

Spring 5-1-2016

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## Recommended Citation

Lew, Dana, "Histological Verification of Electrode Placement in Single Unit Recorded Rats" (2016). *Honors Scholar Theses*. 483.  
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*Histological Verification of Electrode Placement in Single Unit Recorded Rats*

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## Introduction

The Hippocampus is a region of the brain that encodes episodic memories (“what”, “where”, and “when” of an experience) and is important for spatial navigation (Vago & Kesner, 2008). Hippocampal pyramidal cells, also known as “place cells”, show spatial tuning. This means that they fire in spatially restricted fields known as “place fields”. Together these place fields generate a representation of the environment (O’Keefe & Nadel, 1978). In a familiar environment place cell patterns remain relatively constant, but firing patterns can be altered, or “remapped” in response to changes in the environment or behavioral contexts (Markus et al., 1995). CA1 cells show gradual changes in firing activity when small environmental variations occur. CA3 cells remain consistent in response to slight environmental changes, but completely remap in response to extreme environmental changes (Guzowski et al., 2004).

Many aspects of the functional workings of the hippocampus remain unknown, but recent studies indicate that the different sub-regions of the hippocampus are intricately interconnected including extensive CA3 to CA3 connectivity and connectivity between CA1 and various areas of the brain and hippocampus (Strein et al., 2009). A slice from the rat dorsal and ventral hippocampus with the regions labeled can be seen in Figure 1 (Jones et al., 2007). The hippocampus is shaped like a banana with a

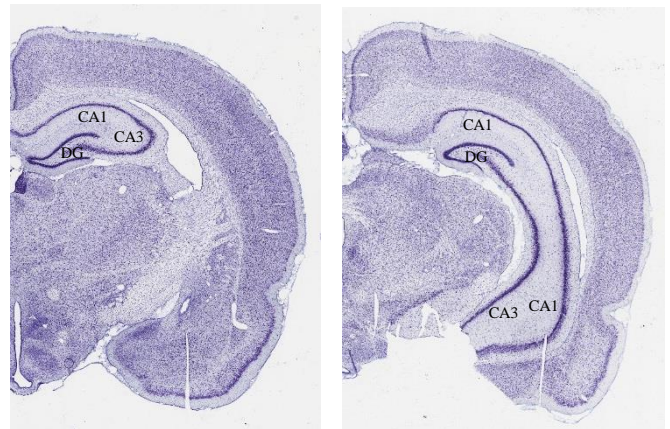


Figure 1:  
Slices from the rat dorsal hippocampus (left) and ventral hippocampus (right) with CA1, CA3, and the Dentate Gyrus (DG) regions labelled.

dorsal region which is close to the septum and top of the brain, and a ventral region which is more lateral and near the bottom of the brain. Currently there is little known about remapping in the ventral hippocampus. The majority of the previous studies have investigated the rodent dorsal hippocampus because it is much easier to target using electrodes or cannula.

There are several theories about the functional organization of the hippocampus. Some studies suggest a dorsal-ventral dichotomy which means that the dorsal hippocampus has greater sensitivity to spatial information, and the ventral hippocampus has a greater role in emotional processes (Moser & Moser, 1998). Other studies suggest a less absolute, gradient-like, division of the functional differences (Strange et al., 2014). The ventral hippocampus has been shown to have fewer place cells with larger place fields than the dorsal region. This suggests that the ventral hippocampus is less sensitive to small changes in the environment (Strange et al., 2014). A better understanding of CA1, CA3, and ventral hippocampal pyramidal cell remapping will help us functionally dissociate the subregions within the hippocampus.

This experiment was designed to determine the functional remapping in the dorsal and ventral hippocampus based on firing from single neurons in freely behaving animals. It is necessary to implant tetrodes in to the rat brain to measure single cells firing in response to novel and conditioned stimuli. We hypothesized that the ventral place cells will remap to a lesser degree compared to those in the dorsal region when the animal is placed in a novel environment. This study will shed light on the functional connections between the hippocampal subregions which create the brain's navigational system by studying the differences in firing at the cell level in response to new and familiar stimuli.

