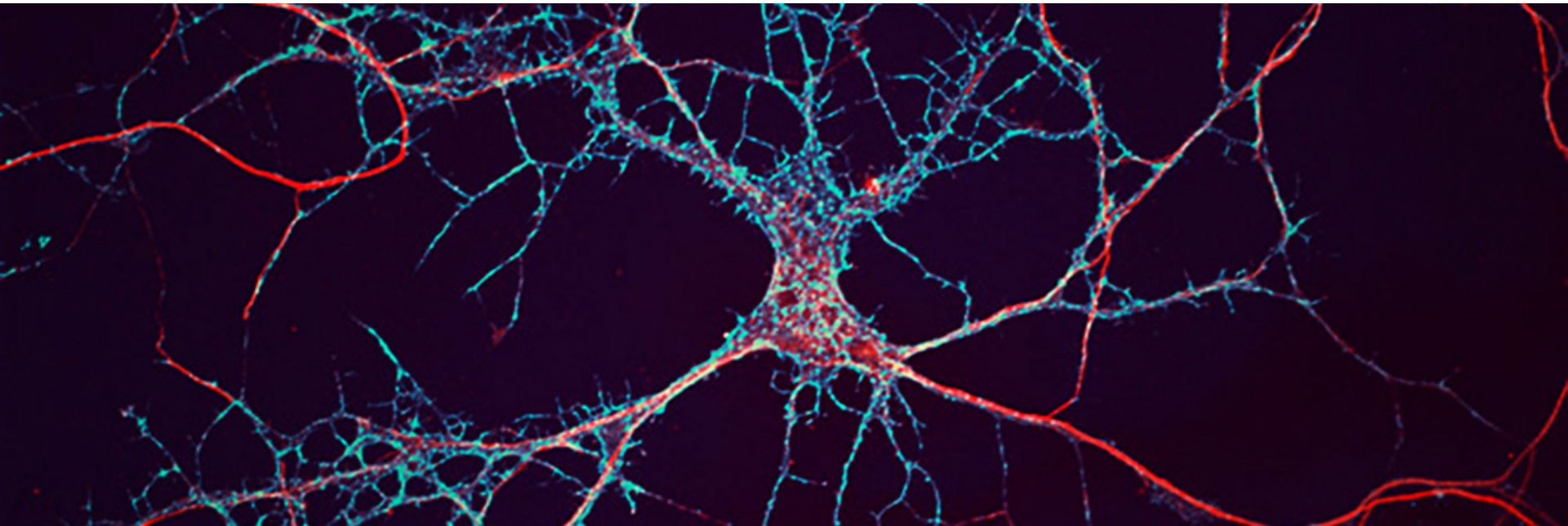
# Applied Neuroscience

**Columbia Science Honors Program**

**Spring 2017**

## Sensory Systems and Neural Circuits I

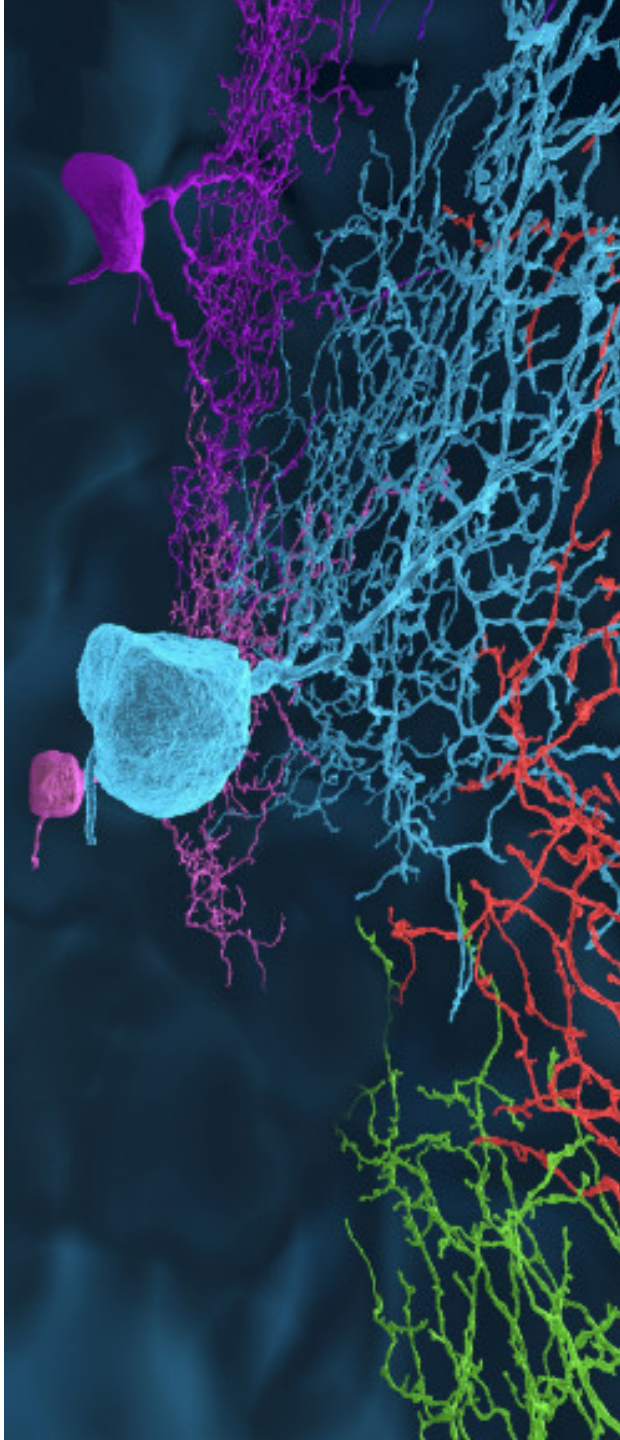


# Sensory Systems and Neural Circuits

**Objective:** Experimental Design in Neuroscience

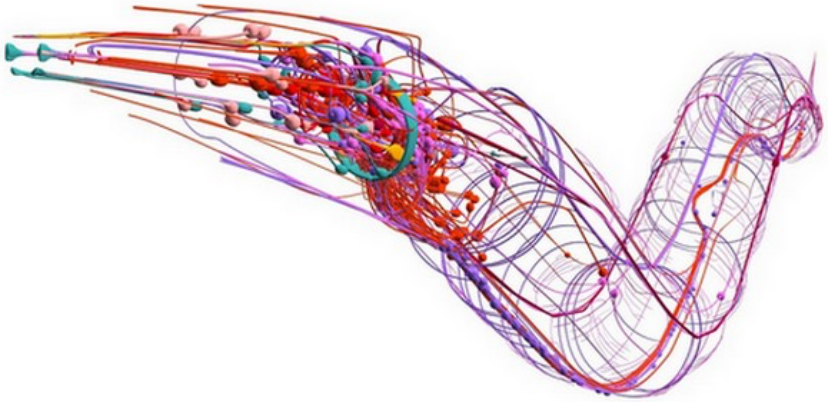
**Agenda:**

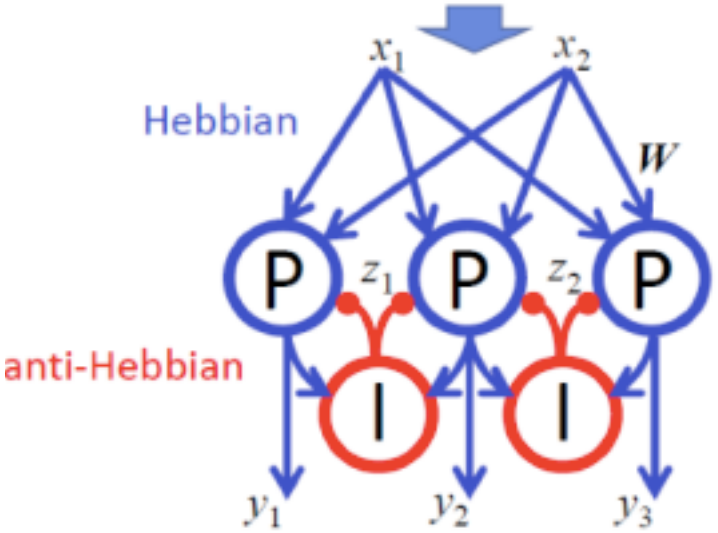
1. Neural Circuits
   - Anatomy
   - Physiology
   - Manipulations
2. Sensory Systems
   - Visual System
   - Auditory System



# Reverse Engineering the Brain

**Connectomics** → **Theory**






**Connectomes** are brain wiring diagrams at the synapse level and requires serial electron microscopy.

**Why Theory?**
While we understand neuron physiology, there is no accepted algorithmic theory of neural computation.
With theory, we test hypotheses on neural connectivity.

# Neural Circuits

**Neural Circuit:** Functional entity of inter-connected neurons that is able to regulate its own activity using a feedback loop

**Features:**

1. Neurons do not function in isolation
2. Neurons are grouped according to function
3. Synaptic connections define the circuit
    - Types of Connections
        1. Axon-Dendrite
        2. Neuron-Muscle
    - Types of Neurotransmitters Used
    - Location and Length of Connections

# Neural Circuits

| Type | Examples | Neural Activity | Synaptic Signaling | Non-Synaptic Signaling |
|---|---|---|---|---|
| Acute (Fast) | Vision, Reflexes | Short window | Yes | No |
| Chronic (Slow) | Hunger, Mood | Long window | Yes | Yes |

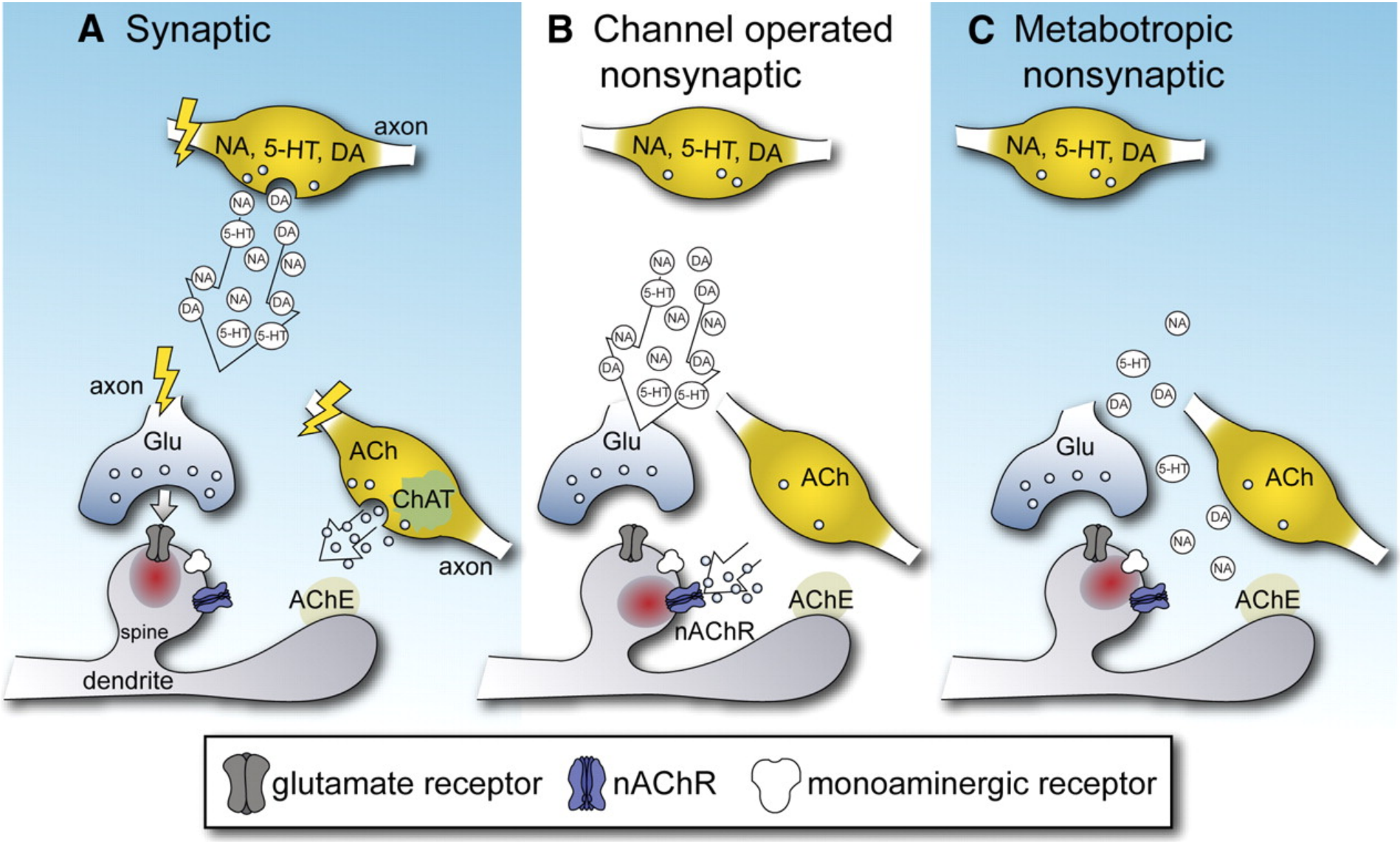
**Acute Neural Circuits:**

- Stimulus-dependent (clear causality)
- Fast responses require fast signaling, which implies anatomical synaptic connections

**Chronic Neural Circuits:**

- State-dependent (unclear causality as multiple stimuli may act together to elicit the behavior)
- Slow responses suggest non-synaptic signals

# Non-Synaptic Signaling



# Why Study Invertebrates?

| Model | Correlated Neurons | Causal Neurons | Neural Circuit |
|---|---|---|---|
| Vertebrates | Yes | Sometimes | No—<br>• Too many neurons<br>• Neurons and connectivity varies |
| Invertebrates | Yes | Yes | Yes—<br>• Fewer neurons<br>• Stereotyped neurons and connectivity |

# Neural Circuits

| Vertebrates | Invertebrates |
| --- | --- |
| 1. Identify a stimulus-dependent behavior of interest (i.e. object attraction in mice).<br>2. Drop an electrode into a part of the brain, present the object to the mouse. See if neurons respond.<br>3. Inactivate neuron to show neuron is part of circuit.<br>• By studying many parts of the brain, many neurons will be found. However, we will not know the gaps between neurons.<br>• The same neuron is hard to identify across animals. | 1. Identify a stimulus-dependent behavior of interest (i.e. heat avoidance in fly).<br>2. Active neuron of interest with optogenetics. See if behavior is produced.<br>3. Inactivate neuron with optogenetics to show neuron is part of circuit.<br>• Neurons are countable (302 in *C. elegans* and 135,000 in *Drosophila melanogaster*)<br>• Neurons are **stereotyped** across individuals and have **reproducible connectivity.** |

# Neural Circuits

Genes and molecules are conserved from invertebrates to vertebrates. Neuroscientists believe that neural circuit principles will be as well.

> *"You have made your way from worm to man, and much within you is still worm."*
>
> *F. Nietzsche, Thus Spoke Zarathustra*

# To Identify Neural Circuits

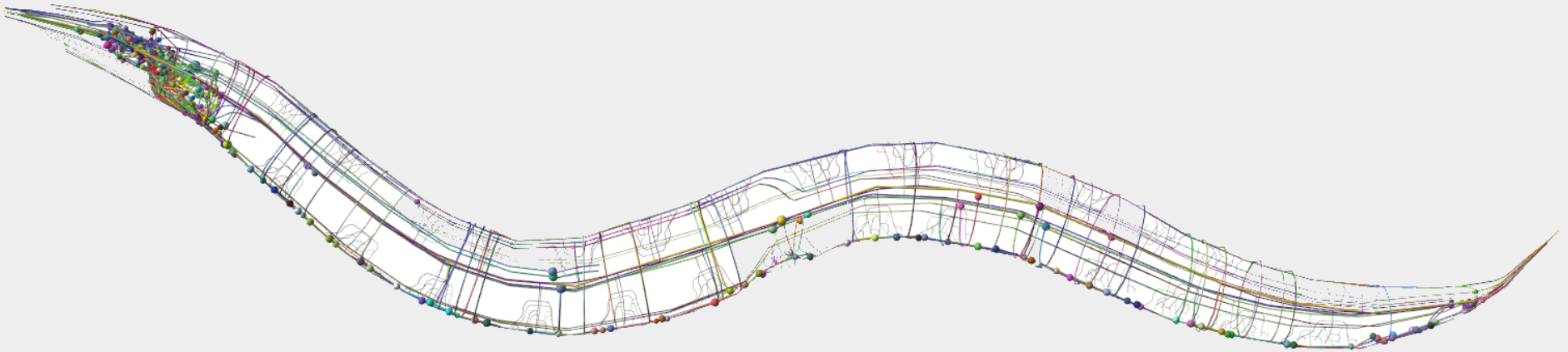
**Step 1:** Find one neuron involved in the circuit.

**Step 2:** Find additional functional neurons.

**Step 3:** Confirm role of all neurons in signal.

**Step 4:** Organize neurons by *epistasis analysis.*

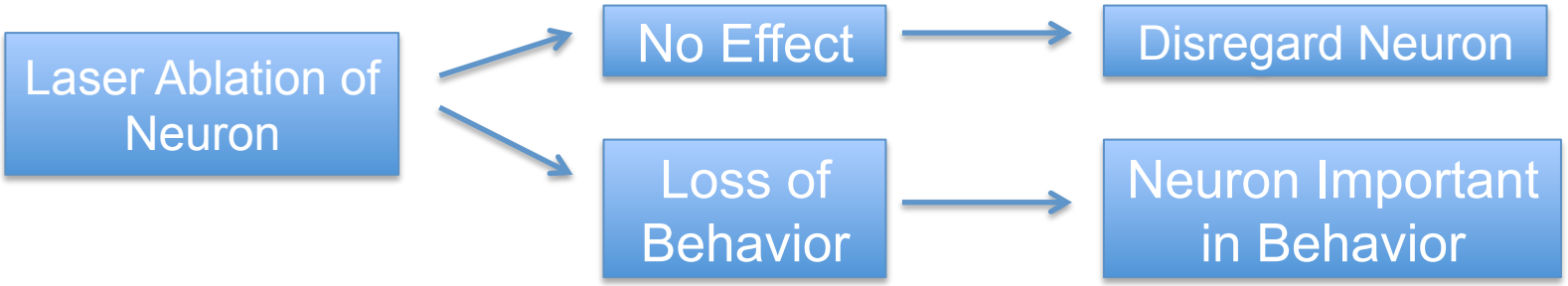
**Step 5:** Draw Circuit.



