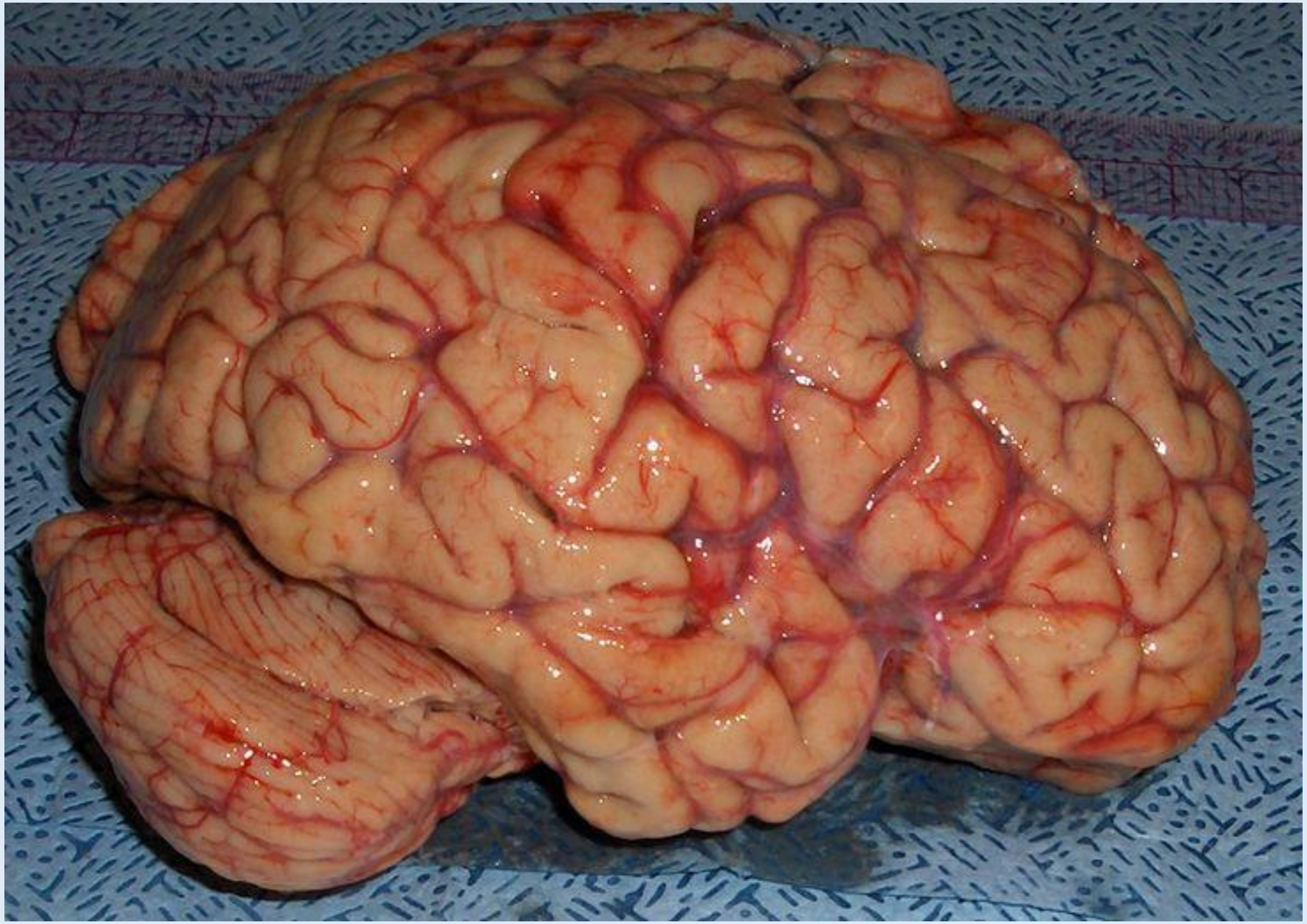
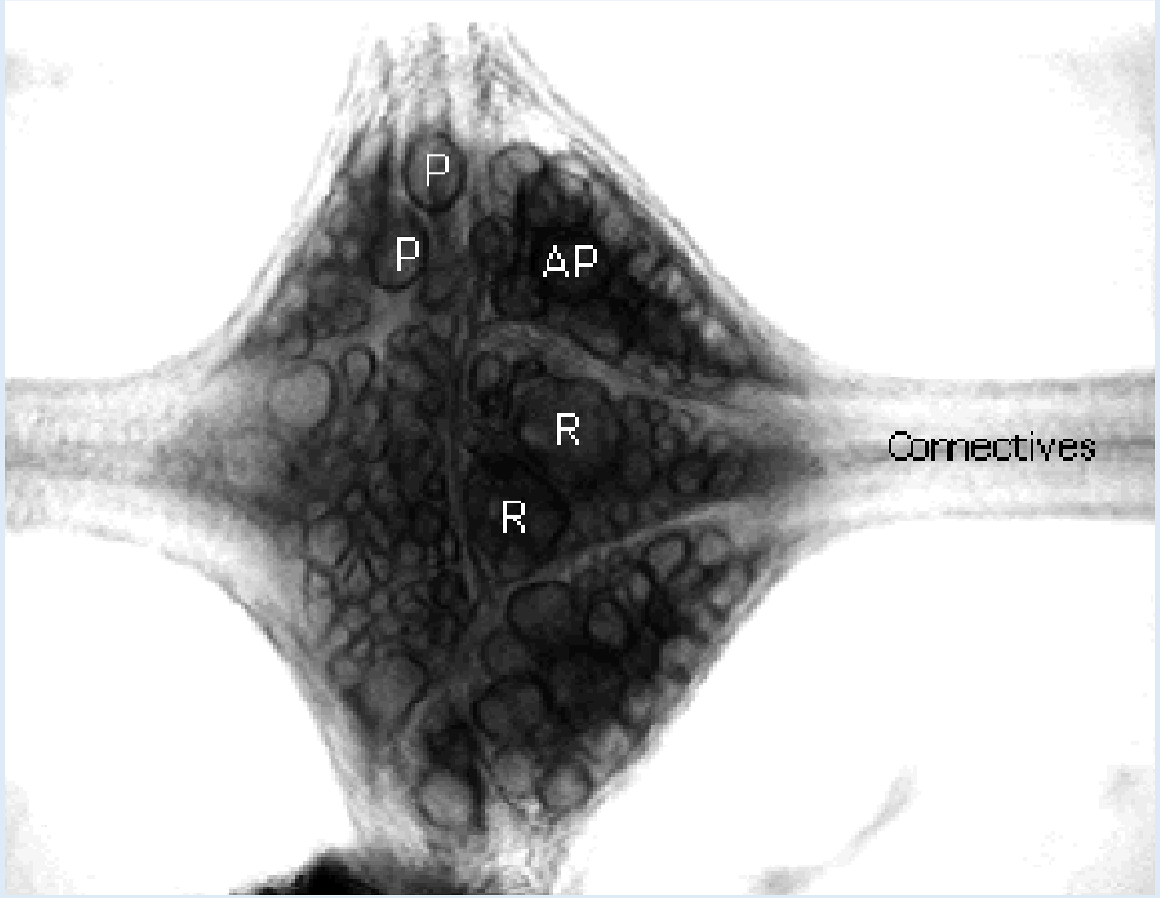
How we study the brain: a survey of methods used in neuroscience

