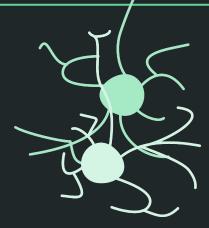
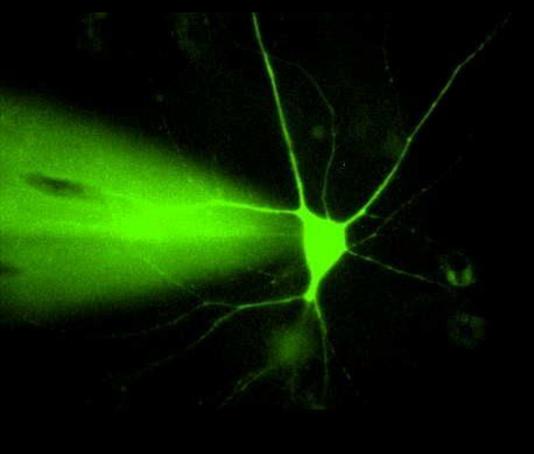
Recording electrical signals from genetically-identified neurons

NGP Bootcamp 2020





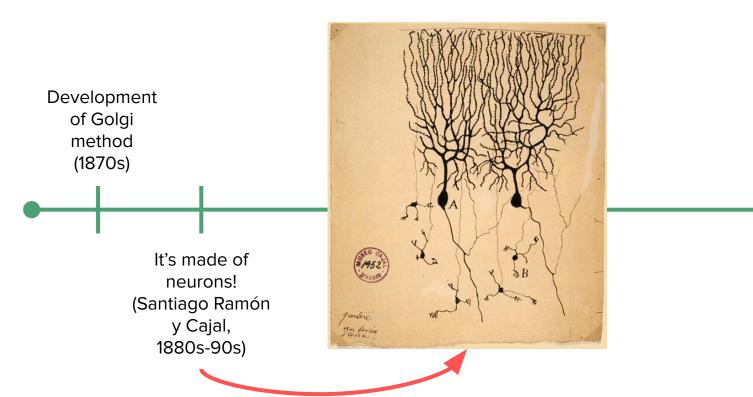
What are you wondering about this image?

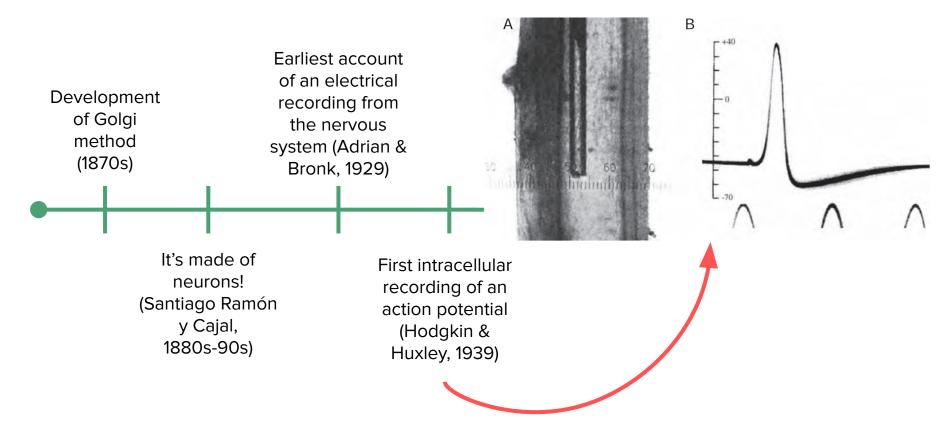
Respond in the chat box!