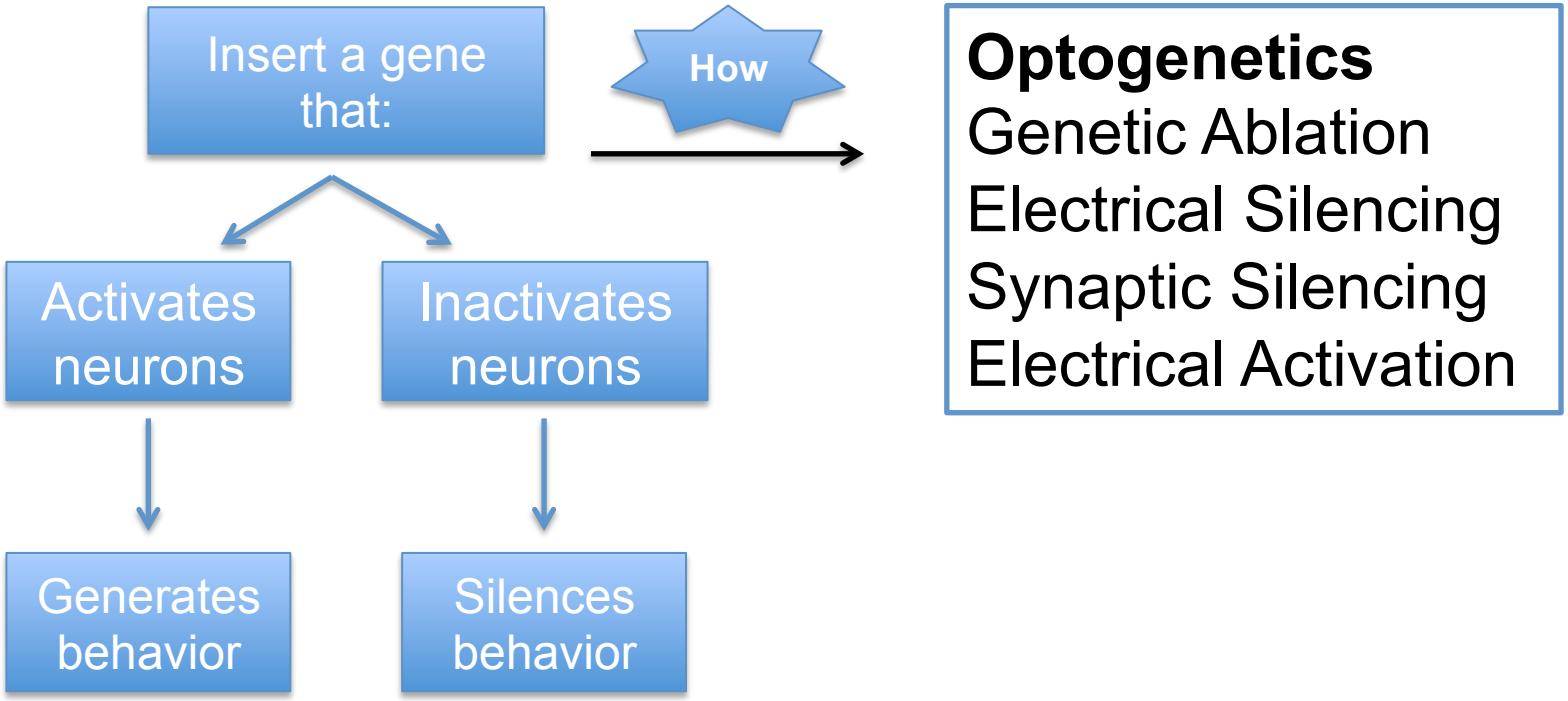
# Step 1: Find one neuron involved in the circuit.

## A. Use systematic inactivation or activation

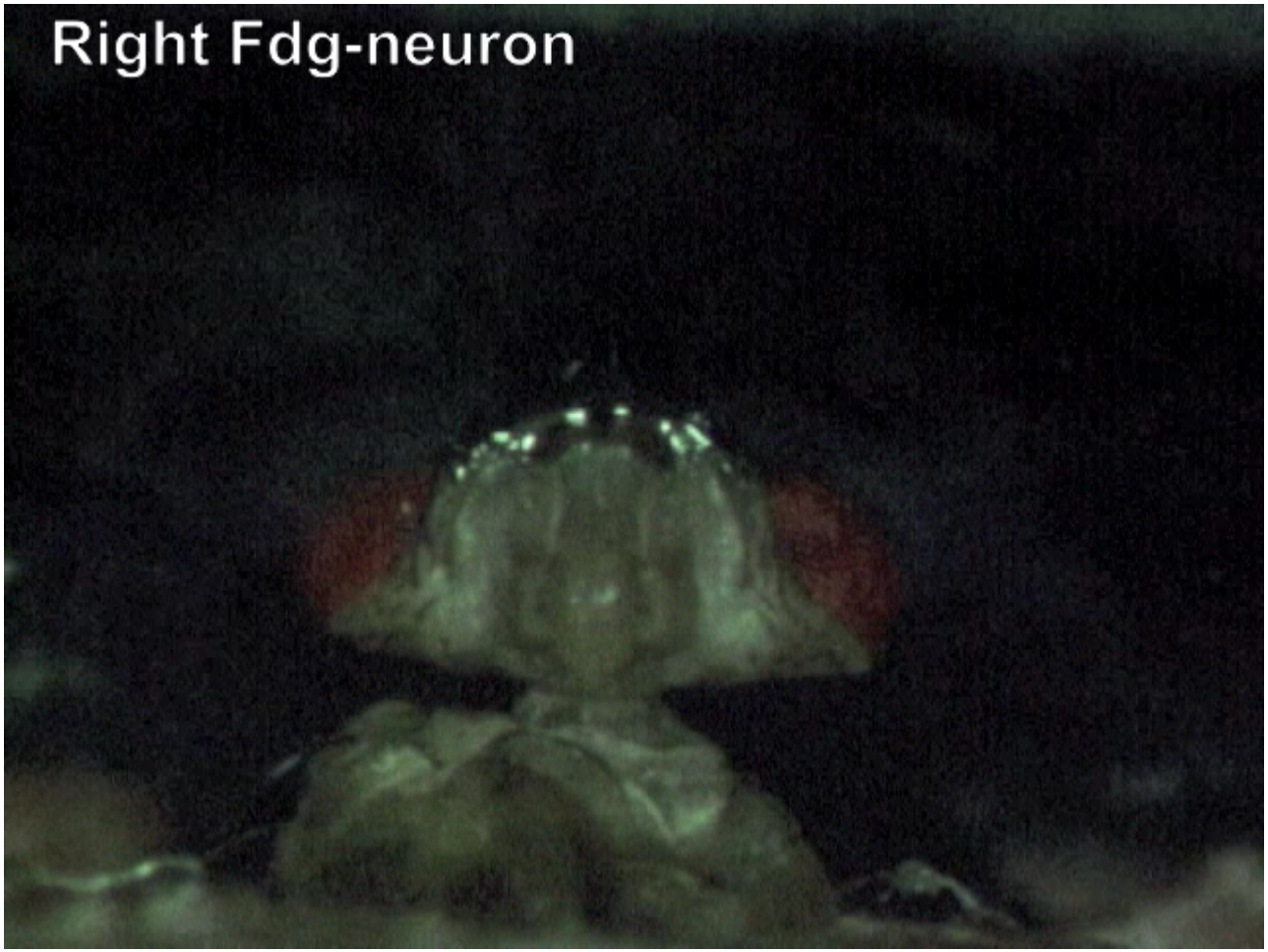
A. Use a laser to ablate neurons




B. Use a transgene to activate or inactivate neurons



# Step 1: Optogenetic Activation of Neurons

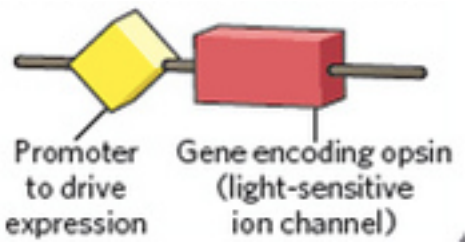


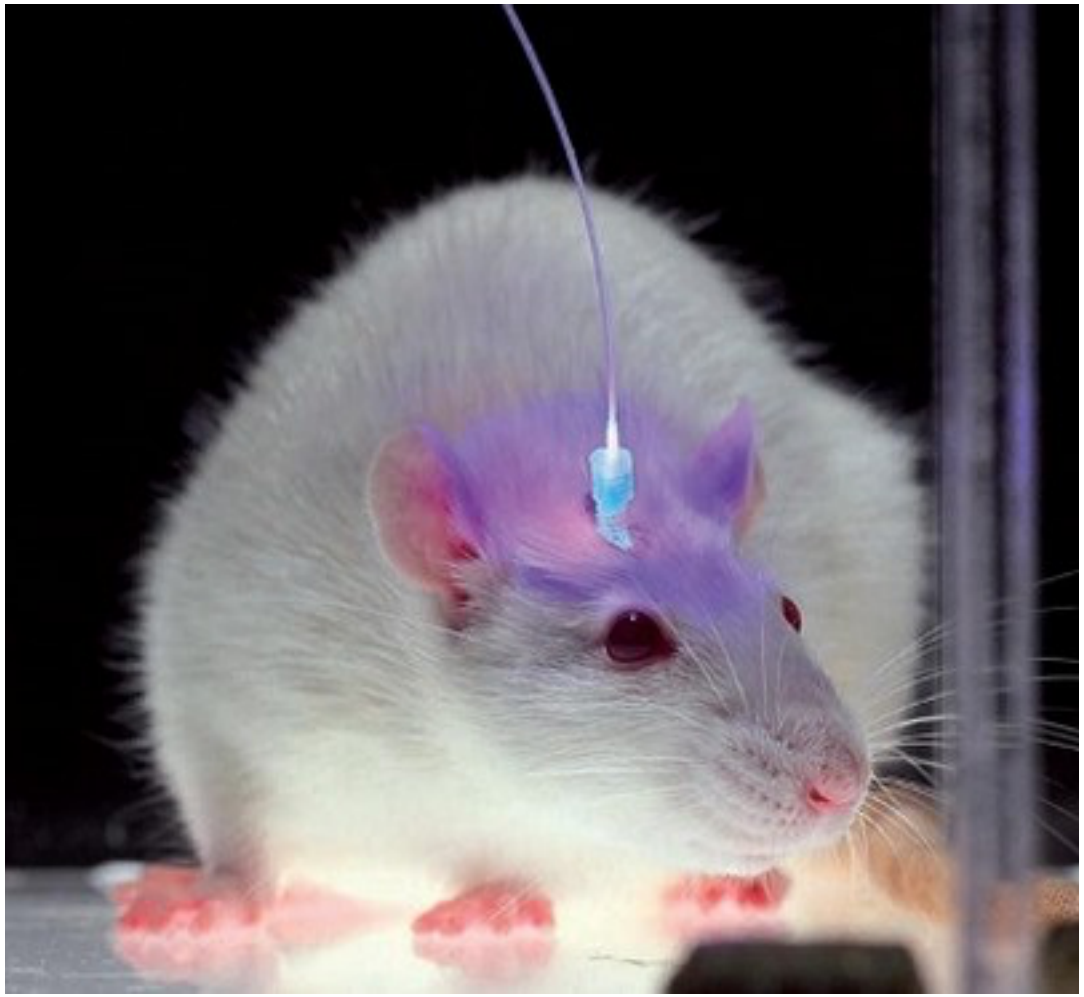

**Proboscis**

Optogenetic activation of an Fdg-neuron induces proboscis extension and pump movement in flies

# Optogenetics

1. Piece together genetic construct.
2. Insert construct into virus.
3. Inject virus into animal brain. Opsin is expressed in targeted neurons.
4. Insert fiber-optic cable plus electrode.
5. Laser light of specific wavelength opens ion channel in neurons.



*Laboratory of Karl Deisseroth, Stanford University*

# Optogenetics

# Test Your Understanding

Select the statement that is false:

A.
Optogenetics is a technique that manipulates neural activity using light.

B.
Exogenous ligands are chemicals not made in the body that can bind to receptors in our nervous system.

C.
Ionotropic receptors are mechanically-gated.

D.
Influx of $Ca^{2++}$ promotes exocytosis of neurotransmitters in the axon terminal.

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**C.**
**Ionotropic receptors are mechanically-gated.**

D.
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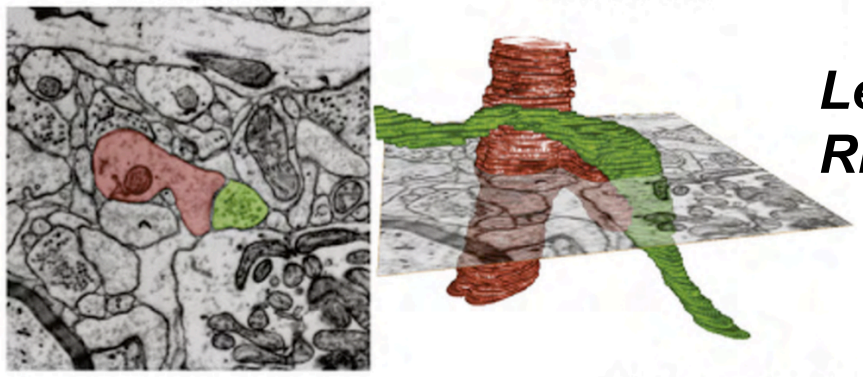
*Explanation:*

Ionotropic receptors are ligand-gated.

# Step 2: Identify additional functional neurons.

A. Continue to use systematic inactivation or activation
B. Identify connectivity of neuron of interest. See if activation or inactivation illustrate function.
    1. Use serial electron microscopy to trace cell processes



***Left:*** 2-D Cross-Section
***Right:*** 3-D Reconstruction

    2. Use a transgene to map connections (trans-synaptic virus)

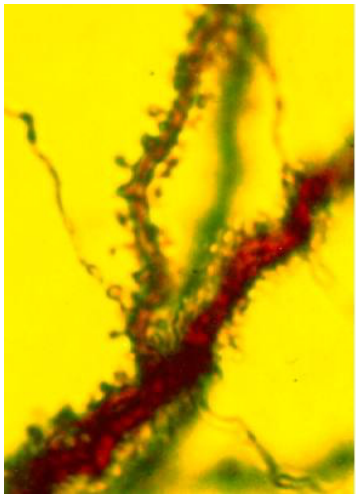
**Caveat:** This may not work. A functional connection may not act through an anatomical synapse. Instead, the circuit may rely on neuropeptides or hormones that are secreted and diffuse to the cells that they affect.

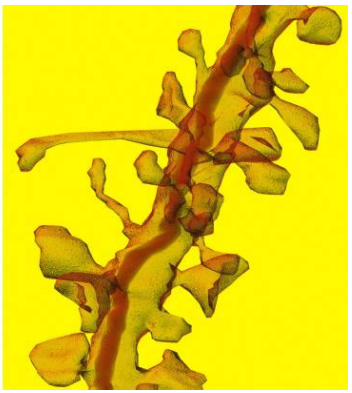
# Tools for Anatomy

Anatomy provides information on the **structure and connectivity of the nervous system.**

| Electron Microscopy | Light Microscopy |
|---|---|
| • Best technique for determining synaptic connectivity<br>• High spatial resolution<br>• *Caveat:* No ability to see changes over time as sample must be fixated. | • Simpler than EM<br>• Can be done with dye injection or transgene (i.e. GFP)<br>• If a pre-synaptic protein is tagged with GFP, then synapses can be visualized.<br>• *Caveat:* No way to know what synapse is connected to. |

*Dendrite Morphology*





# Tools for Anatomy

Anatomy (connectomes) do not provide information about function:

***Anatomy enables neuroscientists to generate hypotheses for circuit function.***

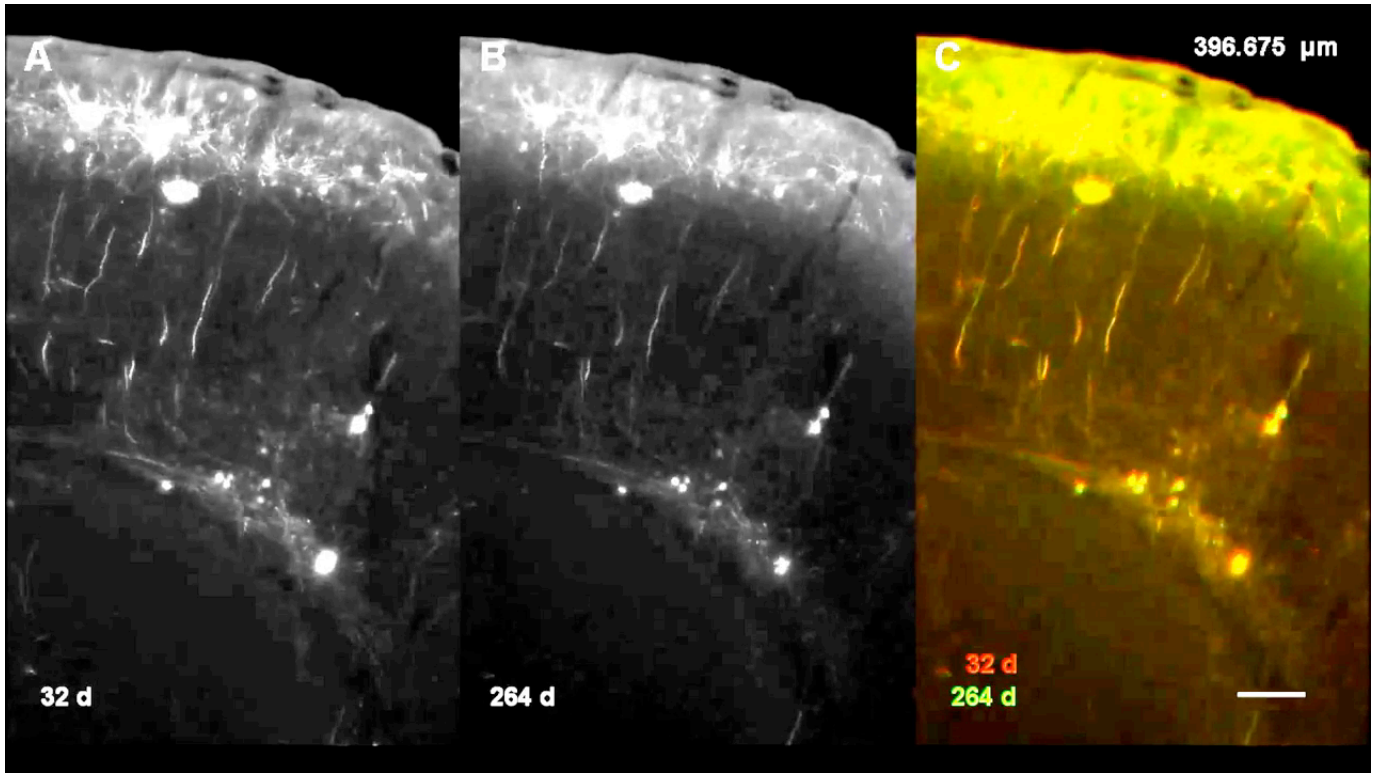
*Caveats:*

- Even if two neurons synapse, it doesn't mean these two neurons **act together** to perform a function.
- Even if two neurons are **not** anatomically connected, it doesn't mean they're not **functionally connected.**
  - A form of non-synaptic signaling may exist, implying a chronic circuit (slower signal).

