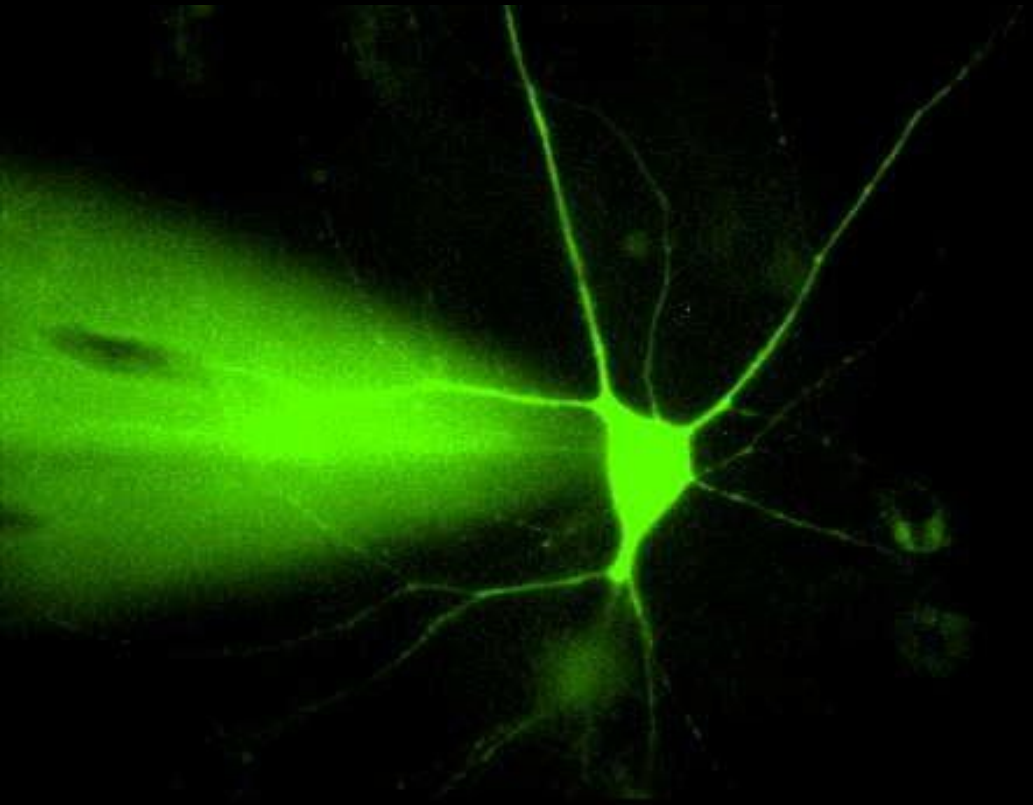
Recording electrical signals from genetically-identified neurons

BIPN 162



What are you wondering about this image?

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
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Neural communication is dependent on the movement of ions (i.e. electricity)

Luigi Galvani was the first to demonstrate that the electricity in the frog was the same as the electricity elsewhere (e.g., in lightning), in an experiment in 1791 (depicted at right).

Neurons have a negative **resting membrane potential** which change during **graded** or **action potentials**.

These are electrical signals that we can measure.

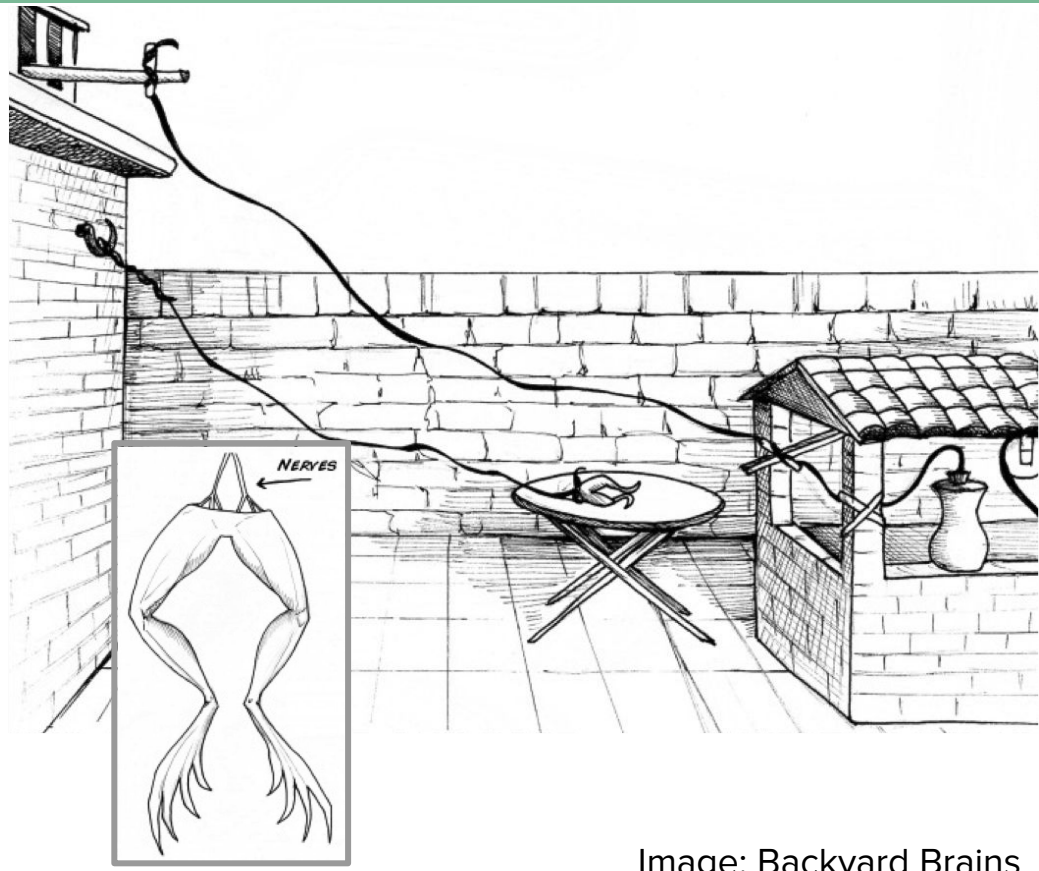
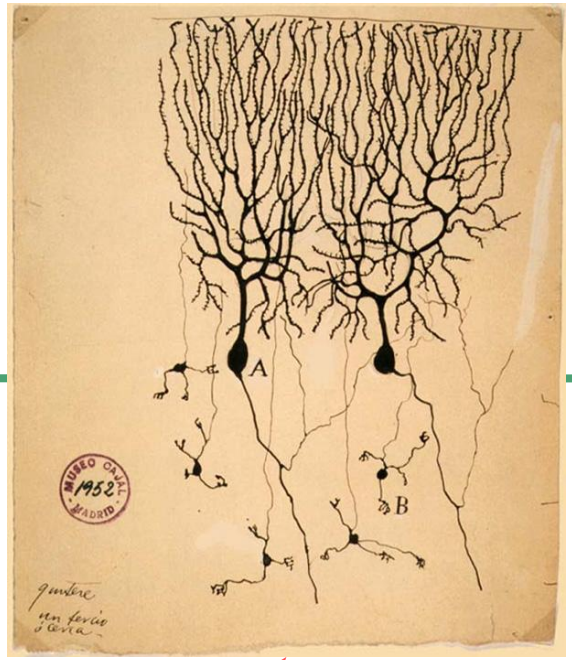


Image: Backyard Brains

Our evolving understanding of the nervous system & our ability to study it

Development of Golgi method (1870s)

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



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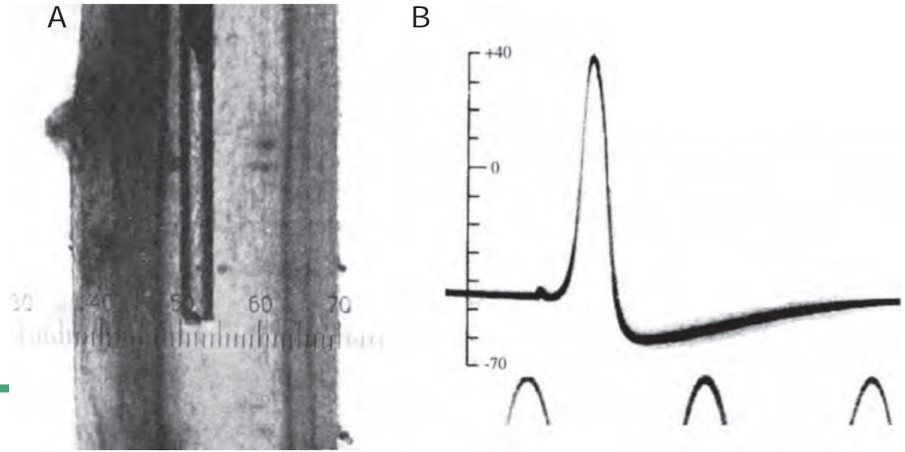
Earliest account of an electrical recording from the nervous system (Adrian & Bronk, 1929)

First intracellular recording of an action potential (Hodgkin & Huxley, 1939)



