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

Section 1: A deeper dive into neuron morphology & What is a cell type?

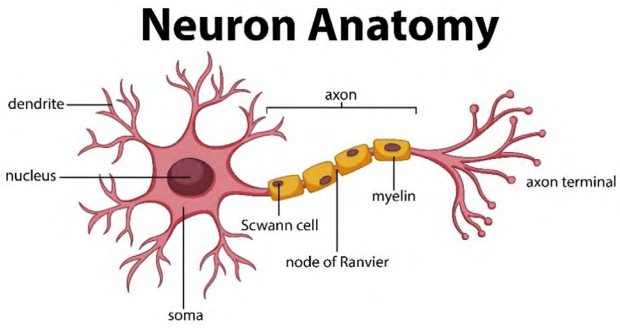
Introduction and goals for students

- In this section, you'll see how actual neurons look very different from the textbook diagram. You will learn and recognize the key morphological differences between axons and dendrites.
- This section will briefly describe why cell types are important.
- You will explore the Allen Cell Type Database to compare the morphology of excitatory versus inhibitory neurons.
- You will reconstruct neurons using the Mozak citizen science game.

Textbook vs. reality

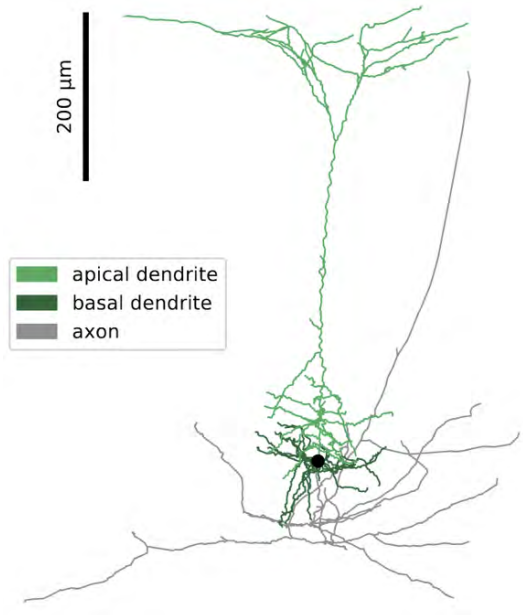
Student question: In the space below, draw to the best of your ability a neuron. Include labels for soma, axon, and dendrite.

Does your neuron drawing look similar to this picture?



This model of neuron is commonly used in neuroscience textbooks, from middle school to even higher college level textbooks. If you search images labeled “neuron” online, most diagrams will look similar.

However, that is a very inaccurate representation of a real neuron. Below is a reconstruction of real mouse neuron from scientists at the Allen Institute. In this reconstruction, the soma is marked with a black circle, two types of dendrite in shades of green, and axon in gray. Note the differences in dendrites’ length around the soma, the multiple branches of the axon and general scale of the parts of neuron.



Student questions

What are some main differences between the textbook diagram of a neuron compared to the real-world reconstruction or model of a neuron?

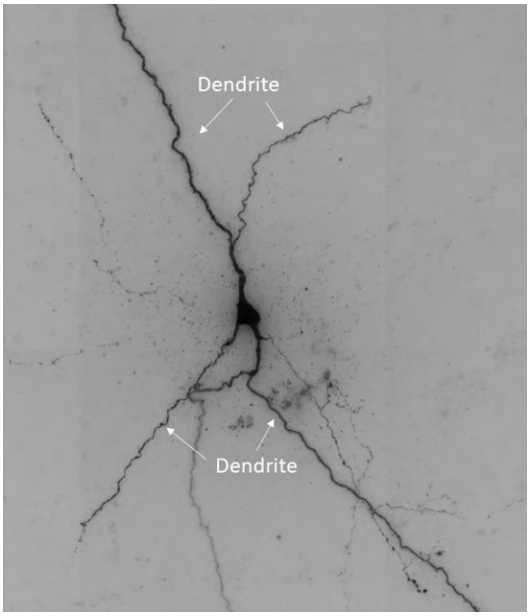
What are advantages of using the textbook or more basic diagram compared to the reconstruction?

What are disadvantages and drawbacks of using this textbook diagram compared to a more accurate reconstruction?

When neuroscientists talk about neuron morphology, we often look at the features of the dendrite, axon, and soma. Here are more accurate distinctions and definitions of the dendrite, axon and soma.

Dendrites are thick branches (compared to axons) that exit the soma and receive synaptic input.

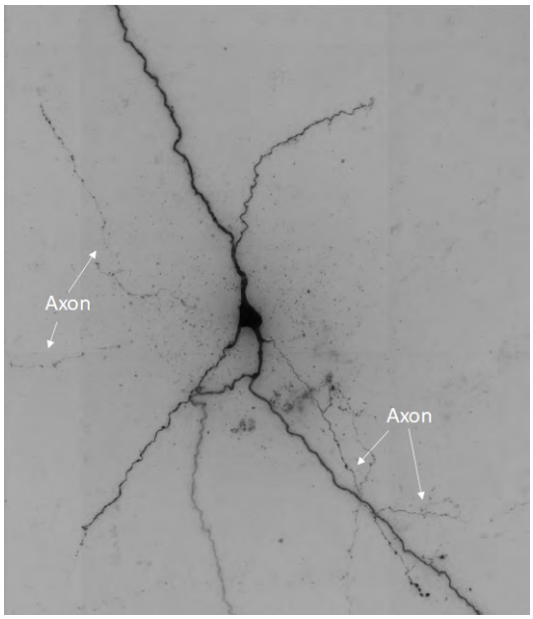
- Defining features include the number and length of its neurites and dendritic branch complexity. (The term neurite is used to refer to any neuronal branch.)
- Dendrite length and extent tends to be smaller compared to the cell's axon.
- Dendrites can have spines, which are small protrusions, that receive synapses from other cells, or instead have very few or no spines giving them a smooth appearance.
- The image below is of a human neuron with a dendrite that has few to no spines.



This image is from a image stack, which is a series of images of a cell that are taken at different depths. Taken together, the stack provides a 3D view of the cell. While we are showing you a single image from the stack here, scientific analyses will use the full stack.

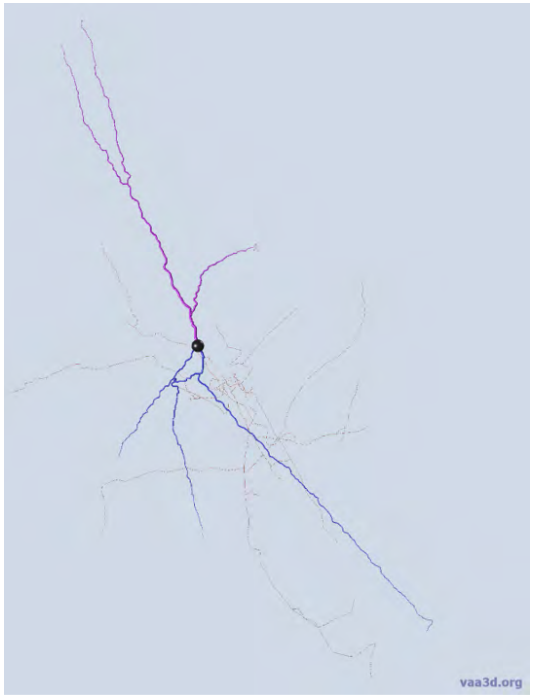
Axons are very complex, extensive, and can form thin branches that transmit signal through action potentials.