*Even though the connectome for C. elegans was determined in 1970, its nervous system was not well understood for the reasons above.*

- Researchers need to determine the **functional routes.**

# Step 2: Viral Tracing

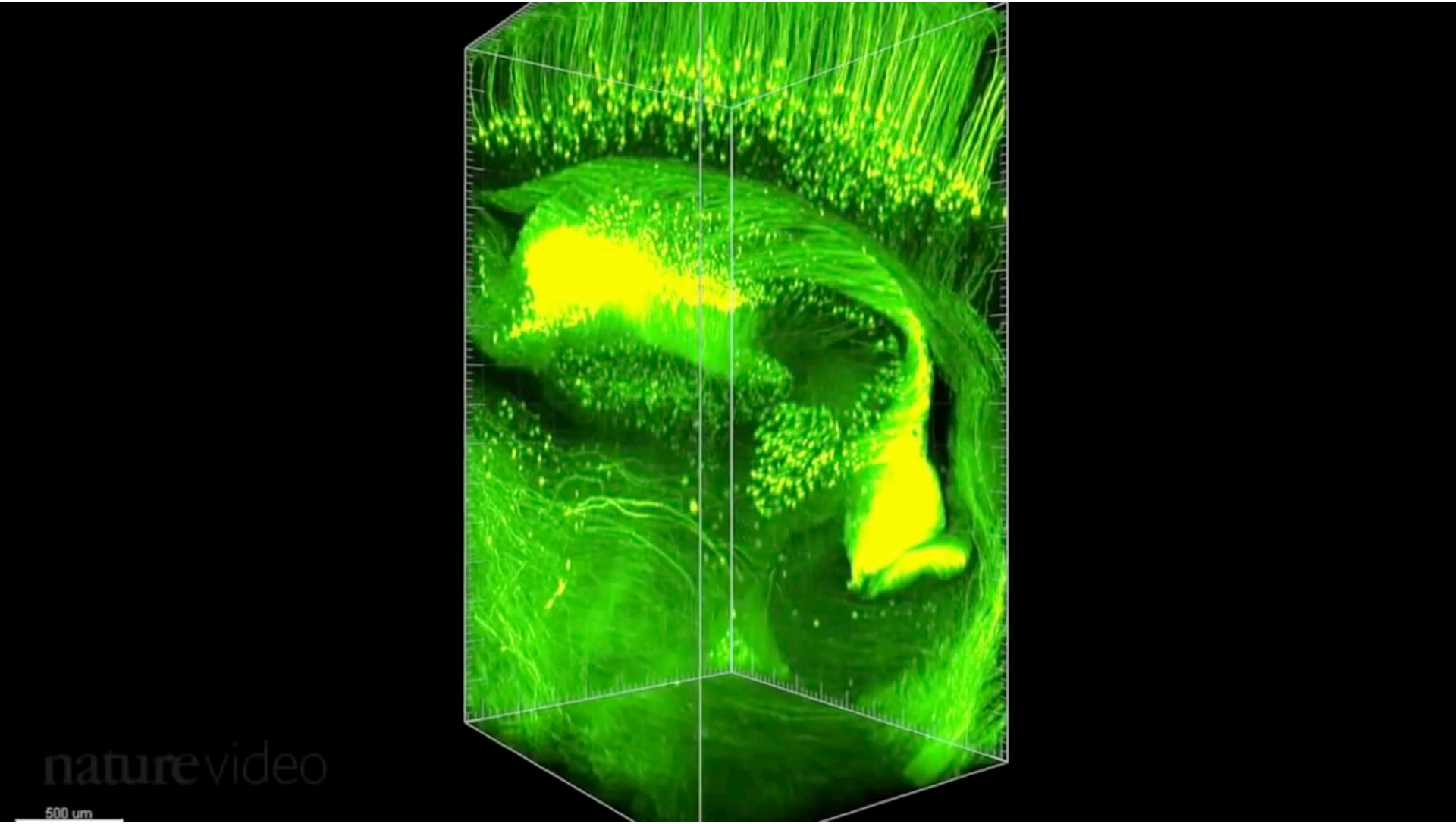



**Anterograde Transport:** tracer moves from soma to synapse, uses **kinesin** to move viruses along axon in anterograde direction

**Retrograde Transport:** tracer moves from synapse to soma, uses **dynein** to move viruses along axon in retrograde direction

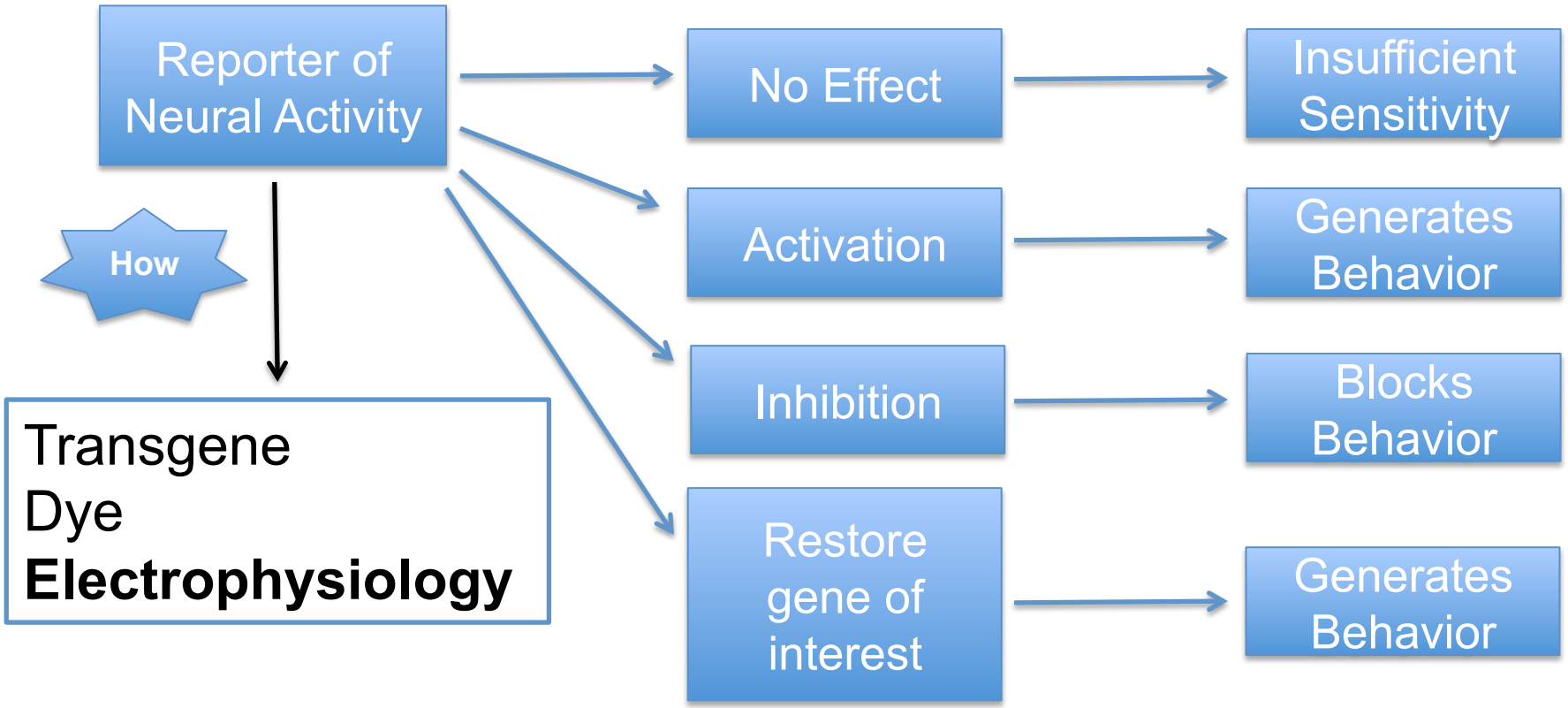
**Dual Transport:** combines above methods to determine both the inputs and outputs of neuronal circuitry

# Step 2: CLARITY



# Step 3: Confirm the role of identified neurons in generation of behavior

A. Use a reporter of neural activity in response to stimulus



# Tools for Physiology

Physiology captures molecular signals that underlie neural circuit function:

*Caveat:*

- Not all neurons signal by action potentials (i.e. neurons in retina and non-spiking interneurons in invertebrates)

There are two ways to do recordings:

1. **Extracellular recording:** electrode outside neuron
2. **Intracellular recording:** electrode inside neuron

**Paired Recording:** technique in which one inserts electrodes into pre-synaptic neuron and post-synaptic neuron simultaneously

1. Inject de-polarizing current to activate pre-synaptic neuron.
2. Observe effect on post-synaptic neuron:
    - Excitatory synapse results in EPSP
    - Inhibitory synapse results in IPSP

# Tools for Physiology

## Paired Recordings

*Caveats:*

- Difficult in practice

Alternative: **ChR2-assisted circuit mapping**

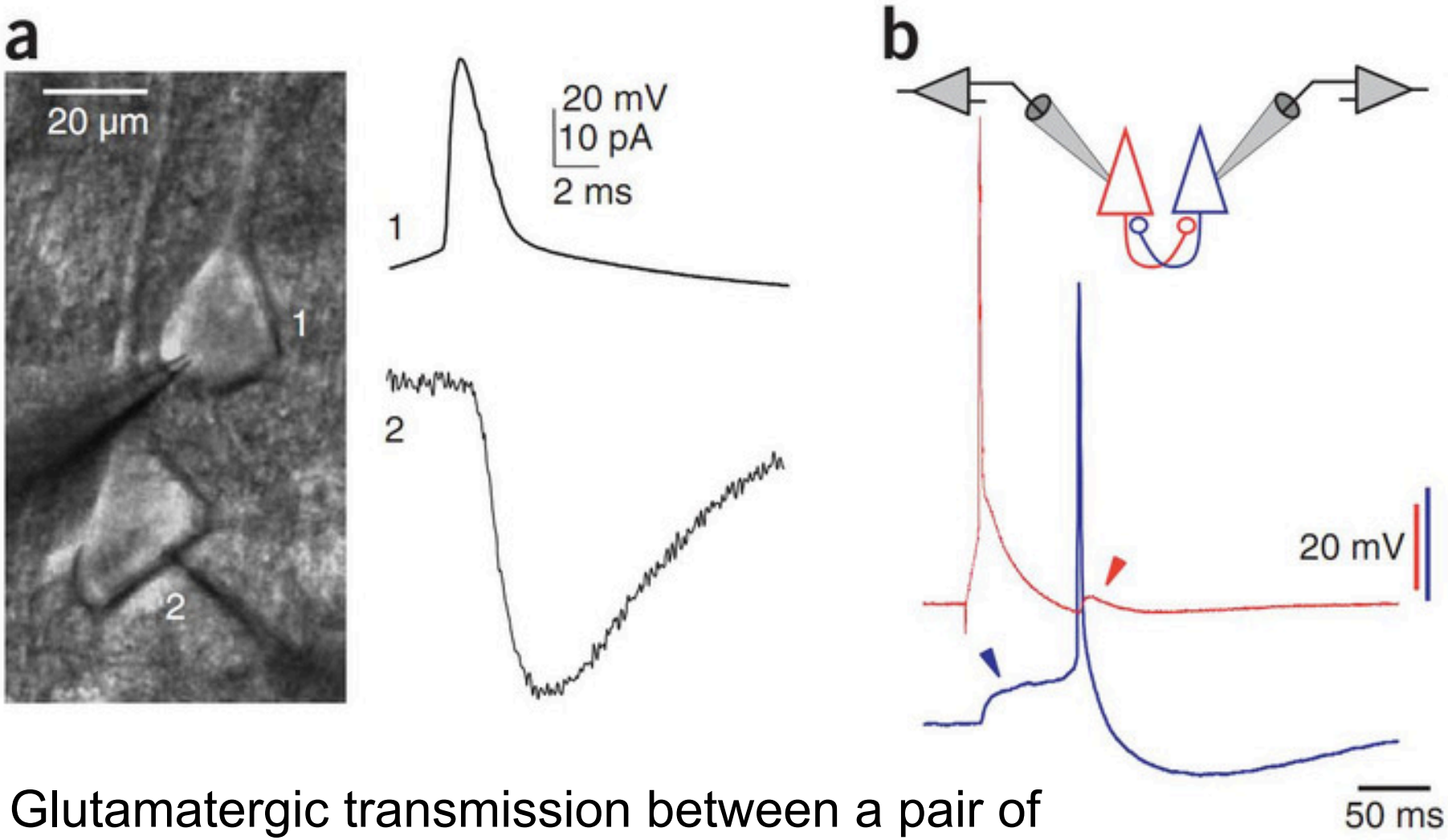
Pre-synaptic neuron is defined by ChR2 expression.
Post-synaptic neuron is defined by targeted patching.
*ChR2 is transported into axons where it can transduce photo-stimulation into action potentials. This method provides a way to study long-range connections in brain slice preparations (axons can be stimulated even when severed from parent somata).*

- Recordings are best done in awake, behaving animals but this is not possible because of movement

Alternative: **Fictive Behavior**

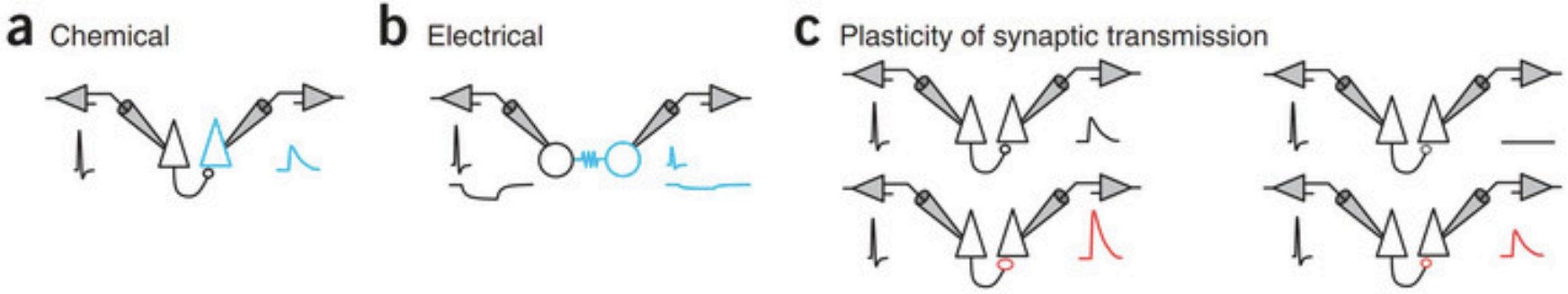
Nerve fibers that connect to muscle are cut (prevents motion) in live animals.

# Step 3: Connectivity by Electrophysiology




Glutamatergic transmission between a pair of Layer V Pyramidal Neurons

# Step 3: Overview of Electrophysiology




**A. Chemical Synapse**

Action potential (**black**) triggered in pre-synaptic neuron evokes an EPSP (**blue**) in post-synaptic neuron.

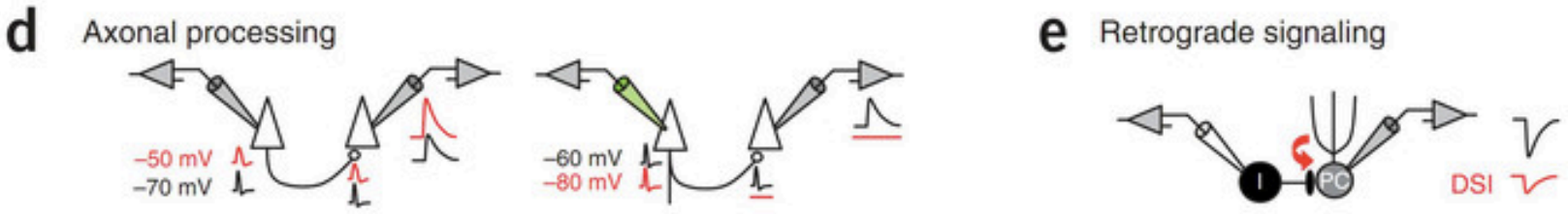
**B. Electrical Synapse**

Action potential (**black**) in first neuron produces attenuated voltage signals (**blue**) in second cell.