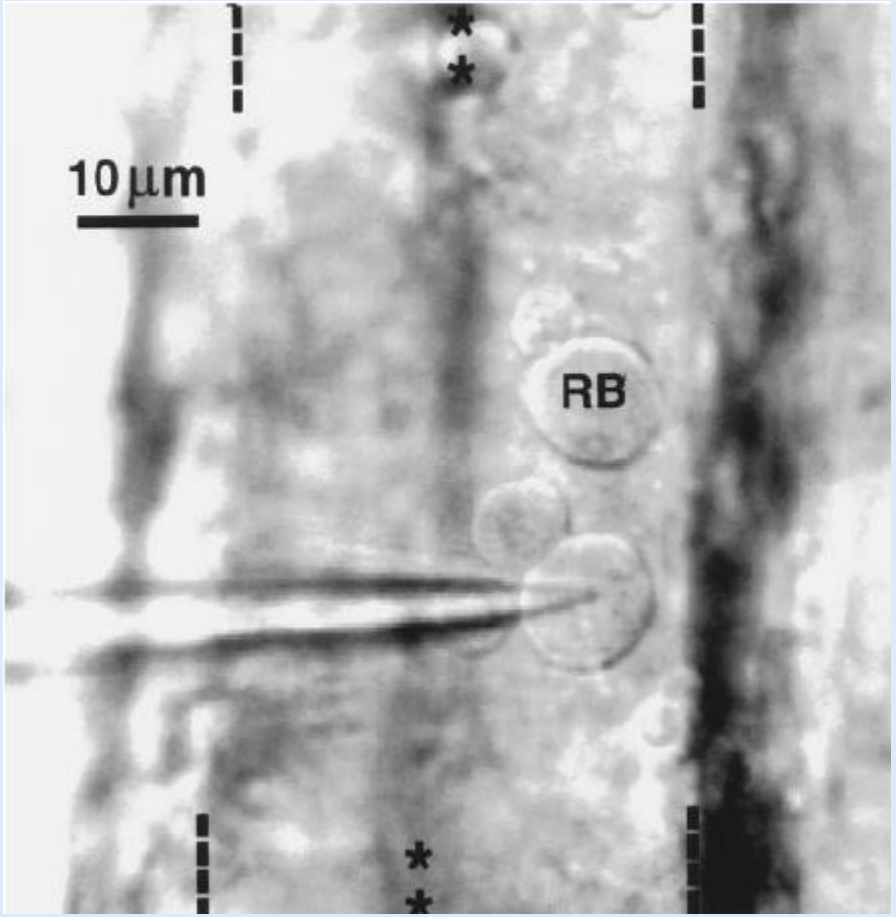


Preparing living neurons for recording

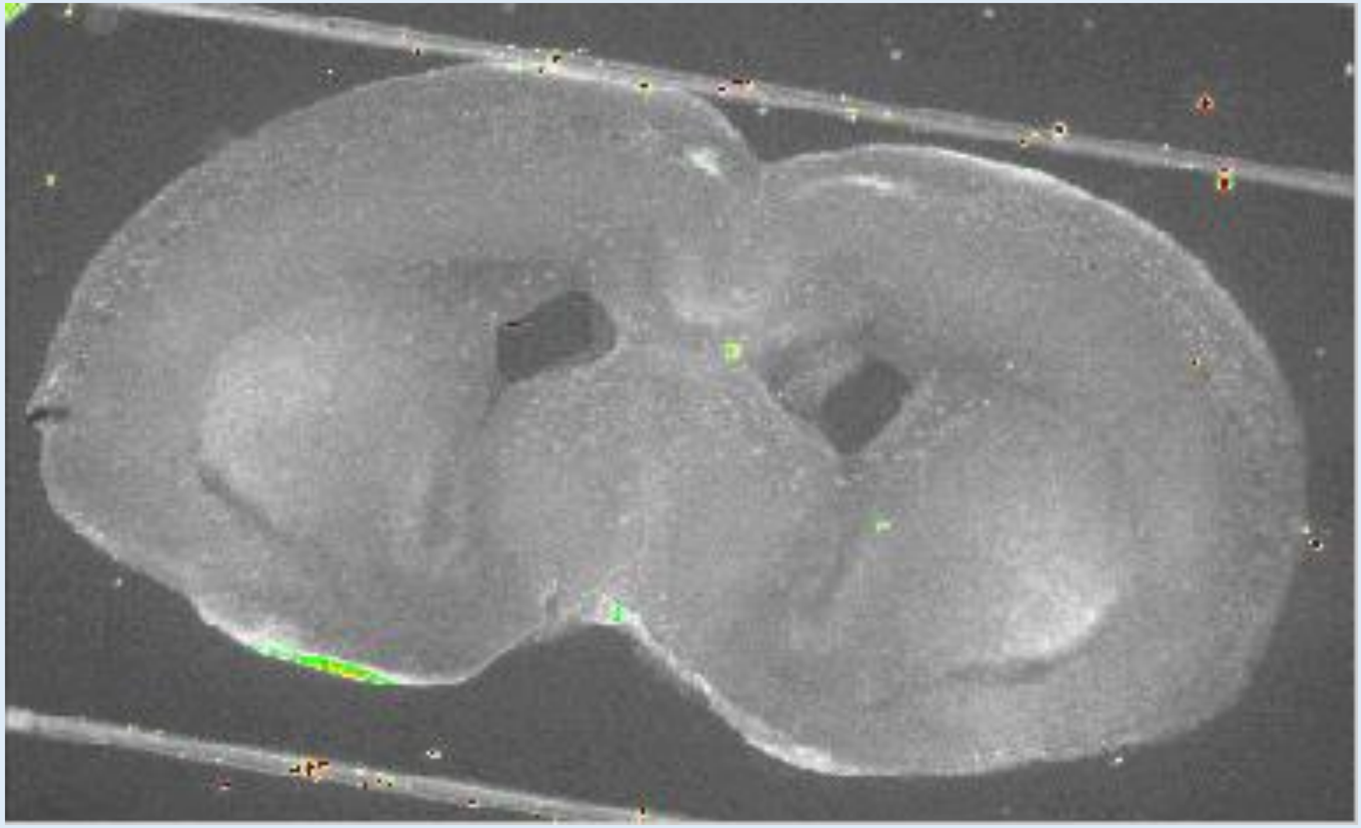
Large identifiable neurons in a leech



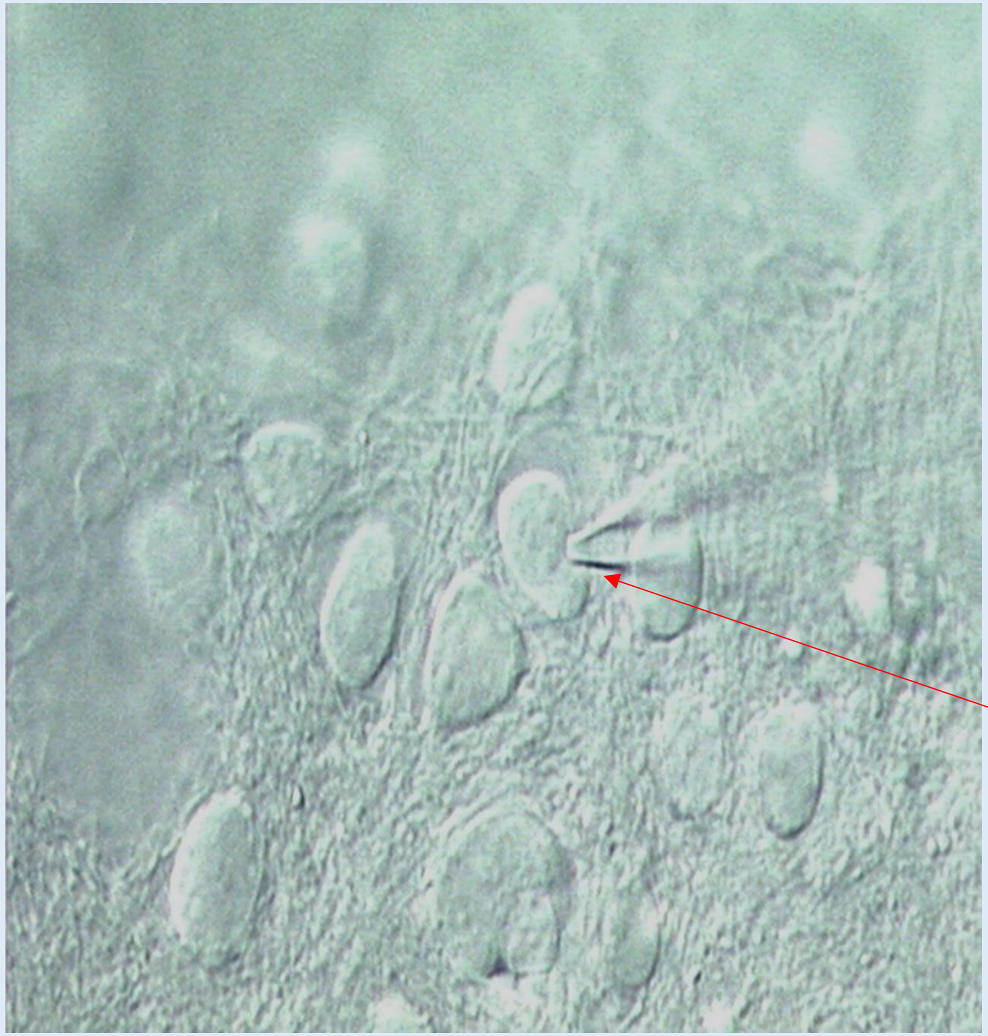
Rohon-Beard neurons in a frog spinal cord



Living slice of a mouse brain



Neurons visible in a living slice of a mouse brain

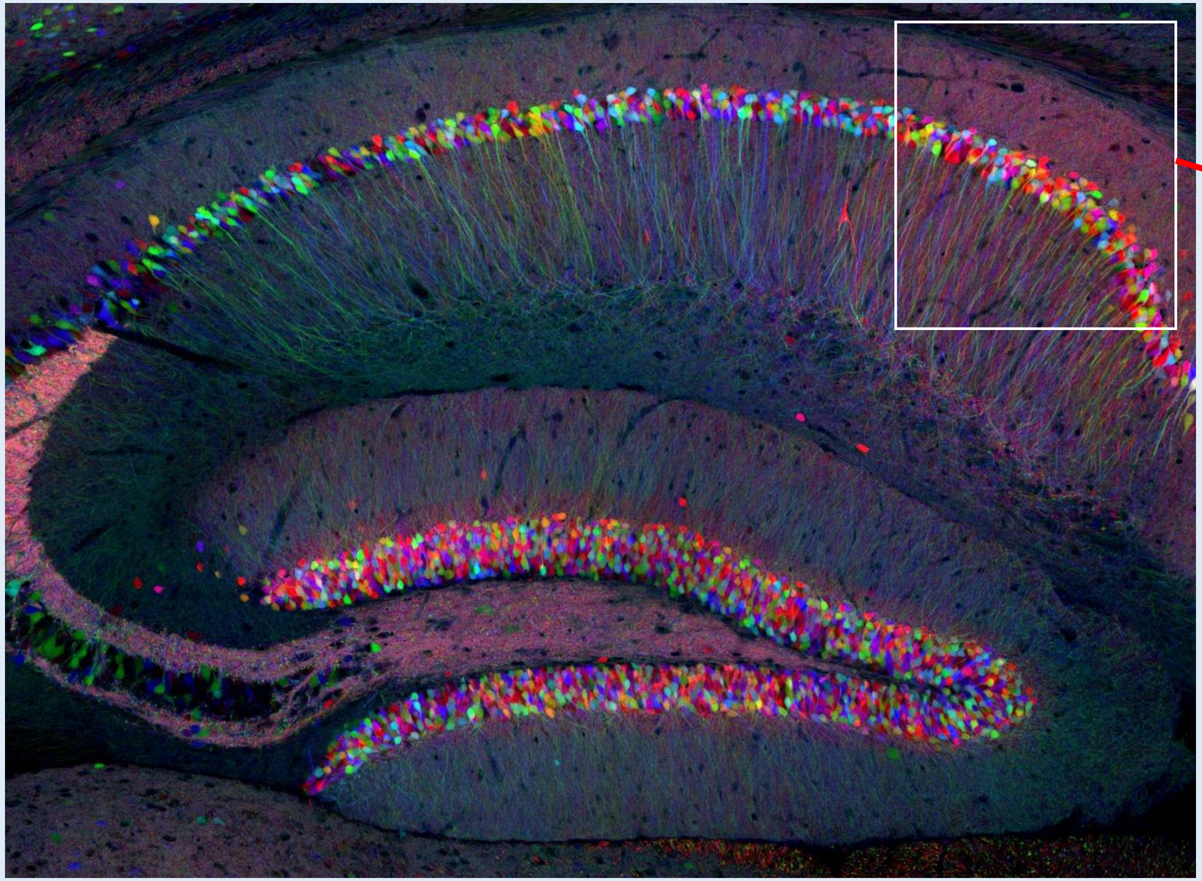


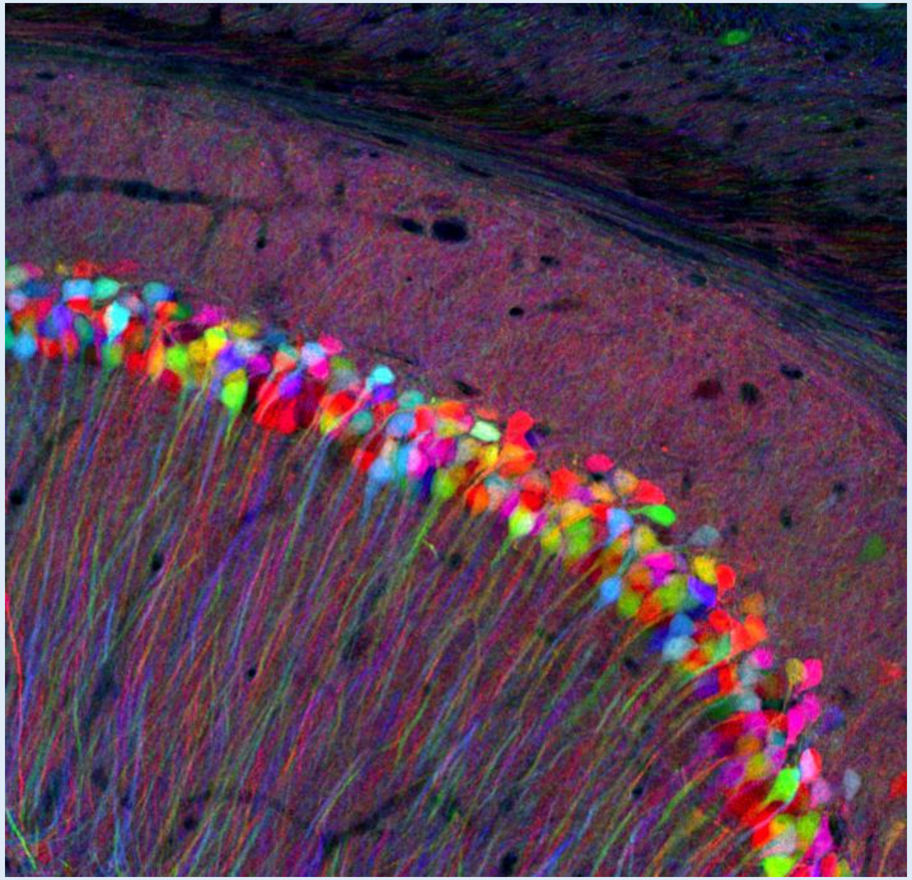
Electrode to record electrical signals from a single neuron

Making living neurons visible and identifying them by type

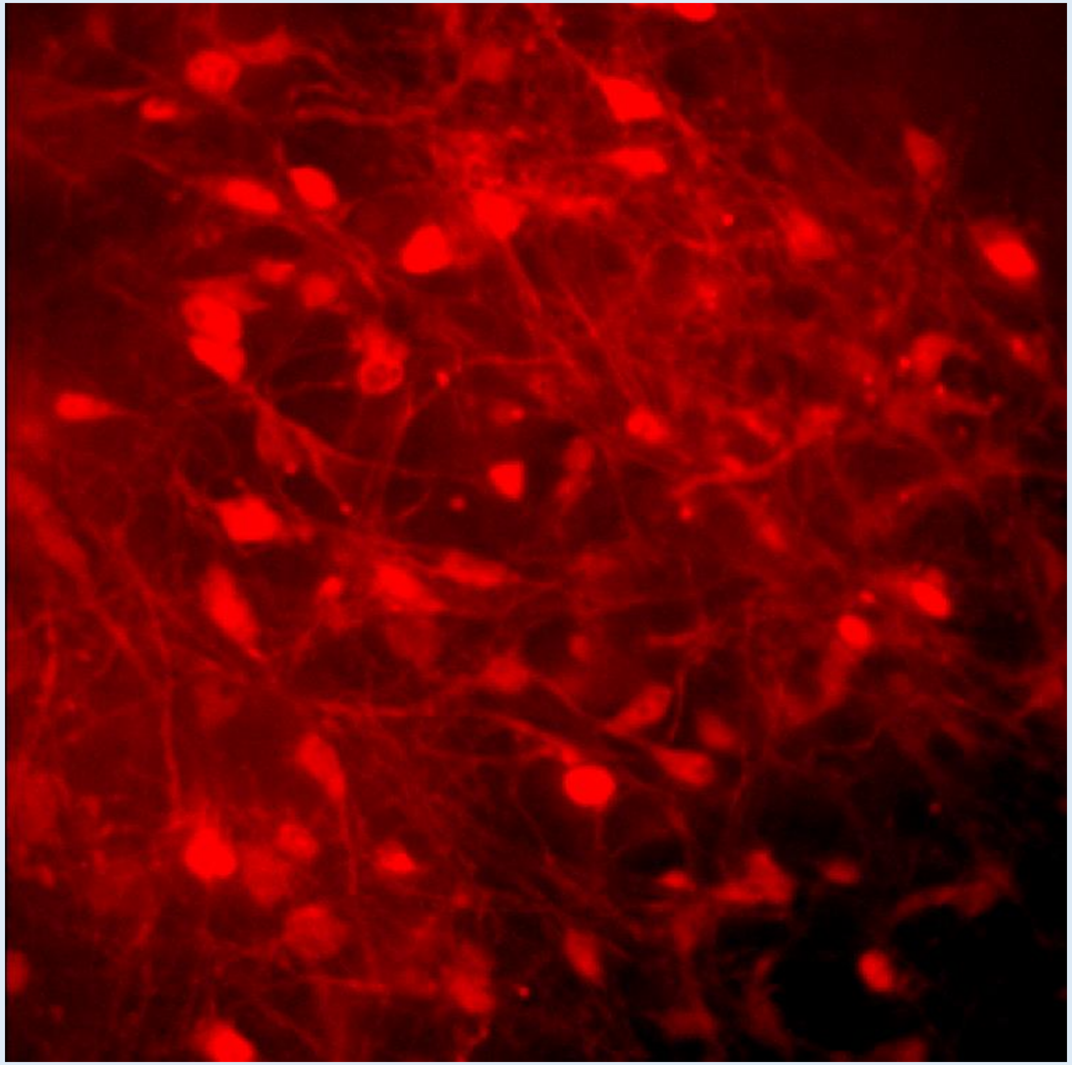
Labeling neurons: ‘Brainbow’