- The length of the axon can vary from micrometers to meters, and it can spread across the entire brain.
- The branching pattern of axons are distinct as branch patterns vary more compared to dendrites.
- The density and distribution of a cell's axon can vary both across and within brain regions, depending on the cell type. For example, in human & mouse visual cortex, the axon of the same cell type will vary greatly depending on which cortical layer(s) of the cortex the soma is in. Cortical layers are the distinct layers of the outer cortex of the brain that are arranged from Layer 1 (superficial) to Layer 6 (deep).
- Axons can be wrapped in myelin, which acts like insulation on a wire. This can increase the speed of an action potential. Axons that travel between brain regions are often myelinated, likely to improve the speed and reliability of signal transmission.
- The image below is the same human neuron but with the axon labeled. Note how much thinner axons are compared to dendrites.



Somas vary greatly in shape and size. The soma is the rounded central hub seen in this cell image. Defining where the soma ends, and axons and dendrites begin can be difficult and nuanced. The soma does contain all the standard organelles in any animal cell.

A reconstruction is a 3D representation of a neuron. Specifically, a reconstruction is a 3D drawing of a cell, drawn in a computer program for the purpose of analysis. Scientists can extract quantitative numerical values from these reconstructions and then run various statistical tests to help better understand the shapes of multiple neurons. Here is reconstruction of the same cell above using our in-house software.



Reconstruct neurons alongside scientists at mozak.science

Mozak is a scientific discovery game about neuroscience. 3D image stacks of neurons are posted on the website so players can reconstruct neurons and capture their morphology. An informational guide to playing Mozak, as well as general information about gameplay can be found on the website. Players from beginner to expert levels of experience can participate in general tracing or enter different challenges.

Mozak is also an important tool for our research pipeline. Each player's tracing is used to find the most agreed upon path for each neuron, which is then reviewed by our team of reconstructors. The final reconstruction is a joint contribution from citizens and researchers who work together. The neuroscience being done at the Allen Institute for Brain Science depends on these citizen science contributions from the Mozak game players.

Why do we still have humans tracing neurons instead of using a more automated method?

Well, picture a tree. And another tree. And another. And another. There's no limit to how many different types of trees your brain can picture, and you know they are all trees. You can also see an image of a tree you've never seen before and still know it is a tree. However, for computers, recognizing that a new image is of a known object is still a difficult task. You have had firsthand experience with this problem whenever a website asks you to verify if you are human by having you click on all the images that contain a given object. This task is simple for you, but not for a computer.

In the field of neuroscience, we are still training our computers to recognize cell's features to distinguish between axon vs dendrite vs image artifacts. However, computers are still not as accurate as a human, so we continue to trace our neurons semi-manually.

Participate in reconstructions

Visit Mozak.science and play the game.

How did tracing firsthand change your outlook on neuronal morphology?

Why do you think using 3D image stacks is an important factor for tracing?

What are some differences you noticed between dendrite and axon appearance?

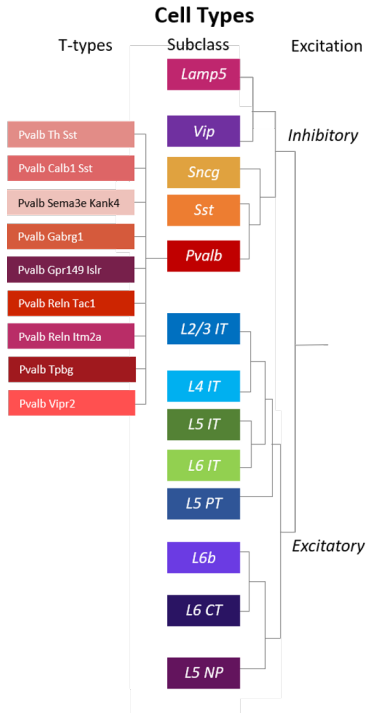
What did you find the most difficult part about tracing? Why do you think tracing is difficult for computers?

What are cell types?

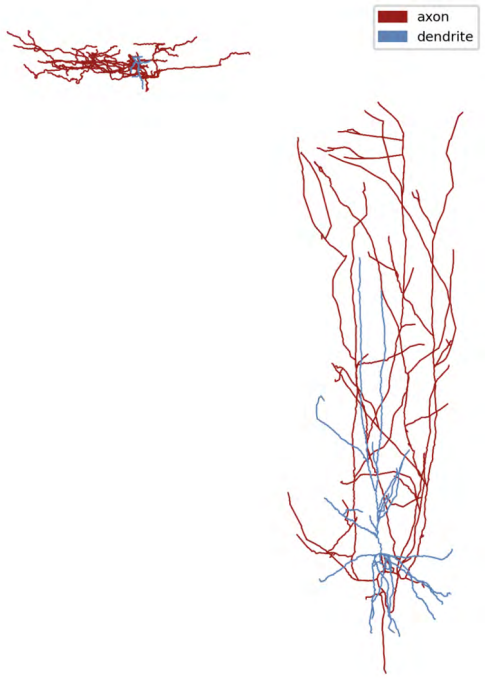
As you can see from looking at accurate models of neurons and reconstructing using Mozak, neurons come in many different shapes and sizes. This leads to the question of how to accurately describe and compare neurons to each other.

One approach is to categorize neurons into "types" based on whether they have certain features or values. A cell can be classified by more than one feature, or modality, such as the RNA transcripts it expresses, the neurotransmitters it releases, its electrical responses, as well as its shape. These modalities have different ranges of complexity. Classifying neurons into different cell types provides a framework through which to understand where these types are found in the brain and how they might function in a circuit. However, it is still an ongoing discussion today as to which modalities to use, which classifications to use, and how to update these labels as scientists uncover more details.

Below is a diagram that shows a few different levels that scientists use to classify neurons. Starting on the right, you can see cells are divided into excitatory or inhibitory by which type of neurotransmitter they release. Next, cells are further divided into subclasses, which are determined by the main protein an inhibitory cell expresses, or by the layer and axon pattern for excitatory cells. Last, we show T-type or transcriptomic types, which show even more detail about the RNA transcripts a cell expresses (see section 2 for more information about T-types).



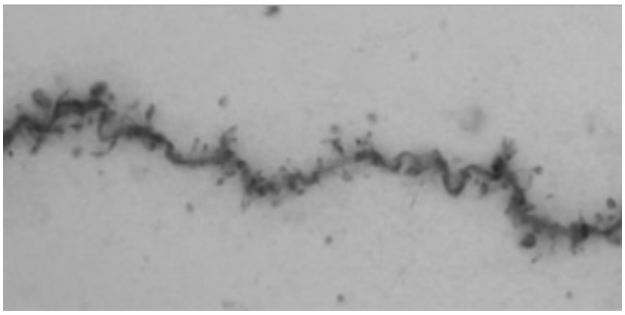
Using morphological descriptions, textbooks originally classified neurons as being bipolar, unipolar and multipolar cells, based on the overall shape. However, for most scientific purposes, defining a cell as just bipolar, unipolar and multipolar is not detailed enough. For example, the two neurons below could both be defined as multipolar, but you can clearly see their structures are very different from each other.

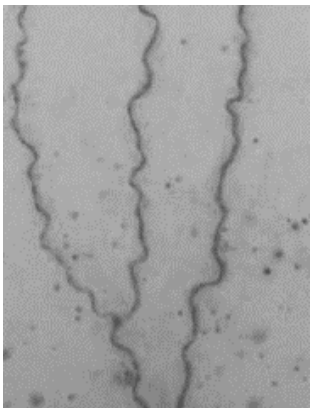


Neuron morphology, or the shape of a neuron, is often a defining feature of a cell type, and as shown is more complex than the usual textbook definition. Your nervous system performs many complex functions; therefore, it makes sense that the neurons involved also have complex structures. Throughout all biology, function and structure are intertwined.