**C. Plasticity of Synaptic Transmission**

In control, pre-synaptic neuron evokes an EPSP in post-synaptic cell (left) or no response (right) in case of silent synapse. After **potentiation** (**red**), the efficacy of synaptic transmission is enhanced.

# Step 3: Overview of Electrophysiology



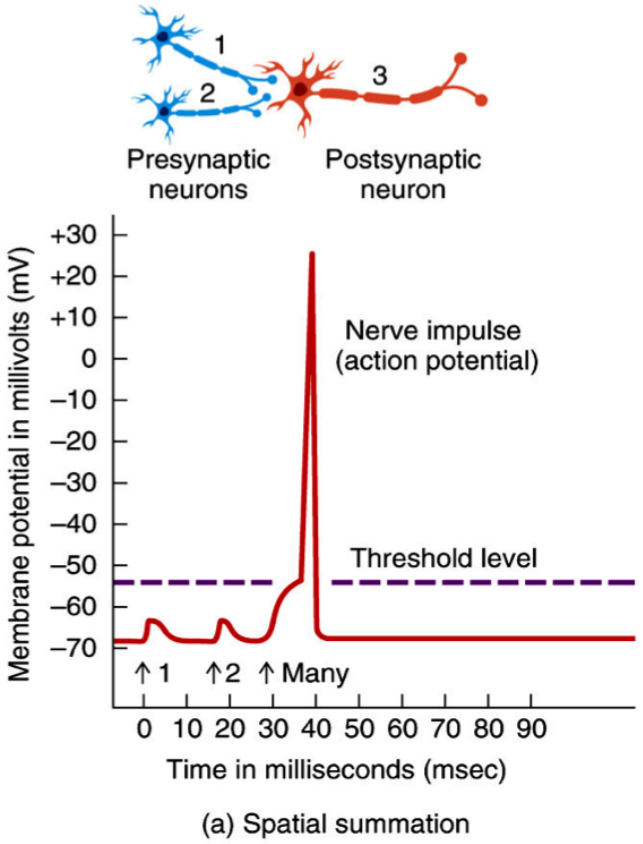

## D. Axonal Processing

Pre-synaptic membrane potential-dependent axonal integration (left) and conduction failure (right).
*Pre-synaptic spike fails to propagate in axon when it is evoked from a hyper-polarized potential (-80 mV).*

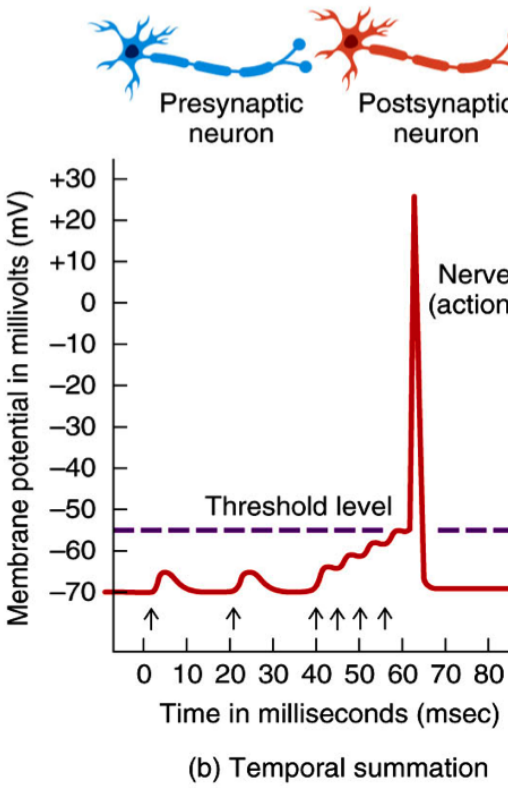
## E. Retrograde Signaling

Retrograde signaling established between an interneuron (I) and a Purkinje cell (PC).
**Depolarization-Induced Suppression** (DSI) of inhibition. Red arrow indicates the release of endocannabinoids from PC to pre-synaptic terminal of interneuron.

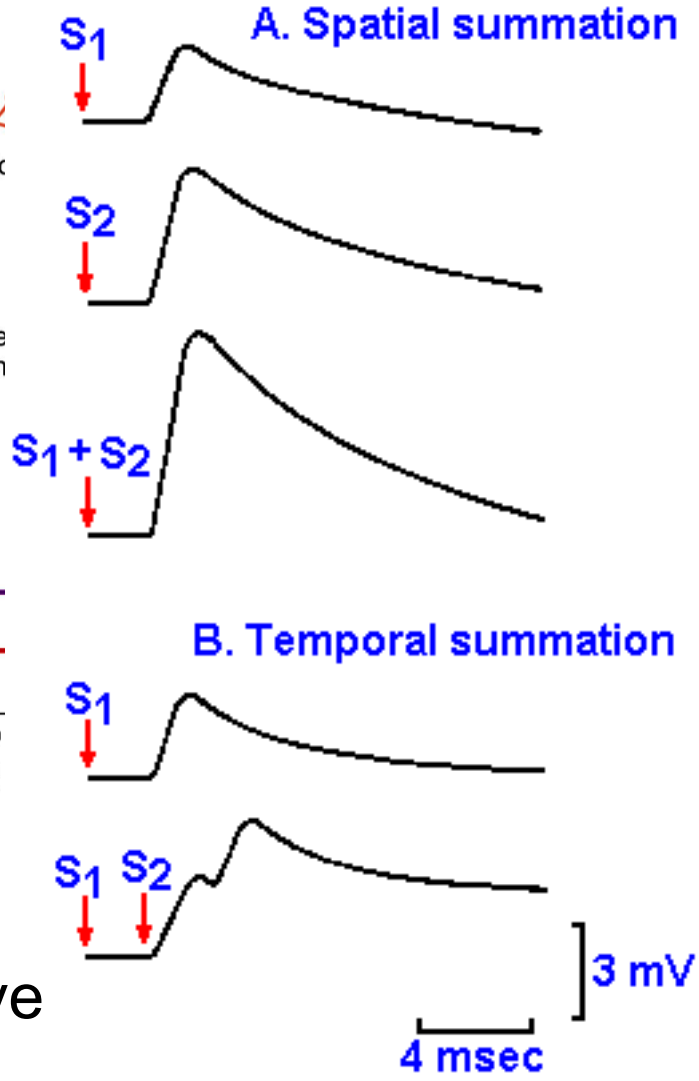
# Summation




(a) Spatial summation




(b) Temporal summation




**Spatial Summation:**
Occurs when post-synaptic potentials arrive near same location

**Temporal Summation:**
Occurs when post-synaptic potentials arrive near same time

# Test Your Understanding

1. The process by which a neuron summates synaptic excitation and inhibition is called:

A. Plasticity
B. Integration
C. Convergence
D. Pulse Frequency Modulation
E. Dis-inhibition

2. In the nervous system, the strength of the stimulus is coded into:

A. Frequency of Action Potentials Generated
B. Amplitude of Action Potentials Generated
C. Both Frequency and Amplitude of Action Potentials Generated

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# Tools for Imaging

## Paired Recordings

*Disadvantages:*

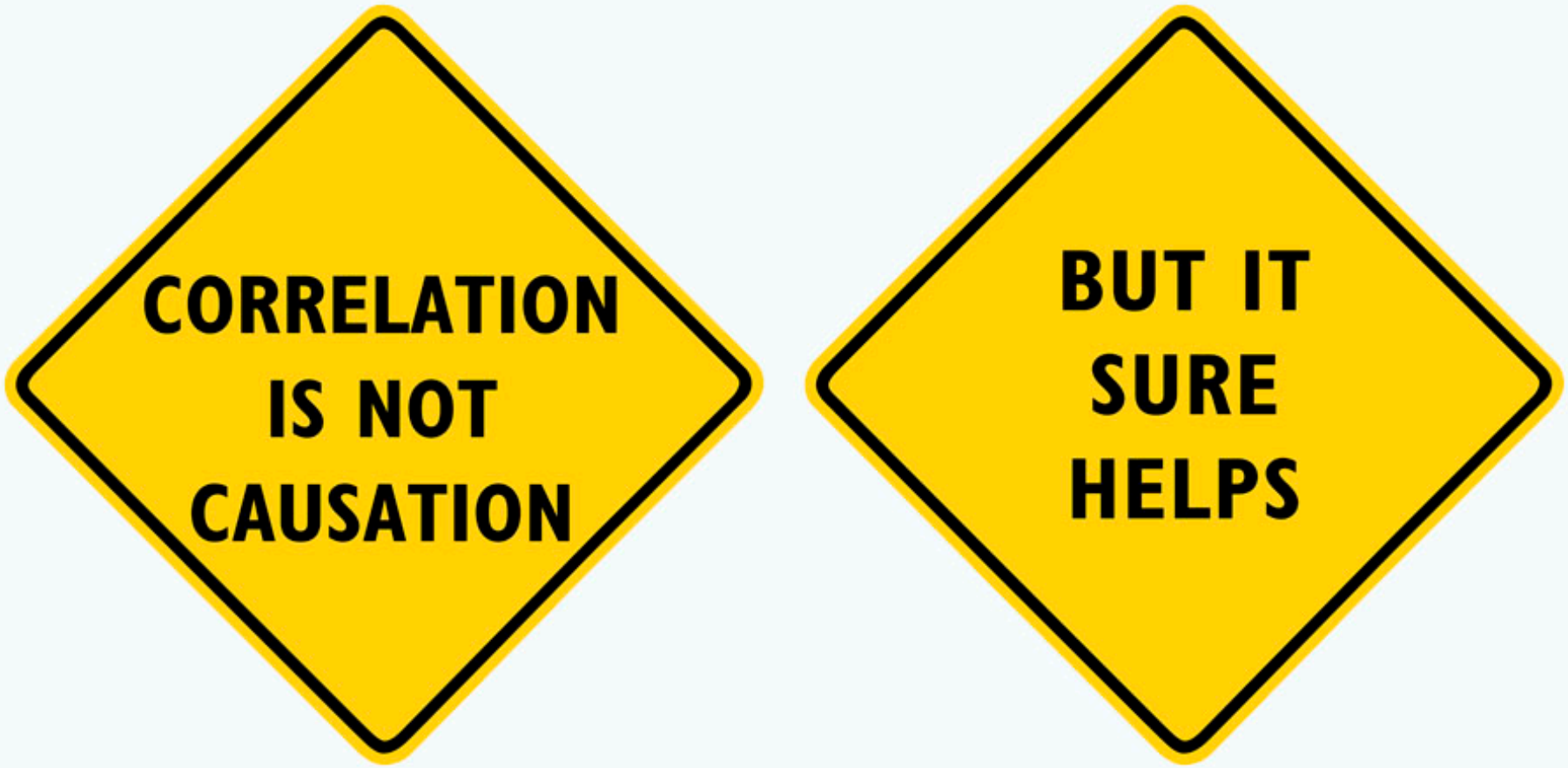
- Can only measure electrical signals
- Lacks spatial resolution (difficulty in localizing current)
- Low through-put (takes time to set up recording and can only do small number of neurons at once)

Physiology provides temporal resolution and anatomy provides spatial resolution.

## Imaging Techniques

- **Calcium Sensors**
  GCaMP is used to monitor calcium activity of many neurons at once.
- **Voltage Sensors**
  Voltage sensors change fluorescence intensity in response to changes in voltage across the membrane.

# Correlation v. Causation




*How can we manipulate physiology to show causal function in neural circuits?*

# Step 4: Organize Neurons by Epistasis Analysis

**A. Inactivate a pair of neurons together and observe whether the behavioral defect is enhanced.**

1. If enhanced, neurons likely function in parallel.
2. If not enhanced, neurons function in series.

**B. Activate a pair of neurons together and observe if behavior is enhanced over a single activation.**

1. If enhanced, neurons likely function in parallel.
2. If not enhanced, neurons likely function in series.

**C. Activate neuron X and see if neuron Y responds.**

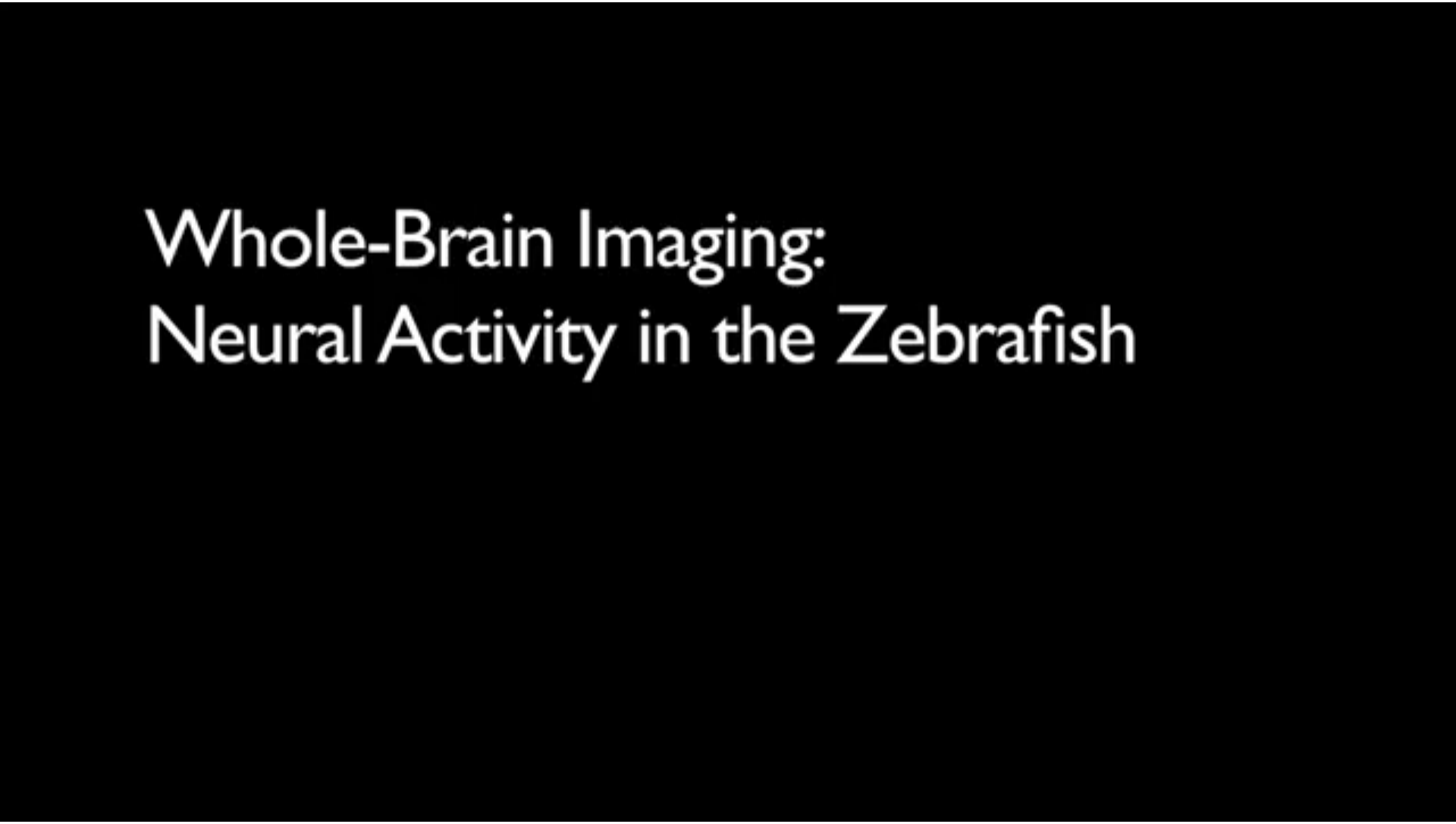
1. If it does, neuron Y is downstream of X.
2. If it does not, neuron Y is upstream of or in parallel with X.

**D. Activate neuron Y and see if neuron X responds.**

1. If it does, neuron Y is upstream of or in parallel with X.
2. If it does not, neuron Y is downstream of X.

*Inactivation experiments show necessity.*
*Activation expressions show sufficiency.*

# Neural Activity Imaging



# Inactivation Experiments

To show a neuron is *necessary* for a behavior, one needs to illustrate that the loss of that neuron results in *partial or complete loss of behavior*.

**Techniques**

A. Laser Ablation
B. Synaptic Silencing
   - Transgenic method where protein blocks chemical synaptic transmission in neuron
     (i.e. tetanus toxin, genetic mutants)
   - If synaptic silencing doesn't result in loss of behavior, neuron may receive input directly from environment or gap junctions.
C. Electrical Silencing
   - Hyper-polarize the neuron through current injection
   - Halo-rhodopsin and Archae-rhodopsin (Arch)

# Activation Experiments

In some cases, inactivation won't show any effect because there is *redundancy* in the circuit. An activation experiment will overcome this problem.

