

Lecture & Lab: Cell Types - Transcriptomics, Morphology, and Electrophysiology

This instructor guide contains:

- Overview
 - Summary
 - Outline
 - Learning Objectives
 - Required Instructor & Student Materials
 - Prior Knowledge Requirements
- Supplementary Materials
 - Optional Pre-Reading/Background Information
 - Optional Test Questions
- Lecture Notes
- Extension 3 Handout

OVERVIEW

Summary: This slide deck is intended to provide a high-level overview of cell types in the brain and the modern research of single-cell RNA sequencing. It is accompanied by a virtual lab exercise that mimics how scientists use open science tools and databases in their research process. The lecture is divided into one main section (Cell Types & Transcriptomics), followed by two optional extensions (Morphology and Electrophysiology). Each division contains a corresponding virtual lab activity that mimics how scientists use public databases and tools to enhance their research.

These slides are built to be modular, allowing you to incorporate which parts into your class.

Outline:

- **Base Lesson: Cell Types & Transcriptomics**
 - Part 1: Cell Type Overview
 - Part 2: Patch-Seq Overview (optional supportive slides)
 - Part 3: Transcriptomics Overview
 - Part 4: Color Analogy
 - Part 5: Lab Activity – Exploratory
- **Extension 1: Morphology**
 - Part 1: Morphology Overview
 - Part 2: Cortex Anatomy & Tilt (optional supportive slides)
 - Part 3: Lab Activity – Comparing
- **Extension 2: Electrophysiology**
 - Part 1: Electrophysiology Overview

- Part 2: Violin Plots (optional supportive slides)
- Part 3: Open Science & Public Tools (optional supportive slides)
- Part 4: Lab Activity – Investigating

Base Lesson can be done independently. **Extensions 1 and Extension 2 require the Base Lesson** to be taught first to explain cell types and how transcriptomics is what our cell types are grounded on. You may select to do Base Lesson, Base Lesson + Extension 1, Base Lesson + Extension 2, or Base Lesson + Extension 1 + Extension 2.

Estimated duration per section/extension: 60 minutes (smaller classes, ~20 students) to 120 minutes (larger classes, ~100 students)

Learning objectives:

Base Lesson: Cell Types & Transcriptomics

- Describe what a cell type is, how to define it, and why it is important.
- Comprehend the multiple modalities definition of cell types, and critically analyze the challenges with this approach.
- Conceptualize the various levels of categorizing cell types.
- Explain transcriptomics/morphology/electrophysiology; illustrate a selected cell type's transcriptomic profile and how it relates to the cell's function.
- Reflect on the current challenges in the neuroscience field related to the definition and application of cell types.

Extension 1: Morphology

- Summarize the evolution of measuring and describing neuron morphology.
- Critique the historical naming of neuron morphology.
- Explain how histograms visualize the density of axon and dendrite.
- Attribute the different morphological properties of GABAergic and glutamatergic neurons.
- Create morphological histograms and compare across multiple datasets.

Extension 2: Electrophysiology

- Explain where the standard action potential curve fits in when looking at firing responses.
- Interpret the intrinsic firing properties of firing response.
- Attribute the different electrophysiology properties of GABAergic and glutamatergic neurons.
- Analyze violin plots and interpret raw electrophysiology data to draw independent conclusions.
- Critique the scientific field on the ability for data to be open via availability and accessibility.

Mode of instruction:

PowerPoint **lecture** with discussion questions throughout. Lab activity requires a **computer** with internet access but no special software.

Instructor materials for this lecture:

- PowerPoint
 - For more details about the slides, see the notes section in the PowerPoint file. For ease of access, the same notes are added to the bottom of this document.

Student materials for this lecture:

- Laptop or computer with internet access
- (Extension 1: Morphology) Paper and writing implements
- (Extension 2: Transcriptomics) Handout for lab activity, which can be shared digitally. Content is also in the slides.

Prior knowledge required for this lesson:

For Base Lesson (Cell Types & Transcriptomics): Basic cell biology of cell structures, the central dogma of biology, and what glutamatergic & GABAergic means in neurons.

For Extension 2 (Morphology): What neurons are, the parts of neurons, basic neuroanatomy.

For Extension 3 (Electrophysiology): Basic of action potentials – this extension is designed to expand standard action potential lessons that you have taught previously.

