Improving Electroencephalograph Probe Placement with Digital Signal Processing

Nicholas Hayes
Principal Investigator: Dr. Annabelle Singer

Singer Laboratory for Neuroscience, Wallace H. Coulter Department of Biomedical Engineering
Georgia Institute of Technology
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Faculty Signatures

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Abstract

Theta waves (theta), sharp wave ripples (SWRs), and spikes are common waveforms that appear in local field potentials (LFPs) within the rodent brain. LFPs are collected using an electroencephalogram (EEG) probe in vivo, while a rodent navigates a virtual reality (VR) environment. Prior to experimentation and data collection, the probe must be positioned into the CA1 region of the hippocampus. Current probe placement techniques rely on manual interpretation of waveform data to predict probe proximity to the hippocampus, which can be imprecise. Improper placement of an EEG probe can cause damage to rodent neurons and create experiment delays. To mitigate these risks, we developed a MATLAB script that assesses behavioral changes in theta, spikes, and SWRs from raw LFPs as a functions of probe depth to automatically predict correct termination location within the rodent hippocampus. We show the success of the depth prediction algorithm in n = 6 different trials against manual placement by showing no significant difference (p = 0.747, unpaired two-tailed t-test) between actual and predicted depth values.

Keywords: rodent hippocampus, virtual reality, electroencephalograph, probe, algorithm, automation, theta waves, sharp-wave ripples, spikes, and local field potentials
Introduction

Background

In hippocampal neuroscience, theta waves (theta), sharp wave ripples (SWRs), and spikes are common waveforms that appear in varied magnitudes in local field potentials (LFPs) within the rodent brain. Theta, which has frequencies of 4 to 12 Hz, occurs during movement. Conversely, SWRs trigger during sleep and immobility and are characterized by frequencies of 100 to 200 Hz. Spikes have frequencies between 500 to 5,000 Hz and can occur during movement or sleep. These waves are collected through an electroencephalogram (EEG), which is performed using a probe in vivo while a rodent navigates a virtual reality (VR) environment.

VR technology has been used for several decades to study spatial navigation in rodents. Thurley et al present a thorough review of VR from the 1980s to 2016, which discusses the most common VR setup consisting of a 270° screen, projector, and free moving ball suspended on a cushion of air. In this design, rodents are head-fixed to the VR rig, which facilitates collection of LFP signals in vivo by minimizing unintended EEG probe displacement. Milk rewards are also commonly given to rodents for completing specific tasks within the simulation to reinforce learning. Projected environments can also be altered to evaluate different components of rodent memory, including spatial navigation, decision making, and sensory processing, making experiments involving VR simulation often advantageous to real world (RW) environments.

In contemporary rodent VR studies, silicon probes are frequently used to detect LFPs. Buzaki et al explore the process of assembling a silicon probe for detecting LFPs in rodent brain. Probes are made by diffusing boron, silicon dioxide, silicon nitride, and silicon dioxide into a silicon wafer. Contact sites, which collect LFPs, are made of gold and iridium. Multiple contact sites can be found on each probe, which allows data from several depths to be collected simultaneously. This structure is advantageous for analyzing waveform patterns and changes throughout several brain layers. Buzaki’s probe measures 5 to 15 μm thick, which is necessary to limit tissue displacement and damage during probe placement. Buzaki presents unfiltered
LFP data taken \textit{in vivo} from CA1 to the dentate gyrus to illustrate the effectiveness of the silicone probe design. However, no quantitative analysis of LFP behavioral changes as a function of depth are presented, and SWR and theta are not categorized or separated from the data provided.

Theta and SWR behavior within the hippocampus has been qualitatively explored by previous studies. Bragin et al study the behavior of theta in rodents during exploratory activity and sleep using a silicon probe \textit{in vivo} within the hippocampus from CA1 to the dentate gyrus\textsuperscript{4}. Theta, which occurs at 4 to 12 Hertz, is most active during exploratory awake activity, and is replaced with irregular waveforms during sleep\textsuperscript{4}. From the LFP data extracted, Bragin notes that theta increases as a function of depth within hippocampal layers and a gradual phase shift of 30 to 90 degrees from CA1 to the dentate gyrus. While Bragin et al quantify changes in power and phase of theta within the hippocampus, no LFP data from regions above the hippocampus is discussed or proposed. Additionally, Klausberger et al investigated the behavior of SWRs and theta in anesthetized mice by probing GABA-releasing interneurons within the CA1 region of the hippocampus using a glass probe\textsuperscript{5}. This study characterizes the frequency range and shape of theta and SWRs in the rodent hippocampus and compares the firing behavior of parvalbumin-expressing basket cells, axo-axonic cells, and Oriens-lacunosum-moleculare (O-LM)\textsuperscript{5}. Quantitative analysis is conducted to determine differences in SWR and theta firing between three different interneurons, and the characteristic shapes of SWRs and theta in the CA1 region are shown.