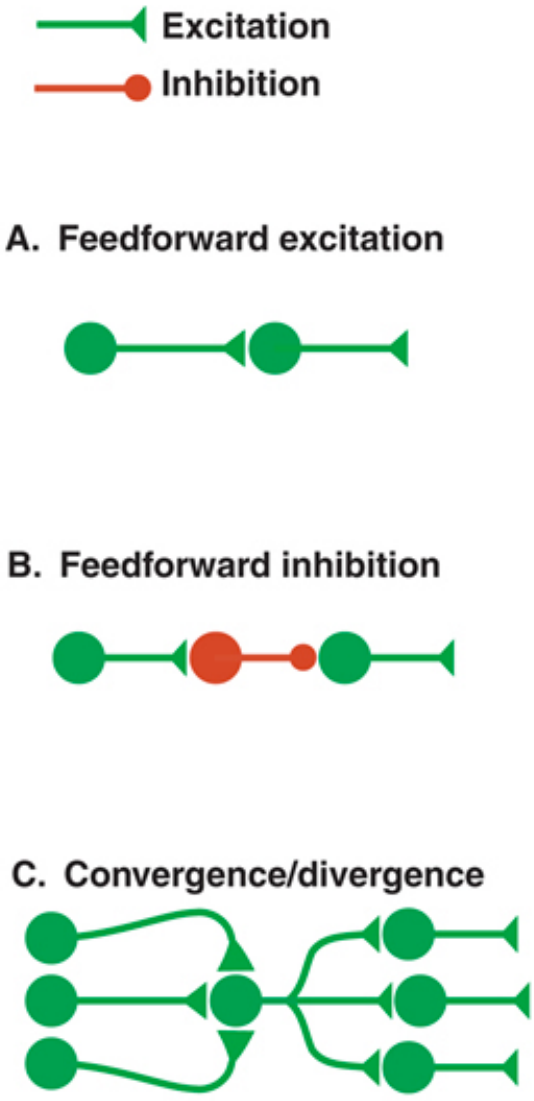
**Techniques**

A. Restore genetic function in neuron
   - Rescue experiment (i.e. NT synthesis)

B. Electrical Activation
   - De-polarize the neuron through current injection
   - Channel-rhodopsin 2 (ChR2)

*If both activation and inactivation experiments are done and result in the expected effect on behavior,* ***and*** *a physiological response is observed, it is reasonable to conclude that neuron of interest acts in neural circuit.*

# Neural Circuit Motifs




## A. Feed-forward Excitation

Allows one neuron to relay information to its neighbor. Long chains of these can be used to propagate information through the nervous system

## B. Feed-forward Inhibition

A pre-synaptic cell excited an inhibitory interneuron, which then inhibits the next follower cell. This is a way of limiting excitation.

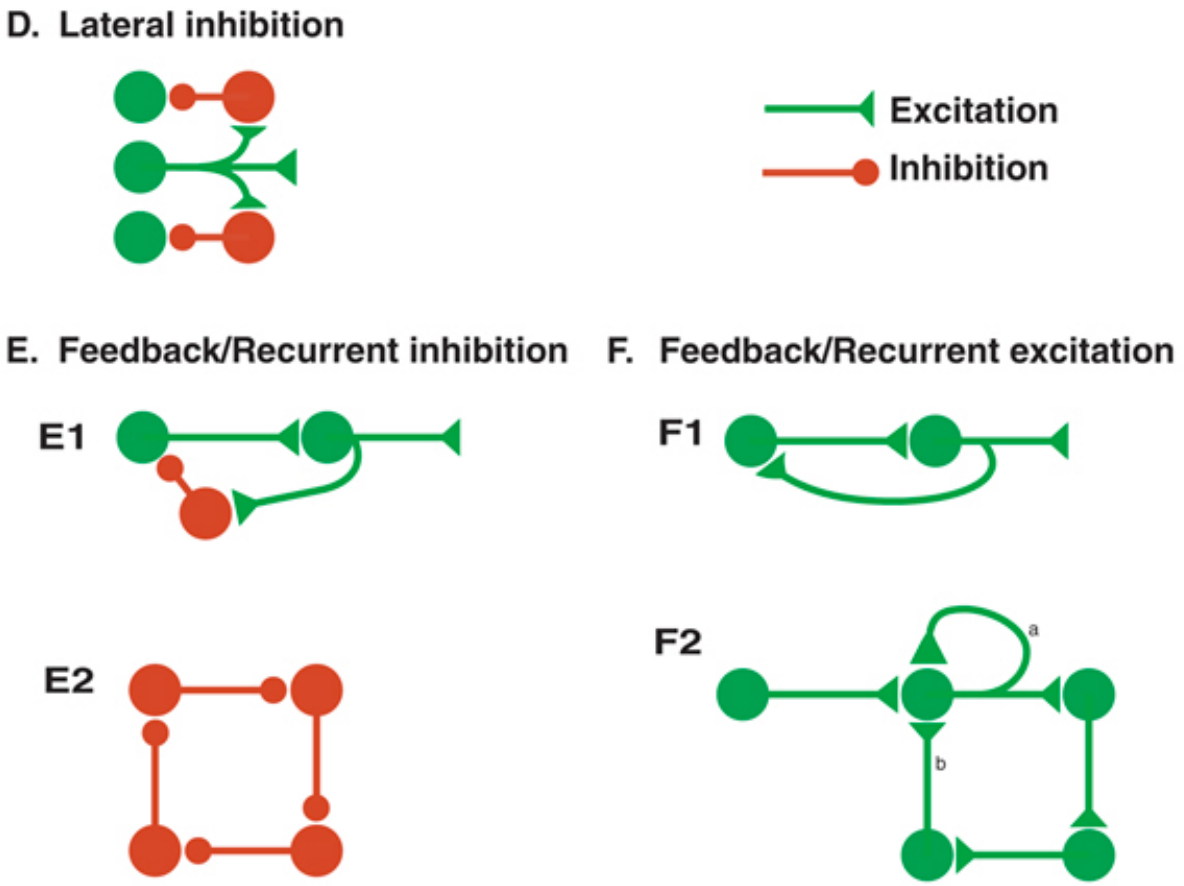
## C. Convergence/ Divergence

One post-synaptic cell receives convergent input from a number of different pre-synaptic cells and any individual neuron can make divergent connections to different post-synaptic cells.

***Divergence*** *allows one neuron to communicate with many neurons in a network.*

***Convergence*** *allows a neuron to receive input from many neurons in a network.*

# Neural Circuit Motifs




**D. Lateral Inhibition**
A pre-synaptic cell excites inhibitory interneurons, which inhibit neighboring cells in the network.

**E. Feedback/ Recurrent Inhibition**

**F. Feedback/ Recurrent Excitation**

# Test Your Understanding

This term best represents a neural circuit where one pre-synaptic neuron synapses with several post-synaptic neurons in order to amplify a sensory signal:

A. Feed-Forward Excitation
B. Convergence
C. Divergence
D. Lateral Inhibition

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**C. Divergence**
D. Lateral Inhibition

*Explanation:*

Diverging circuits allows one neuron to communicate with many neurons (i.e. skeletal muscle contractions). On the other hand, converging circuits allows one neuron to receive many inputs (i.e. spinal cord to brain).

# Properties of Neural Circuits

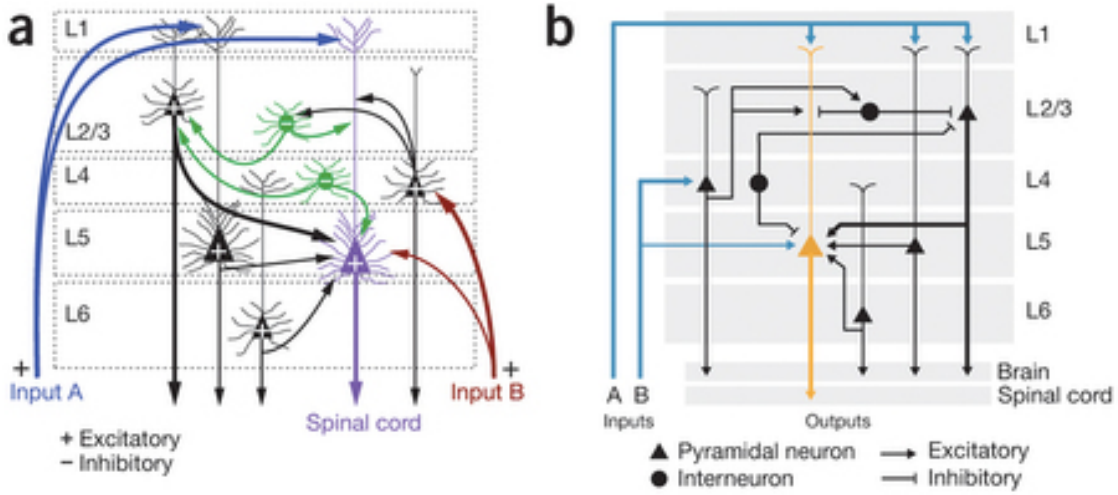
1. Feedback
2. Degeneracy
3. Competition
4. Modularity

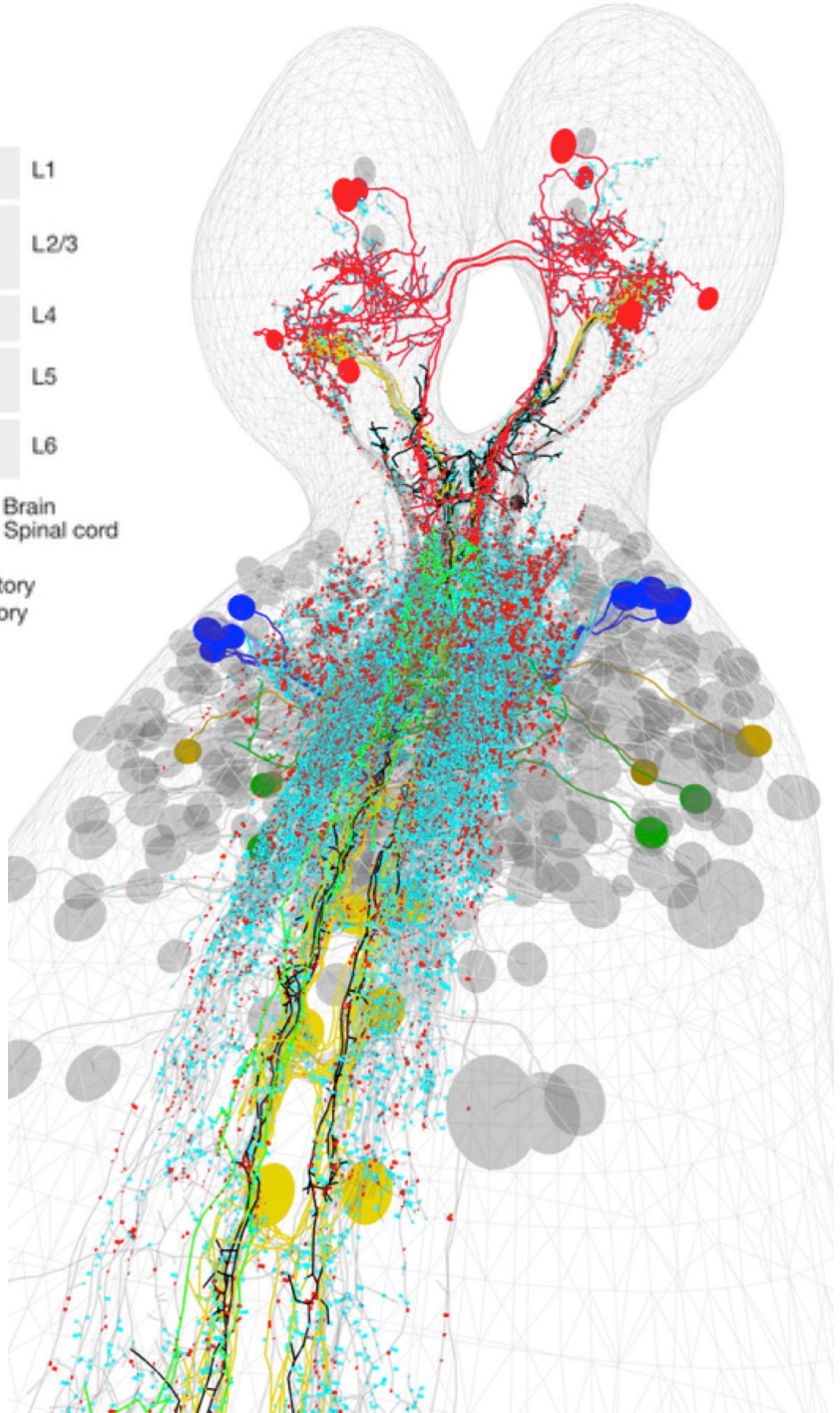
**Degeneracy:** the ability of multiple different configurations or mechanisms to produce the same outcome or serve the same function

**Competition:** small-scale axon elimination during development of nervous system

**Modularity:** permits an organism to process a new input without evolving an entirely novel circuit from scratch (i.e. building diverse objects using existing building-blocks)

# Step 5: Circuit is Complete



*Multi-Sensory Circuits*

# How to Read Scientific Papers

*The Crayfish Escape Lateral Giants As Command Neurons for Escape Behavior (Olson and Krasne, 1981)*

THE CRAYFISH LATERAL GIANTS AS COMMAND NEURONS FOR ESCAPE BEHAVIOR

GENE C. OLSON and FRANKLIN B. KRASNE*

*Dept. of Psychology and Brain Research Institute, University of California at Los Angeles, Los Angeles, Calif. 90024 (U.S.A.)*

# Crayfish Escape Response

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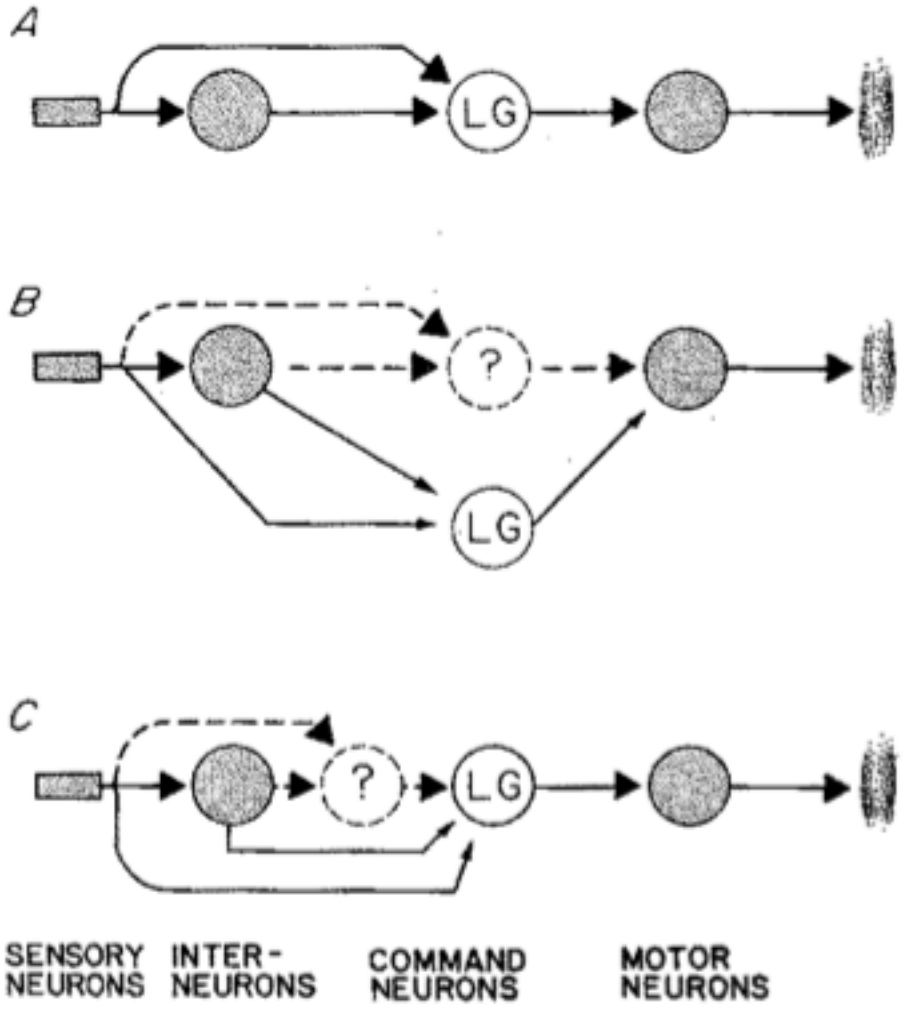


***Figure 1.*** Shading indicates that elements are in parallel. Dashed lines represent hypothesized circuit.
*Assumption:*
*The LG neuron is critical for generation of a tail flip.*

# Crayfish Escape Response

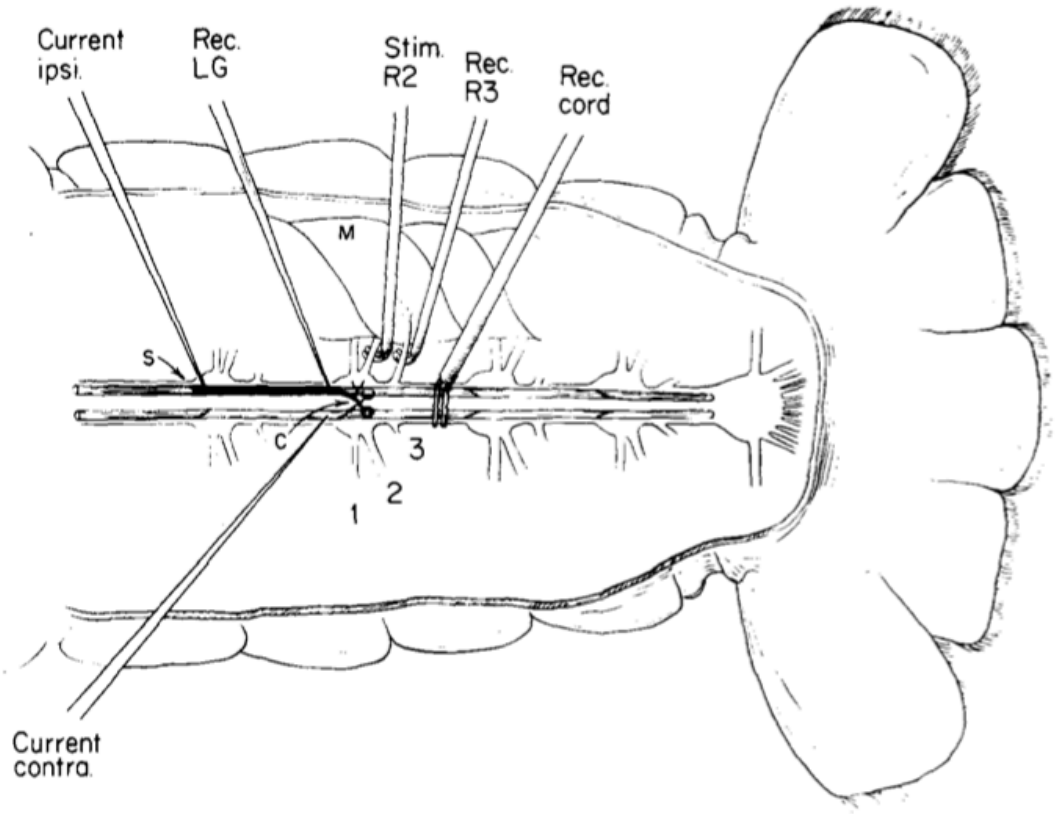


***Figure 2.*** Dorsal view of abdominal nerve cord for illustrating positions for stimulating and recording electrodes (paired recording).