Genetic expression of different ratios of red, green, and blue genetically encoded fluorescent proteins





Labeling neurons by type: Inhibitory neurons labeled with Red Fluorescent Protein

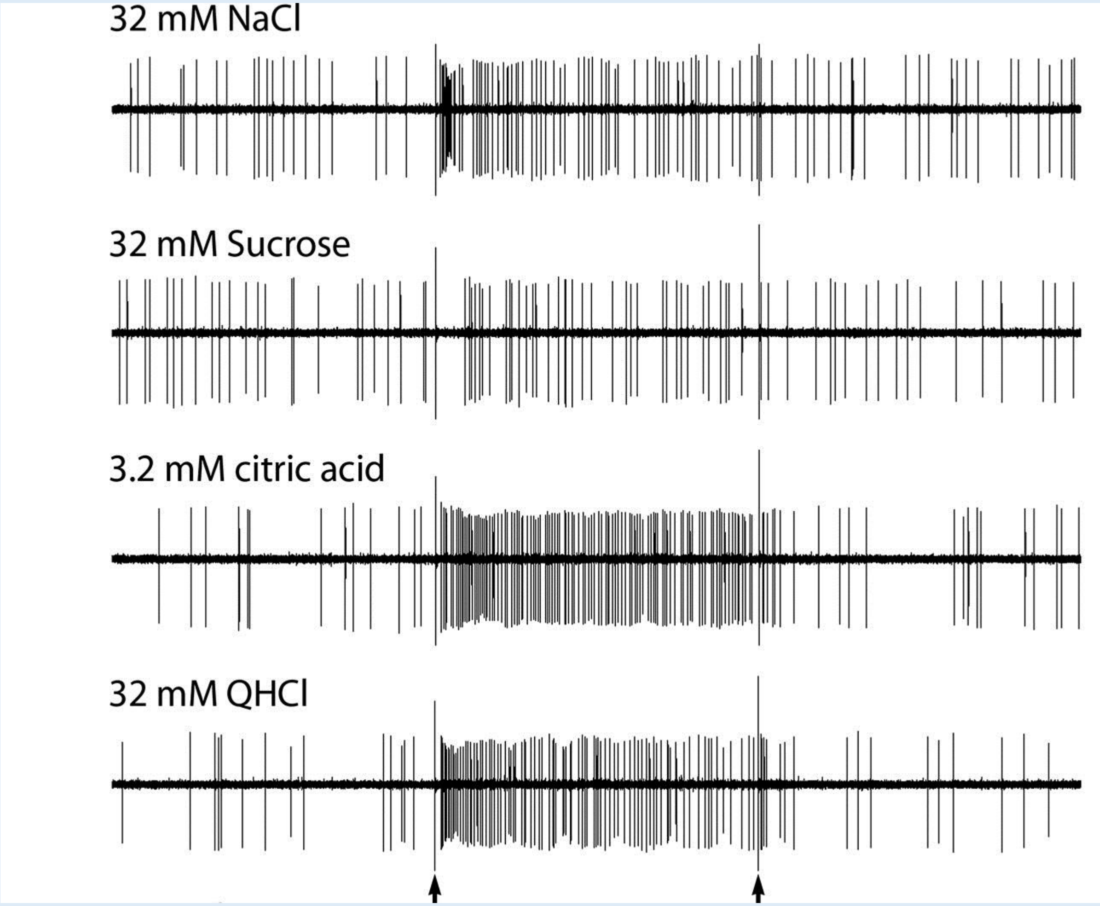


How is this done?

1. Take the gene for a protein unique to these neurons.
2. Extract the DNA sequence that controls the expression of that gene in those neurons.
3. Link the gene for RFP to that sequence.
4. Insert this new construct into the mouse genome and breed this new strain of mouse.

Recording electrical signals from single neurons

Extracellular recording from single taste neurons

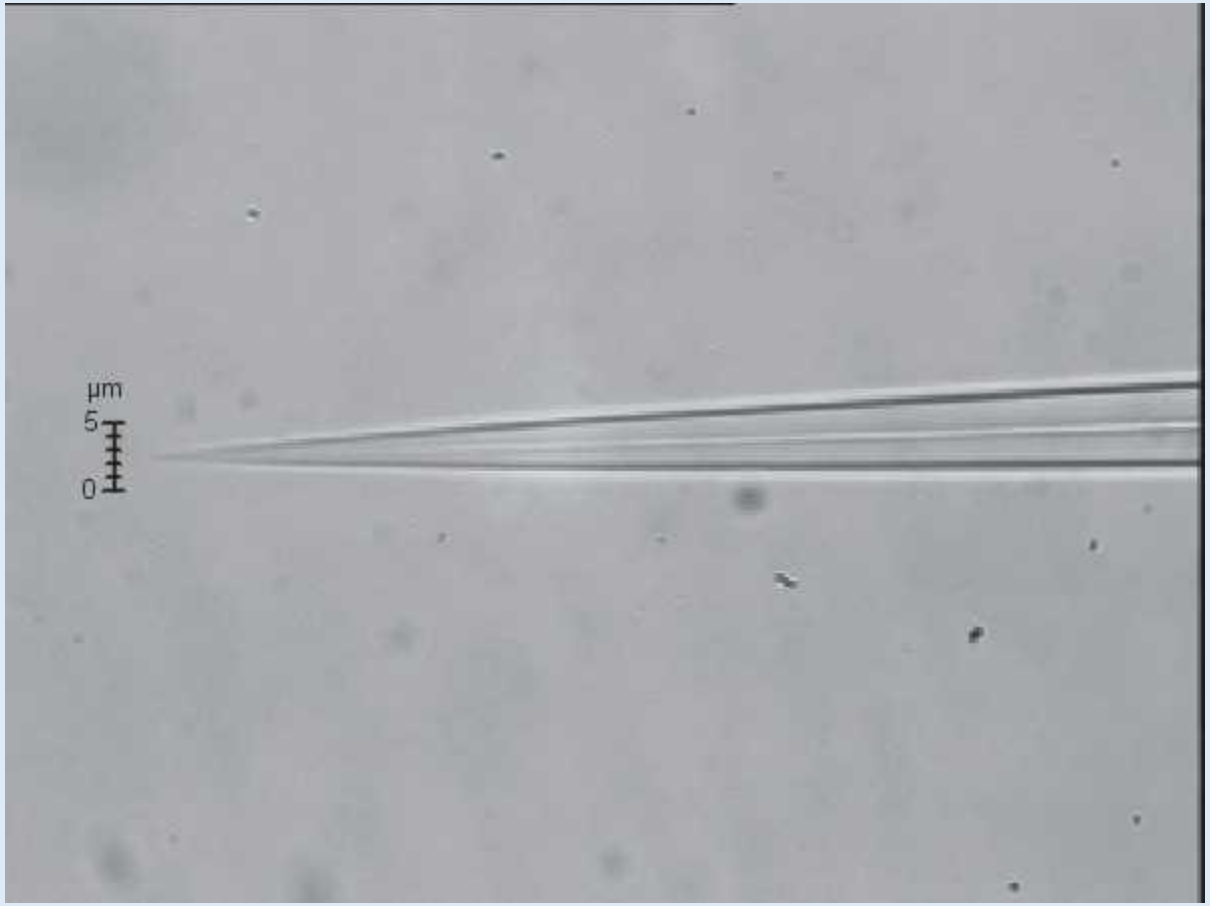


Detects action potentials in response to stimuli.

Shows input/output relations of neurons.

Does not show actual intracellular waveform or mechanisms of signals.

Glass microelectrode for recording from inside a single neuron



The tip is extremely sharp so it can penetrate the cell membrane without injuring the cell.

It is so sharp you can’t see it.

No, really, you can’t see it.

The diameter of the tip is smaller than the wavelength of visible light.

Pipet pulling machine for making these electrodes

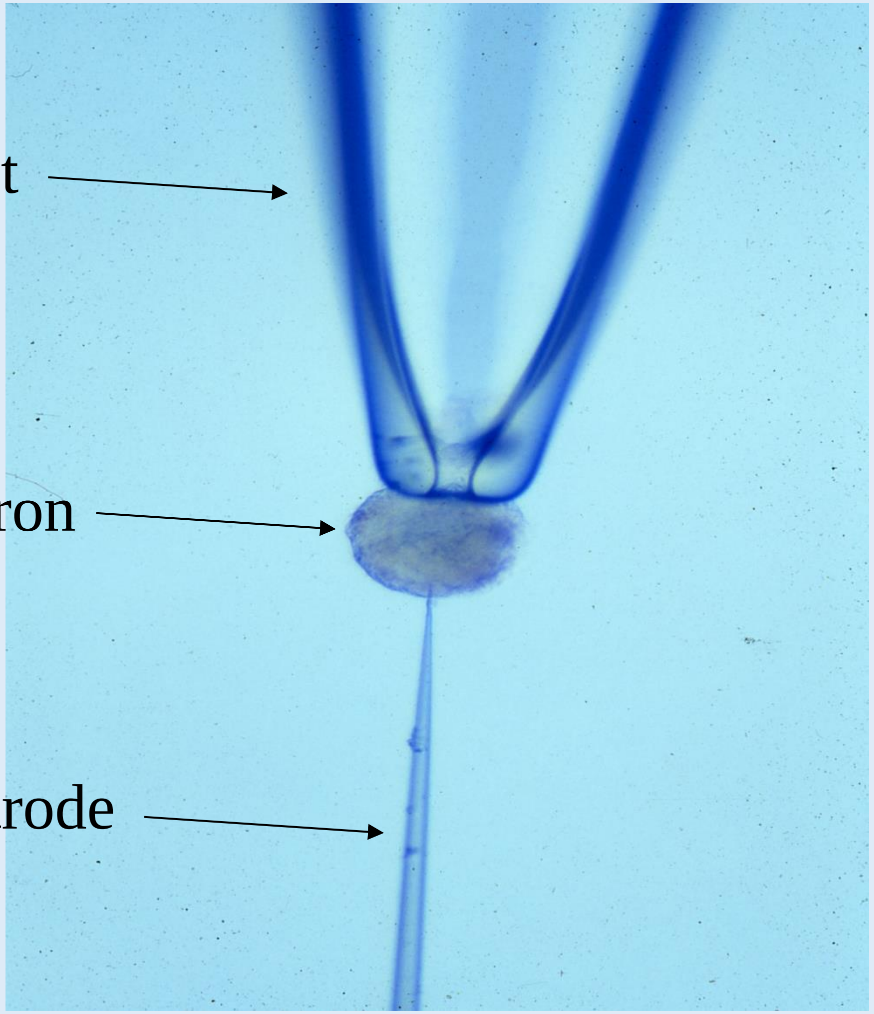
A micromanipulator to position the electrode in the neuron

A vibration isolation table so vibrations don't knock the electrode out of the cell

Large heavy table top

Floats on a cushion of air pumped into the legs

Glass microelectrode inside a single isolated neuron



This is a neurons from a snail.

