Place Cell Responses to Novel Experiences in a Familiar Virtual Environment

Background and Objective

The hippocampus, a structure located in the brain's temporal lobe, plays critical roles in learning and memory. We first began to understand just how important the hippocampus is for memory consolidation from studies of H.M., an epileptic patent who had a bilateral temporal lobectomy procedure performed in 1957 in attempts cure him of his severe seizures [1]. The procedure resulted in the removal of his hippocampus and related structures and left him unable to form new declarative memories (memories of factual information and previous experiences). H.M.'s memories from before the procedure took place, however, were in tact, so his symptoms demonstrated that the hippocampus is essential for the formation of new memories, but not for their long term storage. Following these groundbreaking findings, considerable interest emerged in the hippocampus' role in spatial memory, which provides the contextual background for the formation of episodic memories. Research has shown that the hippocampus uses place cells, which are specific types of hippocampal neurons that fire action potentials when an animal enters a particular location in its environment, to encode spatial information [2]. The specific locations in which place cells fire are known as "place fields." Because certain hippocampal place cells are only active in a particular location, the hippocampus is able to use place cell activity to create a cognitive map of the environment, enabling animals to keep track of their location in space and remember previously visited locations [3]. A recent study by Mao et al. has shown that the rodent retrosplenial cortex also contains neurons that have firing patterns similar to those of place cells found in the hippocampus, providing evidence for the existence of retrosplenial place cells [4]. These firing patterns are thought to encode information that aids in spatial memory, route planning, and navigation [2, 4]. They do this by providing a "memory index" that references to the landmarks and events that occur at each visited location [5]. It is well known that visiting novel locations causes animals to explore, and triggers neural processes that result in enhanced memory storage and consolidation. How novel experiences in familiar locations are processed in the brain, however, is not well understood.

The goal of the proposed project is to study how place cell activity in mice changes in response to new information presented in a familiar location. To do so, we will collect data on mouse hippocampal and retrosplenial place cell activity when a novel stimulus is presented in an otherwise familiar environment. We will use this data to study rate remapping in place cells, which occurs when the rate at which place cells fire changes while the locations of their place fields remains unchanged [6]. In addition, we will correlate place cell activity while the animals sleep to place cell activity while the animals are immersed in a familiar environment performing a behavioral task. Correlations in place cell activity are indicative of memory reactivation, which is the process of replaying memories during sleep [7]. This process is thought to aid in memory consolidation by strengthening the synaptic connections that underlie the memory. With this data, we will test the the hypothesis that memory reactivation and rate remapping in mouse place

cells are greater when an emotional novel stimulus is presented than when a non-emotional novel stimulus is presented in a familiar environment.

Approach

Mice and surgical procedures:

First, transgenic VGAT-Cre, Cre-activated td-Tomato mice (n = 4) will be injected with GCaMP6s in the retrosplenial cortex. GCaMP6s is a genetically engineered calcium indicator protein used in fluorescent imaging. In layer 2/3 pyramidal neurons of the mouse cortex, GCaMP6 has been demonstrated to detect changes in intracellular free calcium concentration, indicative of action potentials, so it will enable us to measure pyramidal cell and interneuron activity during imaging sessions with a two photon microscope [8]. Two to three weeks later, a craniotomy will be made above the retrosplenial cortex. Coverslips will then be attached to the skull over the cranial window so that we will be able to image the exposed portion of the retrosplenial cortex. A headplate will be attached to the skull in the same surgery, so that the mouse can be head-fixed during imaging sessions.

Behavior training:

After 5 days of recovery from head-plate implant surgery, the mice will be trained to run on a wheel (connected to a rotary encoder) that is surrounded by three tablets that use SmoothWalk software to display a virtual environment (Figure 1). As mice run, the wheel's revolutions correspond to their movement within the virtual environment. The virtual environment is a circular track with several prominent objects scattered throughout it in order to identify locations. The virtual environment we are currently using to train mice depicts a classroom setting, so it features several different objects throughout the classroom which are thought to enable the mice to remember specific locations within the environment. In order to be able to detect reliable place cell responses, we will train the mice to traverse the track for several laps (>30) on each day. To motivate the mice to run in the virtual environment, a water reward is dispensed at two fixed reward zones per lap and the mice will be trained to lick at the specific reward zones in order to receive the water rewards. A lick detector will be used to monitor when the mouse's tongue comes into contact with the water dispenser.