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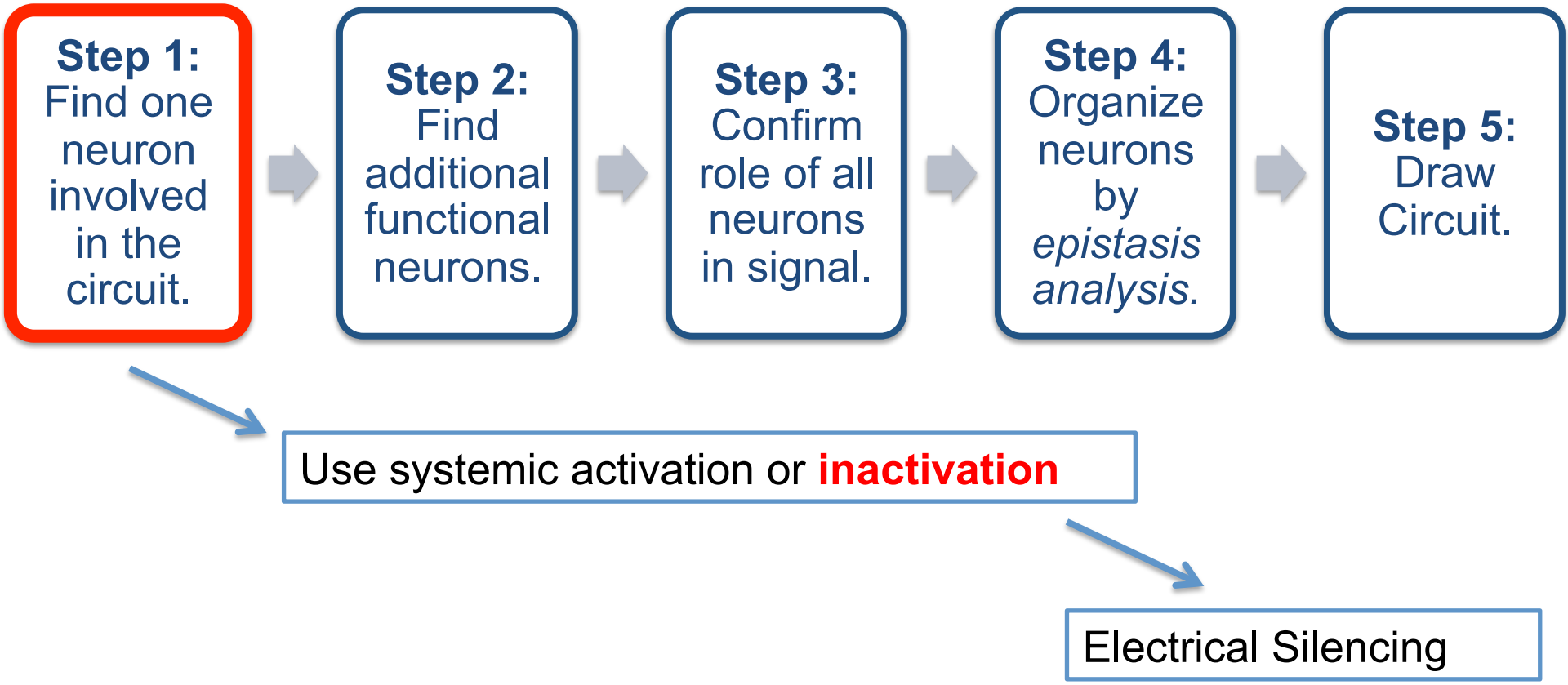

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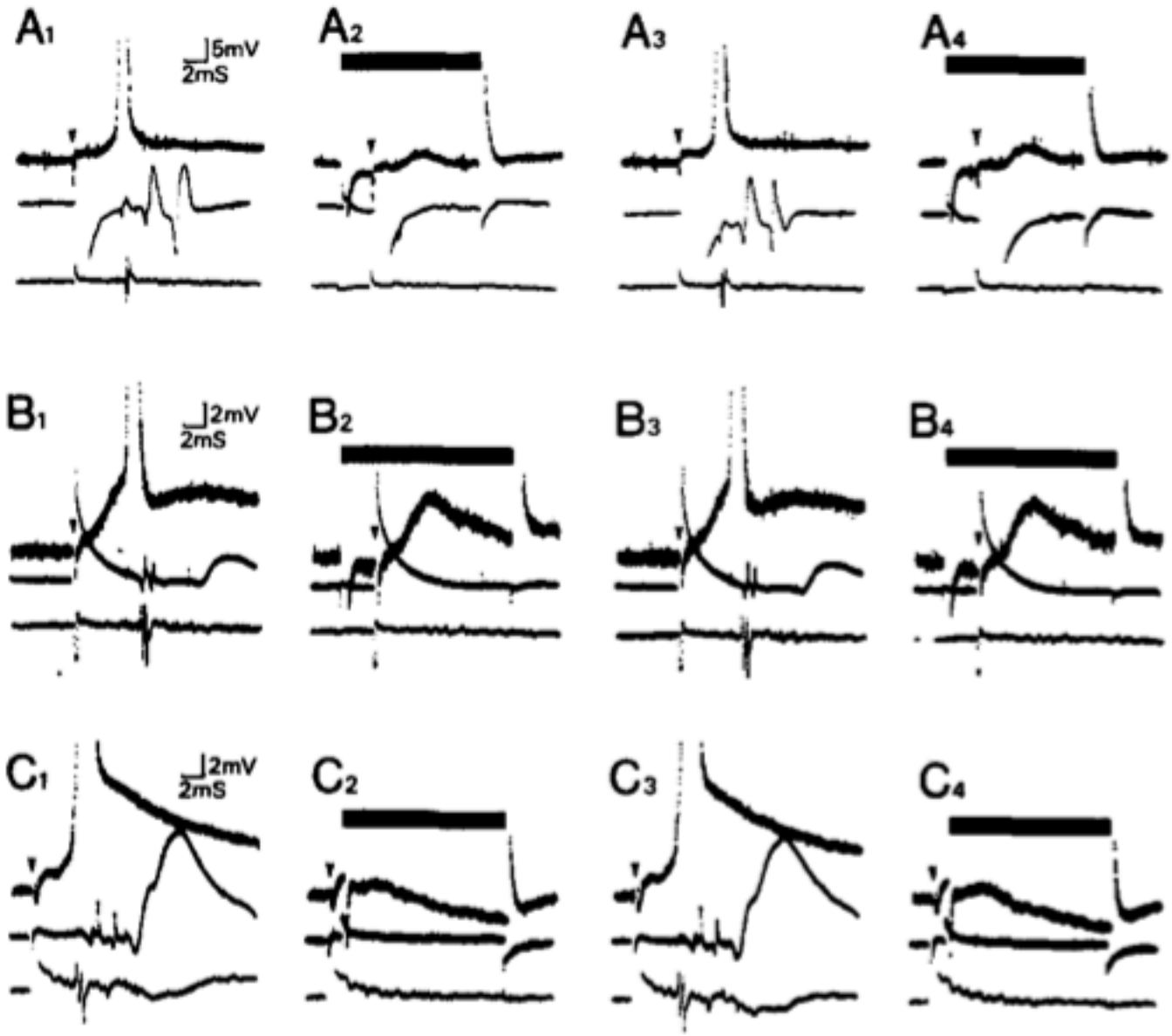
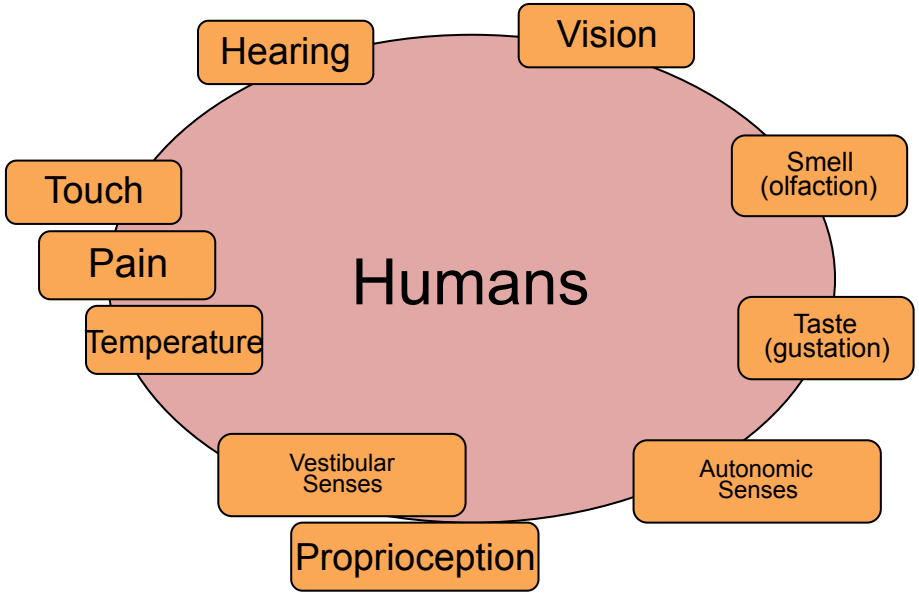
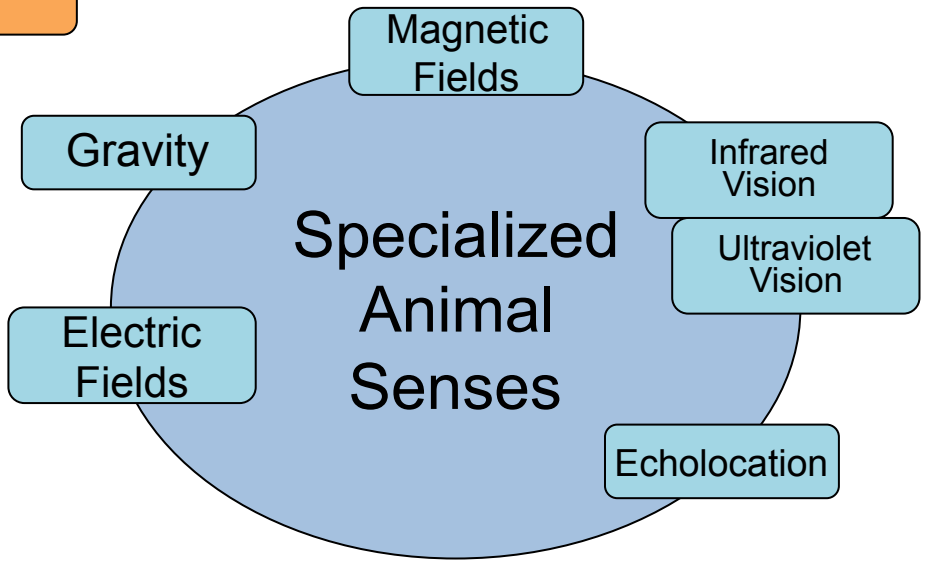



***Figure 3.***
***A.*** *Intracellular recording in LG*
***B.*** *Extracellular recording in Root Area.*
***C.*** *Extracellular recording in dorsal surface of nerve cord.*
***Paired recordings involve a response (shown) and stimulus (second root).***

# Sensory Systems

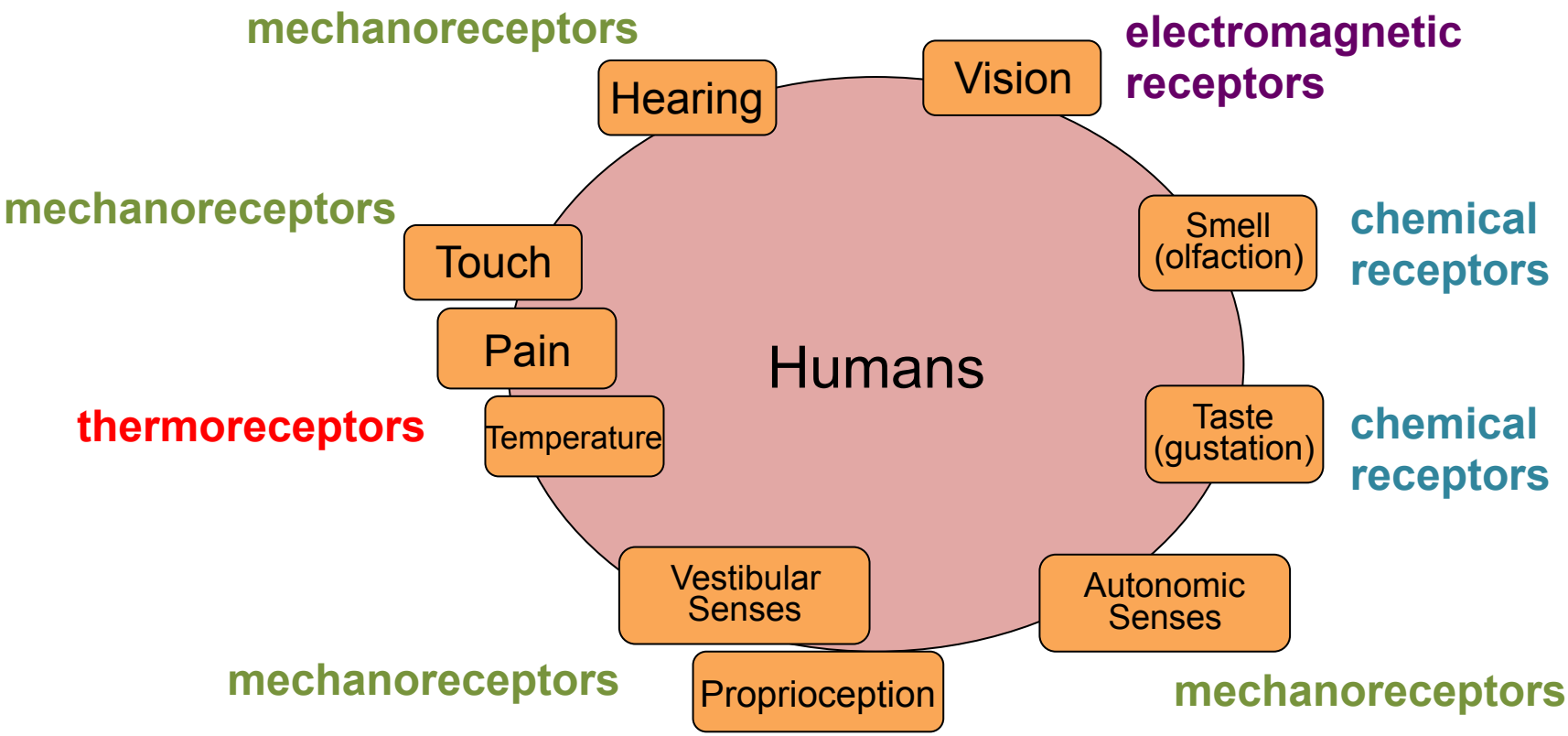
## What are different types of senses?



