Using Extracellular Single-unit Electrophysiological Data as a Substrate for Investigative Laboratory Exercises

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Desirable objectives for laboratory-based science courses include fostering skills in problem solving and reasoning, enhancing data fluency, and encouraging consideration of science as an integrative enterprise. An effective means of reaching these objectives is to structure learning experiences around interesting problems in our own research. In this article, we explore the idea of using extracellular single-unit electrophysiological data as a substrate for student investigatory exercises as a means of achieving many of these objectives. In the article, we provide an overview of extracellular single-unit recording techniques and discuss the organization of single-unit data files. In addition, we describe a multi-week module recently administered in an intermediate-level laboratory course and provide suggestions both for more limited exercises and for more advanced projects. Finally, we describe a companion website that provides to instructors considering implementing similar exercises access to a variety of resources, including software, sample data, and additional information.

Key words: data fluency; neuroscience education, neurophysiology, problem-based learning.

As science educators, we share a core responsibility to prepare the next generation of scientists and consumers of science. There has long been interest in evaluating how we meet this challenge, and numerous reports (e.g., McCray et al., 2003) have stressed the benefits of adopting outcome-based strategies in developing science courses and curricula. What do we want our students to learn? And, how will we assess whether they have achieved the desired outcomes? Answers to these questions can guide decisions concerning the structure and content of our courses and curricula.

As the neurosciences have flourished in recent decades, interdisciplinary neuroscience programs have become part of the landscape at many institutions with strong undergraduate missions, and discussion concerning best educational practices has become increasingly visible within the neuroscience community. As we consider our teaching objectives, it is both useful and important to consider desired learning outcomes. While specific learning outcomes vary as a function of student needs, course content, and institutional mission, consensus has begun to develop around a set of desirable features for courses in undergraduate neuroscience curricula (e.g., Ramirez, 1997; Wiertelak, 2003). Our courses should both capture and generate interest in neuroscience and should promote understanding of scientific inquiry as a way of learning about the world. They should emphasize investigatory, inquiry-based exercises that allow students to test their conceptual knowledge and to develop skills in problem solving, reasoning, and arguing from evidence. Our courses should encourage students to consider the interdisciplinary nature of neuroscience and should encourage integrative thought that transcends traditional disciplinary boundaries. They should promote data fluency and the development of computational skills necessary for working with increasingly complex concepts and patterns of data. In addition, our courses should broaden exposure to research methodologies and should link those methodologies to solving real problems in both basic and applied contexts.

An effective means of reaching many of these objectives is to structure learning experiences around interesting problems in our own research. Here, we describe rationale and methods for using extracellular single-unit electrophysiological data in undergraduate laboratory-based courses. Although the collection of single-unit data may not be a reasonable endeavor for most students in undergraduate neuroscience laboratory courses, we argue that projects involving data analysis can be effectively implemented. A task that all scientists confront is to utilize data to advance meaningful ideas, and single-unit data can provide a substrate for students to learn about this creative process. Broadly speaking, the assignment is this: Use a train of action potentials to advance an interesting idea about how the nervous system represents and processes information.

In this paper we describe how single-unit data is recorded and how it is organized in a typical data file. We provide an example of a multi-week module suitable for intermediate-level laboratory courses and suggestions for more limited exercises and more advanced projects. In addition, we describe a recently implemented web site that provides instructor resources, including data files, links to analysis programs, and more detailed descriptions of analytic techniques (see “Companion website” section).

Extracellular single-unit electrophysiology

Single-unit electrophysiological recording techniques provide a unique and powerful window through which to observe the functioning brain. Single-unit recording involves sampling the activity of single neurons, or small clusters of neurons, using an array of microelectrodes implanted in the brain. When recordings are conducted during the performance of tasks that engage observable
sensory or behavioral processes, the contribution of the sampled cells to processing task-relevant information can be evaluated.

Perhaps the best-known studies using extracellular single-unit recording techniques to examine aspects of neural information processing in the mammalian brain were conducted by Hubel and Wiesel (described by Hubel, 1982). All serious students of neuroscience are familiar with how these and other early researchers mapped the functional organization of the visual system, demonstrating the relationship between receptive field properties and the laminar and columnar architecture of primary visual cortex. Indeed, single-unit recording has been integral to an enormous range of research aimed at examining how the nervous system represents and processes information. This research tackles such exciting issues as the neural representation of space (O'Keefe & Dostrovsky, 1971; Taube et al., 1990), working memory and executive function (Fuster & Alexander, 1971; Funahashi et al., 1989; Miller, 2000), and reward (Schultz, 2006). Even with the recent proliferation and enhancement of advanced functional imaging techniques, single-unit recording has remained the approach of choice where fine temporal and spatial resolution of neural signals is required during ongoing behavior.