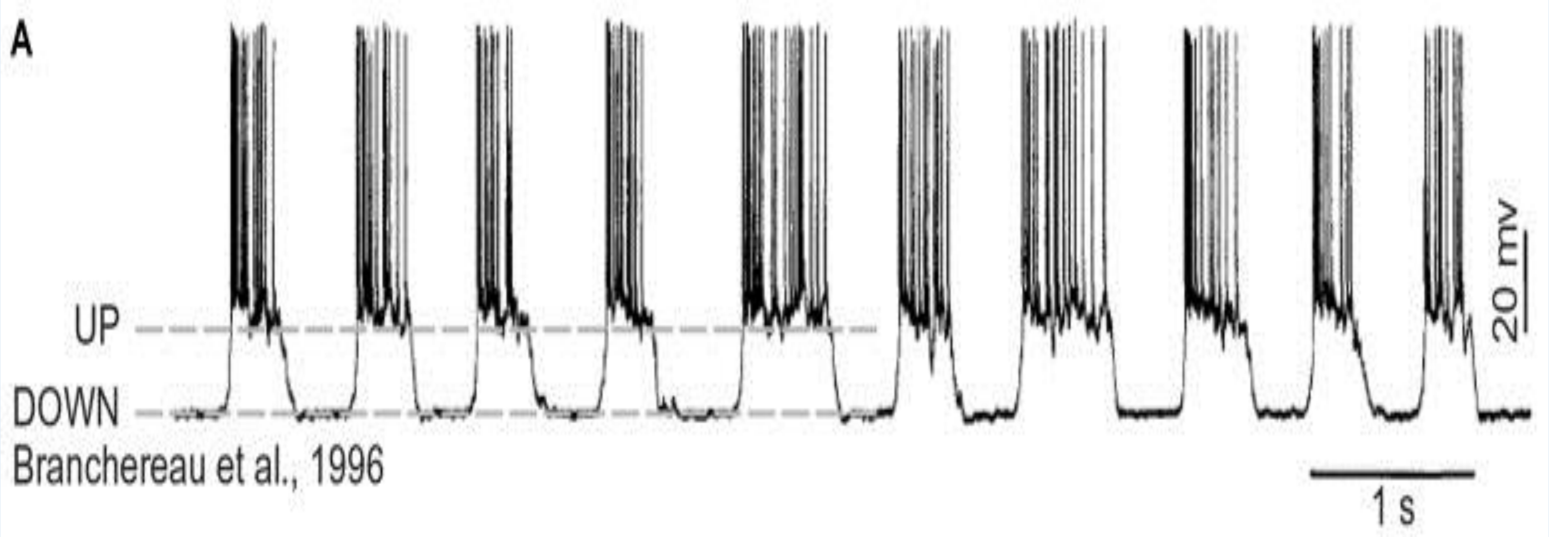
It was dissected out using a hair from a blonde newborn baby. (Blonde hair is finer.)

This was done in my lab.

The hair was taken from my daughter.

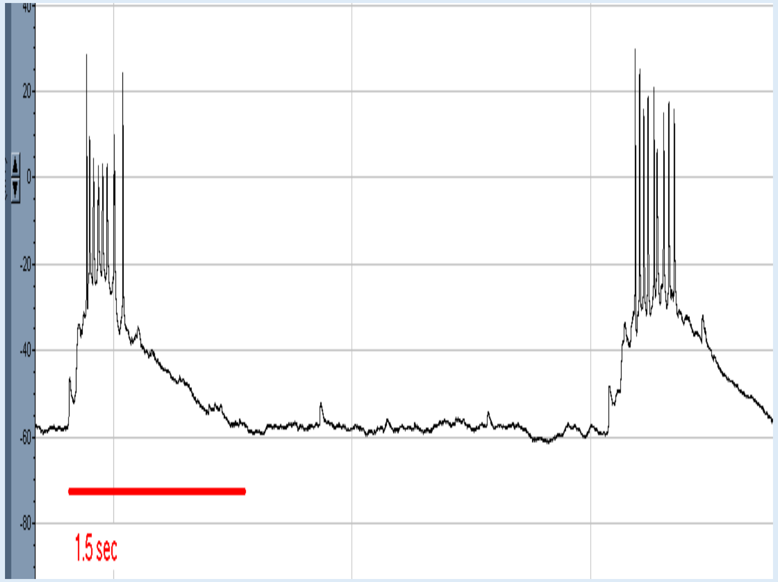
(Scientists know no limits.)

Intracellular recording from single brain neurons



Shows actual intracellular voltage. Notice gaps in action potentials are caused by negative deflections of intracellular voltage.

Intracellular recording from single brain neurons in a brain slice

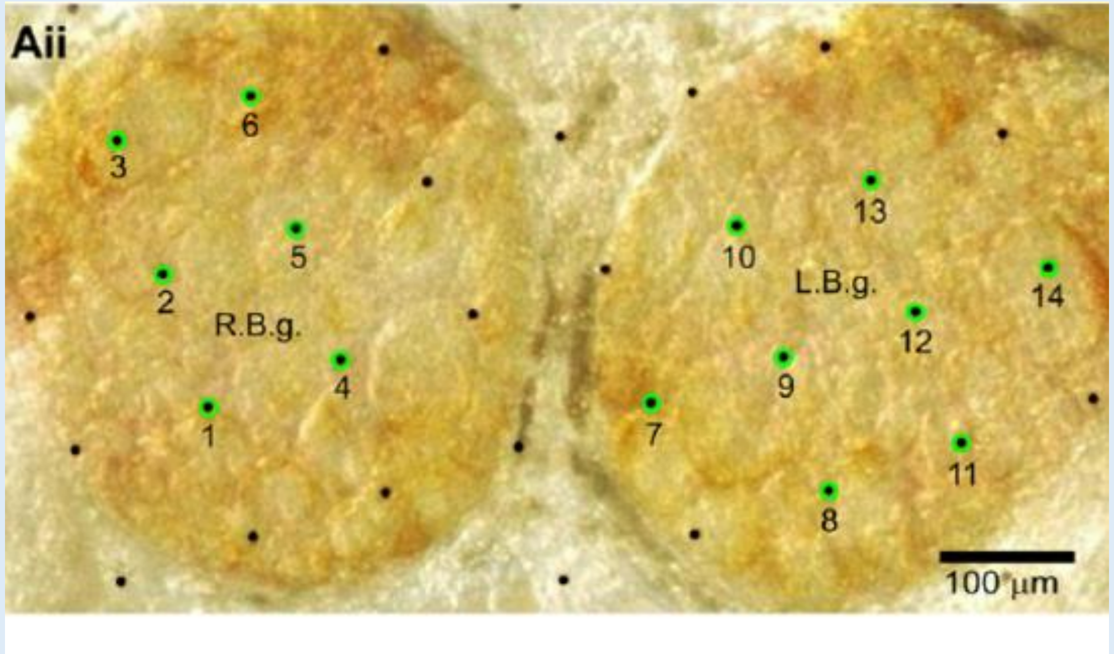


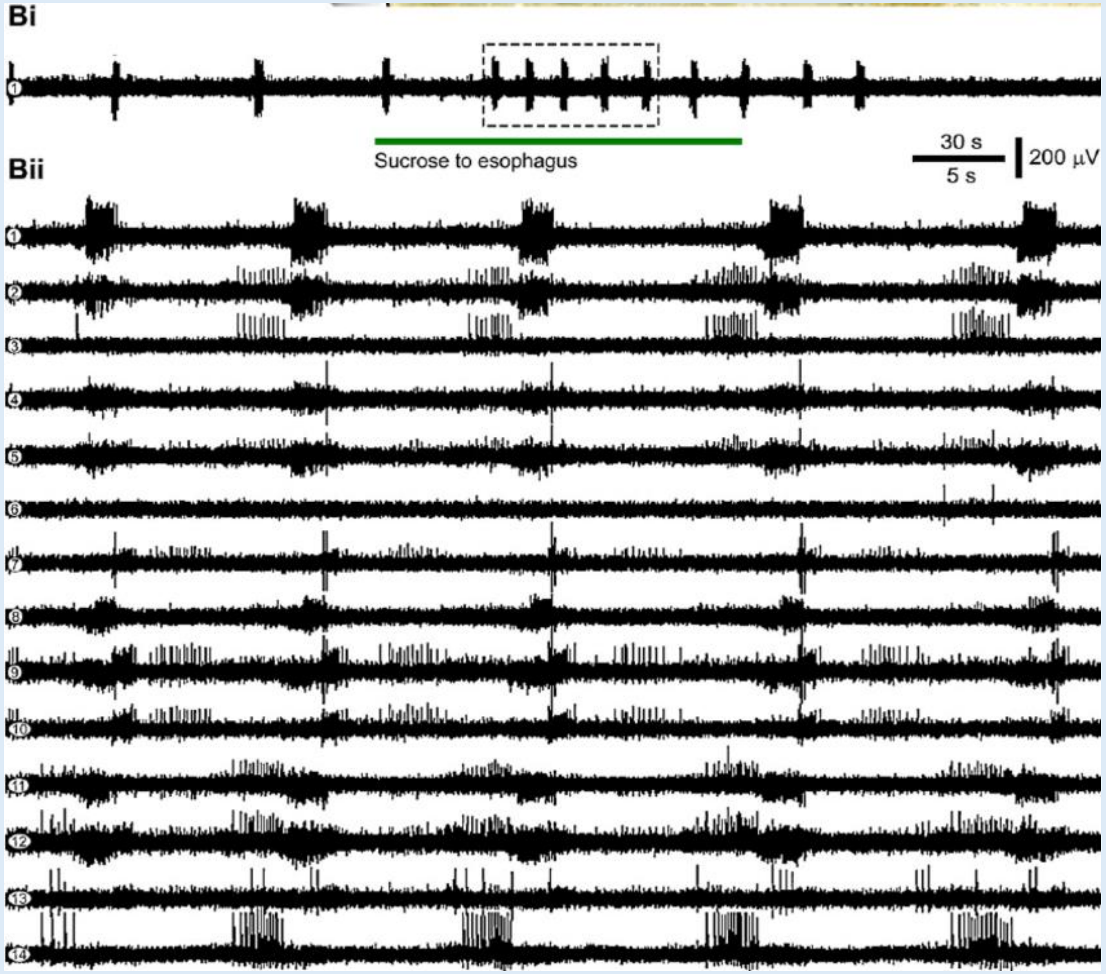
Actual bursting in a brain neuron from previous slide.

Recording electrical signals from many neurons

1. Electrical methods
2. Optical methods

Multi-electrode array recording





The bursts you see control contractions of the esophagus in a snail when it's swallowing.

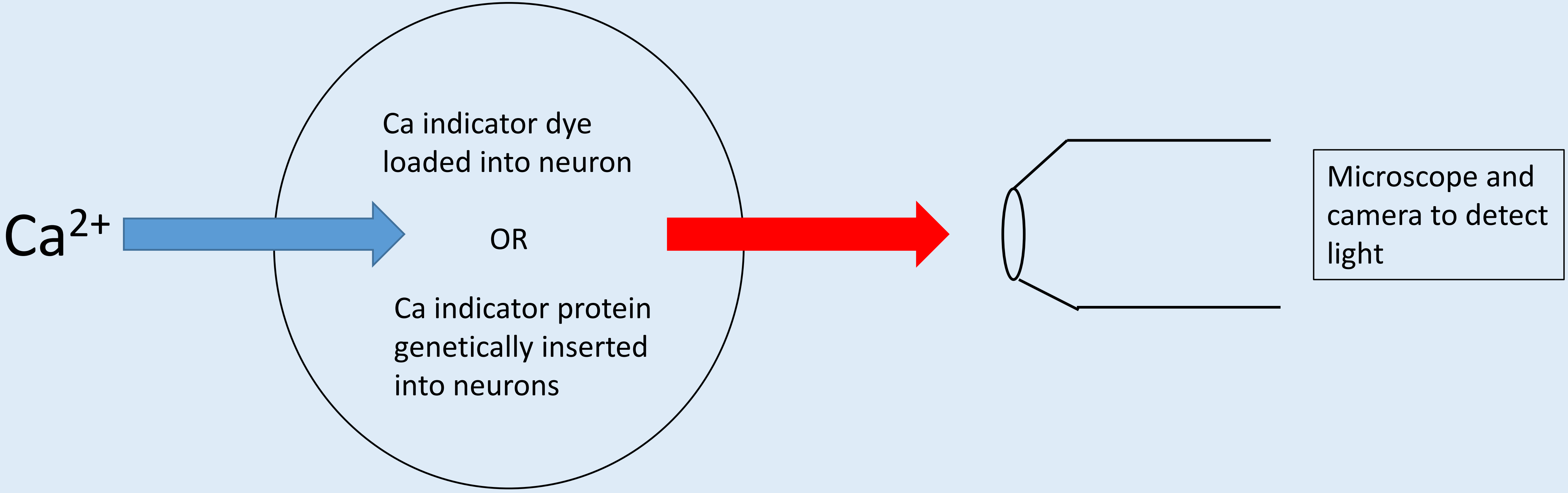
Recording from many neurons: multi-electrode arrays