SUPPLEMENTARY MATERIALS

Optional Pre-Reading & Background Information (for either yourself or students)

- Scientific American: *A Cell Atlas Reveals the Biodiversity Inside Our Head*
<https://www.scientificamerican.com/article/a-cell-atlas-reveals-the-biodiversity-inside-our-head/>
- Cell Review: *What is cell type and how to define it?*
[https://www.cell.com/cell/pdf/S0092-8674\(22\)00783-8.pdf](https://www.cell.com/cell/pdf/S0092-8674(22)00783-8.pdf)
- YouTube Playlist: Any or all of these videos
<https://www.youtube.com/watch?v=GotdGM81x4A&list=PLN-QyZNMh3PuRrdMrmHgfMnsYKWMCWPTf>

Optional Test Questions

Base Lesson: Cell Types & Taxonomies

*bold is correct answer

- Choose from these properties that could be used as cell types definition
 - Transcriptome**
 - Genome
 - Cognitive
 - Anatomical
- Studying transcriptomics is better for studying cell types than genomics for all the following reasons except:
 - DNA would be the same for every cell
 - Measure gene expression per tissue
 - DNA is more commonly studied than RNA**
 - Ribosomes transcribes the RNA for protein expression
- What benefit does single-cell RNAseq have over bulk RNAseq?
 - Single-cell RNAseq is faster
 - Single-cell RNAseq maintains individual cells' transcriptomic identity**
 - Single-cell RNAseq measures only selected genes
 - Single-cell RNAseq is cheaper
- Cell types are classified into 3 hierarchical levels. What is the correct hierarchy of these classifications, from the most general to the most specific?
 - Class, Subclass, Cell Types**
 - Cell Types, Subclass, Class
 - Cell Types, Class, Subclass
 - Subclass Class, Cell Types
- List a challenge in the neuroscience field when it comes to cell types research and how you would fix it.

[here are some potential problems, as long as the student tries to write solution, then it is correct as we want to encourage new ideas]

 - Problem: Getting everyone to agree on names. Potential solution: conference where we people meet and agree, or a standards body like the International Astronomical Union for naming space objects
 - Problem: People use different modalities to study cells. Potential solution: have a "Rosetta stone" where we connect all modalities together
 - Problem: It is expensive to study a single cell type. Potential solution: have free, open science data like the Allen Institute

Extension 1: Morphology

*bold is correct answer

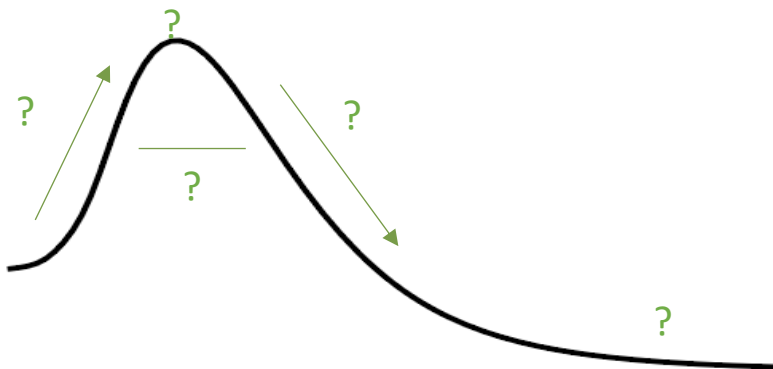
- Modern neuroscience studies neuron morphology in the following ways except:

- a. **Qualitatively describing the features of the neuron**
 - b. Quantifying numerical features of the neuron
 - c. Look at the main protein expression
 - d. Look at the projection pattern
2. Histograms are the way to quantify the shape of a neuron by:
 - a. Measuring dendrite density over layers
 - b. Visualizing how deep the neuron is across layers
 - c. The tilt of the neuron
 - d. **A & B**
 - e. All above
 3. Cell types being named after transcriptomic properties is beneficial because:
 - a. Gives credit to the scientists who discovered it
 - b. Distinctive visual features is an easy way to identify a cell
 - c. It has already been adopted across the entire scientific community
 - d. **Helps brings consensus to cell type label**

Extension 2: Electrophysiology

*bold is correct answer

1. Label the 5 features of the action potential



2. GABAergic and glutamatergic neurons are distinctly different in all the following ways except
 - a. **GABAergic firing patterns are more homologous with each other while glutamatergic has more diverse range**
 - b. Glutamatergic axons project the entire brain, while GABAergic are connected locally
 - c. At the subclass level, GABAergic are defined by the main protein expression while glutamatergic are defined their projection pattern
 - d. GABAergic are inhibitory neurons while glutamatergic are excitatory neurons