However, analysis of waveform changes at different depths is not conducted, as the study is restricted to the CA1 region.

EEG probes can damage neurons and inhibit hippocampal functions if placed too deep, so precise positioning is critical\textsuperscript{3}. Damage from incorrect probe placement can invalidate data and disqualify rodent subjects, which can severely impede data collection\textsuperscript{3}. To detect LPFs, probes are lowered into the hippocampus manually with the use of a microdrive\textsuperscript{3}. During descent, the proximity of the probe to the hippocampus is approximated by manually interpreting theta, SWR, and spike behavior from raw LFP output\textsuperscript{5} using an oscilloscope.

\textbf{Purpose}

To decrease the setup time and risk associated with rodent electrophysiology experiments, we seek to automate EEG probe placement by developing a probe termination depth prediction script in MATLAB. Since numerical changes in waveform behavior have not been established by previous studies, we first performed digital signal processing on existing theta, SWR, and spike data to relate waveform behavior to probe depth. These relationships were then used to create an algorithm with six threshold parameters. We hypothesize that this MATLAB algorithm can successfully predict probe termination depths with no significant difference in depth values when compared to manually interpreted termination depths in a simulated environment.
Methodology

Initial Data Collection

A NeuroNexus A1x32-Poly3-5mm-25s-177 probe was used to collect LFP data from three different male 5X-FAD rodents in a non-stimulation VR environment for 5 trials. The probe had 32 independent channels that captured LFP data simultaneously at different depths (Figure 1). Channel data for each trial was captured, yielding a total of 192 channels for analysis. Data collection information for each trial is shown in Table 1.

Table 1: Data Collection Information

<table>
<thead>
<tr>
<th>Mouse Identifier</th>
<th>Trial Number</th>
<th>Date of Recording</th>
<th>Trial Length (s)</th>
<th>Probe Sample Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>W1</td>
<td>1</td>
<td>March 20, 2017</td>
<td>304.46</td>
<td>2,000</td>
</tr>
<tr>
<td>W2</td>
<td>2</td>
<td>March 29, 2017</td>
<td>1,186.82</td>
<td>2,000</td>
</tr>
<tr>
<td>W3</td>
<td>3</td>
<td>March 29, 2017</td>
<td>1,174.40</td>
<td>2,000</td>
</tr>
<tr>
<td>W4</td>
<td>4</td>
<td>April 5, 2017</td>
<td>1,313.72</td>
<td>2,000</td>
</tr>
<tr>
<td>W5</td>
<td>5</td>
<td>April 19, 2017</td>
<td>1,360.12</td>
<td>2,000</td>
</tr>
<tr>
<td>W6</td>
<td>6</td>
<td>May 16, 2017</td>
<td>1,259.25</td>
<td>2,000</td>
</tr>
</tbody>
</table>

Mouse identifiers, trial numbers, recording dates, trial lengths, and probe sample rates are shown for each trial. All data for this study was collected with a NeuroNexus A1x32-Poly3-5mm-25s-177 probe across 32 channels for the durations shown.

Table 2: Generating Mean Slope Ranges for Theta, SWRs, and Spikes

<table>
<thead>
<tr>
<th>Trial</th>
<th>SWR Slope (µV/mm)</th>
<th>Theta Slope (µV/mm)</th>
<th>Spike Slope (µV/mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18.41024</td>
<td>369.7033</td>
<td>206.2607</td>
</tr>
<tr>
<td>2</td>
<td>367.004</td>
<td>413.8805</td>
<td>119.7069</td>
</tr>
<tr>
<td>3</td>
<td>138.5688</td>
<td>217.8403</td>
<td>157.8839</td>
</tr>
<tr>
<td>4</td>
<td>387.1452</td>
<td>607.8322</td>
<td>357.9685</td>
</tr>
<tr>
<td>5</td>
<td>434.9082</td>
<td>335.8711</td>
<td>536.6417</td>
</tr>
<tr>
<td>6</td>
<td>125.4364</td>
<td>55.21399</td>
<td>361.8525</td>
</tr>
<tr>
<td>Mean</td>
<td>245.2455</td>
<td>333.0536</td>
<td>290.0524</td>
</tr>
<tr>
<td>SD</td>
<td>157.1204</td>
<td>170.2184</td>
<td>143.3602</td>
</tr>
</tbody>
</table>

