Instructor Guide:
Neurons: Beyond the Textbook

Resource structure

In this lesson, students will learn about the concept of cell types and how they are defined, gain a more realistic perspective of neuron morphology, and develop a deeper understanding of how neuron features are analyzed. Students will explore neuron morphology and, using (free, online) software, will reconstruct neurons that will ultimately be included in a larger dataset. Students will also be guided in exploring Allen Institute for Brain Science's open Mouse Patch-Seq dataset to see how neurons vary across transcriptomic types and cortical layers and next steps on how to conduct independent research.

This resource is divided into three sections, which build on each other to provide students with independent and challenging work. Section 1 can be completed as a standalone lesson, for more advanced students add Section 2, and then for students interested in pursuing independent work add Section 3. All parts of this lesson can be done virtually.

In the first section, students will learn what actual cortical neurons look like and how they differ from the standard model textbooks present. Students will also learn the importance of neuron morphology to help define cell classes and types. Students will use Mozak (an online game) to trace neurons themselves, and see how scientists use these tracings (reconstructions) to analyze a cell's morphology (shape). Throughout this section, there are questions for students to reflect on what they have learned and how their previous ideas of neuron morphology may have changed.

In the second section, students will explore published neuron reconstruction data and see that neurons in different cortical layers and transcriptomic cell classes look distinct. (Neurons can be classified as belonging to a transcriptomic class or T-type by the RNA transcripts they express.) Students also explore the Mouse Patch-seq dataset, which includes multimodal data for each cell.

The final section is a guided independent project for students to explore the diversity of neuron morphologies by conducting their own research with options to explore more of our datasets and/or use our more advanced free reconstruction software.

Recommended grade level

Section 1: Highly motivated high school students, such as those in AP Biology, IB Biology, AP Research, or Anatomy and Physiology courses, to introductory-level college neuroscience students
Section 2: Introductory to intermediate college neuroscience students
Section 3: Intermediate to advanced college neuroscience students, graduate students

Learning goals

Section 1:
- Students will be able to draw a realistic representation of a neuron
- Students will be able to explain why scientists look to neuron morphology as a modality for defining cell types

Section 2:
- Students will be able to interpret previously collected neuron morphology data
- Students will begin to explore the different methods of neuron features analysis

Section 3:
- Students pursuing a senior thesis can follow our research plan guide in using morphology as a research topic.

Student knowledge

Students should already have a basic understanding of:
- The major components of a neuron (axon, dendrites, soma) and their basic function
- Basic genetics (i.e., what is a gene)
- General cell anatomy (i.e., the nucleus)

Students do not already need knowledge of:
- Transcriptomic cell types
- Advanced brain anatomy (i.e., specific brain region locations or detailed cortical layers)
- Functional properties of a neuron (i.e., how it fires an action potential or any neuron electrophysiological properties)
- Statistics

Outline for teachers

Section 1

Suitable for introductory-level college neuroscience and for some advanced and motivated high school students, such as those in AP Biology, IB Biology, AP Research, or Anatomy and Physiology courses. This section is also used as a prelude to the more challenging sections 2 and 3.

- This lesson provides a deeper, more accurate representation of neuron morphology.
- This section asks students to review what they know about neurons already.
- Students gain first-hand experience tracing (reconstructing) neurons using a freely accessible website.
- Students will be introduced to the concept of cell classification and cell types.
- Students will explore morphological differences between excitatory and inhibitory cells.

Section 2

Suitable for introductory to intermediate college students, and used as prelude to the more challenging section 3.

- This section explores the historical and current perspective on cell naming (nomenclature).
- This section describes an approach to quantitatively analyzing cell morphology.
- Students will learn about transcriptomic types and how they relate to the morphology of the cell.
- Students will examine the morphology of 2-3 cells of their own choosing in the Allen Cell Types Database.

Below are example specimen IDs for 'Exploring Data' subsection.

Comparing by cortical layer

- Layer 1: 823719680
- Layer 2/3: 804977071
- Layer 4: 605060256
- Layer 5: 760011270
- Layer 6a: 692565557
- Layer 6b: 688115867

Comparing by T-type

- Sst: 863604233
- Lamp5: 711483278
- Vip: 601506507

Comparing by both cortical layer and T-type

- Vip Layer 1: 760316107
- Vip Layer 2/3: 831988252
- Vip Layer 4: 657184390
- Vip Layer 5: 700413526

Section 3

Suitable for intermediate to advanced college students who wish to conduct their own independent research project.

- This section provides guidance in how to conduct one's own independent research project,This section provides ideas for the project goals and ways to obtain data.
- This section provides links to helpful research papers & advice how to read research papers.