Image: De Konick Lab

Objectives for today

- Understand how we can record electrical activity from the nervous system
- Connect the features of action potentials to their function
- Identify ways that we can target our recordings to specific neurons
- Introduce the Allen Cell Types database



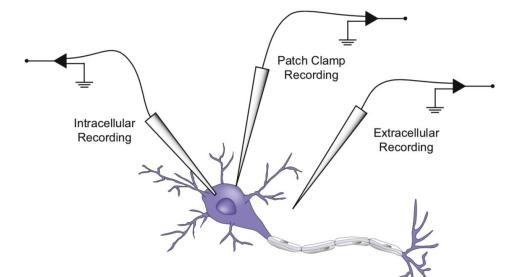


Development of Golgi method (1870s) Earliest account of an electrical recording from the nervous system (Adrian & Bronk, 1929)

Patch clamp electrophysiology developed (Neher & Sakmann 1976)

It's made of neurons! (Santiago Ramón y Cajal, 1880s-90s)

First intracellular recording of an action potential (Hodgkin & Huxley, 1939) **Electrophysiology** is the study of the electrical activity of nerve cells



Three main types:

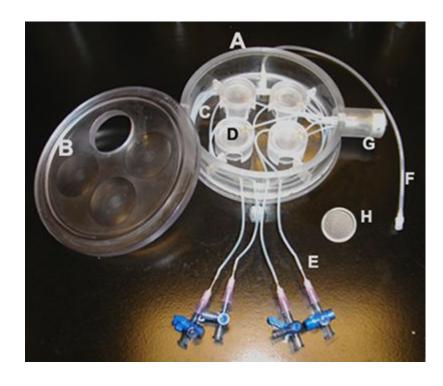
- Extracellular recording: electrode is placed just outside of the cell
- 2. **Intracellular recording**: electrode is inserted into the cell
- 3. **Patch clamp recording:** electrode forms a tight seal with a patch of the membrane (**Cell Types data**)

Patch clamp electrophysiology

- Enables very precise measurement of either whole-cell activity or specific ion channels by "patching" onto the membrane
- Uses a large diameter pipette (1-3 μ m) to press against the membrane of the neuron, rather than pierce into it
- Can be performed in various configurations:
 - Whole-cell patch clamp: electrode is contiguous with the cell membrane, best signal-to-noise of these recording techniques
- Allows for voltage clamp or current clamp recording configurations
 - **Voltage clamp**: change voltage across cell membrane, record current
 - Current clamp: change how much current is injected, and enable voltage (membrane potential) to vary

Slice physiology: in vitro recording from brain slices in a dish

- The brain is extracted and immediately sectioned with a vibratome
- These sections can be kept alive in a dish with artificial cerebral spinal fluid
- Using slice physiology, one can study the electrophysiology of single cells or the connectivity in a circuit by stimulating other cells
- Often, slice physiology is combined with optogenetics or pharmacological blockers to identify/block specific neurons and channels



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Development of Golgi method (1870s) Earliest account of an electrical recording from the nervous system (Adrian & Bronk, 1929)

Patch clamp electrophysiology developed (Neher & Sakmann 1976) Targeting
neurons
using
transgenic
mice
(early 2000s)

It's made of neurons! (Santiago Ramón y Cajal, 1880s-90s)

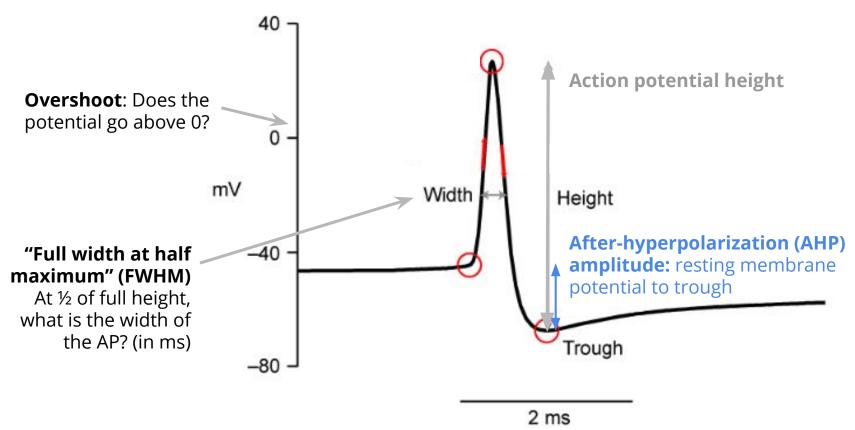
First intracellular recording of an action potential (Hodgkin & Huxley, 1939) Estimate of about 1000 different cell types in the brain (Crick 1994; Stevens 1998)