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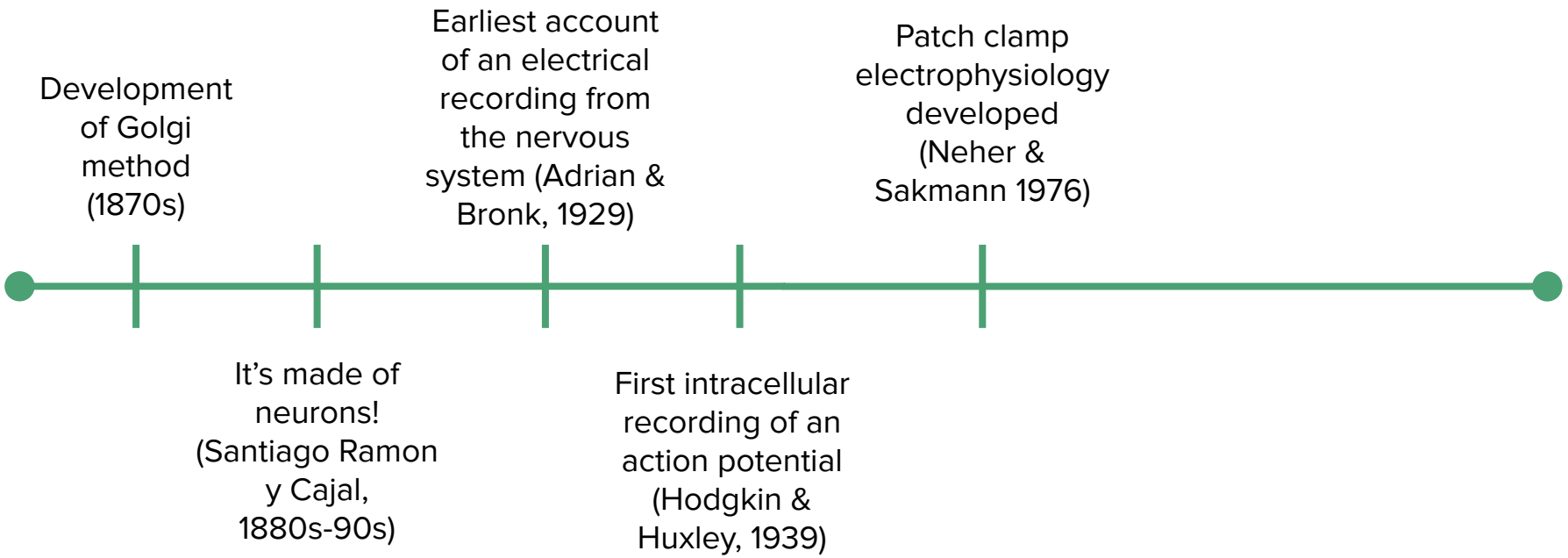
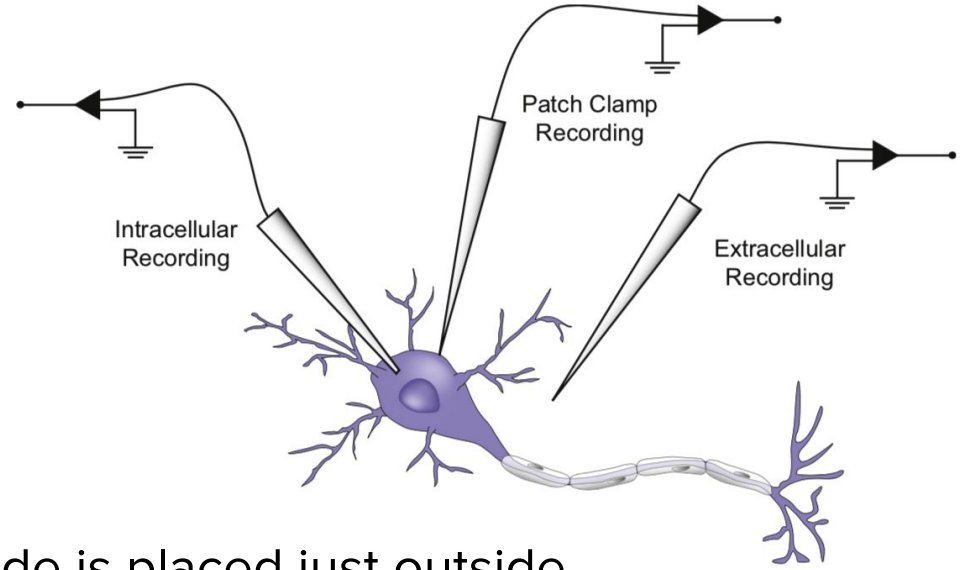


Electrophysiology is the study of the electrical activity of nerve cells



Three main types:

1. **Extracellular recording:** electrode is placed just outside of the cell **(Neuropixels data)**
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 larger diameter pipette (1-3 µm) than sharp electrode intracellular recording 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

Intracellular & patch clamp recordings *can* be performed *in vivo*, but it's quite difficult!

These recordings are more often performed *in vitro*, in a brain slice.

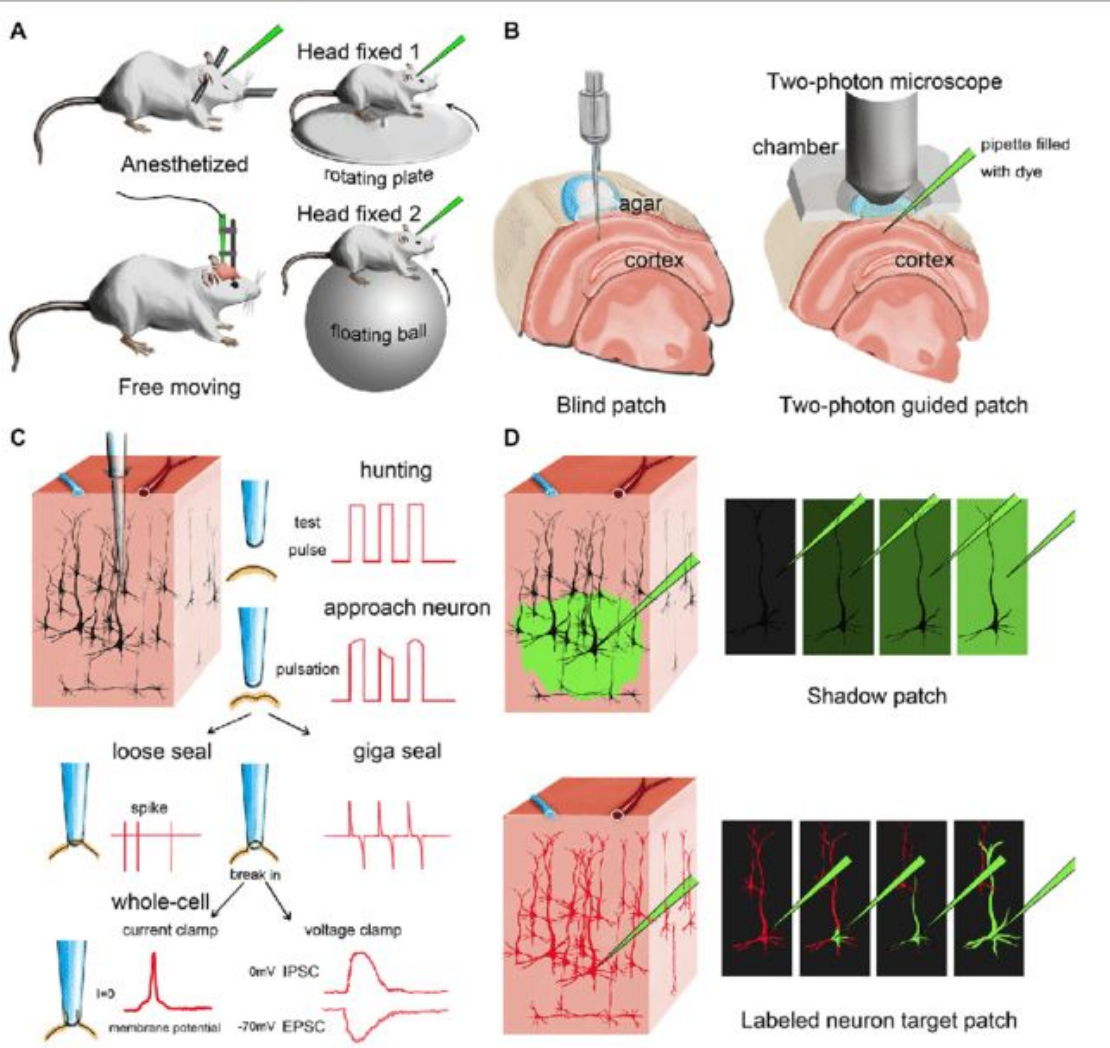
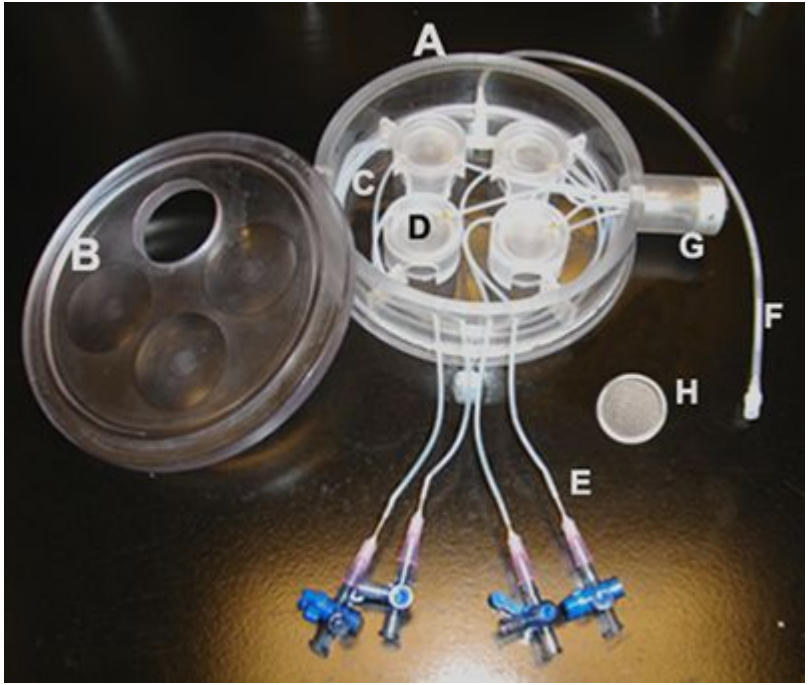
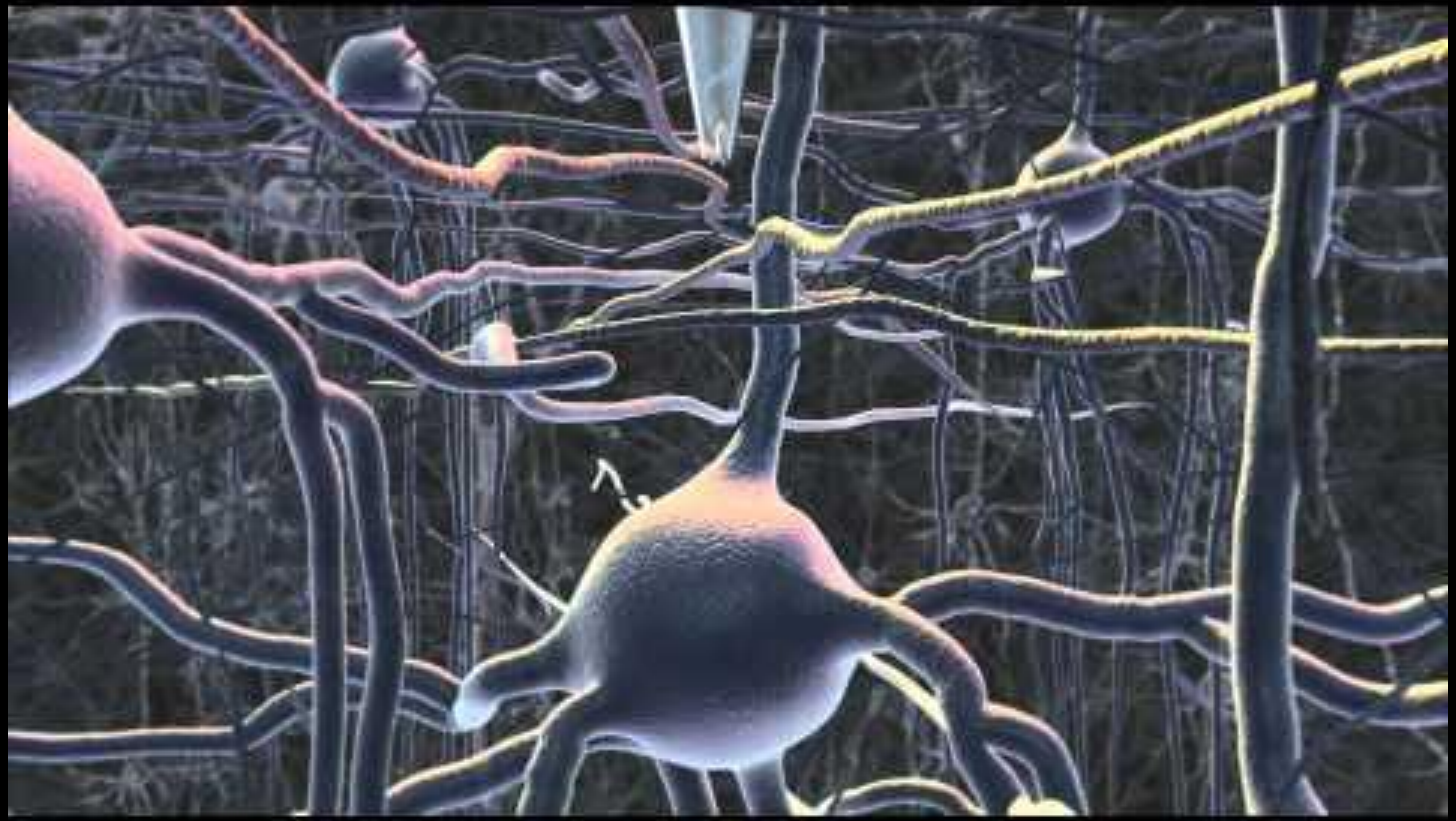


