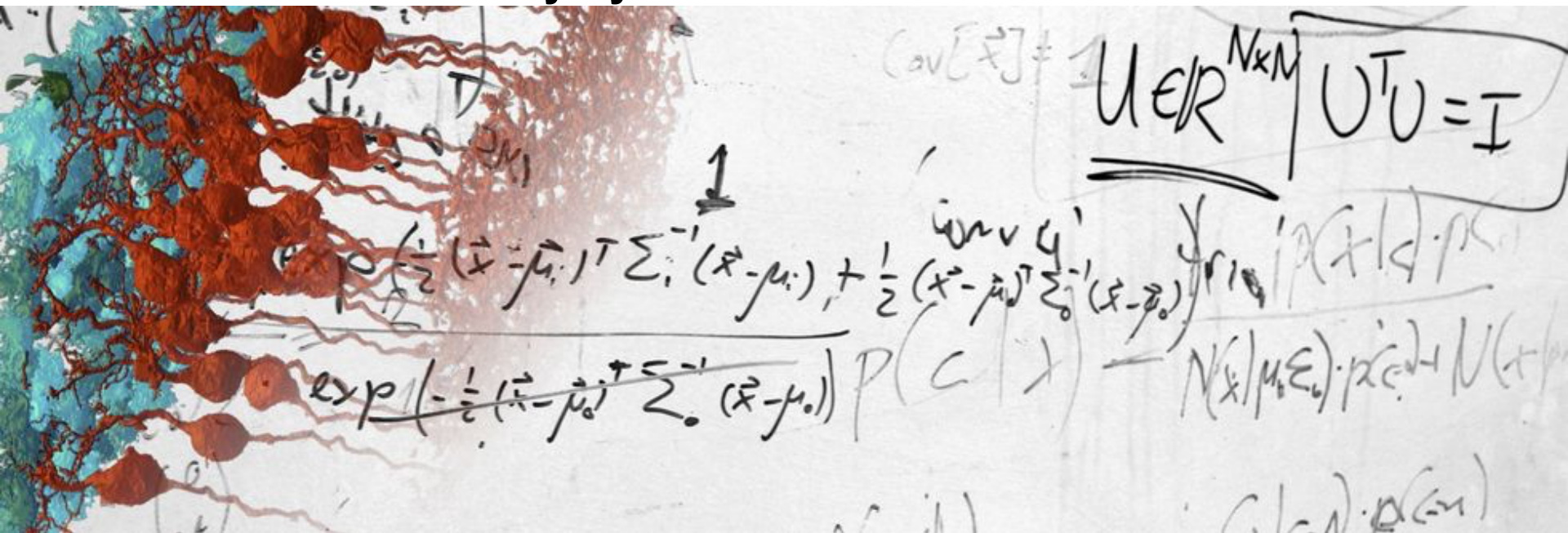
Applied Neuroscience

Columbia Science Honors Program

Fall 2016

Sensory Systems and Neural Circuits



Introduction to Neural Circuits

Objective: Sensory Systems and Neural Circuits

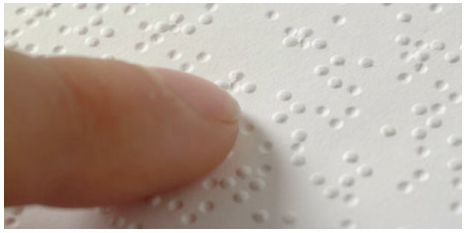
Agenda:

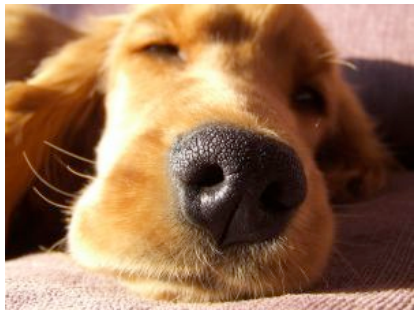
1. Sensory Systems
   Visual System
   Audition
2. Introduction to Neural Circuits
   Neural Circuit Identification
   Electrophysiology

Sensory systems

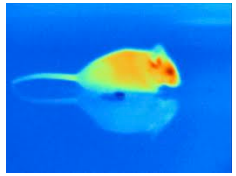
How do we sense the world?

Take different cues from our environment to make sense of the world around us.







Sensory systems

What were some of the earliest senses?

Prokaryotes (single-celled organisms) could detect a variety of stimuli:

1. Chemicals
   - Food and metabolites
   - Noxious molecules
2. Osmolarity
3. pH
4. Pressure
5. Gravity
6. Magnetic fields
7. Temperature

Sensory systems

Hydra: simple prokaryote

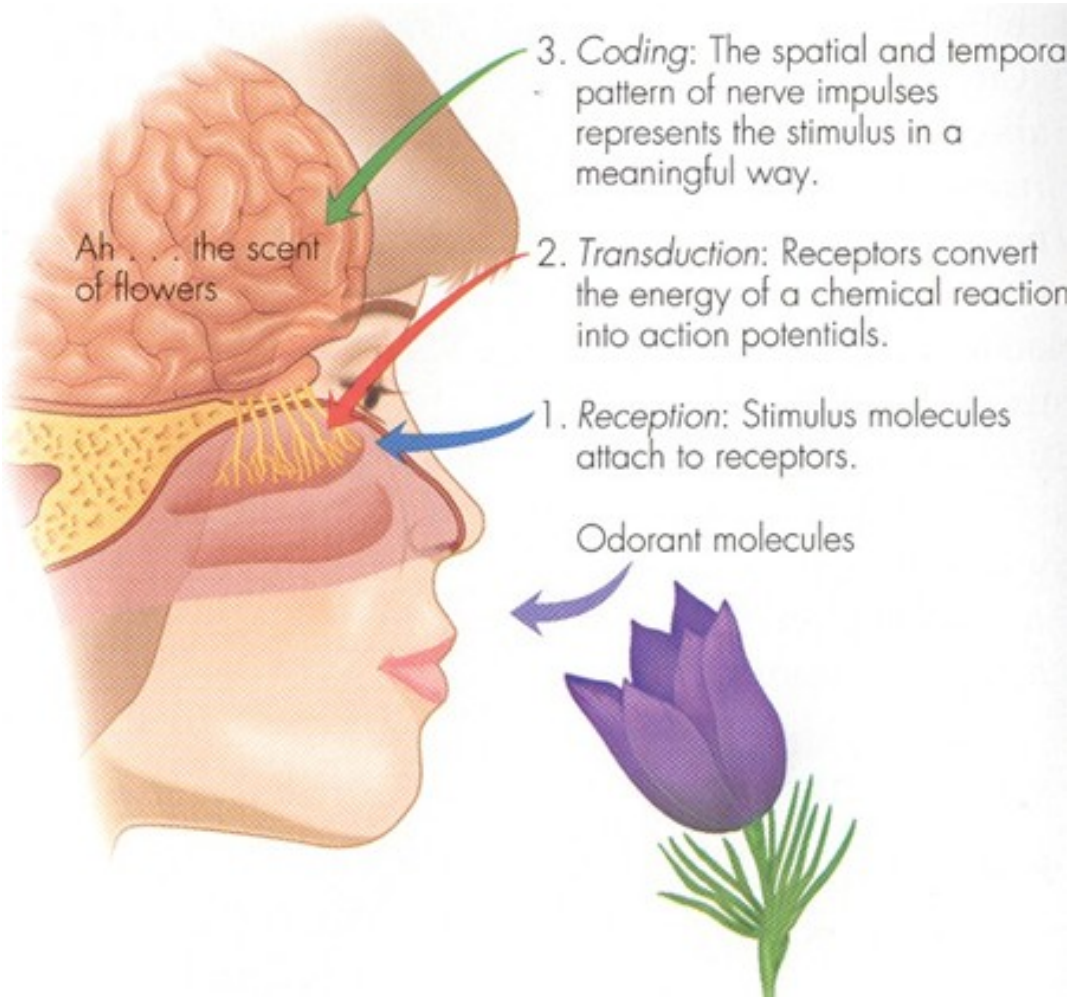
Sensory systems

How do sensory systems work?

Neurons in sensory regions of the brain respond to stimuli by firing action potentials after receiving a stimulus.

Each sensory system follows a specific plan:

1. Reception
2. Transduction
3. Coding

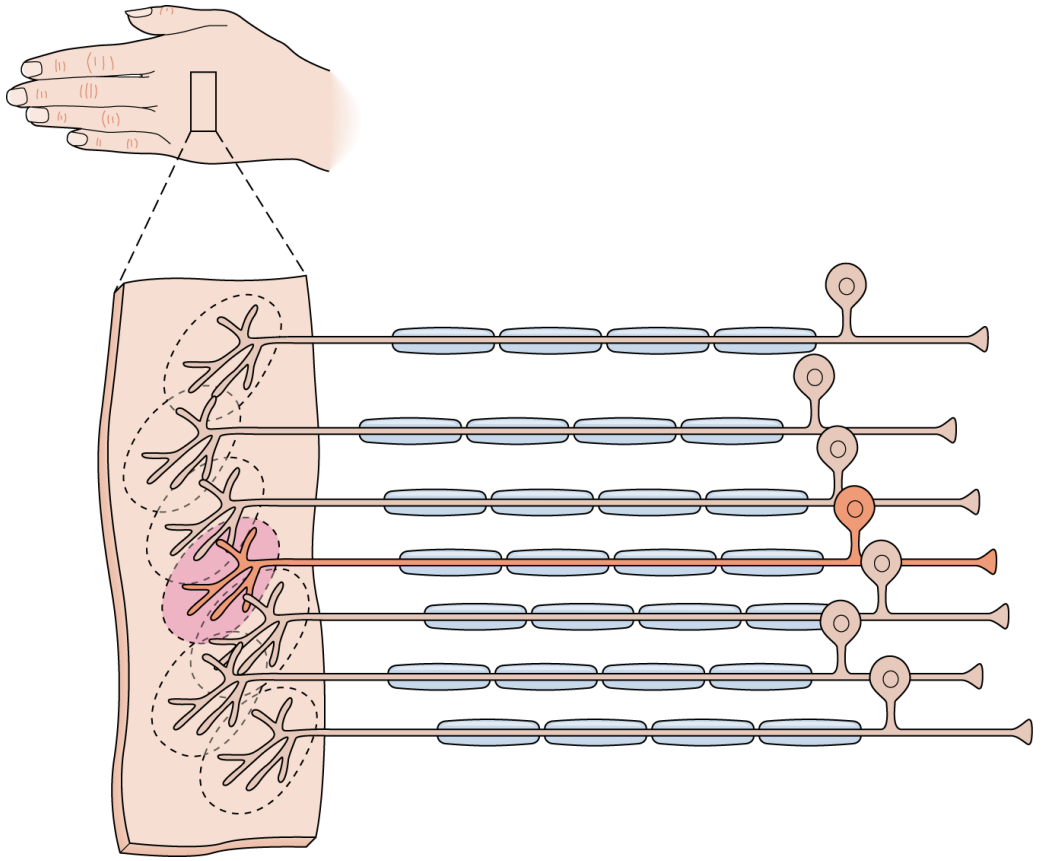


Sensory systems

What are the primary attributes of sensory stimuli?

There are four specific attributes:

- Modality
- Intensity
- Location
- Duration

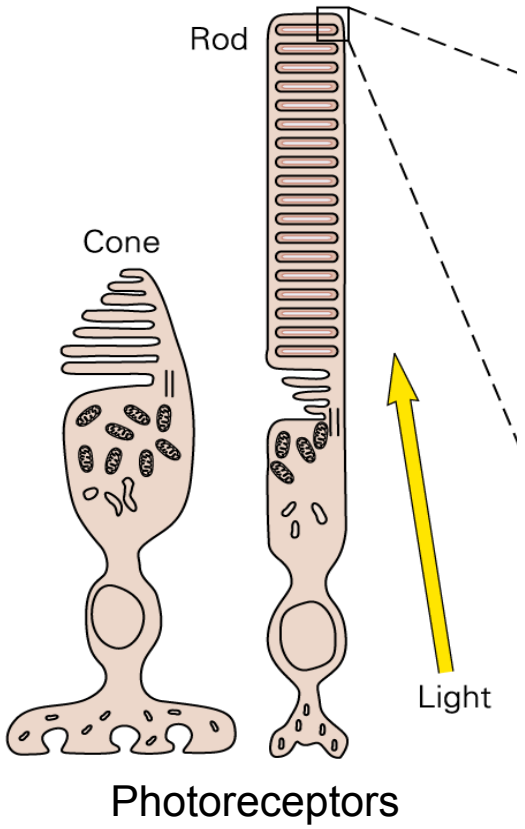


Location: translated to the brain via **receptive fields**

Sensory systems

What are some additional attributes of sensory stimuli?

- Receptor cell specialization
  - Unique cell type
  - Compartmentalization
  - Sensitivity to specific stimuli or energy
- Transduction
- Receptive Fields
- Adaptation and Habituation



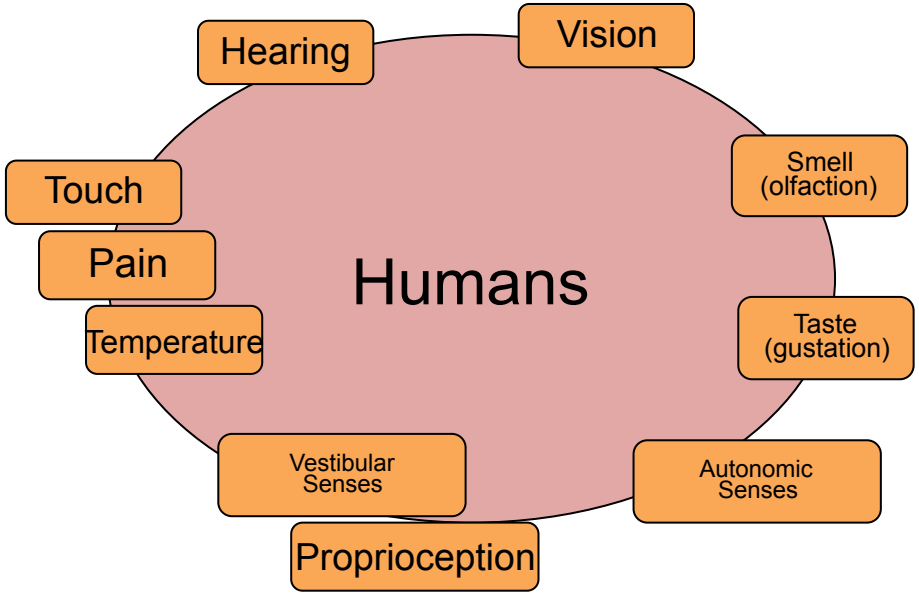
Sensory systems

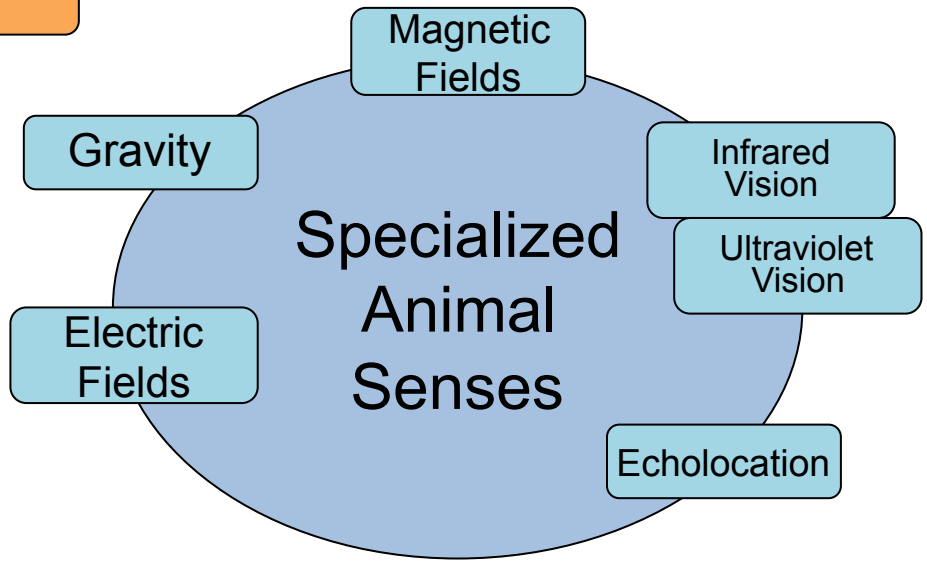
What are the requirements of a sensory system?

1. Needs to have different receptors to discriminate among different forms of energy
2. Needs to discriminate between different intensities of stimulation
3. Needs to respond reliably
4. Needs to respond rapidly
5. Needs to suppress extraneous information

Sensory systems

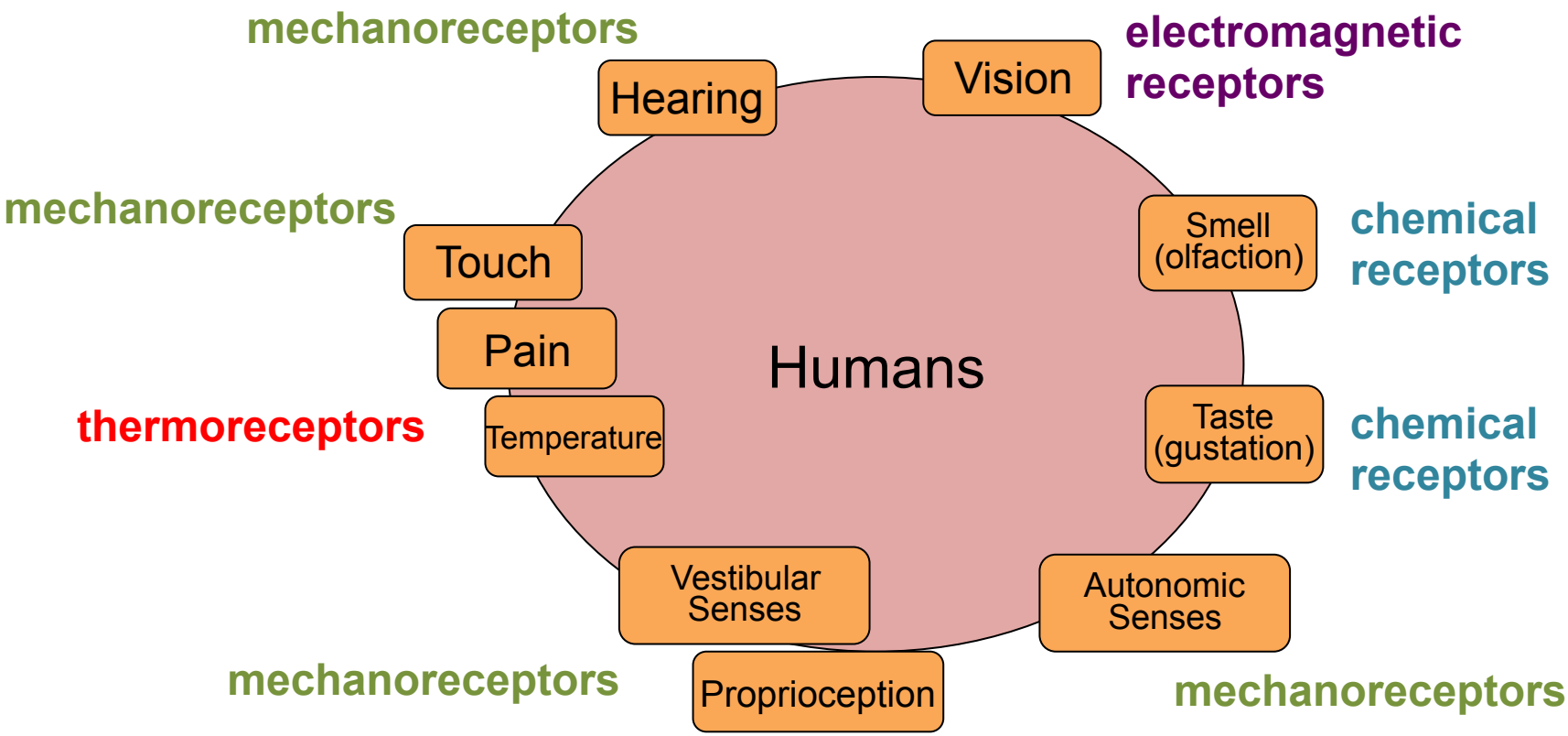
What are different types of senses?





Sensory systems

How can senses be divided into classes?