Neurons can be classified in a variety of ways

- Function
- Location
- Transmitters
- Shape
- Gene/protein expression
- Projections
- Intrinsic (electro)physiology

intrinsic electrical properties of a neuron (e.g., resting membrane potential)

Single action potential features

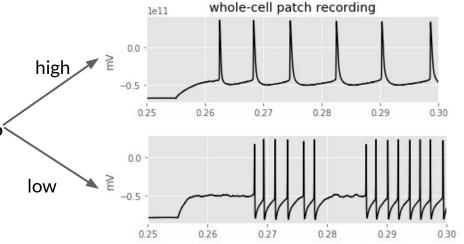


In addition, we can characterize single cells by:

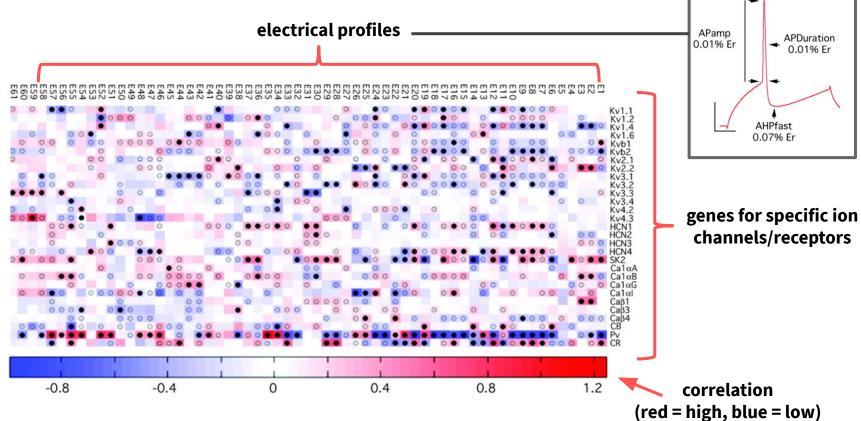
Resting membrane potential: typically around -60 mV, but it depends on the cell

Minimum current to elicit an AP (rheobase)

Upstroke-downstroke ratio: The ratio between the absolute values of the AP peak upstroke and the AP peak downstroke.



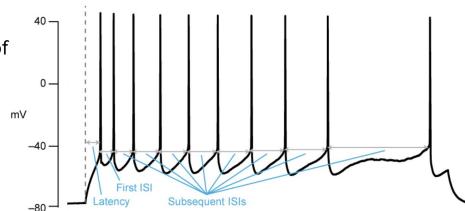
Ion channel composition changes the electrical properties of a cell (Toledo-Rodriguez et al., 2004)



Action potential train features

Several APs elicited from short injection of current

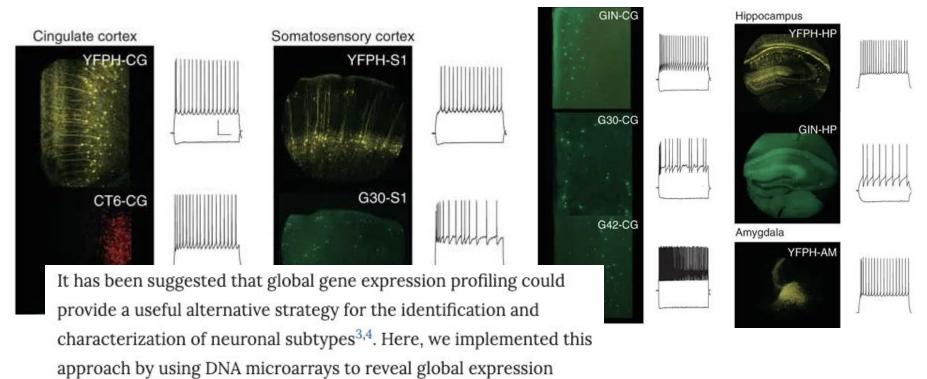
- Interspike interval (ISI): could be defined for any two spikes, but most commonly it is the first and last
 - If the ISI is very short, the sequence of spikes is called a **burst**
- Adaptation rate: $\frac{1}{N-1} \sum_{n=1}^{N-1} \frac{ISI_{n+1} ISI_n}{ISI_{n+1} + ISI_n}$
- Latency: time from stimulus to first spike
- Spike rate: how many spikes/second (on average)?