Image demonstrating ways to patch *in vivo* from Functional dissection of synaptic circuits: in vivo patch-clamp recording in neuroscience.

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





Automated whole-cell patch clamp electrophysiology of neurons in vivo
(note: this is a useful visual demonstration, but most patch clamping is *not* done automatically)
https://www.youtube.com/watch?v=mF7Vd5olw18

Match the following:

a. Easiest to perform in intact living animals
b. Great signal-to-noise ratio
c. Allows you to measure current changes across the membrane

1. Whole-cell patch clamp
2. Voltage clamp configuration
3. Extracellular electrophysiology

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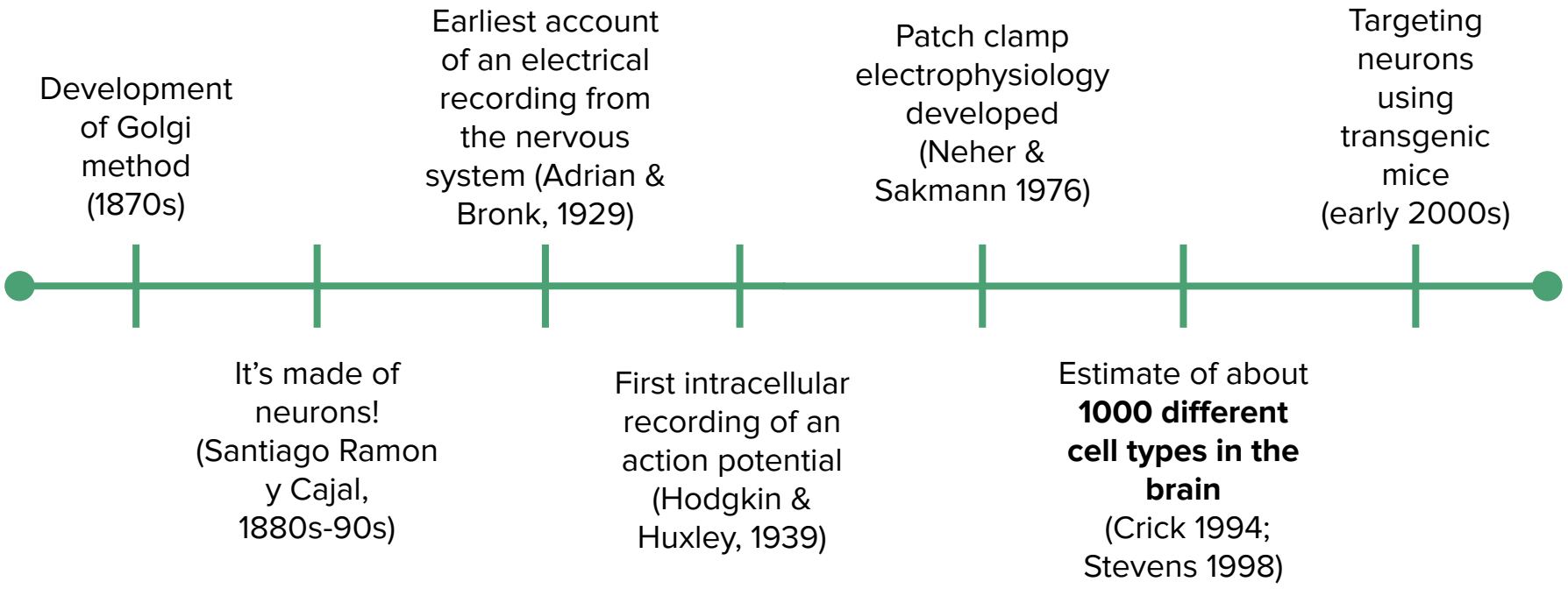
Our evolving understanding of the nervous system & our ability to study it



Neurons can be classified in a variety of ways

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

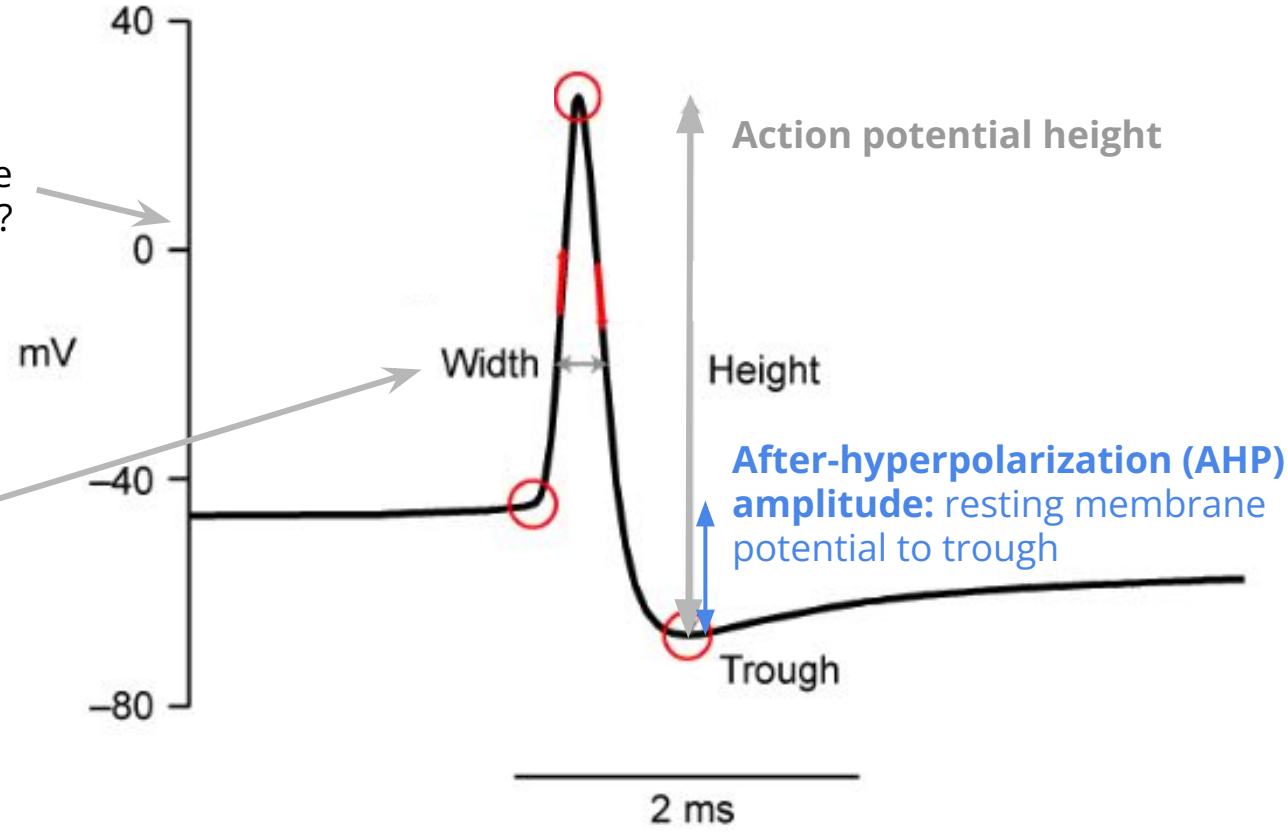
intrinsic electrical properties of a neuron (e.g., resting membrane potential)

* much more coming about this

Single action potential features

Overshoot: Does the potential go above 0?