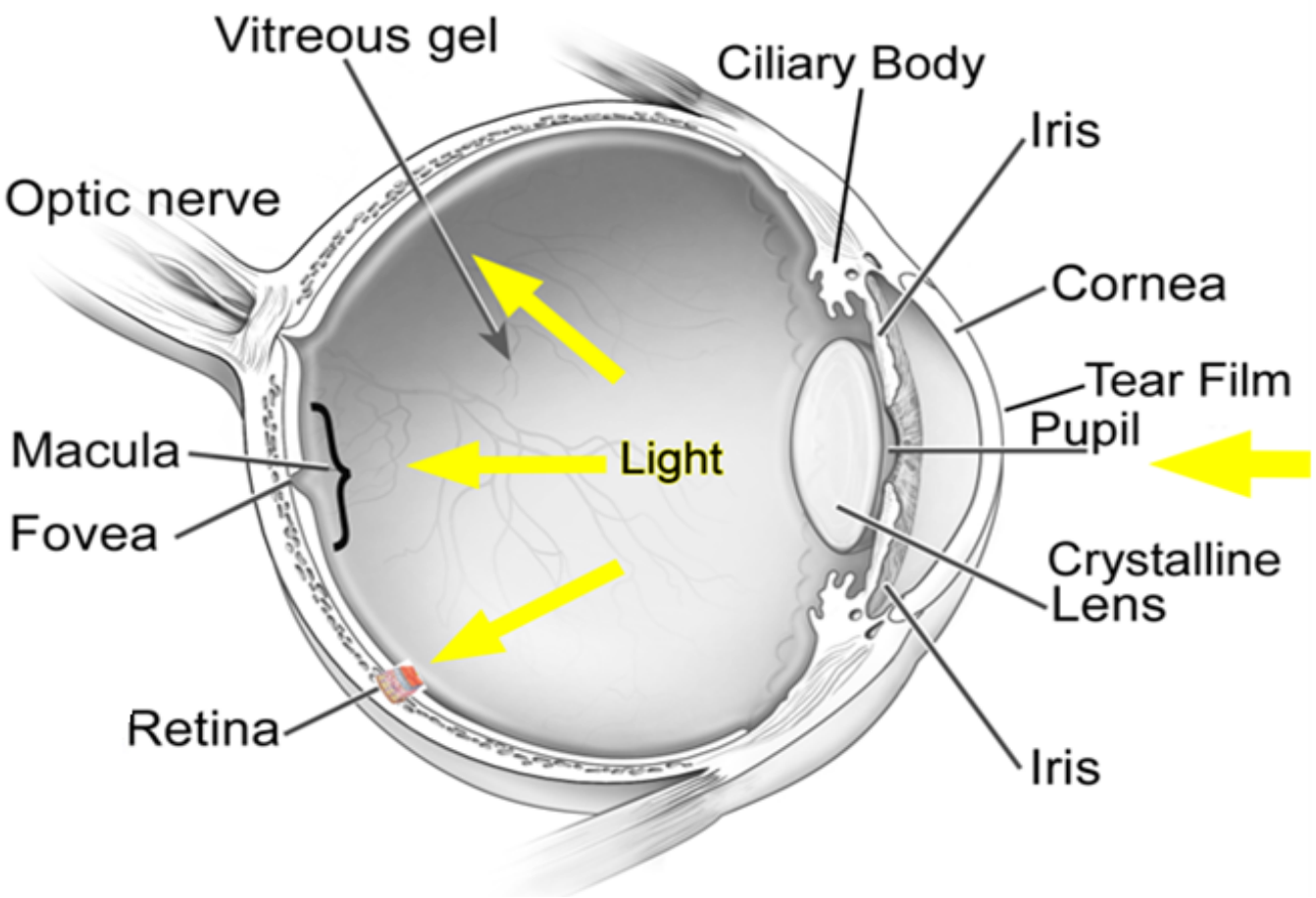


Visual System

Agenda:

1. The Eye
2. The Retina
3. Visual Transduction by Rhodopsin
4. Information From Retina to Primary Visual Cortex

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

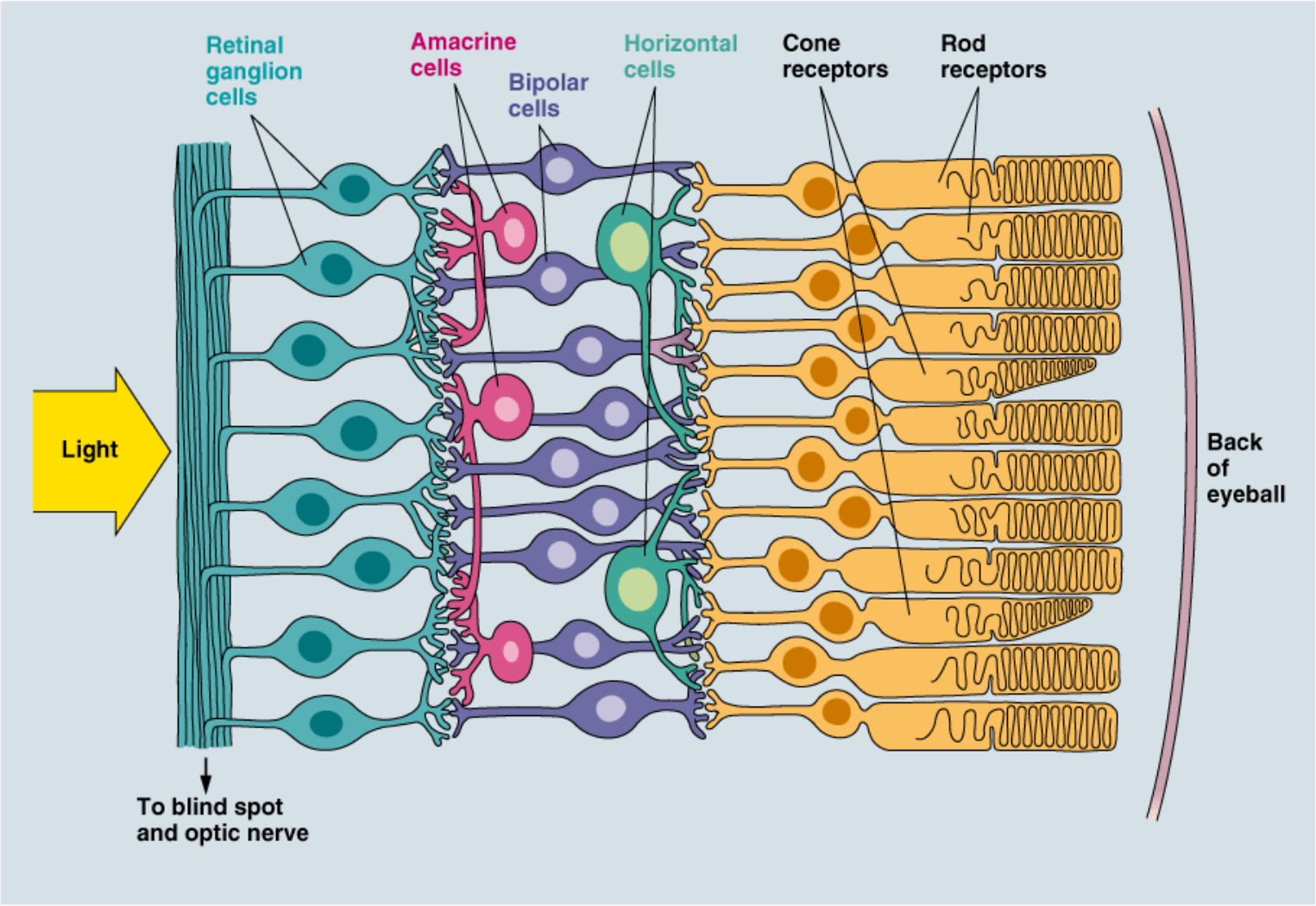
Binocular Disparity: Most mammals have two eyes on the front of their heads, rather than one on each side.

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

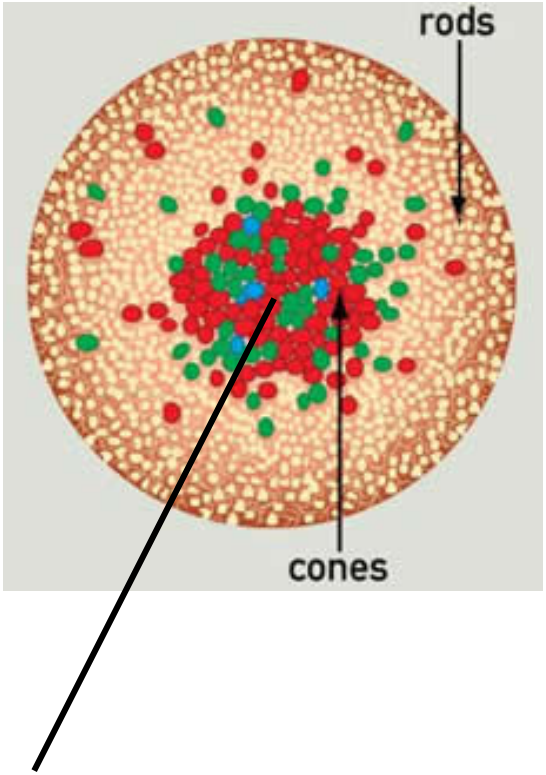
Monocular clues about size and shape are used in perceiving depth.

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

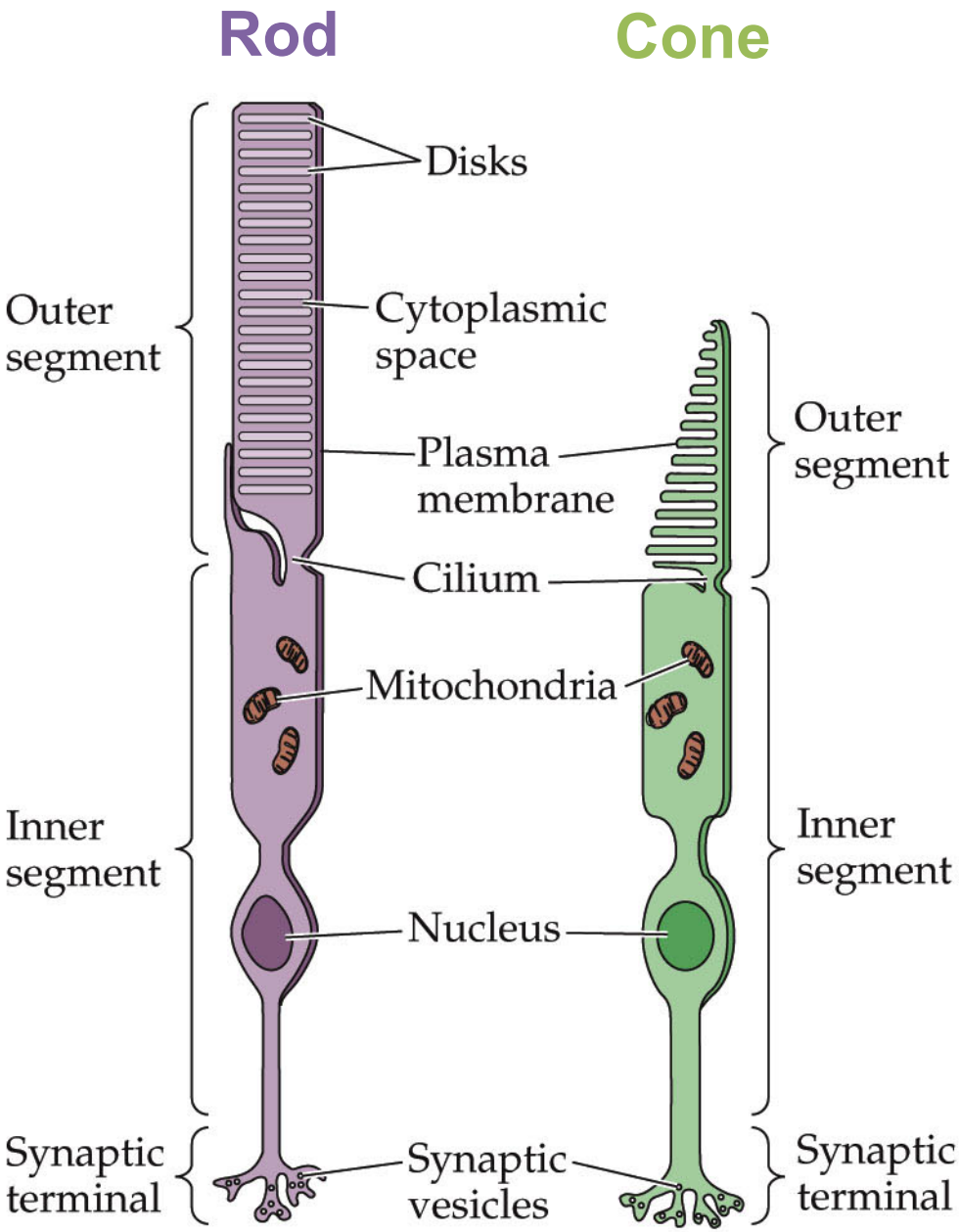
The optic disk has no receptors, creating a blind spot.

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:

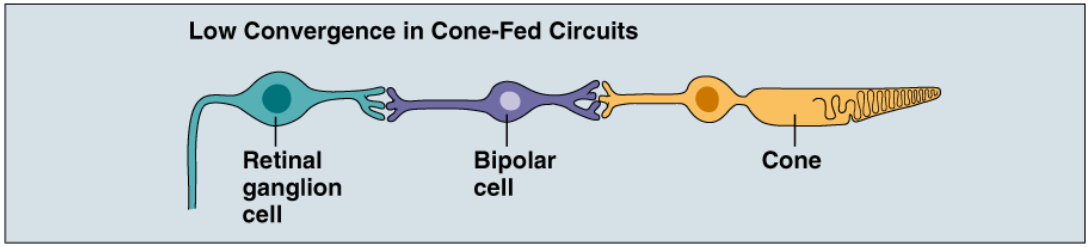
1. **Photopic System**

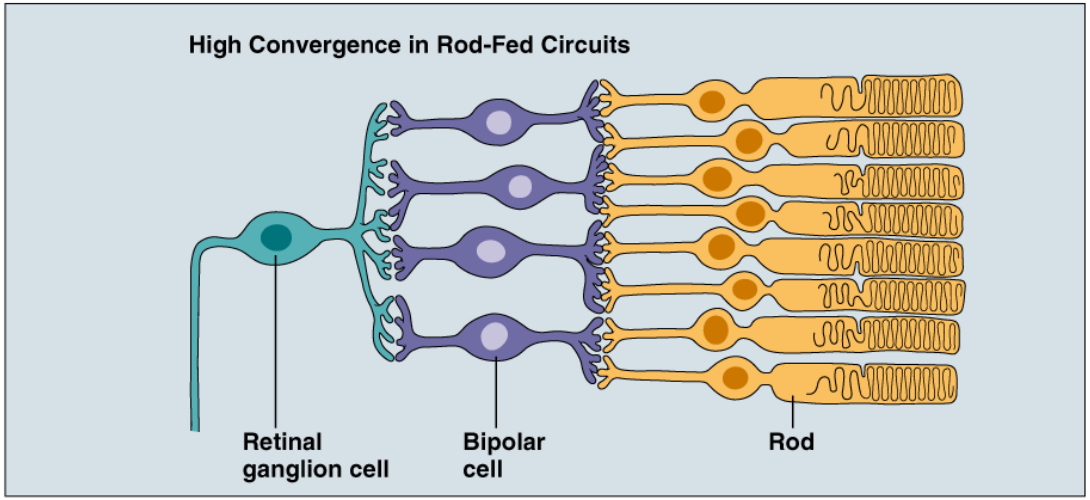
Functions in lighted conditions for high-acuity vision (Cones)

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.

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.

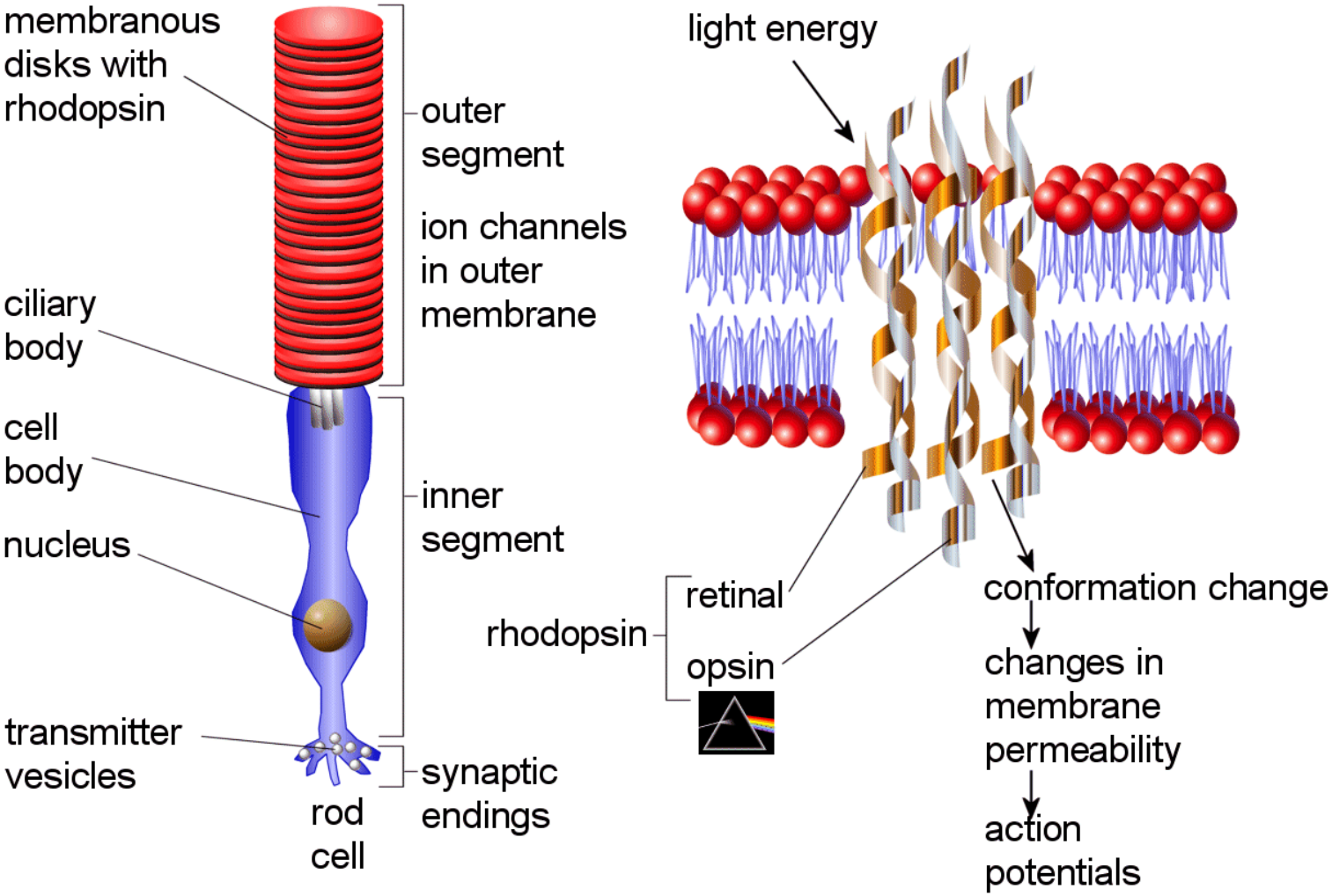
Why does bleaching occur?

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

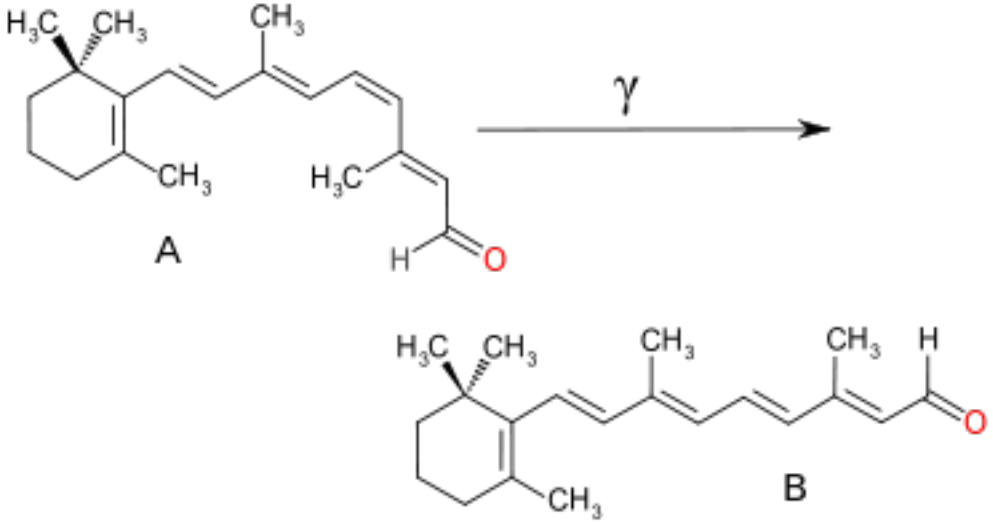
What happens when rhodopsin is totally bleached?