1 second current step

Different neurons can have dramatically different firing patterns & action potential shapes

differences among 12 distinct neuronal populations. These were



Width of the waveform controls Ca⁺² influx and EPSP (excitatory postsynaptic potential) amplitude

Plot with TEA/without TEA

Total Calcium Influx (% Control)

Total Calcium Influx (% Control)

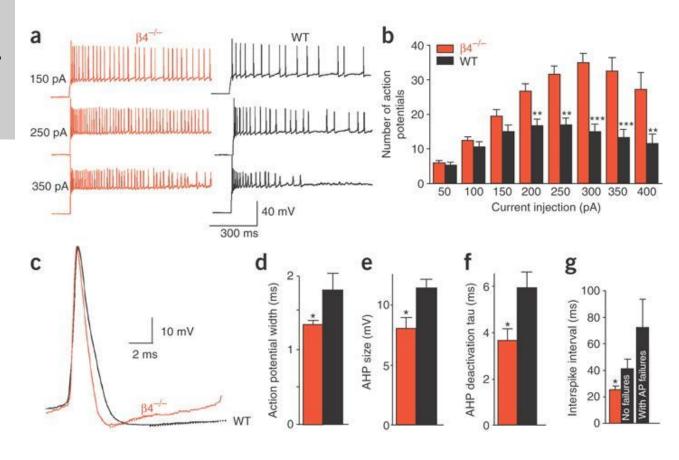
Total Calcium Influx (% Control)

Total Calcium Influx (% Control) "The finding that even 400small changes in spike eak EPSC (% Control) width influence -Ş⊣ 300neurotransmitter release suggests that altering the Ю presynaptic waveform 200may be an important Ь ЮН means of modifying the D a strength of this synapse." 100-100-100 120 140 160 100 120 140 160 AP Width (% Control) AP Width (% Control) Use TEA to broaden AP

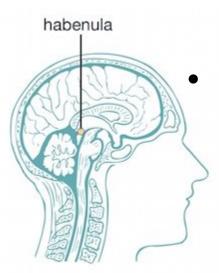
Sabatini & Regehr, 1997 http://www.jneurosci.org/content/17/10/3425

Action potential features have real implications for neural computation

Removing one subunit (β4) of a receptor (BK, a K⁺ channel), causes seizures, possibly by changing the action potential shape of neurons in the hippocampus (Brenner et al., 2005)



Bursting neurons in depression



- The lateral habenula (LHb) inhibits dopamine & serotonin neurons (reward!), and may be involved in depression
 - Ketamine may help by slowing down the lateral habenula (Yang et al., 2018)

In depressed animals, the LHb cells are *too bursty* — because their resting membrane potential is lower, they're more likely to burst

- Some voltage-gated channels (on specific neurons) are triggered by *hyperpolarizing* currents (I_h), enabling cations to enter the cell
- Turns out, it is glia that is removing K⁺ from around the cell, driving the resting membrane potential **down** (Cui et al., 2018)

For a great write-up about these studies: https://medium.com/the-spike/a-new-prime-suspect-for-depression-4a4607a870b0 https://www.brainpost.co/weekly-brainpost/2018/2/19/ketamine-blocks-burst-firing-to-provide-depression-relief

