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Pandemic Teaching: Using the Allen Cell Types Database for Final Semester Projects in an Undergraduate Neurophysiology Lab Course

Yi-Yun Ho[1], Andrea Roeser[1], Gwenda Law[2], and Bruce R. Johnson[1]
[1]Department of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853; [2]Department of Biomedical Engineering, Cornell University, Ithaca, NY 14853.

We designed a final semester research project that allowed students to apply the electrophysiological concepts they learned in a lab course to propose and answer experimental questions without access to laboratory equipment. We created the activity based on lesson plans from Ashley Juavinett and the Allen Institute for Brain Science (AIBS) Allen SDK online examples. An interactive graphic interface was added for students to explore and easily quantify subtle neuronal voltage changes. Before starting the final project, students had experience with conventional extracellular and intracellular recording techniques to record and analyze extracellular action potential firing patterns and intracellular resting, action, and synaptic potentials. They demonstrated their understanding of neural signal transmission in required lab reports using data they gathered before the pandemic shutdown. After students left campus, they continued to analyze data and write lab reports focused on neuronal excitability in snail and fly neurons with data supplied by the instructors. For their final project, students were challenged to answer questions addressing neuronal excitability at both the single neuron and neuronal population level by analyzing and interpreting the open-access, patch clamp recording data from the Allen Cell Types Database using code we provided (Python/Jupyter Notebook). This virtual final semester project allowed students to ask real-world medical and scientific questions from "start to end". Through this project, students developed skills to navigate an extensive online database and gained experience with coding-based data analysis. They chose neuronal populations from human and mouse brains to compare passive properties and neuronal excitability between and within brain areas and across different species and disease states. Additionally, students learned to do simple manipulations of Python code, work remotely in teams, and polish their written scientific presentation skills. This activity could complement other remote learning options such as neuronal simulations. Few online sources offer such a wealth of neuroscience data that students can use for class assignments, and even for research and keystone projects. The activity extends the traditional material often taught in upper-level neuroscience courses, with or without a laboratory section, providing a deeper understanding of the range of excitability properties that neurons express.

Key words: electrophysiology, coding, open access database, Allen Institute for Brain Science, Allen Cell Types Database, Python, Jupyter Notebooks

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The COVID-19 pandemic outbreak in March 2020 caused normal life to halt. Schools and universities closed their campuses and courses were abruptly hosted online. These events not only had a huge impact on people's mental health and personal finances but also completely changed the way education was conducted (Akyildiz, 2020). Moving any course from in-person to online is daunting, however it is especially challenging to convert lab courses to virtual and still maintain the features of inquiry driven exploration (Hanzlick-Burton et al., 2020). Neuronal simulation programs can help teach basic neurophysiological concepts to students in an online, interactive way (Meir, 2021; Heitler, 2021), but without access to lab equipment, students lose many opportunities to understand how science works. This paper demonstrates how we designed an investigative research project for student groups in a lab course without access to lab equipment. We used an online database to offer the students a virtual taste of scientific experimentation and the opportunity to analyze and interpret experimental data.

Although this project was designed as a virtual alternative to a lab-based research project, it is still a gateway to teach data analysis and computational thinking, and a good introduction to online open access resources. Compared to many coding-based assignments, this project teaches data analysis with a focus on answering research questions and completing a research project. Thus, we focused less on coding skills and more on understanding the data through visualization, forming hypotheses from observations and verifying them through data analysis and interpretation, and supporting the findings with relevant research literature.

This project was designed as a final lab group project in a neurophysiology lab class (BioNB/BME/ECE 4910, Principles of Neurophysiology) in the spring semester of 2020 at Cornell University (Ithaca, New York, United States). This lab course is an advanced level undergraduate and beginning graduate neuroscience course. Students were expected to have previously taken or be concurrently enrolled in an introductory neuroscience course such as "Introduction to Neuroscience" (BioNB 2220 at Cornell University). There were 27 matriculated students and two graduate student auditors: three fully enrolled students were graduate students, fourteen were seniors, eight were

- **Allen Cell Types Website Interface:**
  - **Part I**: Getting familiar with Allen Cell Types Database (single student exercise)
- **Jupyter Notebook Interface** (lab group exercises)**:**
  - **Part II**: Measuring intrinsic electrical properties with an interactive graphic interface
  - **Part III**: Acquiring parameters of individual neurons with the *EphysSweepFeatureExtractor* from Allen SDK through entering cell IDs and injection current IDs, and editing variables
  - **Part IV**: Comparing electrophysiological parameters of different neuronal populations

Table 1. Outline of the Final Project. The final project was composed of four parts and required working with two different interfaces. The instruction and code are included in the first part of Supplementary Material and can be downloaded from https://github.com/yi-yun-ho/Pandemic-neurophysiology-teaching-with-the-Allen-Cell-Types-Dataset.git.

juniors, and two were sophomores. Sixteen enrolled students were biological science and related majors and eleven were engineering or physics majors. Before the course was interrupted by the pandemic and students left campus in mid-March 2020, students gained some proficiency with intercellular and extracellular recording techniques to explore neuronal excitability and synaptic transmission in non-vertebrate model systems (crayfish, snails, fruit flies and the algae *Chara*) (Johnson et al., 2014; Wyttenbach et al., 2014). If classes had remained in-person, the rest of the semester's labs included Crawdad lab manual exercises to examine action potentials in Chara, measure passive membrane properties, excitability and neuromodulation of excitability in snail neurons (Wyttenbach et al., 2014), and exercises examining optogenetic activation of behavior and synaptic transmission in fly larvae (Pulver et al. 2011; Vilinsky et. al 2018). To compensate for the students not being on campus to collect their own data for the remaining labs, the instructors provided data for the students to write their lab reports. The data found online from Allen Institute for Brain Science (AIBS) that was used for the final project most closely resembled the intracellular snail neuron data.

Normally as a final semester project for the class, small teams of students design lab experiments and work together to examine underlying mechanisms of neuronal activity. With the class online during final project time, we redesigned the last assignment to focus on data analysis, interpretation, and presentation skills using open-source data. Therefore, this activity can also teach sophisticated data analysis, computational thinking, and utilizing online databases for research projects in in-person courses. Considering accessibility and relevancy, we used the Allen Cell Types Database from the Allen Institute for Brain Science (AIBS) (Allen Institute for Brain Science, 2015a; Casimo, 2021) because this open database contains abundant whole cell patch clamp recordings (2333 recordings) from mice and

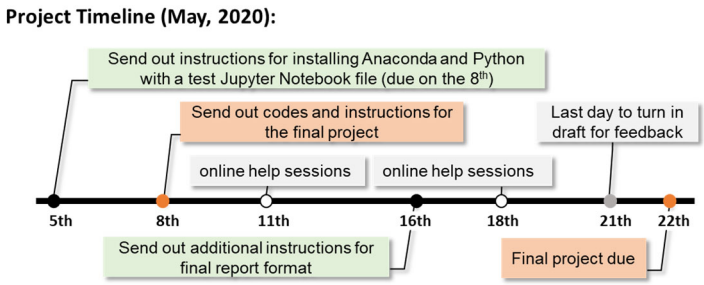

Figure 1. May 2020 Final Project Timeline. Key events of the implementation are shown. Orange shading: Important deadlines; Green shading: Material delivery dates; Light grey shading: Help sessions.

human neurons. The recordings are from different cell types, as well as from various brain areas, cortical layers and disease conditions. The numerous ways to categorize the cell types allowed students to compare electrophysiological properties of neurons from different types that interested them. This database can be accessed easily through the Allen Cell Types Database website and by programming with the Allen Software Development Kit (Allen SDK), which provides a free and easy gateway to analyze data using Python code.

