time spent in the training quadrant (chance = 25%; $t_{(23)} = 2.39$, $p < 0.05$), but not in time spent in the precise platform location (chance = 4%; $t_{(23)} = 1.29$, $p > 0.1$).

Remote memory.—For the rats that had surgery 1 month after training, the MEC lesion group again performed worse than the SHAM group, spending less time in the target quadrant (MEC mean ± SEM: 30.16 ± 3.09%; SHAM mean ± SEM: 46.69 ± 7.03%; $t_{(14)} = 2.15$, $p < 0.05$) and less time in the precise platform location (MEC mean ± SEM: 2.84 ± 0.54%; SHAM mean ± SEM: 12.53 ± 2.76%; $t_{(14)} = 3.45$, $p < 0.01$) during the retention probe trial (Figure 2). The SHAM group showed above chance-level performance in the time spent in the training quadrant (chance = 25%; $t_{(7)} = 3.9$, $p < 0.05$) and in the precise platform location (chance = 4%; $t_{(7)} = 3.09$, $p < 0.05$). The MEC group, however, was not above chance-level performance in either the time spent in the training quadrant (chance = 25%; $t_{(7)} = 1.67$, $p > 0.1$) or the precise platform location (chance = 4%; $t_{(7)} = -2.17$, $p = 0.07$).

Trace Fear Conditioning

Recent memory.—When rats had surgery 1–3 days after fear conditioning and were then placed back into the same context in which they were previously shocked, the cumulative amount of freezing at the end of the trial differed between MEC and SHAM groups. MEC rats showed less freezing in response to the context than SHAM rats (MEC mean ± SEM: 28.03 ± 4.46%; SHAM mean ± SEM: 50.04 ± 5.63%; $t_{(44)} = 3.09$, $p < 0.01$; Figure 3A). When placed in a new context, MEC rats also showed less freezing in response to the tone (MEC mean ± SEM: 21.43 ± 3.39%; SHAM mean ± SEM: 40.61 ± 6.59%; $t_{(44)} = 2.65$, $p < 0.05$; Figure 3B), even though there was no difference between groups in baseline freezing to the new context before the tone ($p > 0.1$).

Remote memory.—When rats had surgery 29–31 days after fear conditioning and were then placed back into the same context in which they were previously shocked, the amount of freezing was marginally different between MEC and SHAM groups. MEC rats showed less freezing in response to the context than SHAM rats (MEC mean ± SEM: 18.99 ± 4.67%; SHAM mean ± SEM: 50.12 ± 13.78%; $t_{(14)} = 2.14$, $p = 0.051$; Figure 3A). When placed in a new context, MEC rats also showed less freezing in response to the tone, even after this longer training to surgery interval (MEC mean ± SEM: 22.01 ± 10.04%; SHAM mean ± SEM: 60.17 ± 9.52%; $t_{(14)} = 2.76$, $p < 0.05$; Figure 3B). The impaired freezing was

specific to the tone/fear association because there was no difference between groups in baseline freezing to the new context before the tone ($p > 0.1$).

Novel Object Recognition

Preference for the novel object did not differ between MEC and SHAM groups (MEC mean ± SEM: 65.99 ± 5.18%; SHAM mean ± SEM: 73.85 ± 4.97%;, $t_{(13)} = 1.09$, $p > 0.1$; Figure 4). Both of these values were above 50% chance (MEC: $t_{(6)} = 3.09$, $p < 0.05$; SHAM: $t_{(7)} = 4.80$, $p < 0.01$). Because the two groups did not perform differently on the recent memory test, the remote memory test was not performed.