Given the impact that single-unit recording research has had on our understanding of the nervous system, it is regrettable that most undergraduate students typically have little exposure to it beyond lecture hall discussion of sensory receptive fields. This situation is unfortunate, but understandable, especially at primarily undergraduate institutions, where resources are often too limited to initiate active electrophysiological research programs. Even at institutions with productive research laboratories, only a small number of undergraduate students actually have the opportunity to learn about single-unit recording first-hand. Given the substantial amount of time needed to acquire skills necessary for the collection, analysis, and interpretation of electrophysiological data, some investigators are reluctant to involve undergraduates in their research. Because it is not usually economically viable (nor, arguably, ethical) to equip teaching laboratories not linked to ongoing research programs with the latest single-unit recording equipment, most neuroscience students graduate with only casual appreciation of the role that this research has had in shaping our view of the nervous system.

**Single-unit data**

In order to make use of single-unit data in analysis exercises, students need to understand how it was collected and how it is organized in a data file (see Figure 1). Recordings are conducted with a microelectrode that permits one to monitor voltage in a small volume of neural tissue. Voltage varies across time, and the waveforms of these voltage deflections (or “spikes”) that exceed an experimenter-determined amplitude for a brief duration (typically <1 msec) are saved along with the time of their occurrence. Thus, raw single-unit data consists of a set of waveforms. These waveforms include action potentials, and waveforms that look annoyingly like action potentials (or “noise”).

Once data has been collected, waveform discrimination software can be used to visualize and plot in feature space various measured and derived waveform characteristics (for example, spike amplitude, spike width, etc.; Figure 1A). Waveforms with similar characteristics tend to form discrete clusters and can be isolated or “cut” (hence the term “cluster cutting”). If the characteristics of a given cluster are consistent with being generated by a single neuron, they are assigned to a “unit,” a putative distinct cell. In this way, spikes generated from several different cells can be isolated on a single microelectrode during a given recording session. Thus, the initial stages of manipulating single-unit data involve detecting and sorting spike waveforms and removing any “noise” from the signal.

After spikes have been sorted and assigned to units, data are typically reduced to an array of spike “timestamps” relative to the onset time of the recording session. In addition, various experimenter-controlled events (tones, rewards, etc.) or recorded behavioral events (lever presses, saccades, head positions, etc.) can be saved in similar fashion. Reduced single-unit data files, therefore, simply consist of a collection of single-unit and session event timestamp arrays (Figure 1B). Armed with these data files and knowledge of how they were collected, one can address a range of questions related to how the neural tissue at the microelectrode tip processed information relevant to the behavior in which the subject was engaged. Analyses can be conducted to explore patterns of behaviorally relevant activity exhibited by individual cells, cell pairs, and small clusters of cells.

**Laboratory-based exercises utilizing single-unit data**

A major goal of education in the sciences, indeed across the curriculum, is the promotion of data fluency. Fueled in part by advances in computing technology, today’s scientists and science consumers are confronted with increasingly complex forms of data. Single-unit data can serve as a substrate for the development of skills in assembling, understanding, and extracting meaning from large data sets. There are several features of spike data that make it interesting in terms of promoting data fluency. First, if recording is conducted for any appreciable amount of time, an individual data file can become very large. Most undergraduate students have had no exposure to working with such large data files. Second, although data files are large, those including processed data are relatively simply organized as a series of timestamps. Students can readily appreciate how data collected from a single unit is represented in a file and how individual files are scaled up as additional units or experimental events are added. Third, a distinguishing feature of single-unit data is combined spatial and temporal resolution that far
exceeds other types of behavioral neurophysiological data such as electroencephalography (EEG), magnetoencephalography (MEG), or functional neuroimaging (Churchland & Sejnowski, 1988). Thus, laboratory exercises that involve single-unit data provide a unique and concrete means for students to bridge levels of nervous system organization, from cellular to behavioral and cognitive levels.

In addition, such exercises provide an excellent means of promoting integrative thinking, as making sense of this data in reduced form often requires bringing together ideas from cognitive and behavioral psychology, neuroanatomy, neuropharmacology, neurophysiology, and computer science. These exercises can be used to introduce basic concepts in sensory or behavioral neurophysiology or as entry points to more advanced projects in computational neuroscience.