After testing the animals were sacrificed and their brains were analyzed to show the regions of the hippocampus from which we were recording. Histological verification was

necessary to ensure that regions which we recorded from are in fact CA1 in the dorsal and the ventral hippocampus. Without this final step our results would be inconclusive because there would be no way to where specifically we recorded from. Histological verification of results can also impact future surgical protocols to better aim electrode implantation toward our target region.

## Methods

One Fischer-344 rat was trained to run between two alternating arms on a seven arm radial maze in a constant environment. The rat was food deprived to 85% of its weight to motivate the animal to run for chocolate sprinkles placed at the end of each arm. When the animal reached criteria of 50 runs within 50 minutes a Microdrive was surgically implanted for single-unit recording. The Microdrive was made of 16 tetrodes, 8 directed to the dorsal hippocampus, and 8 directed towards the ventral hippocampus. The tetrodes were protected by guide cannula, and the tip location was variable allowing us to record from several different cell layers.

The animals were given a one week recovery period prior to retraining on the radial arm maze. Retraining ensured that the animals still had full cognitive function after surgery, and serves as a control to verify that surgery was not causing undue physical or cognitive harm to the animals. After retraining the place cell activity was recorded in dorsal and ventral CA1. Recordings were taken when the animal was in the home cage as well as using a familiar or novel trajectory. For session 1 the animal was exposed to a familiar trajectory then allowed to rest. After the period of rest the animal navigated either the familiar trajectory again or a novel trajectory for the second session. The location and direction of the animals head was recorded

using an overhead tracking system which locates the position of the array based on light emitted from infrared diodes attached to the head stage.

Neural data was recorded and analyzed using a digital Lynx acquisition system (Neuralynx). The spiking cluster signals from each tetrode were amplified 1000-10000x and band-pass filtered at 1- 1000 Hz. The data was subsequently stored and filtered for additional analysis. Individual units were isolated using a spike parameter cluster separation method. Place fields were identified by locating specific clusters that are statistically significant and place field remapping was determined by measuring correlations between firing patterns during the novel and familiar trajectory recording sessions.

Following the final recording parameter the animals were then euthanized using carbon dioxide. The animal's chests were opened using parallel sagittal cuts to allow access to the heart. The descending aorta was clamped to prevent blood flow to lower areas of the body. A pump was inserted through the left ventricle into the ascending aorta and the right atrium was cut allowing saline to clear the anterior region of the body from blood. 3.7% Formalin was then pumped through the body to fixate the brain tissue. The general set up of the perfusion is

seen in Figure 2 (Gage et al., 2012). The head was removed with a guillotine and the brain was extracted immediately using a rongeur. The brain was placed in a vial containing a 3.7% Formalin solution. The brain was kept in Formalin for 24 hours to allow the tissue to set and become firm enough to slice.

After 24 hours, the brain was prepared for slicing by washing with Phosphate Buffered Saline for 60 minutes in three 20 minute intervals. The fibrous

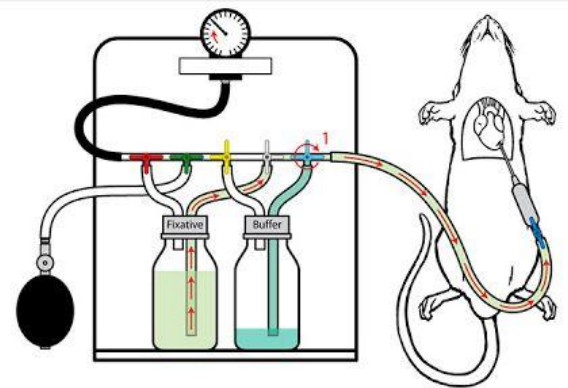


Figure 2:  
Example set up of perfusion in which the buffer and fixative can be pumped through the systemic circulation via the left ventricle. In our set up a clamp was used to prevent perfusion of the lower portion of the body, and a mechanical pump was used.

casing of the brain, the brainstem, and the anterior portion of the prefrontal cortex were removed to allow access to the hippocampus. The brain was mounted on the vibratome stage and held in place by glue and 3% agarose gel. The vibratome was set to slice the brain in 27 micron sections, and after removal of the agarose the slices were mounted in sets of six on subbed slides. After drying the slides were stained using thionine stain which marks cell bodies and cover slipped using DPX mountant.