As an open-source database which contains abundant high-quality datasets, AIBS has been used by many educators for designing lessons and assignments for students ranging from high school to graduate students (Allen Institute, 2021; Chu et al., 2015; Gilbert, 2018; Goller and Casimo, 2020; Grisham, 2009; Grisham et al., 2010, 2012, 2017; Jenks, 2009; Juavinett, 2020a, 2020b; Ramos et al., 2007; Ryan and Casimo, 2021; Shelden et al., 2019). Many lessons utilize datasets from the Allen Brain Atlas to examine neuronal morphology and patterns of genetic expression. Most focus on transcriptome datasets, and only very few use the electrophysiology data (Juavinett, 2020a, 2020b). It is worth noting that the Allen Cell Types Database has also been used by many researchers for scientific publications (Billeh et al., 2020; Huang et al., 2021; Kalmbach et al., 2018; Mosher et al., 2020; Nandi et al., 2020; Schneider-Mizell et al., 2020). Thus, it opens the possibility for advanced students to work beyond the assignments and publish novel research findings, such as through a keystone project.

The AIBS electrophysiology dataset was especially appropriate for the final projects of our course because the mechanisms and the variability of neuronal excitability are main themes of the class.

The class learning objectives of this online, final research project were:

- Students will consolidate their understanding of intracellular electrophysiological recording techniques.
- Students will strengthen their understanding of neuronal excitability parameters and recognize that principles of neuronal excitability apply to all animals.
- Students will ask and answer real-world medical and scientific questions through a virtual taste of a "start-to-end" research project.

Figure 2. Screenshot of Allen Cell Types Database Website (http://celltypes.brain-map.org/data). After selecting a neuron and clicking "Electrophysiology" on the lower left of the neuron block in the front page of the website, the patch clamp recording and morphology of the selected neuron are displayed. Different sweep numbers indicating different injection currents can be selected and the corresponding recording trace is displayed.

- Students will be introduced to and practice working with an online open-access database and simple computer programming (Python).
- Students will demonstrate their ability to write a rigorous scientific report as a final project paper.

The in-person learning objectives missing from a virtual project are similar to those of online simulations. These include learning to work in in-person teams, learning practical skills such as instrumentation use, preparation dissections, how to set up, conduct, and trouble-shoot experiments, realizing that real experiments do not always produce explainable results, and coping with biological variability (Heitler, 2021). However, through this virtual project, our students gained the opportunity to study vertebrate data (mice and human) they could not record in class, and to analyze large datasets for the statistical power to draw stronger conclusions in their final reports. In addition, practicing advanced data analysis and basic coding skills were important components of this project.

METHODS

General Programming Note

Considering some students lacked previous programming experience, we ran the Python program on a Jupyter Notebook (https://jupyter.org/). This platform supports a simple layout and an intuitive code running interface where results are displayed adjacent to the code. We provided the code and instructions (Supplementary Material part 1). This can also be downloaded from https://github.com/yi-yun-ho/Pandemic-neurophysiology-teaching-with-the-Allen-Cell-Types-Dataset.git. The code is adapted partially from AIBS Allen SDK (2015) online examples and from lesson

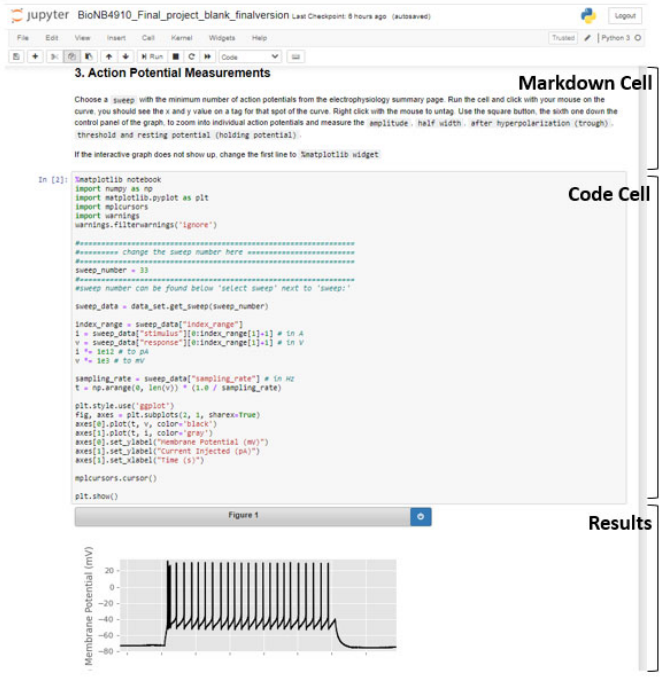

Figure 3. Layout of Jupyter Notebook Interface. Using our final project file as an example, Jupyter Notebook supports markdown cells for writing instructions and code cells for writing code for implementation. After running the code cell, a result is displayed immediately after the code cell. Code was modified from Allen SDK examples (2015).

plans created by Dr. Ashley Juavinett (Juavinett, 2020a; https://sites.google.com/ucsd.edu/neuroedu/). To ensure students of all coding levels could run the code, they were only asked to fill in or replace parameters such as cell ID, input current ID (or sweep ID as used by Allen Cell Types Database), or electrophysiological parameters.

To bridge the students' pre-pandemic data analysis experience from using a graphic interface in LabChart software (ADInstruments, Dunedin, New Zealand) to data analysis using Python code, we introduced a similar interactive graphic interface for students on a Jupyter Notebook. This allowed them to zoom into a graph and move the cursor to a point of interest to display the x and y coordinates simply by controlling their mouse. We considered that the previous familiarity with this data analysis method would help reduce any anxiety for students using Python code for the first time. This feature not only provides an intuitive way of measurement, but it also allows students to explore electrophysiological features that are subtle and thus difficult to quantify and extract through code, such as voltage sag and post-inhibitory rebound. In addition, this quantification method requires students to know important voltage locations for measurement. For example, they must discern the two cursor placements to properly measure action potential width, and where in the voltage response to a current to measure voltage changes to compute input resistance and the membrane time constant. This further strengthened students' understanding of electrophysiological properties by focusing their attention on key excitability properties of the neuron.