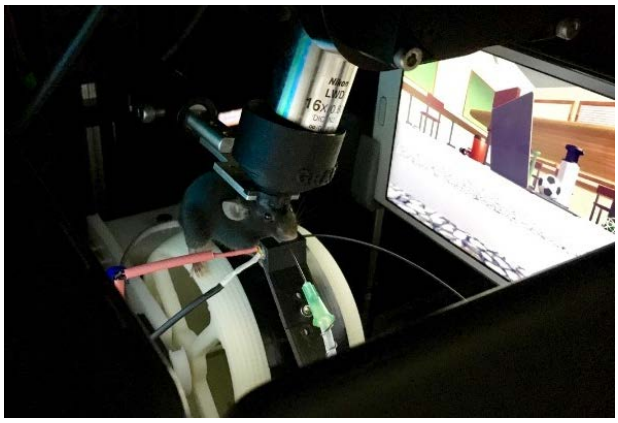

Figure 1. A mouse is head-fixed while running on a wheel, surrounded by tablets that display a virtual environment. In front of the mouse, a lick detector is positioned near the mouth to track when the mouse licks for rewards. Above the mouse's head, a two-photon microscope is positioned over the cranial window in order to image cells.

Place cell imaging:

After one month, when mice become familiar with the task, we will use two photon imaging to begin recording data from place cells as the animals run in the familiar virtual environment. Data on the animals' running speeds and the positions/timing of their licks for the water rewards will also be collected. Once we have data on the mouse's behavior in a familiar environment, we will introduce a novel stimulus during one lap of running, or for one minute if the mouse freezes. The novel stimulus will either be emotional (in this case it will be a cat to elicit an emotional response, such as fear) or non-emotional, such as an inanimate object. For each running session, both in the familiar environment and in the environments with novel stimuli, we will also record from place cells during periods of sleep or rest that immediately precede and follow the running sessions. We will analyze the recorded data from the running sessions and sleep sessions to determine the degree of correlation of place cell activity between the two.

We will assess the effect of the type of novel stimulus presented (emotional or non-emotional) on remapping and memory reactivation in mice by comparing place cell activity upon exposure to a novel stimulus to place cell activity in a familiar environment. This will enable us to assess the magnitude of reactivation and remapping when an emotional compared to a non-emotional novel stimulus is presented. In addition, we will compare the behavioral data on running speed and lick positions in each virtual environment in order to assess the effect of unexpected, novel stimuli on mouse behavior in the virtual reality environment.

Expected Outcomes

I expect that there will be a greater degree of memory reactivation following exposure to the novel, emotional stimulus than following exposure to the familiar environment and non-emotional stimulus, based on prior research indicating that memory consolidation occurs to a greater extent for emotionally charged experiences [9]. I also expect that running speed in the environment presenting the emotional stimulus will decrease compared to running speed in the familiar environment or the environment presenting the non-emotional stimulus. A decrease in running speed is anticipated because when animals detect novelty, they start to explore the novel object/environment, which results in slower running. In addition, the emotional response to the stimulus could include fear responses such as freezing, which would result in lower running speeds.

Specific Responsibilities

In testing this hypothesis, I will be assisting in training the mice until they become familiar with running on the wheel in the virtual environment. I will also be responsible for testing the behavioral effects of the novel emotional vs. non-emotional stimulus and participating in data analysis.

Timeline

Fall Quarter	Winter Quarter	Spring Quarter
- Rodent behavioral training - Collect behavioral data	- Rodent behavioral training - Collect behavioral data and begin collecting imaging data	- Data analysis - Present results at the UCI Undergraduate Research Symposium

Itemized Budget

3D printing of the running wheel at the campus computer lab…….…….. $250
Thorlabs hardware for running wheel assembly ……...……...…….……. $350
Synapsin-GcaMP6s viral vector from Penn Vector Core……..……. $300
Printing a poster for presentation at the annual symposium……... $100
Total Expenses: $1000

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