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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 is role in navigation and place memory.

1. 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, Hopkins, & Squire, 2003; Squire and Bayley, 2007). This phenomenon of temporally graded retrograde amnesia has been demonstrated in animal models (for review, see Squire, Clark, & Knowlton, 2001, 2004; Frankland and Bontempi, 2005), with the consistent exception of rats tested in the Morris watermaze (Bolhuis, Stewart, & Forrest, 1994; Mumby, Astur, Weisend, & Sutherland, 1999; Sutherland et al., 2001; Clark, Broadbent, & Squire, 2005a, 2005b; Martin, de Hoz, & Morris, 2005). Hippocampal lesions in rats, even 14 weeks after watermaze training, impairs memory retrieval (Clark et al., 2005a, 2005b). 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, Broadbent, & Squire, 2007).

More recent work has begun to focus on structures outside the hippocampus in adjacent brain regions, such as entorhinal cortex. One

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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 h of watermaze training showed impaired memory retention for the previously learned platform location (Steffenach, Witter, Moser, & Moser, 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.

2. Materials and methods

2.1. 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, $n = 8$).

2.2. 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).

2.3. Behavioral testing

2.3.1. 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, Thomson, Hall, Morris, & Salter, 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, Moser, & Andersen, 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