The slides were analyzed using a LAS Leica microscope at 4x magnification. The final location of the tetrodes located and recorded. The tetrode placement from previous days was calculated to correlate tetrode placement with cluster data. This correlation ensured that the place cells found were from the CA1 region of the dorsal and ventral hippocampus.

This study has been approved by IACUC. My responsibilities included training animals, perfusions, tissue extraction, tissue slicing, tissue mounting, tissue staining, and histological analysis.

## Results

We were able to locate the tetrode tracks after the final recording day and use that location to map the tetrode location on previous days. The ideal locations were determined such that the tetrode would record from the CA1 region of both the dorsal and ventral hippocampus. The Microdrive allowed the tetrode to be moved a measurable amount each day. The ability to move the electrode allowed us to advance deeper in to the brain and have a higher probability of reaching the region that we were aiming for. There were three guide cannula implanted which held the tetrodes. The first tetrode was placed in dorsal CA1 (dorsal #1). Place fields and remapping have both been seen in dorsal hippocampus, so this tetrode was placed both to collect

data and act as a control. There were two cannula implanted in to the ventral hippocampus CA1. The first ventral electrode (ventral #1) was placed closer to the midline and more posterior in the ventral hippocampus. The second ventral electrode (ventral #2) was placed more laterally and more anterior compared to ventral #1. By placing these two tetrodes in different locations we were better able to understand the functional organization of the hippocampus. This also allowed us to study whether different areas of the ventral hippocampus show place cell remapping.

In the following figures image A was taken from a rat brain stereotaxic atlas (Jones et al., 2007). These images show the ideal location of the tetrode in the hippocampus. Images B-D show slices from our experimental animals. Several slices are show to better depict the movement of the electrode track through the brain.

Following histological analysis it was found that the dorsal tetrode was not in contact with the hippocampus. This tetrode was not implanted deeply enough in to the brain, explaining why we did not record any place cells from the dorsal tetrode. The placement of the dorsal tetrode can be seen in Figure 3.