DISCUSSION

Previous research has shown that the MEC is involved in spatial memory acquisition (Hales et al., 2014) and recollection-based, non-spatial recognition memory (Sauvage and Eichenbaum, 2010), but not in acquiring other hippocampus-dependent tasks, such as novel object or location recognition or context fear conditioning tasks (Hales et al., 2014). However, the involvement of the MEC in retrieving memories that were acquired recently or remotely had not been thoroughly explored. The current study examined whether lesions of the MEC disrupt recently or remotely acquired hippocampus-dependent memories using both spatial and nonspatial tasks. In the watermaze task, rats that received lesions 1–3 days after training were impaired, supporting previous research (Steffenach et al., 2005). Rats were also impaired when they received MEC lesions one month after training (Figure 2). During trace fear conditioning, rats that received MEC lesions 1–3 days after conditioning showed reduced freezing relative to sham rats when exposed to either the conditioning context or the associated tone (Figure 3). When rats received surgery one month after conditioning, MEC lesioned rats still showed reduced freezing when exposed to the associated tone and also showed marginally reduced freezing to the conditioning context. Finally, when tested on the novel object recognition task, rats that received MEC lesions 1 day after being familiarized to an object showed similar preference for the novel object as sham rats, suggesting that MEC lesions did not impair novel object recognition, even when lesioned soon after learning.

Although this is the first study to examine the importance of the MEC for remote spatial memory retrieval, hippocampal involvement has been widely examined. Despite evidence of temporally graded retrograde amnesia following hippocampal damage in both human and animal model studies (Kapur and Brooks, 1999; Squire et al., 2001; Manns et al., 2003; Squire et al., 2004; Frankland and Bontempi, 2005; Squire and Bayley, 2007), many studies have reported ungraded retrograde deficits on tasks involving spatial memory, such as the standard watermaze (Bolhuis et al., 1994; Mumby et al., 1999; Sutherland et al., 2001; Clark et al., 2005a) as well as other spatial memory tasks, including the annular watermaze (Hollup et al., 2001; Clark et al., 2005a) and the dry-land Oasis maze (Clark et al., 2005a). Even when rats are extensively trained on the watermaze task starting at a young age and learn the task months before surgery, hippocampal lesions still impair spatial memory retrieval (Clark et al., 2005b). In addition, visually cuing the platform location did not

prevent or rescue the impairment in spatial memory retrieval following hippocampal lesions, regardless of the age of the memory (Clark et al., 2007).

Other studies have examined whether the lack of a temporal gradient in the retrograde memory impairment was due to the method for lesioning the hippocampus. Given the robust retrograde deficit resulting from conventional lesions, researchers examined the effect of using reversible lesions. Intact spatial memory has been reported in mice when reversible disruption of hippocampal function occurred 30 days after training on a radial arm maze spatial discrimination task (Maviel et al., 2004) and when CaMKII was disrupted in the entorhinal cortex three weeks after training on the watermaze task (Yasuda and Mayford, 2006). However, reversible disruption of hippocampal function in rats was found to impair remote spatial memory in the watermaze (Riedel et al., 1999; Micheau et al., 2004; Broadbent et al., 2006). These results involving reversible lesions suggest that the impairments seen in remote spatial memory in the watermaze are independent of whether the rat received conventional or reversible lesions of the hippocampus.

Another possibility for why hippocampal lesions result in ungraded remote spatial memory deficits is that large hippocampal lesions include damage in adjacent areas outside of the hippocampus or indirectly disrupt function in these areas. Martin et al. (2005) examined this possibility by comparing the deficits resulting from partial versus full hippocampal lesions. They found that the extent of hippocampal damage does not account for the flat gradient as even partial lesions of the hippocampus still impair remote spatial memory retrieval (Martin et al., 2005; Clark et al., 2005). Recently, Ocampo et al. (2017) addressed this possibility by restricting the lesion to only include area CA1, the output of the hippocampus to cortex. They reported that selective lesions of area CA1 caused similar recent and remote memory deficits in the watermaze task (Ocampo et al., 2017). These studies report that even small, selective lesions that are within the boundaries of the hippocampus still result in ungraded retrograde memory deficits on spatial memory tasks.