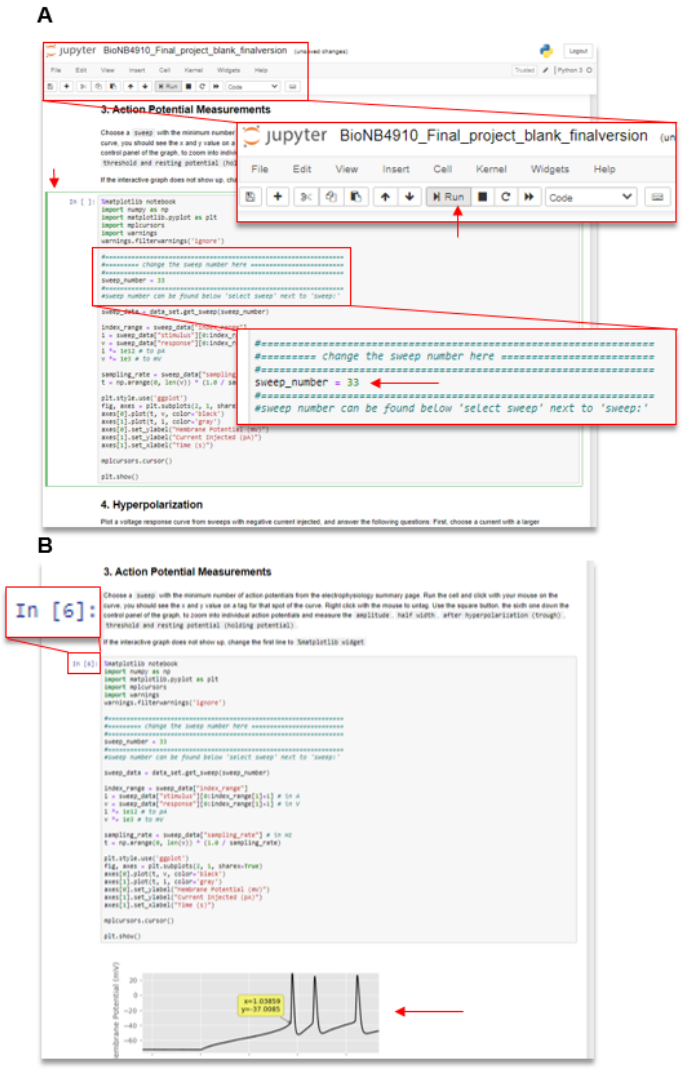

Figure 4. Steps of Running Jupyter Notebook. *(A)* Students first typed in sweep number for the trace they chose (the middle red box), then clicked on the selected code cell (cell was marked by green edging after clicking), and finally hit the "Run" button in a tool bar on the top (the top red box). *(B)* After running, a number appears in the blank brackets next to the code cell (as shown in the red box) and the results are displayed after the code cell (as indicated by the red arrow).

Organization of the Project

The final project contained four progressive parts (Table 1). The complete instructions and the code are included in the first part of the Supplementary Material. The first part was designed to familiarize students with the Allen Cell Types Database (2015) website and ensure both Python and Jupyter Notebooks were correctly installed. Although students worked in small groups, we first asked them to individually use a provided Jupyter Notebook file to plot a neuron's action potential frequency-to-injected-current curve (f-I curve) by manually entering the corresponding firing rate to input currents shown on the Allen Cell Types Database (2015) website. In the second part, students measured action potential parameters and passive membrane properties from single neurons with the

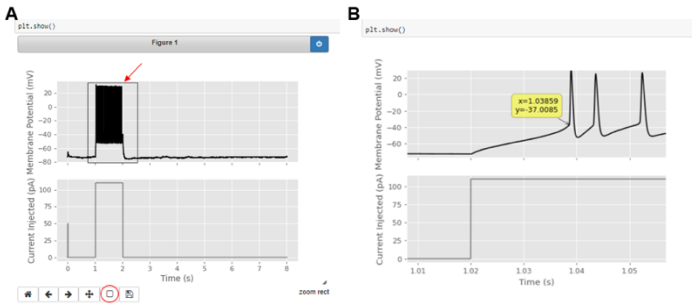

Figure 5. Demonstration of the Interactive Graphic Interface. *(A)* Using Matplotlib backend %matplotlib notebook or %matplotlib widge, an interactive graph was created with a tool bar for manipulating the graph underneath. The square button in the tool bar below the graph (red circle) allows students to zoom-in to a region on the graph (red arrow). *(B)* This figure shows how an interactive cursor (mplcursors.cursor()) works in Jupyter Notebook. When clicking on the graph generated by the code, a yellow tag appears displaying the x, y values of the point.

interactive graphic interface. They compared these results with averaged population parameters calculated automatically by the AIBS provided code and by the Allen SDK "EphysSweepFeatureExtractor" module in the third part of the project. Finally, students compared firing and membrane properties of different neuronal populations by visualizing the distribution of data with adapted Python code (Juavinett, 2020a, 2020b) and with statistical tests.

The 27 students formed 12 groups, each group containing 2-3 students. They were encouraged to form a cross-disciplinary group mixing Biology with Engineering and Physics students.

For parts I, II and III: groups characterized the neuronal properties of different single neurons (one for each group member) of different types, subtypes, or with an identified human disease. For part IV: groups chose different cell populations (one for each group member) to compare. For example, human cells with epilepsy vs. tumors, mouse neurons from different brain regions, aspiny vs. spiny neurons, or neurons with different neurotransmitters. They were required to turn in their report in a Journal of Undergraduate Neuroscience Education (JUNE) template with proper citations and references.

Project Timeline

On May 5, 2020, we sent students a test file containing part I of the final project and instructions for installing Anaconda and Python (Figure 1). Anaconda is a distribution of the Python programming language. With Anaconda, many commonly-used Python packages and Jupyter Notebook are downloaded automatically so students do not have to install required packages or libraries separately. The test file familiarized students with the AIBS website and ensured the correct installation of Python and Jupyter Notebooks. Each student was required to individually submit the f-I plot produced by the code before the group received the instructions and codes to fully implement the final project, thus helping ensure every student in a group could contribute.

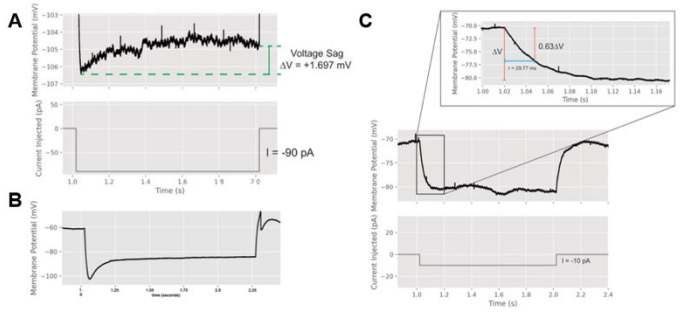

Figure 6. Characterizing the Voltage Responses to Hyperpolarization. Students used a large current injection (30mV change) to observe any voltage sag (*A*, upper graph) and post-inhibitory rebound *(B)*, and a small current injection (5-10mV change) to measure the time constant (C, upper graph). The lower graphs in (A) and in *(C)* show the current injected. Adapted from students' final report with permission.

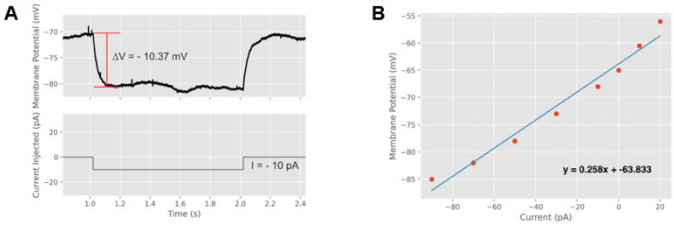

Figure 7. Students Demonstrated Two Methods for Measuring Input Resistance. Students compared the input resistance measured with two methods: (A) measuring the voltage change under a small hyperpolarizing current injection and (B) calculating the slope of a V-I curve. Adapted from students' final report with permission.