https://www.youtube.com/watch?v=lfNVv0A8QvI

Hipocampal place cells

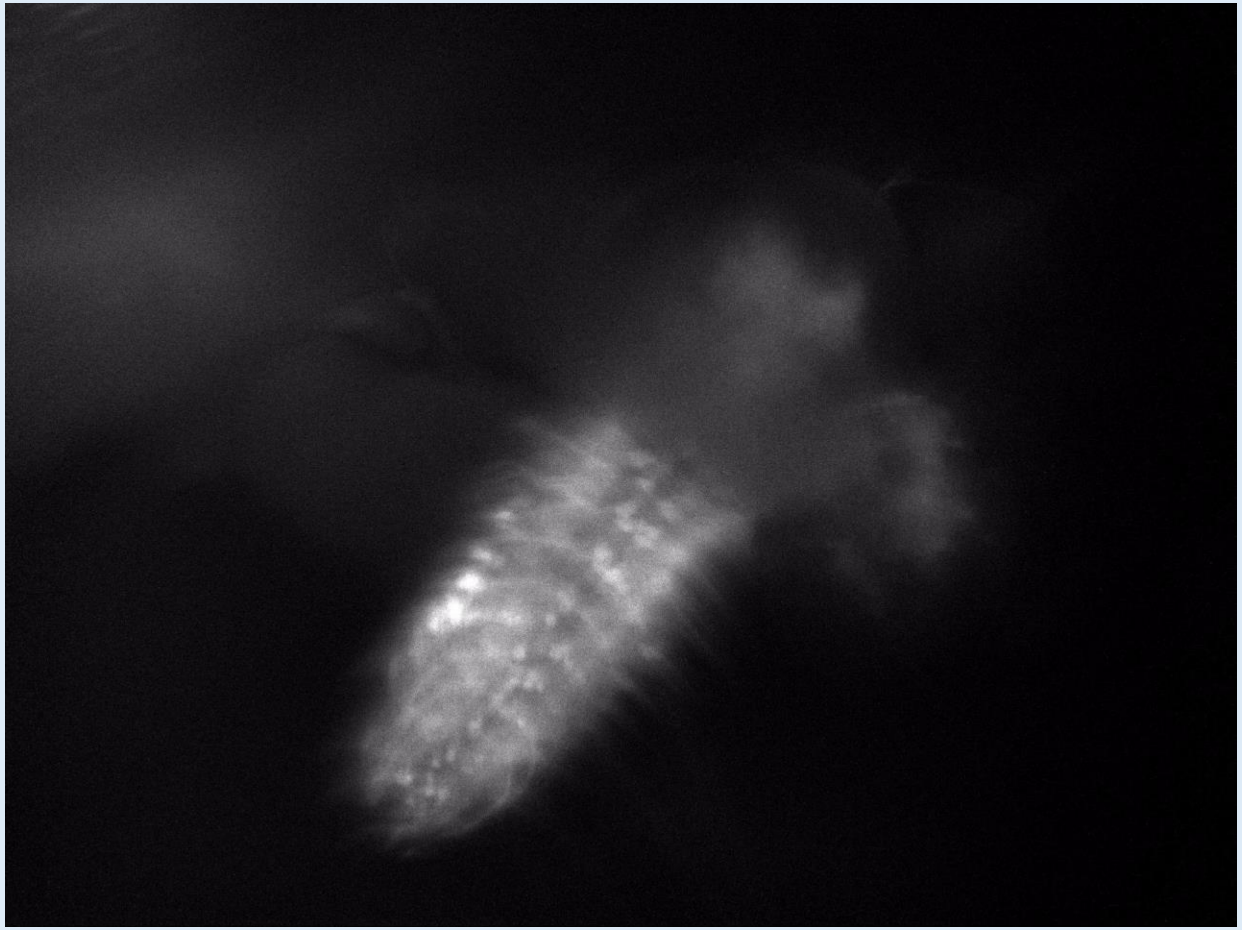
Recording from many neurons: Calcium imaging

Calcium enters neurons when they are active.
Certain molecules emit light in presence of calcium (Ca indicators)

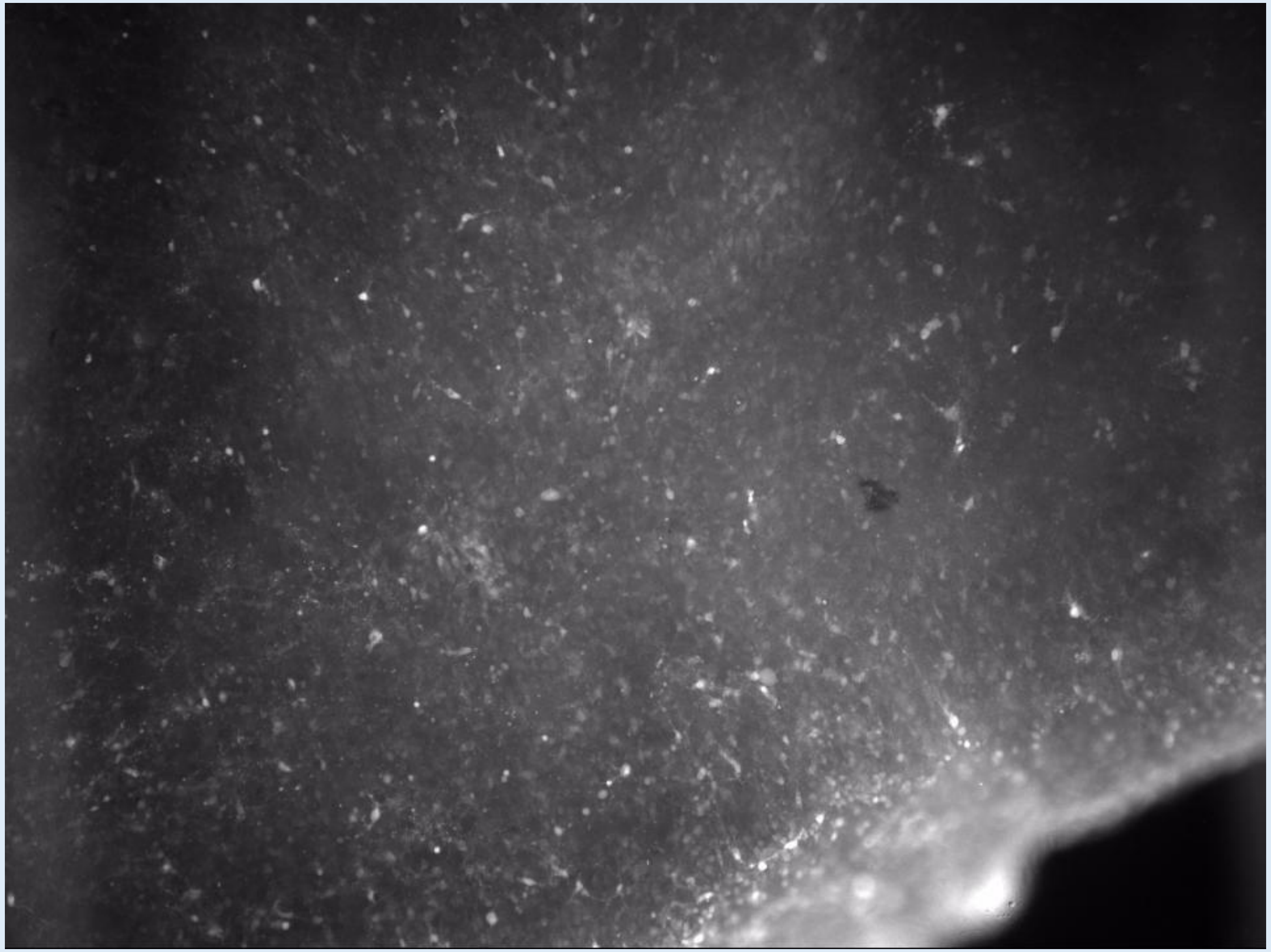


Calcium imaging of motor patterns during fly larva crawling

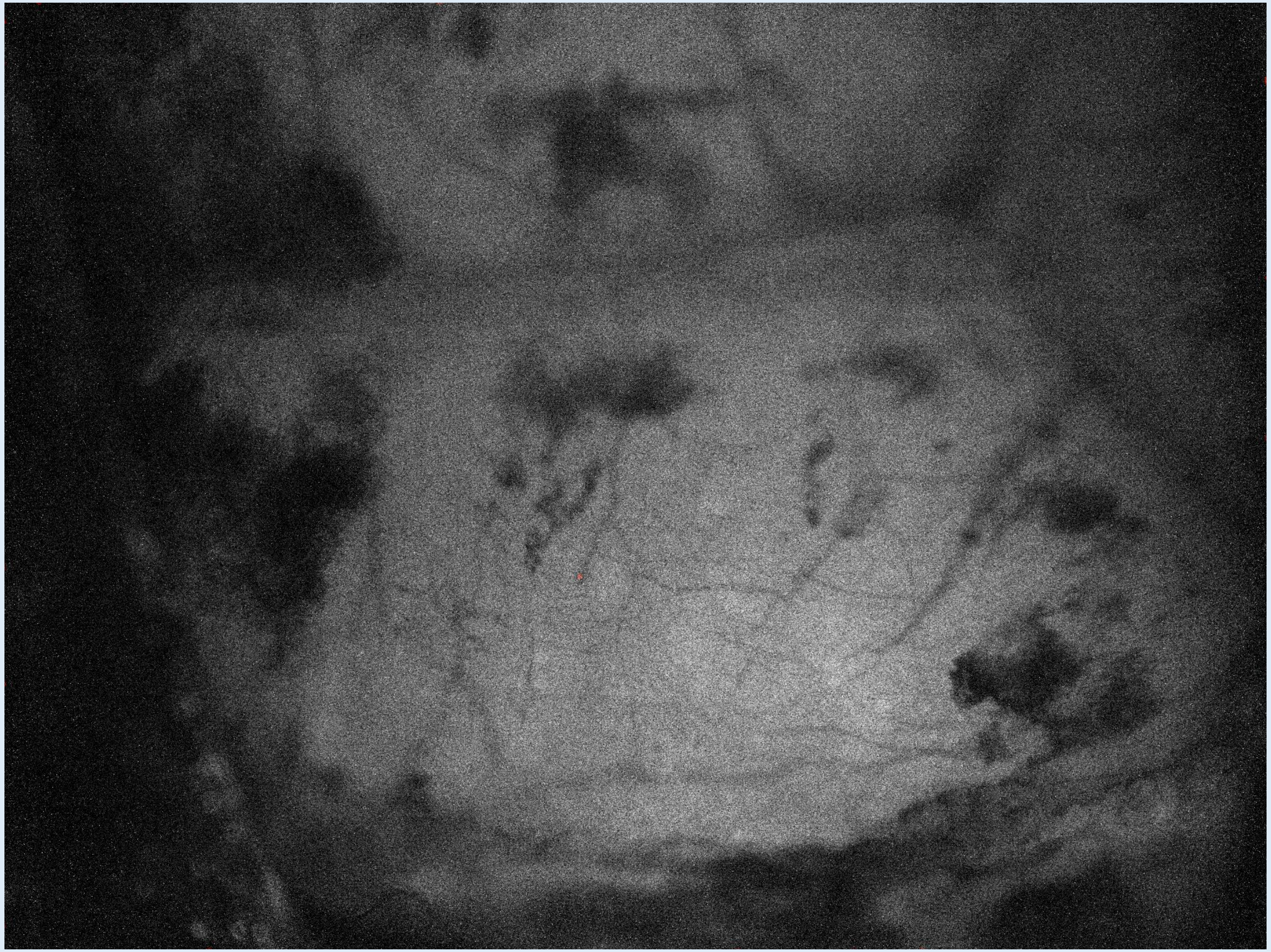
(The calcium dye was expressed only in neurons that control muscles.)