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



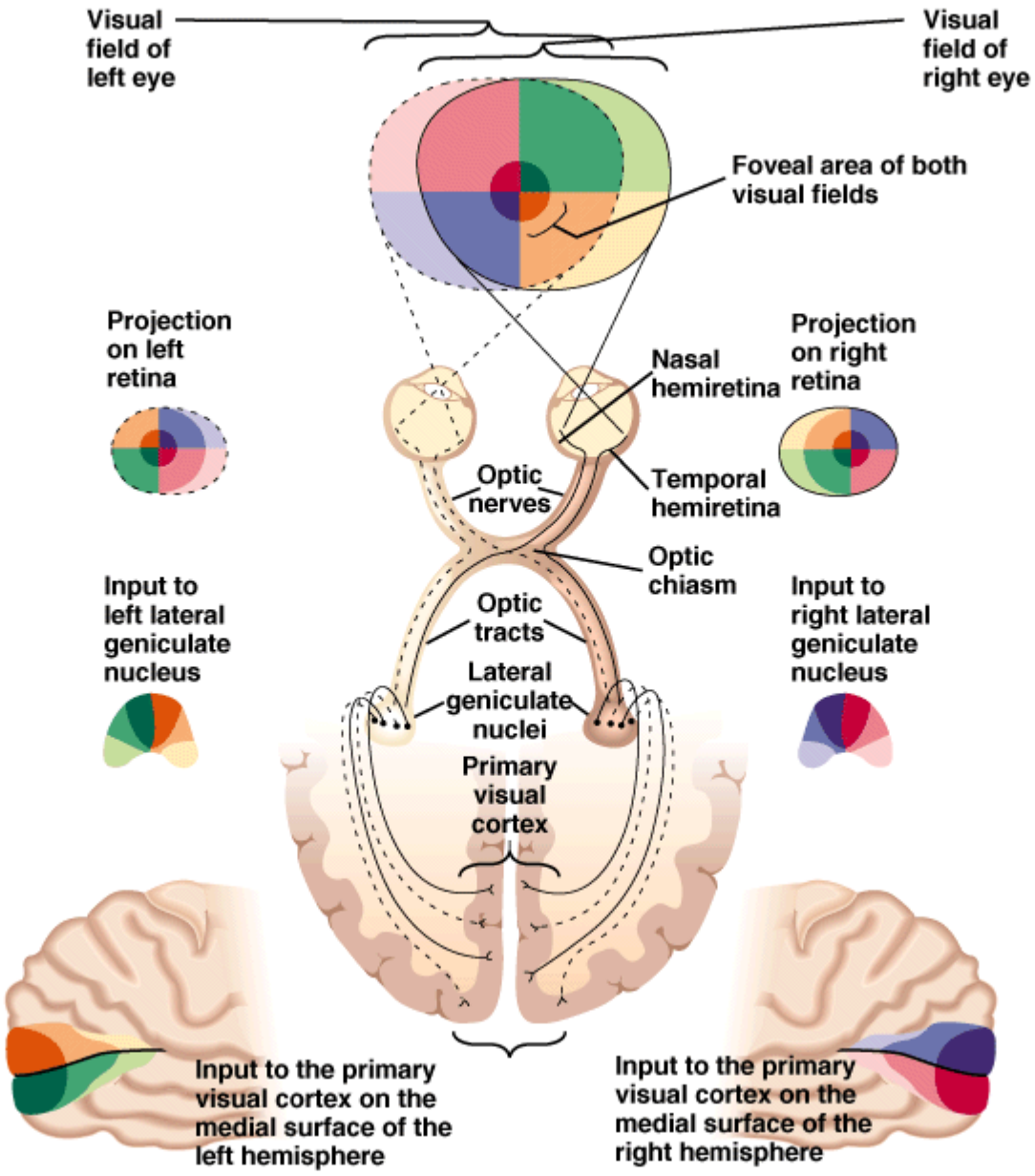
Rhodopsin: G-Protein Linked Receptor (GPCR) that responds to light by initiating a cascade of intra-cellular chemical events

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:

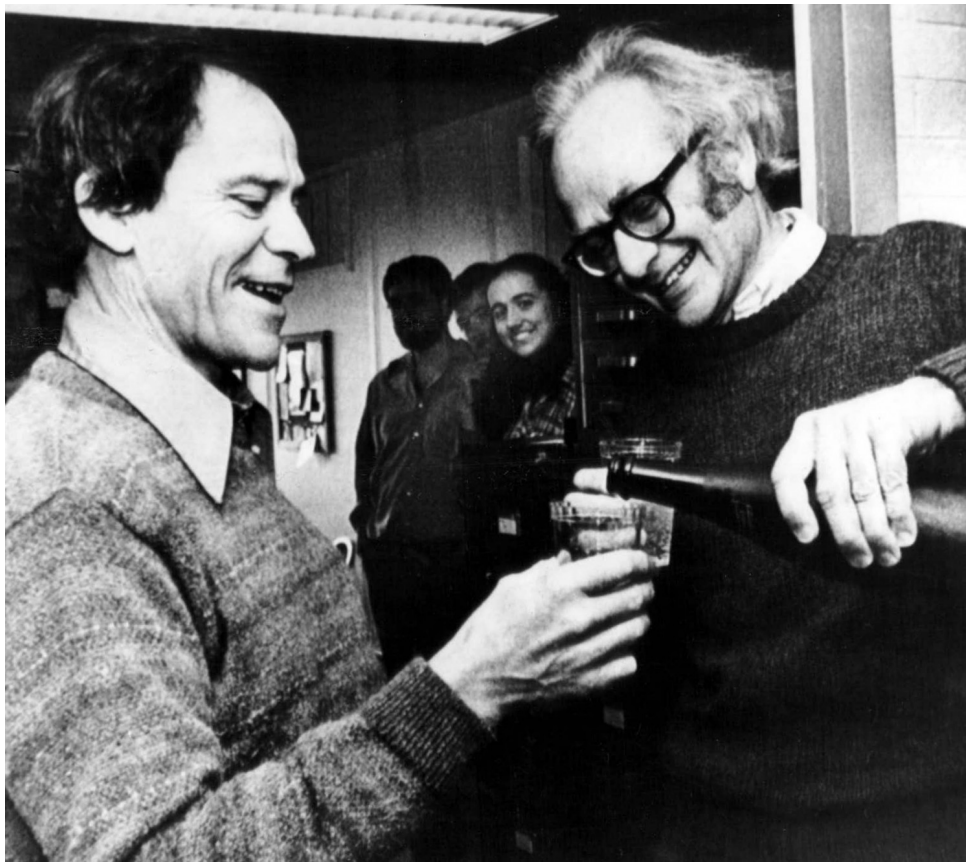
1. **Parvo-cellular Layers (P Pathway)**

   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.

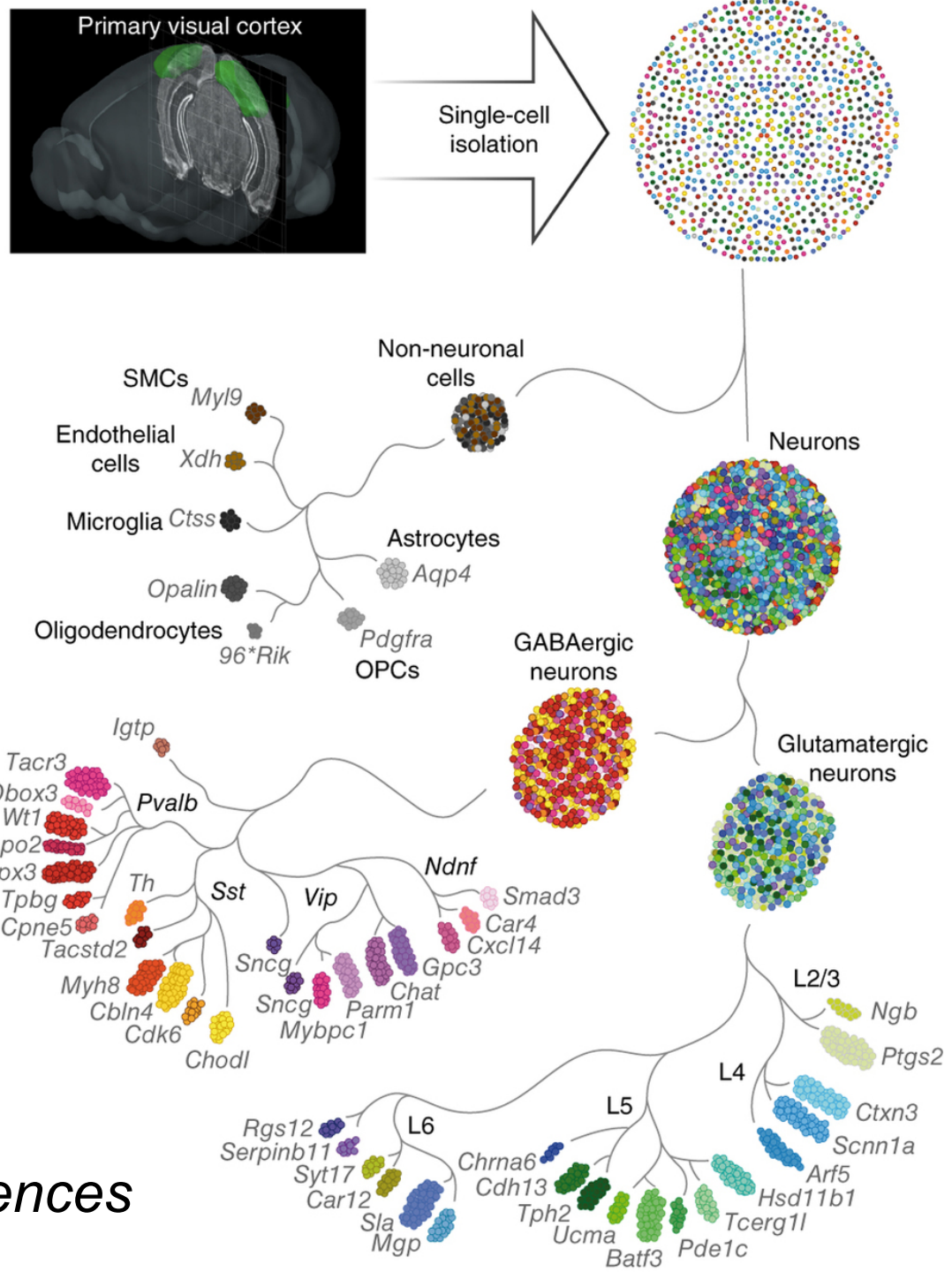
The Neural Basis of Perception



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.

Classification of Cortical Cells in Visual System

1. Single-Cell Gene Expression Profiling through use of Transgenic Mouse Lines
2. Unsupervised Cluster Analysis of Genetic Data



Allen Institute of Brain Sciences

Auditory System

Objective: Understand how the auditory system works

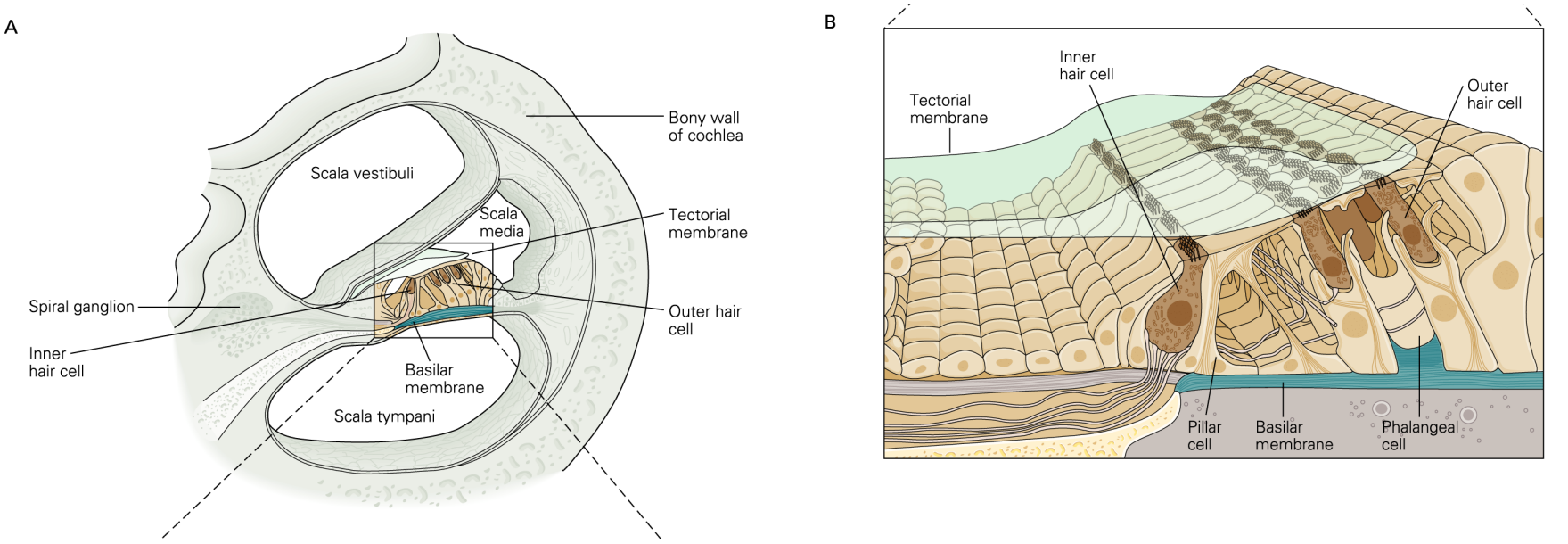
Agenda:

1. Structure and function
   - ❖ Cochlear implants
2. Pathways
   - ❖ Drosophila courtship case study

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

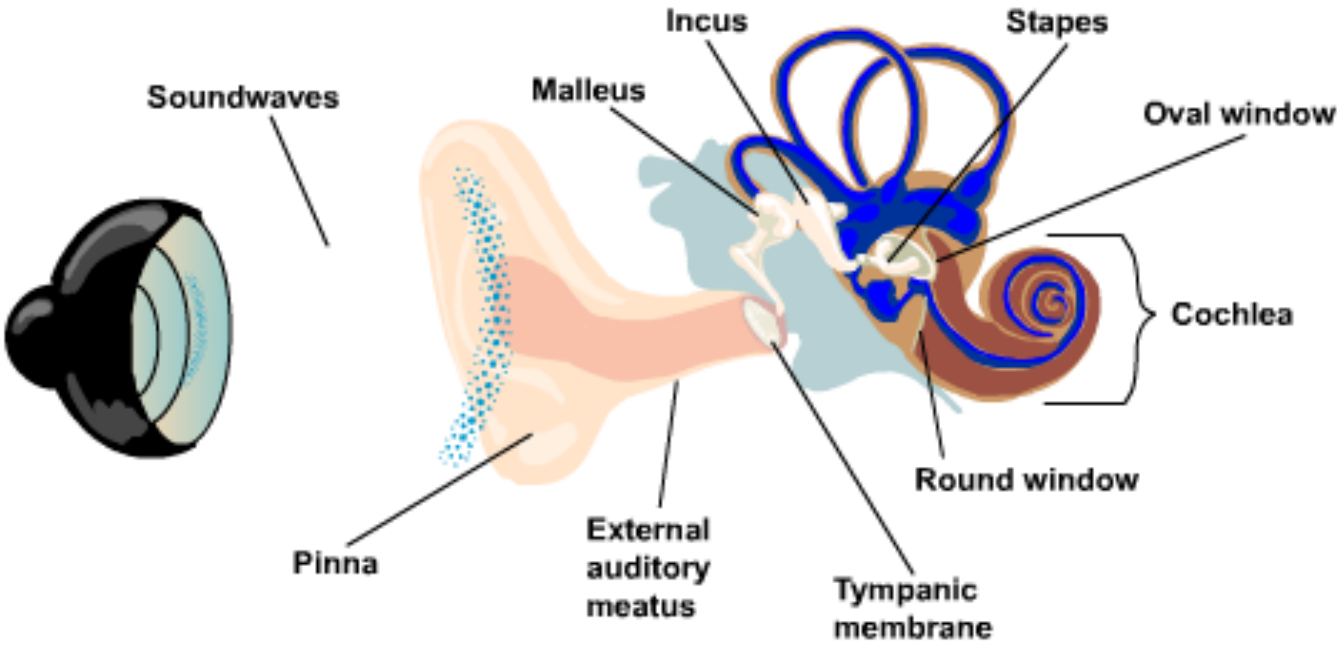


Auditory system

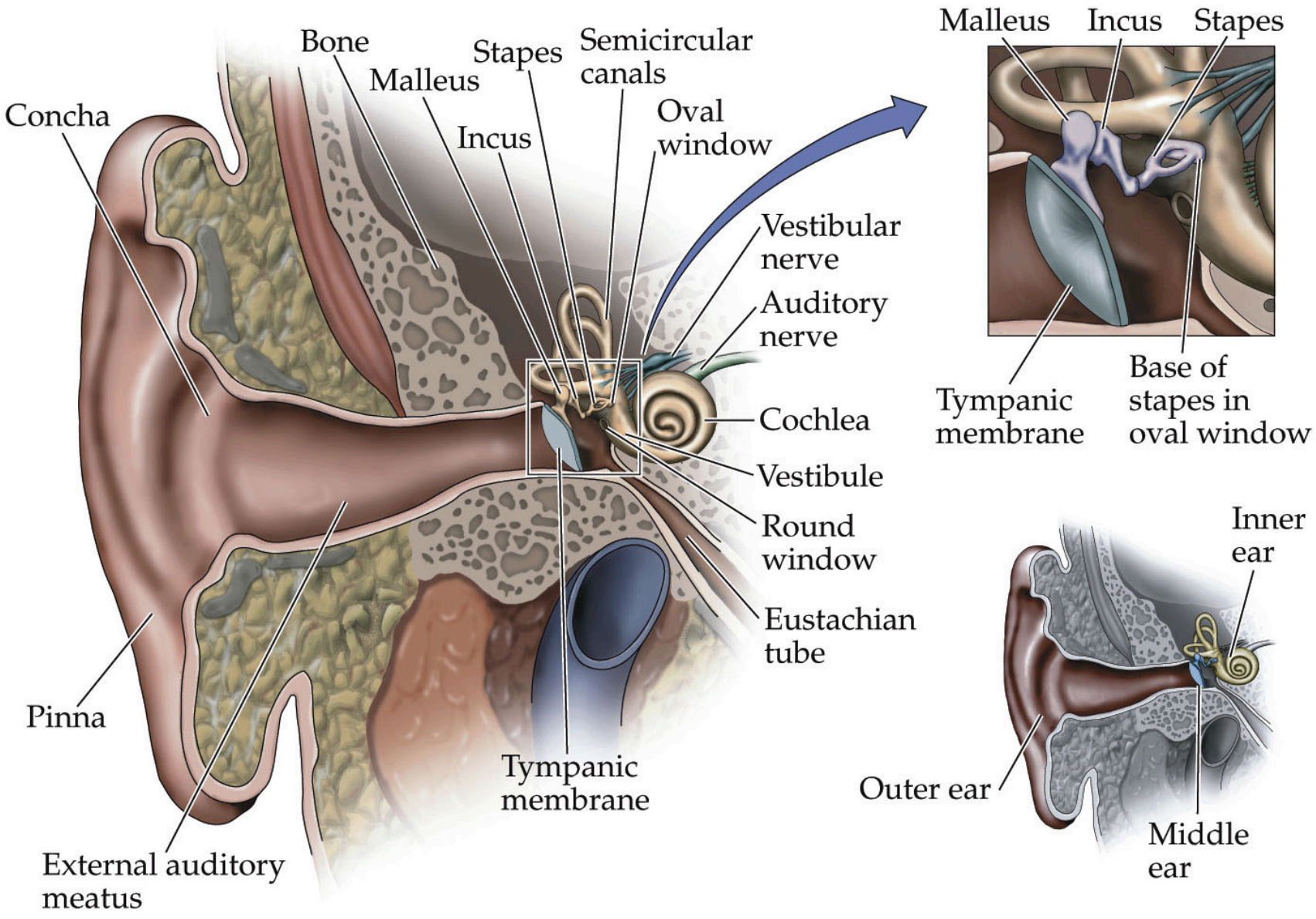
What is sound?