During trace fear conditioning, rats with MEC lesions showed reduced freezing to the conditioning context and to the associated tone for both recent and remote memory groups. These temporally ungraded retrograde deficits are similar to those seen following selective CA1 lesions (Ocampo et al., 2017), but differ from earlier studies examining dorsal hippocampal lesions only (Quinn et al., 2008; Beeman et al., 2013). As a whole, the field of research examining hippocampal involvement in context and trace fear conditioning remains uncertain (Clark 2010; Broadbent & Clark, 2013). What is notable about the current study is that MEC lesions alone impair the retrieval of fear memory for the tone. While many studies emphasize the role of the MEC in spatial processing and memory (Parron et al., 2004; Hafting et al., 2005; Steffenach et al., 2005; Sargolini et al., 2006; Solstad et al., 2008; Krupic et al., 2012; Hales et al., 2014), retrieving the associated fear memory for a tone does not require spatial processing, but instead requires temporal processing in order to associate the temporally discontiguous tone and foot shock. This finding offers new insight into the functions of the MEC and its involvement in temporal aspects of memory processing. These finding are consistent with reports that MEC layer III input into the hippocampus is important for the acquisition of temporal association memory (Suh et al., 2011; Kitamura et al., 2014). In addition, recent research has suggested that the MEC plays a role in

hippocampal dependent temporal processing (Schlesinger et al., 2015; Robinson et al., 2017). However, the role of the MEC in retrieval of memory requiring the association of discontiguous stimuli has not been previously explored.

Rats that received MEC lesions one day after being familiarized to an object did not show any impairment in later recognizing that object, as measured by increased exploration of the novel object. These results are similar to those reported when rats received MEC lesions prior to being familiarized to the object (Hales et al., 2014), suggesting that the MEC is not involved in the formation or retrieval of object recognition memory. Although there is controversy concerning the involvement of the hippocampus in object recognition memory (see Squire et al., 2007; Clark, 2013, for review), the novel object recognition task has been found to be vulnerable to hippocampal damage or disruption in various species, including rodents (Clark et al., 2000; Gaskin et al., 2003; Broadbent et al., 2010; Cohen et al., 2013; Hales et al., 2015), monkeys (Pascalis and Bachevalier, 1999; Zola et al., 2000; Nemanic et al., 2004), and humans (McKee and Squire, 1993; Pascalis et al., 2004). The intact performance of MEC lesioned rats on the NOR task is notable both in how it sets the functions of the MEC apart from the function of the hippocampus and in how distinct these results were from the robust deficits seen in the watermaze and TFC tasks.

In summary, MEC lesions disrupted place memory in the watermaze task and context and trace fear memories. MEC-lesioned rats were impaired at retrieving both recent and remote memories on these tasks. In contrast, rats did not show any impairments in novel object recognition after receiving MEC lesions. Together, these findings suggest that like the hippocampus, the MEC may produce navigational impairments that prevent the expression of otherwise intact spatial memory and that the MEC plays a more complex role in spatial as well as temporal aspects of recent and remote memory retrieval, which extends beyond its more established role in navigation and place memory.

Acknowledgments

We thank Laura Johnson, Maya Sapiurka, Sarah Saturday, Patricia Cintura, and Terence Ellis for technical assistance. This work was supported by a Medical Research Service of the Department of Veterans Affairs grant, a NSF Temporal Dynamics of Learning Center grant (UCSD), and a NIH R01 grant (NS086947). This work was also supported by the College of Arts and Sciences at the University of San Diego. The authors declare no competing financial interests.

