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Spike-LFP Correlation Within the Turtle Electrophysiology Project

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Spike-LFP Correlation Within the Turtle Electrophysiology Project

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Aaron Foote is a senior majoring in Physics and minoring in History at Washington University in St. Louis. His interest in electrophysiology and neurophysics began when he participated in Dr. Ralf Wessel’s Biophysics Laboratory class, and he continued to work with Professor Wessel during the summer. Aaron plans to pursue a Ph.D. in Biophysics or Biomedical Engineering after graduation.

KEY TERMS

• Action Potential
• Electrophysiology
• Local Field Potential
• Python
• Turtle Electrophysiology Project
• Visual Cortex

ABSTRACT

Correlated neural activity within the brain results from the organized behavior of thousands of neuronal cells. In this study, we examine the coordinated neural response to a stimulus in the visual cortex of turtles (Chrysemys scripta). We seek to test the relationship between the low frequency local field potential (LFP) and the action potential spikes of individual neurons by directly measuring electrical activity in the visual cortex. There are two contributions to the local field potential, the correlated activity of nearby neurons and the synaptic input into local neurons. If a correlation between action potentials and local field potentials exists, then we seek to understand the sources of the correlation. Fully exploring this correlation is made easier by the use of the Turtle Electrophysiology Project, an experiment and data management system that automates much of the data collection and analysis required. Additionally, we incorporate a spike detection program called Spikepy in order to further interpret our data. The spike – LFP relationship is examined through the creation of analysis scripts in python that draw on libraries of code from the Turtle Electrophysiology Project as well as the detection and filtering abilities of Spikepy. We found that there is a strong correlation between the local field potential and action potential spikes measured in the visual cortex, bringing us closer to understanding the nature of correlated neural activity.

FACULTY MENTOR: RALF WESSEL PH.D.
ASSOCIATE PROFESSOR OF PHYSICS

Professor Wessel’s NeuroPhysics group seeks to delineate principles of visual information processing at the level of spatiotemporal network dynamics in optic tectum and visual cortex. The central component of the NeuroPhysics research program consists of in vitro electrophysiological recordings of cortical activity in the turtle eye-attached whole-brain preparation in response to computer-controlled visual stimulation of the retina. The synergy of advanced neurotechnology, comparative in vitro physiology, and physics-inspired theory provides a fertile opportunity to advance our understanding of cortical microcircuit function.

ACKNOWLEDGEMENTS

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Jacques Jordan Lamothe, a junior majoring in Political Science and Economics
INTRODUCTION

Though the field of neuroscience has made great advances since its inception, researchers continue to grapple with unanswered questions. The process through which photons colliding with optical nerves in the back of the eye are converted into mental images remains poorly understood. Neuroscientists are currently trying to map the specific regions of the brain that contribute to this process and the type of electrical activity that the process generates. Uncovering the relations between the brain’s different electrical signals is critically important to the success of this research.

Within layered groups of neurons, such as those in the brain’s visual cortex, a stimulus generates several types of electrical signals. In order to better understand complex neurological phenomena, such as vision, one must look at the relationship between all types of signals, not just the individual features themselves. One such signal is an action potential, a rapid depolarization and repolarization of an individual neuron’s cellular membrane used to transmit information across a cell. This registers as a high frequency (~1000 Hz) deflection spike when measured via an electrode near the polarizing neural tissue. Another feature, the local field potential, or LFP, is a low frequency (~300 Hz) deflection caused by synchronized electrical activity across a region of neural tissue. The LFP amplitude increases during high levels of correlated activity and during periods of increased synaptic input into the nearby neurons.

To measure this activity, we analyze the synchronized neural response to stimuli in the visual cortex of turtles (*Chrysemys scripta*). While similar experiments have been performed with other small animals, the turtle has a biological mechanism that enables their brains to continue functioning without oxygen for extended periods of time. In practice, this makes data collection less time sensitive, as we are still able to evoke a signal after the turtle brain has been separated from the body’s oxygen supply for some time. We gathered two kinds of data from the turtle, searching recordings and visual recordings. Searching recordings are traces of spontaneous electrical signals that are not generated by a controlled stimulus. Visual recordings, however, are collections of responses to a specific visual event such as a flash of light.

