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Research report

Place cell activation predicts subsequent memory

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HIGHLIGHTS

- Rats performed both continuous and delayed spatial alternation tasks.
- Many but not all hippocampal neurons had distinct firing patterns in the two tasks.
- In the delay task, place cells more strongly differentiated left and right turn trials.
- In the delay task, place cell activation level predicted subsequent memory accuracy.
- This "subsequent memory effect" complements findings in fMRI studies in humans.

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ABSTRACT

A major quandary in memory research is how hippocampal place cells, widely recognized as elements of a spatial map, contribute to episodic memory, our capacity to remember unique experiences that depends on hippocampal function. Here we recorded from hippocampal neurons as rats performed a T-maze alternation task in which they were required to remember a preceding experience over a delay in order to make a subsequent spatial choice. As it has been reported previously in other variations of this task, we observed differential firing that predicted correct subsequent choices, even as the animal traversed identical locations prior to the choice. Here we also observed that most place cells also fired differently on correct as compared to error trials. Among these cells, a large majority fired strongly before the delay or during the retrieval phase but were less active or failed to activate when the animal subsequently made an error. These findings join the place cell phenomenon with episodic memory performance dependent on the hippocampus, revealing that memory accuracy can be predicted by the activation of single place cells in the hippocampus.

1. Introduction

Several recent studies have characterized place cells in rats performing maze tasks where they are required to remember the preceding episode in order to select a correct path on the next trial [1–7]. These studies have shown that ensembles of hippocampal neurons distinguish different routes animals take as they pass through the same places, and therefore predict past and future spatial choices. In addition, in some of these studies place cells similarly predicted routes associated with occasional mistakes [3,8]. These findings are consistent with the observation that place cells can maintain their spatial firing patterns, and do so associated with behavioral choices, when critical maze cues are removed [9]. The combined findings from these studies suggest that errors are not due to "forgetfulness", i.e., failure to retrieve a memory representation, but rather to retrieval of the incorrect spatial representation. However, this conclusion is not consistent with the results of functional imaging studies on humans showing that a high level of hippocampal activation during encoding or retrieval predicts accurate subsequent memory, whereas subsequent errors are predicted by a low level of hippocampal activity [10,11]. These studies suggest that errors are associated with a failure to encode or retrieve a hippocampal memory representation. Here we examined whether hippocampal activity at the level of individual neurons also predicts subsequent memory in rats performing a memory task that depends on hippocampal function.

2. Methods

2.1. Subjects

The subjects were seven male Long-Evans rats weighing between 350 and 400 g at the time of electrode implantation. The rats were allowed ad libitum access to food for the duration of the experiment, but were restricted to 30 min of water per day on the day before each training, testing, and recording session. If no testing or recording was to take place the following day, water was available ad libitum for 24 h. The rats were housed singly and kept on a 12 h/12 h light/dark cycle. Recording and testing were carried out during the light phase of the cycle, and rats were tested

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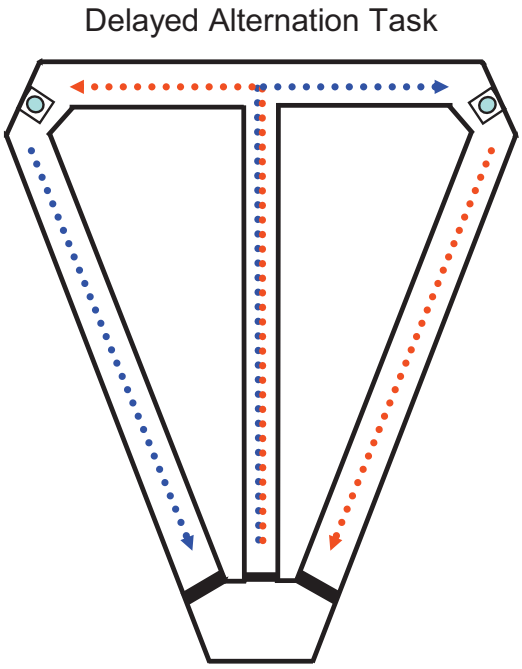

Fig. 1. The delayed spatial alternation task. Blue: path on left to right (LR) trials; red: path on right to left (RL) trials.

approximately 5 days/week. The experiment was conducted in accordance with guidelines set forth by the National Institutes of Health, and protocols were approved by the Boston University Charles River Campus Institutional Care and Use of Animals Committee.

2.2. Apparatus

The modified T-maze apparatus used (Fig. 1) was constructed of wooden runways 8 cm wide with wooden walls 2 cm high. Both the walls and floor were painted black. The central runway that comprised the stem of the T was 130 cm long, and additional wall strips were added to this portion of the maze to narrow its' width to 7.5 cm. A crosspiece 94 cm long formed the choice arms. The distal ends of the choice arms were connected to the base of the stem by additional runways. Small Plexiglas wells (6.35 ×3 6.35 cm square plaques with circular depressions of radius of 1 cm and maximum depth of 0.5 cm) were recessed into the floor at the end of each choice arm at the points marked by circles in Fig. 1. Water was delivered to the wells via an 18 gauge cannula hooked up to a reservoir via tubing and under the control of solenoid valves activated by hand-operated switches. The T-maze was elevated 80 cm from the ground on pillars that had inside reverse guillotine doors for controlling the rat's access to the arms of the maze. The maze was surrounded by black curtains on three sides (the fourth side was partially open to the remainder of the room), and several large, high-contrast, distinctive visual cues were attached to the curtains. The platform and cues remained at the same location relative to each other and to the remainder of the environment throughout the experiment.