1. Sound is a series of pressure changes in the air
2. It can vary in frequency and intensity over time

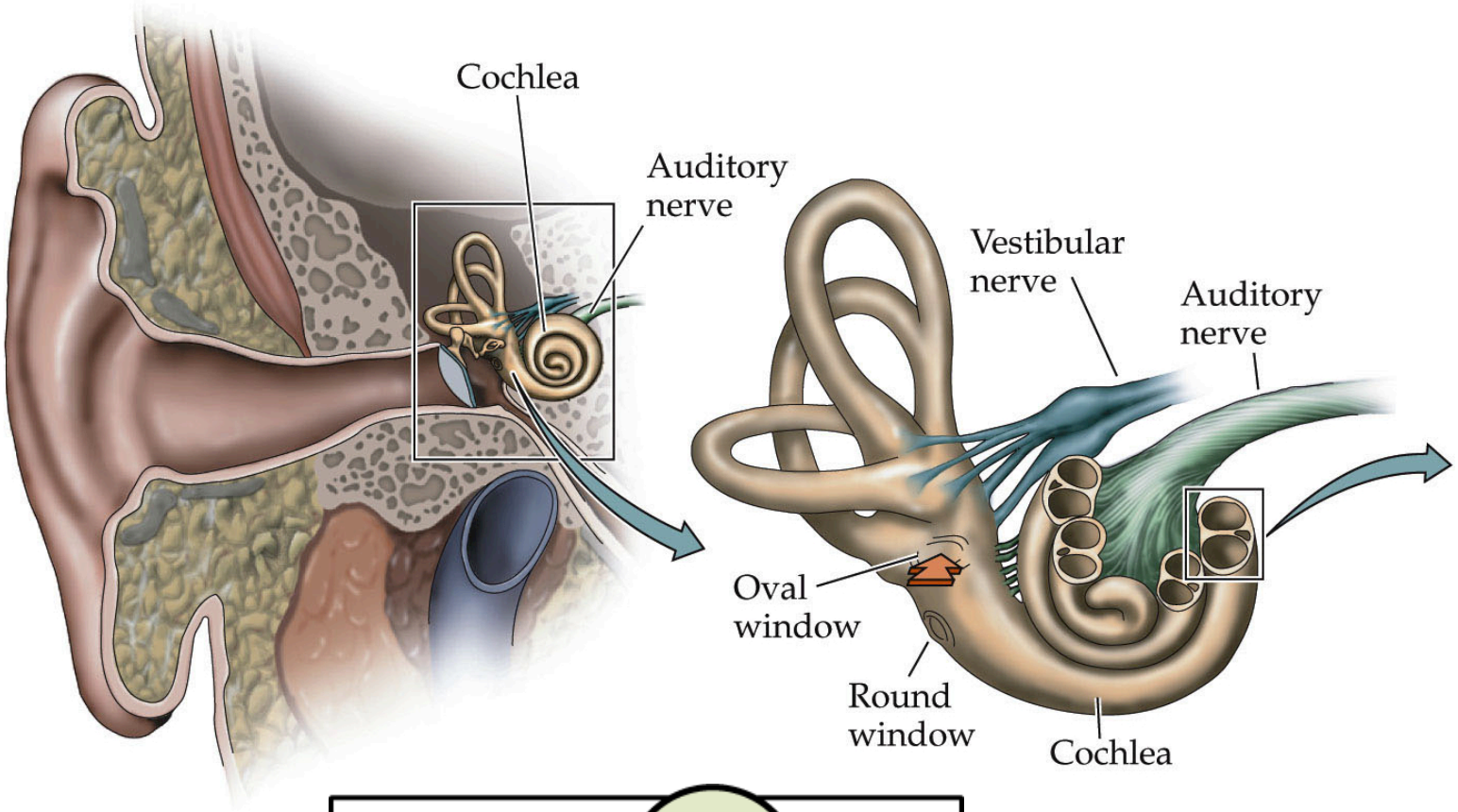
How is it processed?

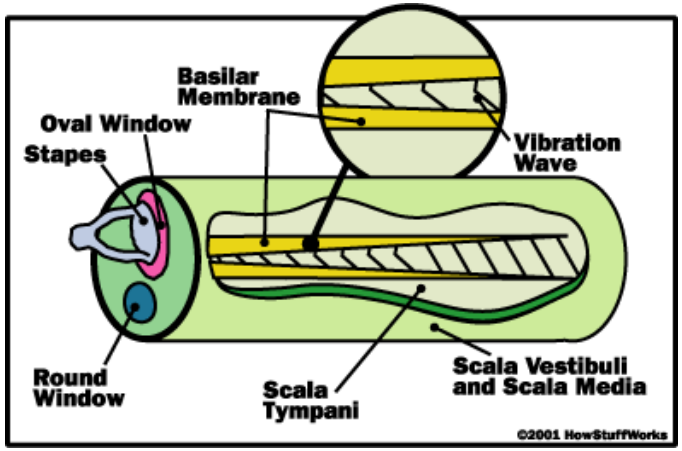


Human ear


Concha
Bone
Malleus
Stapes
Semicircular canals
Incus
Oval window
Vestibular nerve
Auditory nerve
Cochlea
Vestibule
Round window
Eustachian tube
Pinna
Tympanic membrane
External auditory meatus
Malleus
Incus
Stapes
Tympanic membrane
Base of stapes in oval window
Inner ear
Outer ear
Middle ear

Cochlea


Cochlea
Auditory nerve
Vestibular nerve
Auditory nerve
Oval window
Round window
Cochlea


Basilar Membrane
Oval Window
Stapes
Vibration Wave
Round Window
Scala Tympani
Scala Vestibuli and Scala Media
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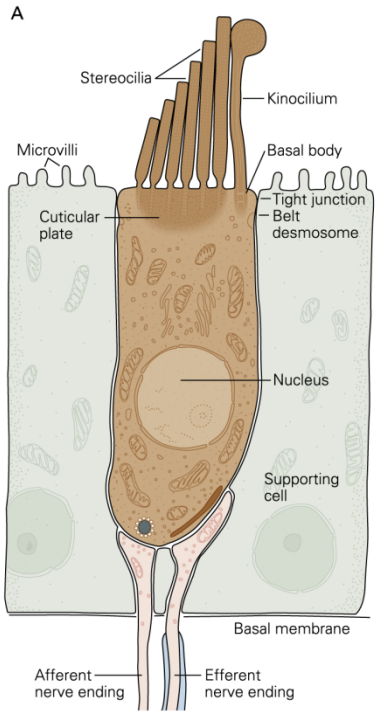
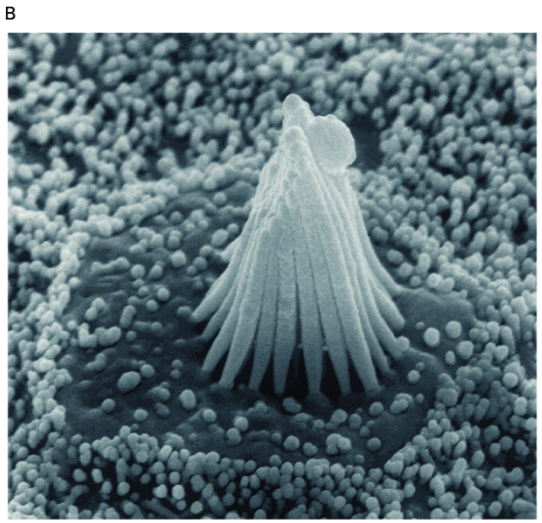
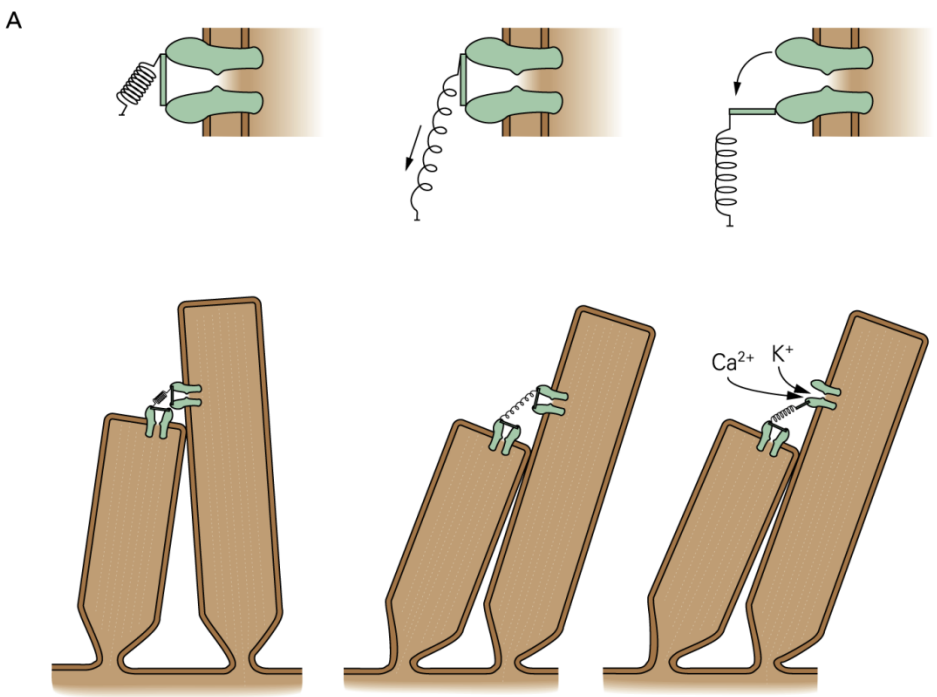
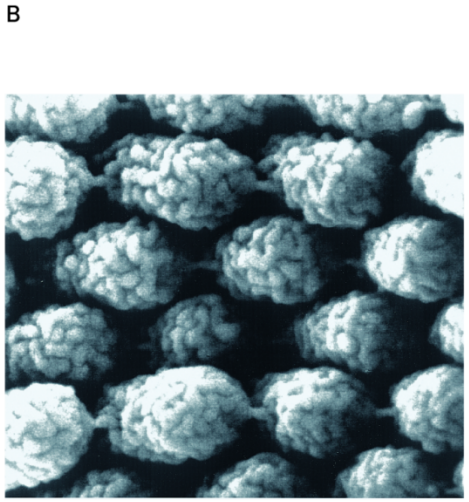
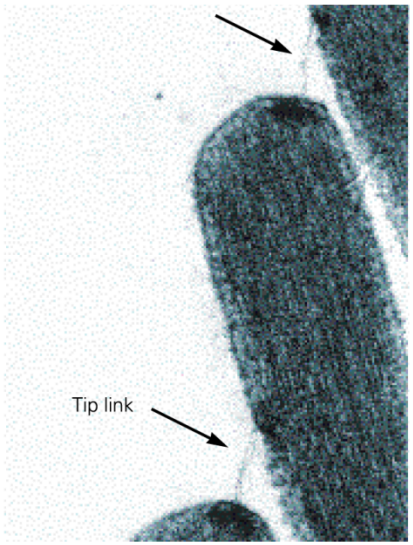
Cochlear hair cells