Objectives for today

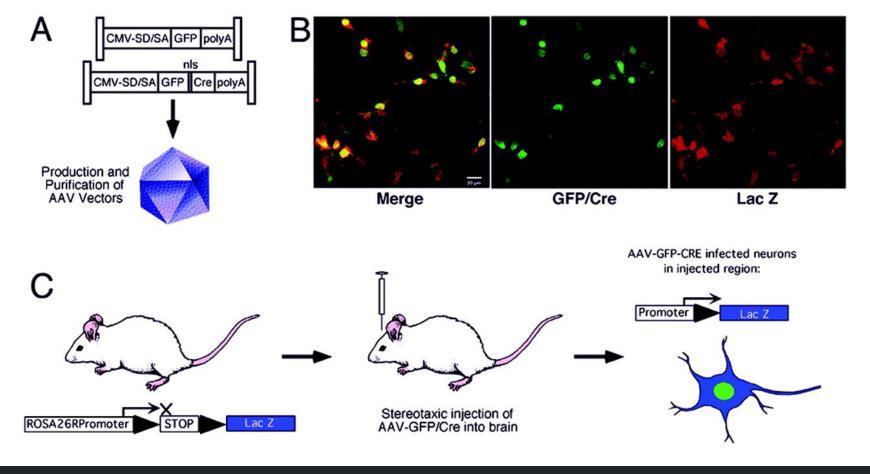
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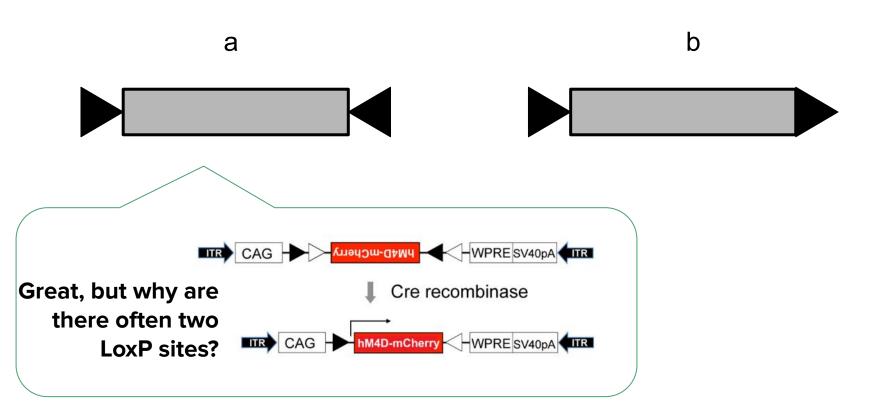
As measured by microarrays, RNAseq, etc. or by identifying cells by expression of *one* particular gene

How can we specifically target cells for electrophysiology experiments by their genetic identity?