3. Why do scientists practice open science?
 - a. Their funders require it
 - b. Promote transparency
 - c. Increase reuse
 - d. To profit
 - e. A & B only
 - f. A & B & C only**
 - g. All the above

4. (Open-ended): List two benefits and two challenges of open science
 - a. Potential benefits: comply with federal requirements, encourages reuse, helps increase equity & lower barriers, helps fix reproducibility crisis, helps solve complex problems
 - b. Potential challenges: expensive, takes dedicated staff, unsure how to retroactively fix old data, not glamorous, takes a significant amount of time, requires upkeep with updates

BASE LESSON: CELL TYPES & TAXONOMIES, PART 1: CELL TYPES OVERVIEW – LECTURE NOTES

Slide #	Notes
2	<p>Common metaphor for describing cell types is parts list – and that part list because knowing what “healthy part” is necessary to figuring out what part is responsible for causing a disease/disorder</p>
3	<p><i>Slide has animation</i></p> <ul style="list-style-type: none"> • The diversity of brain cell types was one of the earliest observations in modern neuroscience and continues to be one of the central concerns of current neuroscience research. • The taxonomy of cell types in the brain is ultimately required to understand how neural circuits evolved to underpin complex behaviors. • In broad terms, evolution is closely tied to genetics, development and environment. And took half billion years to assemble these neurons, that can be classify as set of cell types. • Cell Types can be categorized by their properties that influence a cell’s spiking behavior, and therefore affect the organism’s behavior & function as whole.
4	<p><i>Slide has animation</i></p> <ul style="list-style-type: none"> • The task is similar to Y maze where the mouse goes left then is trained to go right. What is recorded in blue and red is the firing rating of the neurons for the given cell type. <p>Summary from Figure 3 Kim 2016</p> <ul style="list-style-type: none"> • Raster plots and spike density functions during four different behavioral stages (trial onset, sample target, delay, and reward; the animal’s approximate position at the onset of each stage is indicated by gray shading on top) • The trials were divided into correct left choice (deep blue), incorrect left choice (light blue), correct right choice (deep red), and incorrect right choice (light red) trials and plotted separately.
5	<ul style="list-style-type: none"> • 84 donors having range of AD severity, all studied within the medial temporal gyrus • Each bar is a different cell type to measure the difference between abundance of said cell type in AD vs healthy

	<ul style="list-style-type: none"> You can notice how different severity of the cell types effects differently - if you group all the cells by the subclass
6	<i>Slide has animation</i>
7	<p><i>Slide has animation</i></p> <ul style="list-style-type: none"> Historically, anatomy & morphology were the standard for cell types. Cajal, Golgi, Nissl and many other scientists have been drawing neurons for over 200 years. As technology advanced, we added electrophysiology via methods such as patch-clamp. Technology is still advancing, and in future we hope to add connectivity as another cell type defying property, but we are not quite there. Cornerstone of defining cell types though is gene expression data.
8	<p><i>Slide has animation</i></p> <p>Optional discussion question: what do you think will be the next property in studying cell types? One answer is proteomics = study of proteins</p>
9	<ul style="list-style-type: none"> We want to combine all those properties to have multi-modal definition We measure gene expression via RNA sequencing (RNA-seq). Four key strengths of RNA sequencing (Zheng 2019): <ul style="list-style-type: none"> Can identify transcripts in species without genomic sequences Less noise than other microarray-based methods More sensitive than microarray-based methods Wider application than other microarrays We have single-cell RNA-seq datasets for 10^5 neuron & non-neuronal cells across mouse (Tasic 2018). What is very interesting about RNA datasets that it sorts cells very well, more than you expect In total, we measure all the other properties of anatomy, morphology, electrophysiology, connectivity and RNA sequencing to have multi-modal definition of cell types.
11	<p>Patch-clamp: good overview here: https://www.leica-microsystems.com/science-lab/life-science/the-patch-clamp-technique/</p> <p>Patch-Seq overview: https://www.jneurosci.org/content/41/5/937 Huge driving force in the Patch-Seq wide widespread use is the increased resolution to RNA sequencing</p>