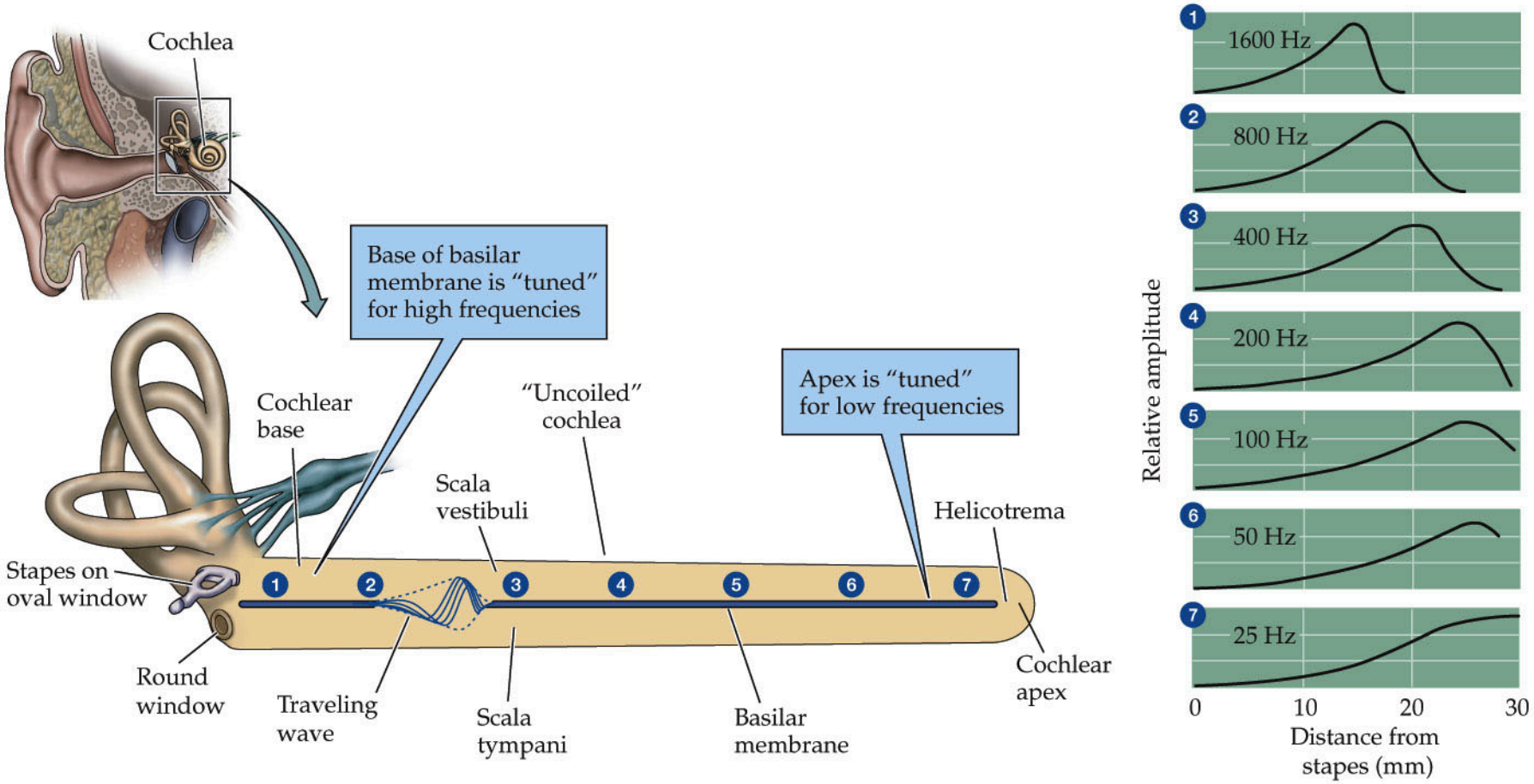




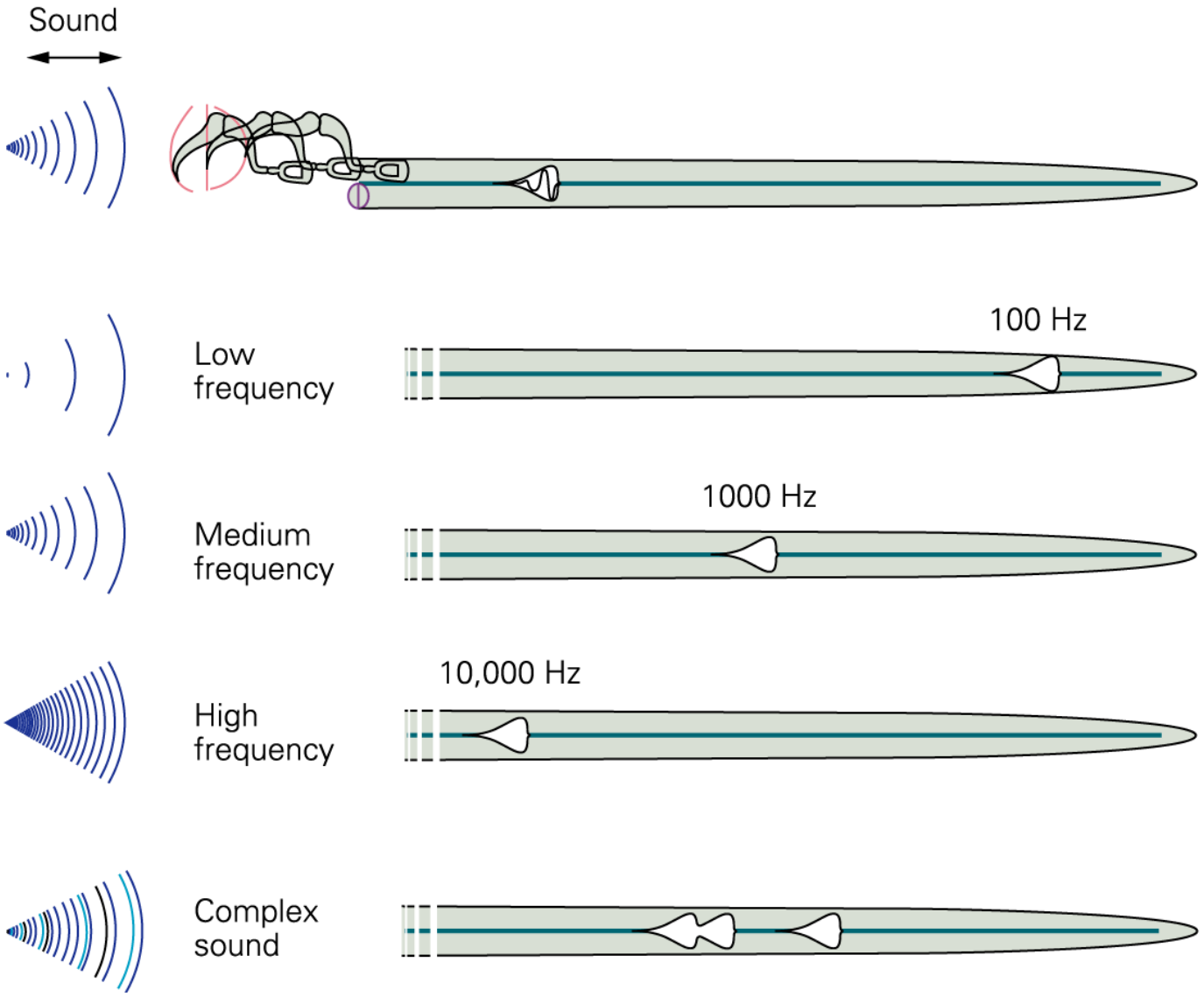




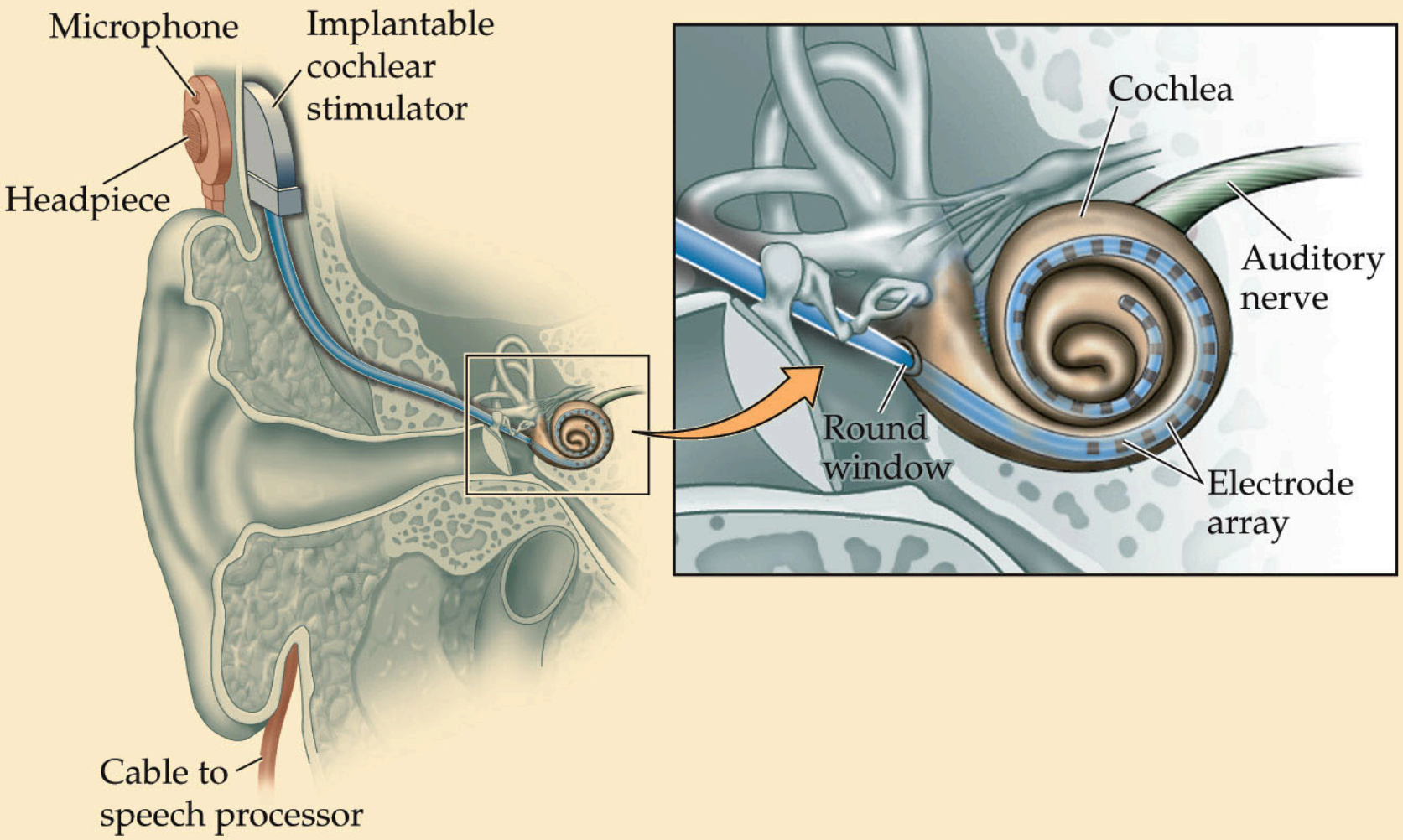
Signal transduction in the cochlea



Frequency differences



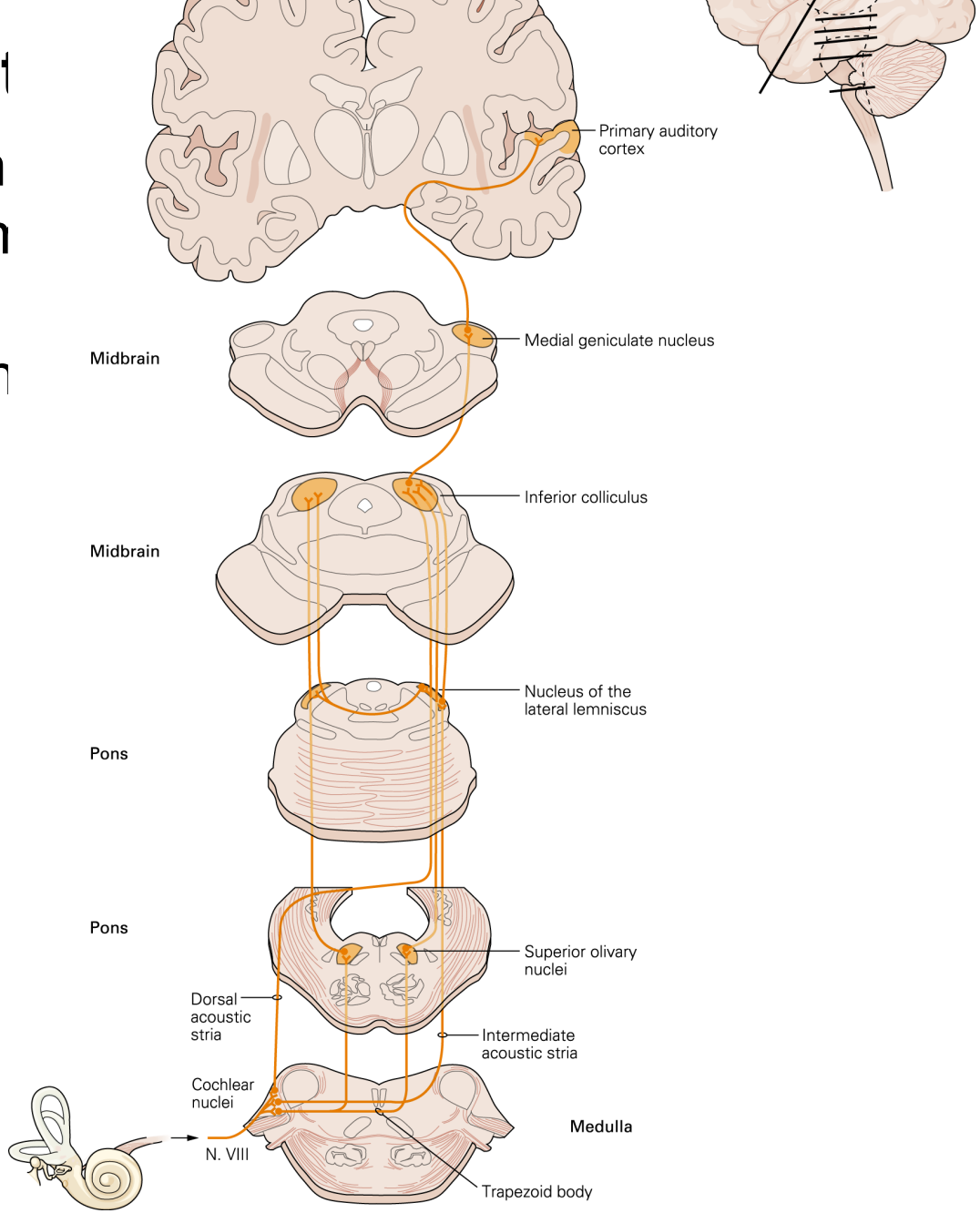
Application: cochlear implants



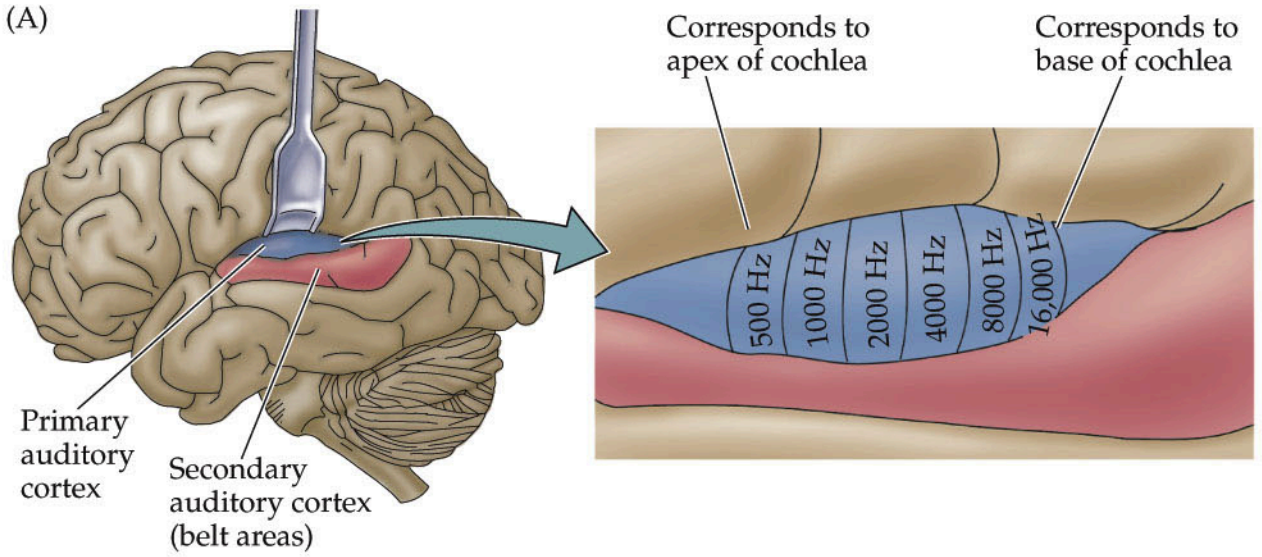
Auditory syst

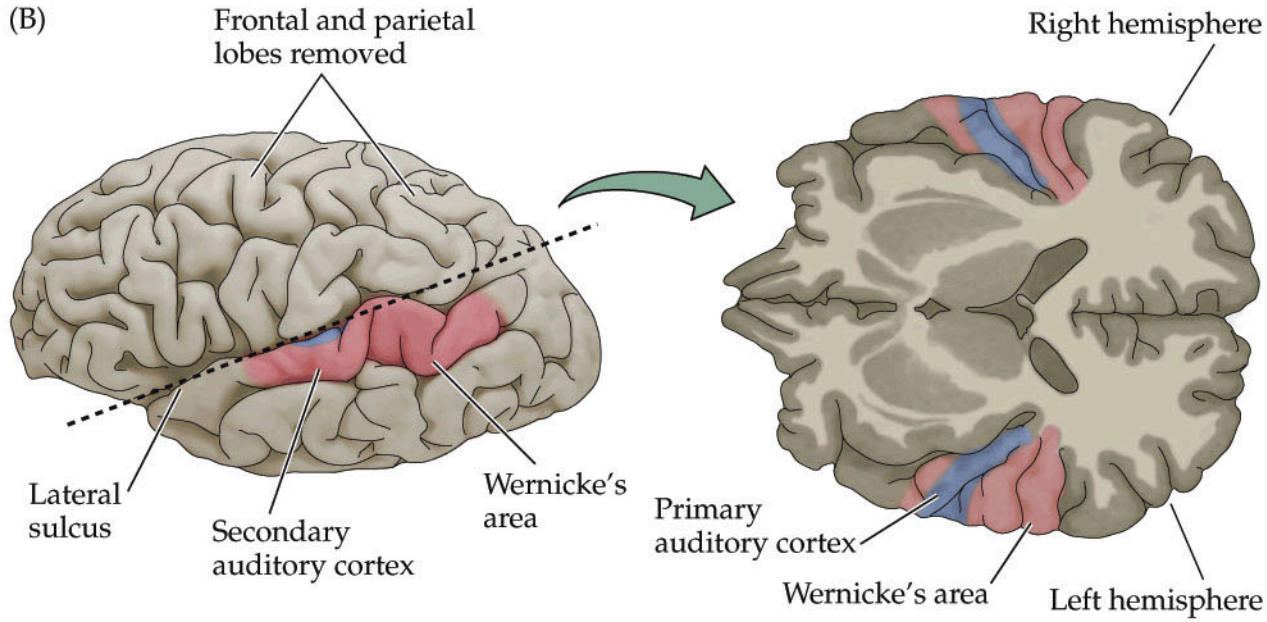
Connections in
auditory systen

Here is the gen
organization:

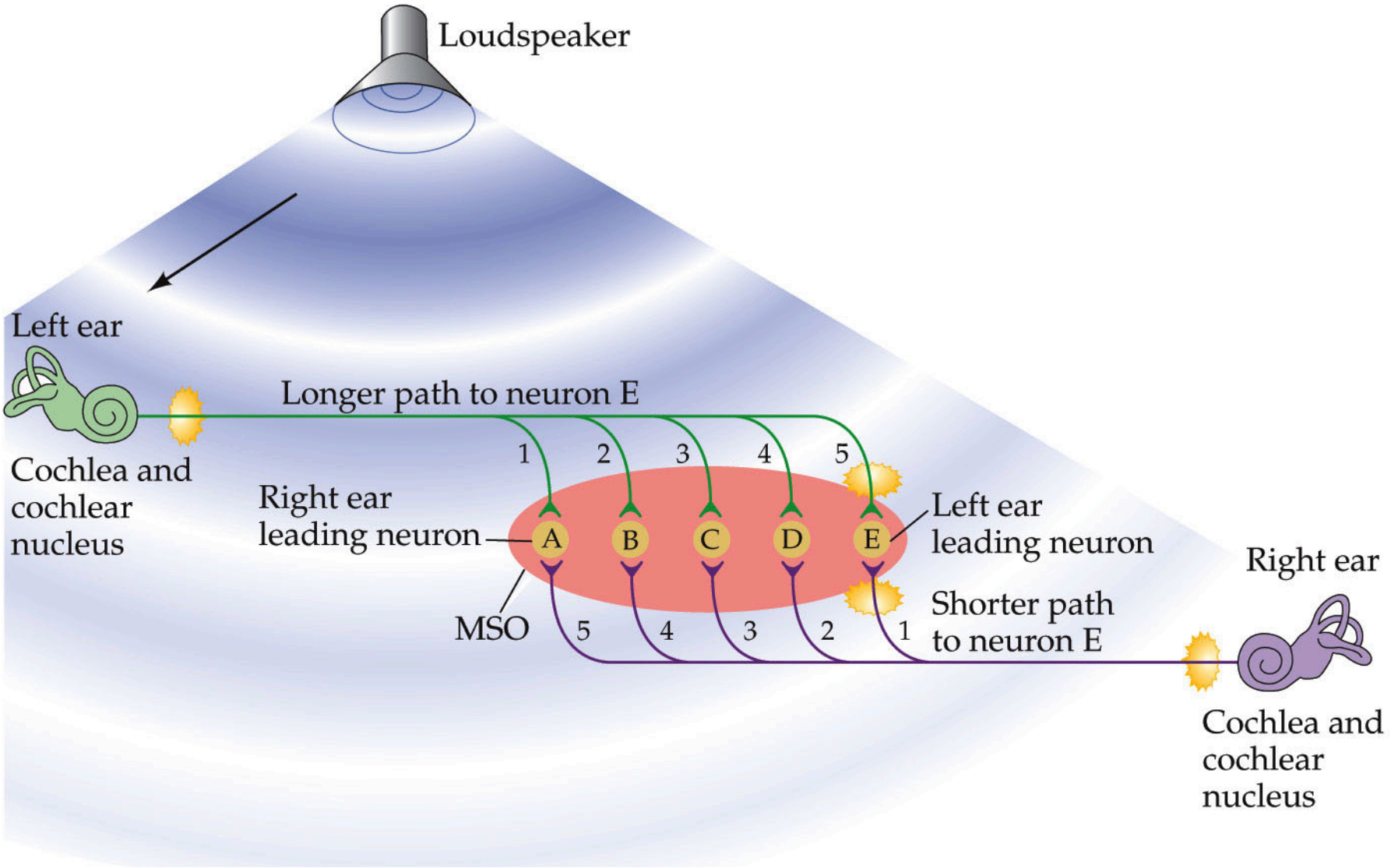


Sound in the cortex





Timing and sound localization



Drosophila courtship case study

Vol 458|12 March 2009|doi:10.1038/nature07810 nature

ARTICLES

The neural basis of *Drosophila* gravity-sensing and hearing

Azusa Kamikouchi[1,2,3]*, Hidehiko K. Inagaki[2]*†, Thomas Effertz[1,4], Oliver Hendrich[1,4], André Fiala[4,5], Martin C. Göpfert[1,4] & Kei Ito[2]

The neural substrates that the fruitfly *Drosophila* uses to sense smell, taste and light share marked structural and functional similarities with ours, providing attractive models to dissect sensory stimulus processing. Here we focus on two of the remaining and less understood prime sensory modalities: graviception and hearing. We show that the fly has implemented both sensory modalities into a single system, Johnston's organ, which houses specialized clusters of mechanosensory neurons, each of which monitors specific movements of the antenna. Gravity- and sound-sensitive neurons differ in their response characteristics, and only the latter express the candidate mechanotransducer channel NompC. The two neural subsets also differ in their central projections, feeding into neural pathways that are reminiscent of the vestibular and auditory pathways in our brain. By establishing the *Drosophila* counterparts of these sensory systems, our findings provide the basis for a systematic functional and molecular dissection of how different mechanosensory stimuli are detected and processed.

The fruitfly *Drosophila melanogaster* responds behaviourally to gravity and sound. When tapped down in a vial, the flies tend to walk up against the Earth's gravitational field, a directed behaviour that is known as negative gravitaxis or anti-geotaxis[1–4]. When exposed to male courtship songs, females reduce locomotion whereas males start chasing each other, forming so-called courtship chains[4,5]. Both *Drosophila* gravitaxis and sound communication have long been prime paradigms for the genetic dissection of behaviour[1–5], but the underlying sensory mechanisms are poorly understood. The human ability to sense gravity and sound relies on specialized vestibular and auditory organs in our inner ear[6,7]. In the fly, the ability to hear has been ascribed to the antenna[5,8–14]: the club-shaped third segment and the distal arista (formed by the fourth to sixth segments) of the antenna sympathetically vibrate in response to acoustic stimuli and, analogous to our eardrum, serve the reception of sound[12,14]. Vibrations of this antennal receiver are picked up by Johnston's organ (JO), a chordotonal stretch-receptor organ with ~480 primary sensory neurons in the second segment of the antenna (Fig. 1a). These JO neurons have also been surmised to have a role in gravity sensing[2,15]. The antennal receiver of the fly is predicted to deflect in response to gravitational forces (see Supplementary Information footnote 1), but physiological evidence exploring the role of JO neurons in gravity sensing has not been reported so far.

Here we examine the role of *Drosophila* JO neurons in gravity and sound detection. It has been shown that the JO neurons of the fly can be anatomically categorized into five subgroups, A–E, each of which targets a distinct area of the brain[13]. Whether this anatomical diversity is paralleled by function, however, has remained unclear[16]. We show that JO neuron subgroups are functionally specialized in that they preferentially respond to distinct types of antennal movement. We further show that this functional diversity reflects distinct behavioural requirements, with different JO neuron subgroups being needed for the response of flies to gravity and sound. These neural subgroups differ genetically and feed into distinct neural pathways in the brain. We have traced these newly identified sensory pathways and provide tools to dissect their function.

Monitoring neural activities in JO

To assess directly neural activities in *Drosophila* JO caused by the antennal receiver movement, we have developed a live fly preparation that affords access to intracellular calcium signals in JO neurons through the cuticle of the antenna (Fig. 1a, b). An intact fly was mounted under a coverslip with the first and second antennal segments immobilized to prevent muscle-based antennal movements. The antennal receiver was kept freely moving, as was confirmed by laser Doppler vibrometric measurements of their mechanical fluctuations[17]. We mechanically actuated the antennal receiver by means of electrostatic force[17–19] (Fig. 1a and Supplementary Fig. 1a), and expressed a genetically encoded calcium sensor in JO neurons via the yeast-derived *GAL4/UAS* gene expression induction system, in which expression of reporter genes fused under *UAS* is activated specifically in the cells that express Gal4 (ref. 20). To distinguish mechanically evoked calcium signals from possible movement artefacts, we used the sensor cameleon 2.1 (Cam2.1)[21,22], which allows for ratiometric measurements of calcium-induced fluorescence resonance transfer (FRET) between enhanced cyan fluorescent protein (eCFP) and enhanced yellow fluorescent protein (eYFP).