The Turtle Electrophysiology Project, or TEP, is a database system that provides an organized framework that stores and analyses our recordings. Written primarily in python, it enables the user to form collections of searching or visual recordings and tag them with useful notes. The TEP also contains libraries of code for organizing, analyzing, and plotting results from data. The collections can all be created and edited using Search GUI program that is built into the database. Similarly, the python based application Spikepy is a useful tool for analyzing spikes and electrical traces. It provides a flexible framework that can be modified through plug-ins to perform a wide variety of functions, depending on the user’s needs.

METHODS AND PROCEDURES

Our study combines biophysical methods with software infrastructure to test the relationship between spikes and LFP’s. To gather tracings, we begin by isolating the turtle eyes, optic nerves, and brain from the body through a lengthy dissection
process. The turtle is anesthetized to facilitate the removal of the head from the body. Excess bone and muscle are carefully cleared from around the brain and optical tissues. The Dorsal Ventricular Ridge (DVR) of the brain is cut and held open in order to expose the visual cortex without damaging optical neurons. The brain is secured into a specially-designed recording chamber, with the visual cortex open to the air. By inserting glass electrodes into neural tissue, we can take both extracellular and intra-cellular recordings from within the exposed visual cortex. The process of gathering data is largely controlled by a Lab View program within the TEP, enabling the user to change the type and duration of any visual stimuli. In order to gather visual recordings, one must choose a protocol that defines the characteristics of the stimulus delivered. Stimuli can range from flashes of light of varying color and duration to colored dots moving across a screen, each potentially producing a different stimulus.

Every recording taken from within the visual cortex is arranged into a collection. It is important that all data within a collection is gathered from the same location and electrode depth within the visual cortex in order to minimize variability within a collection. There are a number of ways to test the spike-LFP relationship, but they are all generated in a similar manner using python scripts. The TEP already contains libraries of code that are useful for spike detection and LFP analysis, in addition to Spikepy. Beginning with libraries of code, one can modify programs to perform slightly different functions or return the processed data in a more useful format. For example, the existing code in the database can be modified to analyze tetrode recordings instead of single electrode recordings, providing new opportunities for further analysis. The user must also write supplementary code that opens the correct files for each program and scales the script so that it can process data from a large number of recordings. Once the analysis script has been completed, it must be tested to ensure that it performs the desired functions.

In our experiment, we seek to test for the existence of a spike-LFP correlation through a multi-step process using a python analysis script. This first step of the process is to filter the raw voltage traces with low frequency and high frequency filters in order to separate the action potential spikes and the local field potential. This process is diagramed below.

**Figure 1**

The raw trace shown on the left is filtered using a high pass filter and a low pass filter, producing the two modified traces on the right. The top right recording contains only low frequency local field potential deflections. Any low frequency activity has been removed from the figure on the bottom right so that only high frequency action potential spikes remain. The spikes are identified by the heavy lines on the bottom of the figure.
Separating the local field potential trace and the spike trace is essential in order to accurately quantify the number and magnitude of the characteristics of interest. For both filtered traces, we divide the recordings into one second intervals to expose any potential associations between the LFP and spikes. For the trace containing only high frequency spikes, we use a Spikepy plug-in to detect any action potential spikes that may occur within a one second interval from the trace. This plug-in identifies any spike that is more than six standard deviations above the mean value of the trace and outputs the number of spikes found as a list. Each element in the list indicates the number of spikes detected in the corresponding one second interval. For example, for a ten second recording, Spikepy would output a list of the following form:

\[ [0, 2, 1, 0, 0, 1, 0, 0, 0, 3] \]

With this sample result, we see that there are no spikes in the first one second interval of the trace, two spikes in the second interval, one spike in the third, and so forth.

For the filtered trace containing the low frequency LFP features, we create a script to calculate the area underneath the recording in order to measure the magnitude of the local field potential. We examine the absolute value of the filtered trace to measure the magnitude of the LFP, and calculate the total area under the trace for each one second interval. For a ten second trace, a possible output for this area program would be in the form:

\[ [0.015, 0.0334, 0.0445, 0.0254, 0.0245, 0.027, 0.01, 0.020, 0.0323, 0.0415] \]

In this case, the area under the trace on the first one second interval would be 0.015 mV/s and so forth. We set both the spike detection and area portions of our analysis script to output the relevant data as a list in order to best observe any correlations between the two.