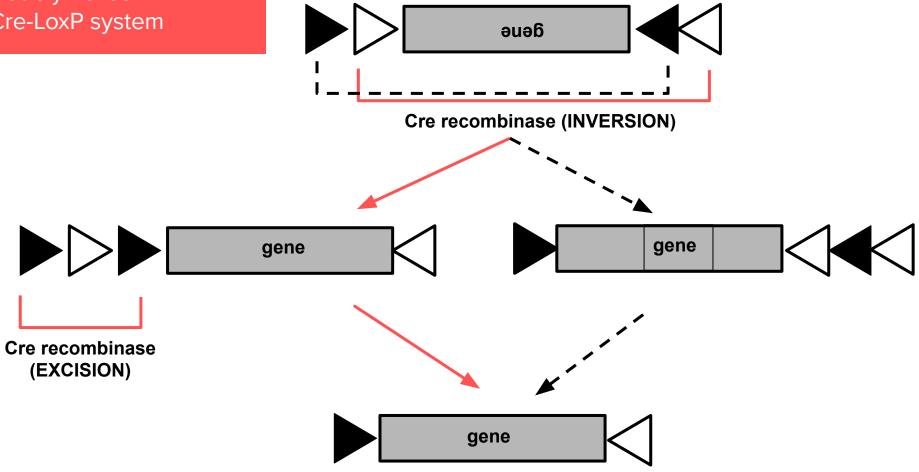


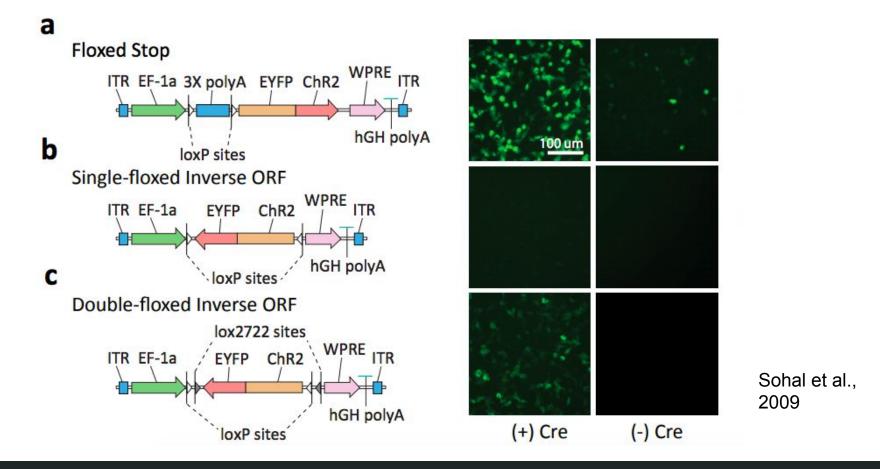
Adeno-associated viruses (AAVs) & binary expression systems (Cre/LoxP) can be used to express genes in specific cells (Kaspar et al. 2002)

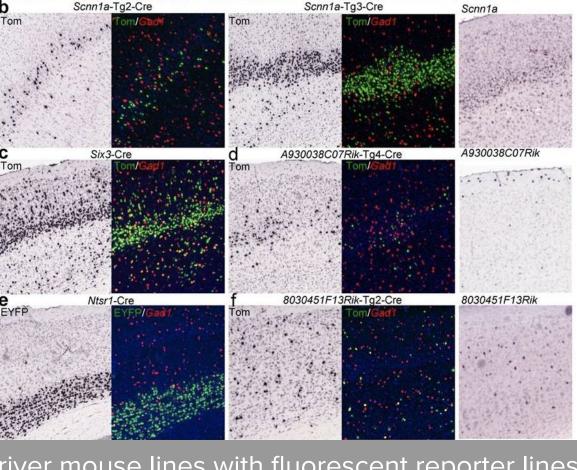
Which of these configurations results in an inversion?



Doubly-floxed Cre-LoxP system





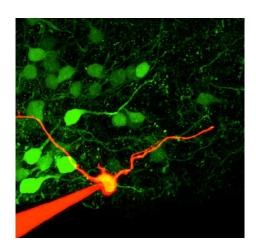


Scnn1a-Tg3-Cre

Madisen et al., 2010

Crossing Cre-driver mouse lines with fluorescent reporter lines marks specific subsets of cells

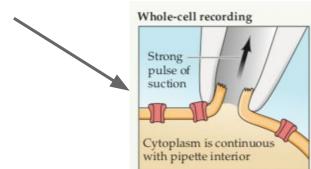
Cre-driver lines enable targeting of a gene of interest



Hypothalamic neurons were identified under a 2-photon microscope, to target a GFP+ neuron with whole-cell patch clamp. Red-fluorescent dye is included to allow the visualization of the neuronal structure. (Kong Lab, Tufts)

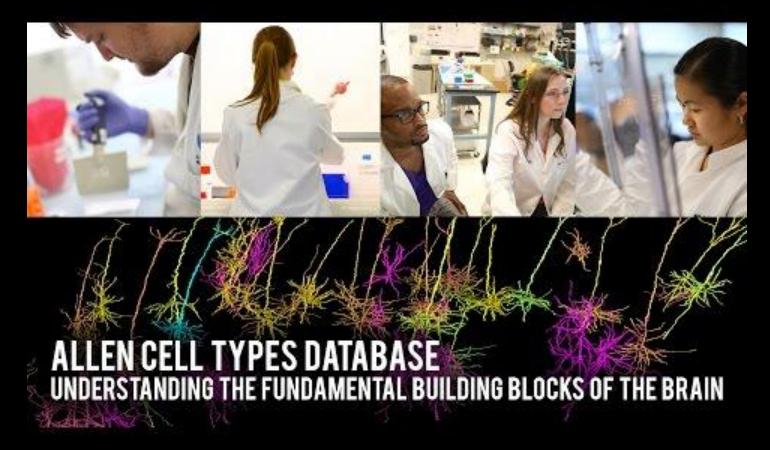
- In the Allen Brain Cell Types dataset, they expressed a fluorescent protein (e.g., GFP) in specific cells, using the binary Cre-LoxP expression system.
- This enables them to target specific cells with their electrode in whole-cell patch clamp configuration