"Full width at half maximum" (FWHM) At ½ of full height, what is the width of the AP? (in ms)



In addition, we can characterize single cells by:

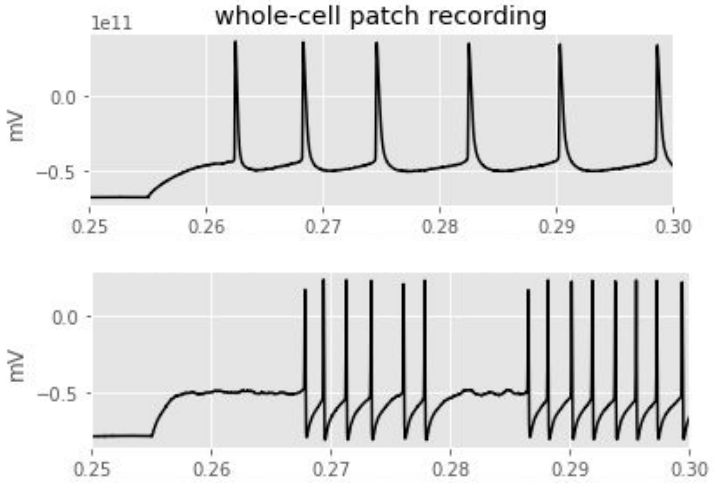
Resting membrane potential: typically around -60 mV, but it depends on the cell

Minimum current to elicit an AP (rheobase)

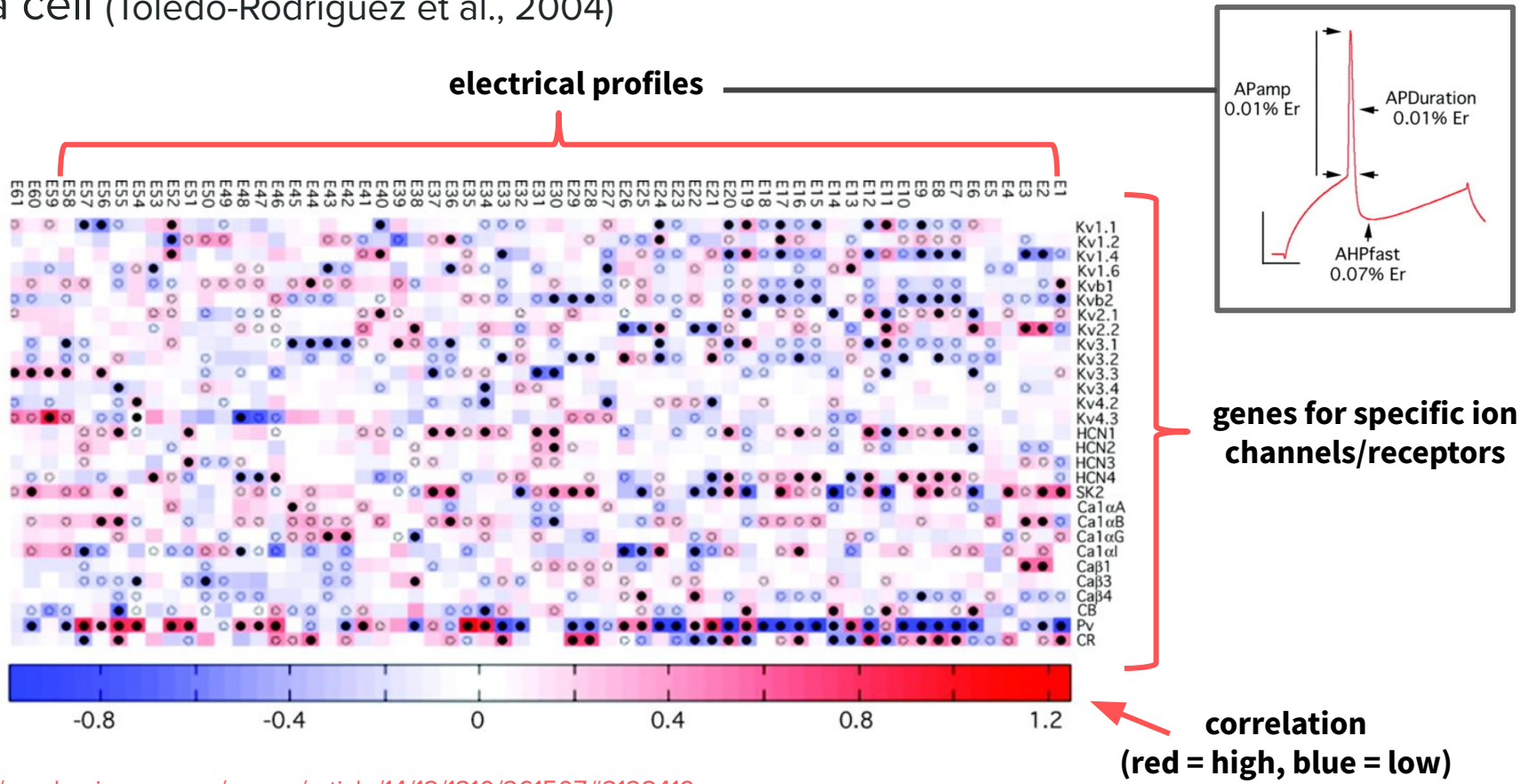
Upstroke-downstroke ratio: The ratio between the absolute values of the AP peak upstroke and the AP peak downstroke.

high

low



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

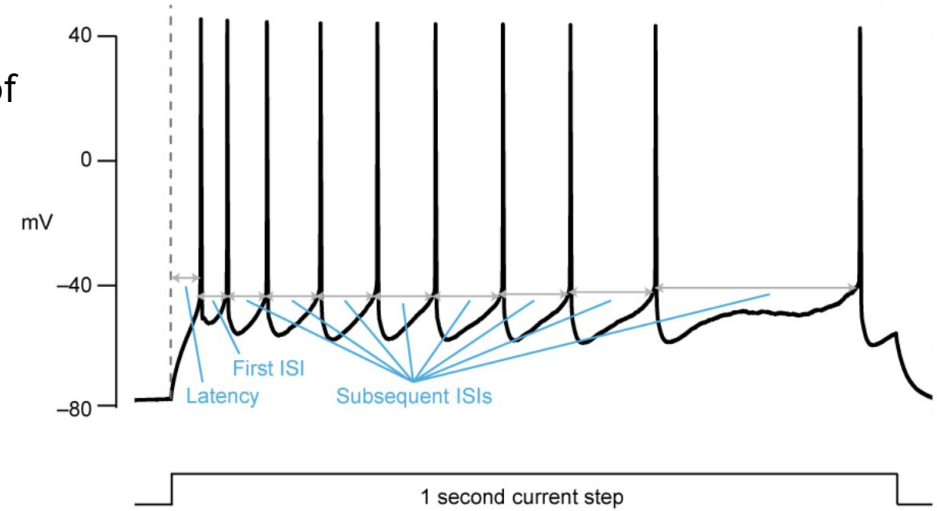


https://academic.oup.com/cercor/article/14/12/1310/261507#3128419

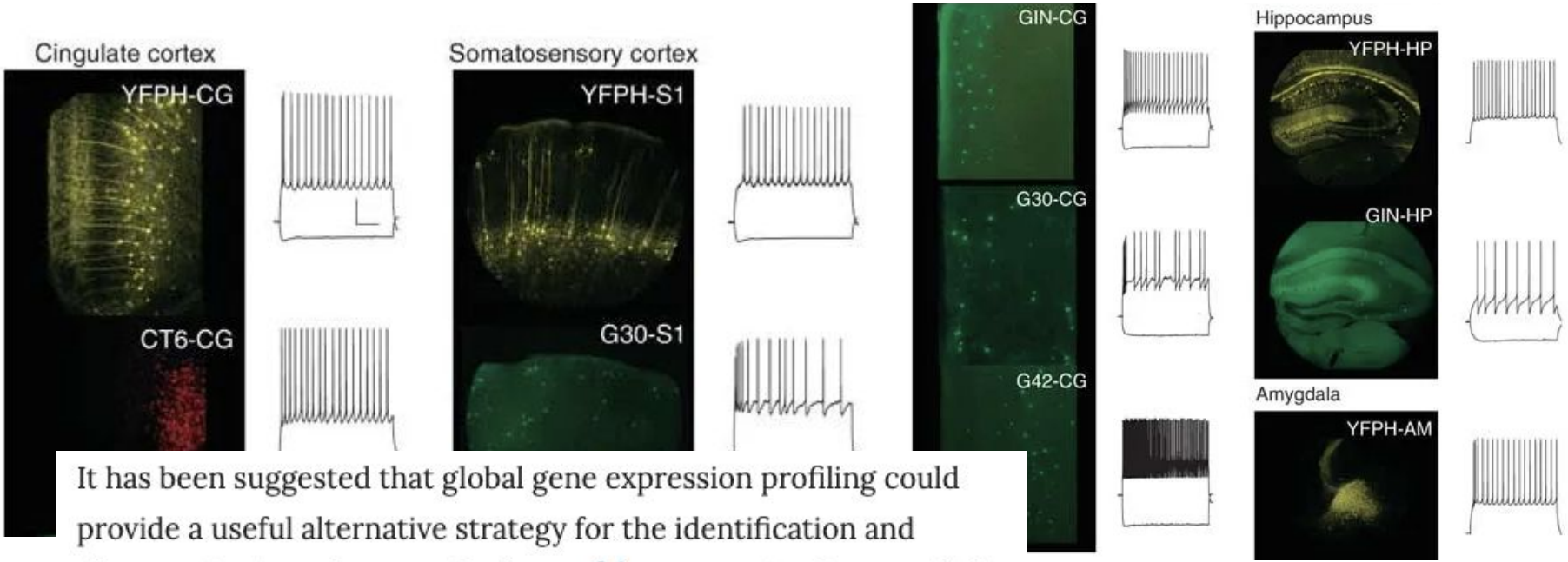
Action potential train features

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)?