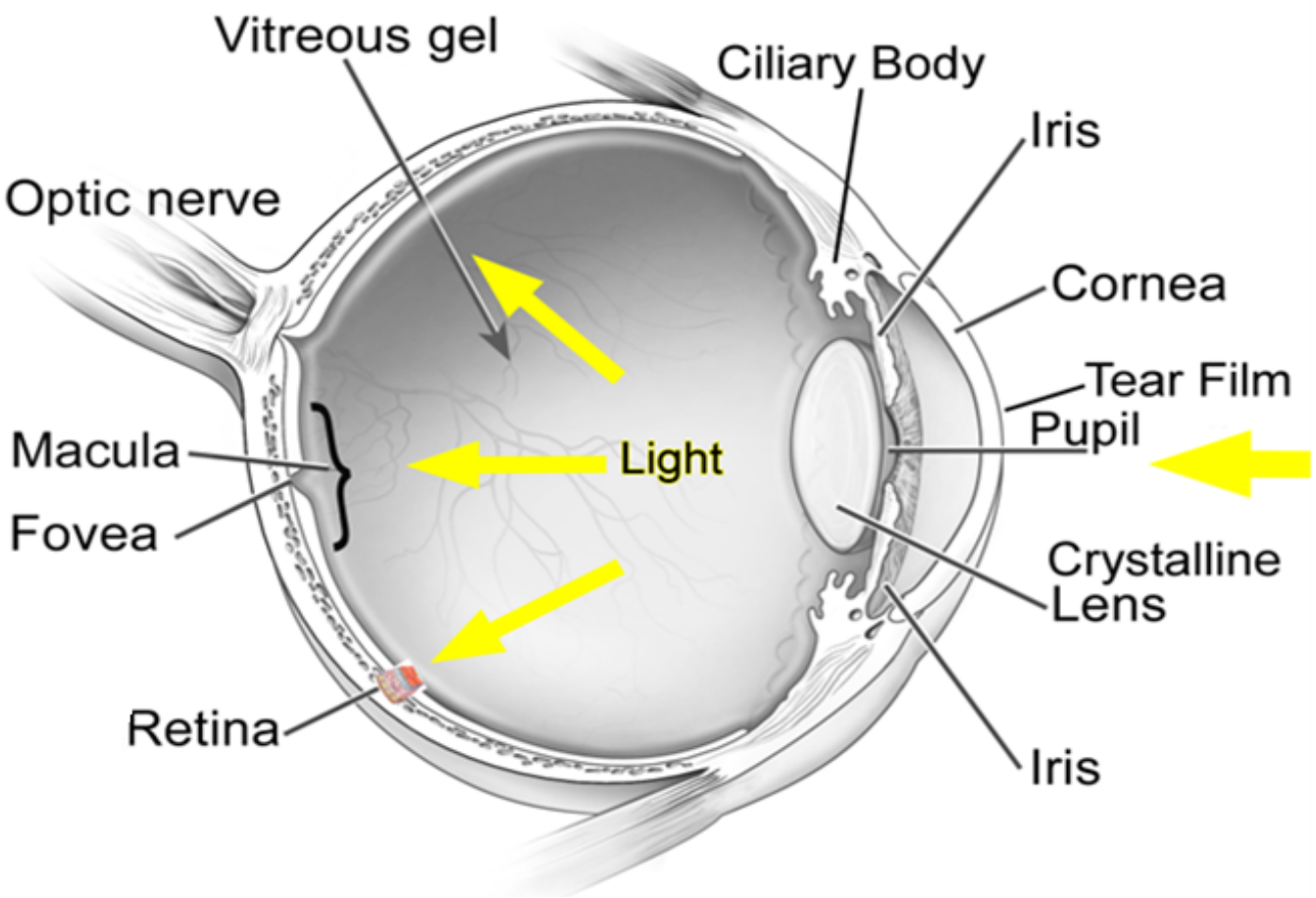



# Sensory Systems

## How can senses be divided into classes?



# The Eyes




Pupil size is a compromise between sensitivity and acuity

**Visual Processing:** sequence of steps that transforms information from the eye to the brain for cognitive processing

# The Eyes

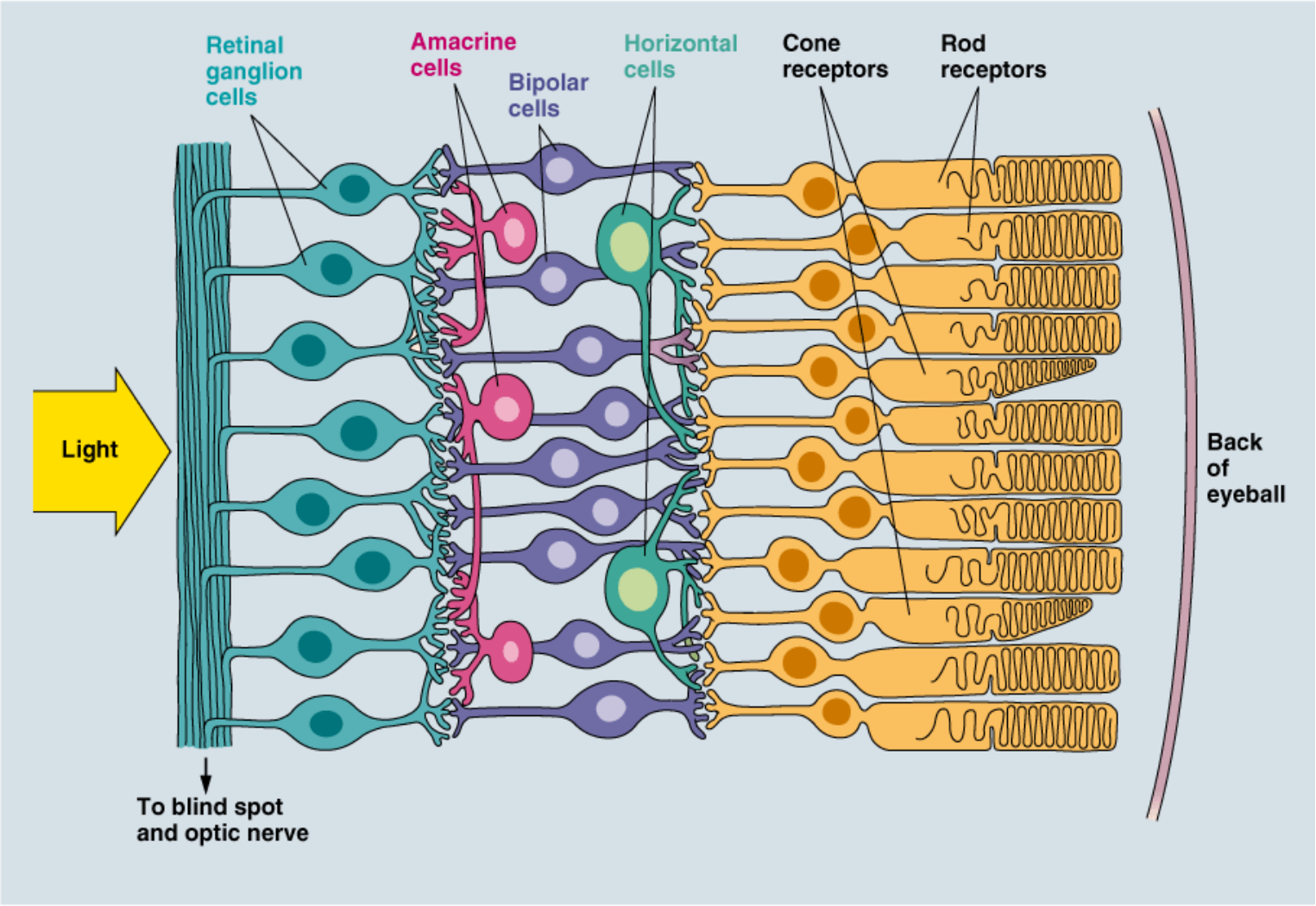
**Binocular Disparity:** Most mammals have two eyes on the front of their heads, rather than one on each side.

*This cuts down the field of view but ensures that most of what is seen is seen through both eyes.*

**Stereopsis:** Impression of depth that is perceived when a scene is viewed with both eyes

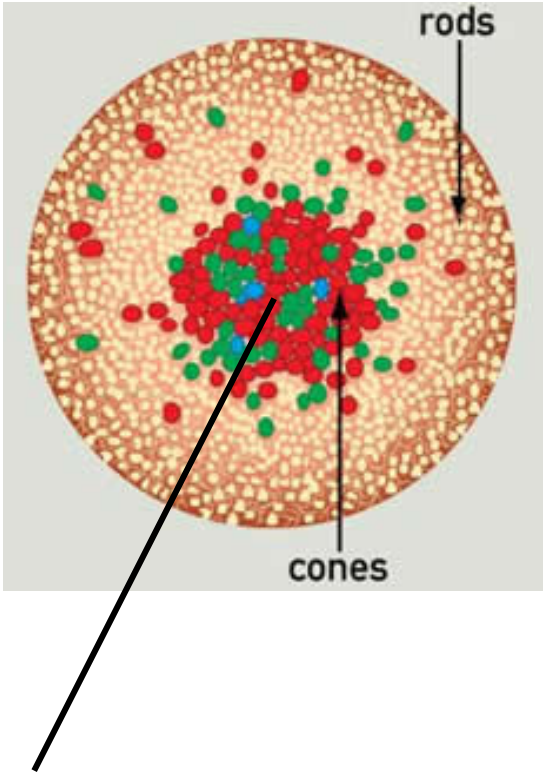
*Monocular clues about size and shape are used in perceiving depth.*

# The Retina




Receptor is **farthest from light**. *What does this imply? Incoming light is distorted by four layers of neurons prior to* reaching receptors

# The Retina




**Optic Disk:** Region of axons of retinal ganglion cells penetrate the retina and exit the eye

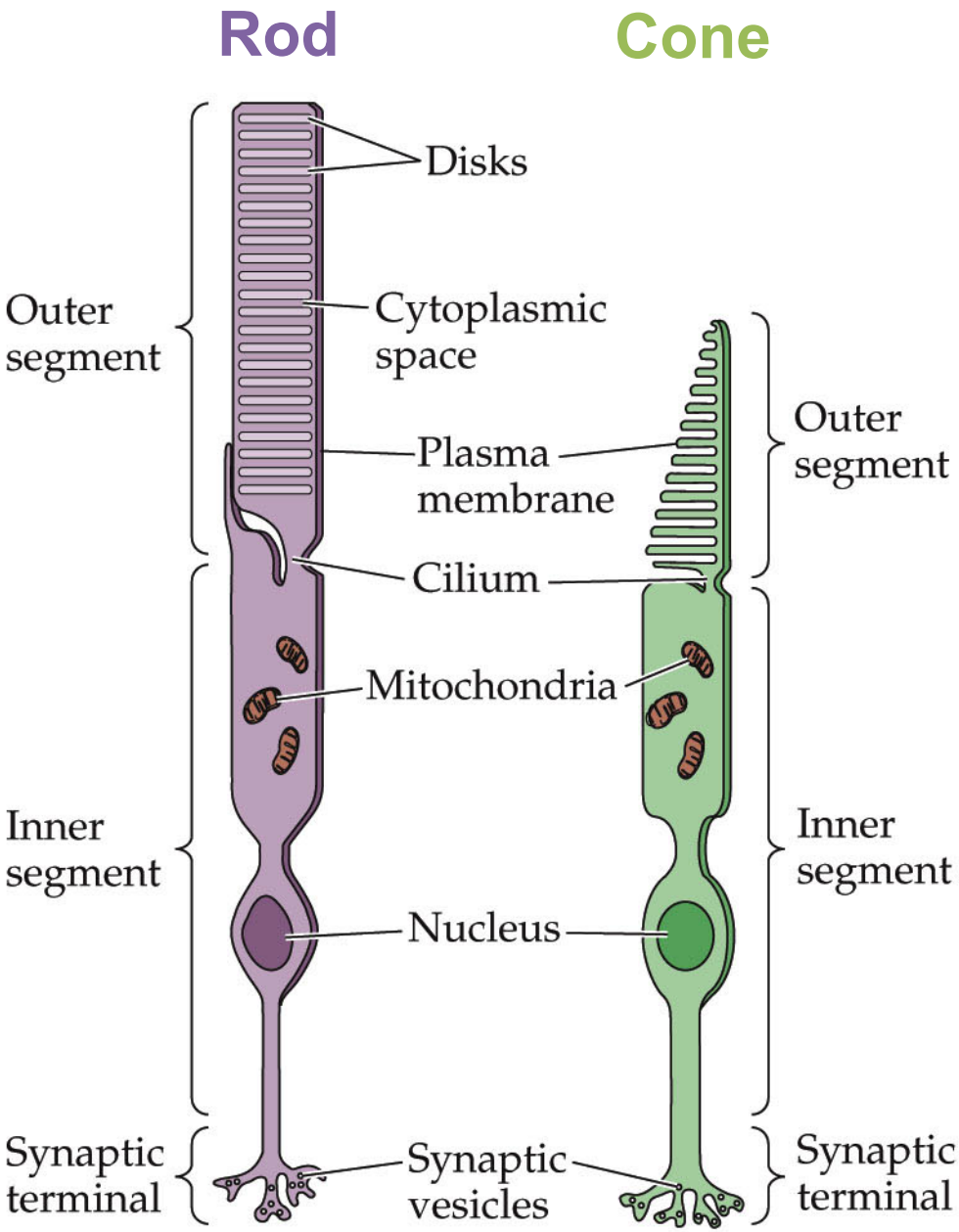
*The optic disk has no receptors, creating a blind spot.*

**Completion:** Our visual system is able to use visual information furthered from receptors around optic disk to complete visual image.

**Fovea:** center of retina and only part that is capable of mediating high-acuity vision. *Why?*

The axons of the retinal ganglion cells are thinnest over the fovea and the light is distorted less before reaching the layer of receptors.

# The Retina




***Duplexity Theory of Vision*:**

*In a sense, we have two visual systems:*

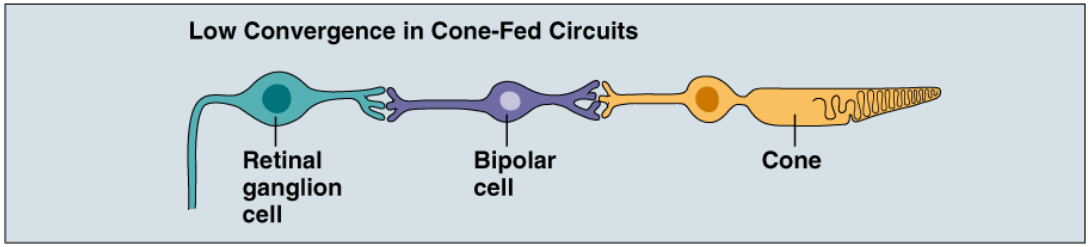
1. **Photopic System**

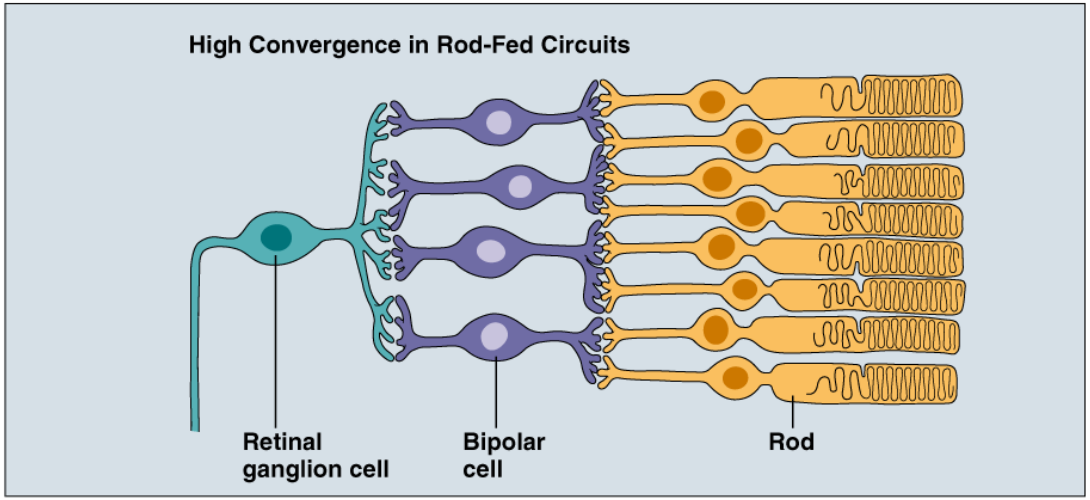
Functions in lighted conditions for high-acuity vision (Cones)

2. **Scoptopic System**

Functions in dim light for low-acuity vision (Rods)

# The Retina




**Photopic System** has **low sensitivity** with information from few receptors combined at next cell level (**low convergence**).
**Scotopic System** has **high sensitivity** with many receptors converging on ganglion cells (**high convergence**).

Only cones in fovea while rods pre-dominate in periphery.

# Test Your Understanding

1. The fovea is the part of the retina that contains photoreceptors called:
    A. Rods
    B. Cones
    C. Amacrine cells
    D. Ganglion cells

2. The rods and the cones synapse directly on:
    A. Amacrine cells
    B. Ganglion cells
    C. Bipolar cells
    D. Horizontal cells

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# The Retina

Our eyes are in continual motion, making a series of
**Fixations:** ~3 per second, connected by
**Saccades:** Rapid movements between fixations

*In this way, neurons of the visual system respond to **change**, not steady input.*

*What we perceive at any instance is the sum of the input that has been received the last few fixations (**Temporal Integration**). This is why images do not disappear when we blink.*

# Visual Transduction by Rhodopsin

**Visual Transduction:** Conversion of Light Energy to Neural Signals by Rods and Cones

*How do rods transduce light?*

1. When light is absorbed by **rhodopsin**, it becomes bleached (removes its color).
2. Bleaching reaction **hyper-polarizes** the rods, which results in a cascade of intracellular events that ultimately results in a neural signal.

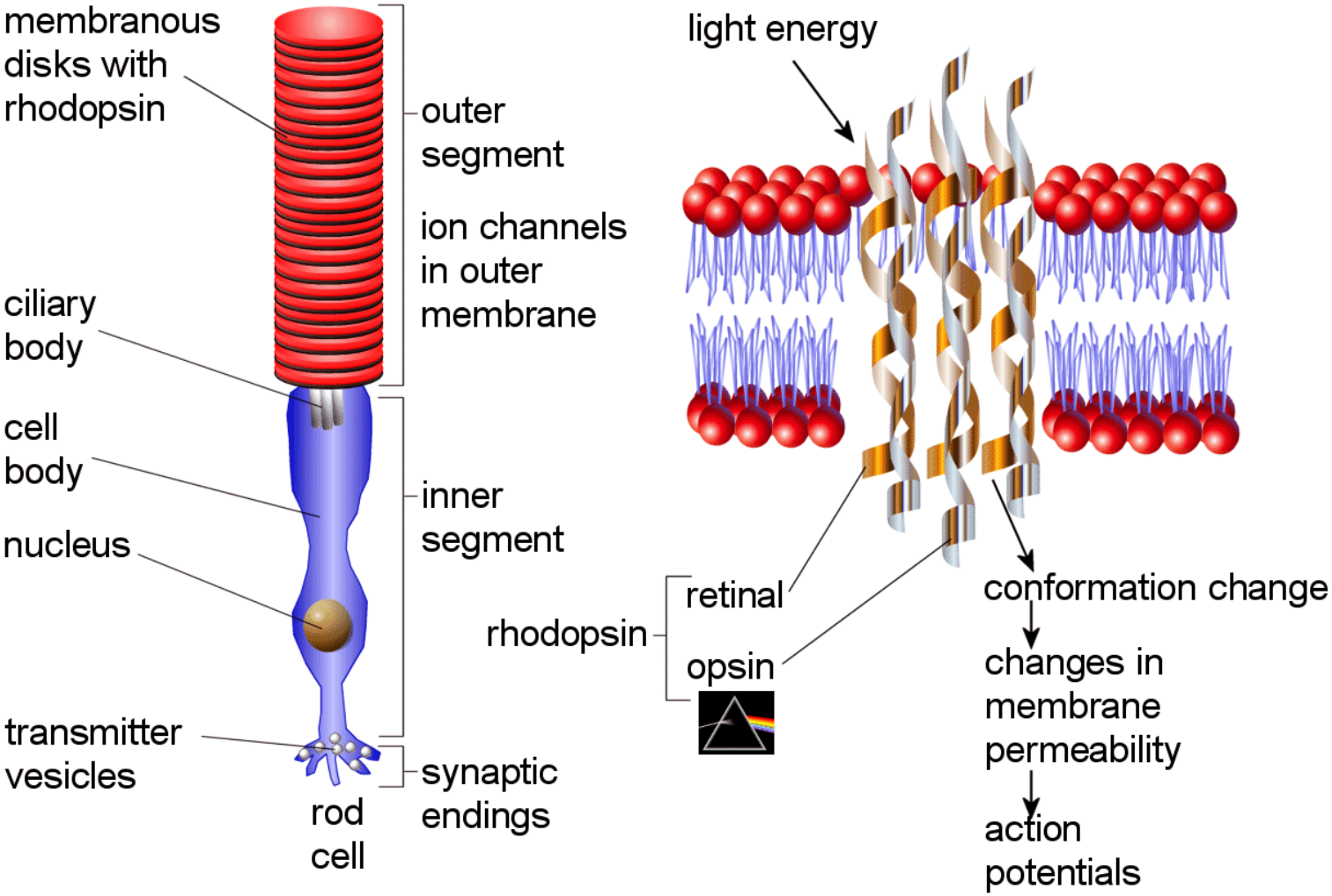
*Why does bleaching occur?*

Two components of rhodopsin, **retinal** and **opsin**, separate in light.