The final portion of our python script plots the elements of the area under the curve list with the elements of the spike detection list on the Cartesian coordinate system. For every one second interval, there is both a spike number and a trace area associated with it. Using the sample ten second recording given above, we would see a plot with ten points with the following coordinates:

\[ [(0, 0.015), (2, 0.0334), (1, 0.0445), (0, 0.0254), (0, 0.0245), (1, 0.027), (0, 0.01), (0, 0.020), (0, 0.0323), (3, 0.0415)] \]
Graphing these sample data points on a trace area vs. spike number plot produces the result below.

**Graph 1**

This graph is a sample of the final output of the analysis script used in our experiment. The vertical axis is the area underneath the signal, which increases during high levels of local field potential activity. The horizontal axis is the number of spikes detected by Spikepy. Each data point represents a one second interval of time, and its position on the vertical and horizontal axes is determined by the signal area and the number of spikes detected on that interval.

This plot contains only ten sample data points, corresponding to 10 intervals of time. When analyzing collections of recordings, however, every brief time interval from every recording within the collection is displayed on the resulting graph. With this large sample size, any correlation between spike number and trace area would theoretically become clear. To quantify the correlation, we determine the line of best fit for the set of data points. We extract the slope of that line as well as how closely the line fits the set of data in order to compare analysis results between collections.

**RESULTS AND ANALYSIS**

**Spontaneous Recordings**

After analyzing a total of seven collections, each containing a minimum of 38 spontaneous recordings, we see a clear pattern emerge. There is a largely linear, proportional correlation between the number of spikes and the area underneath the local field potential. This is consistent with our hypothesis that the action potential spikes and the area underneath the signal are correlated in some way. On the following page are two sample graphs of our data, divided by collection number. In order to be consistent, the collections are organized so that each recording in the collection is taken from the same sample at the same electrode penetration depth.
Graph 2a

Graph 2b
Each data point shown in the plots above corresponds to a one second interval within a spontaneous recording in the indicated collection. Though some collections show more precise results than others, there is a subtle but significant curve at low spike numbers that prevents the data from being completely linear. However, as the magnitude of the spike number and area increases, the graphs each show asymptotically linear behavior. It is also worth noting that the lines of best fit for each data set never cross the origin. Since each point represents a one second interval of time, it is unlikely that the total signal area would be zero or nearly zero over such a long period. This lack of intercepting the origin can be further explained by the fact that, with our method, it is impossible for a trace to output data points with a negative area. These two consequences of the experiment's analysis method could also explain why there is a slight curve at low spike numbers.

We summarize some important characteristics of the best fit lines in the table below.

<table>
<thead>
<tr>
<th>Searching Recording Collection Number</th>
<th>Linear Slope (mV/(s*spike number))</th>
<th>R (Linear Regression Constant)</th>
<th>R^2 (Linear Regression Constant Squared)</th>
</tr>
</thead>
<tbody>
<tr>
<td>26</td>
<td>0.00155</td>
<td>0.87563</td>
<td>0.76673</td>
</tr>
<tr>
<td>27</td>
<td>0.00181</td>
<td>0.81498</td>
<td>0.66420</td>
</tr>
<tr>
<td>28</td>
<td>0.00158</td>
<td>0.95196</td>
<td>0.90622</td>
</tr>
<tr>
<td>29</td>
<td>0.00156</td>
<td>0.93200</td>
<td>0.86861</td>
</tr>
<tr>
<td>30</td>
<td>0.00143</td>
<td>0.94080</td>
<td>0.88510</td>
</tr>
<tr>
<td>31</td>
<td>0.00132</td>
<td>0.93366</td>
<td>0.87172</td>
</tr>
<tr>
<td>34</td>
<td>0.00135</td>
<td>0.92788</td>
<td>0.86096</td>
</tr>
<tr>
<td>Average</td>
<td>0.00151</td>
<td>0.91099</td>
<td>0.83193</td>
</tr>
</tbody>
</table>

**Table 1**

The line of best fit for every collection possesses an R value above 0.8, and if the first two collections are ignored then each line possesses an R value above 0.9. This indicates that the data is fairly precise, and the observed linear trend is significant.

