SUPPLEMENTARY MATERIAL

Mapping human neuron diversity in the search for new therapies

Student learning outcomes:

1. Describe epilepsy and how seizures occur in the brain
2. Discuss how neuronal diversity may impact the therapeutic potential of new drugs
3. Access human neuron electrophysiological, morphological and transcriptomic data from epileptic patients
4. Analyze electrophysiological data from human epileptic patients
5. Discuss the ethical, legal and social implications of this human neuron data set
6. Discuss the advantages & limitations of studying neurons from human patients

Technical skills based LOs...
1. Apply big data from high-throughput experiments to detect patterns and quantify data
2. Identify and test a new hypothesis
3. Apply appropriate statistics to quantitative results
4. Describe practical considerations of data collection in humans
5. Access data from high-throughput experiments on living human brain tissue in the Allen Institute database
6. Interpret basic science data in the context of pre-translational research

Part 1: Pre-Work

1. Read "The Seizures of Epilepsy" excerpt from *Neuroscience Exploring the Brain* by Mark F. Bear, Barry W. Connors, Michael A. Paradiso: The Seizures of Epilepsy_Bear Text (2 pages)
2. Watch the two minute neuroscience video on Epilepsy: https://youtu.be/OGFQhLPaaOQ
3. Read the following excerpts "***Introduction to the case***" and "***Experimental design of the Allen Cell Types Database and your experiment***" to prepare you for our case study. The excerpt also includes links to other required readings as well as supplemental reading suggestions. (~4-8 pages)
4. Answer the "***Pre-work Questions***"

Introduction to the Case

You have just started work in the lab of Dr. Naomi Marsh in the neurology department at the university hospital. Dr. Marsh is conducting research on a new drug that is thought to affect neuronal excitability and she wants to test its effect on the properties of temporal lobe neurons. Temporal lobe epilepsy is the most common form of focal epilepsy, so she is especially interested in testing this compound's effects on cells in that region of the brain.

Dr. Marsh hopes the drug will affect the properties of temporal lobe neurons to reduce the likelihood of a seizure. However, first she needs to gather some baseline data on temporal lobe neurons' excitability, and how these cells may be distinct from cells in other areas of the brain.

You have access to living neurons' data from tissue samples collected from neurosurgery patients for the Allen Cell Types Database, a resource by the Allen Institute for Brain Science. The team examines tissue removed as part of routine neurosurgery for epilepsy and glioblastoma. The tissue included in this dataset is in the path the surgeons have to take to access the pathological site. The seizure focus or tumor from the patient are examined by a pathologist and not included in this dataset. The team has measured these cells' properties and shared them in an open database that you can use to do this experiment.

Experimental design of the Allen Cell Types Database and your experiment:

1. You will access the data at **celltypes.brain-map.org**.
   a. The goal of Allen Cell Types Database is to create a complete census of cell types in the human and mouse brains based on their shape, electrophysiology, and gene expression.
      i. Read this article (1 page) about one research use of the data.
      ii. Read this article (2 pages) about human tissue donors.
      iii. The databases include data from both human and mouse brains, but the regions included in the dataset are different and some of the methods vary. Learn more on the database home page.
2. There are two main datasets in this database, which you can access at the links in the top bar of the page. If you want to read detailed information about the methods used, visit the documentation page.
   a. **RNAseq data**: single-nucleus RNA sequencing (human) and single-cell RNA sequencing (mouse) data recording relative gene expression levels of tens of thousands of genes in tens of thousands of cells. We will not be using this dataset in this experiment, but you can explore more about the methods and interpretation of the data on the dataset homepage.
   b. **Cell Feature Search**: single-cell electrophysiology and morphology data from humans and mice. Each block on the bottom of the page corresponds to one cell and has links to its data.
      i. On the electrophysiology page for each cell, series of standardized stimuli were applied to each cell and its response recorded. Some properties of the cell's electrophysiology were recorded or calculated from the data. For some cells, a computation model of its firing is also available.
      ii. On the morphology page, there are views of the cell in 3D after it was dyed to show the cell more clearly. Full and partial digital reconstructions for the 3D shape of the cell are available, and the reconstructions are labeled with dendrite type, axon, and cell body.
      iii. Some cells have only electrophysiology data, and some cells have both electrophysiology and a morphological reconstruction.