Introduction for instructors

The Allen Institute is a nonprofit biomedical research institute located in Seattle, Washington. Our divisions and programs – the Allen Institute for Brain Science, the Allen Institute for Neural Dynamics, the MindScope Program, the Allen Institute for Cell Science, the Allen Institute for Immunology, and The Paul G Allen Frontiers Group – are dedicated to answering some of the biggest questions in bioscience and accelerating research worldwide. We share all of our data and research findings with the scientific community and general public. Launched in 2003 by founder Paul G. Allen, the Allen Institute is supported by government, foundation, and private funds to enable its projects. The Allen Institute for Brain Science creates large-scale, open datasets that address fundamental questions about the brain's components and functions. These datasets and other tools form the Allen Brain Map, and are publicly available online at brain-map.org.

Key neuroscience concepts underlying this lesson

- Neurons come in many shapes and sizes and are not easily represented by the typical textbook diagram.
- Neuroscientists image and trace (or reconstruct) the processes (axon and dendrite) of neurons filled with dye to visualize and analyze the cell's morphology.
- Neuroscientists often classify neurons using several different features (e.g. neurotransmitter released, transcripts for proteins expressed, electrophysiological responses, and morphology). Neurons are defined as being in an excitatory or inhibitory cell class by the neurotransmitter they release. Neurons can be classified as belonging to a transcriptomic or T-type by the RNA transcripts they express as found by genetic sequencing of the contents of the cell's nucleus.
- Excitatory and inhibitory neurons have distinct morphologies. However, excitatory neurons look similar across species, as do inhibitory neurons.

Allen Cell Types Database (appears in Section 1 and 2)

- This open database of brain cell data contains a survey of biological features (electrophysiology and morphology) derived from a single cell, from both human and mouse. It is part of a project to create a census of cells in the mammalian brain.
- Mouse cells are acquired from selected brain areas in adult mice. Cells are chosen if they are positive for a fluorescent marker expressed by specific cell classes, based on marker genes.
- Human cells are acquired from brain tissue donated by Seattle-area neurosurgery patients and their surgeons, and individual cells are selected based on soma shape and laminar location.
- Researchers attach (patch) electrodes to individual neurons using pipettes and record the electrophysiological properties of the cell. While patching the neurons, they also fill the cells with dye.
- The filled neurons are imaged using light microscopes and the neuron morphology is determined by tracing (reconstructing) the dye-filled neurons from the resulting images.
- This dataset consists of electrophysiological traces (membrane potential and action potential shape) and morphological reconstructions (histograms/distribution patterns of axon and dendrites across cortical layers).

Mozak is a free online game that allows players to trace neurons. The completed neurons get added to the Allen Institute's Allen Cell Types Database and are used in research studies. Tracing neurons takes a long time, but people are very good at the game, so the Allen Institute collaborates with the public via Mozak to add more neurons than can be completed by their team alone.

Mouse Patch-seq dataset (appears in section 2)

- The Mouse Patch-seq database is a summary of triple modality (electrophysiology, transcriptomic, and morphology) data from single cells in mouse primary visual cortex.
- Researchers patch on to individual neurons using pipettes and record the electrophysiological properties of the cell. While patching the neurons, they fill the cells with dye and at the end of recording, they extract the neuron's nucleus using the pipette.
- The filled neurons are imaged using light microscopes and the neuron morphology is determined by tracing (reconstructing) the dye-filled neurons from the resulting images.
- The genetic information from the extracted nucleus is sequenced to determine the RNA transcripts that the cell expresses. This transcriptomic information is then used to classify neurons according to previously established genetic classification. (For more detail on transcriptomic types, please see the supplemental technical background section below).
- This dataset consists of transcriptomic classification of neurons (Transcriptomic/T-Type Assignment), electrophysiological traces (membrane potential and action potential shape), and morphological reconstructions and classifications (histograms/distribution pattern of axon and dendrites across cortical layers).
- When all 3 modalities are compared, cells are assigned to a MET (morphological, electrophysiological, and transcriptomic) type – where cells with similar morphological, electrophysiological, and transcriptomic features are grouped together.

Further technical background

Beyond excitatory and inhibitory classes, neurons can be further divided into subclasses. For example, inhibitory subclasses are defined by the major protein expressed by a group of neurons (Lamp5, Sncg, Vip, Sst, and Pvalb).

Within subclasses, neurons can be further divided into transcriptomic types (T-types). These T-types are originally based on genetic sequencing of the intracellular contents from fluorescently tagged cells from transgenic mouse lines. We find that cells from different T-types will have very distinct axon and dendrite distribution patterns. Furthermore, some T-types are characterized by how their axons project to specific layers.

For more information, please read:
casestudies.brain-map.org/celltax
portal.brain-map.org/explore/classes/nomenclature