BASE LESSON: CELL TYPES & TAXONOMIES, PART 2: PATCH-SEQ OVERVIEW –
LECTURE NOTES

Slide #	Notes
13	<p>Huge driving force in the Patch-Seq wide widespread use is the increased resolution to RNA sequencing</p> <p>Patch-clamp good overview here: https://www.leica-microsystems.com/science-lab/life-science/the-patch-clamp-technique/</p> <p>Patch-Seq overview: https://www.jneurosci.org/content/41/5/937</p>
14-22	<i>Slides to be played sequentially to show Patch-Seq animation</i>
14	Here have example of neuron in some tissue
15	Apply suction to the pipette, which causes the cell membrane to ...
16	... rupture, giving us direct access to the interior of the cell. At this time, the fluid inside the pipette begins to diffuse into the cell
17	<p>And while the cell is filled, we inject electrical current into the cell to characterize its responses</p> <p>In this process we learn things like:</p> <ul style="list-style-type: none"> - How much current is needed to make this cell spike - How fast can it spike - What is the shape of its action potentials
18	After a few minutes, we begin to retract the pipette with the nucleus attached.
19	As we pull away, the cell re-seals around the pipette, leaving its original morphology intact and ready to be imaged.
20	Finally, the nucleus is removed completely and sent to be sequenced.
21	To clarify how the RNA is extracted from nucleus, there are two major methods of extracting (see slide 28), then nucleus is reverse transcribed, we amplify cDNA and can sequence it.

**BASE LESSON: CELL TYPES & TAXONOMIES, PART 3: TRANSCRIPTOMICS
OVERVIEW LECTURE NOTES**

Slide #	Notes
26	<p>Other benefits in doing RNAseq:</p> <ul style="list-style-type: none">• High throughput (millions of cells)• Many features (~30,000+ genes)• Direct links to genome (disease/genetic tools)• Works with postmortem tissue• Universal across organs and species• Many analysis tools
28	<p>Think of cells as fruit. There are two methods in eating that fruit</p> <ol style="list-style-type: none">1. Bulk: blend all that fruit up in smoothie (aka extract transcriptomics from tissue)2. Single cell: keep each fruit as individual piece (aka extract transcriptomics from each cell) <p>Bulk is a cheaper and faster way but you lose the identity of all the fruits you drank. Single is more time-consuming and expensive to have all that fruit but you know each fruit's identity</p> <p>We are focusing in on single-cell method as helps keeps cell's identity.</p>

**BASE LESSON: CELL TYPES & TAXONOMIES, PART 4: COLOR ANALOGY –
LECTURE NOTES**

Slide #	Notes
30-45	<i>Slides to be played sequentially to show color analogy animation, just click after each animation and will play by itself</i>
41	Subclasses are defined as: Inhibitory: canonical marker genes Excitatory: layer specificity and long-range projection patterns
45	Marker genes are not necessarily the most prominent genes. They are a minimal set based on the NS Forest method of marker gene selection.

BASE LESSON: CELL TYPES & TAXONOMIES, PART 5: LAB ACTIVITY
EXPLORATORY – NOTES

Slide #	Notes
49	<p>Some background on the database you are about to explore – more info on slide 99 “Example of multiple groups working together, with all data being open: BICCN”</p> <ul style="list-style-type: none">• Cell Type Knowledge Explorer comes from a multi-group effort to map the primary motor cortex• Motor cortex was chosen as the first area as it is highly conserved across species• In order to make the data more accessible, this research tool – Cell Type Knowledge Explorer was created to help scientist with their research
51	<p><i>Slide has animation</i></p> <p>Have students pair or group up, and let them select a color. Then reveal the corresponding cell type and have them write it down.</p>