The code and instructions for project parts II to IV were sent out May 8th, 2020 to each lab group (Figure 1). Students had two weeks to complete the project and write a group report. During this two-week period, we held two virtual help sessions by video conference to assist with technical issues and provide advice and feedback on the research directions. We sent out additional instructions on the report format and contents on May 16th, 2020 and provided an optional review opportunity for students to turn in a draft of their report for feedback before the final report deadline (Figure 1).

Unfortunately, the short period to design and implement the final semester project did not allow time for the Institutional Review Board for Human Participant Research Office (IRB) at Cornell University to consider an exemption of student assessment from full IRB protocols.

Details of Project Design

Part I

The first part of the project was designed to familiarize students with the Allen Cell Types Database website (http://celltypes.brain-map.org/data, Figure 2) and Jupyter Notebook (Figure 3). The main purpose of part I was to ensure each student could successfully navigate the Allen Cell Types Database website and locate the information needed. Students gained a sense of the rich scope of data available in the Allen Cell Types Database, which helped them develop an interesting research question to explore for the final project.

Students were asked to individually explore the AIBS website, select either a mouse or human neuron of interest, and acquire the corresponding firing rate to various input currents for that neuron through selecting a stimulation sweep number, which corresponds to the amplitude of the stimulus current (Figure 2). Once a sweep number was selected, data from a whole cell patch clamp recording showed the voltage response of that neuron to an input current. Then, the student manually filled in the corresponding firing rate for each input current acquired from AIBS website into the provided Jupyter Notebook file, which allowed them to plot the neuron's f-I curve.

Although students can access and explore many features through the AIBS website interface, more detailed quantification of the patch clamp data cannot be easily implemented without some programming. Here, we used simple coding as a gateway to introduce more advanced data analysis skills. With programming skills, quantification of a large dataset, running statistical analysis, and even modeling can be implemented easily. Learning data analysis and coding skills prepares students for both academic and industry jobs.

We used Jupyter Notebook as the coding interface due to some key advantages. First, it is free. Second, it supports the free programming language Python, which is a widely-used programming language and what Allen SDK supports. Therefore, Jupyter Notebook allowed us to easily adapt the packages and example codes Allen SDK provides to access AIBS data. Third, the interface is intuitive for students without coding experience (Figure 3) through supporting markdown cells where instructors provide directions preceding the code and displaying results immediately after the code cell. This design allows students to relate the instructions to the code and the code to the results.

To run Jupyter Notebook code, students simply click on the code cell and then click the "run" button on the tool bar (Figure 4A, top). In parts II and III of the final project, students were required to enter parameters before running some of the code. For example, a cell ID or an injected current ID (sweep number) (Figure 4A, middle). The edges of the code cell turn green after clicking. Then after successfully running, a number will show up in the blank brackets left of the code cell indicating the completion of code running and a result will be displayed immediately below (Figure 4B). In this example, a firing trace in response to a stimulation current is displayed as a result of code running.

Part II

The second part of the project used an interactive graphic interface to bridge students' experiences in examining the data acquired in the teaching lab through software with analyzing virtual dataset through code. One of the difficulties in data analysis with code is that measurement can be abstract without graphic display, and it can be hard to verify whether the code does what we ask. So, in part II, we introduced the interactive graphic interface on Jupyter Notebook (Figure 4B; Figure 5B)

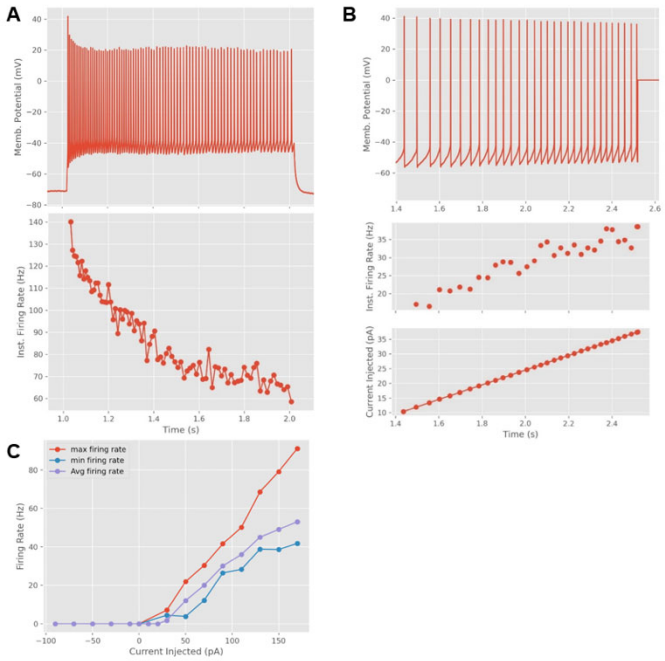

Figure 8. Measuring Instantaneous Firing Rate. Using the Allen SDK (2015) EphysSweepFeatureExtractor to extract time points when action potentials occur, students calculated the instantaneous and average firing rate. This allowed students to observe spike frequency accommodation (*A*, upper: a firing trace in response to long square pulse; lower: corresponding instantaneous firing rate), characterize the firing properties with ramp injection (*B*, upper: a firing trace in response to a ramp current; middle: corresponding instantaneous firing rate to the ramp; lower: the amplitude of injected ramp current), and *(C)* plot an f-I curve to square pulses of different amplitudes. Adapted from students' final report with permission.

allowing students to zoom into the details of a voltage response and characterize action potentials and passive membrane properties quantitively. Students could make sense of quantitated voltage measurements through measuring a voltage response graphically in this intermediate stage before jumping into automatic extraction of time points and parameters with code. The interactive graphic interface is enabled under Matplotlib backend %matplotlib notebook or %matplotlib widge (Hunter, 2007) (Figure 5A). This allowed students to move around the graph and zoom into a region of interest. The interactive cursor is created by the function "mplcursors.cursor( )" (Lee, 2016) (Figure 4B; Figure 5B). By clicking a point of the graph, the x and y coordinates are displayed (Figure 4B; Figure 5B) and through using these interactive features, each group measured and compared action potential parameters (threshold, peak amplitude, trough, and width) and passive membrane properties (time constant, input resistance, and rheobase) of two to three single neurons (Examples in Figures 5B, 6, and 7). Students were also asked to characterize a neuron by its voltage response to a large hyperpolarization current (30 mV change) by describing any voltage sag (Figure 6A) and/or post-inhibitory rebound (Figure 6B), and by measuring the time constant (Figure 6C) and input resistance (Figure 7A) under a small hyperpolarization current (5-10mV change). This is an example where subtle properties such as voltage sag and post-inhibitory rebound can be difficult to quantify and extract through code but can be relatively easy to measure with an interactive graphic interface. Input resistance was measured once again by calculating the slope of a straight line fitted to a peak-voltage-to-injected-current curve (V-I curve) (Figure 7B). Students were asked to compare and explain the differences between input resistances measured with these two methods in the paper's Discussion section. Students also compared the rheobase measured from step currents and from a ramp current. This challenges students to recognize and think how different measuring or analysis methods can lead to different results.