3. *In order to collect electrophysiology data, the tissue must still be alive. Where does the living human tissue come from?*
   a. The Allen Institute has partnerships with neurosurgeons in the Seattle area who are performing surgery to remove diseased tissue related to epilepsy and glioblastoma tumors. In order to reach a diseased site deep in the brain, the surgeons often have to remove some healthy, unaffected tissue. This tissue needs to be removed as part of the surgery, but is not sent to the pathology

    department along with the seizure focus or tumor for diagnostics. It normally would be thrown away as medical waste. Instead, it is sent to the Allen Institute for scientific use.

    b. Because the tissue comes from the surgical site, the scientists do not control what region of the brain is being sampled. For example, the temporal lobe is the most common site for epilepsy, so there are a large number of samples from this region.

    c. The RNAseq data can be collected with postmortem tissue donations, so more regions are represented.

4. *How is the electrophysiology data collected?*

    a. The electrophysiology team runs individual cells through a standardized routine of stimuli and records the cells' responses. For some cells, another team develops a computational model that captures the cell's responses mathematically and aims to predict how it would respond to other stimuli.

5. *How are the morphology reconstructions made?*

    a. There are several different methods for tracing neurons in three dimensions. When the electrophysiological experiments are done, the cell is injected with a Biocytin stain and a series of 2D images are taken in a stack to form a 3D image of the cell. A scientist then traces and labels the image stack and creates a 3D model of the cell.

6. *All of the brain-map.org datasets are big data, including tens of thousands of cells, genes, or tissue slices. Why big data?*

    a. Large datasets enable more complex, sensitive statistical analyses. Statistical tests using larger datasets are less likely to return false positives or be affected by outliers and artifacts.

    b. The brain is a highly complex system. Each brain region has distinct properties and findings are not necessarily generalizable across the brain. Big datasets that include the whole brain enable comprehensive studies.

    c. Big, comprehensive datasets can capture rare phenomena, such as the rosehip neuron, that may otherwise be missed in smaller samples.

Supplemental Statistical Resources:

We will be performing some basic summary statistics and some statistical tests on our data in this project. If you need to review statistical concepts, here are some resources:

- The following sections of An Introduction to the Science of Statistics: From Theory to Implementation:
    1. Mean (section 2.1.2, page 21)
    2. Standard deviation (section 2.2.2, page 25)
    3. Interpreting P values (section 18.3, page 331)
    4. T test (section 20.5 for two sample t tests, page 368)
    5. Mann-Whitney U test (section 20.9.2, page 378)
- Explanation of Standard Deviation and how to calculate by hand, in a Google sheet or Excel: https://www.biologyforlife.com/standard-deviation.html
- One versus Two Tail Tests: https://www.graphpad.com/guides/prism/8/statistics/one-tail_vs__two-tail_p_values.htm
- Mann-Whitney Test: https://www.graphpad.com/guides/prism/8/statistics/how_the_mann-whitney_test_works.htm?q=man+whitney

Pre-work Questions:

1. Neurons communicate via chemical neurotransmitters. Neurons also rely on their electrical properties to support electrochemical transmission. Illustrate neurotransmission between two neurons accounting for both electrical and chemical properties. Mark where the signal is transmitted by neurotransmitters and where it is carried by electrochemistry. Label major parts of the neurons.

2. Neurons have electrical properties that are related to their membranes and ion channels. Briefly describe the molecular basis of an action potential. Start your description with neurotransmitter binding to a receptor. Include details about the resting state of the cell in your answer.

3. Describe what happens in the brain on a cellular level during a seizure. How are the cells' properties and interactions affected?

4. We can measure many different electrical properties and events in neurons. List two. Describe one of them in more detail - how do you interpret that property of the cell?

5. There is variation between regions of the brain and layers of cortex. Why does that variation matter in the context of studying drug efficacy?

Part 2: Your experiment (in class work)

It's time for you to access the Allen Cell Types Database to do some analysis for Dr. Marsh's research. Refer to the pre-work for details of how this dataset was collected and how to interpret it.

1. Access celltypes.brain-map.org.
2. In-depth protocols can be found on the Documentation tab. Search tips, metadata details, programmatic access (API) instructions, and more website information can be found on the Help tab.
3. Dr. Marsh has assigned you to *evaluate the properties of neurons in the temporal lobe, and determine if they are distinct from the properties of other brain regions.* To start, click on the Cell Feature Search tab and use the checkboxes to filter for only human cells.
   a. Experiment region A: middle temporal gyrus (MTG) 309 cells
   b. Experiment region B: Frontal lobe (FroL) 23 cells
   c. Hint - not sure where a region is located? When you open any cell's data panel, click on the region name in the header to bring up the reference atlas to that area. Or go to atlas.brain-map.org and find Adult Human.
4. From your group's assigned brain region & cells (MTG and/or FroL), claim your cells in the google spreadsheet.