Linear regressions were conducted to compute the average slope for theta, SWRs, and spike data for results for n = 6 files. From these results, the mean was taken to compute the average SWR, theta, and spike slope values across all six files. The data points used to generate a mean slope value and standard deviation for each waveform are shown. These points were incorporated into the probe prediction value as range thresholds, where each threshold was the mean – SD to mean + SD.

Methodology

Initial Data Collection

A NeuroNexus A1x32-Poly3-5mm-25s-177 probe was used to collect LFP data from three different male 5X-FAD rodents in a non-stimulation VR environment for 5 trials. The probe had 32 independent channels that captured LFP data simultaneously at different depths (Figure 1). Channel data for each trial was captured, yielding a total of 192 channels for analysis. Data collection information for each trial is shown in Table 1.

Figure 1: A 32-channel NeuroNexus A1x32-Poly3-5mm-25s-177 probe was used to collect LFP data from three different male FX-FAD mice in n = 6 separate trials.
Preliminary Data Analysis

The raw LFP data was extracted using a MATLAB script and filtered for theta, SWRs, and spikes. Data was from extracted from its native .intan format, imported into MATLAB, converted to .mat format for filtering using a script. From this format, theta bands were isolated at 4 to 12 Hz, spikes were isolated at 500 to 5,000 Hz, and SWRs were isolated at 100 to 200 Hz. The theta, SWR, and spike data were related to probe depth based on channel location. Filtered data for theta, SWRs, and spikes were exported to Microsoft Excel files for future use in algorithm development.

Threshold Criteria Development

The extracted data were evaluated to create threshold criteria for predicting probe depth in MATLAB. Theta amplitude, average theta slope, SWR amplitude, average SWR slope, spike amplitude, and average spike slope were considered as parameters to predict depth. Threshold values for these criteria were established based on the behavior of each of these factors at the termination depths, where the probe had been manually identified as having reached
the hippocampus in each trial. Waveform behavior was assessed in each trial to generate accurate threshold values. Mean slope and standard deviation for theta, SWRs, and spikes was calculated for each of the six data sample files. Ranges were then established based on these values, where the lower threshold was the mean subtracted from the standard deviation and the higher threshold was the mean added to the standard deviation. These values are displayed in Table 2.

Traces of theta, SWR, and spikes were observed across all six files to establish threshold criteria for amplitude. For each file, the amplitudes for theta, SWRs, and spikes at the end depth were evaluated to generate a range of values to act as a characteristic thresholds. Sample traces from this process are shown in Figure 2.

In order to gauge the relative importance of theta, SWRs, and spikes as predictors of depth, the covariance between depth and amplitude was computed for each sample data file. From this process, theta was shown to be the most correlative predictor of depth, with spikes being the second most correlative predictor, and SWRs being the least correlative of the three parameters. To establish weighting criteria, covariance ratios were established based on correlative strength for implementation into the algorithm. The results of this process are shown in Table 3.

**Implementation**

These thresholds were integrated into a MATLAB script that simulates real-time data input by importing simulated LFP data that was evaluated by the script based on the threshold criteria to make a prediction of the appropriate termination depth. Filtered data were imported from Microsoft Excel iteratively to simulate probe descent. Data were assessed against six threshold descent to determine whether or not to move the probe downward. To satisfy the three amplitude criteria, a minimum of three consecutive channels had to exceed the amplitude for theta, SWR, and spikes to compute pass or fail results. For the slope criteria, linear regressions were computed for the channel data for theta, SWRs, and spikes to compute average slopes. These slopes were checked against the threshold criteria values shown in Table 2 to generate pass or fail results. The aggregated results from the six criteria were evaluated using a weighted decision algorithm, where the pass/fail results were combined in a vector.