2.3. Continuous alternation training

Before implantation of the recording electrodes, the rats were shaped in multiple stages to perform a continuous spatial alternation task on the modified T-maze [2]. In the first stage of training, each rat was placed at the base of the central stem of the apparatus, facing the choice arms. Clear Plexiglas barriers were placed such that the rat was forced to traverse the central stem and enter one of the choice arms. After it entered one of the arms, a small drop of water was delivered to the well in that arm. The rat was prevented from retracing its' route on the choice arm, and so then traversed the connecting arm back to the base of the T. At this point a barrier blocked the entrance to the opposite connecting arm, forcing the animal to traverse the stem of the T again. Another barrier blocked the entrance to the previously entered arm, so the rat was then forced to enter the other choice arm, and water was delivered to the well in this arm. This procedure was repeated using barriers to direct the animal's traversals over the stem and to alternate entries into the choice arms, until the animals ran the pattern consistently. In the second stage of training, the use of barriers at the choice point was phased out; each time the rat reached the end of the stem it could enter either arm, but it was rewarded only for alternating arm entries and was not allowed to retrace its steps. In the third stage of training, the barrier forcing the rat into the stem after returning along the connecting arms was phased out. The animals continued to run in a "figure 8"-like pattern despite no barriers, but they were prevented from retracing their steps at any point using reverse guillotine doors built into the maze.

During each subsequent training and testing session, the rats were placed on the central stem with no barriers and allowed to run 15-20 continuous trials. The experimenter remained outside the curtained enclosure throughout the session. The animal's behavior was observed via a video monitor connected to a tracking system. During each trial when the rat made a correct (alternating) arm choice, a drop of water was delivered to the well in that arm after the arm entry. During trials when the animal made the incorrect choice, no reward was provided. Furthermore, no reward was provided even if the rat retraced its steps back to the choice point and entered the other choice arm. Instead, following mistakes the rat was required to continue along the connecting arm, reenter the stem, and make the correct choice on the following trial.

2.4. Delayed alternation testing

To examine hippocampal neuronal firing patterns during performance with an increased memory demand, the rats were tested on a delayed alternation version of the task immediately following the completion of 15-20 continuous alternation trials on each recording session. The delay was imposed by retaining the rat in the start area of the maze using the built-in reverse guillotine doors for 30 s, a period much longer than what has been shown to result in deficits in rats with hippocampal lesions. The doors were not raised until the rat reached the start area of the maze, ensuring that any differential firing during the delayed alternation task on the return arms was not a result of a visual cue provided by the raising of the doors. No food or water reward was given during the delay period. At the end of the delay, the doors were lowered, allowing the rat to leave the start area and make a free choice, with reward given only for alternating the response performed on the previous trial. If the rat made more than two sequential errors, reverse guillotine doors were raised when the rat reached the choice point, forcing a correct response. The delayed alternation trial block contained the same number of trials as was performed in the continuous trials block.

2.5. Surgery

When performance on the continuous alternation reached asymptote, a microdrive array of six 13 μm tetrodes [12,13] was implanted, aimed at area CA1 of the dorsal hippocampus. Rats were anesthetized using isoflurane delivered with 100% oxygen and placed in a stereotaxic instrument (David Kopf Instruments, Tujunga, CA). The skull was exposed, and bregma and lambda were made level. A small hole (~1.5 mm diameter) was drilled over the hippocampus on one side of the skull for the placement of the electrode array, and six additional holes were drilled for the placement of skull screws used for electrical grounds and for securing the microdrive to the skull. The electrode array was implanted just above the dorsal hippocampus at 3.6 mm posterior to bregma, 2.4 mm lateral to bregma, and 1–1.5 mm below the surface of the brain. The cannula was coated with sterile petroleum jelly. Grip cement (Henry Schein Inc., Melville, NY) was used to secure the microdrive to the skull, and to cover the exposed skull.

2.6. Data acquisition

Following a 7-day recovery period, daily screening for unit activity was conducted while the rats were in an opaque rectangular box (61.6 cm long × 43.8 cm wide × 40.0 cm high) that was outside of the T-maze apparatus. If pyramidal cell activity was identified (see Section 2.7), the animal was placed on the T-maze, and unit activity was recorded while the animal performed first the continuous spatial alternation task, followed by the delayed alternation task as described in the section on behavioral training. If no pyramidal cell activity was identified during screening, the rats were allowed to run a session of 20 continuous alternation trials without recording units. The electrode was advanced 40–80 μm after the session and allowed to settle overnight (at least 16 h) before the next recording session.

Neural activity was first passed through a multi-channel unity gain source follower field effect transistor (jFET) that was connected via a fine wire cable to the animal's headstage. It was then passed through an overhead commutator (Biela Development Inc., Gaithersburg, MD), differentially amplified between 5000× and 10,000× (Neuralynx Inc., Tuscon, AZ), band-pass filtered from 600–6000 Hz, and digitized at 28 kHz (Data Translation DT2821, Data Translation Inc., Marlboro MA) using Enhanced Discovery software (Datawave Technologies, Longmont, CO) on a Pentium-based personal computer. One wire from each tetrode was filtered from 1 to 400 Hz and sampled at 1 kHz to record the local field potential. For each recording session, a dedicated electrode that was driven to the corpus callosum served as a reference for differential recording. In some rats, an additional electrode was placed at the hippocampal fissure to record the electroencephalogram, and was filtered and sampled as described above.

The rats' location was recorded using a video camera system (Datawave Technologies) by tracking two incandescent bulbs on the rats' head, with one made brighter than the other by putting a resistor in series between the two bulbs. Position data for each light were sampled at 60 Hz and recorded as x–y coordinates. The coordinates and timestamps were saved to disk with the unit data.