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

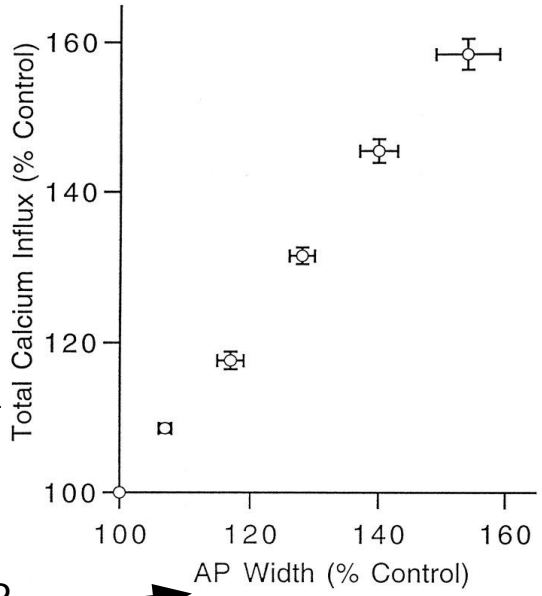


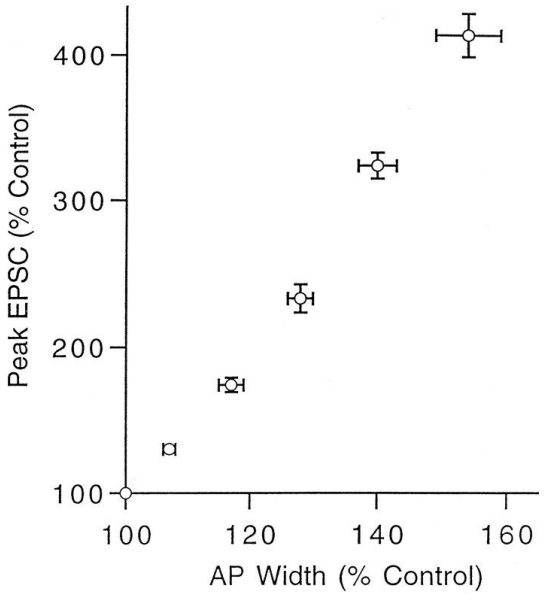
It has been suggested that global gene expression profiling could provide a useful alternative strategy for the identification and characterization of neuronal subtypes[3,4]. Here, we implemented this approach by using DNA microarrays to reveal global expression differences among 12 distinct neuronal populations. These were

Sugino et al., 2005

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

"The finding that even small changes in spike width influence neurotransmitter release suggests that **altering the presynaptic waveform may be an important means of modifying the strength of this synapse.**"



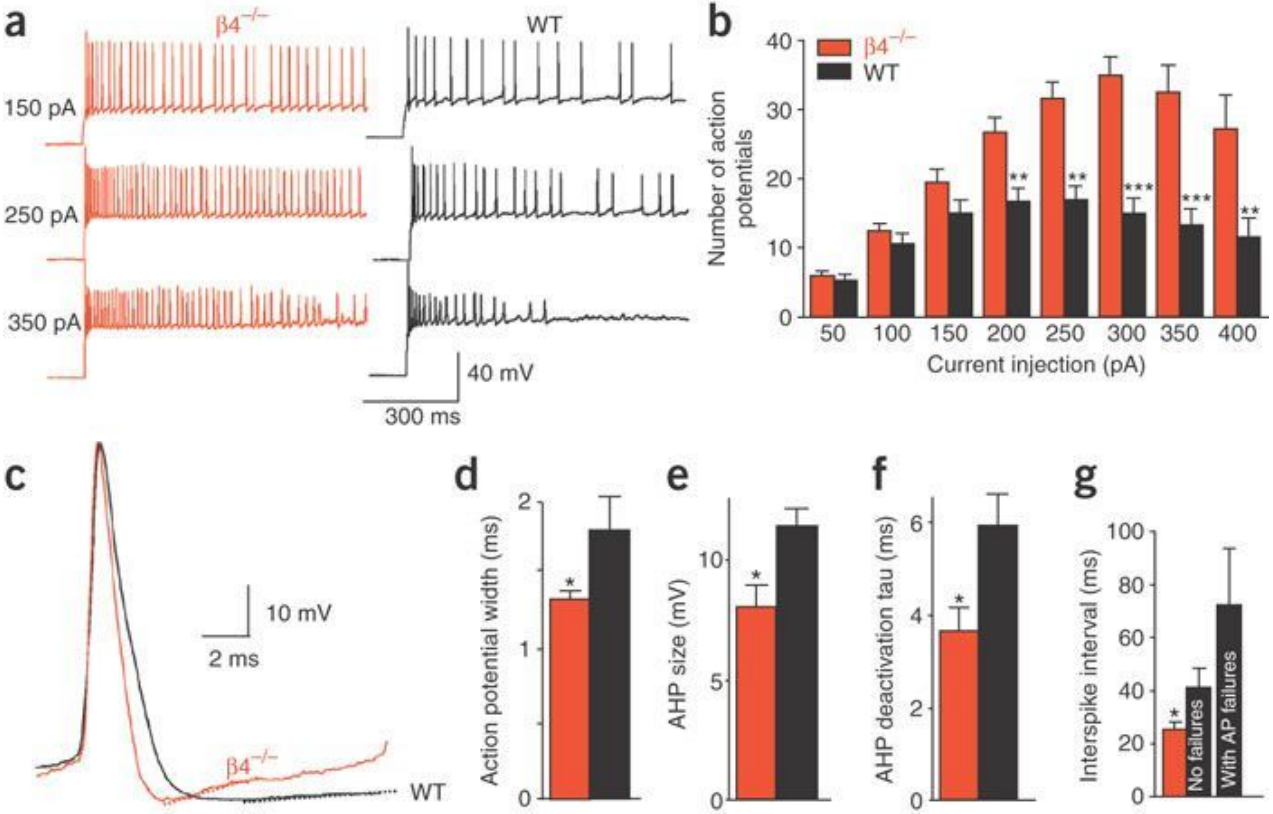


1. Use TEA to broaden AP
2. Plot with TEA/without TEA

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

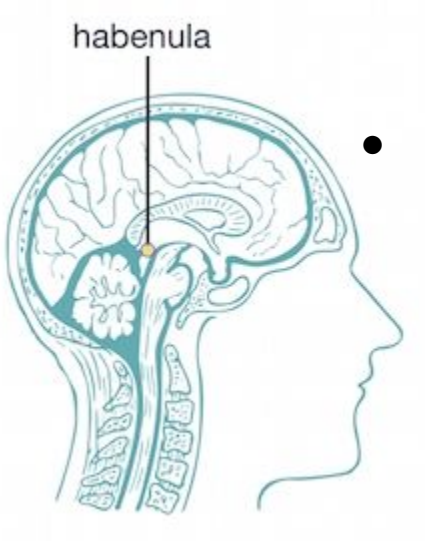
Action potential features have real implications for neural computation

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)



https://www.nature.com/articles/nn1573

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

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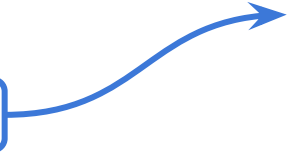
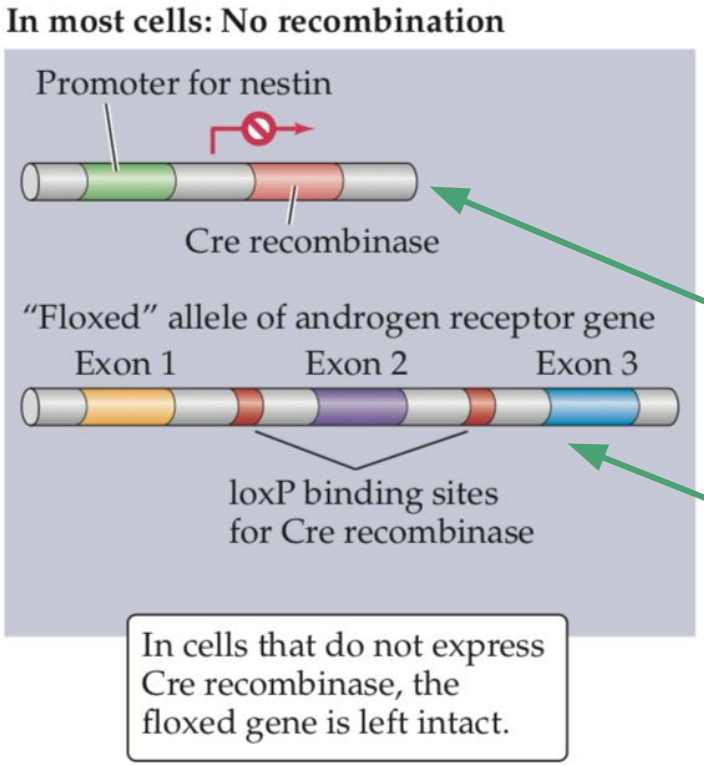


As measured by microarrays, RNAseq, etc. or by identifying cells by expression of *one* particular gene

* much more coming about this

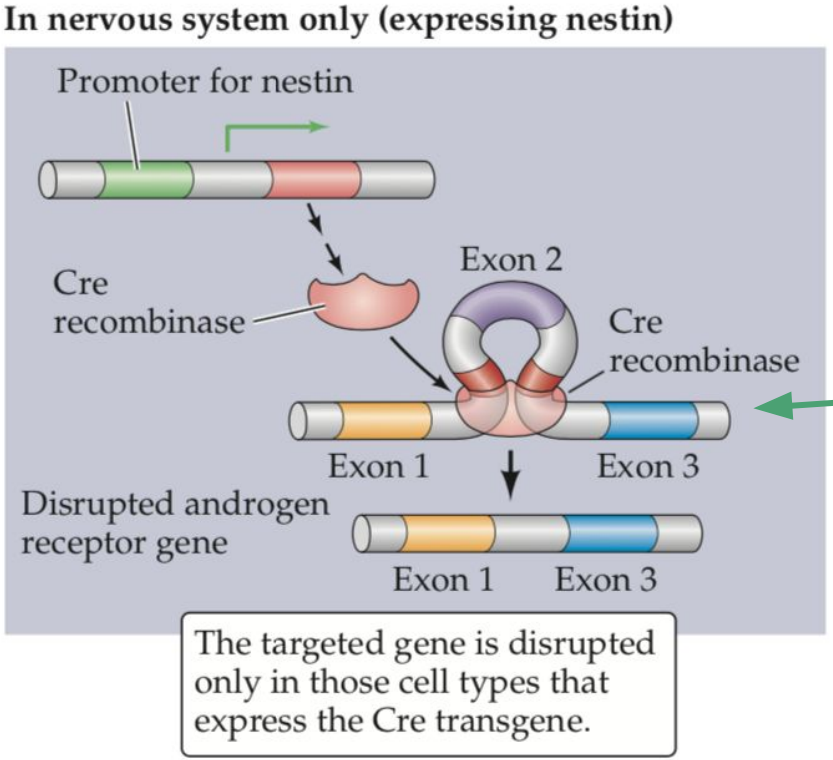
How can we specifically target cells for electrophysiology experiments by their genetic identity?