   a. *This sounds easy but our goal is to analyze every cell in these regions* (all 332 cells).
   b. To do this, we have to work collaboratively and start with the *Cell ID number*.
   c. The figure to the right shows how to search the database for human neurons only in the FroL as an example. 23 cells fit this criteria and the first cell's ID number is boxed in red at the bottom.
   d. Our shared Google sheet is designed to highlight the Cell ID entry if it is identical to an existing ID in the column. *This way we do not duplicate data from the same cells.* In other words, if you enter a cell ID and the cell highlights, someone has already claimed that cell and you need to go find a new one! Make sure you enter your Cell ID and name to claim that cell before you start spending time collecting data. Please do your best to put your cell in numerical descending order.
   e. For each cell, record data in the table here, *starting with the Cell ID number*. Make sure you add your data to the sheet in order of cell ID as much as possible.

5. Each cell by itself does not tell us very much about the cell properties found in this region. To learn more, we will calculate some basic statistics on the cells in your groups.
    a. ***<u>Once the whole class has entered their data, make a copy of the Google sheet for just you and your group to work on in your own shared space</u>***.
6. In the data collection table, you will divide the cells up by brain region and by cortical layer. For each brain region and cortical layer, calculate the mean and standard deviation of the **firing rate** and **resting potential**. Refer to the supplemental pre-reading on statistics for how to calculate and interpret these statistics. Use the built in functions in your spreadsheet program to calculate mean (=average) and standard deviation (=stdev). ***List the means with std devs here in a well organized table:***

7. Compare the two regions using a two-sample Wilcoxon/Mann-Whitney U-test. These are not available as built-in functions in most spreadsheet programs: use <u>this</u> online calculator or enter the formula manually (refer to the pre-reading). Why would we use one of these tests instead of a t-test?

8. Interpret the results of your test. What can you say about the difference between temporal and non-temporal neurons? What can't you say? Think about the sample you were testing when you describe what your results apply to.

9. Using our collected data, ask a new research question and design a way to test the new hypothesis (i.e. identify another comparison you'd like to make). Write out your new research question, hypothesis, and discuss the results of your new comparison.

Part 3: Conclusions and further reflection on experiment

1. Summarize your findings from your experiments. What did you find? What cells and brain regions do your findings apply to? What cells (or conditions) do your findings NOT apply to?

2. Think about the electrophysiology of epilepsy. If you were to conduct the same experiments the Allen Cell Types Database team did on cells that had been taken from a seizure focus, what properties would you anticipate being different? Why?

3. When Dr. Marsh tests her drug on epileptic tissue, what effect on electrophysiological properties should she expect to see if the drug is effective? What about if the drug is not effective?

4. Research some mechanisms of action of anti-epileptic drugs. What do the mechanisms have in common? How is the mechanism of drug action related to the electrophysiology of the cell?

5. We used cells from individuals undergoing neurosurgery for epilepsy or glioblastoma for this experiment. Why did we not use tissue from healthy individuals? Why does this dataset NOT include tissue from the seizure focus/tumor from these individuals?

6. Discuss some of the ethical, legal, or social implications of this human neuron dataset.

7. Identify and describe at least two limitations of studying neurons from living human donors.

8. Discuss a limitation and advantage of alternative options (i.e. studying model organisms such as mice, or developing computer ("in silica") models) to the study of living human tissue for this research.

9. Please give us some feedback.
    a. Did you enjoy this case study? Why or why not?
    b. How could we improve the case?
    c. Were there questions or steps in the experimental procedure that you found confusing?
    d. Are there other research questions you are interested in exploring with this dataset?

Want to learn more? Supplemental Resources:

Johung and Monje. "Neuronal Activity in the Glioma Microenvironment" *Curr Opin Neuro Biol* 2018
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5927594/

Schwartzkroin. "Cellular electrophysiology of human epilepsy" *Epilepsy Research* 1994
https://www.sciencedirect.com/science/article/pii/0920121194900493

Kohling. "Voltage-gated Sodium Channels in Epilepsy" *Epilepsia* 2002
https://onlinelibrary.wiley.com/doi/pdf/10.1046/j.1528-1157.2002.40501.x

Tai et al. "Impaired excitability of somatostatin- and parvalbumin-expressing cortical interneurons in a mouse model of Dravet syndrome." *PNAS* 2014
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4121787/