Here we provide an example of an exercise module carried out in an intermediate-level undergraduate neuroscience laboratory course. The project involved using neuronal data recorded from dorsolateral prefrontal cortex in two rhesus macaques (Macaca mulatta) trained to perform an oculomotor delayed-response (ODR) spatial working memory task. During performance of this task, prefrontal neurons often exhibit alterations in firing rate related to sensory, mnemonic, or motor processes (Funahashi et al., 1989; see Goldman-Rakic, 1996, for a review).

Materials
Four data files comprising sets of spike trains from nine neurons total were used. These files contained a subset of previously published data (Wang et al., 2004). Signals were recorded using a multi-barrel glass microelectrode: a single center barrel used for electrophysiological recording and surrounding barrels used for iontophoretic application of various receptor agonists and antagonists.

Waveform discrimination was performed on standard PCs using OfflineSorter software (OFS; Plexon, Inc., Dallas, TX). Histogram displays of neuronal firing rate and synchrony were generated using Neuroexplorer software (NEX; Nex Technologies, Littleton, MA). Links to these vendors are available on the companion website, along with links to alternative software freely available under GNU General Public License.

Context of the project
The project served as a substrate for laboratory-based activities during the first third of a semester-long course in cognitive neuroscience. This course had not been taught in previous semesters. The course enrolled 18 students, all of whom were in either their junior or senior year. Eleven students were neuroscience majors. The remaining students were either psychology or biology majors. All students had taken two prerequisite courses: an introductory neuroscience course with strong interdisciplinary themes and an introductory psychology course. Fourteen students had taken at least one lab class

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**Figure 1.** Single-unit data collection and data file organization. A, Voltage fluctuations are monitored with microelectrodes positioned in a neural region of interest. Voltage deflections (or “spikes”) that fall within experimenter-determined values for amplitude and width are saved, along with their time of occurrence (or “timestamp”) for further analysis. In this example, waveform “clusters” from the “green” and “red” units are shown on the right following “cluster cutting” isolation, and the spike waveforms of the “green” and “red” units are shown on the left. Note that the waveforms for the two units shown on the left differ significantly in some ways (e.g., the size of the waveform’s valley, or point of lowest voltage, and maximum voltage following the valley). It is these differences that cause the waveforms to form distinct clusters when the waveform characteristics of each spike is plotted as a single point in feature space (as shown on the right), and it is these clusters that represent the spiking activity of single units (i.e., neurons). B, The times of occurrence of spike waveforms are stored in unit timestamp arrays, and the time of occurrence of behavioral and paradigmatic events are stored in event timestamp arrays. Thus, each session data file is reduced to a simple collection of timestamp arrays. These data files form the substrate for investigative laboratory exercises. Screenshots from Offline Sorter (A) and Neuroexplorer (B) using data freely available from Plexon, Inc. (Dallas, TX), printed with permission.
meeting requirements for the neuroscience major: Cellular and Molecular Neuroscience, Cognitive Psychology, or Behavioral Neuroscience. Several students had taken Human Physiology or another relevant biology course.

Relevant classroom activities conducted during the assignment interval included reading and discussion of several chapters from a reader in cognitive neuroscience (Gazzaniga, 1999). In addition, students attended twice-weekly lectures on subjects including an introduction to major themes in cognitive neuroscience, cytoarchitecture of the neocortex, and basic anatomy and physiology of the visual system.

The project
During the first lab meeting, students were informed that they would be given raw electrophysiological data recorded from non-human primates performing a spatial working memory task and that they would be asked to evaluate an idea about the role of a particular class of receptors in modulating the activity of neurons engaged in the task. Prior to being given any further details, however, students engaged two hour-long laboratory-based exercises aimed at developing competence basic single-unit data analytic procedures.

The first exercise required students to perform spike separation techniques using OFS. This software is fully functional with sample data supplied by the vendor and can be freely downloaded from the vendor’s website (http://www.plexoninc.com). Instructions on how to use this software can be found on the companion website. OFS supports a number of spike separation techniques, including manual cluster-cutting in two- or (visually captivating) three-dimensional feature space, waveform-crossing, and several automated algorithms. Students were provided with the sample data file and were asked to use spike-sorting techniques (1) to determine the number of neurons recorded in the data file and (2) to estimate the number of spikes per neuron. The exercise required students to evaluate features of spike waveforms, to learn about data file structures, and to become familiar with and use raster and histogram displays. Students were encouraged to use several sorting methods, and in order to promote discussion on the effectiveness of each method, students worked in groups of two or three and used the same data file.