Cre/LoxP system

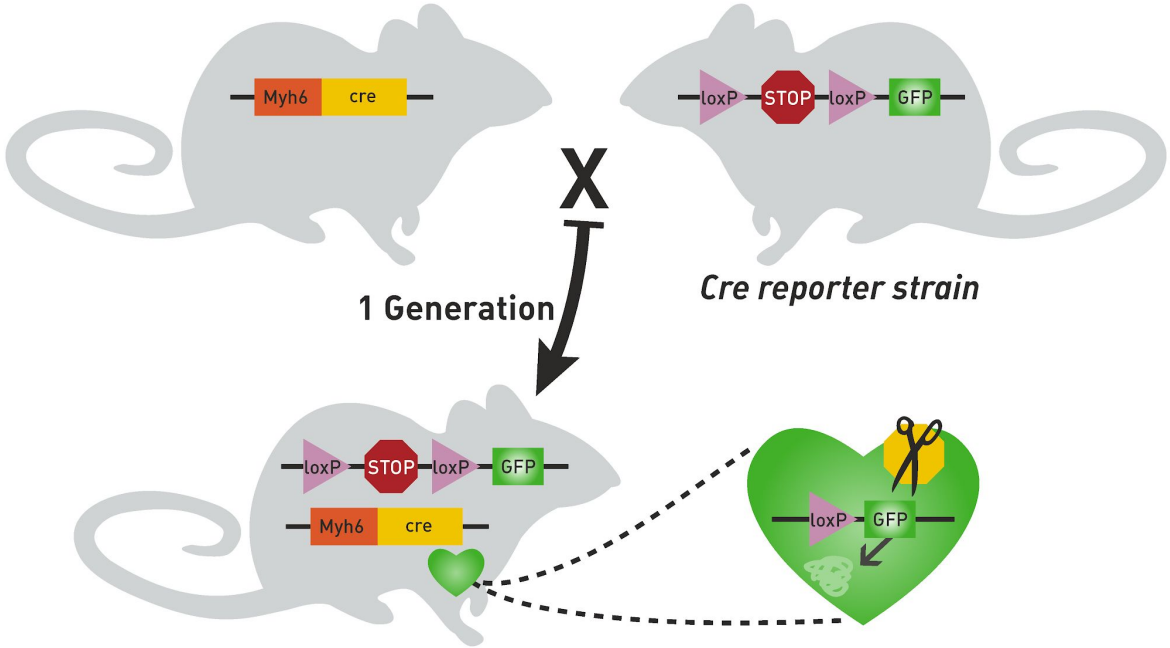


- The Cre/LoxP system enables **conditional knockouts** for specific genes of interest
  - Cre/LoxP found in bacteriophage
  - Commonly introduced in mice to enable a **binary expression system** (on/off)
- **Driver mice** express Cre-recombinase behind a promoter, so that Cre-recombinase is expressed when gene is expressed
- **Reporter mice** contain loxP binding sites around the gene you'd like to remove or flip
  - E.g., a gene to knockout, or a STOP codon
  - Or we can introduce loxP binding sites in a virus

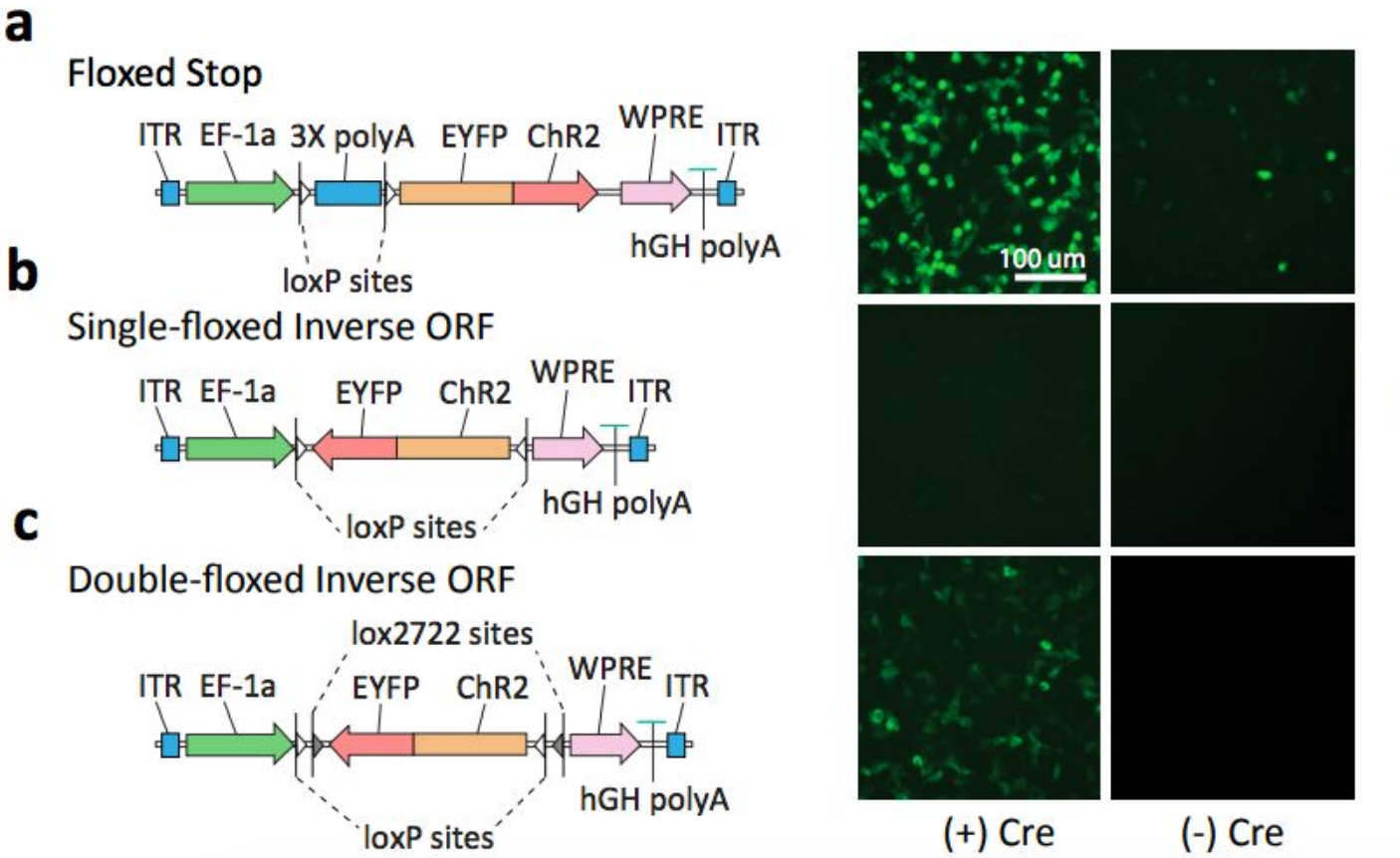
Cre/LoxP system *(continued)*



- During homologous recombination, if Cre-recombinase is expressed, genes between loxP sites are removed
  - Often, loxP sites flank a stop codon (lox-STOP-lox system), that is then removed to enable expression of subsequent genes
  - E.g., if the **blue** gene is a GFP, removal of the **purple** stop codon would enable GFP expression
  - Genes can also be flipped, depending on the configuration of loxP sites.
- This can work for various tissue types, not just neural

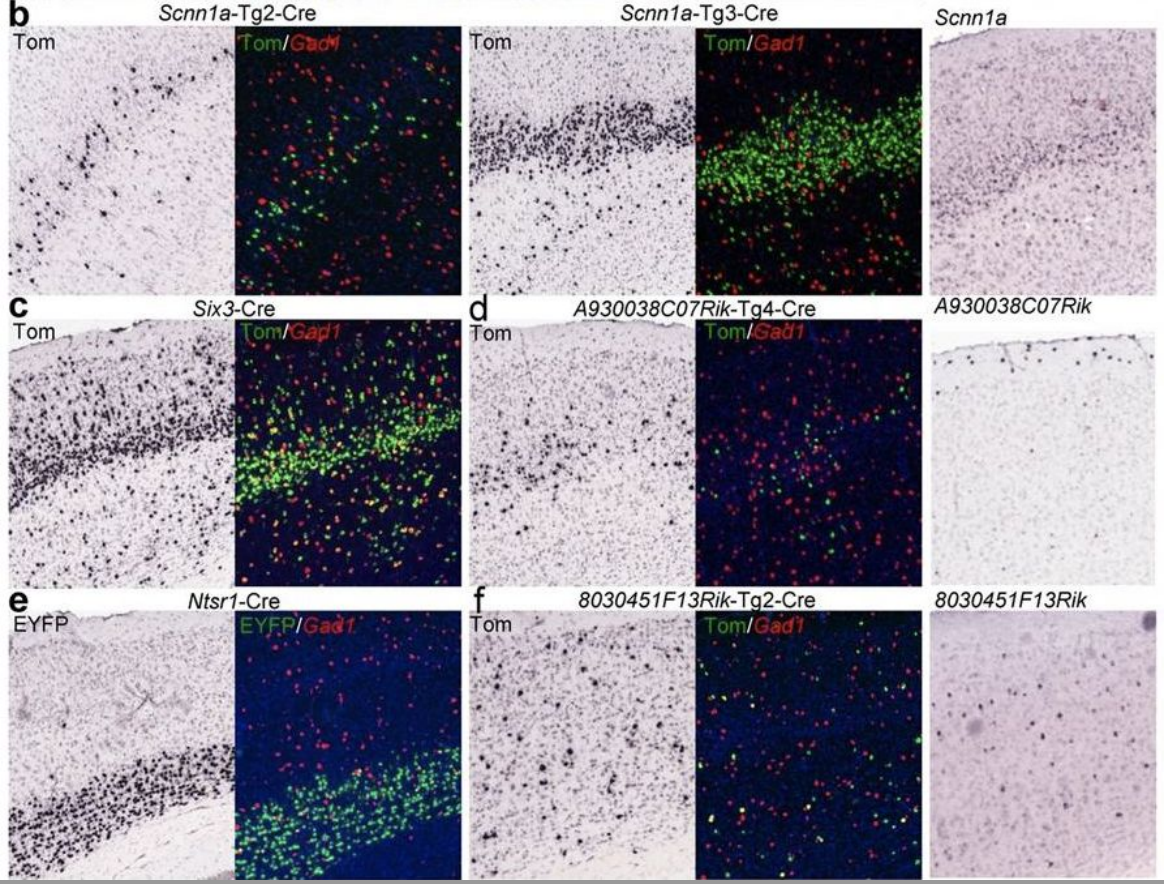


Demonstration of Cre/loxP system to target a gene found in cardiac muscle



Sohal et al., 2009

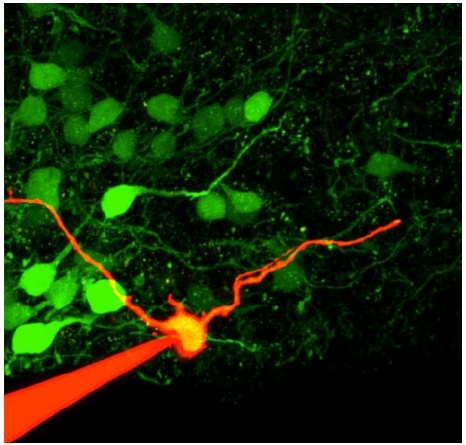
Double-floxed ORF (DIO) system leads to stronger signal with less leak