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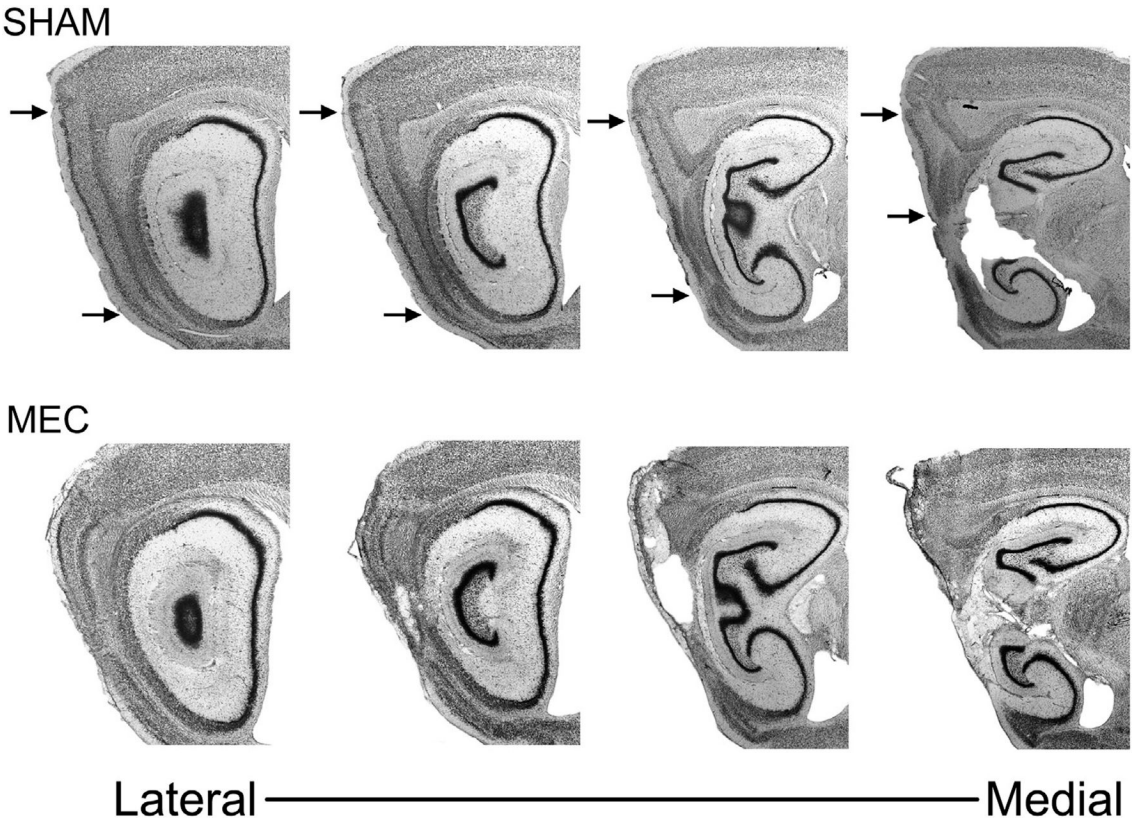

Figure 1. Extent of MEC lesions versus sham tissue

A. Photographs through the rat medial entorhinal cortex (MEC) at three sagittal levels (lateral to medial) for rats with MEC or sham lesions. The letters around the sham tissue section in the top row identifies the orientation of the sections (d, dorsal; v, ventral; a, anterior; p, posterior). The black arrows indicate the dorsal and ventral borders of the MEC. Scale bars below each tissue section indicate 1 mm.