When we expressed *cam2.1* in essentially all JO neurons by means of the *F-GAL4* driver[9] (JO-all > *cam2.1*), antennal movement evoked reciprocal changes in eCFP and eYFP fluorescence (Fig. 1c). These signals were largely reduced when *cam2.1* was expressed in homozygous *nanchung* (nan^{36a}) mutants[9], but not in heterozygous controls (Supplementary Fig. 1b). Like sound-evoked potentials in the antennal nerve of flies[9], mechanically evoked calcium signals in JO neuron somata thus depend on the transient receptor potential vanilloid (TRPV) channel Nanchung, providing additional evidence for the

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Drosophila auditory system: basics

1. Began being studied in the early 1960s
2. Researchers characterized the sounds produced by males during courtship
3. Each species of *drosophila* creates a unique pattern of sounds
4. This is believed to prevent cross-species reproduction and facilitate copulation within species

More recent research

1. Researchers focused on characterizing the courtship song of various species of *drosophila*
2. They use playback to estimate the behavioral preference for a song
3. Researchers also study which genes are important for hearing

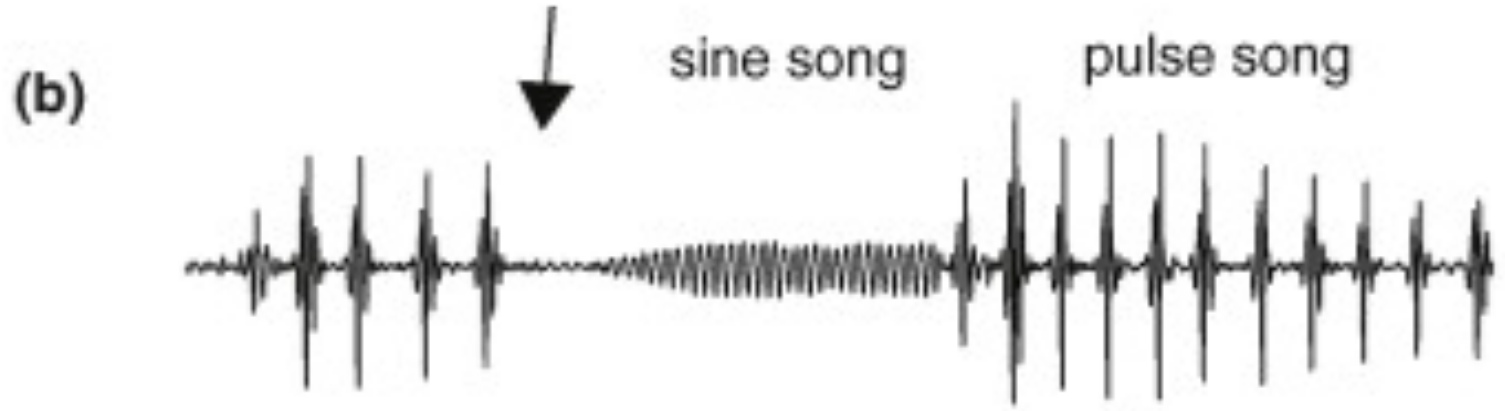
This paper wants to study the mechanisms controlling the production and perception of courtship song.

Why study the fly auditory system?

1. The major acoustic stimulus flies respond to is courtship song
2. Auditory behaviors like courtship and mating are extremely robust in flies
3. Neurons that generate song patterns may be involved in detecting the same signals

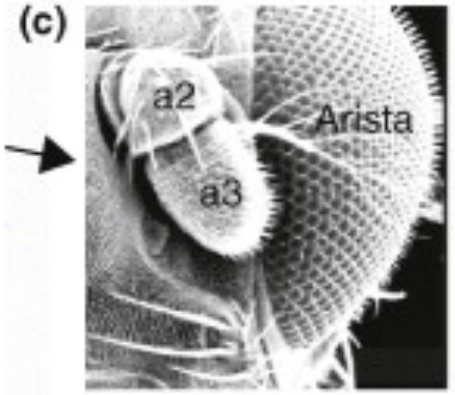
Understanding courtship song

Drosophila courtship songs are made up of two parts: pulse and sinusoidal, and are produced by wing vibration

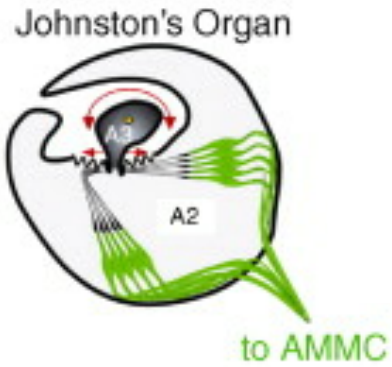


Components of hearing in *drosophila*

1. Antenna
   - Multifunctional: detects odor molecules and can respond to mechanical stimulation
     - This is done using the arista (branched structure that protrudes off the third segment of the antenna)



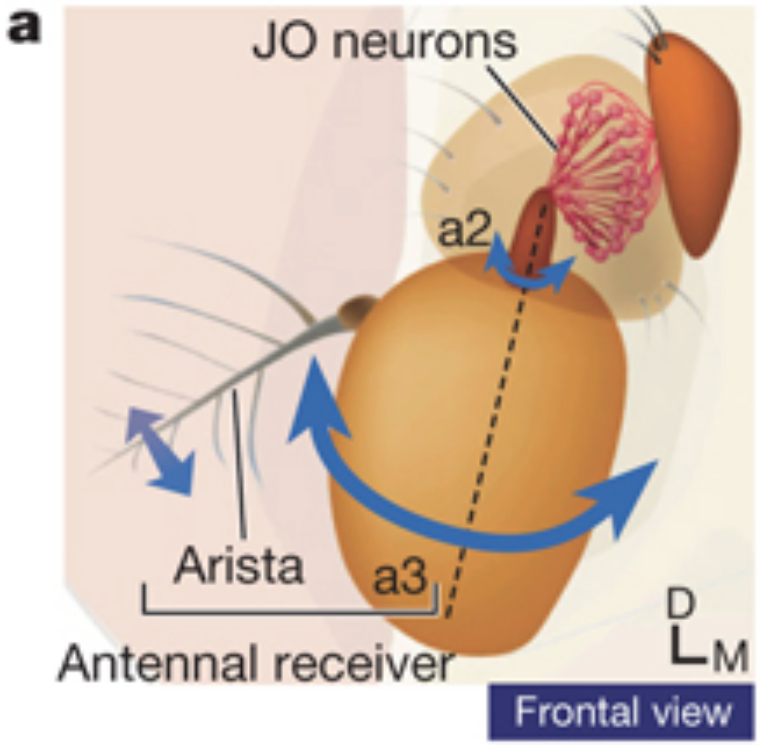
2. Johnston's Organ
   - Made up of ~480 neurons that project along the antennal nerve to the AMMC

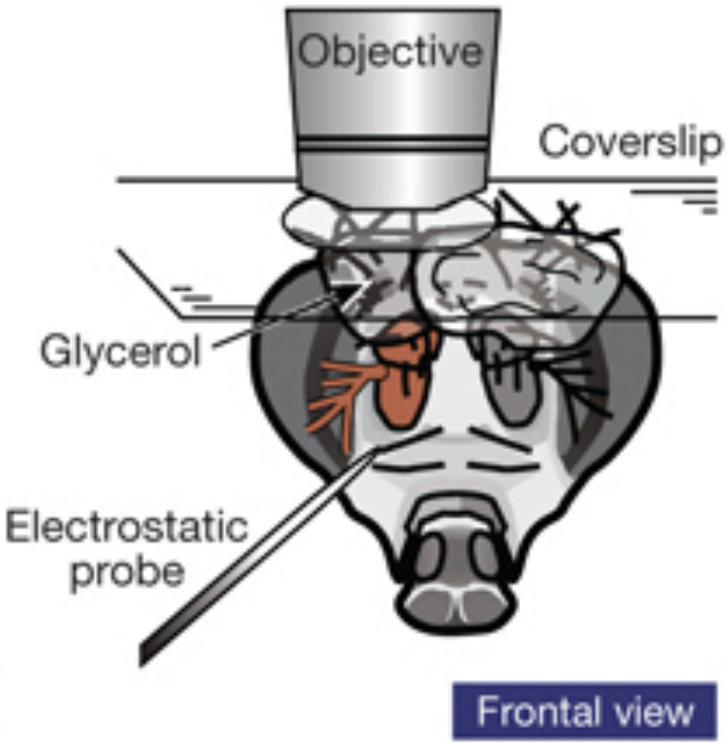


3. AMMC
   1. JO neurons can be subdivided into five groups that target direct zones of the AMMC
   2. Each JO neurons typically innervates one zone of AMMC

Purpose of study

1. It is unclear whether anatomic divisions of the Johnston's Organ (JO) was paralleled by function
2. This study shows that JO subgroups and functionally specialized since they respond to distinct types of antennal movement





Scientific approach

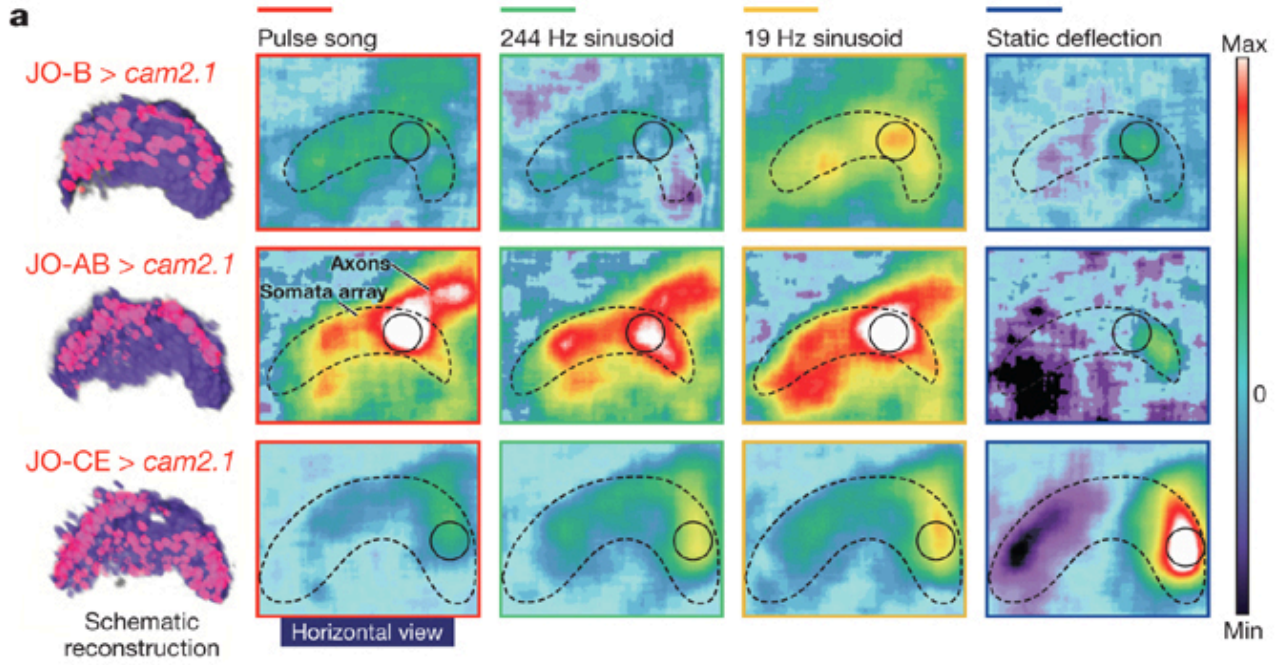
1. Want to test sound (frequency) and gravity perception
   - Sinusoidal and pulse frequencies for courtship song
   - Forward and backward static deflection to mimic effect of gravity

2. Identify candidate organ, genes and neurons to study
   - Since the JO contains 5 separate parts, they want to understand whether physical separateness confers functional distinction

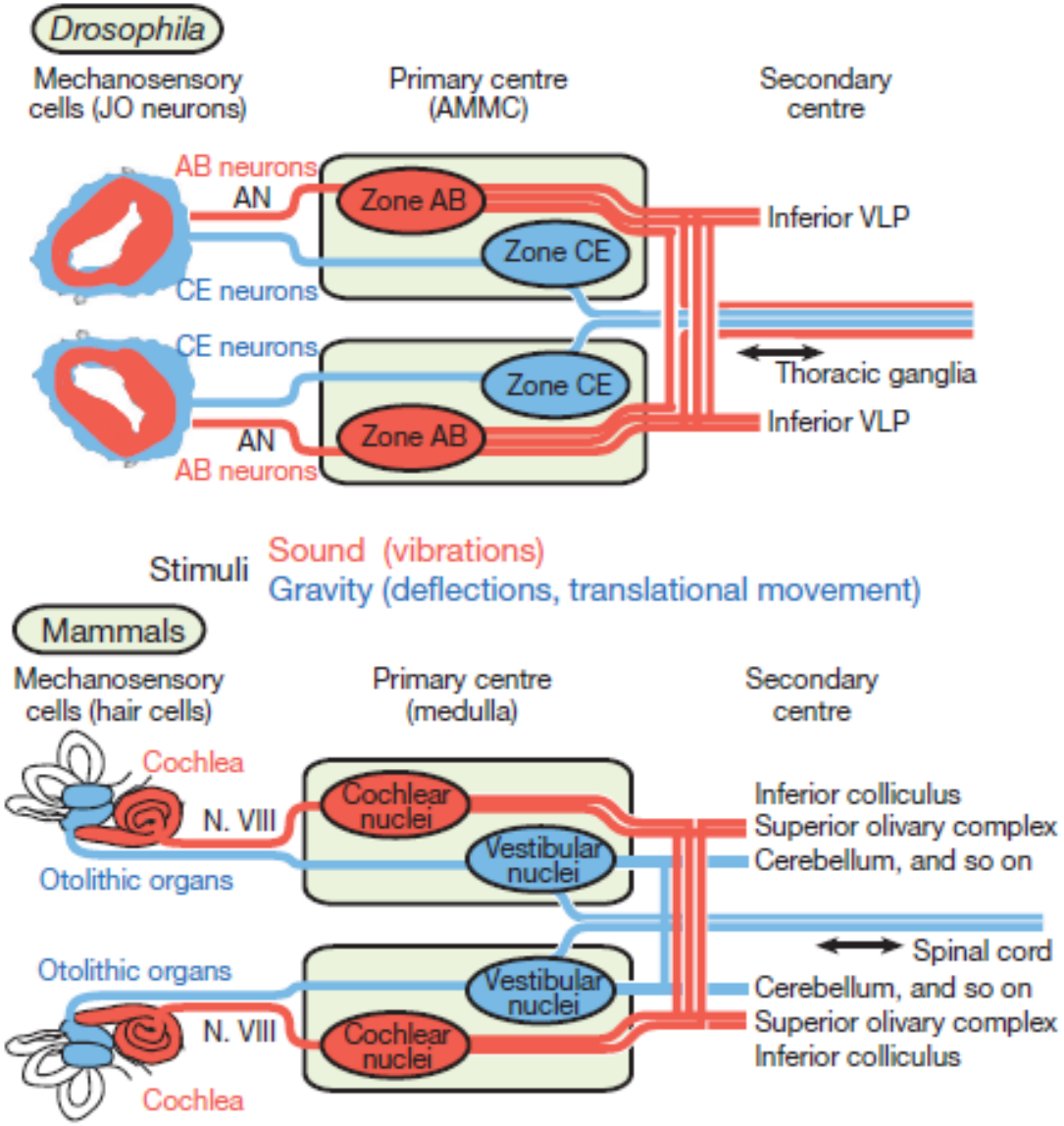
3. Disrupt functionality of some part of the neurons in specific regions while presenting stimuli
   1. Observe response compared to wild-type control
   2. Check what is being disrupted actually causes the observed response

Conclusion

1. AB group
   - Antennal vibrations
   - Sound detection role
     - A = high-frequency vibrations
     - B = low-frequency vibrations
2. CE group
   - Static deflection
   - Gravity sensing

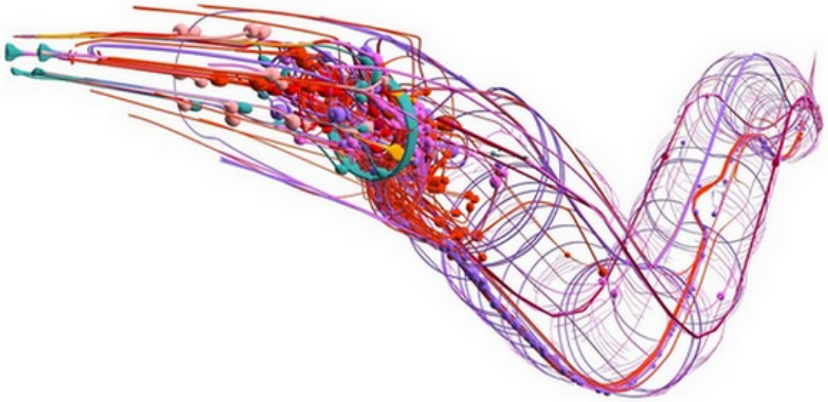


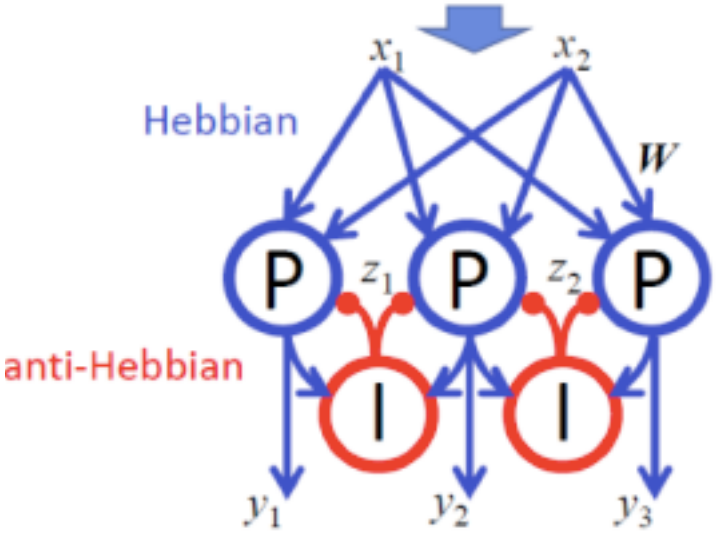
Comparison to mammalian circuits



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

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.

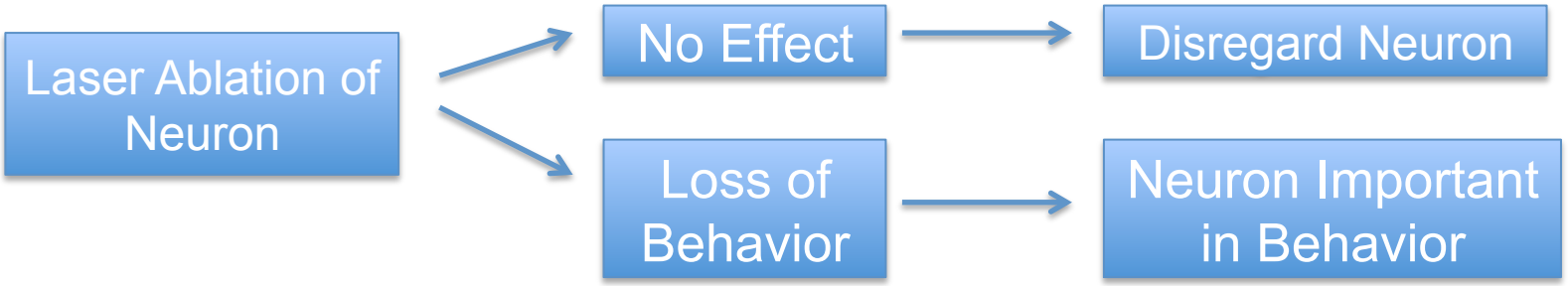
Step 4: Organize neurons by *epistasis analysis.*

Step 5: Draw Circuit.

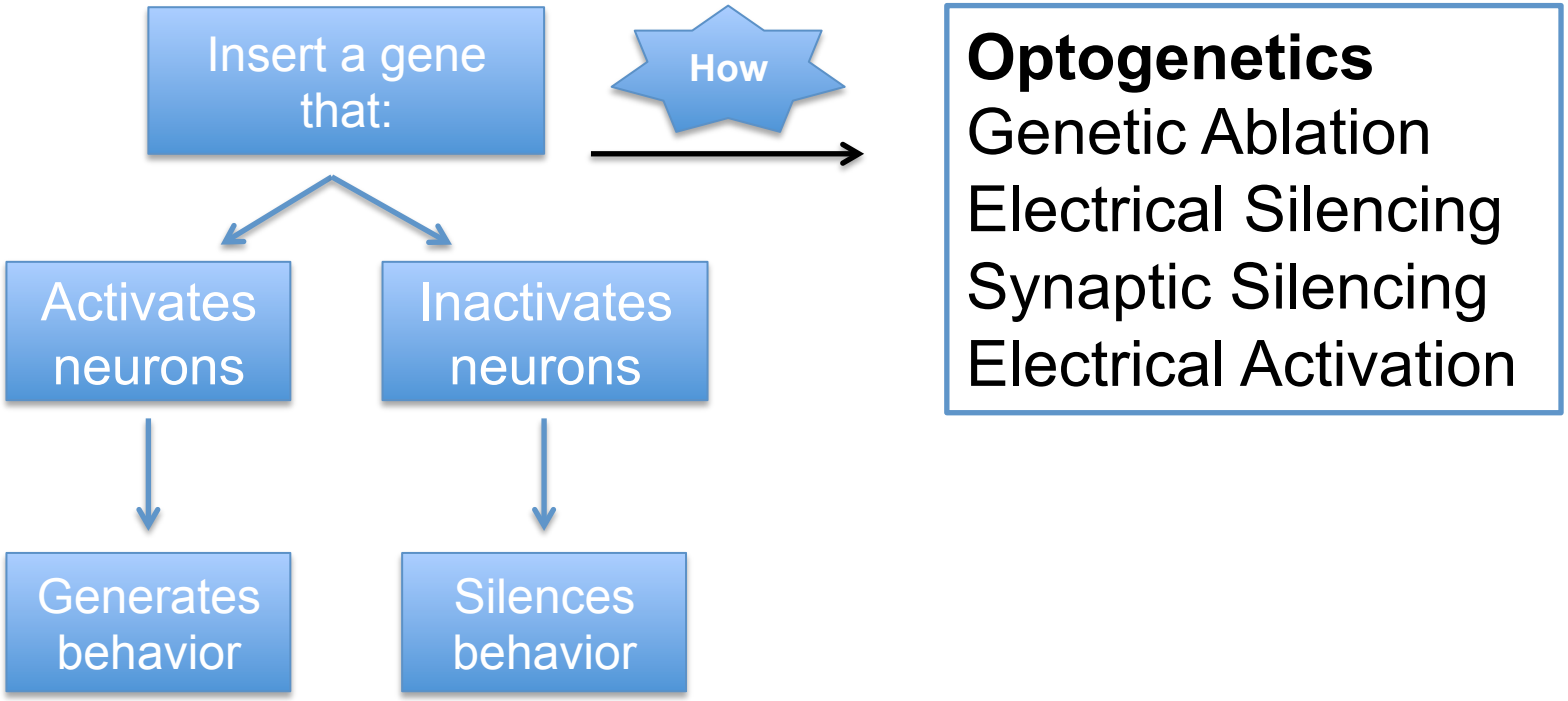
Step 1: Find one neuron involved in the circuit.