 Whole-cell recording



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Introduction to the Allen Cell Types Database

DIx1-CreERT2 Z. Josh Huang

DIx5-CreERT2

Z. Josh Huang

Drd1a-Cre_EY262

Richard Palmiter

Drd2-Cre ER44

Drd3-Cre KI196

Nathaniel Heintz and Charles Gerfen

Nathaniel Heintz and Charles Gerfen



Expressed in strong scattered cells throughout the brain. Expression is more widespread in olfactory bulb and cortical subplate (amygdala).

restricted populations within the medulla, pons, and cerebellum.

These are in situ hybridization images showing location of gene expression

Reporter expression is throughout the cortex, hippocampus, striatum, and other structures. Cre expression is enriched in piriform cortex and st

Cre expression is enriched in layer 2/3 of somatosensory areas and retrosplenial area of cortex; and in restricted populations within the hippoca

Enriched in striatum, restricted populations within cerebellum, cortex, thalamus, medulla, and olfactory areas.

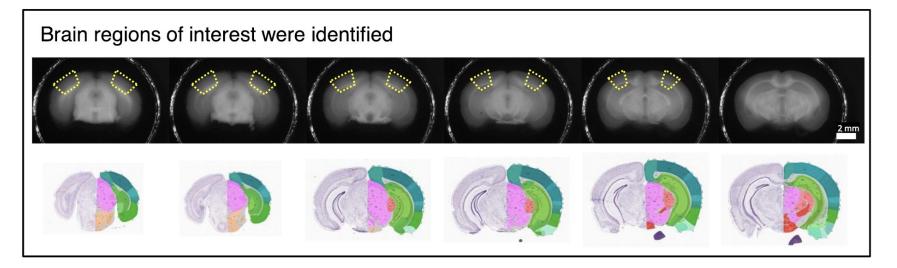
cortical subplate, and striatum.

Allen Brain Institute uses hundreds of different Cre-driver lines

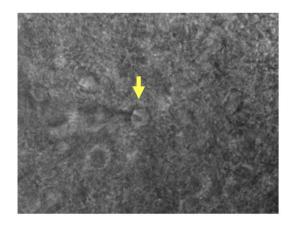
Widespread, interneuron expression throughout cortex, striatum, amygdala, hippocampus, olfactory bulb, hypothalamus, and pallidum.

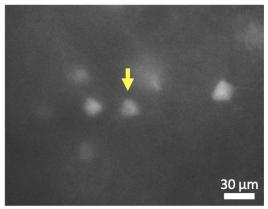
(from http://connectivity.brain-map.org/transgenic)



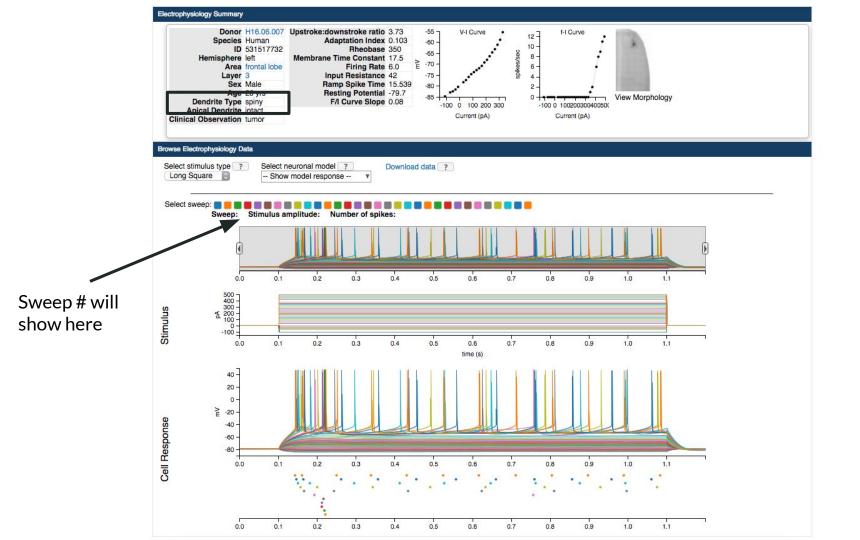


Neurons were targeted and recorded based on 40X brightfield and fluorescent images, to verify targeted neuron.