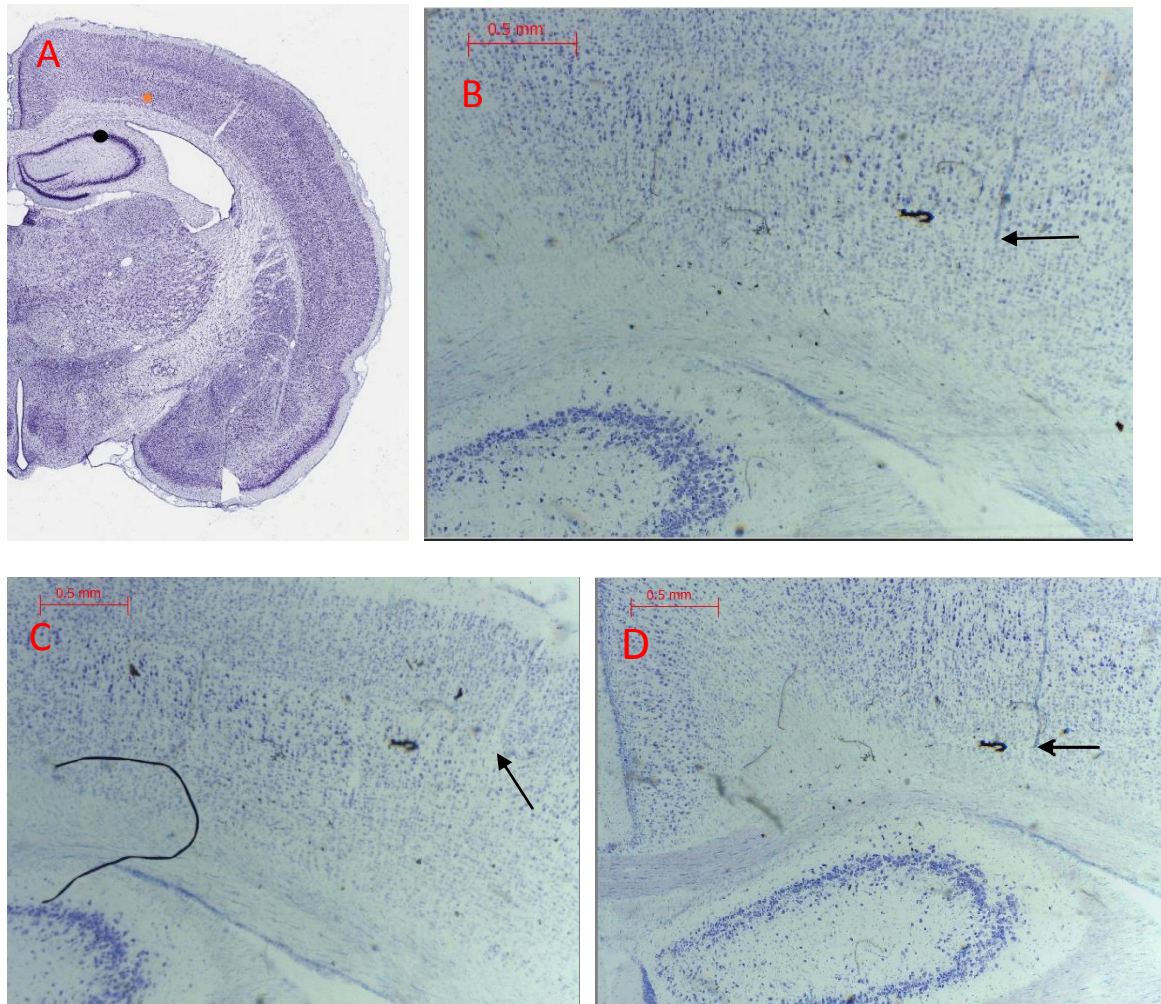


Figure 3: Dorsal Tetrode #1

- A) Ideal and actual locations of the dorsal hippocampus electrode. The ideal location is shown in black. The actual final location is shown in orange.
- B) Raw image of the tetrode track, 54 microns anterior to the final placement. The tip of the electrode is not within the hippocampus. It is denoted by an arrow
- C) Raw image of the tetrode track 27 microns from the final placement of the electrode. The tip of the track is not within the hippocampus. It is denoted by an arrow.
- D) Raw image of the tetrode track. This slice shows the final placement of the tetrode after recording, denoted by the arrow. The tip is not within the hippocampus.

The placement of the ventral #1 tetrode was in the posterior and medial region of the ventral hippocampus. The final placement of the tetrode was in the Dentate Gyrus. While this was not the intended placement of the tetrode, calculations show that the tetrode was within CA1

during some recording sessions, which means that the cells from which we recorded may have been ventral place cells. The final placement of this tetrode is seen in Figure 4.