**Shifted Spontaneous Recordings**

There is a possibility, however, that the time between an action potential spike and a period of increased signal area is synchronized in such a way so that our data could be misleading. For example, if there is an action potential spike 0.5 seconds into the recording, and the associated period of increased signal area does not begin until one second later, then the spike and the correlated LFP area would not be measured in the same time interval. To test for this effect, we altered the python script by shifting the one second interval window by 0.5 seconds. For a recording of duration N seconds, this results in time intervals such as:

$$[(0.5s, 1.5s), (1.5s, 2.5s), (2.5s, 3.5s), (3.5s, 4.5s), \ldots (N - 1.5s, N - 0.5s)]$$
While maintaining every other aspect of our analysis script, we gathered a new set of data with the altered windows. It should be noted that there was an anomaly within Collection 30 that made this shifted windows analysis impractical, so only 6 graphs of searching recordings were recorded.

The trends observed using standard time intervals were repeated when each window was shifted by 0.5 seconds. Though individual values changed, the overall pattern remained consistent with the earlier part of the experiment. The important characteristics of these shifted time windows are summarized in the table below.

Comparing the features of the lines of best fit plotted for the standard windows and the shifted windows shows little difference between the two. As a result, we are confident that the trends observed are significant and worth further examination.

CONCLUSION
The relationship between action potential spikes, signal area, and local field potentials within the visual cortex of turtles is a complicated one. There are multiple reasons for a spike to occur, and the LFP is influenced both by correlated synaptic activity and neural input. These many variables present a challenge when attempting to further understand the nature of the spike-LFP correlation. However, using the Turtle Electrophysiology Project, python plug-ins such as Spikepy, and a unique analysis method, we have observed a pattern between action potentials and the signal area present in spontaneous recordings. The linear trends measured thus far from spontaneous recordings do little to explain the details of that correlation, only that it exists and it is worth studying further. For visual recordings, our methods need to be further modified in order to observe patterns in the spike-LFP correlation. Does the correlation still exist in response to a specific stimulus, or does one feature dominate over the other? How long after a controlled stimulus is there a maximum number of action potential spikes or the greatest amount of signal area?

<table>
<thead>
<tr>
<th>Searching Recording Collection Number</th>
<th>Linear Slope (mV/(s*spike number))</th>
<th>R (Linear Regression Constant)</th>
<th>R^2 (Linear Regression Constant Squared)</th>
</tr>
</thead>
<tbody>
<tr>
<td>26</td>
<td>0.00154</td>
<td>0.86630</td>
<td>0.75048</td>
</tr>
<tr>
<td>27</td>
<td>0.00177</td>
<td>0.81891</td>
<td>0.67061</td>
</tr>
<tr>
<td>28</td>
<td>0.00154</td>
<td>0.94552</td>
<td>0.89400</td>
</tr>
<tr>
<td>29</td>
<td>0.00157</td>
<td>0.95010</td>
<td>0.90269</td>
</tr>
<tr>
<td>31</td>
<td>0.00131</td>
<td>0.92762</td>
<td>0.86049</td>
</tr>
<tr>
<td>34</td>
<td>0.00136</td>
<td>0.92962</td>
<td>0.86420</td>
</tr>
<tr>
<td>Average Values (shifted)</td>
<td>0.00152</td>
<td>0.90635</td>
<td>0.82375</td>
</tr>
<tr>
<td>Average Values (standard)</td>
<td>0.00151</td>
<td>0.91099</td>
<td>0.83193</td>
</tr>
</tbody>
</table>

Table 2
The next step in our research is to analyze visual recordings, which result from specific and controlled stimuli. In these cases, simply dividing the recordings into one second intervals becomes less useful. For visual recordings, we hypothesize that the action potential and LFP area would be correlated to the movement of an object across a screen, a flash of light, or another similarly simple stimulus. As a result, we think it will be necessary to divide visual recordings into longer time intervals based on what kind of stimulation is occurring at that time. We will examine visual recordings that use a series of black dots moving across a white screen as a stimulus. We will divide each recording into four separate intervals: pre-stimulus, stimulus, inter-stimulus, and post-stimulus. The pre-stimulus category is the time interval that existed before any dots began moving, while the stimulus period is any interval of time during which a black dot was moving. The inter-stimulus period is defined as the time between moving dots, and the post-stimulus period is the time after which all dots have stopped moving for that recording. The spike number and signal area across each of these four periods is normalized to one second in order to make comparisons clearer. Therefore, we will output the number of spikes per second and the signal area per second for each type of stimulus interval, graph those normalized values, and examine any trends that occur.

Notes