One important classification of neurons is whether they release neurotransmitter that excites or inhibits their targets. Research has found that excitatory neurons and inhibitory neurons in the cerebral cortex have distinct morphologies in a few key ways. Excitatory neurons typically have a lot of spines on their dendrites. The role of spines is complex, but importantly, they are often the site of excitatory input synapses. On the other hand, inhibitory neurons have far fewer or no spines on their dendrites, giving them a smooth appearance. Excitatory neurons typically send their axons very widely in the cerebral cortex or out of the cortex to other brain regions. Inhibitory neurons typically have axons close to their soma and dendrites, not extending as far.

Below are some examples of excitatory (spiny) and inhibitory (aspiny, sparsely spiny) neurons from mouse cortex. Take some time to examine the shapes of these neurons before proceeding to the next step of this activity.



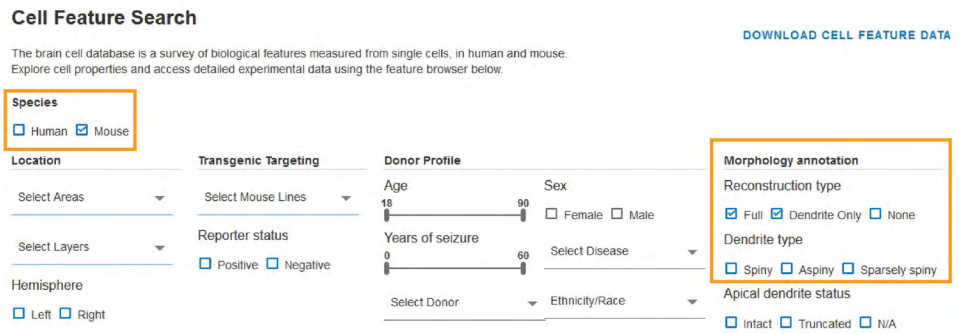


Above: Spiny dendrite.
Right: Aspiny dendrite.

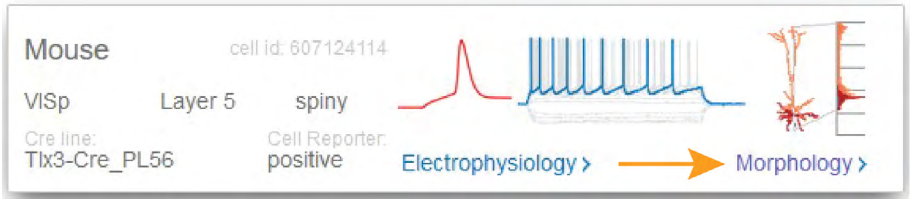
Navigating the Allen Cell Types Database - Morphology/Electrophysiology dataset

Visit the database at celltypes.brain-map.org/data.

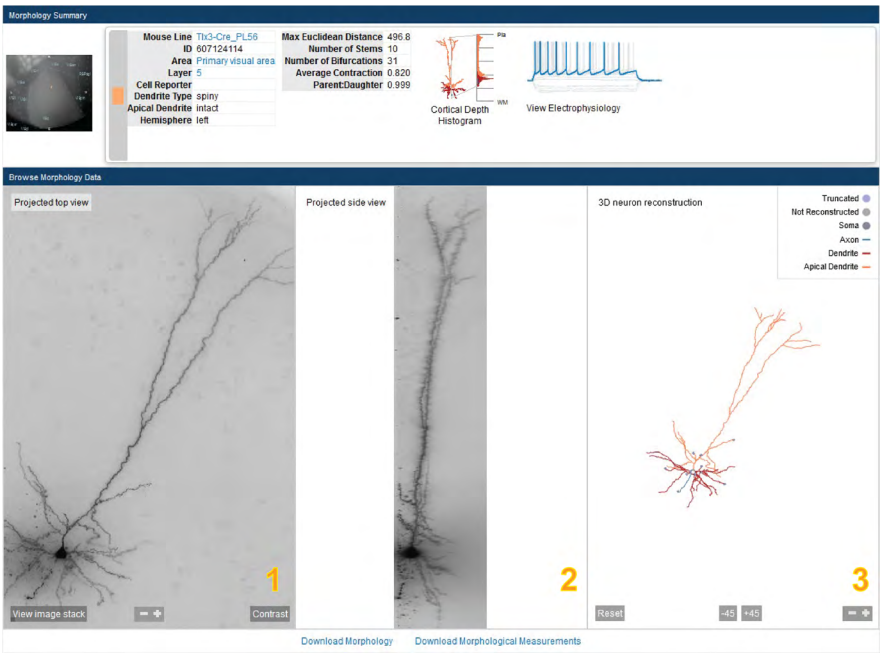
At the top of the main page there are several options for filtering the database, the “Species” and “Morphology annotation” sections marked below by orange boxes are the only ones that will be used for the student questions:



Below the filter options at the top are cell cards with the corresponding cell types data. Each card contains different types of information about the cell. For the purposes of the questions below, focus on the Morphology section only (indicated by an orange arrow). Left click the blue “Morphology>” icon on the right side of the card to expand the morphological features of the cell.



A new window will open with the Morphology summary of the card, where you can see the projected top view (maximum intensity projection, MIP) on the left, the projected side view in the middle, and the 3D neuron reconstruction on the right.



Explore cells from the Allen Cell Types Database

Return to the full list of cells at celltypes.brain-map.org/data. In the filters, under "Species," select mouse. Under "Reconstruction type," select both "Full" and "Dendrite only."

Under "Dendrite type," first select "Spiny" to view excitatory neurons. After viewing some cells, select both "Aspiny" and "Sparsely spiny" to view inhibitory neurons.

What other differences in the shape do you notice between excitatory and inhibitory neurons besides the presence of spines?

Using your knowledge about the function of axons and dendrites and observing the differences in the morphology of excitatory and inhibitory neurons, which would you predict receives information from more cortical layers and which receives information from just a few layers? What do you think this means for the function of the cell?

Now you will compare spiny mouse and spiny human cells.

Return to the filters at the top of the list of cells. Under "Species," select both human and mouse. Under "Reconstruction type," keep "Full" and "Dendrite only" selected. Under "Dendrite type," select spiny.

Note: to differentiate between human and mouse cards, check the species information on the top left of the card.

Do the excitatory (spiny) neurons in human look more similar to the spiny or aspiny neurons of mouse? How are the spiny neurons in human similar to those in mouse? How are they different?

Why do you think excitatory neurons from different species look more similar than excitatory vs inhibitory neurons in one species? What do you think it means about how excitatory neurons function in the brain?

In the space below, draw to the best of your ability a neuron. Include labels for soma, axon, and dendrite.

Go back to your first neuron drawing at the beginning of this lesson. What major changes do you note between your drawings?

Section 2: Naming cell types, quantifying morphology, and multimodal cell types

Introduction and goals for students

- You will learn the complexity in naming neuron types.
- You will learn how histograms are used to quantify neurons for study.
- You will learn about transcriptomic cell types.
- You will observe morphological differences between visual cortex layers, and differences between T-types.
- You will compare differences between cortex layer vs T-types.

As mentioned in section 1, cell types are a useful tool for scientists to classify and compare neurons. The different types of cells are often named based on the feature used to define them. For example, the Parvalbumin (Pvalb) cell subclass contains neurons that express parvalbumin. However, in the past, neurons were named in other ways.