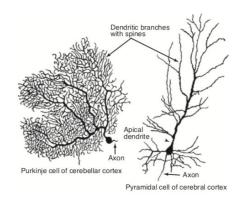


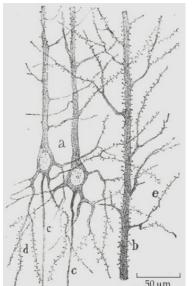


Ramp -	/	Description: Current injection of increasing intensity at a rate much slower than the time constant of the neuron. Details: Ramp of 25 pA per 1 second, terminated after a series of action potentials are acquired.	
Long Square		Description: Square pulse of a duration to allow the neuron to come to steady-state. Details: 1 s current injections from -110 pA (or -190 for some Pvalb neurons) to rheobase + 160 pA, in 20 pA increments. For some human neurons with low IR, steps were from -220 pA in 40 pA increments.	
Short Square		Description: Square pulse brief enough to elicit a single action potential. Details: 3 ms current injections used to find the action potential threshold within 10 pA.	While recording, different current waveforms were
Short Square Hold -60mV Hold -70mV Hold -80mV		Description: Short pulse stimulus with stepped holding potentials. Details: Bias current brings the neuron to steady state potentials of -60 mV or -80 mV. If the neuron rests at -80 mV or -60 mV, the neuron is held at -70 mV.	injected into the cell.
Short Square Triple		Description: Three short pulse stimuli in rapid succession. Details: Three threshold stimuli of 3 ms duration are delivered at decreasing frequencies from ~140 Hz to ~30 Hz.	
Noise 1 & 2		Description: Noise pulses offset with square current injections. Details: Pink noise generated from 2 seeds (1 & 2) scaled to three amplitudes, 0.75, 1, and 1.5 times rheobase. Additional details can be found in the Appendix.	





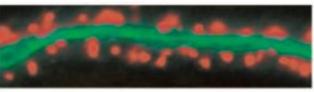




Spiny vs. aspiny dendritic structure

- Dendritic spines are protrusions on the dendrites of some neurons
 - Usually found on excitatory cells (and usually these contacts are excitatory)
 - Most notably found on pyramidal cells
- Spines can change a lot with experience, disease, etc.
- In other words, they are a major site for

synaptic plasticity



actin tubulin

For more: <u>Actin-Based Plasticity in Dendritic Spines</u>

Application programmer interfaces (APIs) provide an **interface** for users to interact with software/data

Software development kits (SDKs) provide a set of tools, libraries, documentation, code samples, etc. that allow developers to create software applications, and often include APIs

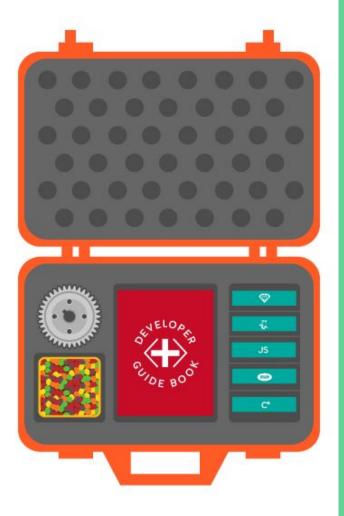


Image: Nordic Apis

To accessing the notebooks for this lecture (and all future lectures):

- Click this link:
 https://datahub.ucsd.edu/hub/user-redirect
 /git-sync?repo=https://github.com/NGP-Bo
 otcamp/Bootcamp2020
- Open the NEU210 container

Resources

<u>Documentation - Allen Cell Types Database</u>

Allen Cell Types Features Glossary

Software Carpentries "Combining Dataframes with Pandas"

Merge and Join DataFrames with Pandas in Python