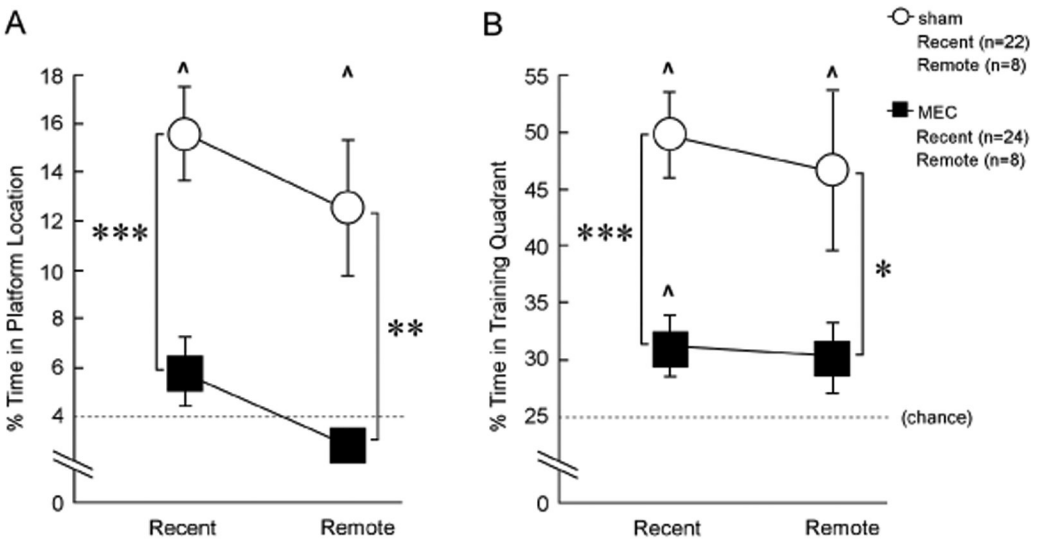

Figure 2. Retrieval of recently and remotely acquired watermaze platform location impaired after MEC lesions.

Probe trial performance measuring the percentage of time that each rat spent in the platform location (A) and in the target quadrant (B) in rats that had received medial entorhinal cortex (MEC) or sham lesions either 1–3 days (recent) or 1 month (remote) after training. Dashed lines indicate chance performance for the platform location and quadrant, which was 4% and 25%, respectively. Rats with MEC lesions were impaired at retrieving the platform location and target quadrant regardless of whether the training occurred 1–3 days before or 1 month before surgery. Sham rats performed above chance for all measures, whereas MEC-lesioned rats only performed above chance in the recent memory condition. Error bars indicate SEM. Asterisks indicate difference from sham group (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Carets indicate performance above chance ($p < 0.05$).

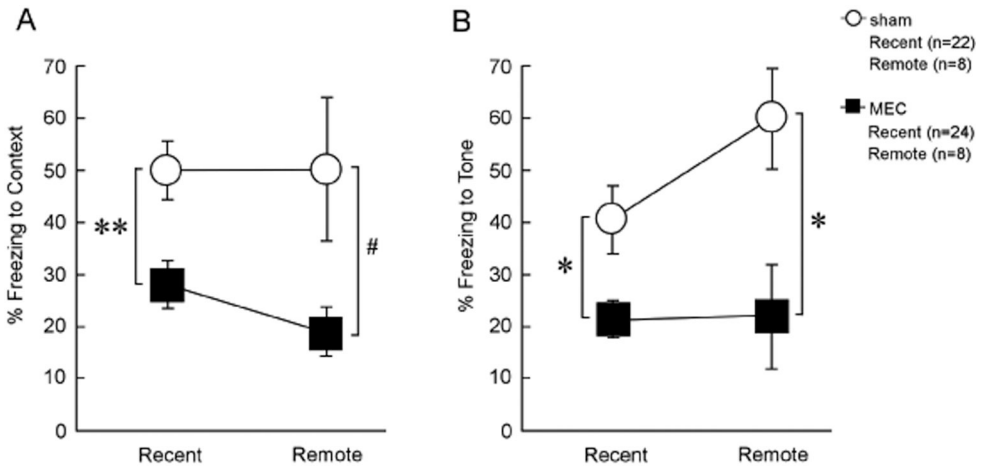

Figure 3. Retrieval of recently and remotely acquired fear memory impaired after MEC lesions.