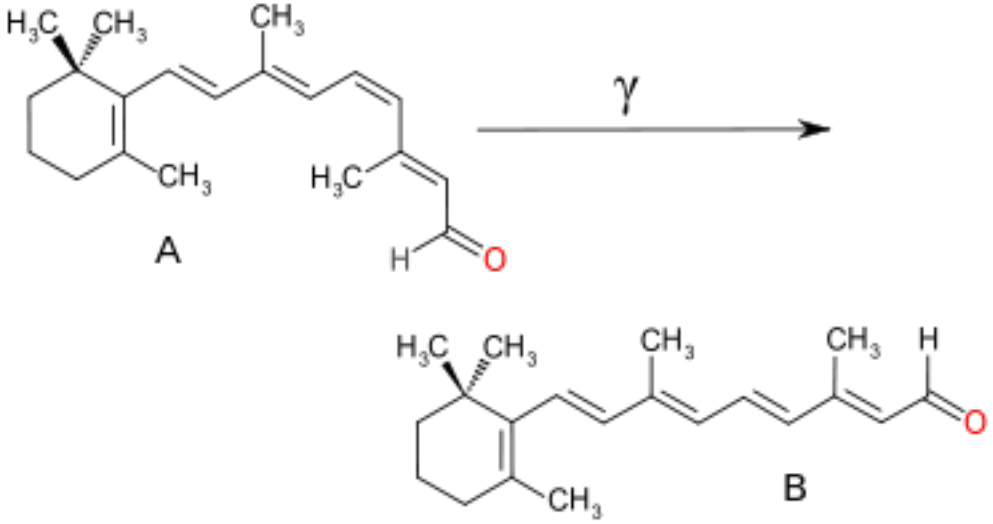
*What happens when rhodopsin is totally bleached?*

Rhodopsin loses its ability to absorb light and transduce a signal. In the dark, however, retinal and opsin re-unite, and rhodopsin regains its color and ability to absorb light and transduce.

# Visual Transduction by Rhodopsin



# Visual Transduction by Rhodopsin



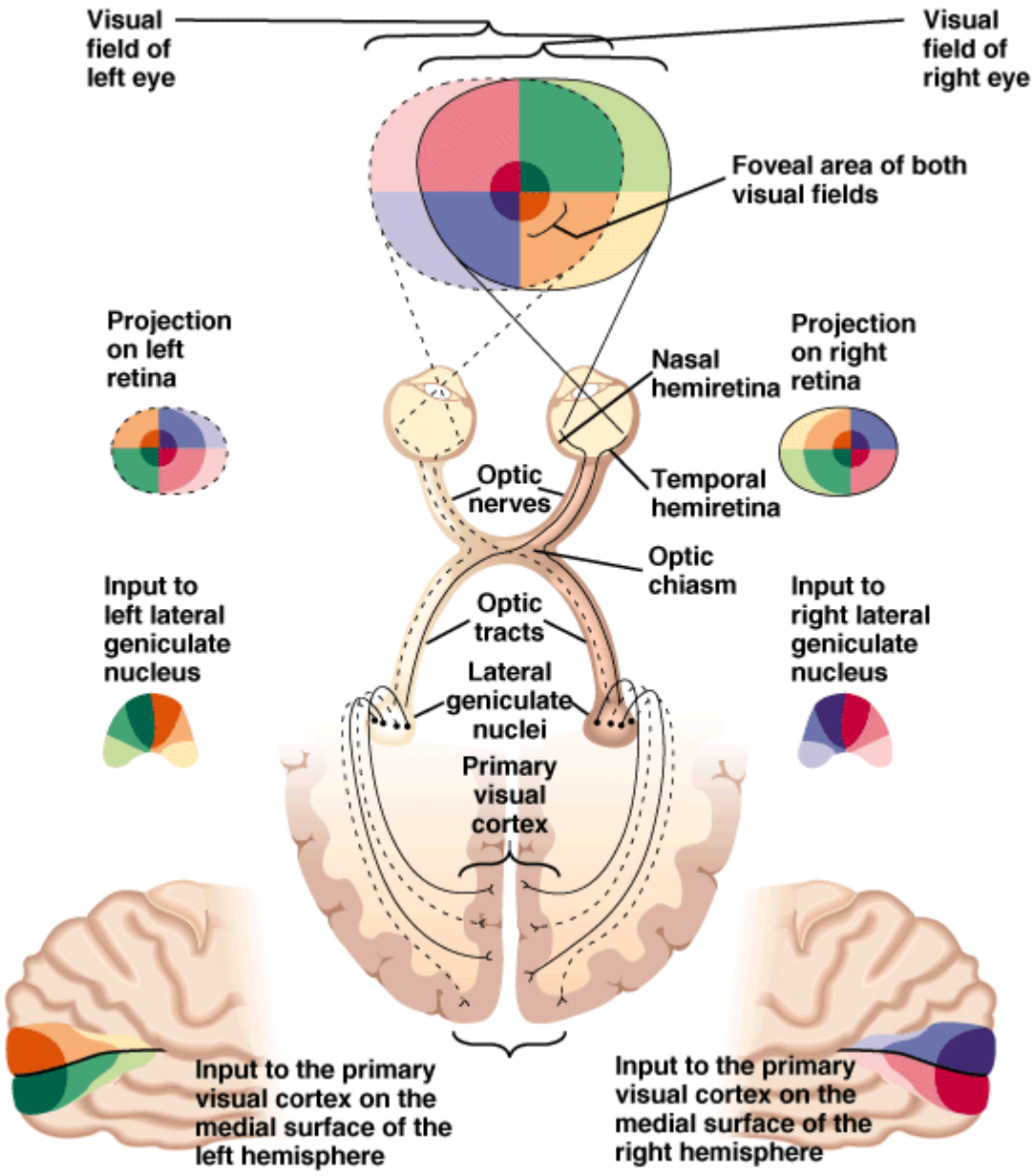

**Rhodopsin:** G-Protein Linked Receptor (GPCR) that responds to light by initiating a cascade of intra-cellular chemical events

Bleaching rods via exposure to light results in an intra-cellular cascade of events that deactivates cGMP, closing $Na^+$ channels, which **hyper-polarizes** the rod cell, and reduces the release of glutamate.

**cGMP:** intra-cellular chemical that keeps $Na^+$ channels partly open when rods are in darkness

*Rods are thus slightly depolarized.*

# From Retina to Primary Visual Cortex




**Retina-Geniculate-Striate Pathway**
Primary Visual Cortex is **striate cortex** below **lower layer IV**. *The stripe is composed of axon terminals from **lateral geniculate nuclei.***

**Retinotopic Layout:** Surface of visual cortex is a **map** of retina.

# From Retina to Primary Visual Cortex

Retina-Geniculate-Striate system consists of two independent channels:

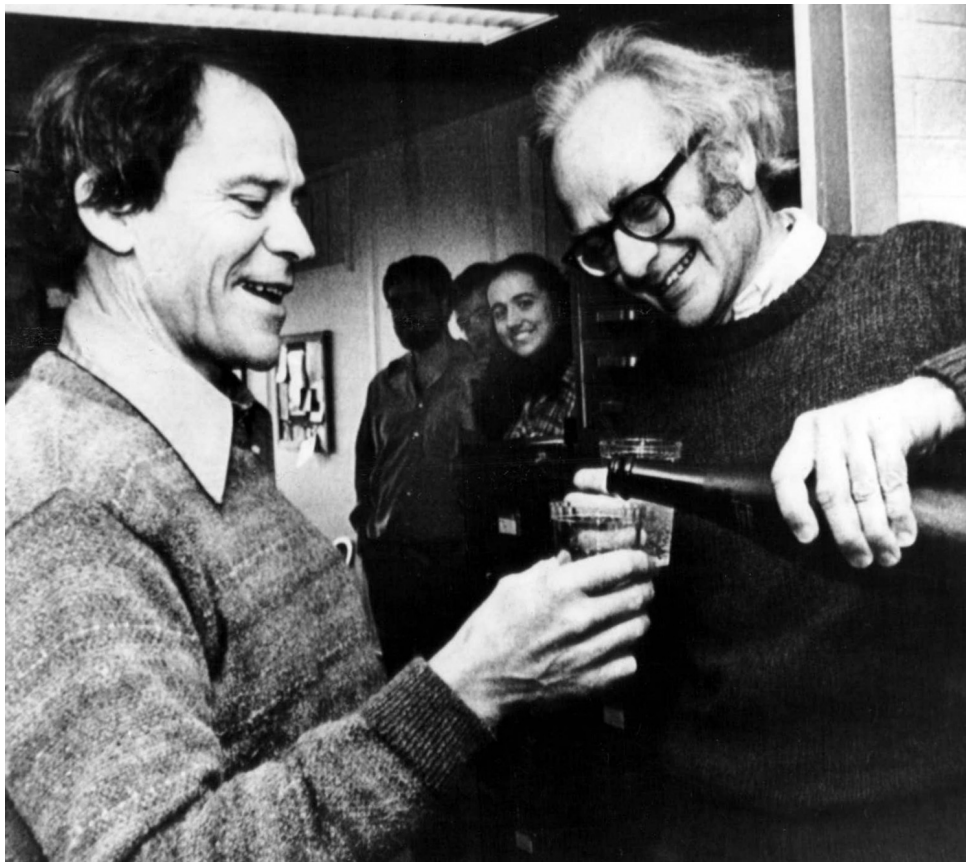
1. **Parvo-cellular Layers (P Pathway)**

   These layers are found in the top four layers of each lateral geniculate nucleus (LGN) and are composed of small body neurons. They are responsive to color, fine detail patterns, and react to slow or stationary objects.

2. **Magno-cellular Layers (M Pathway)**

   These layers are found in the bottom two layers of the LGN, composed of large body neurons, and are responsive to rods and movements.

# The Neural Basis of Perception

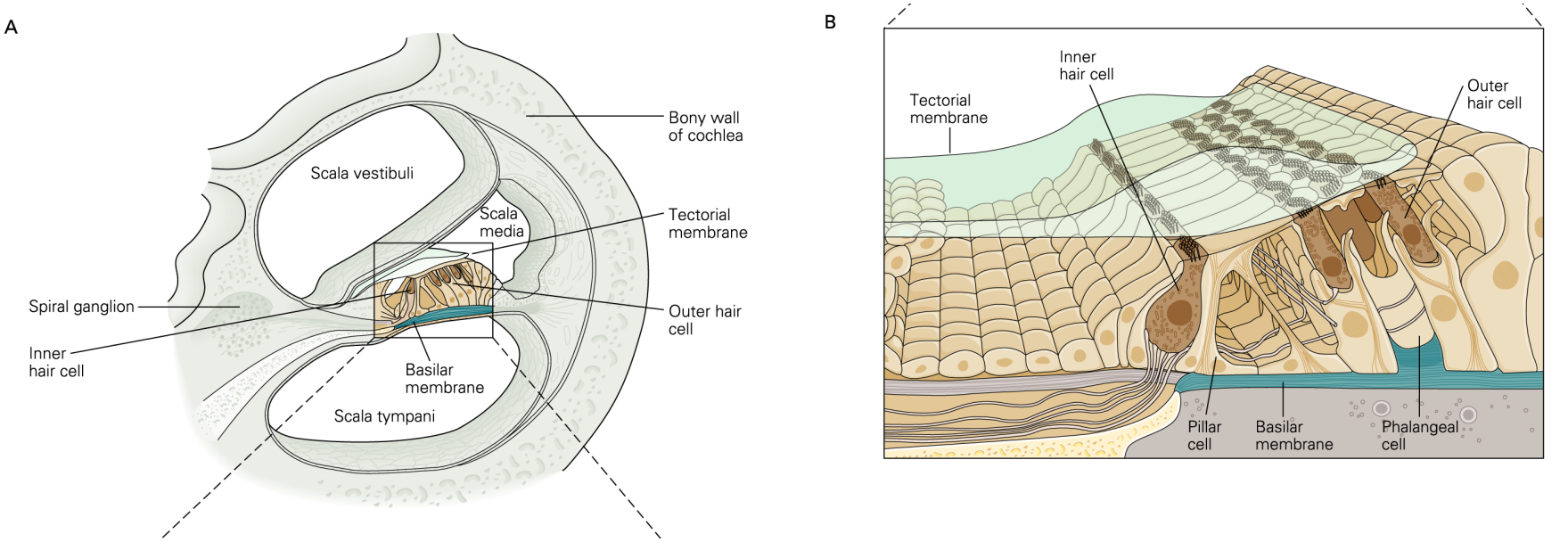


**David Hubel and Torsten Wiesel** shared the *1981 Nobel Prize in Physiology* for their discoveries concerning information processing in the visual system alongside **Roger Sperry**, for his independent research on the cerebral hemispheres.

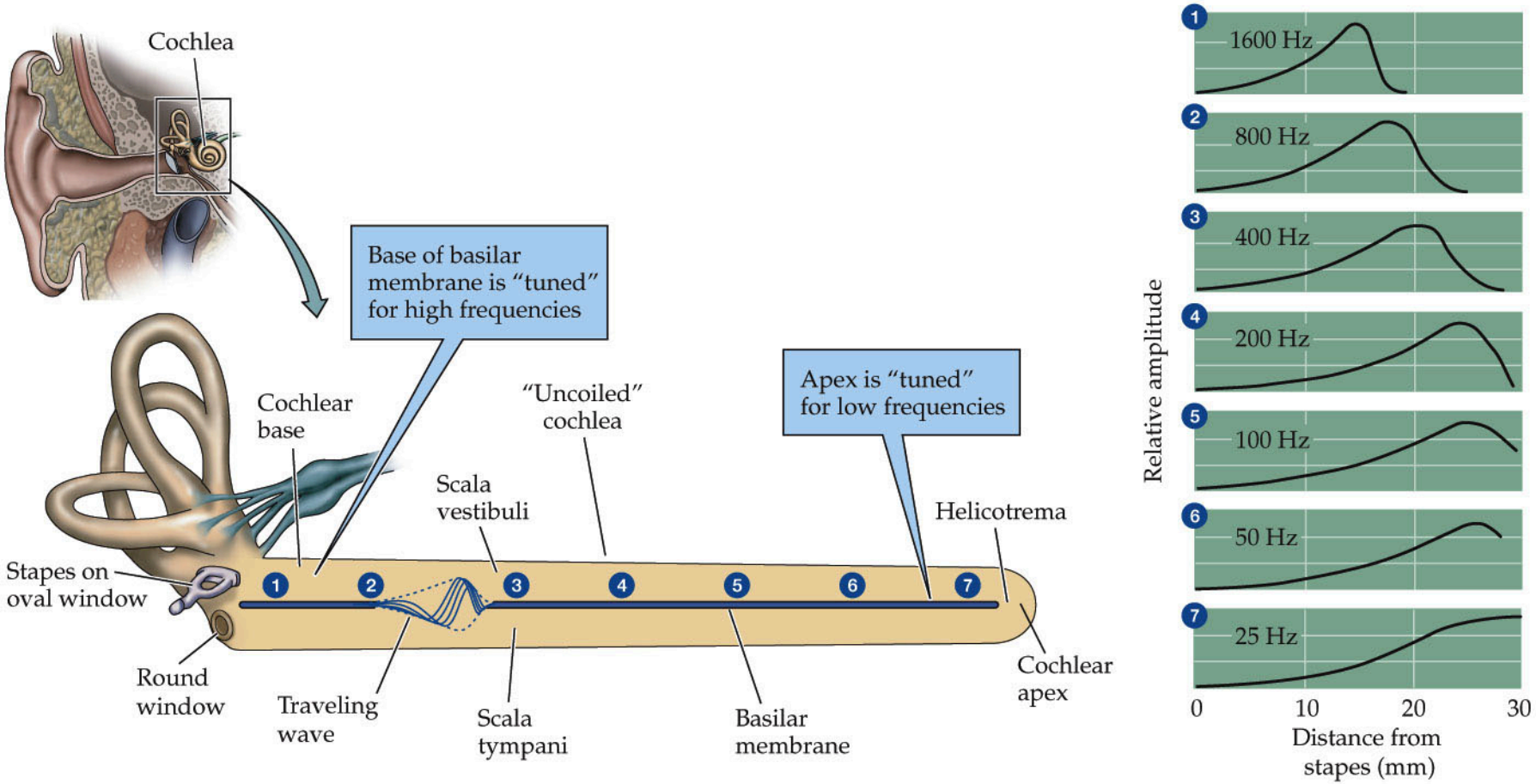
# Auditory System

The **hair cell** is the key structure in the vertebrate auditory system.

1. Used to sense movement in fluid (fluid present in the inner ear)
2. Hair cells in Organ of Corti (in cochlea) respond to sound
3. The fluid, called **endolymph**, has lots of potassium
    - This ionic imbalance provides an energy store, which is used to trigger neural action potentials when the hair cells are moved



# Signal Transduction in the Cochlea



# Next Time:
# Sensory Systems and Neural Circuits II