EXTENSION 3: MORPHOLOGY, PART 1: MORPHOLOGY OVERVIEW –
LECTURE NOTES

Slide #	Notes
57	Size of 2D image is about ~2mm x ~2mm.
58	Here is quick video of 3D reconstructed neuron load on the software
59	Automated software extract the reconstruction file (which is just file of hundreds of points with their xyz coordinates), and the software reads those coordinates to measure morphology features
60	An analogy is we went from describing shapes like Rorschach test to having those shapes described with numerical precision such as length, color shade, height, etc •
61	Automated Python script (that is open source) extract these features from the reconstruction files
62	The histogram is built by adding up the length of axon or dendrite within a small vertical step of cortical space. 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 locations. Neuron histogram on left: one Sst Htr1a cell. Neuron histogram on right: sum of histogram for multiple Sst Htr1a cells, with the dark line within the gradient being the cell on the left exact histogram
63	Glutamatergic neurons don't have prominent gene marker like GABAergic does. ET is updated name for the PT. ET projects bilaterally. IT projects contralaterally (Saiki 2017)
64	Can see how these neurons are glutamatergic but their dendrite morphologies are different. L2/3 IT, or intratelencephalic projecting, neurons mainly have dendritic branches in L1-L2/3, while L4/5 IT neurons have fewer dendritic branches that largely appear in superficial L5. While L5 ET, or extratelencephalic projecting neurons, have many more dendrites, especially in L5. Axon is not measured here as its hard to capture full axon for glutamatergic cells

65	<p><i>Slide has animation</i></p> <p>You may have noticed the axon was missing from previous slide's histograms. As mentioned, glutamatergic neurons project across the entire brain.</p> <p>The image on the left is the morphology of L2/3 IT, while the right image is of multiple whole mouse brain reconstructions, with the bright blue neuron being from the same brain region. [click to start animation], you can see how much the axon is missing from the morphology</p>
66	<p>Sncg was previously grouped with Vip but have found to be genetically distinct</p> <p>Lamp5 was previously named for cells that express 5-hydroxytryptamine receptor 3A but lack Vip ($Htr3a^+/Vip^-$) (Tremably et al 2016, Tasic et al 2018)</p>

**EXTENSION 1: MORPHOLOGY, PART 2: CORTEX ANATOMY & TILT –
LECTURE NOTES**

Slide #	Notes
73	<p>This is a very, very simplified version of fixing tilt with streamline. A lot of this is dependent on the CCF (common coordinated framework), which is a complicated topic within itself. Scientist Lydia Ng has great post about this on our community forum (https://community.brain-map.org/t/ccfv3-highlights-tilting-at-the-cortex/1000) along with our CCF paper (https://www.cell.com/cell/fulltext/S0092-8674(20)30402-5?_returnURL=https%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2FS0092867420304025%3Fshowall%3Dtrue).</p> <p>If you or a student has interest in the intersection of math and anatomy, this is great place for them to research more.</p>

EXTENSION 2: ELECTROPHYSIOLOGY, PART 1: ELECTROPHYSIOLOGY OVERVIEW-
LECTURE NOTES

Slide #	Notes
80	The goal here is to assess a diverse array of electrophysiological features while still enabling comparison across all cells
81	<i>Slide has animation</i> And this is where the familiar action potential curve comes from.
82	Adapting: Fires quickly in the beginning and slows down Burst: Burst of action potentials in beginning, and then normal regular spiking afterwards burst Fast-spiking: High number of action potentials
83	See how there are distinctly different types of firing responses. For Glutamatergic, see qualitatively similar with the majority of adapting and burst firing patterns For GABAergic, see how more diverse in properties - there is still adapting, and burst is slightly different with larger bouts of burst, irregular, and fast-spiking. The fast spiking is most well known in GABAergic.
84	See how the types of firing patterns is even further breakdown when looking at the subclass. Such as Sst Chodl having burst while Pvalb has fast-spiking
85	<i>Slide has animation</i> Go back into zoomed version of the action potential, we can group by class and compare them within in class. We can see how GABAergic have more diversity than glutamatergic. How do we compare that quantitatively?
86	Here are some of the features that can be measured from electrophysiology. There are ~50 features in total that are extracted. All the features are extracted automatically and done in free package on https://github.com/AllenInstitute/ipfx . Highlighting some features important to distinguish cell types: <ul style="list-style-type: none"> • Degrees of sag – mediated by HCN channels • Firing rate - number of action potentials per sec/100 ms • Interspike intervals: time difference between first and last spiking, tells us if cell is accommodating or burst like

	<p>properties.</p> <ul style="list-style-type: none"> • Most important is the kinetics of action potential (aka how wide is the stroke, what is the peak, what is the upstroke and downstroke)
90	<p>Now using cell types with transcriptomic data as anchor and linking it to multiple modalities like ephys, we can ask what genes are responsible for these diverse ephys properties.</p> <p>For example, we know that Na⁺ is responsible for the upstroke and K⁺ is responsible for the downstroke of action potential, we can start asking what subunits of proteins are responsible for this curve. And further explore if the difference in upstroke & downstroke between cell types is from variation in gene expressions, such as those responsible for Na⁺ and K⁺.</p>
11	<p>Patch-clamp: good overview here: https://www.leica-microsystems.com/science-lab/life-science/the-patch-clamp-technique/</p> <p>Patch-Seq overview: https://www.jneurosci.org/content/41/5/937 Huge driving force in the Patch-Seq wide widespread use is the increased resolution to RNA sequencing</p>