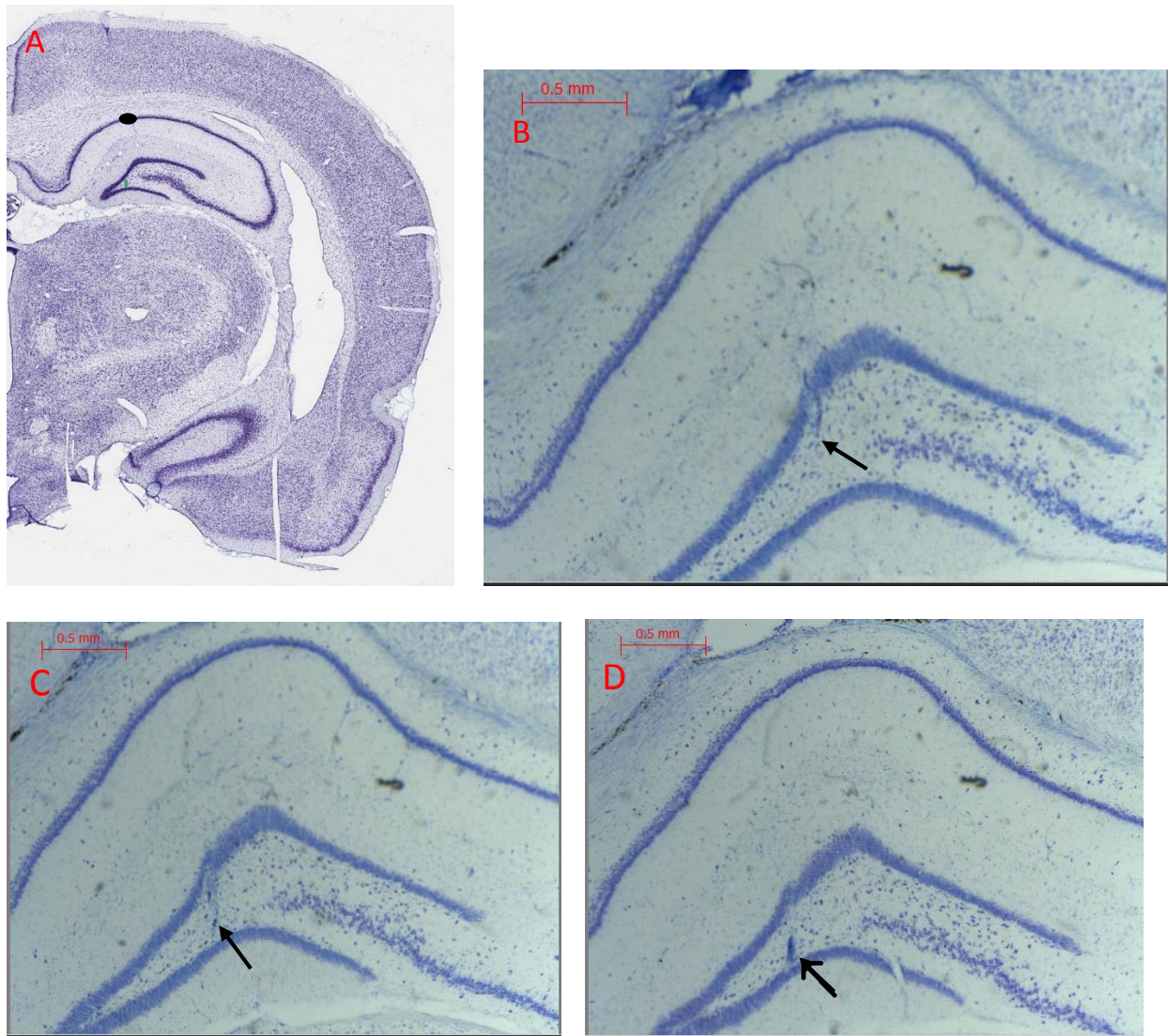


Figure 4: Ventral Tetrode #1

- A) Ideal and actual locations of the first ventral hippocampus electrode. The ideal location is shown in black. The actual final location is shown in green.
- B) Raw image of the tetrode 54 microns anterior to the final placement of the tetrode. The tip is within the Dentate Gyrus region of the hippocampus. The tip is denoted by an arrow.
- C) Raw image of the tetrode 27 microns anterior to the final placement of the tetrode. The tip is within the Dentate Gyrus region of the hippocampus. The tip is denoted by an arrow.
- D) Raw image of the tetrode track. This slice shows the final placement of the tetrode after recording denoted by an arrow. The tip is within the Dentate Gyrus region of the hippocampus.