A. Use systematic inactivation or activation

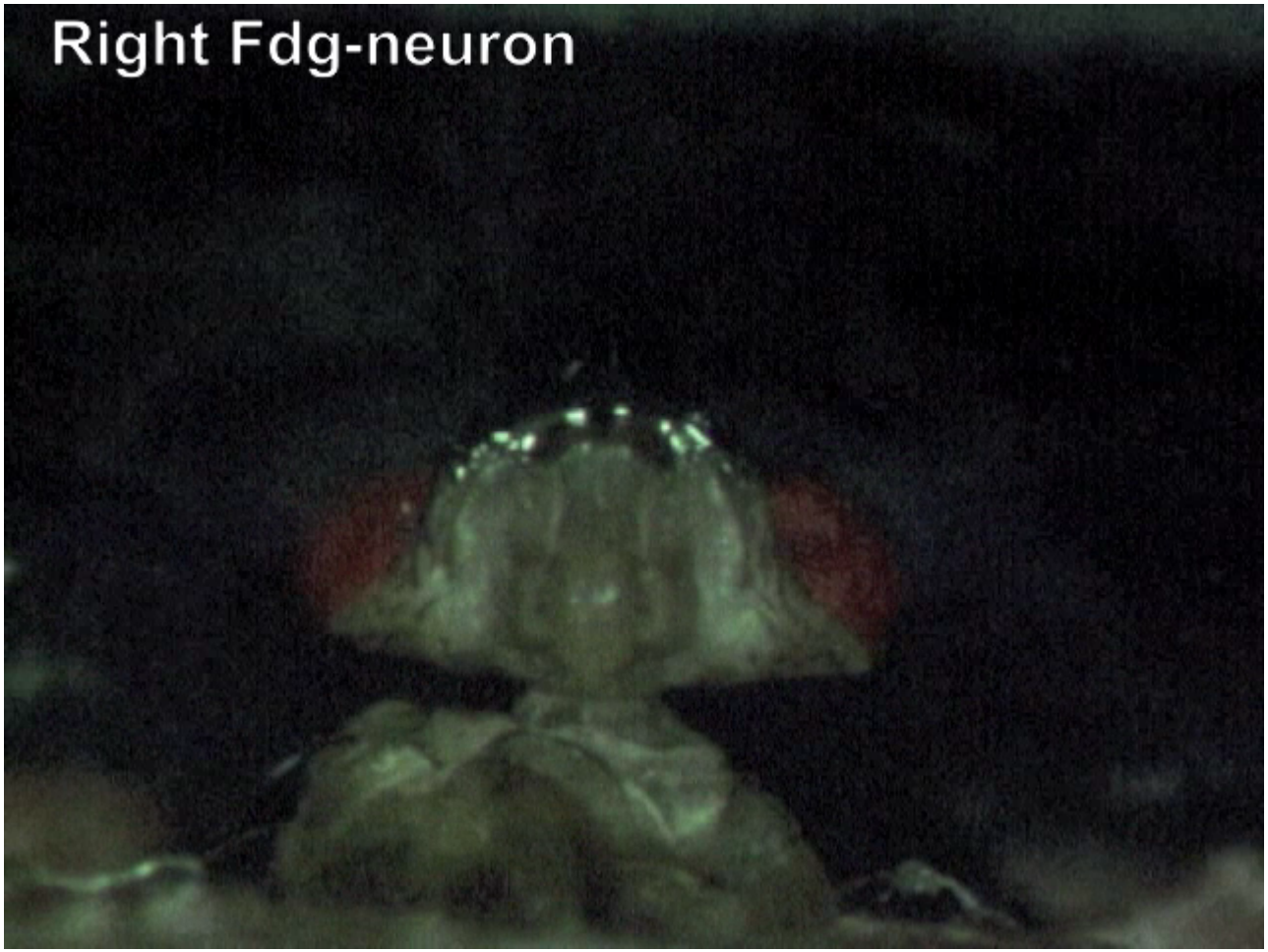
A. Use a laser to ablate neurons



B. Use a transgene to activate or inactivate neurons



Step 1: Optogenetic Activation of Neurons

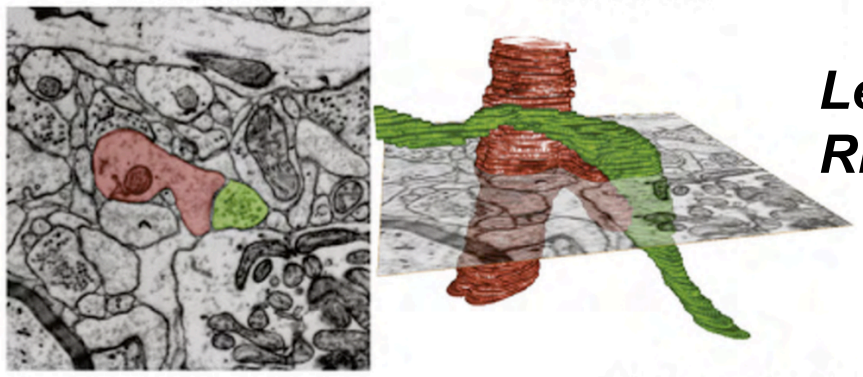


Proboscis

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

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

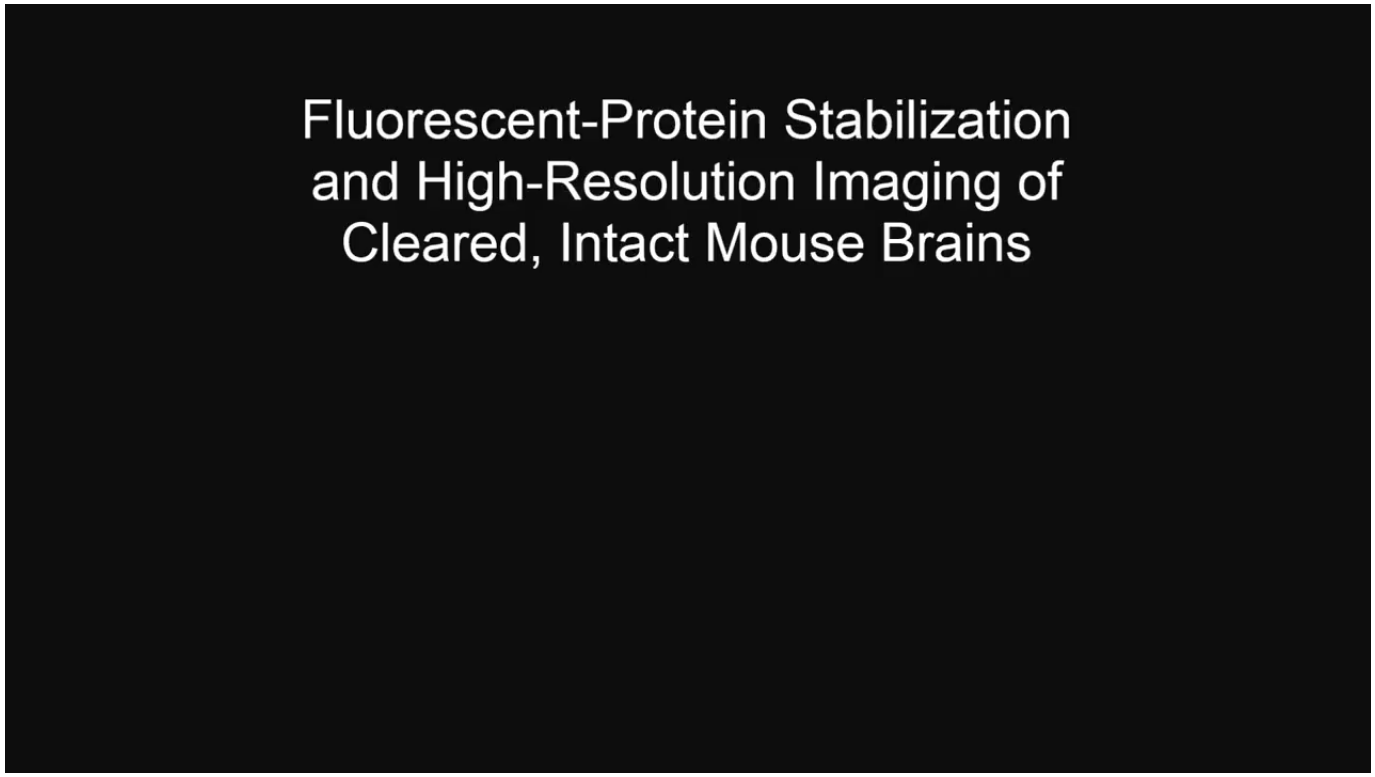


Left: 2-D Cross-Section
Right: 3-D Reconstruction

    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.

Step 2: Viral Tracing

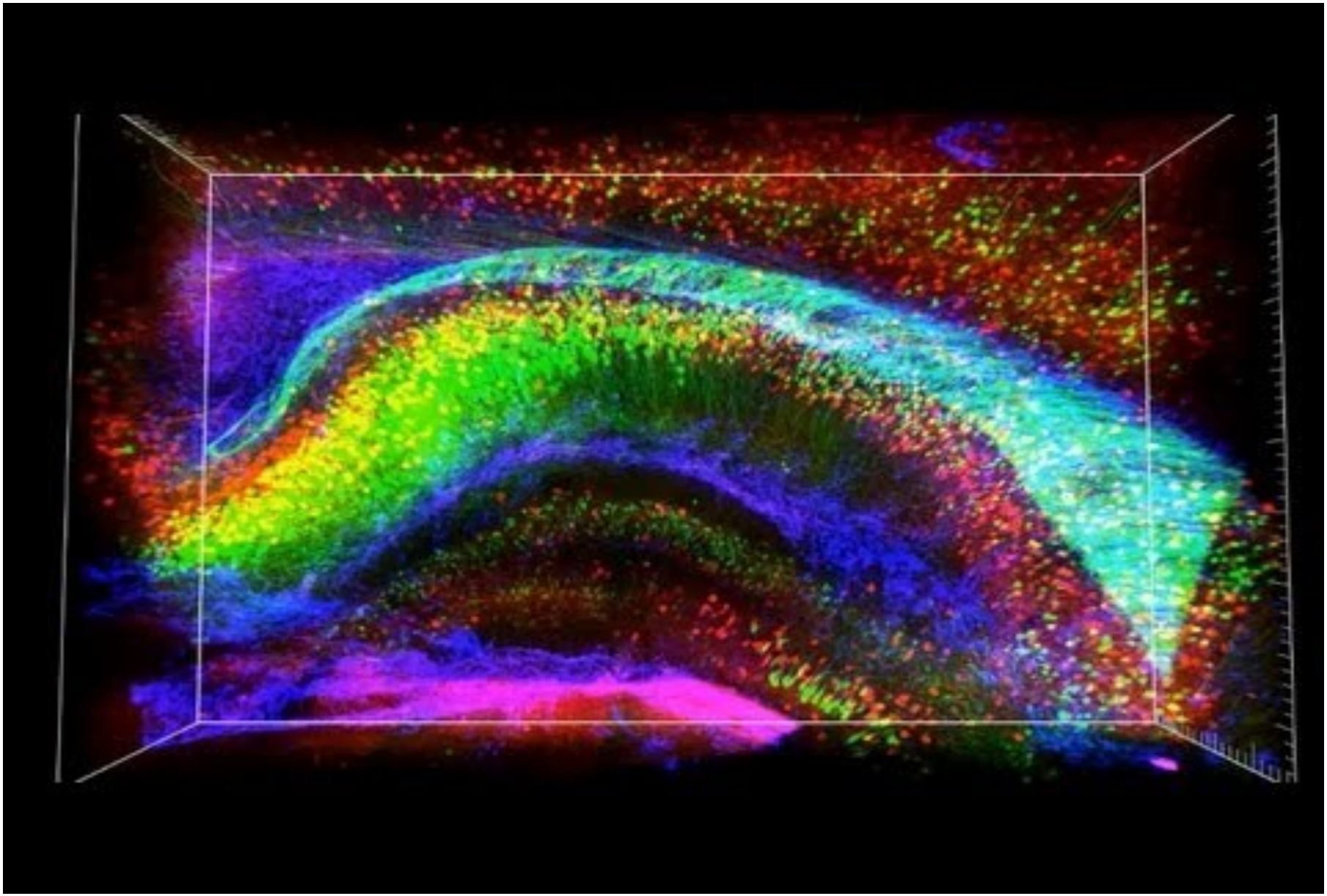


Anterograde Transport: tracer moves from soma to synapse, uses **kinesin** to move viruses along axon in anterograde direction

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

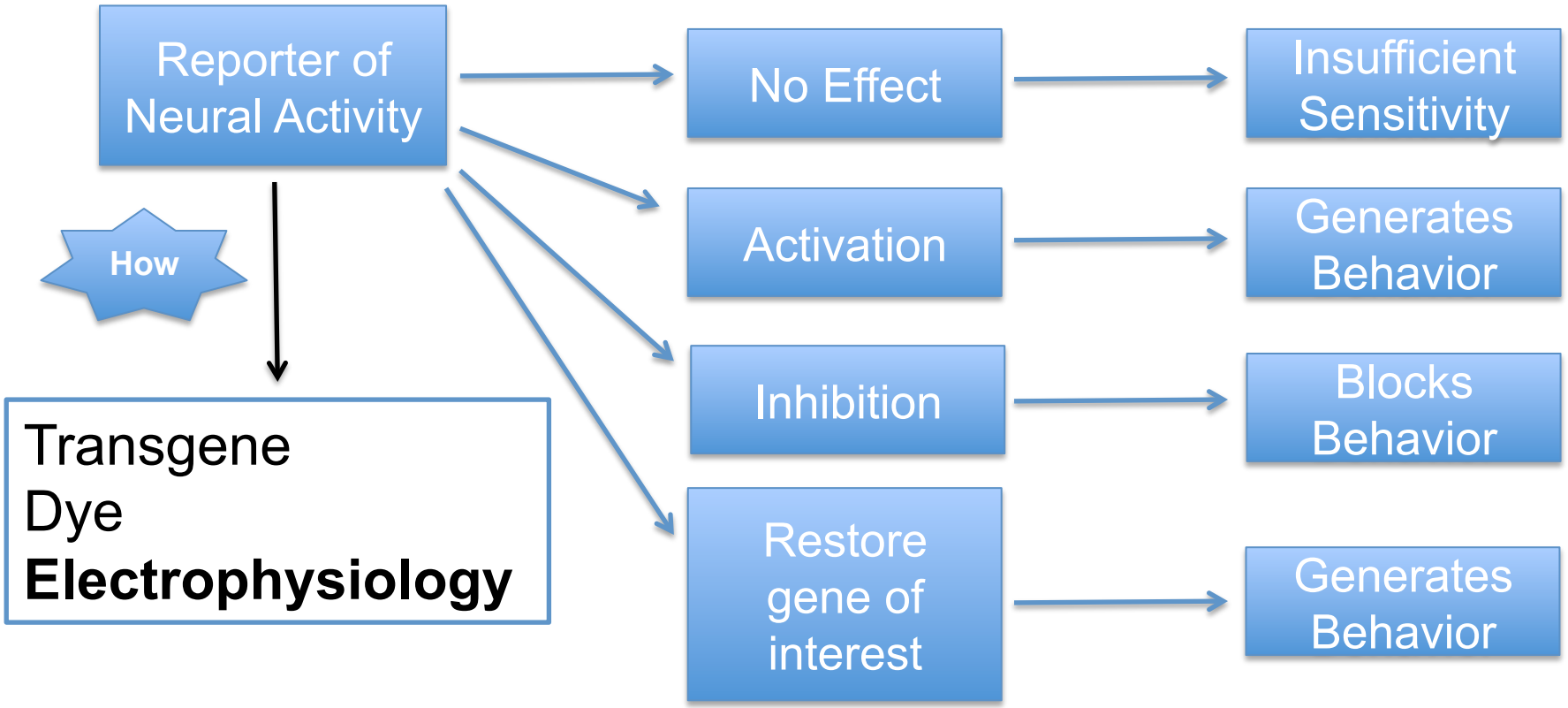
Dual Transport: combines above methods to determine both the inputs and outputs of neuronal circuitry

Step 2: CLARITY

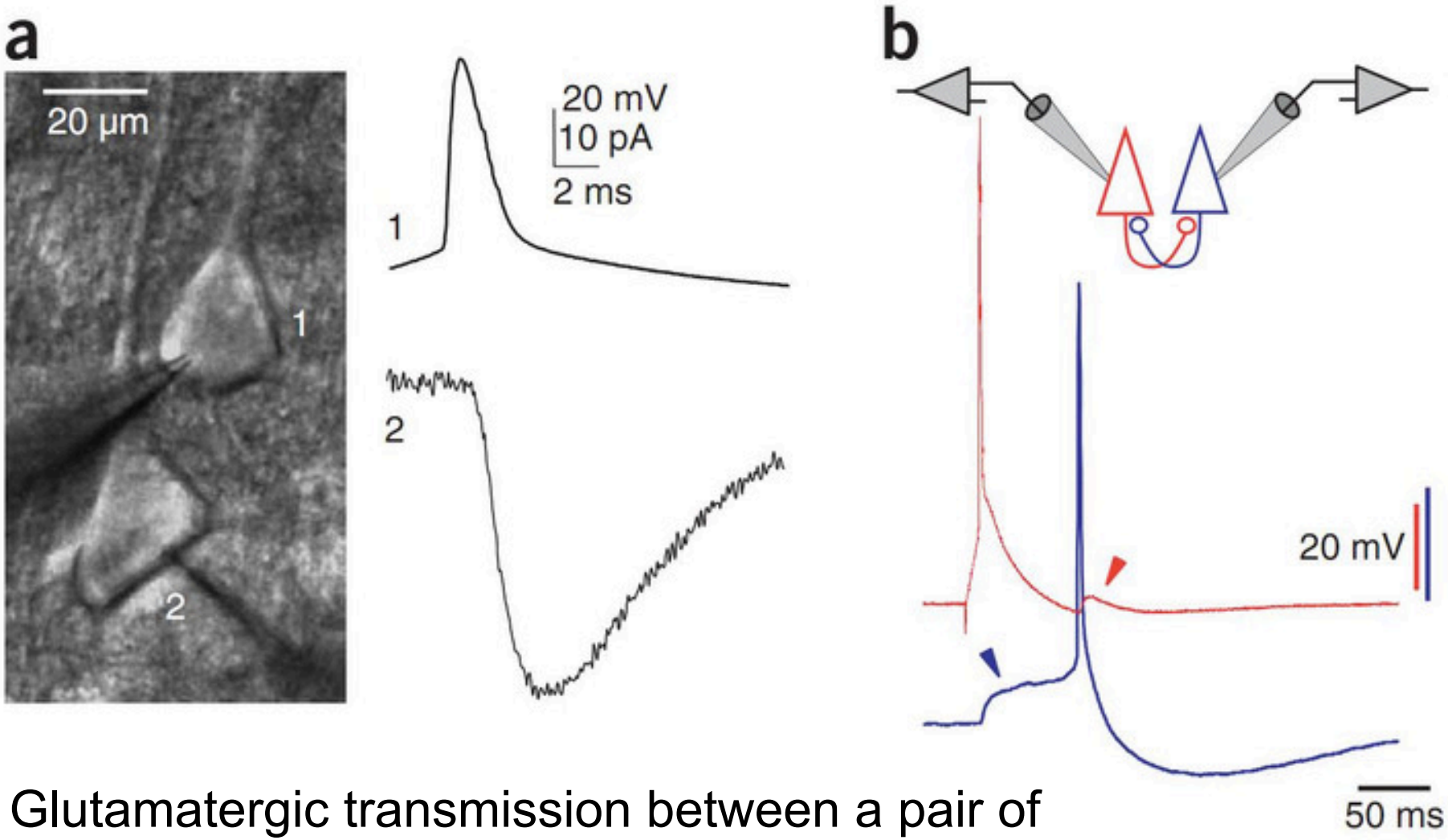


Step 3: Confirm the role of identified neurons in generation of behavior

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

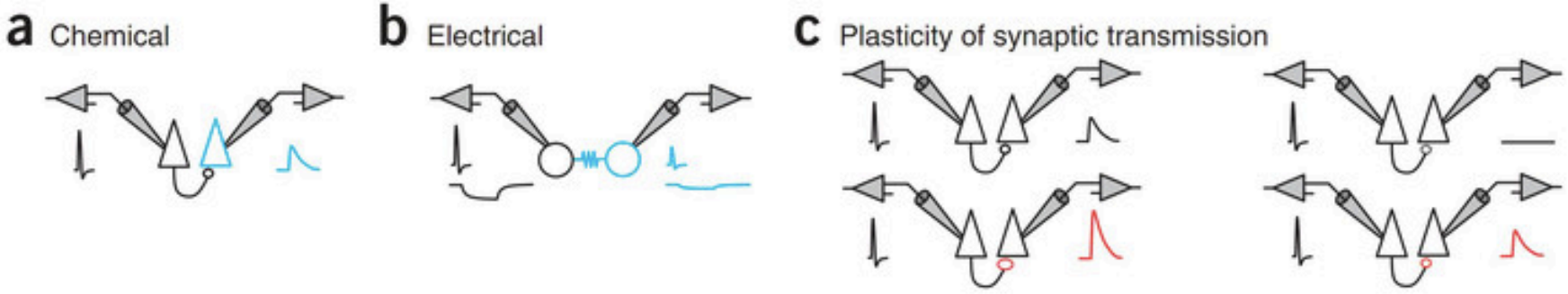


Step 3: Connectivity by Electrophysiology



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.