Madisen et al., 2010

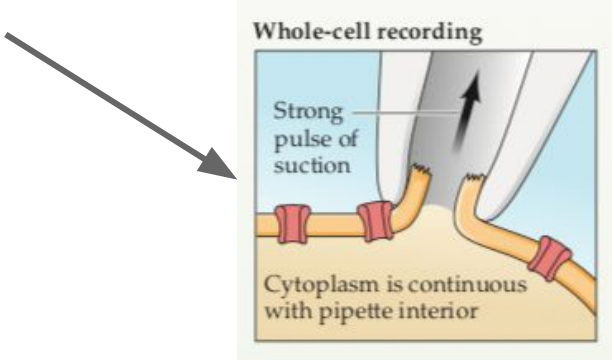
Crossing Cre-driver mouse lines with fluorescent reporter lines marks specific subsets of cells

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**



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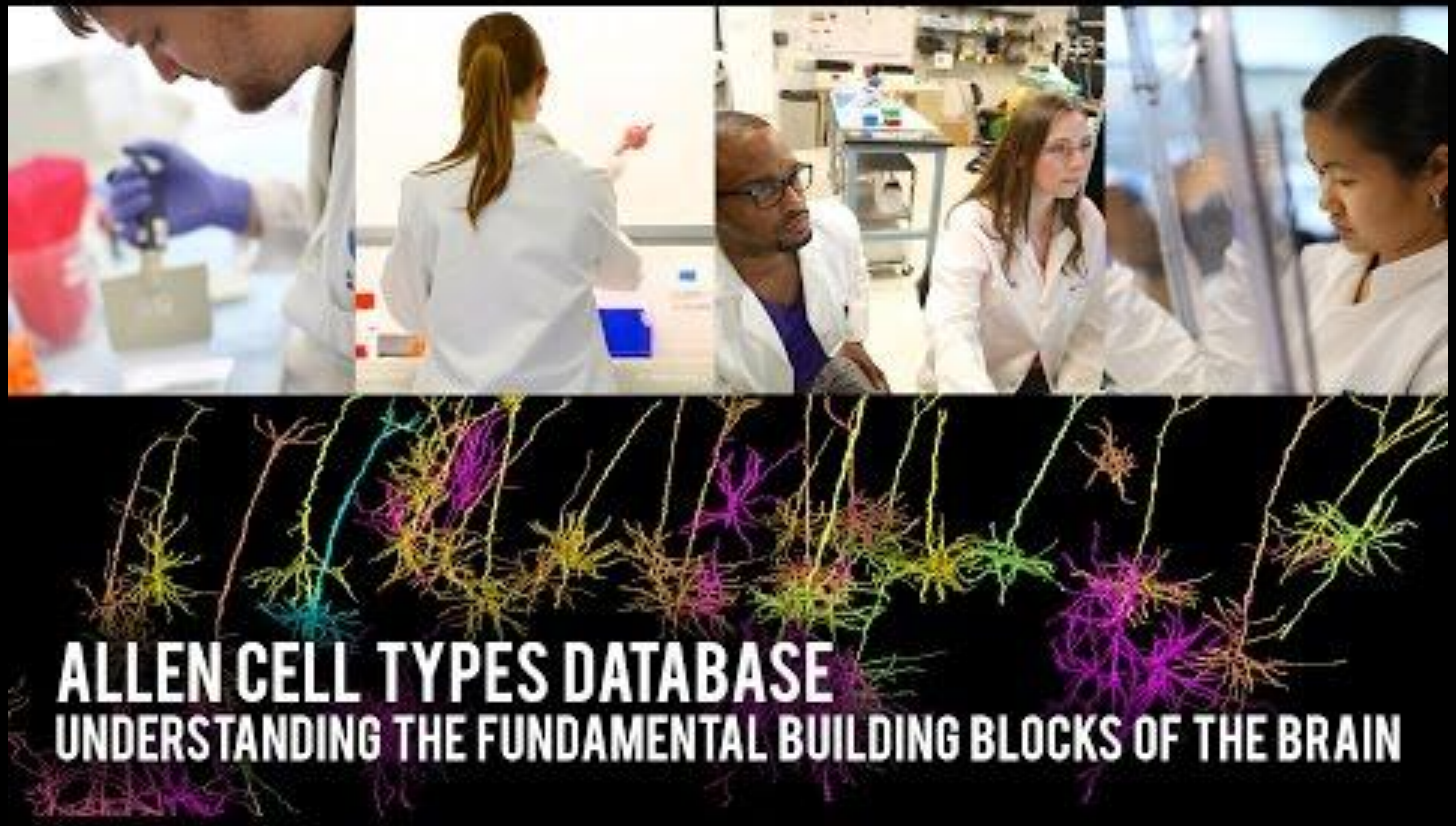
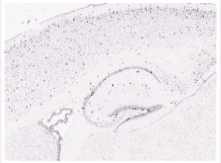
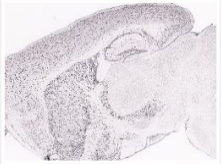
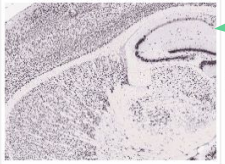
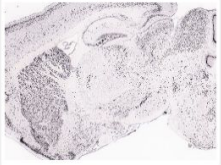


Introduction to the Allen Cell Types Database

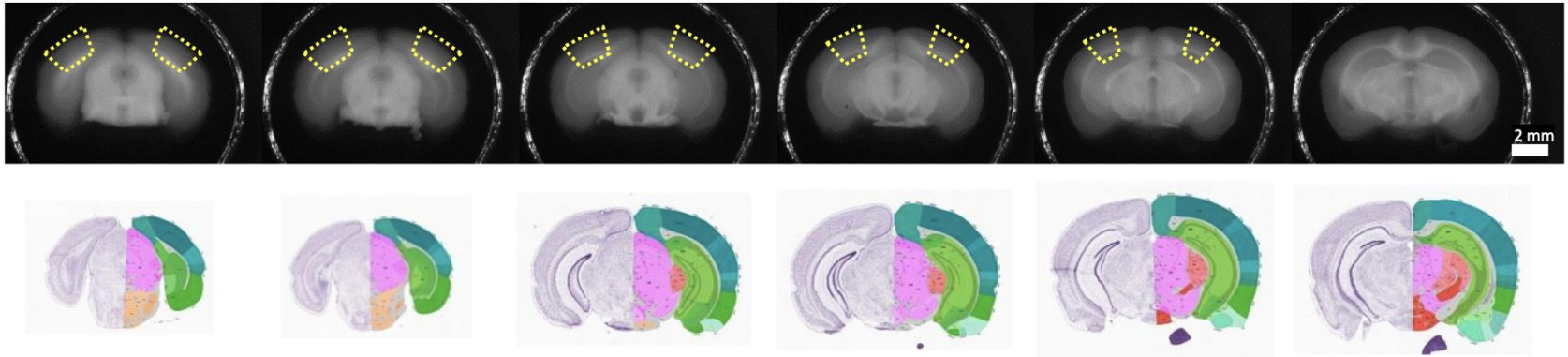
Dlx1-CreERT2 Z. Josh Huang		Expressed in strong scattered cells throughout the brain. Expression is more widespread in olfactory bulb and cortical subplate (amygdala).
Dlx5-CreERT2 Z. Josh Huang		Widespread, interneuron expression throughout cortex, striatum, amygdala, hippocampus, olfactory bulb, hypothalamus, and pallidum.
Drd1a-Cre_EY262 Richard Palmiter		Reporter expression is throughout the cortex, hippocampus, striatum, and other structures. Cre expression is enriched in piriform cortex and str restricted populations within the medulla, pons, and cerebellum.
Drd2-Cre_ER44 Nathaniel Heintz and Charles Gerfen		Enriched in striatum, restricted populations within cerebellum, cortex, thalamus, medulla, and olfactory areas.
Drd3-Cre_KI196 Nathaniel Heintz and Charles Gerfen		Cre expression is enriched in layer 2/3 of somatosensory areas and retrosplenial area of cortex; and in restricted populations within the hippoca cortical subplate, and striatum.

These are *in situ* hybridization images showing location of gene expression

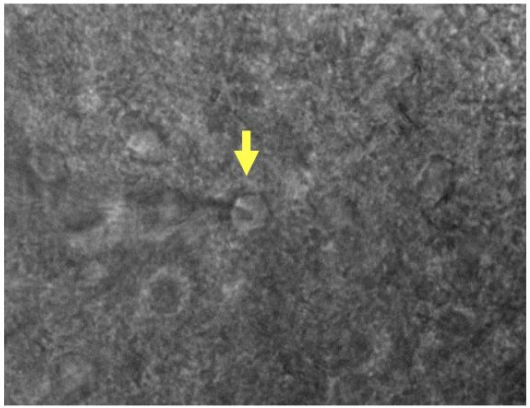
Allen Brain Institute uses hundreds of different Cre-driver lines
(from http://connectivity.brain-map.org/transgenic)

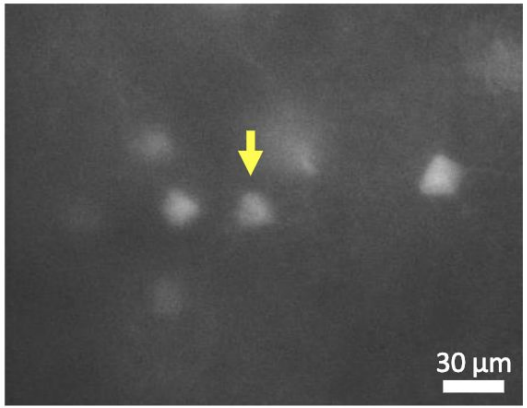

ALLEN INSTITUTE for
BRAIN SCIENCE

Brain regions of interest were identified



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

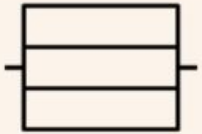




Ramp

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.

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.