The ventral #2 tetrode was found to be in a more anterior lateral region of the ventral hippocampus. The final placement was also within the Dentate Gyrus, but it was again found that the electrode was within CA1 during recording periods. The location of the third tetrode is seen in Figure 5.

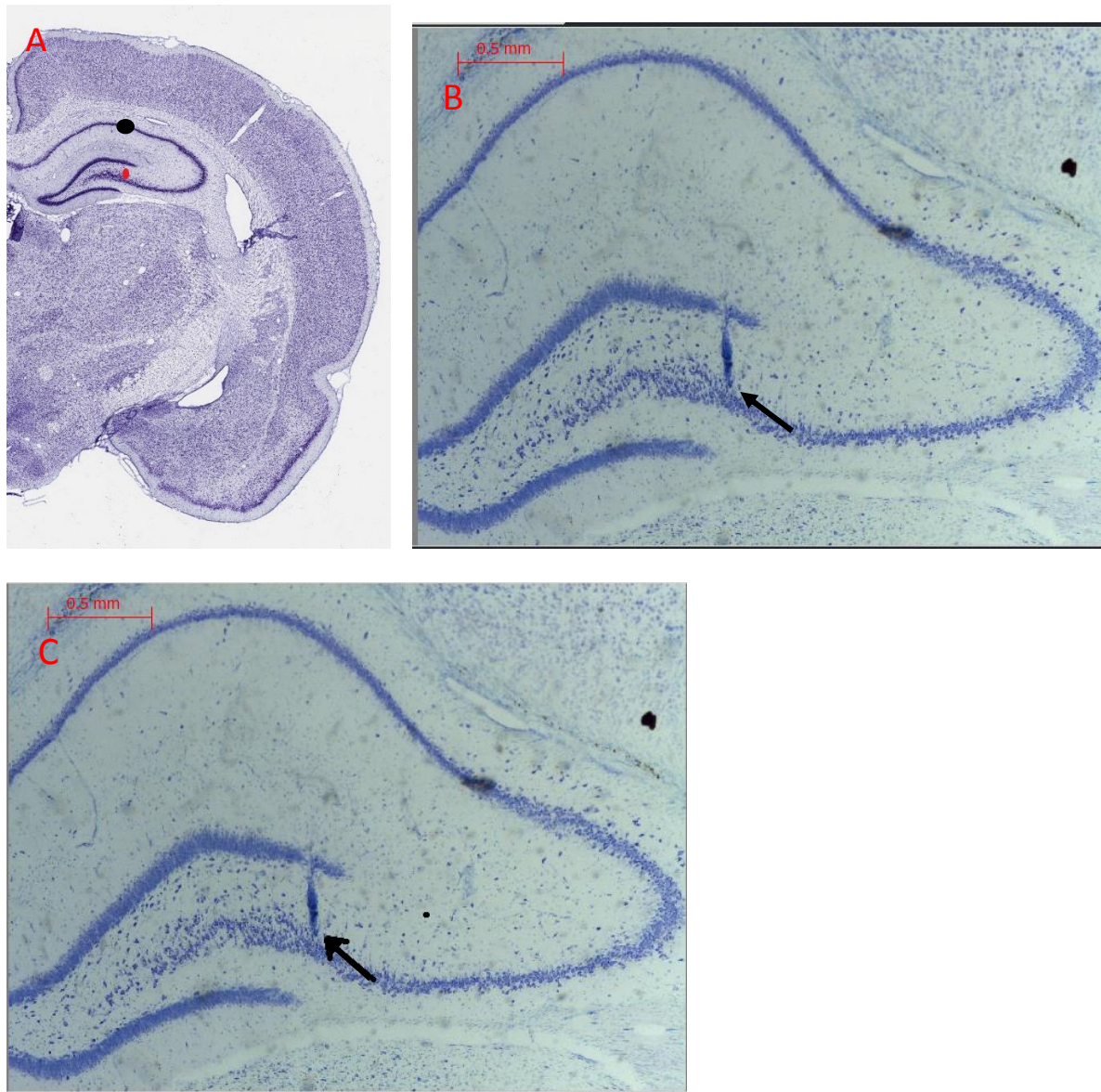
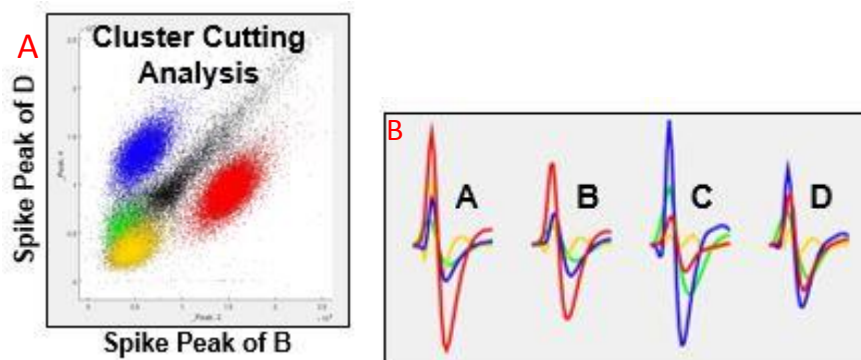