Nuances of Nomenclature and Exploring Data

Historically, neurons with unique or identifiable shapes were often named for the people who first discovered them or for what the cell might have looked like to that scientist. For example, basket cells were named as such because their axons form a very dense ball or basket around the soma. While that name describes some part of the morphology of the cell, it does not provide a complete description of that cell's morphology, including important information such as cell layer.

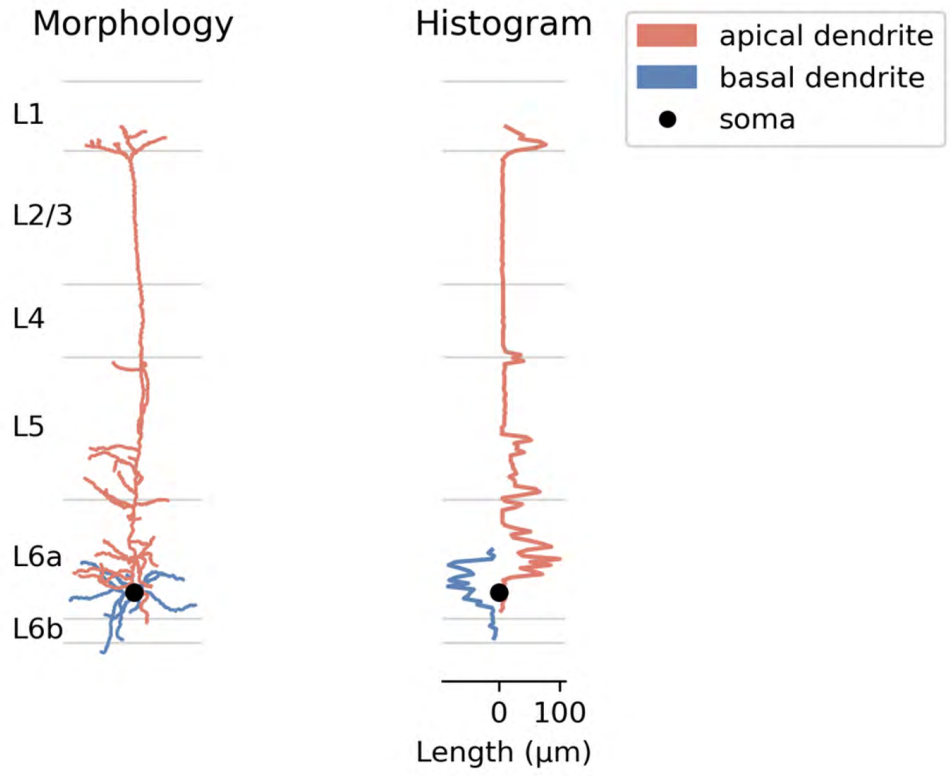
Therefore, neuroscientists are beginning to shift away from these subjective labels to more objective descriptions and quantification, including histograms. The shape of a cell can be described by many quantitative measurements, including but not limited to branch order (number of branch points from soma to tip), thickness of branches, number of tips, number of branches. However, scientists are finding that some of these measurements can vary widely even when comparing two neurons known to be similar (e.g. from the same T-type). One solution to this problem is to use a histogram to represent the data.

A histogram is a way to show how much of something is located along an axis. In this morphology research, we use histograms to quantify and summarize the distribution of a cell's axons and dendrites along the depth of the cortex. Our histogram is built by adding up the length of axon or dendrite within a small vertical step of cortical space. We then plot it relative to the cortical layers. The y-axis on the histogram represents literal space in the brain, and the x-axis represents the total amount of cell length present in that vertical location.

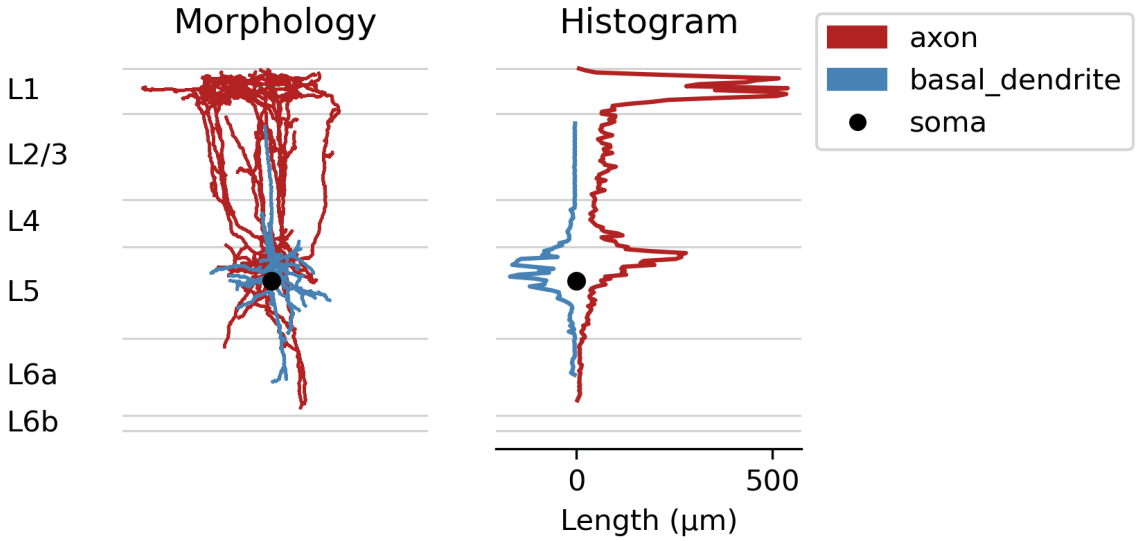
The diagram below shows a cell's reconstruction (on the left) and the same cell's histogram (on the right). The orange line of the histogram represents how much apical dendrite the cell has in each layer.

For example, there is a sideways peak at the bottom of layer 1 (L1), because the cell has more apical dendrite in the bottom of layer 1 compared to layer 2/3 (L2/3), which has near 0. The cell has a soma in Layer 6a, and basal dendrite in Layer 6a and Layer 6b (blue line). If we want to compare how much apical dendrite is in Layer 6a versus Layer 1, we could count the number of branches, but some branches are longer than others. Instead, we can compare the height of the peaks of the histogram to see where this cell has more apical dendrite. This histogram does not show the axon locations, but we can do the same process to quantify axons as well.

By using histograms, neuroscientists can compare multiple neurons at a summary level that captures the broad patterns of the neuron's structure, and important signal does not become lost in superficial differences.



Here is another histogram of an aspiny, inhibitory cell that has no apical dendrite, but does have the axon reconstructed. You can note the high density of axon in layer 1 (L1), therefore the histogram has a huge spike of axon in layer 1. Scientists would look at this histogram and note the second highest spike of axon at the border between layers 4-5. This would be a feature scientists would explore more thanks to this histogram; this feature would of not have been as easily noted if only looking at the reconstruction by itself.



Can you draw a different neuron that would have the same axon and dendrite histogram as this one?

Scientists are now using morphological features, like the histograms above, in combination with other modalities, such as the physiological or genetic attributes of a cell, to further define cell types. One of these other modalities is the transcriptomic data or the RNA that a cell transcribes.

A transcriptome is like a genome: it is a biological record of genetic information, but unlike a genome, which is a record of DNA, a transcriptome is a record of a cell's transcribed RNA. Transcriptomic types (T-types) are a classification system that divides neurons into groups based on the RNA found within the cell. These RNA transcripts are collected by using a very small pipette to attach or "patch" onto the surface of a cell, pulling open a small opening in the membrane. Then the contents of the cell soma are extracted (or pulled out of the soma) using the pipette. The RNA transcripts within the cell are then read (or sequenced).