	Peak	Average Spike Width	Trough	Threshold	Resting Potential
Measurements with a cursor	34.28 mV	.96 ms	-57.5 mV	-48.6 mV	-73.5 mV
Measurements with EphysSweepFeatureExtractor	32.2 mV	.87 ms	-59.7 mV	-42.5 mV	--

Table 2. Verification of Measured Action Potential Parameters. Students compared the parameters of action potentials from a single neuron measured with an interactive graphic interface in part II (shown are parameters from few action potentials students measured) and the mean values calculated by the EphysSweepFeatureExtractor module in part III (mean values of all action potentials generated by the specified neuron by current injections). Adapted from students' final report with permission.

Part III

The interactive graphic interface in part II allowed students to explore and measure the electrophysiological features of single neurons with minimal coding skills, however, quantifying features that require multiple measurements such as spike frequency accommodation can be time-consuming without automatic extraction. By part III, students were expected to be more comfortable with coding after the interactive measurement in part II. Therefore, we introduced the Allen SDK "EphysSweepFeatureExtractor" module, which automatically extracts parameters of electrophysiological properties. This opens the possibility for students to explore more advanced data analysis such as averaging across multiple measurements and neurons, running statistical analysis, and even grouping neurons based on their electrophysiological features. With the time points of action potentials extracted by the module, students calculated the instantaneous firing rate of a voltage response, then plotted the instantaneous firing rate against the largest injected step current, and lastly observed the spike frequency accommodation. The voltage response to the injected current is seen in Figure 8A top, where the interval between action potentials increases over time, with the corresponding decay in firing frequency shown in 8A bottom. Students also observed that in response to the rising phase of a depolarizing ramp current injection (firing to ramp shown in Figure 8B top), the instantaneous firing rate (Figure 8B, middle) to the ramp current injection (Figure 8B, bottom) increases. The ramp current injection experiment and the displayed instantaneous firing rate allowed students to observe the increase in firing rate to an

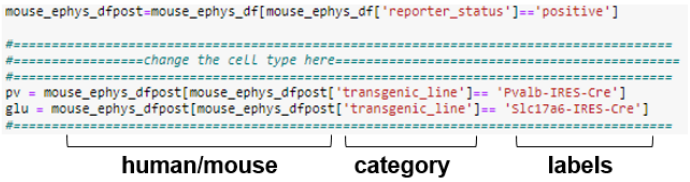

Figure 9. Screenshot of the Code in part IV where editing specific variables was required. Students selected the groups of neurons to analyze through editing these three segments of the code shown in the screenshot: human/mouse, category, and labels. They altered these three segments to specify neurons of different cell types, disease states, cortical layers, dendritic types, or regions of the brain.

increasing current (Figure 8B). They were also asked to describe whether there is a non-linear subthreshold voltage response to a ramp current, indicating activation of subthreshold "booster" currents (McCormick, 2014). Lastly, students generated a f-I curve using the maximum, the minimum, and average firing rate under different amplitudes of long square current injections using code (Figure 8C) and compared it to the f-I curve they acquired manually through the AIBS website in part I of the report. Besides plotting instantaneous firing rate, students also compared the parameters extracted by the EphysSweepFeatureExtractor module to parameters they measured with the interactive graphic interface in part II (Table 2). Students could only measure one action potential at a time in part II, while the parameters given by the EphysSweepFeatureExtractor module are the average values from all action potentials generated by a given current injection.

Part IV

In parts I to III, students measured the electrophysiological properties of individual neurons. It is necessary, however, to characterize features from multiple neurons within a population to address the differences between populations in a statistically meaningful way. In part IV, students compared electrophysiological characteristics between two or more populations of neurons through editing provided code (Figure 9). Students were provided with three example codes, which demonstrated extraction of neuronal groups by their cell or dendritic types, and instructed to plot the distributions of an electrophysiological property on a histogram (Figure 10). The code for this part was adapted from Juavinett (2020a, b). Based on their research interests, students compared properties of brain neurons from: humans to mice; aspiny neurons to spiny neurons; neurons of different cell types, cortical layers, or brain regions; or neurons from human patients with different diseases (examples of neuronal population comparisons student made are shown in Supplementary Material, part 2). It is not clear from the electrophysiological documentation for the Allen Cell Types Dataset if recorded brain neurons were from a tumor or focal point of an epileptic seizure, or from surrounding or covering brain tissue in these areas. Students were asked to use appropriate statistical tests to address the significance of the differences and to explain the ionic mechanisms causing these differences in the

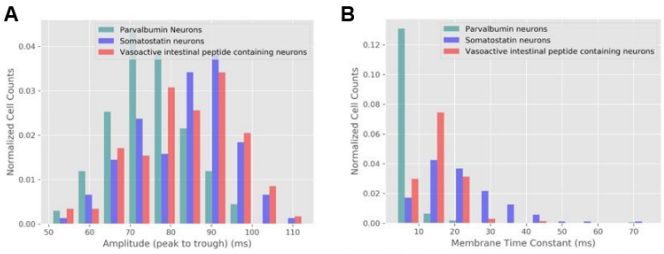

Figure 10. Visualization of Differences in Populations. A student group compared action potential amplitudes *(A)* and membrane time constants I of three major types of inhibitory neurons, parvalbumin neurons, somatostatin neurons, and vasoactive intestinal peptide containing neurons (green, blue, and red). They made these plots by editing code variables and plotting the histogram to visualize the variation found across each electrophysiological property within the population and to compare the range of distribution between each population with code adapted from Juavinett (2020a, 2020b). Adapted from students' final report with permission.

Discussion section of their paper. Students then compared the population results to the results from their single neurons measured in parts I to III. If available on the Allen Cell Types Database, we asked students to present a screenshot of the studied neurons' morphology. Through comparing the results from single neurons to the entire population, we hoped students could appreciate the diversity and variation of neuronal properties within and between defined populations.

Scientific Writing

In addition to measurements and analysis of individual neurons and populations of neurons, each group of students was asked to team-write their final project in a Journal of Neuroscience Education (JUNE) article format, including the usual paper sections and proper journal-specific reference formatting (See part 3 of Supplementary Material for instructions on report writing and part 4 of Supplementary Material for the instructor scoring rubric for final paper grading). The rubric was shared with students before their papers were due. The final paper was worth 30% of the total semester grade. Other class assignments made up the following final grade percentages: lab reports from in-person data gathering and data from instructors (35%); midterm paper, which was an extended revision of a lab report (20%); and short problem sets and neuronal simulation assignments using Neurosim (Heitler, 2021) (15%). The final semester paper and earlier course experience accomplished one of the main objectives of the lab course: for students to become proficient in writing a scientific report. They trained for this by writing several group lab reports throughout the semester and an individual midterm paper in this style.