Figure 5: Ventral Tetrode #2

A) Ideal and actual locations of the second ventral hippocampus electrode. The ideal location is shown in black. The actual final location is shown in red.

- B) Raw image of the tetrode track 27 microns anterior to the final placement of the tetrode. The tip is within the Dentate Gyrus region of the hippocampus. The tip is denoted by an arrow.
- C) Raw image of the tetrode track. This slice shows the final placement of the tetrode after recording denoted by an arrow. The tip is within the Dentate Gyrus.

Using the histological data we were able to verify that the cells we were recording from were in CA1 of the ventral hippocampus. We analyzed our recordings using a method called cluster cutting. The cluster cutting data shown in Figure 6 is a visual representation of the four identified hippocampal pyramidal cells recorded from ventral tetrode #1 on a recording day in which we may have found place fields. Cluster cutting was used to discern the different cells from which we recorded and allow us to be sure that any place fields found were from discrete pyramidal cells. We calculated the location of the electrode on the date of the recording using measurements of the movement of the electrode within the brain each recording day. By calculating the amount that the tetrode was moved between the final placement and the date of our data analysis, we were able to compute the placement of the tetrode on the date that this data was taken from.



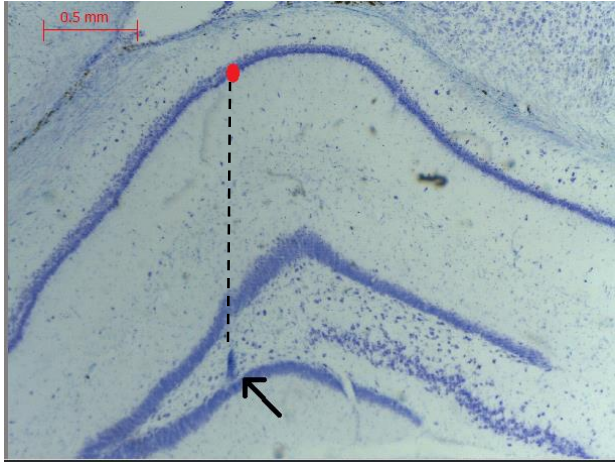


Figure 6:

- A) Four distinct cells identified in a single recording from one tetrode. Black data points represent noise or insignificant data, while the colored data points correspond to the four identified hippocampal pyramidal cells.
- B) Average waveforms of the four cells, separated by electrode. A taller waveform indicates a more powerful signal, corresponding to a smaller physical distance between the source of the signal and the electrode.
- C) Location of the electrode during the recording of shown in Figures 6A and 6B. Final electrode track is denoted by an arrow. The location of the electrode during the recording in Figures 6A and 6B is denoted by the red marker. The dashed line shows the movement of the electrode between the recording day depicted in Figures 6A and 6B and the final recording day. The electrode was within ventral CA1.