Calcium Imaging of P0 Cortical Neurons Reveals Single neuron and cluster activity



Optical recording of neural activity through the skull of a living mouse

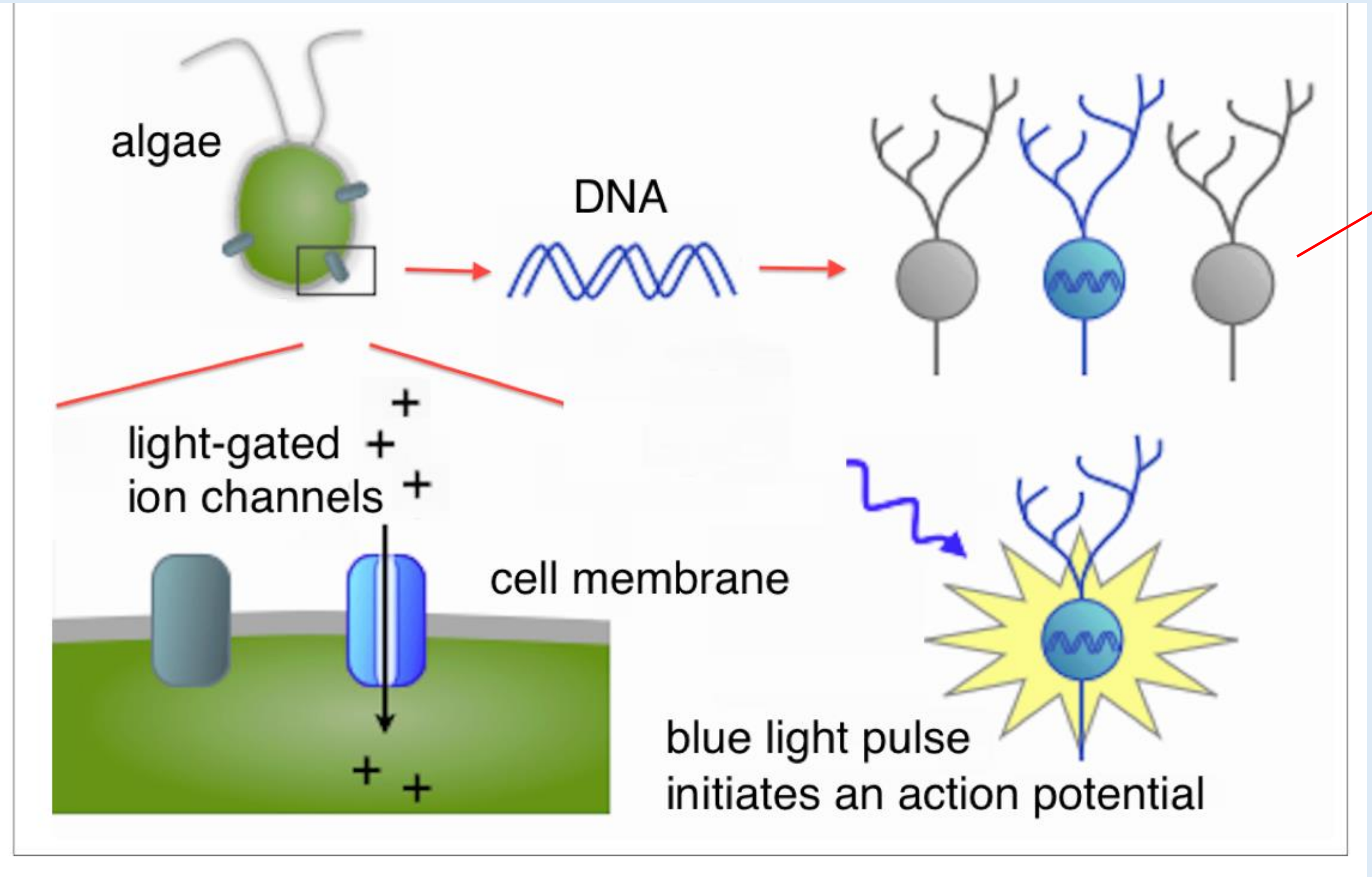


Ai93-Cux2-CRE-CAMK2tet mouse
GCaMP6f targeted to superficial layers
P6

Calcium imaging allows recording of neural activity using

A. Magnetic imaging
B. Light output of specialized molecules
C. High sensitivity amplifiers
D. Sound

Optogenetics: Controlling neurons with light



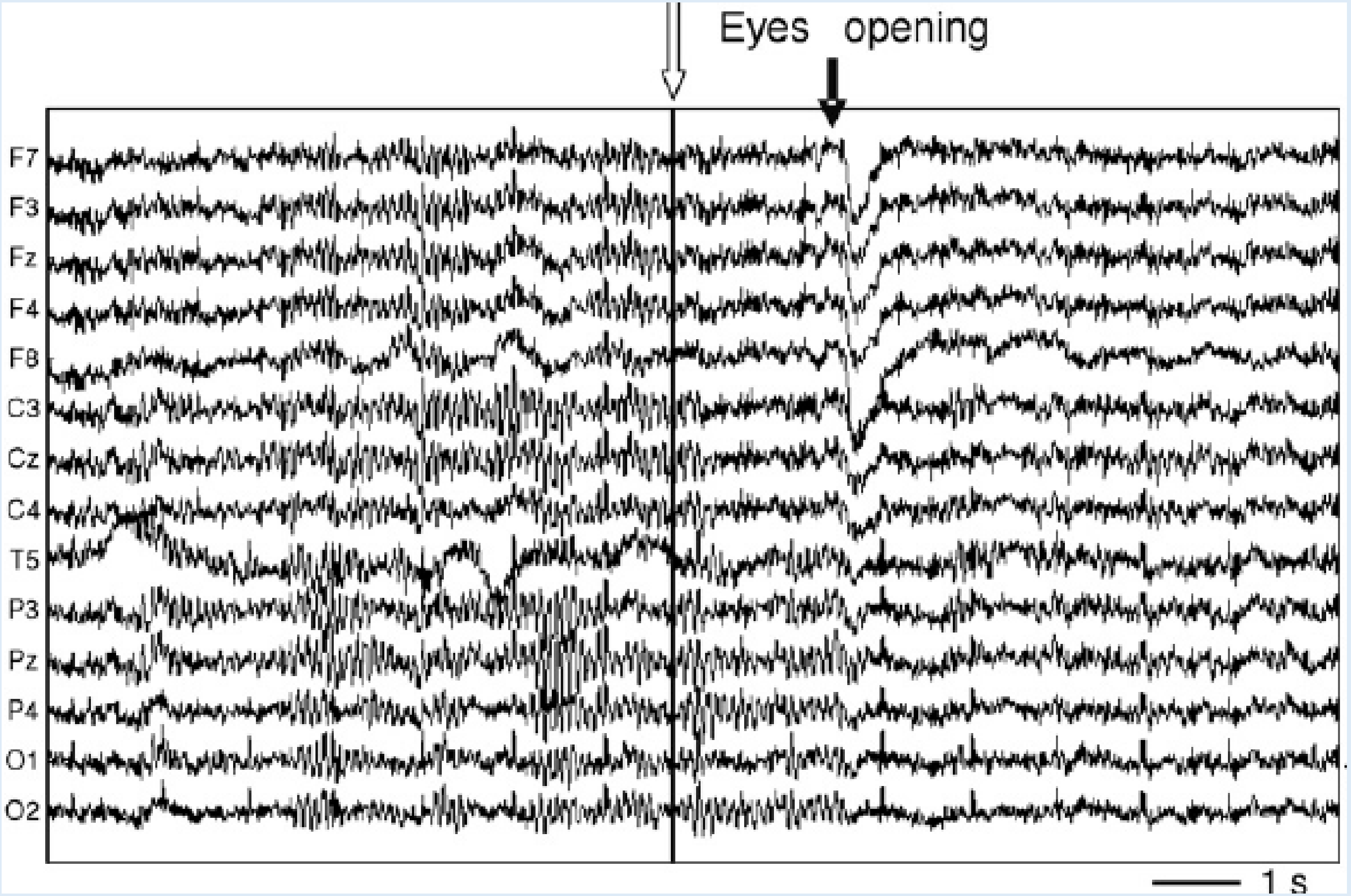
Light-activated ion channels expressed in specific cell types.

Stimulate those neurons in the intact animal with a fiber optic implant.

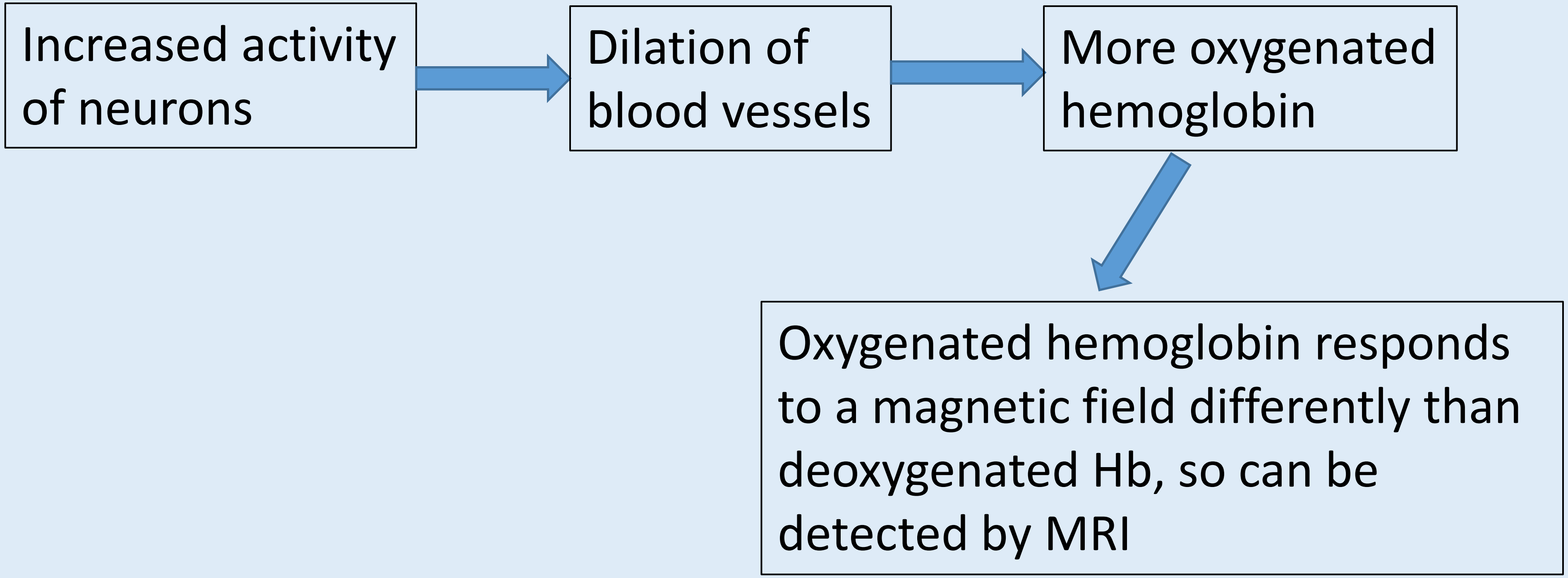
https://www.youtube.com/watch?v=7Mmsah0v9Qc

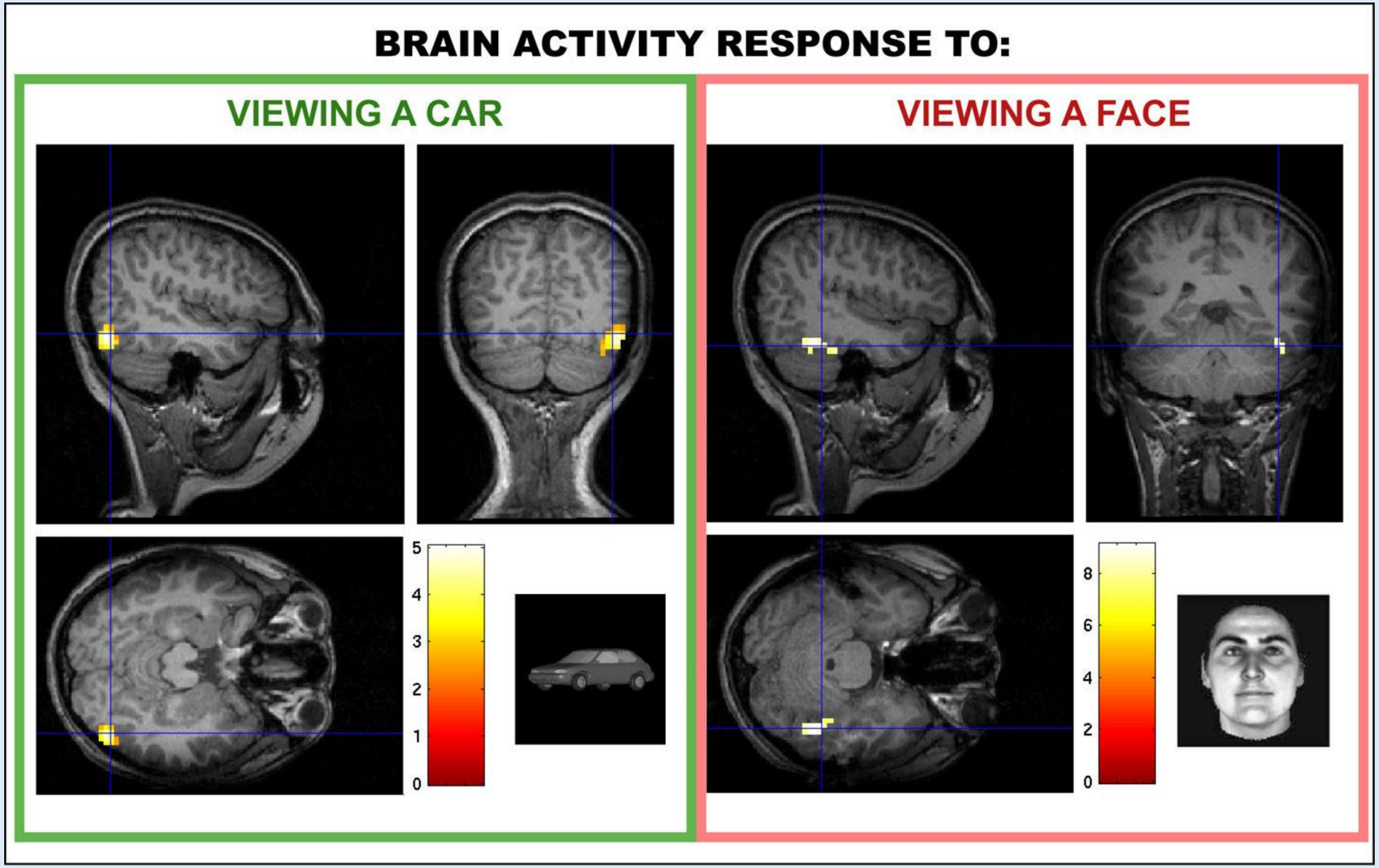
Recording brain activity in humans

Human EEG showing relaxed – attentive transition



Functional MRI (fMRI): Measuring brain activity in awake humans




BRAIN ACTIVITY RESPONSE TO:
VIEWING A CAR
VIEWING A FACE
5
4
3
2
1
0
8
6
4
2
0

Functional MRI (fMRI) monitors brain activity by measuring

A. Changes in electrical fields
B. Changes in blood oxygenation
C. Changes in light emission from the brain
D. Changes in brain light absorbance

Brain-Computer Interfaces: Thought-driven Devices

Signal processing to extract relevant information*

Effector device:

Robotic limb
Wheelchair
Screen cursor
Actual limb (bypassing spinal injury, for example

Record brain activity

* Training required. Signal processing might extract, for example, the EEG signal corresponding to visualization of a limb movement.