In the Results section of their final semester project, students were asked to present their measurements and compare electrophysiological properties between individual neurons in parts I, II and III, and between neuronal populations in part IV, including proper statistical tests. In the Discussion section, in addition to explaining the differences and similarities between single neurons and

Exemplary discussion 1 from a student group:
"The fast-spiking PV interneurons have specific types of voltage-gated channels including the voltage-gated potassium channels (Kv3) that allow for the characteristics of rapid firing of action potentials (Bischop et al., 2012). These include small AP widths as in Figure 45, small time constants as in Figure 47, small adaptation ratios as in Figure 49 for PV-expressing interneurons"

Exemplary discussion 2 from a student group:
"We found that the aspiny neurons had a significantly shorter average inter-spike interval than the spiny neurons, but that the aspiny neurons have a larger spike frequency adaptation index, meaning that they undergo a stronger form of spike frequency adaptation than the spiny neurons. The differences in spike frequency adaptation could be due to a difference in the quantity or type of calcium-activated potassium (or chloride) channels, which are responsible for mediating spike frequency adaptation (Ha and Cheong, 2017).'

Exemplary discussion 3 from a student group:
"Using non-diseased cortical neurons as a source of comparison, the average input resistance found in literature was approximately 38 MOhms (Beaulieu-Laroche et al., 2018)."

Box 1. Exemplary Discussion Examples from Final Student Papers. Student groups used ionic based mechanisms to explain the differences between electrophysiological properties in neuronal populations (examples 1 and 2). In example 3, a student group compared the input resistance they measured (~300 MOhms) to that in the literature. Differences in recording quality, cell type and size, and open rest channels could account for the difference from similar research measurements mentioned in the quote. Adapted from students' final report with permission.

populations by known ionic mechanisms, they were asked to compare their results to what is known in the literature about the excitability properties of the neuron types they examined, and the ionic currents underlying these properties (McCormick, 2014). This challenged students' ability to explain the results and digest literature and gave students a taste of how a research project is completed as real-world scientists.

RESULTS

In the spring 2020 class, all (27/27) students successfully generated a f-I plot in part I by the first deadline and all 12 groups turned in their reports by the last deadline (Figure 1). In their final reports, every group was able to measure the parameters with the provided code and to edit the code in part IV to generate histograms showing the electrophysiological properties of the different populations they studied (example shown in Figure 10). Most groups (8/12) recognized and discussed the variation and diversity of electrophysiological properties within a population defined by species, cell type, disease state, or cortical layer. In terms of interpreting the results, all groups

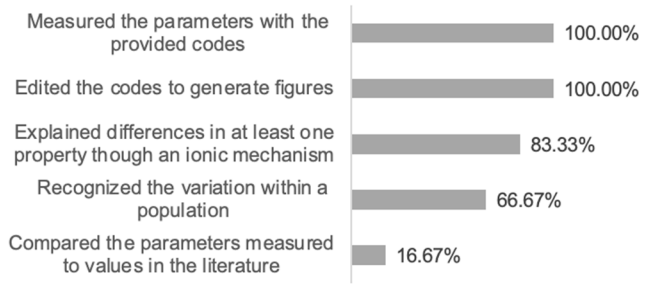

Figure 11. Student Performance on Final Projects Based on Final Group Reports. Numbers show the percentage of complete project outcomes out of total number of groups (12).

described the assigned neuronal excitability parameters, and most groups (10/12) explained differences between neuron types in at least one electrophysiological property (e.g. resting potential, spike frequency accommodation, sag current) through an ionic mechanism. Although all groups used some literature to support their explanations, only two groups (2/12) compared the specific parameters they measured to specific values found in the literature. Box 1 shows selected final paper quotes for examples of students using research literature for discussion of ionic excitability mechanisms and electrical property comparisons. The student final project performance is summarized in Figure 11.

In terms of research focus, half the student teams (6/12) compared the neuronal properties from human samples under different diseases states, and the rest focused mainly on comparisons between spiny and aspiny neurons (5/12), with one team (1/12) comparing neurons from different cortical layers or (2/12) from different cell types (two groups did more than one comparison) (see Supplementary Material part 2 for range of student comparisons of neuron types). All groups turned in a team paper except for one student group of two students who had difficulty communicating virtually and therefore submitted final papers separately.

The most challenging part of this project for students was installing and running Allen SDK in Anaconda, especially for students using a Mac machine (Apple, Inc.). Luckily, we resolved all installation issues through virtual video chat and screen sharing. In the future, to minimize the compatibility issues, we suggest using a cloud hosting service such as Google Collaboratory or Binder, as suggested by Juavinett (2020b).

Some groups did more work than was required by generating additional results or delving deeper into the literature to explore comparisons of ionic current mechanisms of firing properties between neuron types. For example, in addition to the required histograms, a group generated box plots to display the distribution of parameters (Figure 12A-B). The same group also identified where the property of the individual neuron they measured in parts I-III lied within the distribution of the properties of the population (Figure 12C). A few groups included the AIBS dataset morphology of the neurons they studied in their report. From the performance of these outstanding groups, we saw some students grew strong research interests, and the potential of

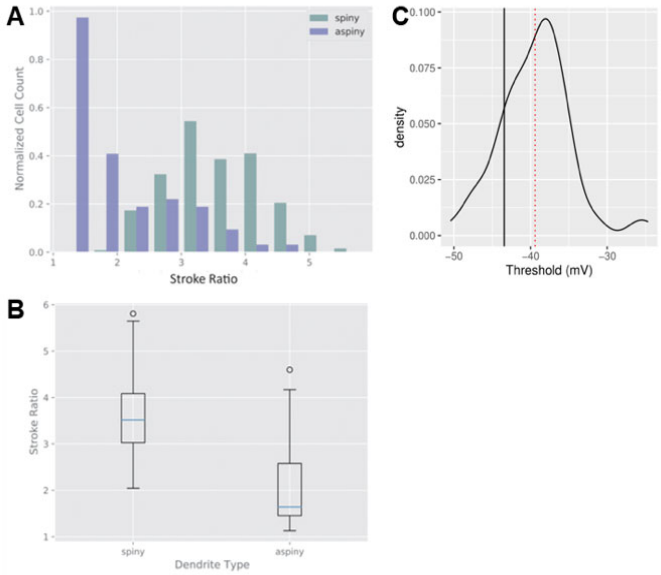

Figure 12. Creativity of Students. *(A-B)* A student group generated a box plot *(B)* in addition to the required histogram to show the differences of stroke ratio between aspiny and spiny neurons. *(C)* The same student group compared the threshold of an aspiny neuron to all aspiny neurons from epileptic patients. The solid curve is the probability distribution of thresholds of all aspiny neurons from epileptic patients. The mean of the population was shown in dotted red line and the threshold value of the single neuron they measured was shown in solid black line. Adapted from students' final report with permission.

students developing a larger independent research project from this activity.

DISCUSSION

The outbreak of the COVID-19 pandemic suddenly moved all Cornell University undergraduate courses to an online platform leaving instructors and students limited time to adjust. After the courses were moved online and the campus emptied on 17th March, the instructors of BioNB4910 had little time to prepare alternative final projects, while managing to hold online lectures and virtual lab sections at the same time. We decided to build on the electrophysiology lesson plan and code created by Juavinett (2020a, b). These lesson plans and the Allen SDK example code (AIBS, 2015) provided us with the foundation to design a new approach to the final class project design in time.