EXTENSION 2: ELECTROPHYSIOLOGY, PART 4: OPEN SCIENCE & PUBLIC TOOLS –
LECTURE NOTES

Slide #	Notes
96	<p><i>Slide has animation</i></p> <ul style="list-style-type: none"> • You found a promising paper from google scholar, you go to open the paper and paywall. And even to make paper open access, often the cost is for the lab to make it available for everyone • Your Institution pays the expensive paywall, and you get the paper. You are excited by one of their findings and want to replicate the experiment, however methods sections are typically very broad and not protocol • Dishearten you decide to just look at the raw data and see if you can learn more that way. However no raw exist, which unfortunately very common
97	<p>Luckily the field is getting better with make data available.</p> <ul style="list-style-type: none"> • Instead of paywalls, more papers are publishing free versions such as on Bio Archive • Growing push to publish full protocols by making white papers and posting SOPs on protocol.io • Database banks like Brain Image Library or NEMO, help folks deposit their datasets
98	<p>However, the next key phase in open science is making sure it is accessible – a lot work still needs to be done here. Accommodations include reduce jargon to visually impaired alternatives.</p>
99	<p>In the end, why does it matter...</p> <ul style="list-style-type: none"> • Ask the class if they heard the reproducibility crisis • Can open discussion how giving free access, lowers the barrier of entry • Ultimately, large complicated problems will not be able to solve without us working together. Hence initiatives like BRAIN is important...

Extension 2 - Lab Activity: Investigating Handout

“These ‘Sst Chodl’ neurons are rare and, based on expression of specific marker genes, correspond to the only known cortical interneurons with long-range projections. Recent studies using the multimodal cell phenotyping method Patch-seq confirmed that ‘Sst Chodl’ cell sets characterized based on morphology and electrophysiology match those defined by transcriptomic profiles.” *Common cell type nomenclature for the mammalian brain* (Miller 2020) <https://doi.org/10.7554/eLife.59928>

This scientific paper states that Sst Chodl has been given a separate subclass compared to normal Sst subclass. This was due to Sst Chodl having distinct different transcriptomic mapping, and other papers finding different electrophysiology features. As a researcher, it is up to you to begin looking at the electrophysiology data to see if you agree or disagree with their statement.

Go to the Cell Type Knowledge Explorer to investigate if you see the same electrophysical differences.

1. Go to Cell Type Knowledge Explorer and find Sst Chodl and Sst subclass in mouse
 - I. What type of firing response does Sst Chodl and Sst subclass have? (adapting, burst, irregular, fast-spiking)

 - II. Compare to the violin plots of Sst Chodl vs Sst subclass. Which characteristic is more distinct? Which is more similar?

2. Pick 3 Sst cell types. What firing response do they have? Compare the violin plots. Are they more or less similar to Sst Chodl? Explain your reasoning

Now you want to investigate the raw data; the following part is about how to download & interact with the raw data. Recommend using Windows and Chrome

1. Use the metadata file to find the file name of the data of interest.
 1. Go to Github link of metadata file and click on the download icon to download the file (.csv). *CSV file is similar to excel file*

https://github.com/AllenInstitute/CTKE_viz/blob/main/ephys/Tolias_m1_patchseq_meta_data.csv

1. Find your cell type of interest by sorting RNA type in column T.
 2. Write down the mouse name (column F) and sample name (column E). You will need to these names to know what raw data to look for.
2. Open the raw data on Dandi
 1. Go back to Cell Type Knowledge Explorer and your cell type of interest. By “Electrophysiology”, go to “Download Data” – this will open a separate page called Dandi where the data is stored
 2. Click on “Files” on the right side to view all the ephys data. Refer to your mouse name (column F) written down
 3. Click on the mouse name. For the sample name (column E) written down, go to “Open With” and select “MetaCell/NWBExplorer”. *This make take a while to load*
 4. Click on the eyeball to view different recordings

What ways would you make the raw data more accessible? Remember, these data sets are large, so consider the manual effort needed as well.