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. Rats received medial entorhinal cortex (MEC) or sham lesions either 1–3 days (recent) or 1 month (remote) post-conditioning. Rats with MEC lesions showed impaired fear memory when exposed to the previously conditioned context or the associated tone regardless of whether conditioning occurred 1–3 days before or 1 month before surgery. Error bars indicate SEM. Asterisks indicate difference from sham group (* $p < 0.05$, ** $p < 0.01$, # $p = 0.051$).

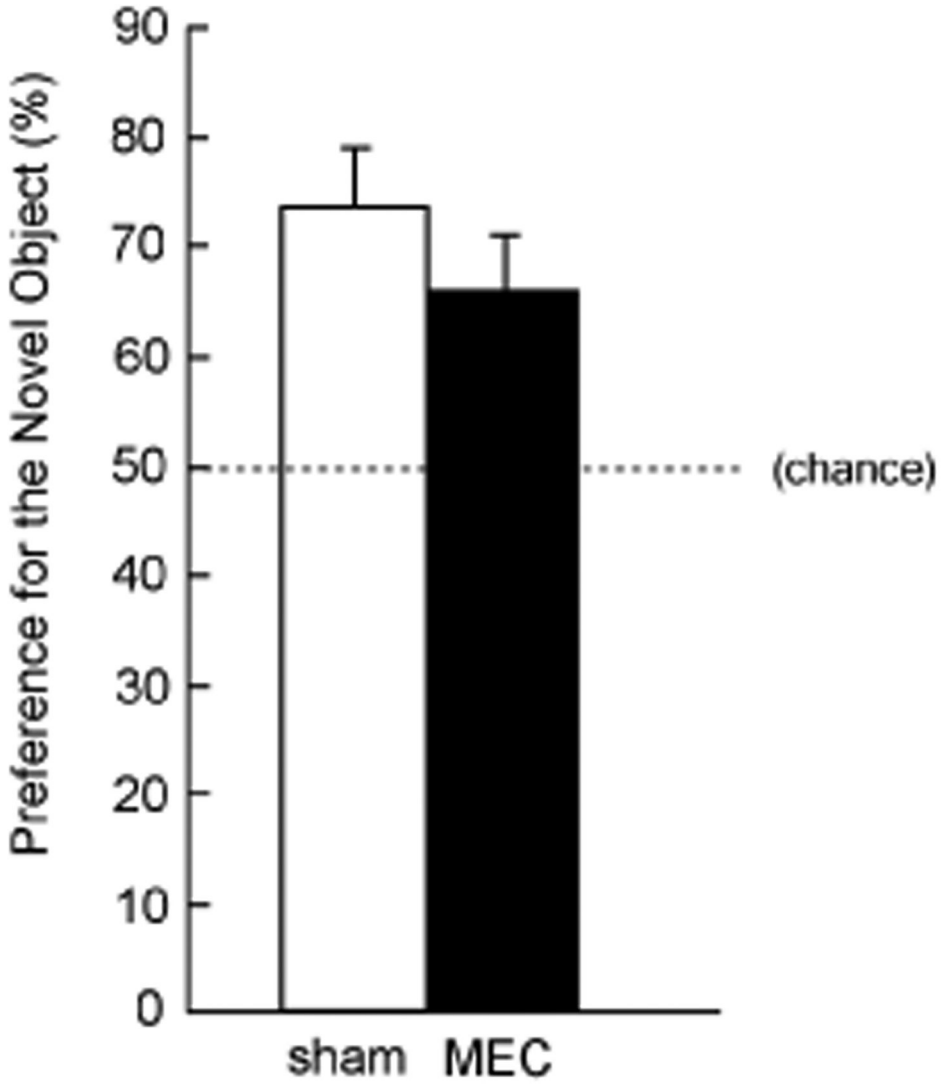

Figure 4. Novel object recognition was not impaired following MEC lesions.

Rats with sham (white bar) and medial entorhinal cortex (MEC; black bar) lesions performed equally and better than chance on the novel object recognition task. Dashed line indicates chance performance of 50%. Error bars indicate SEM.