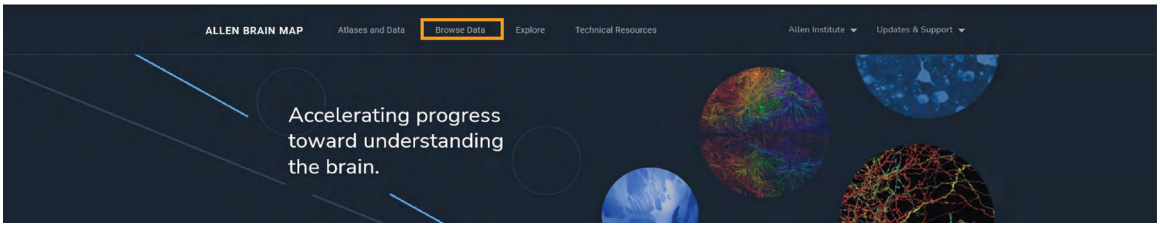
Neurons are categorized into T-types based on which transcripts they expressed and in which quantities. Neurons from the same T-type often have similar morphologies, and neurons from different T-types often have different morphologies. Therefore, the Allen Institute for Brain Science Morphology & 3D Reconstruction team compares the axon and dendrite distributions of neurons both within and across t- types. T-types are a helpful category for comparing morphology because they can be more specific than categories/names used in the past. For example, Martinotti cells are defined by being Somatostatin (Sst) expressing neurons with axon in Layer 1, however, there are multiple T-types with cells that match this description, indicating that Martinotti cells can be further subdivided if you use T-types. This finding shows that it can be helpful to use multiple modalities (morphology & transcriptomics) to define a cell type.

Exploring Data

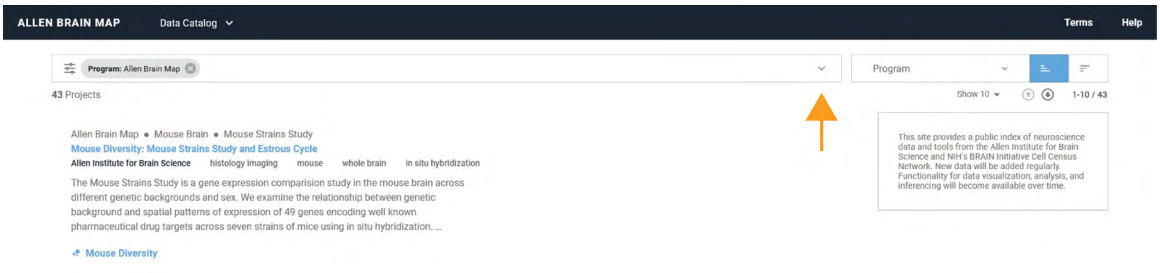
By exploring the database, students will be able to examine cells of different T-types across cortical layers and compare their morphological features.

Visit the Mouse Patch-seq dataset at
knowledge.brain-map.org/data/1HEYEW7GMUKWIQW37BO/summary.

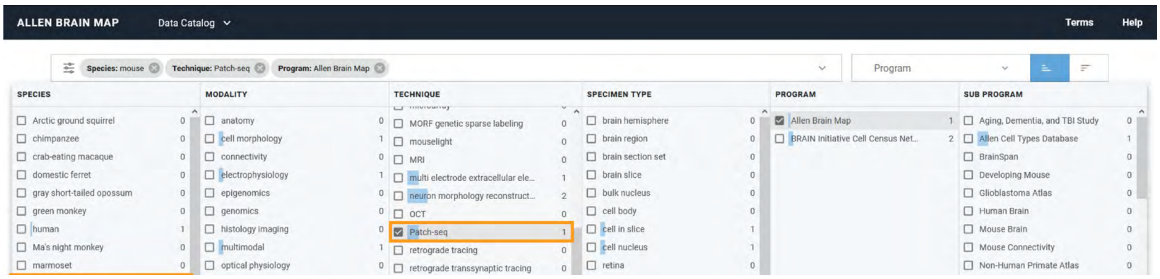
Alternatively, the database can also be reached by visiting brain-map.org. From here, click "Browse data" in the top menu.



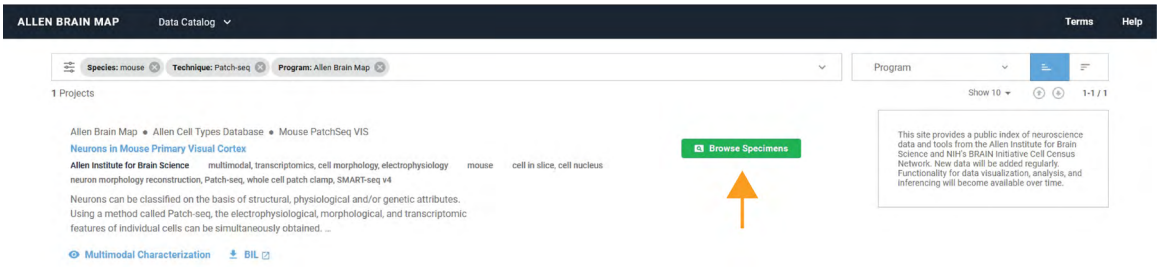
On the top of the Data Catalog page, click on the dropdown menu to the left of “Program.”



Under “Species” select “mouse,” and under “Technique” scroll down to select “Patch-seq.”



Exit the dropdown menu and, to the right of the “Neurons in Mouse Primary Visual Cortex” project, click on the green “Browse Specimens” button to visit the online interactive database. Click on the project name to download the data for offline use.



Database navigation tips

General Navigation

- To see general information and description about the project, navigate using the dropdown menu under Mouse PatchSeq VIS.
- To return to the broader Data Catalog, navigate through the same menu.
- To ask a question about the product or data, click “Help” in the top right of the website and post a question on the Community Forum.
- To return to the Allen Brain Map home page, click the corresponding button in the top left of the website.