The second exercise, conducted one week later, required students to determine whether the neurons in the data file exhibited an alteration in firing rate in relation to a repeated behavioral event. In order to evaluate this idea, students generated standard peri-event firing rate histograms using NEX. As with OFS, NEX is fully functional with sample data, and both software and data can be freely downloaded from the vendor’s website (http://www.neuroexplorer.com). When students were comfortable using the software and had successfully generated several peri-event histograms, they were asked to generate cross-correlation histograms to examine the incidence of synchronous firing between simultaneously recorded pairs of neurons. Both peri-event and cross-correlation techniques have been used widely in the behavioral neurophysiology literature, and both are described in detail on the companion website (see Perkel, et al., 1967a,b, for more thorough consideration of these techniques).

Importantly, during the weekly lab meetings, students evaluated background literature related to the role of prefrontal cortex in performance of the behavioral task through student-lead discussion of several relevant articles (Funahashi et al., 1989; Constantinidis et al., 2001; Wang et al., 2004). Thus, in addition to acquiring skills in analyzing single-unit data, students developed an understanding of the experimental context in which the techniques have been applied.

Following the second laboratory meeting, students were given access to the four primate data files and were asked to use them to evaluate the following proposal: Cholinergic muscarinic mechanisms play a role in maintaining information in spatial working memory. Students were told that methoctramine (a relatively selective M₂-like muscarinic receptor antagonist), had been iontophotically applied mid-way through recording sessions in which the task had been performed. (Students were informed that the actual drug applied was not a muscarinic antagonist; the drug was not revealed for proprietary reasons.) Several relevant resources were made available to students, including a review of muscarinic receptor-mediated signaling mechanisms (Cualfield & Birdsall, 1998). Students were given two weeks to analyze the data and to prepare a lab report detailing their findings and conclusions.

Project evaluation
Students submitted comprehensive reports that required them both to master practical lab skills in working with unit data and to consider their analyses within the context of published research. The quality of student projects was quite high. All students were able to sort waveforms successfully and to generate firing rate histograms that showed the neuronal activity patterns of their cells. Following the two practice labs, all students could explain how to use OFS and NEX to work with the sample data, and all could produce accurate firing rate rasters and histograms from raw, unsorted data (Figure 2). Students reported that having an opportunity to practice using software with sample data files before being given actual data was useful.

Although students were able to conduct isolated analyses quite easily, most encountered difficulty in interpreting their results and in preparing their reports. There were two characteristic problems. First, most students generated relatively weak hypotheses. Signaling mechanisms of muscarinic receptors had not been explicitly discussed in class, and students mentioned that it would have been useful to generate a list of relevant questions and testable hypotheses as a group before considering the data. In fact, with any unit data, a range of questions could be asked – some more easily addressed than others. It would be advisable to encourage students
to consider simple questions first (“How many cells are in the file?”, “How many appear to fire in relation to a measured behavioral response?”) and to ask more complicated questions after these initial questions have been answered.

Second, although all students knew how to conduct the appropriate analyses, a number failed to present their analyses in a concise, organized and appropriate fashion. For example, one report included several pages of graphs and tables for each cell in the sample. Another report included only a raster display for a single cell during a single trial. Although most reports were organized well, more time could have been spent during lab meetings discussing how to select and present appropriate analyses that support drawn conclusions. In future iterations of this exercise, students will be encouraged to consider more carefully the rationale for the selection of particular analytic tools and figures in published background readings.

CONCLUSIONS

We have outlined an exercise designed to introduce undergraduate neuroscience students to extracellular single-unit electrophysiology. As this exercise and similar exercises require analysis but not acquisition of single-unit data, they can be readily implemented in a broad range of settings. Such exercises can be used to meet a variety of pedagogical objectives, including fostering interest in scientific inquiry, promoting integrative thought across traditional disciplinary boundaries, and enhancing data fluency. The use of single-unit data can expose students to methods of inquiry and a world of knowledge not traditionally tapped in undergraduate laboratory courses, and knowledge and skills gained by students can enrich their perspectives of the functioning brain. Given the remarkable extent to which single-unit data has increased our understanding of neural processes to date, we believe students will appreciate, and be excited by, this opportunity to learn about this important approach, in particular, and neural processing, in general.

COMPANION WEBSITE

http://www.macalester.edu/nrp/

To provide support to instructors interested in exploring the use of single-unit data in laboratory courses, we have established a companion website. A number of data files along with descriptions of the studies for which they were collected are currently available on the website for free download and more will be posted as they become available. Student-generated descriptions of recording procedures and analytic tools are also posted.

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