Computational methods in neuroscience

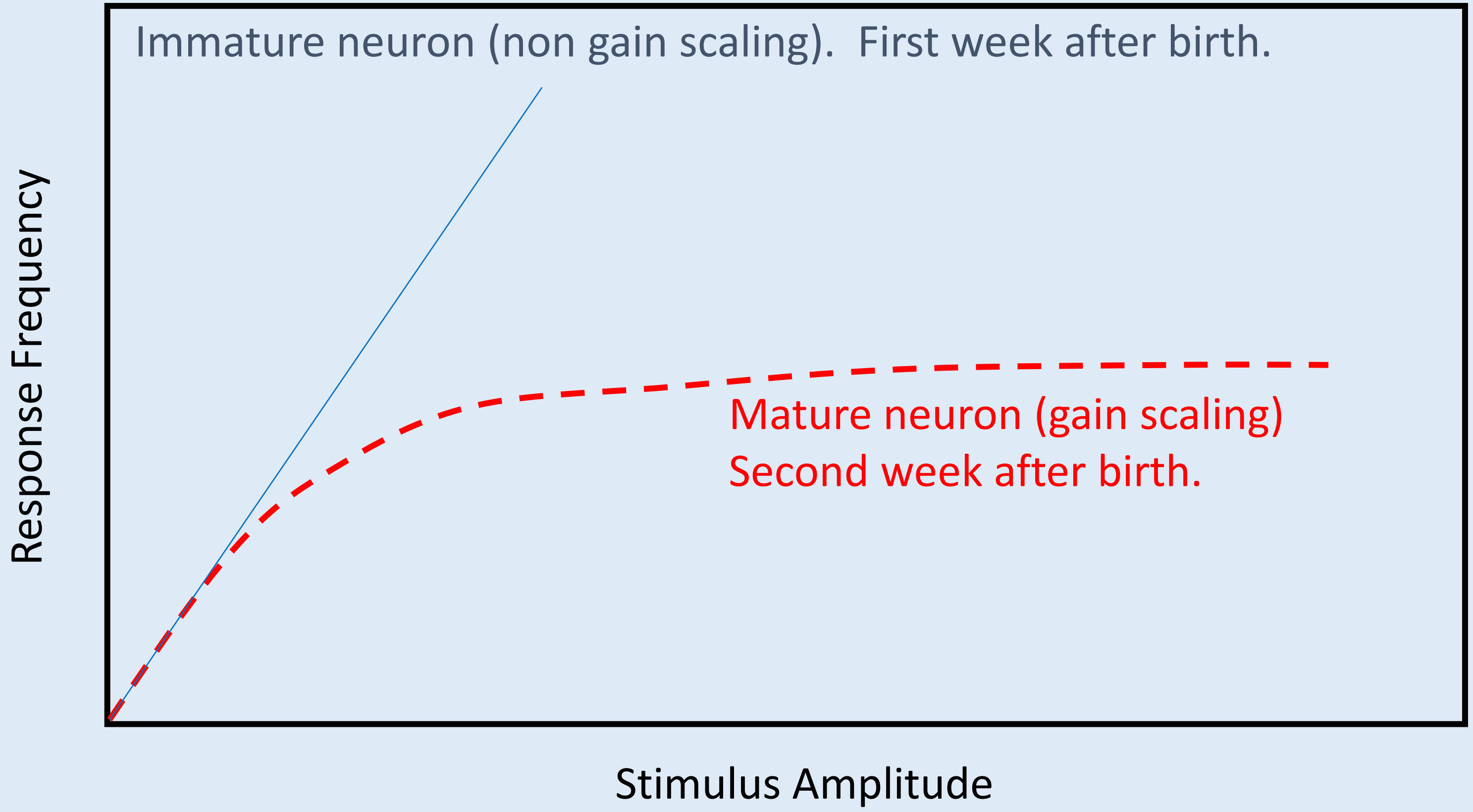
Two (of many) uses of computational methods:

1. Predict how a collection of connected neurons (‘circuit’) behaves based on experimentally measured properties of the individual neurons.
2. Make a model neural circuit based on known parameters and see what it can learn.

An example from my lab

Mature mouse brain neurons adapt to increasing stimulation. The frequency of their action potentials does not increase linearly as the stimulus gets larger. Immature neurons haven't developed this property yet.

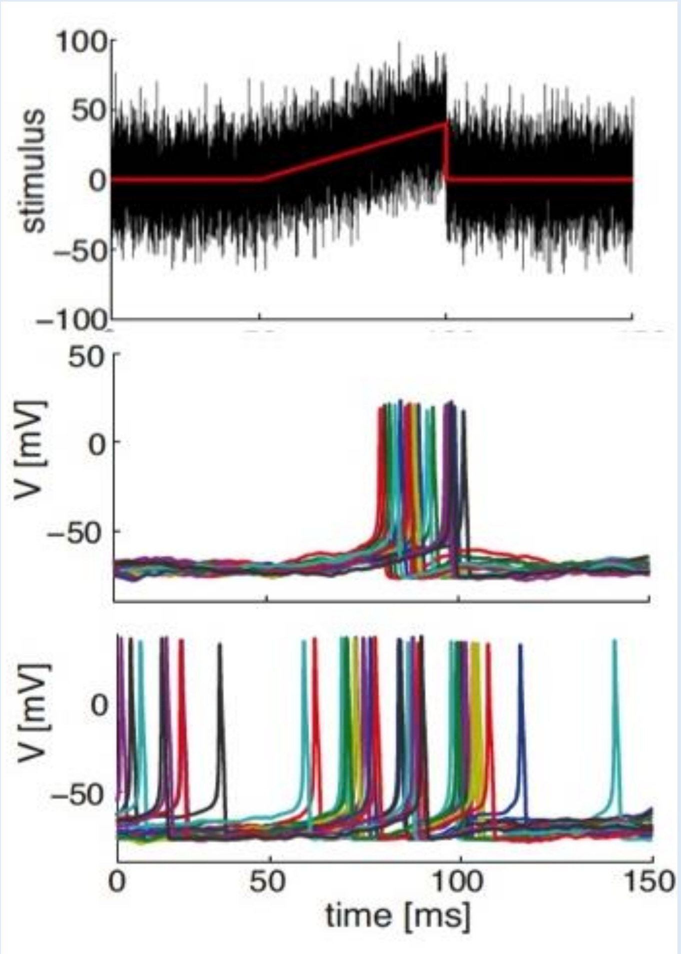
Immature neurons engage in synchronized, low-frequency activity.
Mature neuron engage in non-synchronized, higher-frequency activity. (Except during seizures!)
Are these two set of facts related?



Models of neurons and circuits reveal possible functions of lack of gain scaling in immature cortical neurons

Model neurons without gain scaling synchronize firing to noisy ramp stimuli (i.e. they respond to the ramp).

Model neurons with gain scaling do not synchronize firing to noisy ramp stimuli (i.e. they respond to the noise).

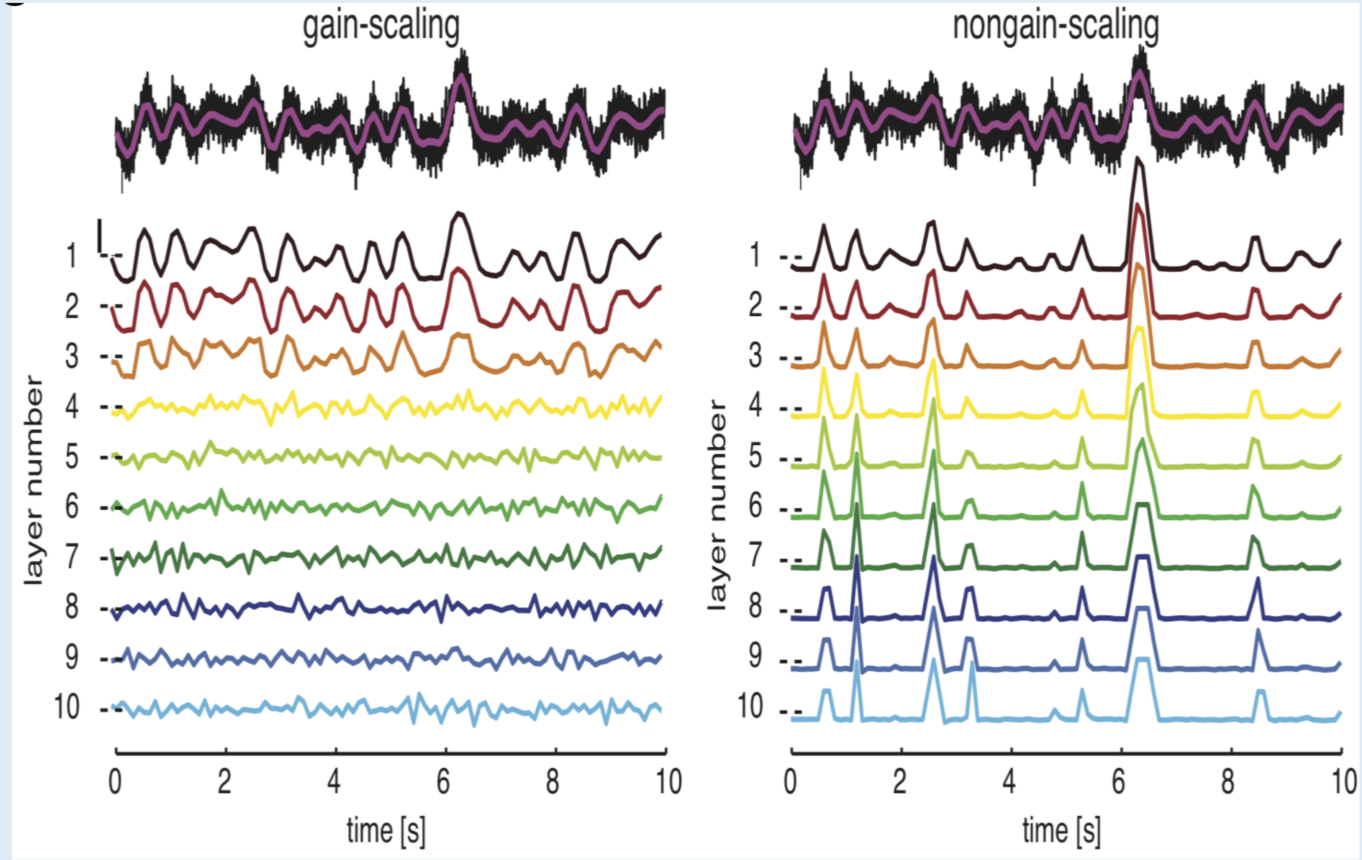


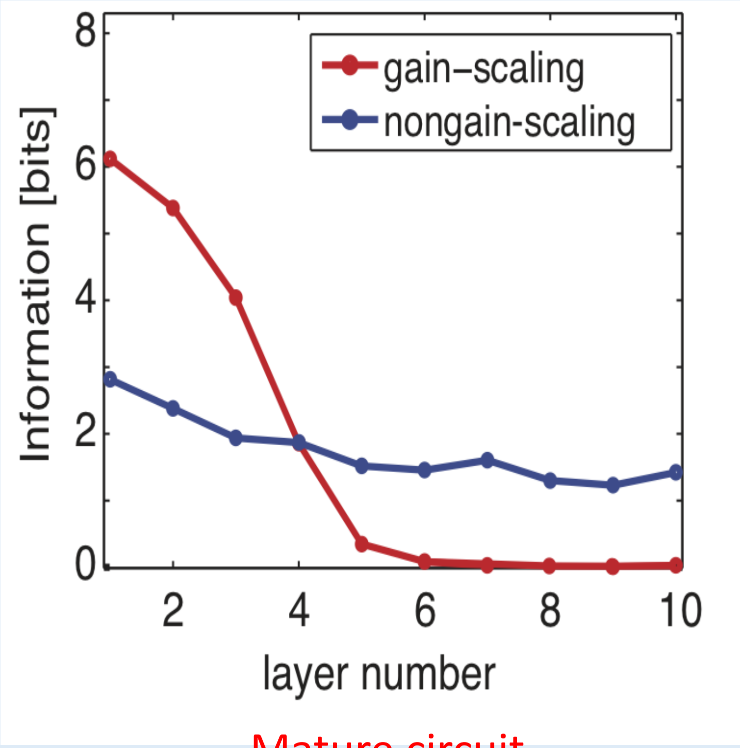
Noisy ramp stimulus

Immature model neuron

Mature model neuron

Model circuits of neurons that lack of gain scaling show better transmission of low-frequency information across synapses