### Conclusions/Implications

This project involved extensive histological analysis of the rat hippocampus. My work focused on histological verification of the tetrode placement including perfusion of animals, extractions of brains, brain slicing, mounting, staining, and analysis. Analysis included using LAS Leica software to locate the final placement of the electrode. The final placement allowed us to calculate the location of the electrode on each recording day. This knowledge is a crucial verification step ensuring that our data is from place cells within CA1.

Recording from the ventral hippocampus is extremely difficult. The ventral hippocampus is very deep within the rat brain, making implanting tetrodes accurately very difficult. Due to the difficulty involved there are very few labs attempting to record from place cells within the

ventral hippocampus. We found that both of the ventral hippocampus electrodes were recording from the CA1 region of the ventral hippocampus, but in the future we would like to record from deeper areas of the ventral hippocampus. The ventral hippocampus is a large structure and our electrodes could have been implanted in an area which would allow them to travel deeper in to the structure while recording from ventral CA1, as seen in Figure 7. In order to do this we will try to implant the cannula more laterally. By implanting further from the midline we may be able to collect data from place cells throughout different areas of CA1 in the ventral hippocampus.

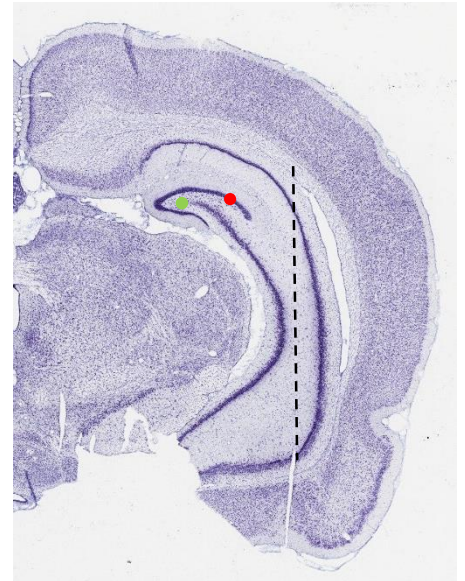


Figure 7:  
The green and red dots show the final placements of our electrodes in this study. The dashed line shows an ideal electrode track which may be used for a future study.

Remapping is known to occur in the dorsal hippocampus when the animal is placed in a new environment and a place cell previously found to fire in one location begins to fire in a new location. Place cell remapping is relatively unexplored in the ventral hippocampus due to the difficulty involved in implanting tetrodes to the ventral hippocampus. The ventral hippocampus is much deeper within the brain, and therefore well aimed cannula implantation is challenging. This study showed that ventral hippocampal single unit recording is feasible, and that significant single unit recordings can be measured within the ventral hippocampus. We hope to be able to use data collected from ventral CA1 to support ventral hippocampal remapping.

In addition to the modified placement of the ventral tetrodes, in the future we would like to repeat our study with more animals to create greater statistical significance. In future animal surgeries we will attempt to have better electrode placement in the dorsal hippocampus.

Recording from the dorsal hippocampus can strengthen our data. Because the dorsal hippocampus is much better understood than the ventral hippocampus, recording neuronal outputs from both areas simultaneously will allow us to verify our recordings. This may also help us to better understand the communication between the dorsal and ventral hippocampal subregions. We are also interested in studying the CA3 region of the hippocampus. This region has extensive connectivity both internally (CA3 to CA3) and to CA1. Recording from CA1 and CA3 cells simultaneously could further illuminate the functions of these areas as well as elaborate on the functionality of the hippocampus as a whole.

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