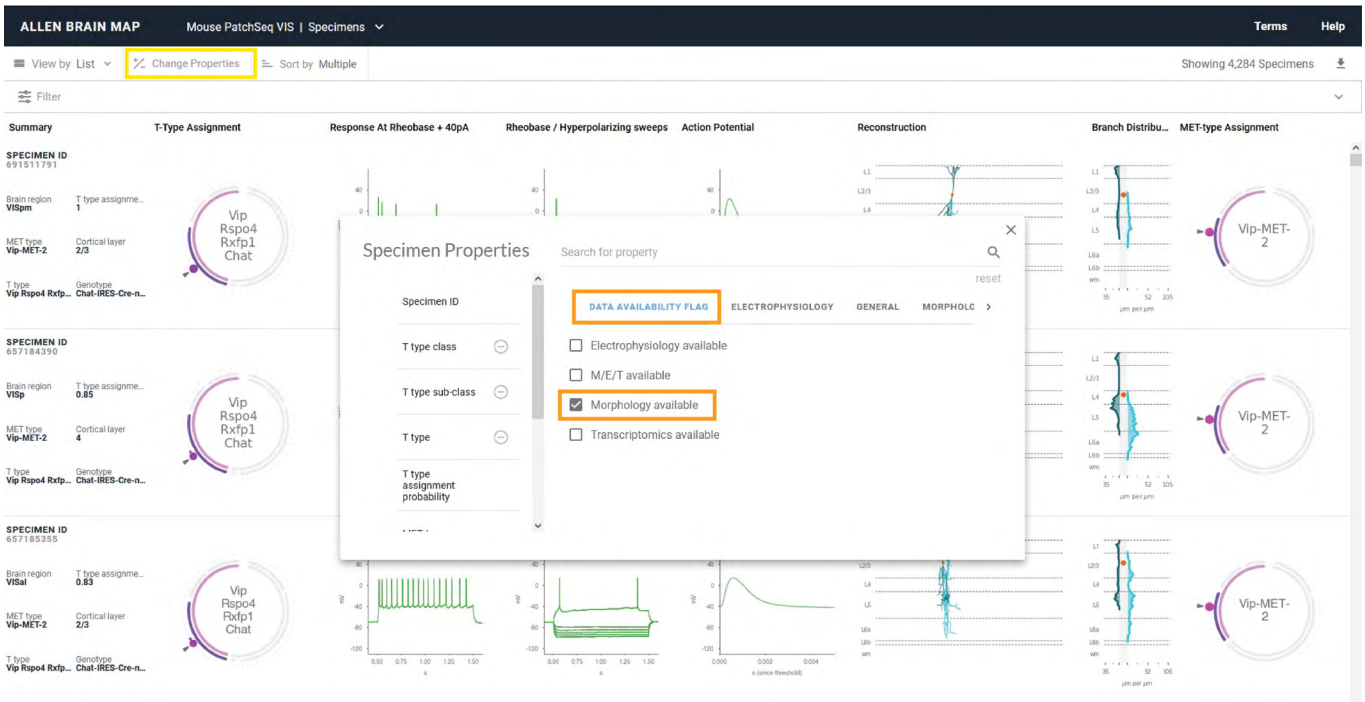
Change Properties – Choose which specimen properties to see and filter with.

- Hover over any property to see a brief explanation.
- Search for any specimen property.
- Select (or de-select) a property from any available category. Selected properties will appear in the list view to the left of the selections.
- Click-and-drag properties in the list view to change the order they appear in.
- Hover over any property in the list view to see a hide button. Click the hide button to remove that property from the list view.
- When you close "Change Properties" window by clicking the "x" on the top right, the selected properties will appear in the "Filter" panel, in the selected order.

Filters

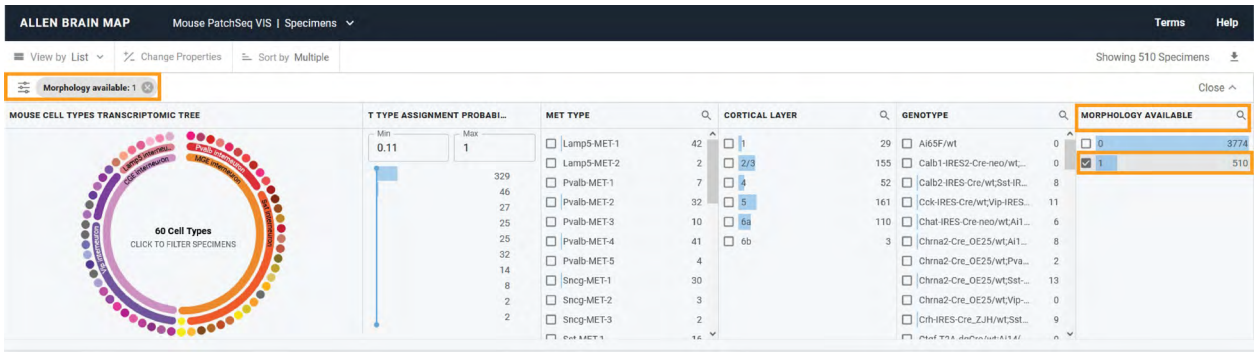
- Open the filter panel by clicking the button in the top section of the website.
- The properties you selected previously will appear here, in the selected order.
- Click any value of the selected properties to filter for specimens with that value.
- The current filter selections will be reflected in a row above the filter panel.
- The total # of specimens matching the current selection is shown in the top right of the website.
- Close the filter panel by clicking the filter button again or the close button at the bottom of the panel.
- Any filter selection can be de-selected by clicking the value again. Alternatively, all selections for a property can be de-selected by clicking the "x" next to the property name in the row above the filter panel.

To start, select Change Properties (yellow). Under Data Availability Flag (orange), check Morphology available (orange). Press x to close the pop up.



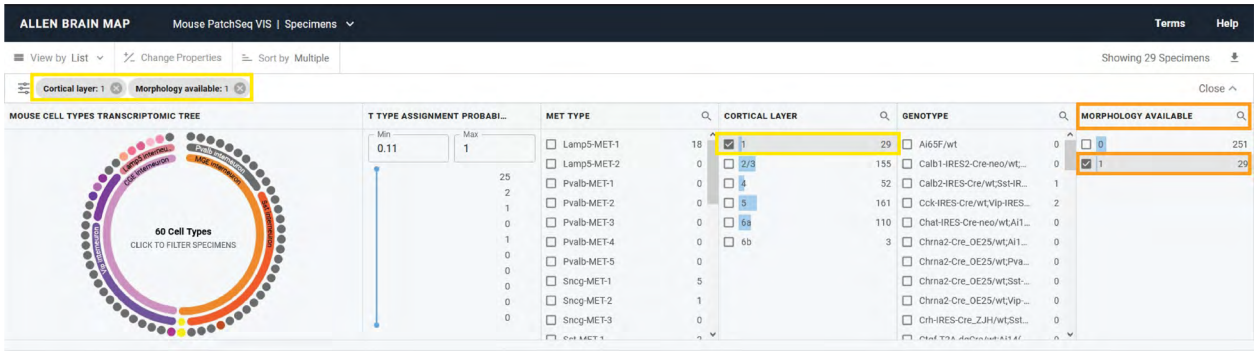
Open the Filter panel by clicking anywhere on the bar with the filter icon on the left and the arrow on the right.

Under Morphology available select 1. Close the Filter panel.



Comparing by cortical layers

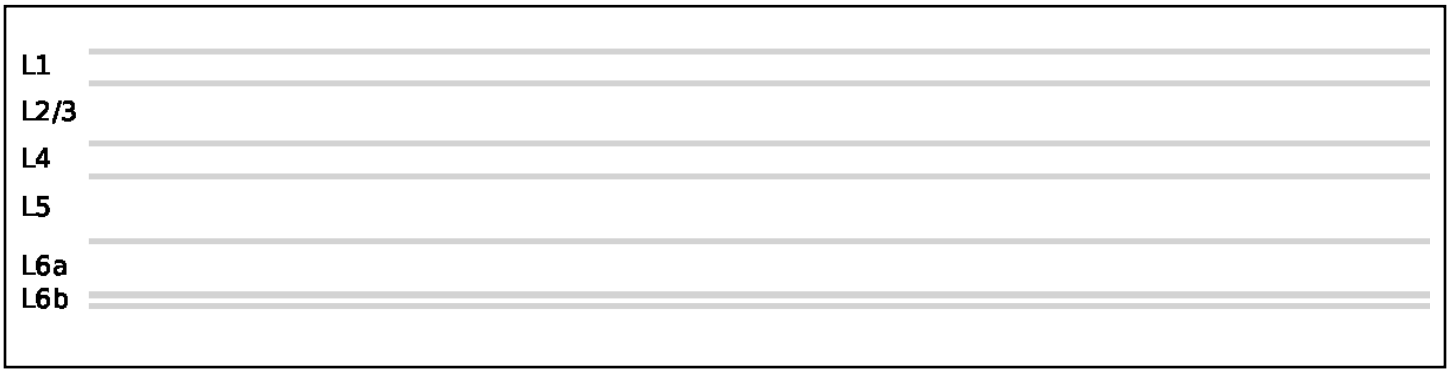
Select Filter. Under Cortical Layer, select Layer 1 (middle yellow box).