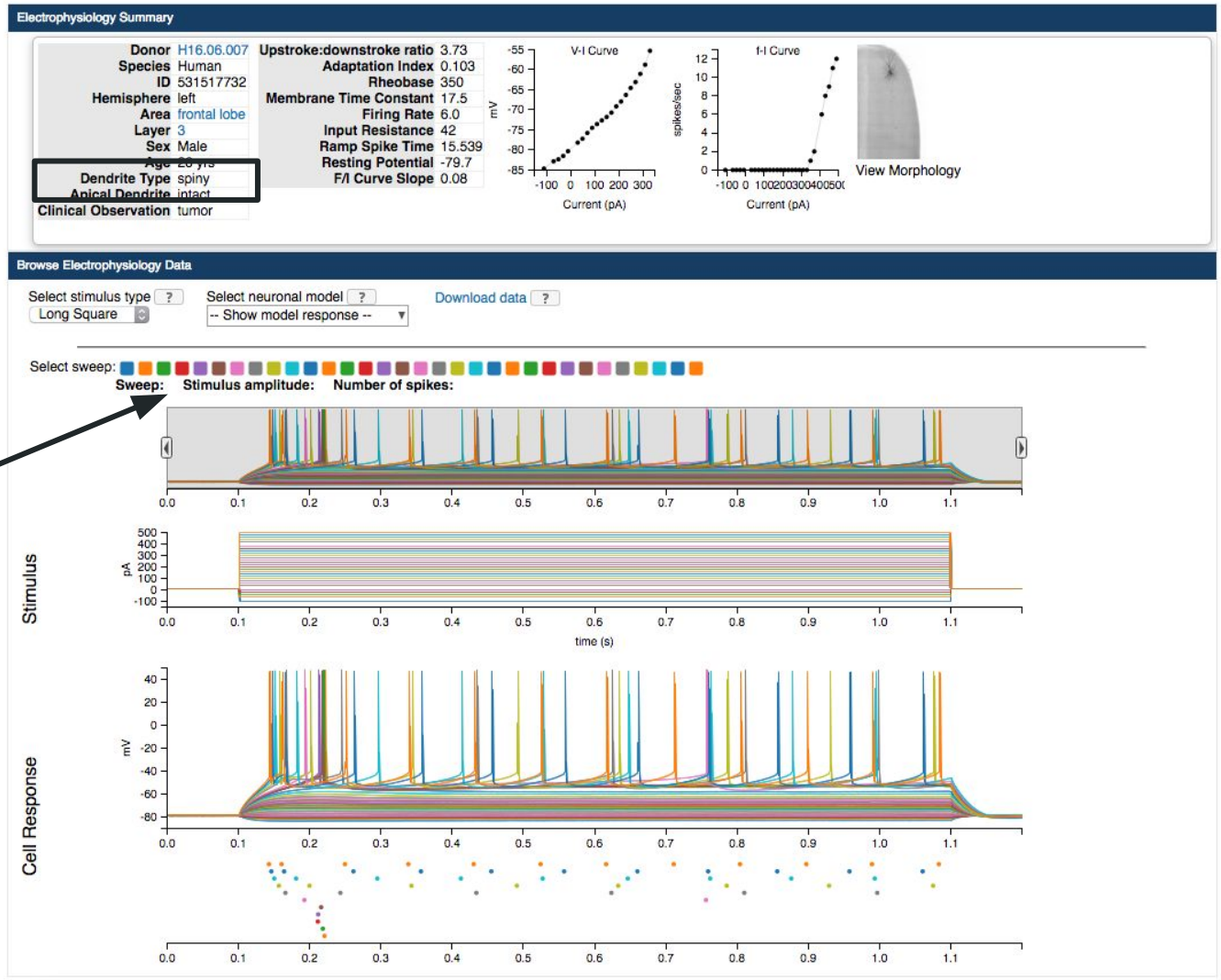
Short Square
Triple

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.

While recording, different current waveforms were injected into the cell.

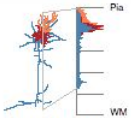


Sweep # will show here

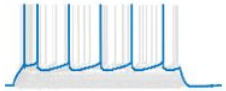
Morphology Summary

Donor	H16.06.007
Species	Human
ID	531517732
Hemisphere	left
Area	frontal lobe
Layer	3
Sex	Male
Age	26 yrs
Dendrite Type	spiny
Apical Dendrite	intact
Clinical Observation	tumor

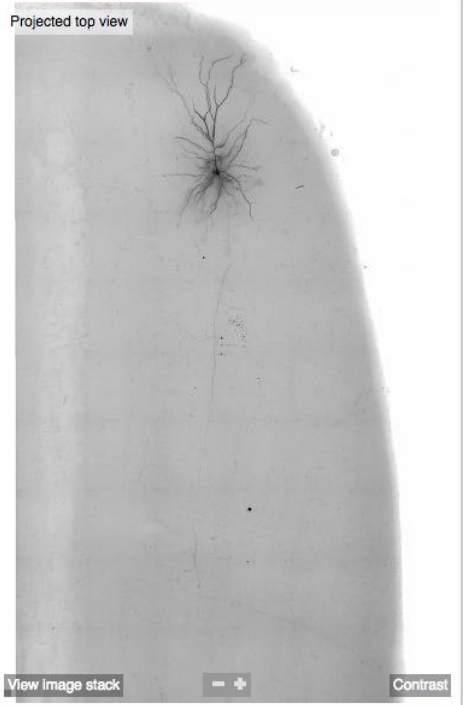
Max Euclidean Distance	491.9
Number of Stems	7
Number of Bifurcations	42
Average Contraction	0.846
Parent:Daughter	0.932

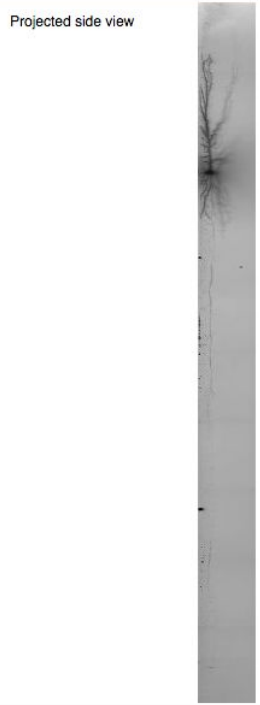


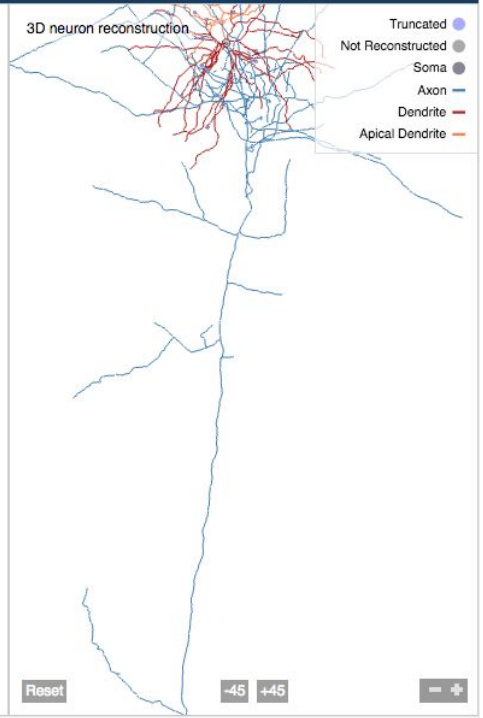
Cortical Depth Histogram


View Electrophysiology

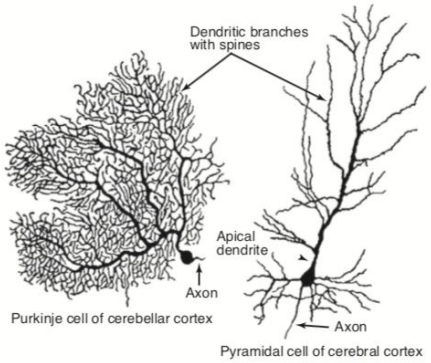
Browse Morphology Data





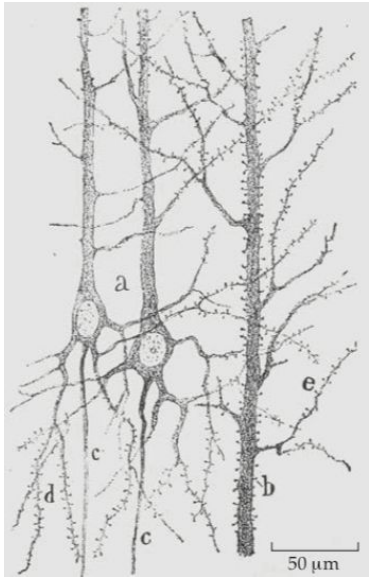


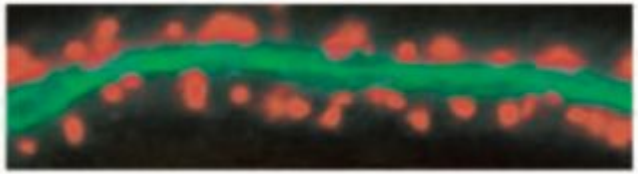
Download Morphology Download Morphological Measurements



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: https://science.sciencemag.org/content/290/5492/754

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

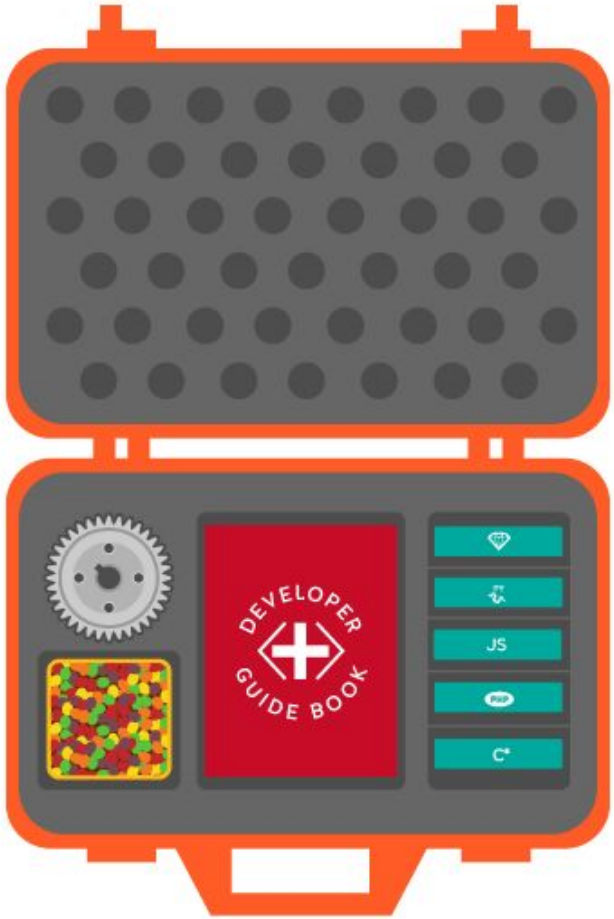


Image: Nordic Apis

Resources

Documentation - Allen Cell Types Database

Allen Cell Types Features Glossary

Software Carpentries “Combining Dataframes with Pandas”

Merge and Join DataFrames with Pandas in Python