From this solid foundation, we introduced an interactive graphic interface on Jupyter Notebook as a link between what the students already used in the lab to the online coding interface and as an intermediate stage between phenomena observation and abstract analysis and coding. The interactive measurement can strengthen students' understanding of electrophysiological properties through hands-on experiences (Rogers and Scaife, 1998) where here students' "mouse" measured voltage responses. On the other hand, data analysis through automatic extraction with EphysSweepFeatureExtractor from Allen SDK enabled multiple measurements and thus the calculation of the instantaneous firing rate, quantification of spike frequency accommodation, and changes in firing frequency due to a ramp current injection. Compared to Juavinett's (2020b) lesson plan, we emphasized less on the teaching of coding, and more on electrophysiological properties and the process of completing a full research project with an online database.

Although the project was designed to teach students to complete a full research project from question to analysis to interpretation of results without being in the lab, it can also be a gateway to programming and data analysis for biology students without programming backgrounds. Python is a widely-used programming language in the field of data science and neurobiology (Muller et al., 2015). Learning Python prepares students with in-demand skills for neuroscience research and for data scientist positions (Hoy, 2021). Many useful data analysis (such as NumPy and Pandas) and visualization packages (such as Matplotlib, ggplot, and Seaborn) have been created for this open-source program, and students can easily find free online tutorials (Python Software Foundation, 2021; GeeksforGeeks, 2021; Morris, 2021; Varoquaux et al., 2020), or other useful resources if encountering technical issues (see George et al., 2021).

This project can also be incorporated into a lab-based course during in-person teaching. A few possible ways of doing this include: (1) asking students to compare invertebrate data they recorded in lab to vertebrate data downloaded from Allen Cell Types Database, (2) using data from Allen Cell Types Database to form and test hypotheses for their lab-based research project, and (3) applying the data analysis methods and Python code to analyze their own lab-generated datasets.

Based on students' final reports, all groups completed the tasks designed in the project and some groups even went beyond and conducted extra analyses (Figure 12) and wrote more extensive discussions (Box 1). However, we recognize that it may be challenging for students to work on a group project virtually, especially given the stress caused by the COVID pandemic. Two groups reported a team member not participating much in the project and one group broke apart and submitted individual papers. In future, we will add more progress check points for each individual before deadlines and try to meet with each group during the process to ensure the engagement of every student.

Technical limitations including unstable internet service and problems with microphones and cameras can be an issue for working as a team virtually. Luckily, no students reported encountering technical issues. As all students ran Jupyter Notebook locally on their own machines and only downloaded Anaconda and a few files, the requirement of steady internet connection was greatly reduced. While using a web-based hosting service reduces the compatibility issue of having different machines, it also creates a higher demand for a steady internet connection.

Although all groups were able to complete the measurement tasks, only limited firing properties were explained by each student group using proper ionic mechanisms. In future iterations, we will put more emphasis on interpreting the data by providing more specific instructions on what we expect. We will also emphasize the importance of comparing measured data to known values in the literature.

In addition to what is presented here, more analyses of neuronal excitability can be included in the project. For example, one can plot the correlations of two electrophysiological properties. It is also possible to cluster neurons into groups based on their electrophysiological properties and to see if these clusters overlap with any known labels, such as neurons of certain cell types (Gouwens et al., 2019).

This paper demonstrates a way for students to work on group electrophysiology research projects at home, and a way to learn data analysis with research projects. We show how to provide a research-like experience and also teach electrophysiological concepts with an online database, therefore opening the possibility of research projects for undergraduate-level electrophysiology courses without student access to lab equipment.

REFERENCES

Allen Institute for Brain Science (2015a) Allen Cell Types Database. Available at http://celltypes.brain-map.org/data.

Allen Institute for Brain Science (2015b) Allen SDK. Available at http://allensdk.readthedocs.io/en/latest/.

Allen Institute for Brain Science (2015c) Allen SDK examples. Available at http://alleninstitute.github.io/AllenSDK/_static/examples/nb/cell_types.html#Cell-Types-Database.

Allen Institute (2021) Education Outreach. Available at https://alleninstitute.org/about/education-outreach/.

Akyıldız ST (2020) College students' views on the pandemic distance education: A focus group discussion. Int J Tech Edu Sci (IJTES) 4(4):322-334.

Beaulieu-Laroche L, Toloza E, van der Goes M S, Lafourcade M, Barnagian D, Williams Z M, Eskandar E N, Frosch M P, Cash S, Harnet, M T (2018) Enhanced dendritic compartmentalization in human cortical neurons. Cell 175(3):643–651.

Billeh YN, Cai B, Gratiy SL, Dai K, Iyer R, Gouwens NW, Abbasi-Asl R, Jia X, Siegle JH, Olsen SR, Koch C, Mihalas S, Arkhipov A (2020) Systematic integration of structural and functional data into multi-scale models of mouse primary visual cortex. Neuron 106(3):388-403.

Bischop DP, Orduz D, Lambot L, Schiffmann SN, Gall D (2012) Control of neuronal excitability by calcium binding proteins: a new mathematical model for striatal fast-spiking interneurons Front Mol Neurosci 5:78. doi: 10.3389/fnmol.2012.00078

Casimo (in press) Enhancing student research experiences with open data from the Allen Brain Map. J Undergrad Neurosci Edu.

Chu P, Peck J, Brumberg JC (2015) Exercises in anatomy, connectivity, and morphology using Neuromorpho. org and the Allen Brain Atlas. J Undergrad Neurosci Edu 13(2):A95-A100.

GeeksforGeeks (2021) Learn Python Tutorial. Available at https://www.geeksforgeeks.org/python-programming-language/learn-python-tutorial/.

George P, Sandström M, Birdsall M (in press) TrainingSpace: Neuroeducation without borders. J Undergrad Neurosci Edu.

Gilbert TL (2018) The Allen brain atlas as a resource for teaching undergraduate neuroscience. J Undergrad Neurosci Edu 16(3):A261-A267.

Grisham W (2009) Modular Digital Course in Undergraduate Neuroscience Education (MDCUNE): a website offering free digital tools for neuroscience educators. J Undergrad Neurosci Edu 8(1):A26-A31.

Grisham W, Schottler NA, Valli-Marill J, Beck L, and Beatty J (2010) Teaching bioinformatics and neuroinformatics by using free web-based tools. CBE—Life Sci Edu 9(2):98-107.

Grisham W, Korey CA, Schottler NA, McCauley LB, and Beatty J (2012) Teaching neuroinformatics with an emphasis on quantitative locus analysis. J Undergrad Neurosci Edu 11(1):A119-A125.

Grisham W, Brumberg JC, Gilbert T, Lanyon L, Williams RW, and Olivo R (2017) Teaching with big data: report from the 2016 Society for Neuroscience Teaching Workshop. J Undergrad Neurosci Edu 16(1):A68-A76.

Goller CC and Casimo K (2020) Does Organelle Shape Matter? A High-throughput (HT) Imaging Case Study. Available at https://drive.google.com/file/d/18IF6Xg7yPJRQIvkSs_DsuAXZ1ivtSg4N/view.

Gouwens NW, Sorensen SA, Berg J, Lee C, Jarsky T, Ting J, Sunkin SM, Feng D, Anastassiou CA, Barkan E, Bickley K (2019) Classification of electrophysiological and morphological neuron types in the mouse visual cortex. Nature Neurosci 22(7):1182-95.