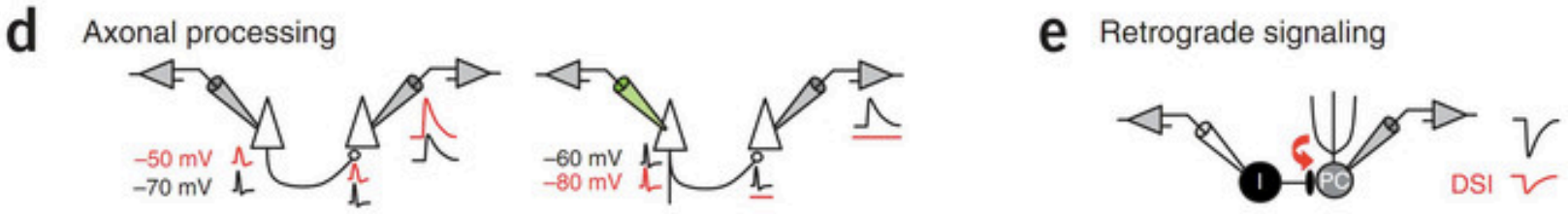
B. Electrical Synapse

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



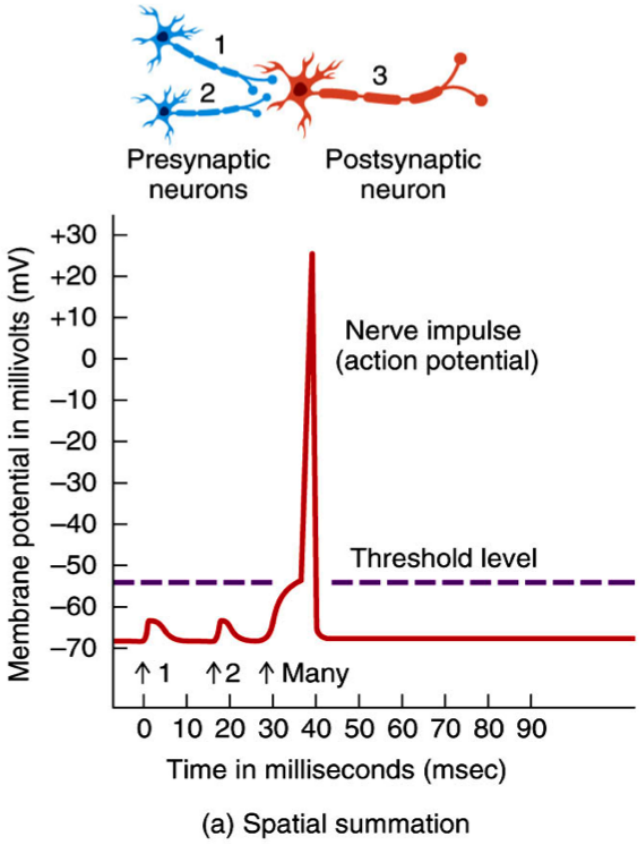
D. Axonal Processing

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).

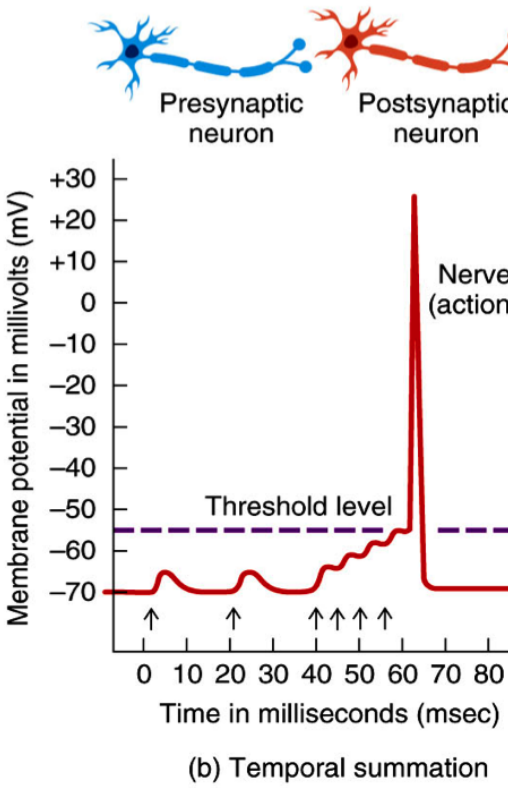
E. Retrograde Signaling

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.

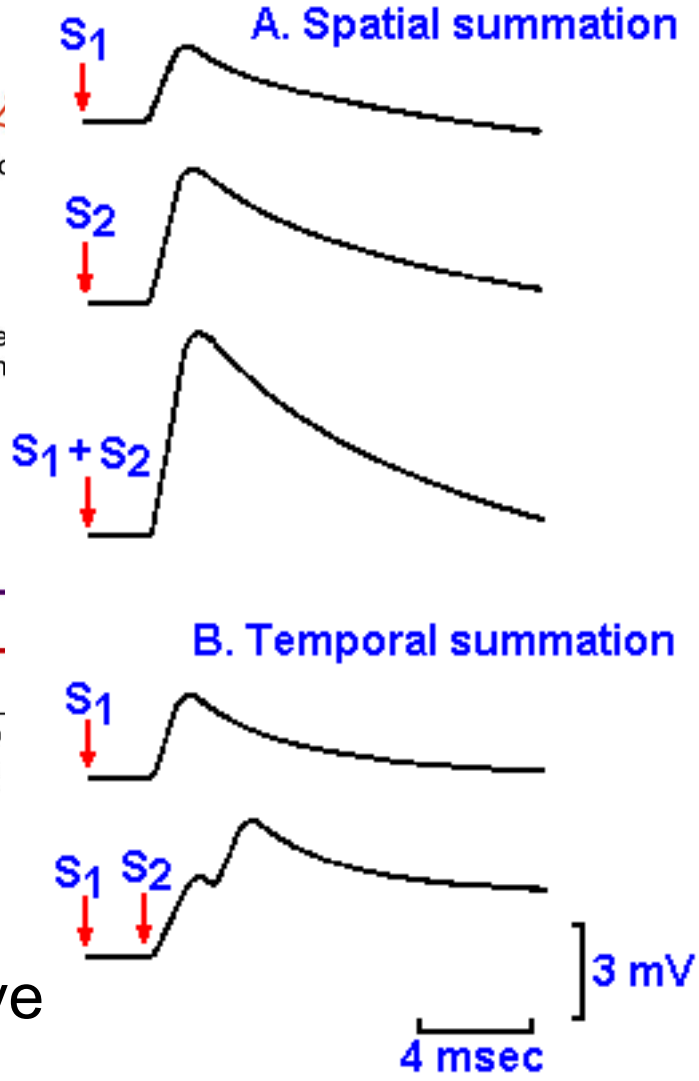
Summation



(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

Review of Content

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

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.

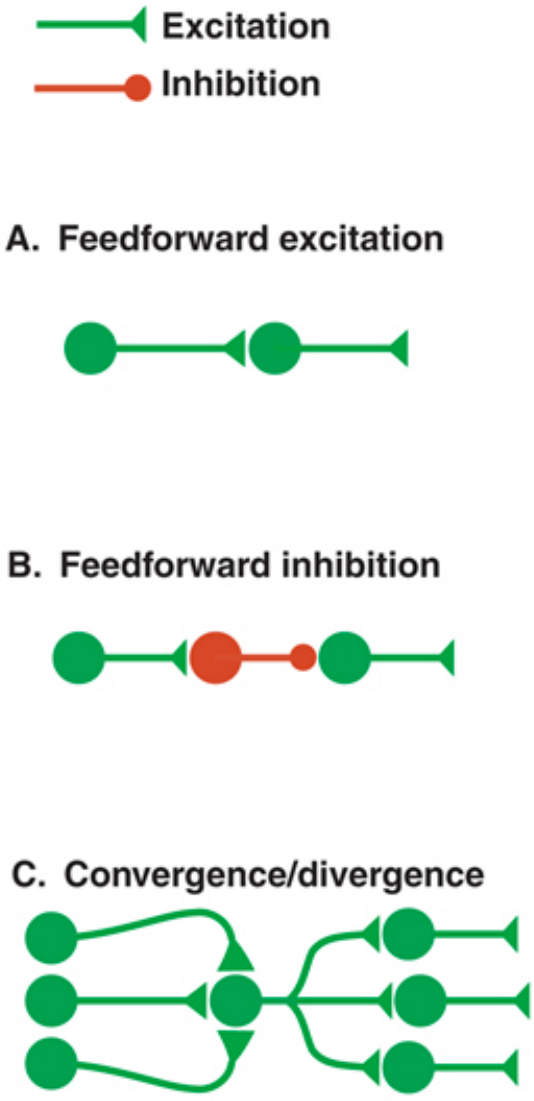
C. Activate neuron X and see if neuron Y responds.

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.

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.

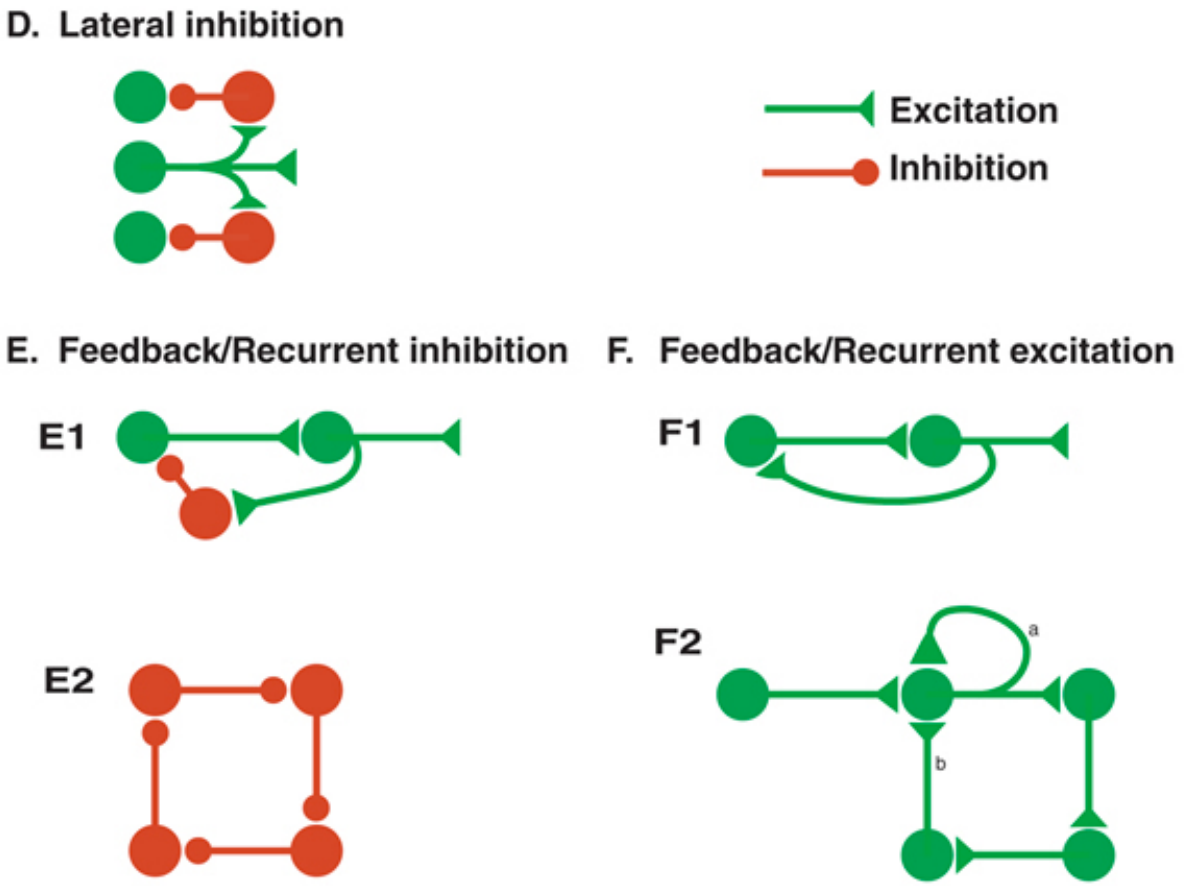
C. Convergence/ Divergence

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

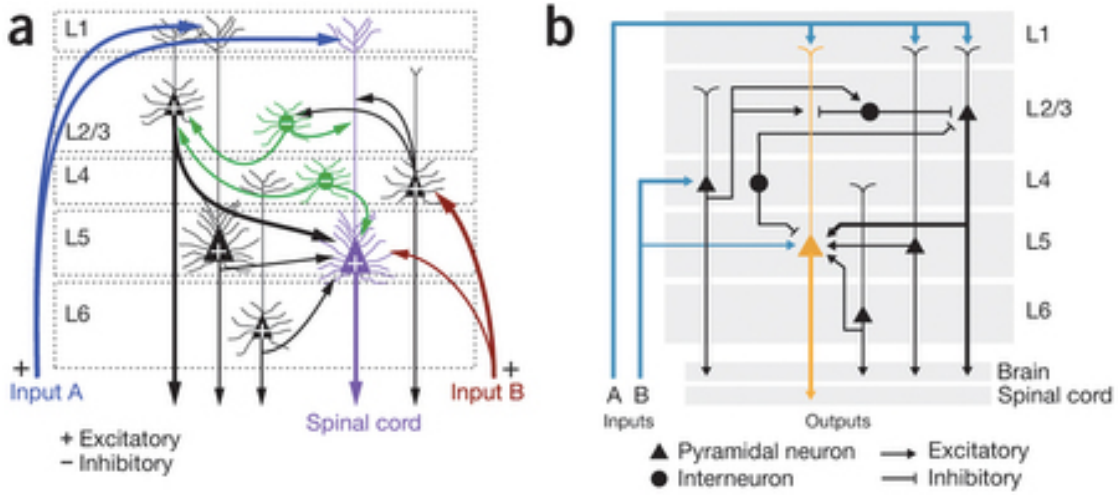


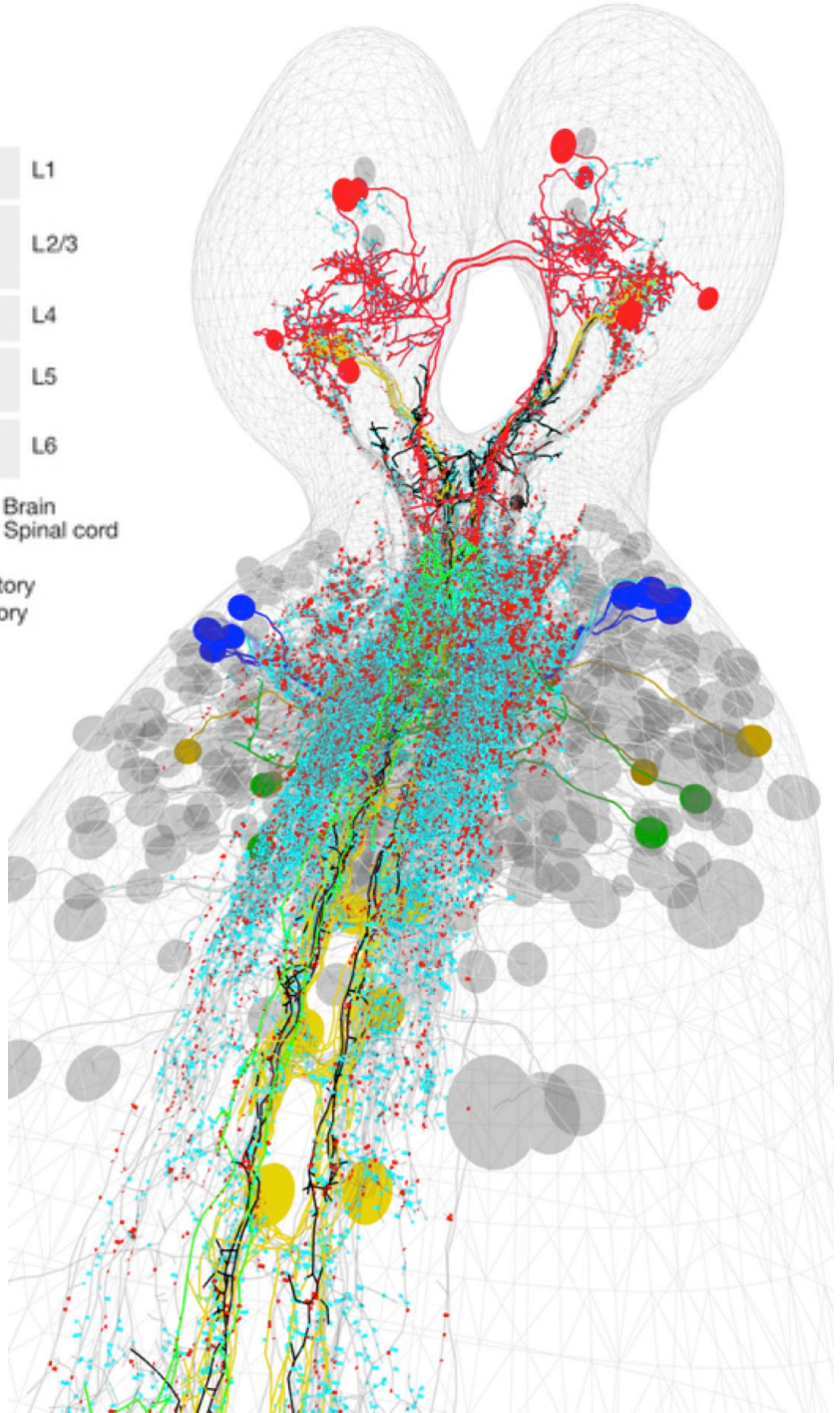
D. Lateral Inhibition
A pre-synaptic cell excites inhibitory interneurons, which inhibit neighboring cells in the network.

E. Feedback/ Recurrent Inhibition

F. Feedback/ Recurrent Excitation

Step 5: Circuit is Complete





Multi-Sensory Circuits