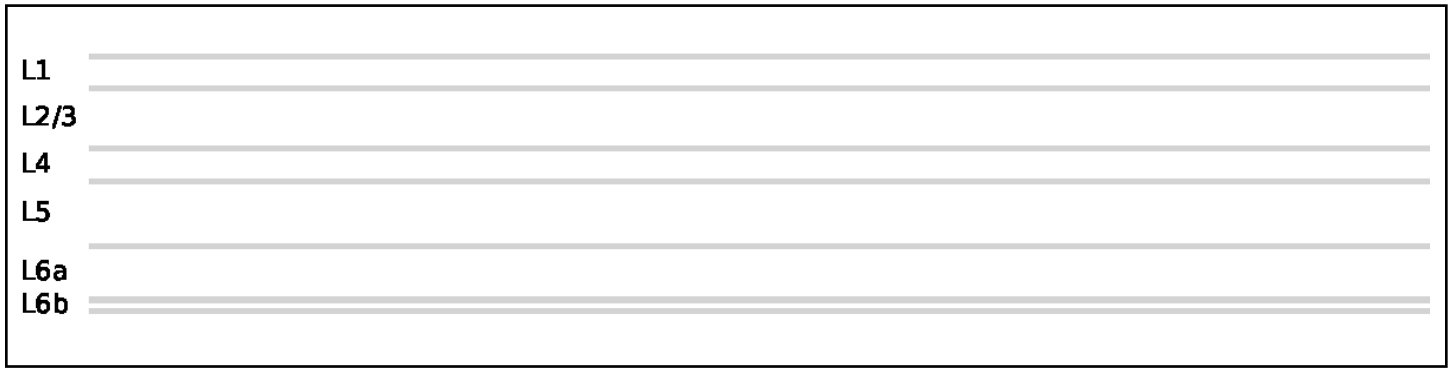


Choose one cell from the list of cells in layer 1. Note the details for that cell in the table below. Do a quick sketch of the reconstruction on one of the blank layer diagrams below. (Remember to label which cell in the table corresponds to which sketch.) Be sure to match the relative location in each layer of axon, dendrite, and soma. Bright blue is axon, dark blue is dendrite, orange dot is soma.

Repeat this process for one cell from each cortical layer.

Cortical layer	1	2/3	4	5	6a	6b
Specimen ID						
Brain region						
T-type						
MET type						





What differences in axon location do you notice across the cells of different layers?

What differences in dendrite location do you notice across the cells of different layers?

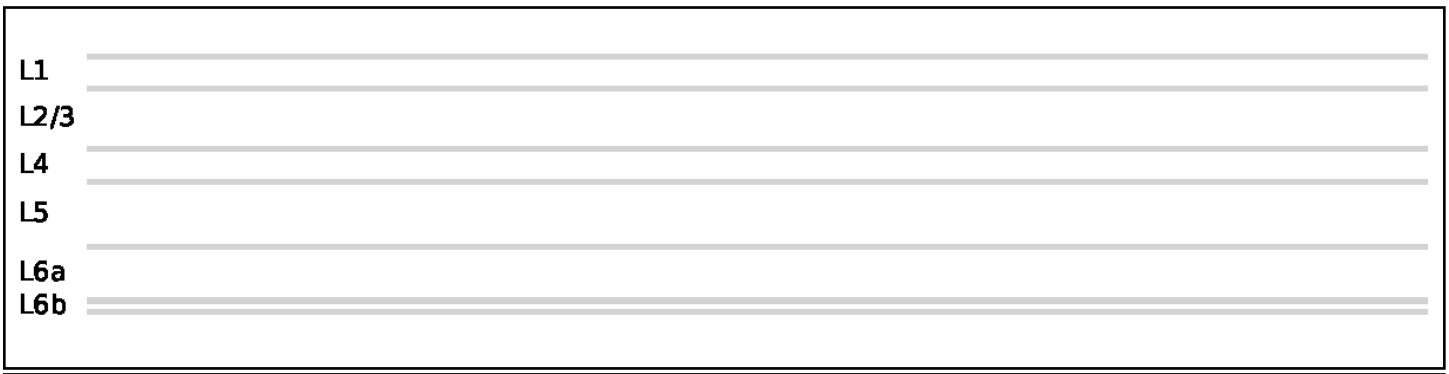
Is there any layer that is very different from the others?

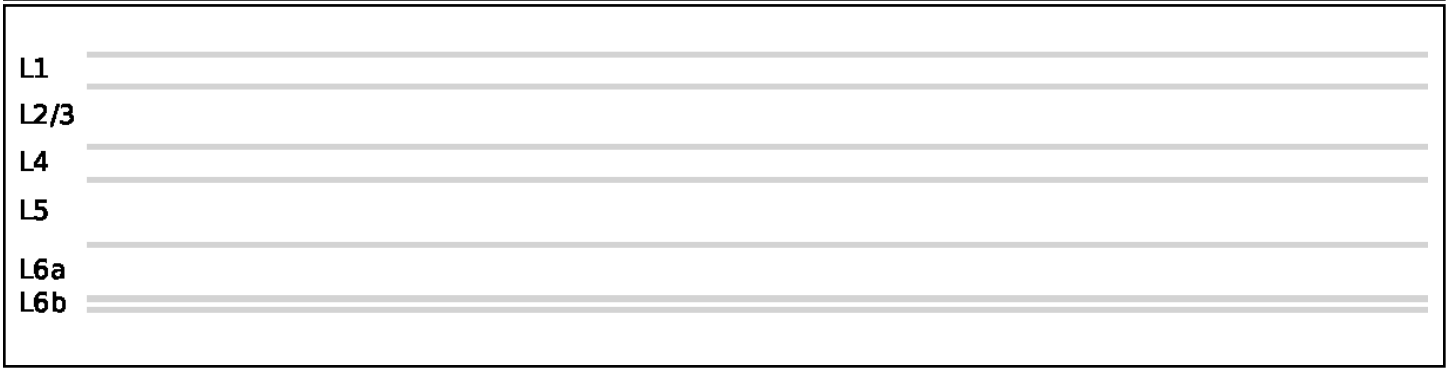
Comparing by T-types

De-select all cortical layers from the filters. Click on the wheel at the left to filter by T-type. Note: when hovering, it will show how many cells are available.

Choose any one T-type. Note the details for that cell in the table below. Do a quick sketch of the reconstruction on one of the blank layer diagrams below. (Remember to label which cell in the table corresponds to which sketch.) Be sure to match the relative location in each layer of axon, dendrite, and soma. Repeat this process with the other T-types.

T-type	VIP	Lamp5	Sst	Pvalb	Sncg
Specimen ID					
Brain region					
Cortical layer					
MET type					





What differences in axon location do you notice across the cells of different T-types?

What differences in dendrite location do you notice across the cells of different T-types?

How do the T-types compare to one another overall?