Figure 3: Algorithm Overview

and the weights shown in Table 3 were applied to generate numerical scores for each waveform using Equations 1.
Equation 1: Waveform (φ) Score

\[
\phi \text{ Score} = \phi_w \phi_i + \phi_w \phi_s
\]

\(\phi_w = \text{covariance weight ratio}\)

\(\phi_i = \text{amplitude indicator}\)

\(\phi_s = \text{slope indicator}\)

If the summed weighted scores for theta, SWRs, and spikes generated in Equation 1 were greater than Equation 2, probe descent was terminated and a predicted depth value was assigned. A summary of this process is shown in Figure 3. This process was conducted \(n = 6\) times to generate predicted termination depths, which were compared against actual termination depths.

Equation 2: Score Threshold

\[
\text{Score Threshold} = \theta_w + \sigma_w + \omega_w
\]

\(\theta_w = \text{theta covariance weight ratio}\)

\(\sigma_w = \text{spike covariance weight ratio}\)

\(\omega_w = \text{SWR covariance weight ratio}\)

Results

Difference in Predicted Termination Depth versus Actual Termination Depth

The algorithm prediction results for all six trials are shown in Figure 4. The actual value represents the termination depth that was manually assessed for each file, while the predicted depth represents the depth predicted by the algorithm. An unpaired two-tailed t-test was used to evaluate the difference between the predicted and actual depth groups. There was found to be no significant difference (p-value = 0.747) between these two groups.
**Percent Error for Predicted Termination Depth versus Actual Termination Depth**

When the predicted termination depths generated from the algorithm was compared to the actual termination depths, the resulting mean percent error across the six samples was 6.55 percent with a standard deviation of 5.58 percent.

**Discussion**

**No significant difference in predicted probe depth versus actual probe depth**

There was no significant difference between the algorithm’s predicted depth and the actual termination depth across the six samples. The combination of covariance weighting, mean slope threshold ranges, and amplitude threshold ranges seems to be an effective method for predicting probe depth given filtered theta, SWR, and spike data inputs.

**Field Significance & Applications**

This study represents the first quantification of specific threshold ranges for theta, SWR, and spike behavior at the hippocampal surface. Previous studies have qualitatively depicted waveform behavior around the CA1 region of the hippocampus, but none have quantitatively explored amplitude and slope behaviors during probe descent.

This is also the first documented process of simulated probe placement with the use of an algorithm. The current standard in the field is to manually lower EEG probes into the rodent hippocampus by manually interpreting an oscilloscope. The degree of accuracy achieved in this study illustrates the potential for automated scripts to assist in probe placement in rodent neuroscience to reduce experimental setup time and neuronal damage during probe descent.

**Conclusion**

In alignment with our primary hypothesis, no significant difference was found between the predicted and actual depth values. This finding illustrates the potential capabilities of an automated probe placement system, which could eventually replace manual probe placement techniques and allow for safer and faster rodent electroencephalography in the field.

**Future LabView Integration**

Future studies should expand on the techniques developed here by integrating probe prediction software with hardware capable of moving a probe based on algorithm results. To further evaluate the effectiveness of the MATLAB algorithm, a LabView script to control probe depth based on waveform data inputs could be created. This script would iteratively control the z-orientation of an EEG probe based on input from a MATLAB script based on the established threshold criteria. To simulate real-time data input from a rodent, a simulated raw LFP data would be imported into MATLAB, where data for theta, SWRs, and spikes would be isolated through filtering. The behavior of these waveforms would then be assessed against the threshold criteria to make a prediction of probe proximity to the hippocampus. The outcome
of this decision would be used to iteratively control probe descent. Percent error would then be calculated between the actual and predicted probe placement to evaluate the success of the algorithm. After verified success with simulated data, this design could then be directly applied during a live electrophysiology trial on a head-fixed rodent.

**Future Algorithm Improvements & Optimizations**

The algorithm could be optimized over a broader range of samples and probes. This study was limited in sample number, using a sample size of n = 6 due to restrictions on data availability. Ideally, large aggregates of theta, SWR, and spikes would be assessed and processed through the algorithm to generate a broader sense of its effectiveness. Additionally, future versions of the algorithm could require fewer data points to compute threshold parameters. The current version generates parameter values by assessing waveform behavior across the entire trial duration, which would not be practical to use in a real-time data acquisition environment. The algorithm should also be integrated with other types of probes to determine what necessary adjustments would need to be made to optimize the process for different probe designs and setups. Different material compositions and channel arrangements would likely greatly influence the effectiveness of the algorithm, which would need to be addressed to provide a broad solution for probe placement.

**References**