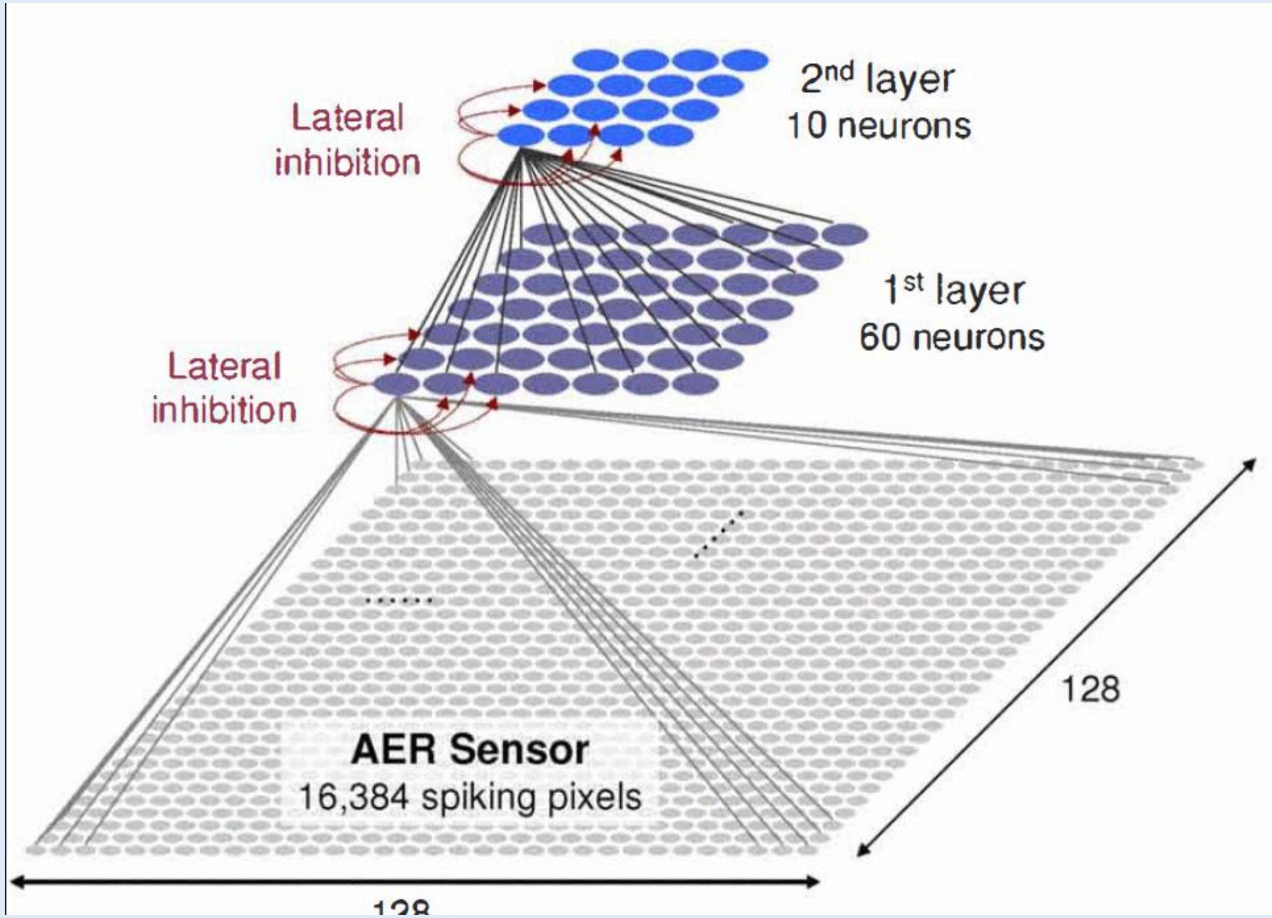
Mature circuit

Immature circuit

Mature circuit

Immature circuit

Undirected learning in a model neural network

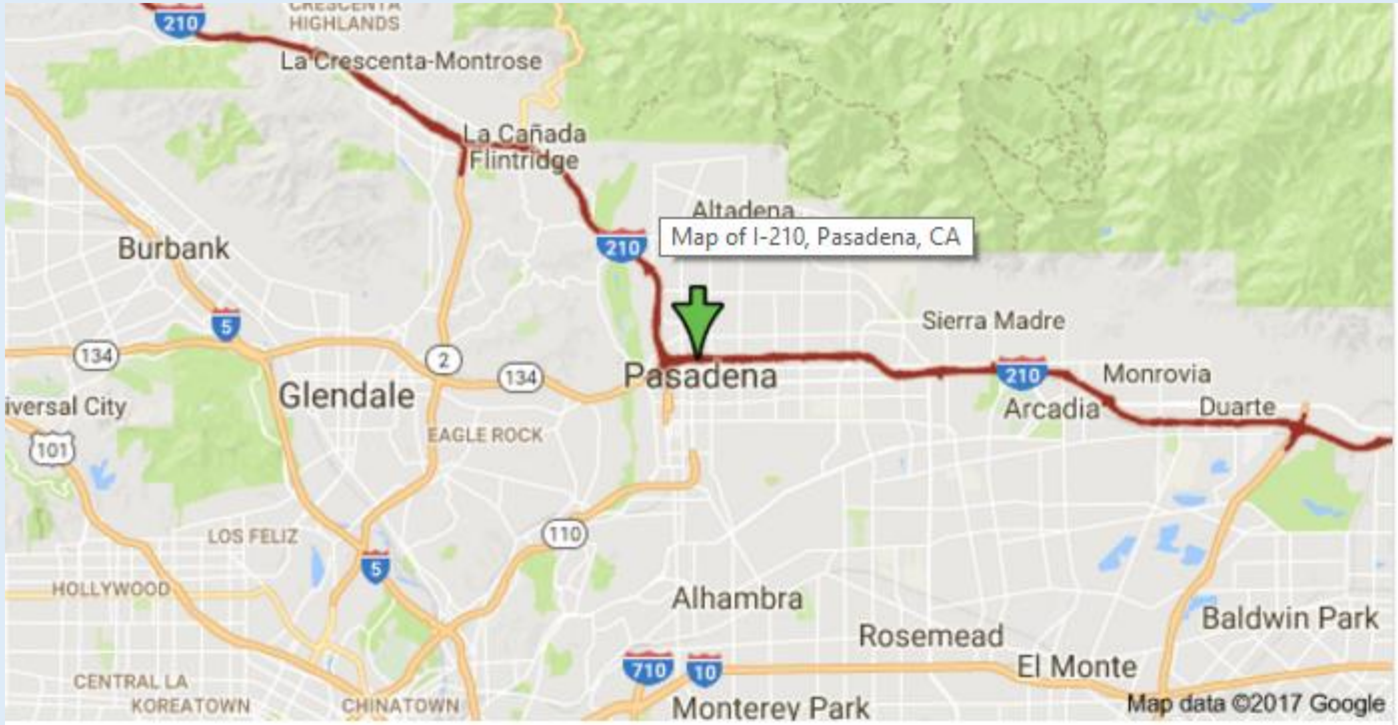


These 10 neurons receive information from layer 1. They interact with the same rules as brain neurons

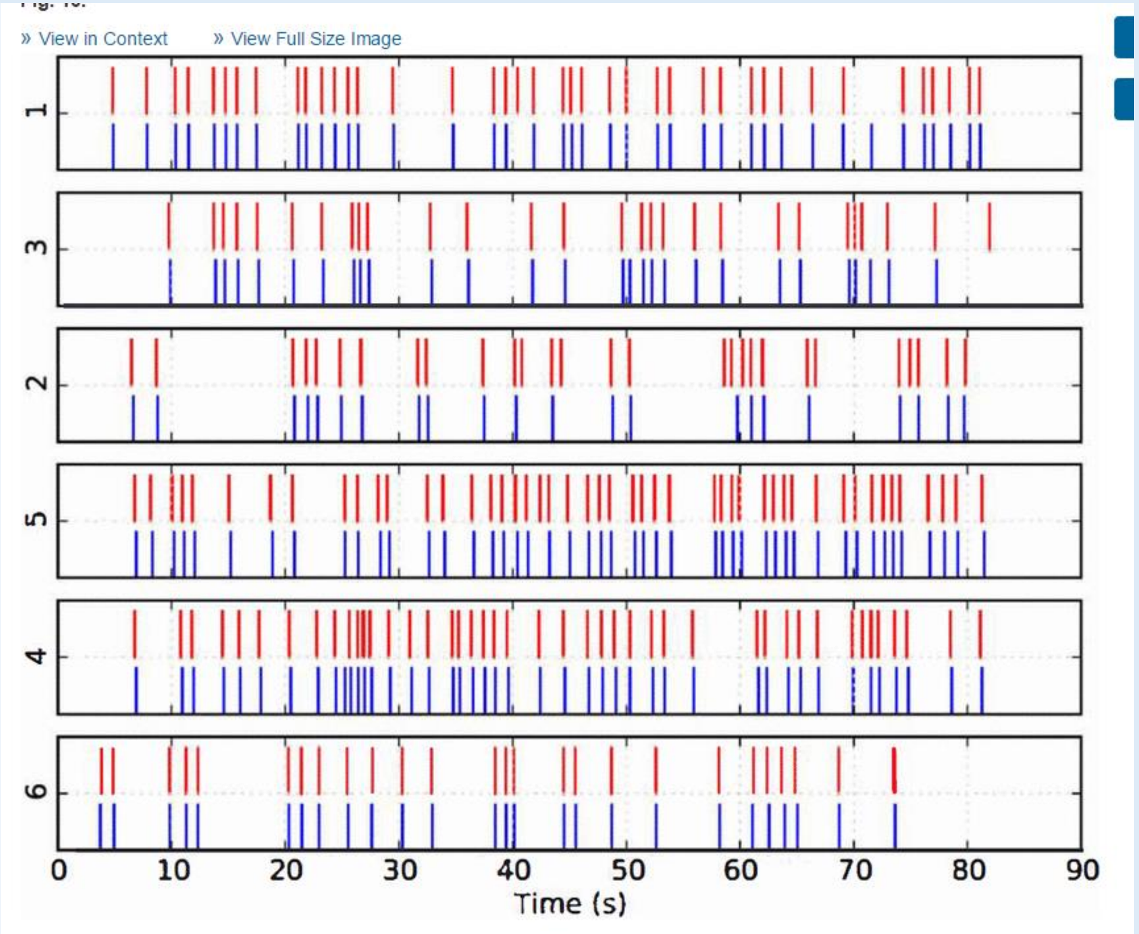
These 60 neurons receive information from the retina. They interact with the same rules as brain neurons

Artificial retina
This sees the film of cars on the 210 freeway

Now, show the ‘retina’ a film taken from an overpass on the 210 freeway in Pasadena.
Don’t instruct the model network in any way. Just let the normal rules of how neurons communicate through synapses and how synapses change over time (learning) work. Do this for <u>only 10 minutes.</u>
Now look at electrical activity of the 10 output neurons in Layer 2…



They have learned to count the cars in each lane of the freeway!



Manual count of cars in each lane

Spiking activity of different neurons in output layer