Ha GE, Cheong E (2017) Spike frequency adaptation in neurons of the central nervous system. Exp Neurobiol 26:179–185. Available at https://pubmed.ncbi.nlm.nih.gov/28912640.

Hanzlick-Burton C, Ciric J, Diaz-Rios M, Colgan W III, Gage GJ (2020) Developing and implementing low-cost remote laboratories for undergraduate neurobiology and neuroscience courses. J Undergrad Neurosci Educ 19:A118-A123.

Heitler WJ (in press) Neurosim: some thoughts on using computer simulation in teaching electrophysiology. J Undergrad Neurosci Educ.

Hoy RR (2021) Quantitative skills in undergraduate neuroscience education in the age of big data. Neurosci Letts 759:136074. https://doi.org/10.1016/j.neulet.2021.136074

Huang J, Sheng J, Wang D (2021) Manifold learning analysis suggests novel strategies for aligning single-cell multi-modalities and revealing functional genomics for neuronal electrophysiology. bioRxiv. doi:10.1101/2020.12.03.41055

Hunter JD (2007) Matplotlib: A 2D Graphics Environment. Comput Sci Eng. 9(3):90-95.

Jenks BG (2009) A self-study tutorial using the Allen Brain Explorer and Brain Atlas to teach concepts of mammalian neuroanatomy and brain function. J Undergrad Neurosci Edu 8(1):A21-A25.

Johnson BR, Wyttenbach RA, Hoy RR (2014) The crawdad project: crustaceans as model systems for teaching principles of neuroscience. In: Crustacean Experimental Systems in Neurobiology (Wiese K, eds) pp 285-295. Berlin, Heidelberg: Springer.

Juavinett A (2020a) Open Neuroscience Education. Open Neurosci Educ Available at https://sites.google.com/ucsd.edu/neuroedu/.

Juavinett A (2020b) Learning How to Code While Analyzing an Open Access Electrophysiology Dataset. J Undergrad Neurosci Edu 19(1):A94-A104.

Kalmbach BE et al (2018) h-Channels contribute to divergent intrinsic membrane properties of supragranular pyramidal neurons in human versus mouse cerebral cortex. Neuron 100(5):1194-1208.

Lee A (2016) mplcursors – Interactive data selection cursors for Matplotlib. https://mplcursors.readthedocs.io/en/stable/#.

McCormick DA (2014) Membrane Potential and Action Potential. In: From Molecules to Networks, 3rd edition (JH Byrne, R Heidelberger, MN Waxham, eds) pp 351-376. Amsterdam, Netherlands: Academic Press.

Meir E (in press) Designing a simulation lab: The process that led to Action Potentials Explored and Extended, two simulation-based neurobiology labs. J Undergrad Neurosci Educ.

Morris C (2021) Kaggle: Learn Python. Available at: https://www.kaggle.com/learn/python.

Mosher CP, Wei Y, Kamiński J, Nandi A, Mamelak AN, Anastassiou CA, Rutishauser U (2020) Cellular classes in the

human brain revealed in vivo by heartbeat-related modulation of the extracellular action potential waveform. Cell Rep 30(10):3536-3551.

Nandi A, Chartrand T, Van Geit W, Buchin A, Yao Z, Lee SY, Wei Y, Kalmbach B, Lee B, Lein E, Berg J, Sümbül U, Koch C, Tasic B, Anastassi ou CA (2020) Single-neuron models linking electrophysiology, morphology and transcriptomics across cortical cell types. bioRxiv. doi: 10.1101/2020.04.09.030239

Muller E, Bednar JA, Diesmann M, Gewaltig MO, Hines M, Davison AP (2015) Python in neuroscience. Front Neuroinform 9:11.

Pulver SR, Hornstein NJ, Land BL, Johnson BR (2011) Optogenetics in the teaching laboratory: using channelrhodopsin-2 to study the neural basis of behavior and synaptic physiology in *Drosophila.* Adv Physiol Educ 35:82–91.

Python Software Foundation (2021) The Python Tutorial. Available at https://docs.python.org/3/tutorial/.

Ramos RL, Smith PT, Brumberg JC (2007) Novel in silico method for teaching cytoarchitecture, cellular diversity, and gene expression in the mammalian brain. J Undergrad Neurosci Edu 6(1):A8-A13.

Rogers Y, Scaife M (1998) How can interactive multimedia facilitate learning. In: Intelligence and Multimodality in Multimedia Interfaces: Research and Applications (Lee J, ed). Menlo Park, CA: Association for the Advancement of Artificial Intelligence Press.

Ryan J, Casimo K (2021) A Course-Based Research Experience Using the Allen Brain Map: From Research Question to Poster Session. J Undergrad Neurosci Edu 19(2):A240-A265.

Schneider-Mizell CM et al (2020) Chandelier cell anatomy and function reveal a variably distributed but common signal. bioRxiv. doi: 10.1101/2020.03.31.018952

Shelden EA, Offerdahl EG, Johnson GT (2019) A Virtual Laboratory on Cell Division Using a Publicly-Available Image Database. CourseSource. https://doi.org/10.24918/cs.2019.15.

Varoquaux et al. (2020) Scipy Lecture Notes. Available at http://scipy-lectures.org/index.html. doi: 10.5281/zenodo.594102

Vilinsky I, Hibbard KL, Johnson BR, Deitcher DL (2018) Probing synaptic transmission and behavior in *Drosophila* with optogenetics: A laboratory exercise. J Undergrad Neurosci Educ 6(3):A289-A295

Wyttenbach RA, Johnson BR, Hoy RR (2014) Crawdad: An Online lab manual for neurophysiology. Sunderland, MA: Sinauer Associates, Inc.

Received September 19 2021; revised November 5, 2021; accepted November 12, 2021.

ACKNOWLEDGEMENTS

This work was supported by Department of Neurobiology and Behavior, Cornell University. The authors thank students and undergraduate teaching assistants in BioNB/BME/ECE 4910 Principles of Neurophysiology (Spring, 2020) for their hard work under difficult, pandemic conditions and Dr. Ashley Juavinett for advising our project design and sharing her lesson plan.

Parts I to Part III were adapted from the AIBS code (http://alleninstitute.github.io/AllenSDK/_static/examples/nb/cell_types.html#Cell-Types-Database). Part IV was adapted from code written by Professor Ashley Juavinett at UCSD (https://github.com/ajuavinett/CellTypesLesson/blob/master/CellTypesNotebook.ipynb).

Undergraduate Teaching Assistant (TA) Gwenda Law contributed a great amount of the code in a very short time frame, and trouble-shot the code to make sure it ran smoothly. Undergraduate TAs Molly Smullen and Gwenda Law wrote the instructions for Anaconda installation. The grading Rubic for the final paper was originally designed by Dr. Joannalyn Delacruz while a graduate teaching assistant for BioNB/BME/ECE 4910.

Address correspondence to: Dr. Bruce R. Johnson, Department of Neurobiology and Behavior, 129 Garden Ave. 1134 Comstock Hall, Cornell University, Ithaca, NY 14853. Email: brj1@cornell.edu