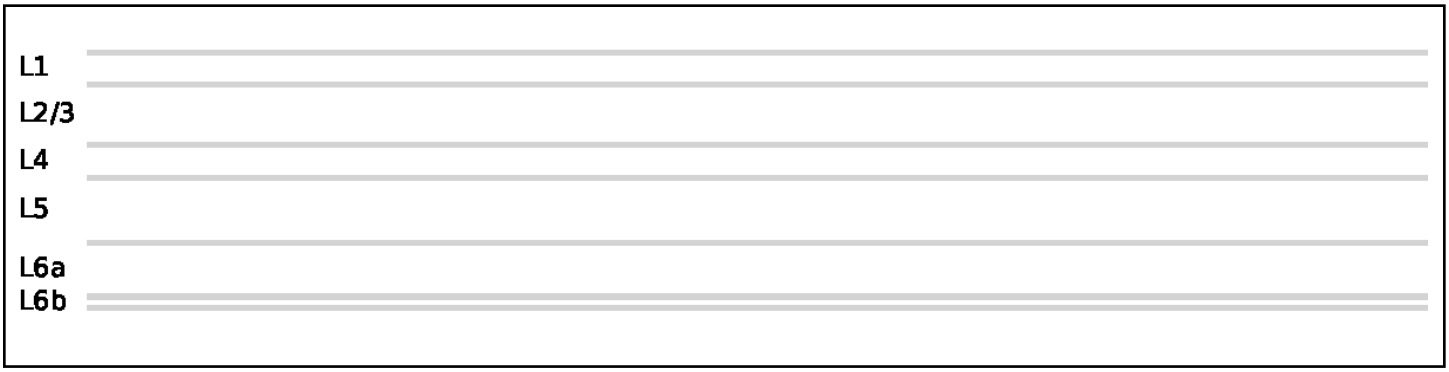
Comparing by both layers and T-types

De-select all cortical layers and T-types from the filters. Choose a T-type from these options: Vip, Lamp5, Sst, and Pvalb.

Your T-type: _____

Set the T-type filters to your cell type. Note the details for three cells of that type from three different cortical layers. Do a quick sketch of the cells on the layer diagram below.

Cortical layer			
Specimen ID			
Brain region			
MET type			



Name two differences you observed between cells of the same T-type from different layers.

1. _____

_____

2. _____

_____

What are the similarities between the cells across layers?

Do you think there is a clear answer whether T-types or layers are more influential on morphology? Explain your answer.

In this activity, we have not discussed electrophysiology at all, but we often use this modality to define cell types. Based on what you know about electrophysiology and cell type definitions, how do you think adding electrophysiology would impact cell typing?

Section 3: Independent research guide

Introduction and goals for students

- This is a basic guide to conducting your own morphology research project using open data from the Allen Institute for Brain Science.
- You will read scientific papers to better understand the foundational knowledge surrounding morphology - suggested review papers are listed below.
- You will learn how to plan a scientific project like scientists do.

Morphology Background & Research Plan

What you will study will be determined by your interests. Hopefully, Section 2 of this lesson helped you see which variables, such as cortical layer & T-types, could influence the morphology of neuron. Using the open Mouse Patch-Seq data found on **brain-map.org**, explore additional questions for your project.

Think about any details that stood out to you. Were there any cell features you found particularly interesting? What are some morphological qualities you could further explore? Consider possible comparisons you could make.

The Allen Institute shares its data freely and publicly so scientists, the public, and students like you can use it in their work. You may also have access to other morphology data through your research mentors.

Here are some papers to give you more background information.

Scientific articles:
Gouwens, N.W., Sorensen, S.A., Berg, J. et al. (2019). Classification of electrophysiological and morphological neuron types in the mouse visual cortex. Nat Neurosci 22, 1182–1195. https://doi.org/10.1038/s41593-019-0417-0

Gouwens, N. W., Sorensen, S. A., et al. (2020). Integrated Morphoelectric and Transcriptomic Classification of Cortical GABAergic Cells. Cell, 183(4), 935–953.e19. https://doi.org/10.1016/j.cell.2020.09.057

Review articles:
Petilla Interneuron Nomenclature Group, (2008). Petilla terminology: nomenclature of features of GABAergic interneurons of the cerebral cortex. Nature reviews. Neuroscience, 9(7), 557–568. https://doi.org/10.1038/nrn2402

DeFelipe, J., et al. (2013). New insights into the classification and nomenclature of cortical GABAergic interneurons. Nature reviews. Neuroscience, 14(3), 202–216. https://doi.org/10.1038/nrn3444

Here are some ideas of the types of research questions you could use in this project:

- Is there a difference between the axon distribution patterns of specific cell classes in human vs mouse?
- How does the morphology of cells within a given T-type change depending on soma depth across layers vs. within a layer?
- How does soma shape and size relate to neuron morphology?
- What are the morphological differences in cells labeled 'sparsely spiny' compared to 'aspiny'?
- How does the angle of an excitatory neurons' apical dendrite differ depending on cell soma location, particularly in Layer 6b?

Once you have decided on your question, determine your research plan. This plan will include your hypothesis, reference material supporting your hypothesis, the variables you are testing, what your controls are, and how you will measure or quantify group differences.

Here are examples for the plan if you wanted to ask the question: **how do layers in visual cortex influence apical direction of excitatory neurons with somas in layer 6b?**

Background reading: For this research question, you would need to read textbooks or papers that explore what cortical layers are, how they form, and what their importance is. Then you could read about the formation and function of apical dendrites. Next, you could read any papers that study apical dendrite morphology, such as Ledergerber and Larkum 2010 (pubmed.ncbi.nlm.nih.gov/20881121).

Research question: For this example, we already have a research question. Make sure that your research question is testable, meaningful, and has not already been answered by prior work.

Variables: For this research question, you might examine cell soma location (layer) as the independent variable and apical dendrite angle as the dependent variable.

Measuring your variables: For this research question, you might measure the branch length and branch order of apical dendrites of different cells.

Collecting your data: You can use the Allen Institute's open morphology data - both the raw data and the reconstructions - in your independent research. You can download completed reconstructions from **celltypes.brain-map.org/data** in .swc file format. For this research question, we will need reconstructed cells and we will measure and record branch length and branch order.

If you want to generate your own reconstructions of your own data or from unreconstructed cells from the Allen Institute, see our free, open-source reconstruction software along with instructions and protocols to reconstruct at **brain-map.org/explore/toolkit/morpho-reconstruction**. You may have access to a lab or work with an investigator who is willing to share image stacks of neuron morphology you can use, or you can use Allen Institute data with no special permission.

Analyzing your data: For this research question, you will need to quantify branch length and order. See **brain-map.org/explore/toolkit/morpho-reconstruction/vaa3d-mozak/** for guidance on how to run analyses on your swc files to calculate branch length and comparison.

Conclusions: What conclusions would you draw after comparing the morphology of different cells? What future experiments would you suggest to further test your conclusions.

Use the worksheet on the next pages to help you outline your research plan and figure out how to collect your data.

After your analysis is complete, write down your results and present either as a paper or a scientific poster!

The Allen Institute supports researchers and students like you to present research using its data at conference or publish their findings in journals. Please see the Terms of Use (alleninstitute.org/legal/terms-use) for details on how to properly use our data and Citation Policy (alleninstitute.org/legal/citation-policy) for how to cite it. If you publish using our data, we invite you to share your work by email at communications@alleninstitute.org or on social media (@alleninstitute).

Initial research question: _____

Background research: List the papers you plan to read to help you refine your question, learn the context of your work, and develop your hypothesis. This will include both scientific and review articles.

Refined research question: _____

Hypothesis: _____

Variables (note whether each variable is independent or dependent): _____

_____

_____

Measuring your variables (what will you measure for each): _____

_____

_____

Collecting your data - how will you collect your data? How will you keep track of it so it can be analyzed later?

Analyzing your data - what analyses do you plan to run and how will you run them (i.e., with what software)?

Conclusions: