Historical notes:

Unlike other fields in Biology that are organized around a specific set of techniques such as molecular biology and genetics, an important feature of neurobiology is that it is a multidisciplinary field and neurobiologists may be trained as biochemists, physiologists, anatomists, a behaviorist, mathematician or a molecular biologist..

Our current views about nerve cells, the brain, and behavior have emerged over the last century from a convergence of five experimental traditions:
Anatomy , embryology, physiology, pharmacology, and psychology, using two major approaches **reductionist (or bottom-up) and holistic (top down).**

Types of models reflect the main experimental traditions

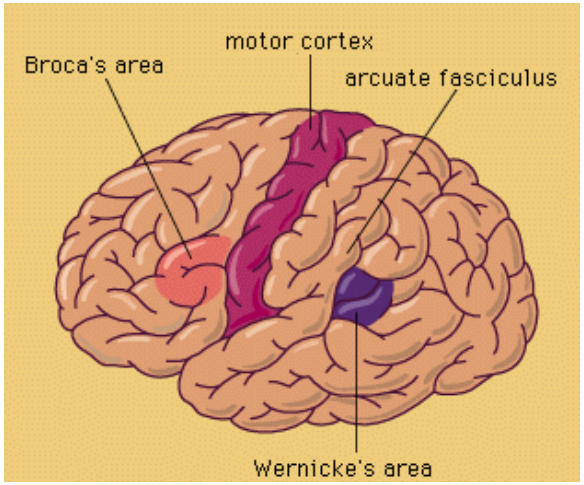
Mechanistic/biophysical -> Reductionistic approach
Phenomenological -> Holistic Approach

19th century controversy between

holistic traditions maintained that the brain was a homogeneous organ with no specific subparts

whereas ,

localizationists (e.g., Paul Broca) argued that the brain is organized in functionally distinct areas, each specialized in specific mental operation.

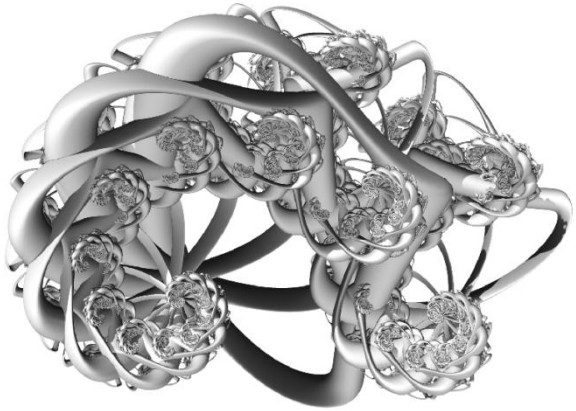


The controversy was temporarily summarized with its finding of a language area,

but the issue isn't settled insofar as the brain as a whole is a highly connected organ at every level from the individual neuron to the hemispheres.

Holistic traditions:

Gestalt psychologists (beginning of 20th century) , developed theoretical and methodological principles to define holistic approach in cognitive psychological research.



They described perception to be more than the sum of its parts examined in isolation, requiring not only to understand properties of individual elements but studying how the entire brain reconstruct the external world.

With the advent of high quality brain imaging, the holistic methods available to the nineteenth century clinical neurologist, based mostly on the detailed study of neurological patients with defined brain lesions, were enhanced dramatically by the ability to examine cognitive functions in intact behaving normal human subjects.

reductionist traditions:

Anatomy : Golgi and Ramon y Cajal made the first detailed descriptions of nerve cells

Camillo Golgi was nominated for the Nobel Prize in Physiology or Medicine as early as 1901, when the first prize was awarded. After that, his name came up every year until 1906, when he was awarded the prize together with Santiago Ramon y Cajal.

reductionist traditions:

Anatomy : Golgi and Ramon y Cajal made the first detailed descriptions of nerve cells

Camillo Golgi:

Developed a way of staining neurons to reveal their structure.
He believed in the **syncytium theory:** namely, that nerve cells are connected through a diffuse network of interconnected cytoplasm.

Ramón y Cajal:

• Mapped out the neural circuits of many brain areas in numerous animal species –vertebrates and invertebrates – including the neuron types in each brain area, and their inputs and outputs.
• He did this in normal and pathological brains; and across developmental stages.
• He mapped neural circuits based on structure alone – and without the use of anterograde or retrograde tracers, which were unknown at that time: A tour de force, and a classic example of inferring function from structure.

• Ramón y Cajal is regarded by many as the founder of Neuroscience.

• Ironically, Ramón y Cajal contradicted Golgi's theory via extensive experimental studies which all used the Golgi staining method…
• But: The finding of electrical synapses, made of gap junctions, suggests that Golgi
was not totally wrong, after all.

Some more things about Cajal..

He didn't like authority (Example: At age 11 he was imprisoned for destroying the town gate with a homemade cannon)

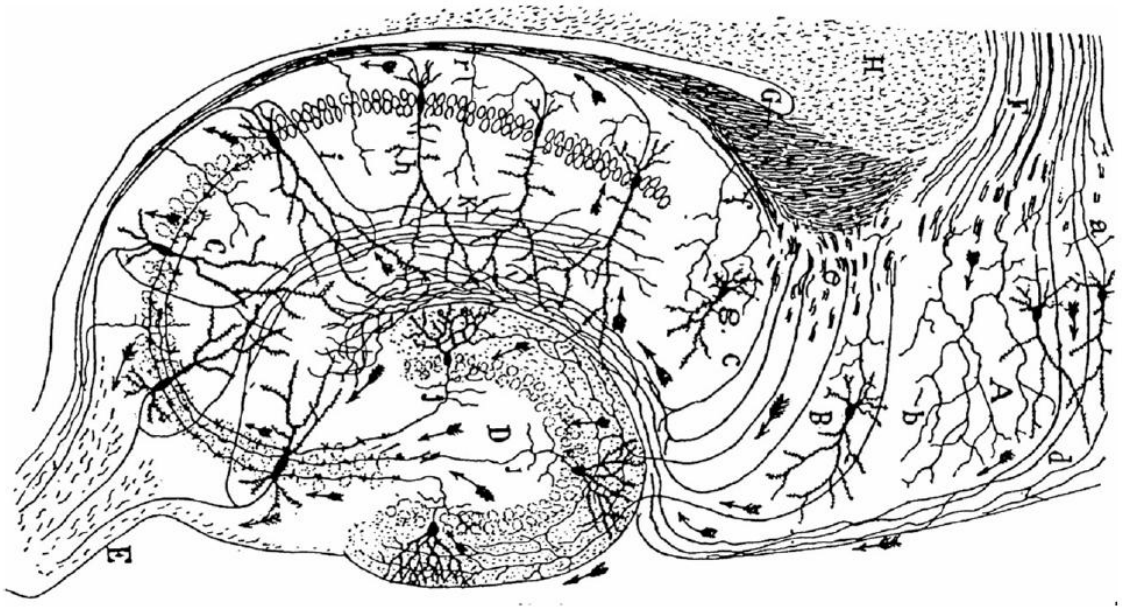
He was an excellent gymnast

For a time he was a shoemaker

And then a barber

And he wrote science fiction stories under his pseudonym "Dr. Bacteria"

THEN He suggested that the neuron was the anatomical and functional unit of the nervous system

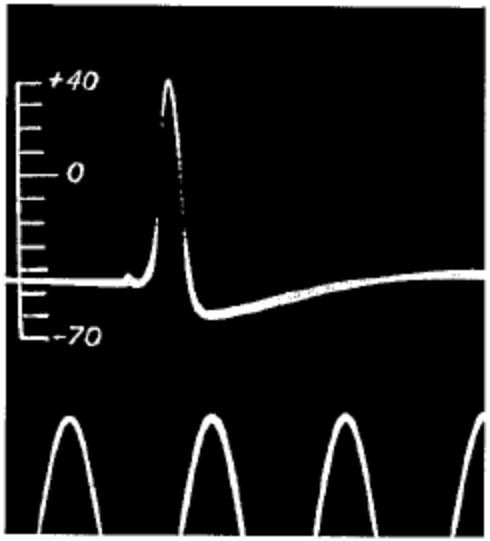


Example: Drawing of the neurons, their interconnections, and flow in information in the rodent hippocampus (by **Ramon y Cajal ,**1911).

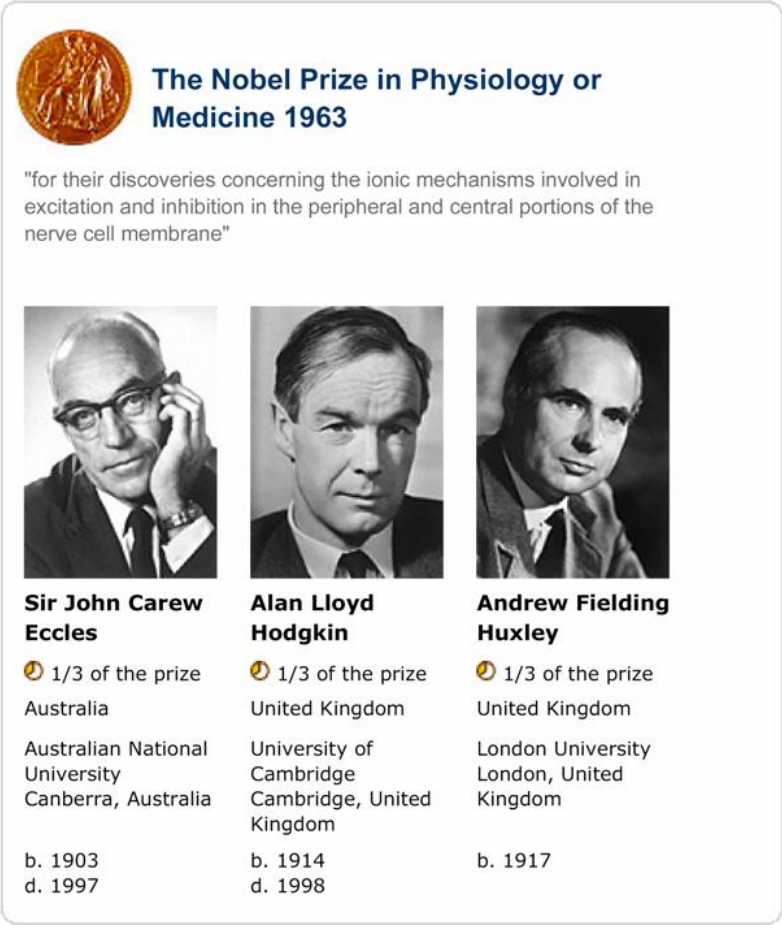
Reductionist traditions:

Physiology :

In 1937 Sir **Alan Lloyd Hodgkin** found that an action potential generates a local flow of current that is sufficient to depolarize the adjacent region of the axonal membrane, in turn triggering an action potential (Hodgkin, 1937).



The historic recording with a capilary pipette place across the membrane of a squid giant axon (From Hodgkin and Huxely 1939)



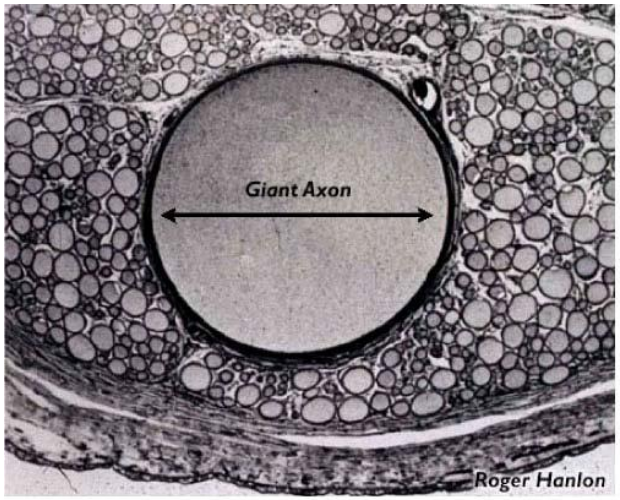
The Nobel Prize in Physiology or Medicine 1963

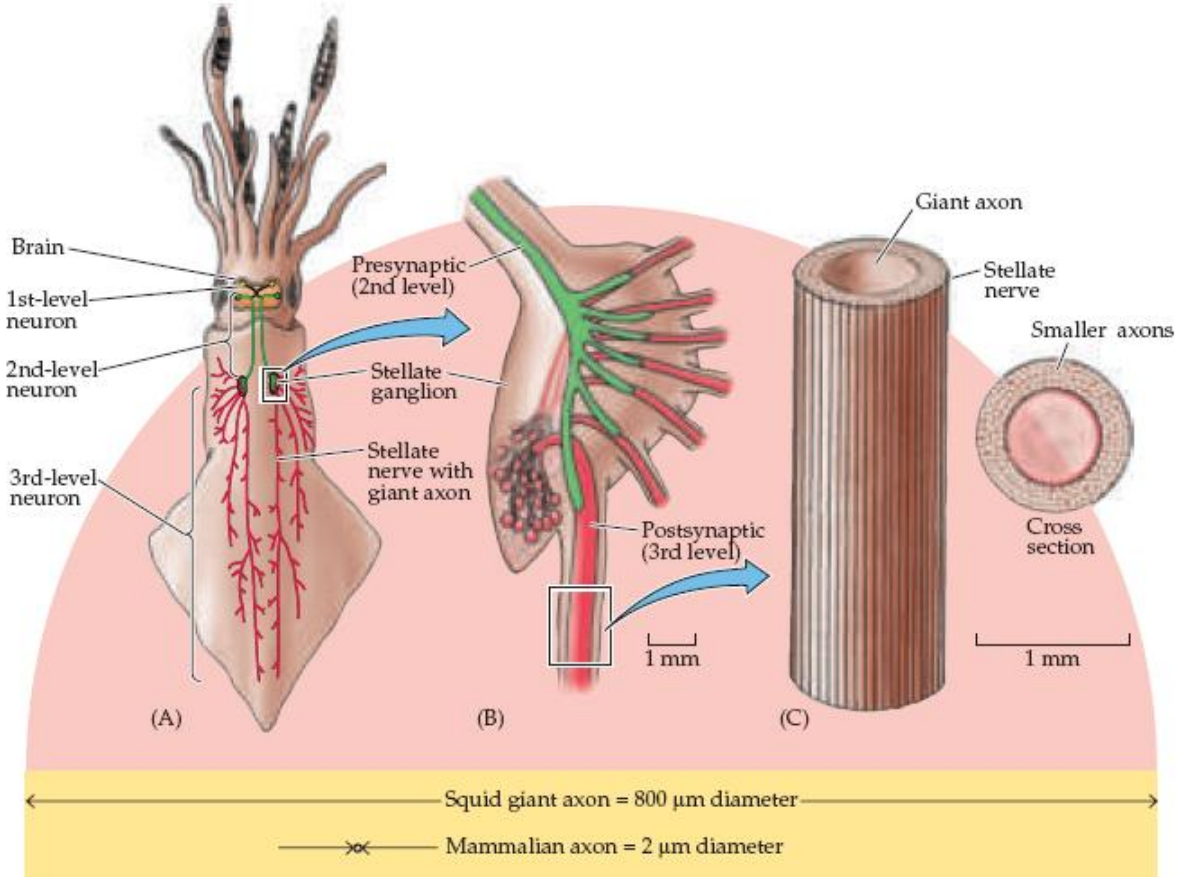
"for their discoveries concerning the ionic mechanisms involved in excitation and inhibition in the peripheral and central portions of the nerve cell membrane"

Sir John Carew Eccles	Alan Lloyd Hodgkin	Andrew Fielding Huxley
1/3 of the prize	1/3 of the prize	1/3 of the prize
Australia	United Kingdom	United Kingdom
Australian National University Canberra, Australia	University of Cambridge Cambridge, United Kingdom	London University London, United Kingdom
b. 1903 d. 1997	b. 1914 d. 1998	b. 1917

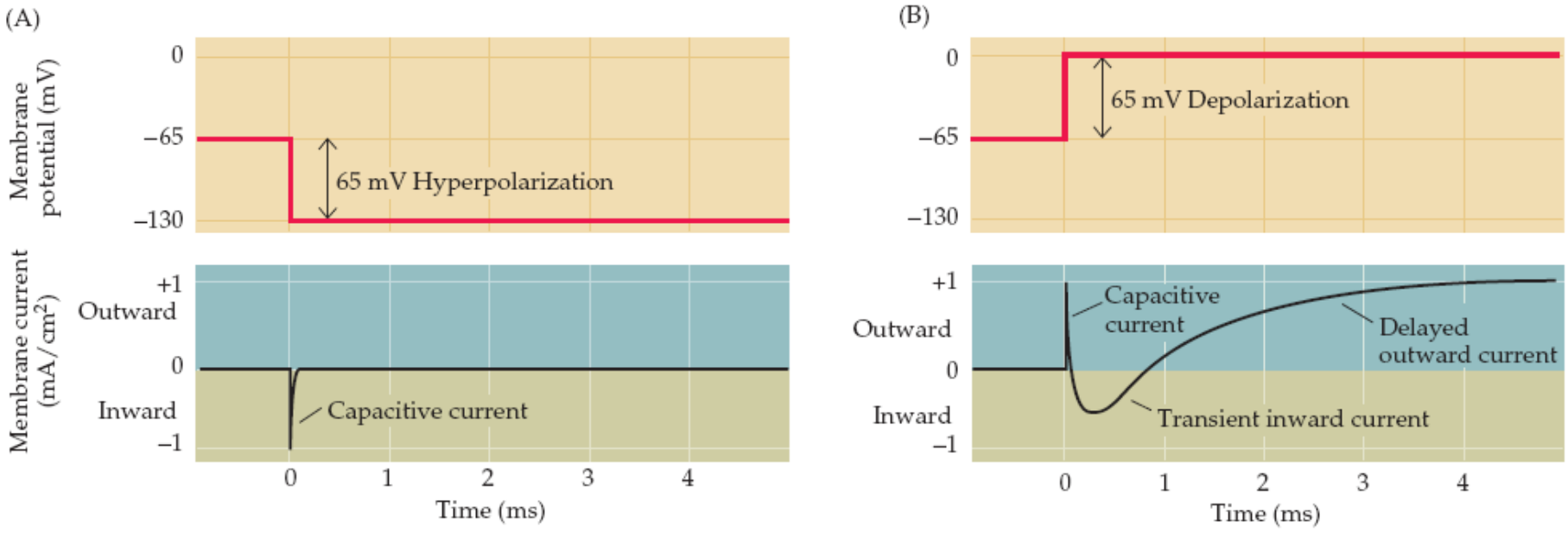
They used the Giant squid axons to measure current - voltage relations in neurons

Action potentials travel faster in a larger axon than in a smaller one
The squid have evolved the giant axon to improve the speed of their escape response

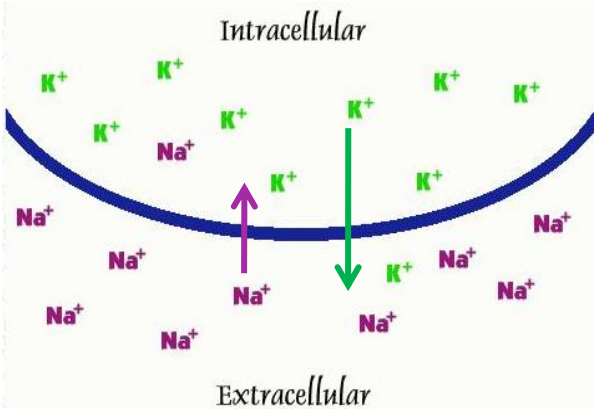




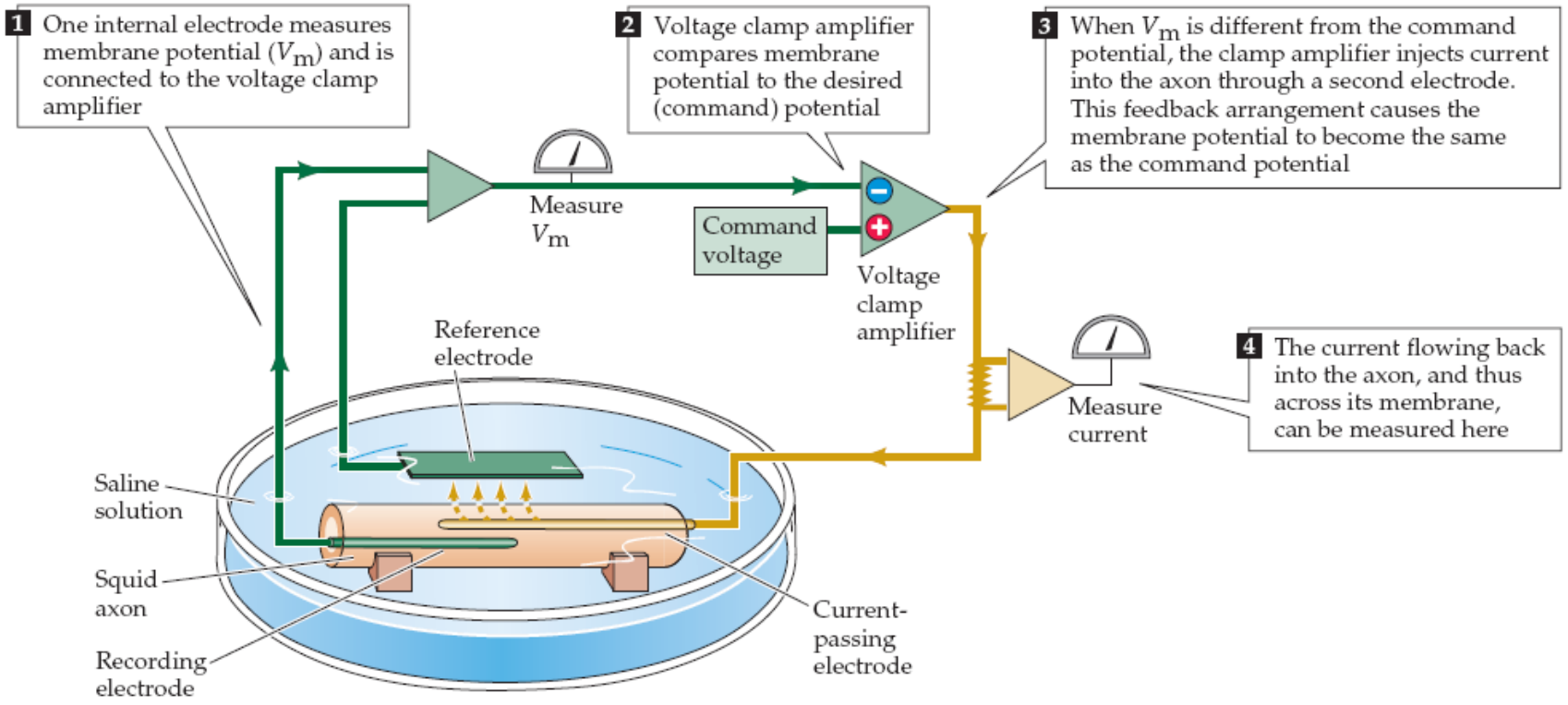
Hodgkin and Huxley showed that step depolarizations of the squid axon trigger an inward current followed by an outward current.



By using ionic substitution they demonstrated that this net current could be separated into two distinct components, a rapid inward current carried by Na^+ ions, and a more slowly activating outward current carried by K^+ ions.

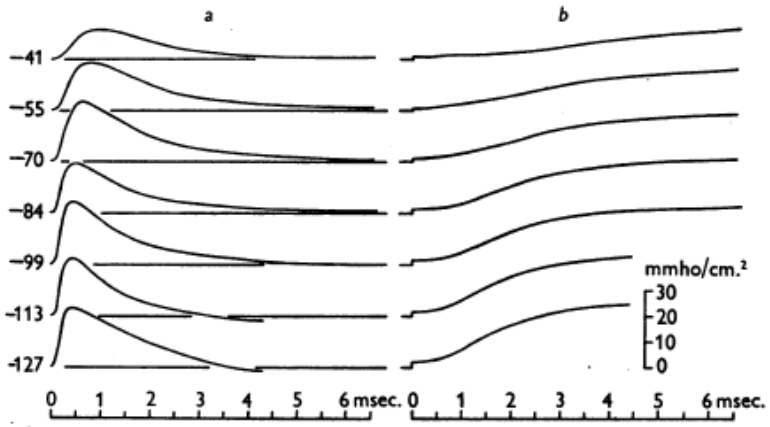


They developed voltage clamp protocols to manipulate the voltage across the cell's membrane and study current-voltage relationships.

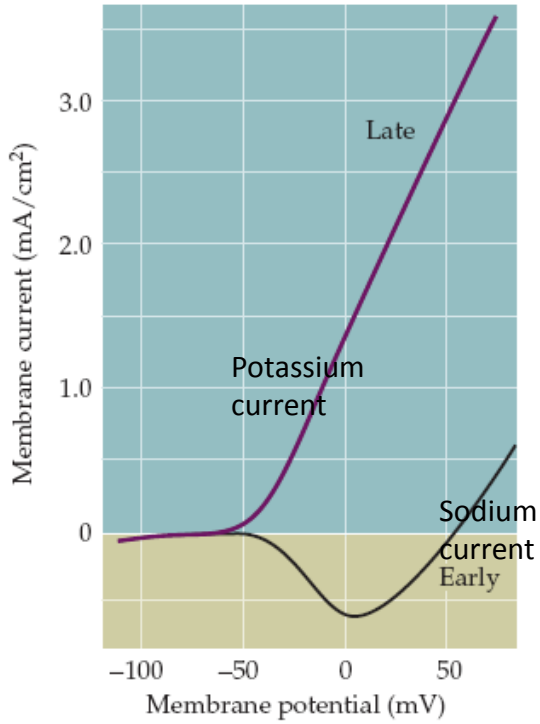


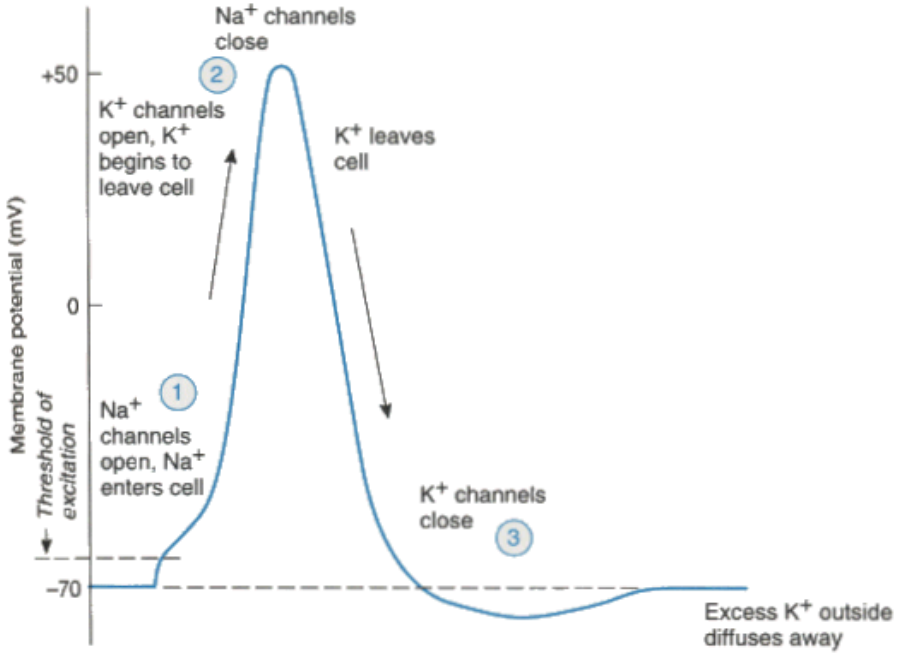
(Voltage clamp was invented by Kenneth Cole and George Marmountin the 1940's)

They concluded that these two currents result from independent permeability mechanisms for Na + and K+ with conductances changing as a function of time and membrane potential



In H&H papers the direction of Sodium (a) and Potassium (b) currents and voltages are flipped: voltage is the displacement from rest (with negative sign)





They characterized ionic conductance and the specific current flows during an action potential.

They developed an empirical representation of the experimental data in a quantitative model, the first complete description of the excitability of a single cell.

Reductionist traditions:

The patch clamp technique : Single channel recording

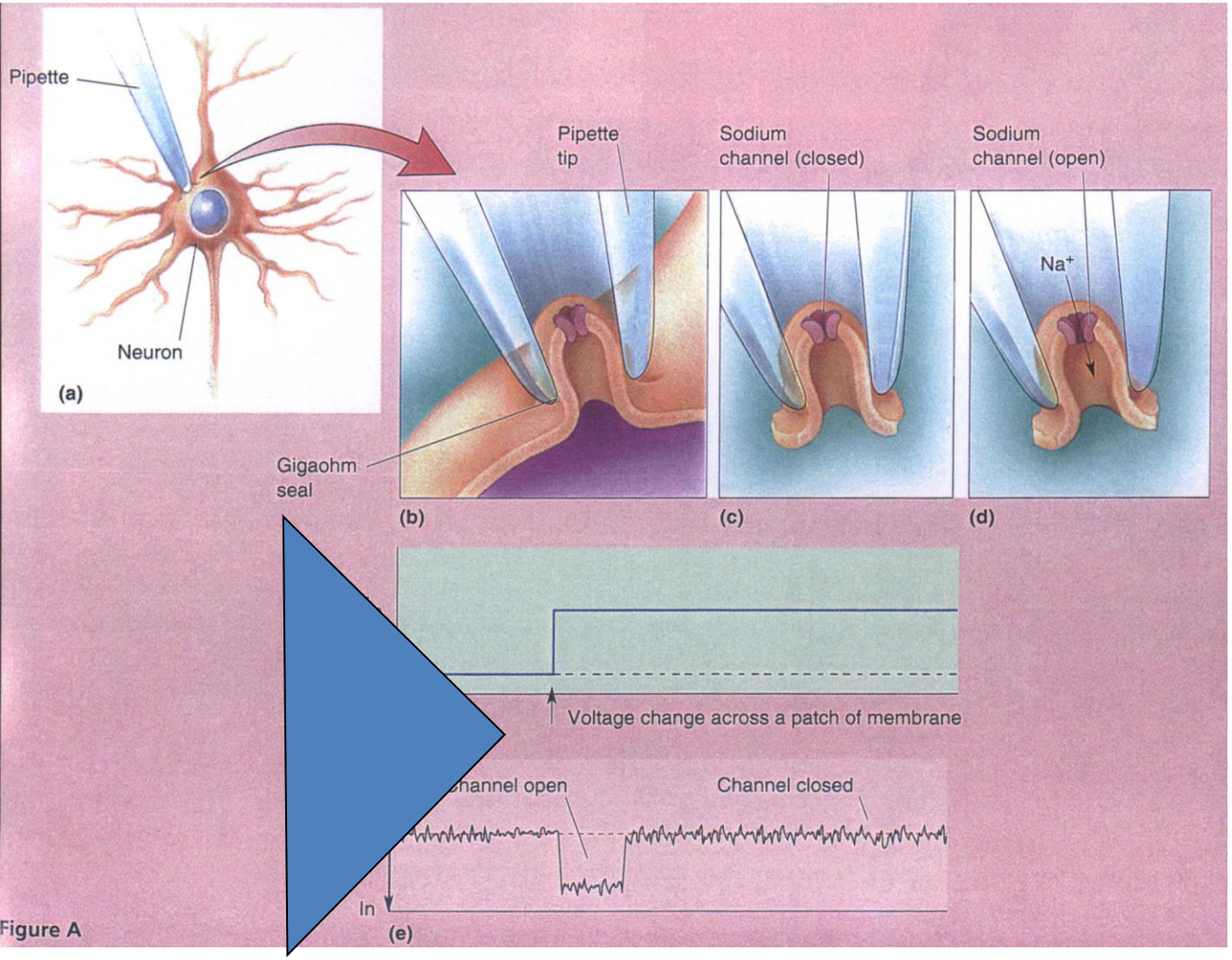
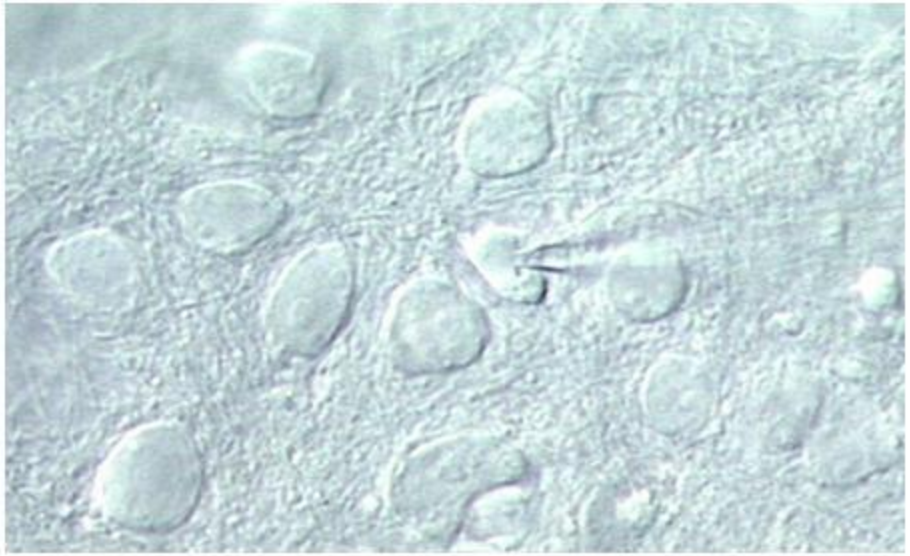
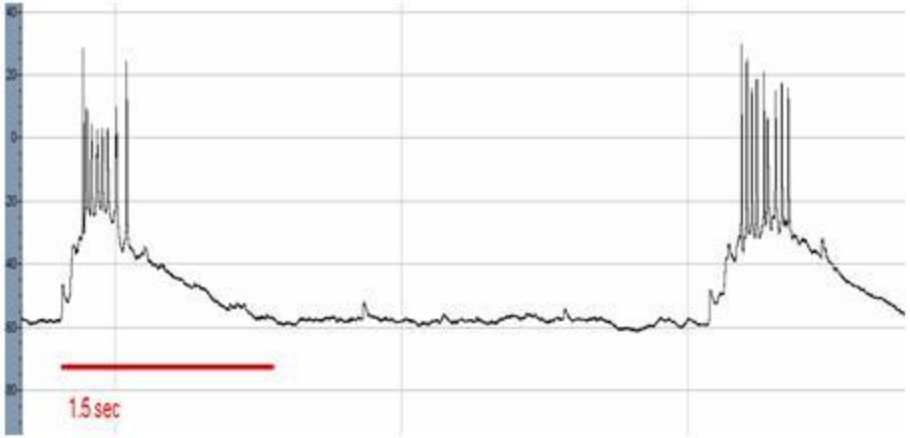


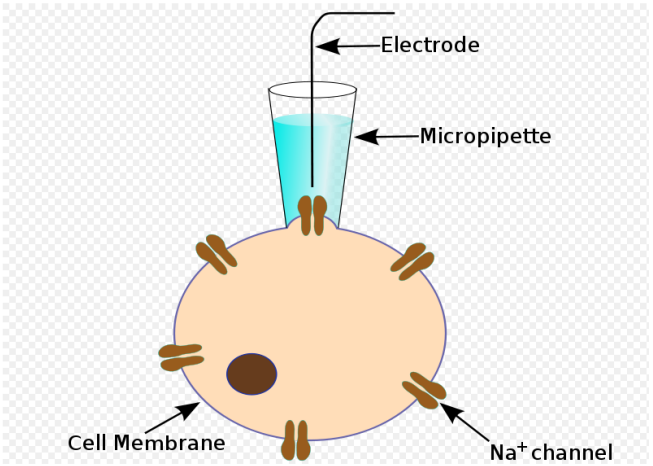
Figure A

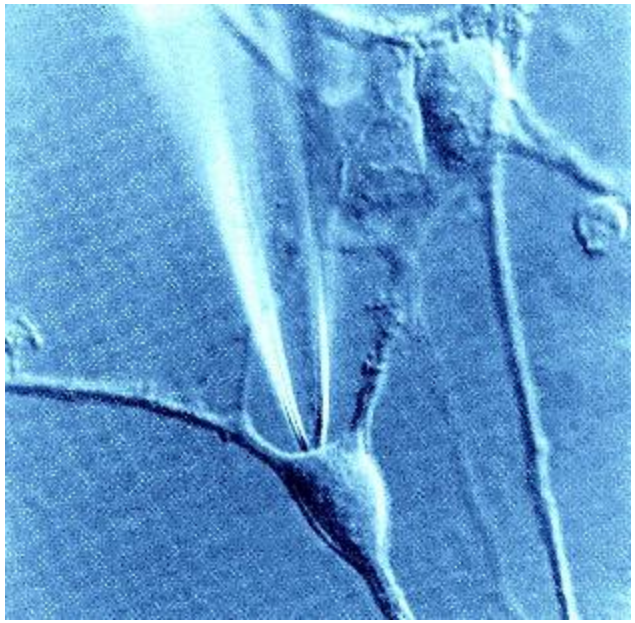
Erwin Neher (left) and Bert Sakmann in their laboratory (1985).

Modern electrophysiology the patch clamp





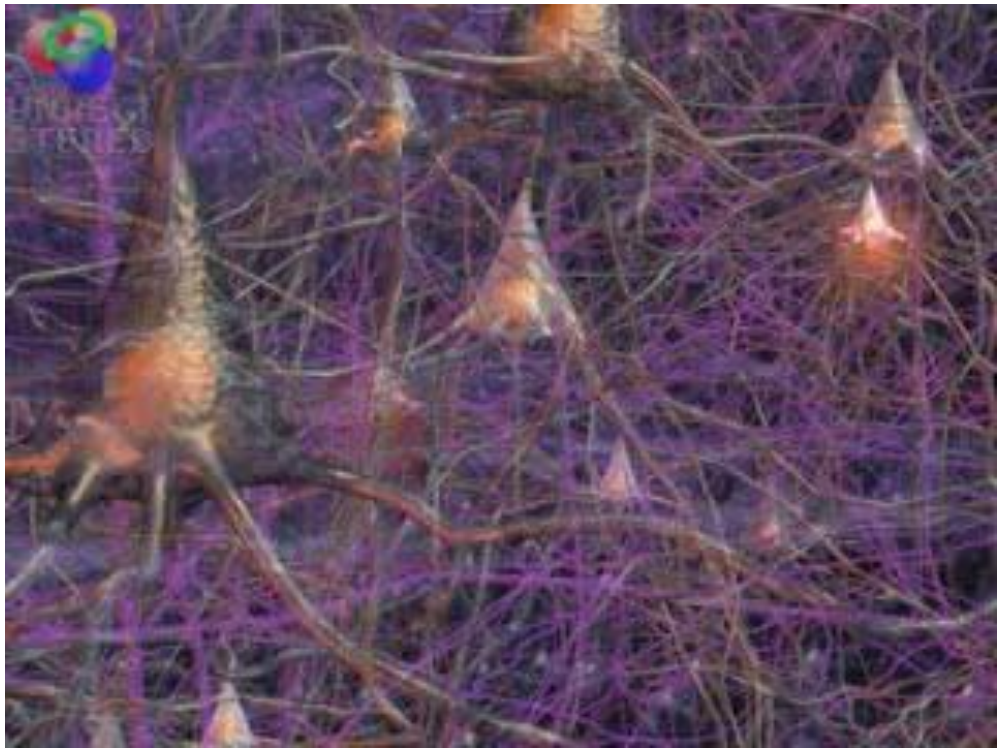




Many of current challenges in neuroscience is in developing new strategies for **combining reductionist and holistic approaches** to provide a meaningful bridge between molecular mechanism and mental processes

This is due to

The enormous complexity of neuronal systems



However, many of current challenges in neuroscience is in developing new strategies for **combining reductionist and holistic approaches** to provide a meaningful bridge between molecular mechanism and mental processes

This is due to

The enormous complexity of neuronal systems

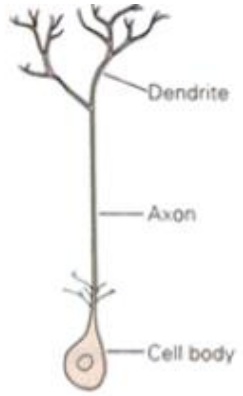
The simplicity of mechanisms, preserved across different scales, systems and organization levels

Computational tools enhancing our ability for cross scaling

Complexity

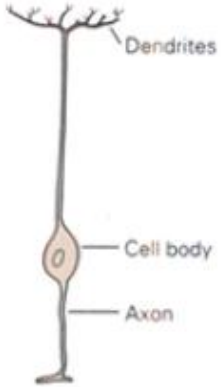
Thousand different types of nerve cells

Unipolar Cell



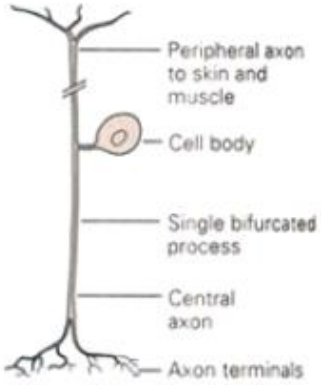
Invertebrate neuron

Bipolar Cell



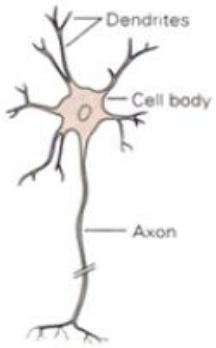
Bipolar cell of retina

Pseudo-unipolar Cell

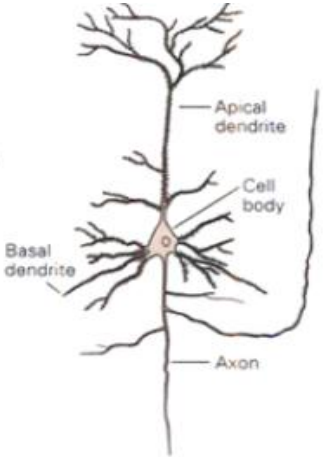


Ganglion cell of dorsal root

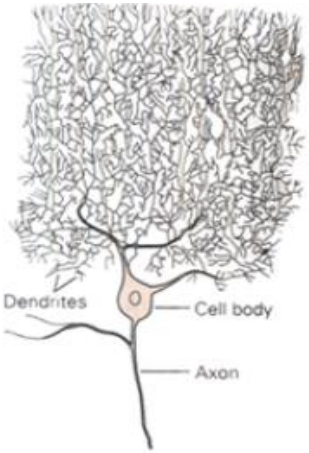
Three types of multipolar Cells



Motor neuron of spinal cord



Pyramidal cell of hippocampus



Purkinje cell of cerebellum

Complexity

Thousand different types of nerve cells

Recent years provide increasing evidence that glia can directly modulate the function of neurons.

Each using a mixture of analog and digital coding at the same time

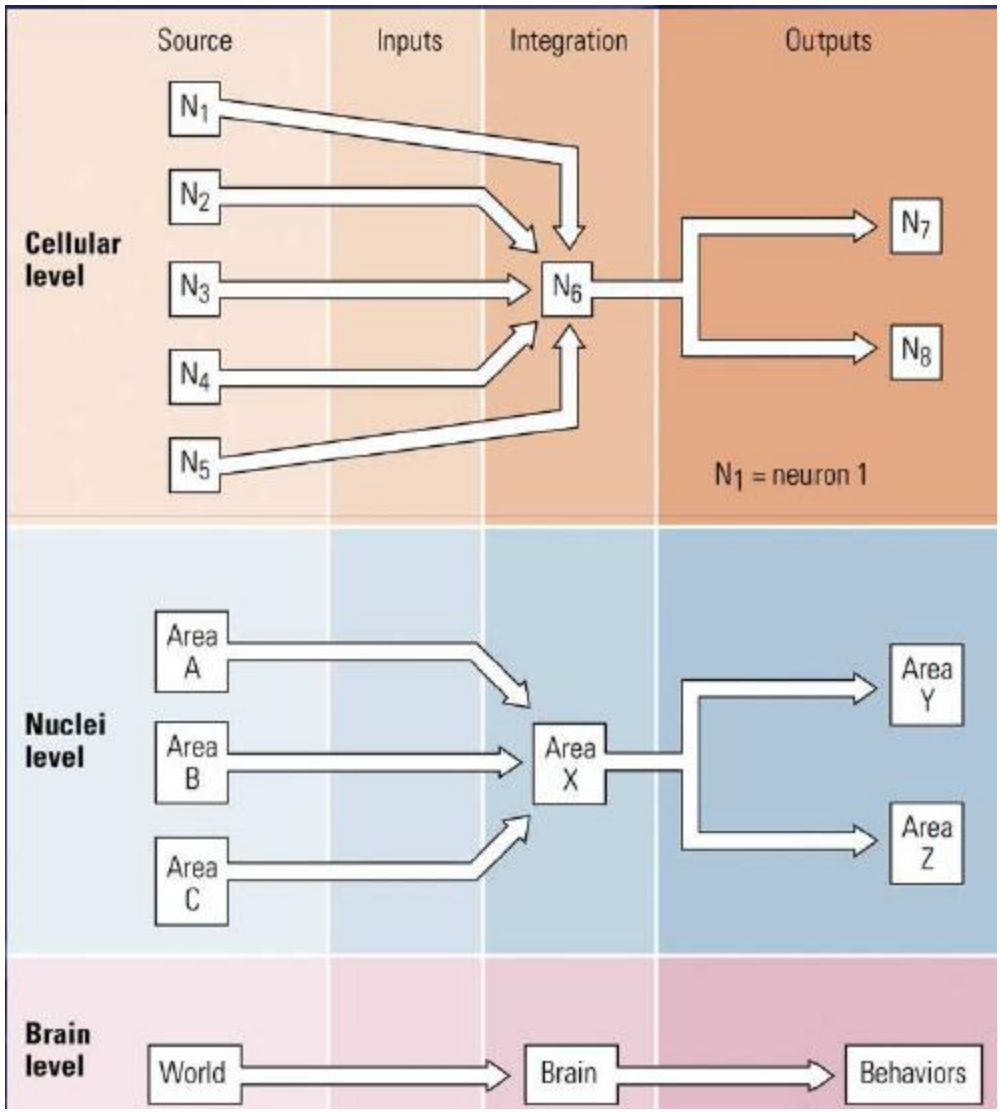
And can carry up to a million synapses

Dendrites are not passive collectors of signals – they don't simply add the excitatory and inhibitory inputs – they process the information
Some can generate "calcium spikes" and also there actually can be electrical connections (special synapses)

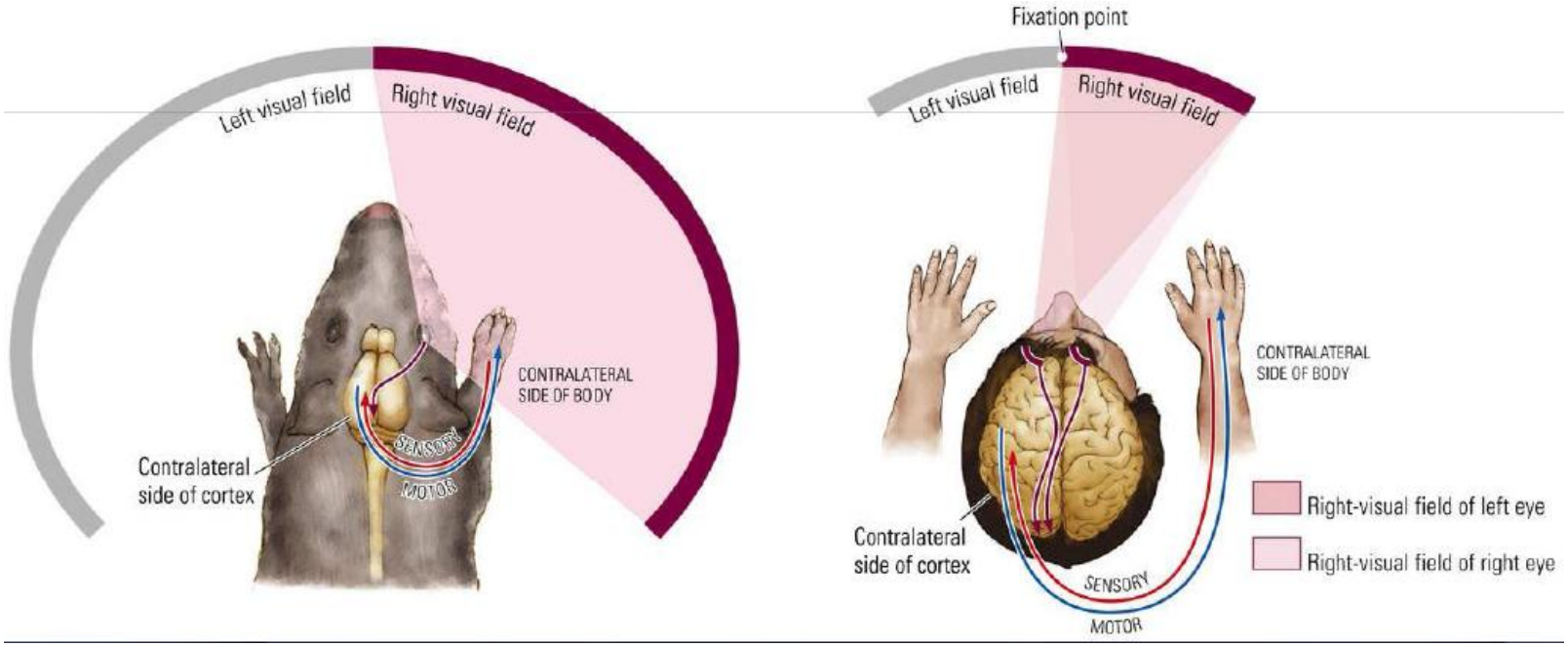
Large variety of ion channels on a neuron's surface with specific ion-selectivities, kinetics and thresholds determines membrane conductance and potential

Even if a repeating scheme of brain sequence processing is

In->Integrate->out



Many of the Brain's Circuits Are Crossed



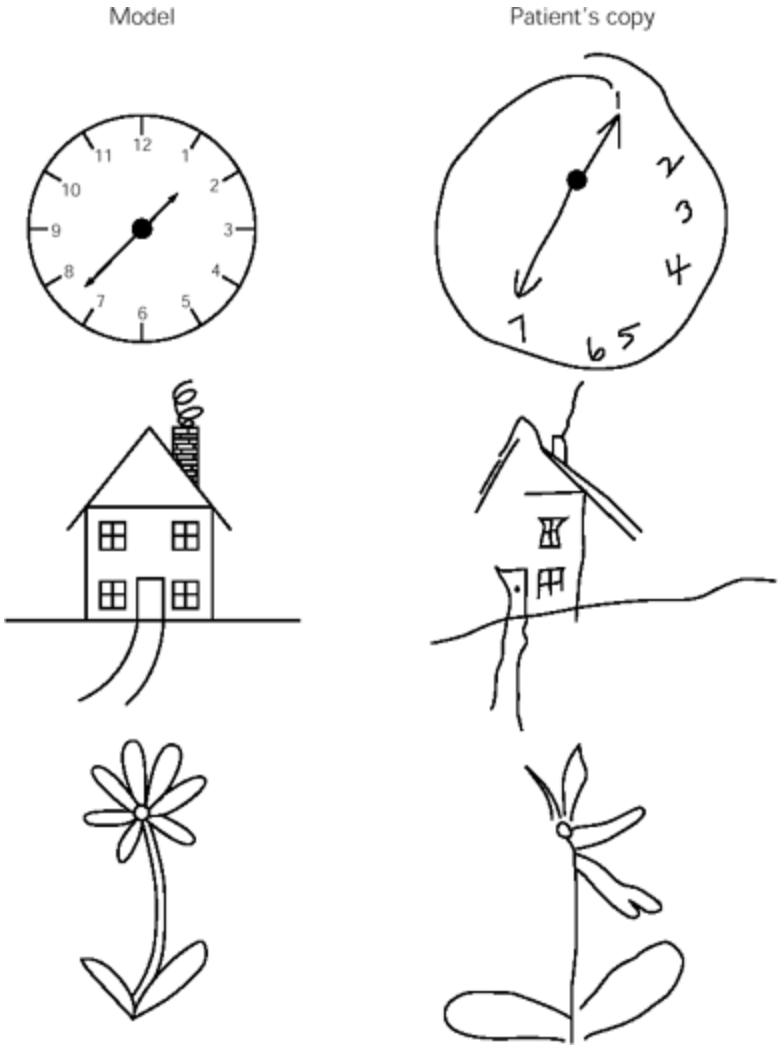
For example: Left Motor Cortex controls the right part of the body, while Right Motor Cortex controls the left part of the body.

Sensory areas of the brain are also primarily contralateral.
TWO COMMENTS:
* Symmetric brain areas in both hemispheres are interconnected via the corpus callosum and additional commisures: Thus, under normal conditions, information reaches both sides of the brain.
* In split-brain patients, Roger Sperry described asymmetries in some high cognitive tasks (language – left hemisphere, visuospatial – right hemisphere)

A note about brain cross laterality…

The principle of contralateral control holds also for some higher brain areas: For example, attempt to copy the model drawing revealed severe unilateral neglect, in a patient with lesions in the *right posterior* parietal cortex.

* Function is specific to brain areas and also to hemisphere.
* Asymmetry: Unilateral neglect primarily follows right-hemispheric lesions.

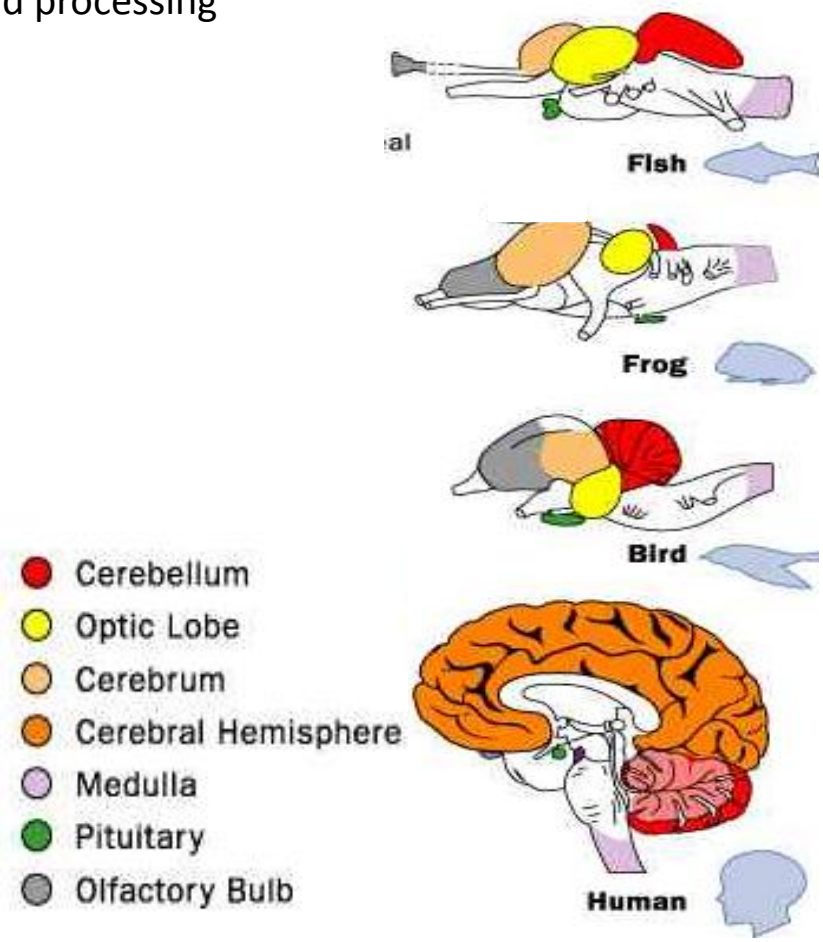


The Central Nervous System functions in multiple levels

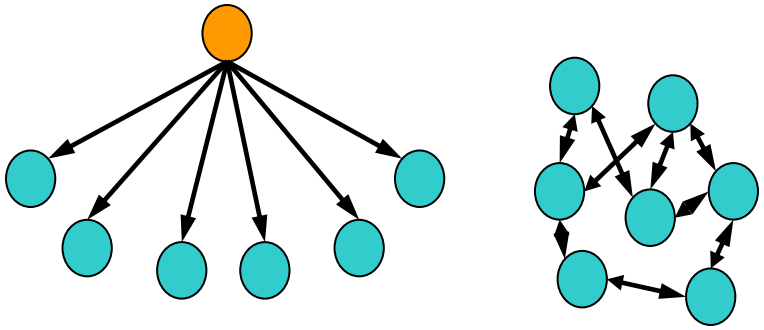
– "Descent with Modification" During evolution, new brain areas were placed on top of older ones

– Newer brain levels added increased control and processing

– Levels work together to produce behavior



Brain systems are organized both Hierarchically and in Parallel:



Functions in the brain are both localized and distributed

Because each function (e.g., language) has many aspects, it is not surprising that these aspects reside in widely separated areas of the brain

Binding Problem:
Because a single sensory event is analyzed by multiple parallel channels that do not converge onto a single brain region, there is said to be a problem in binding together the segregated analyses into a single sensory experience

i.e when we view a blue square and a yellow circle, some neurons signal in response to blue, others to yellow, and others to the shapes. how the brain ensure that the sensing of blue is coupled to that of a square shape?

The good news is:

the fundamental principles are simple,
highly conserved and ubiquitous

Example of conserved principles:

All animals share the same basic toolkit

Not only that the molecular and cellular properties of cells, and neurons among them, are highly conserved
(evolved even before multi-celled animals evolved)

The same principles and toolkits appear in different organizational levels
(molecular, cellular, network, tissue, organism)

Principles

Feedback Loops
Fast positive feedback
Slow negative feedback
Their balance leads to adaptive responses

Synchronization of coupled elements (activity from collective responses of ion channels, synapses, cells, tissues)

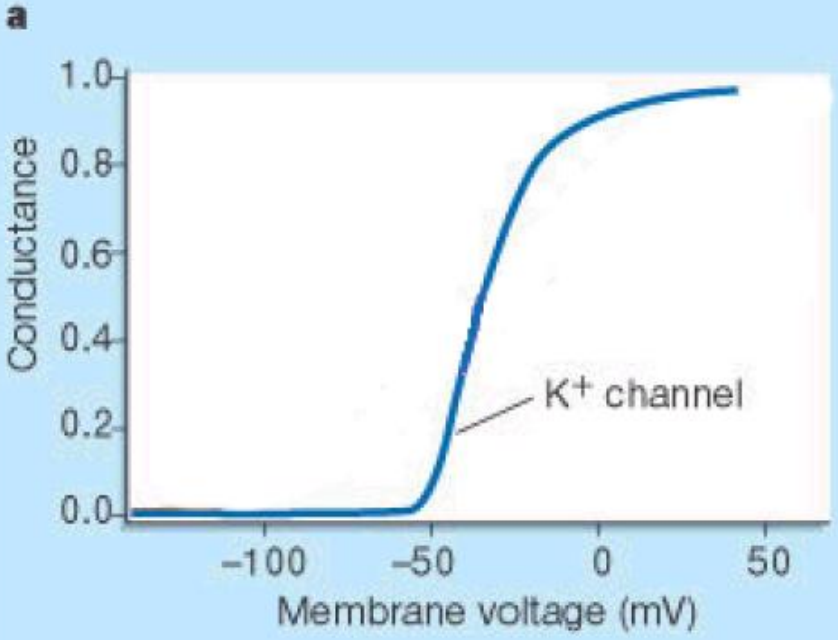
Oscillations

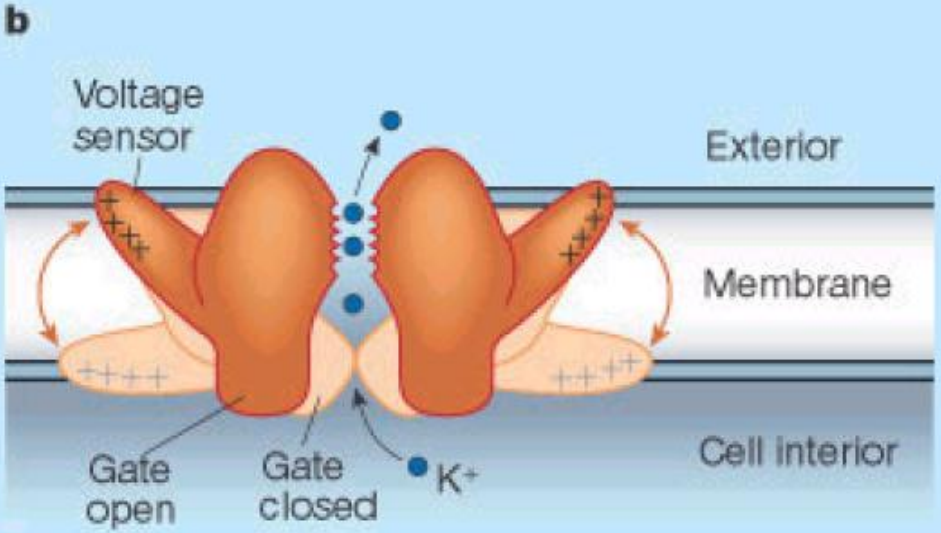
On the Molecular Level

Ion Channels:

- Neural activity results from transferring local stimuli into a pattern of ion fluxes
- The relevant response is mediated through timed (synchronized) opening and closing of ion channels

Voltage-gated channels:





Sigworth, F.J. (2003) *Nature* 423, 21-22

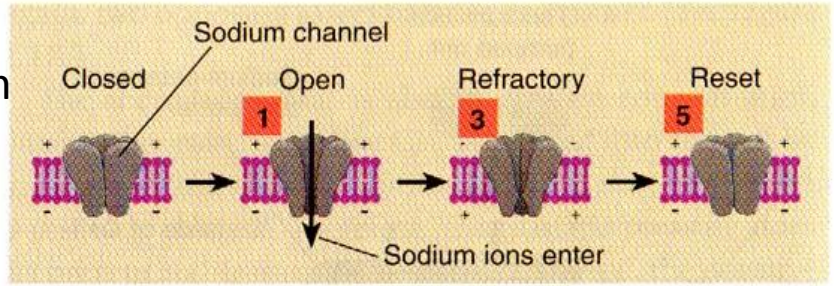
Voltage-gated channels:

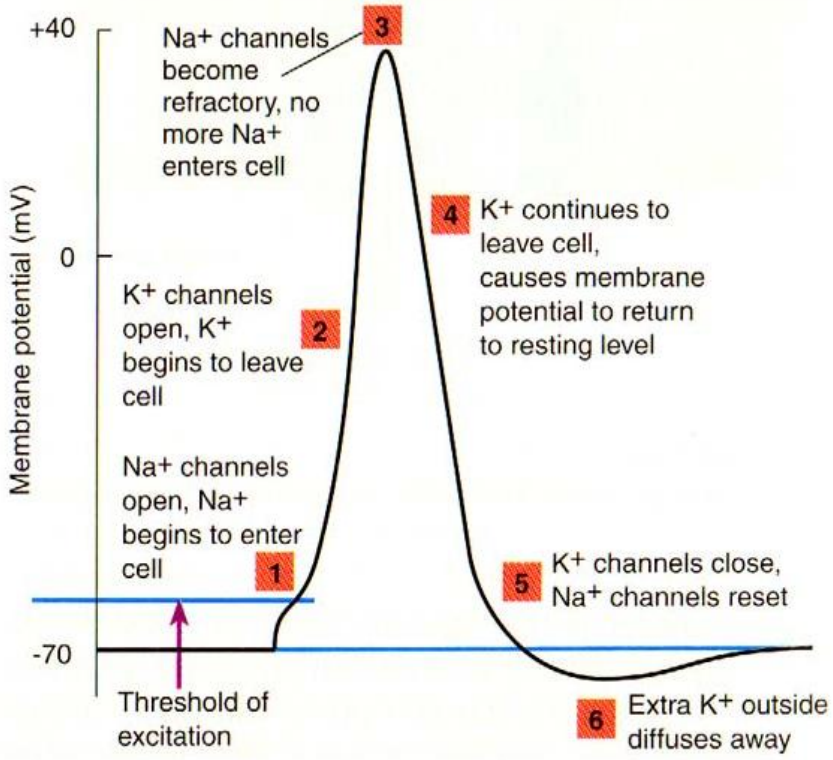
- There are at least 50 basic types
- They can be very specific about which ions they allow to pass
- Vary considerably in amount of charge required to activate them
- Some open when membrane is depolarized, some when it is hyperpolarized
- Important differences in the time-course of opening
- With a mix of voltage-gated channels neurons can perform almost any response

Voltage-gated channels

These facilitate the spread of information – the action potential

Stimulus causes sodium channels to open

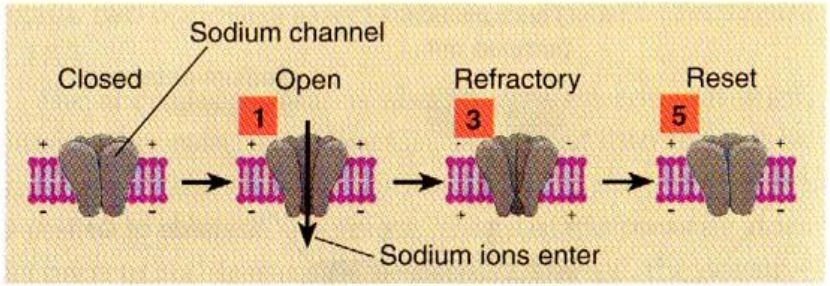


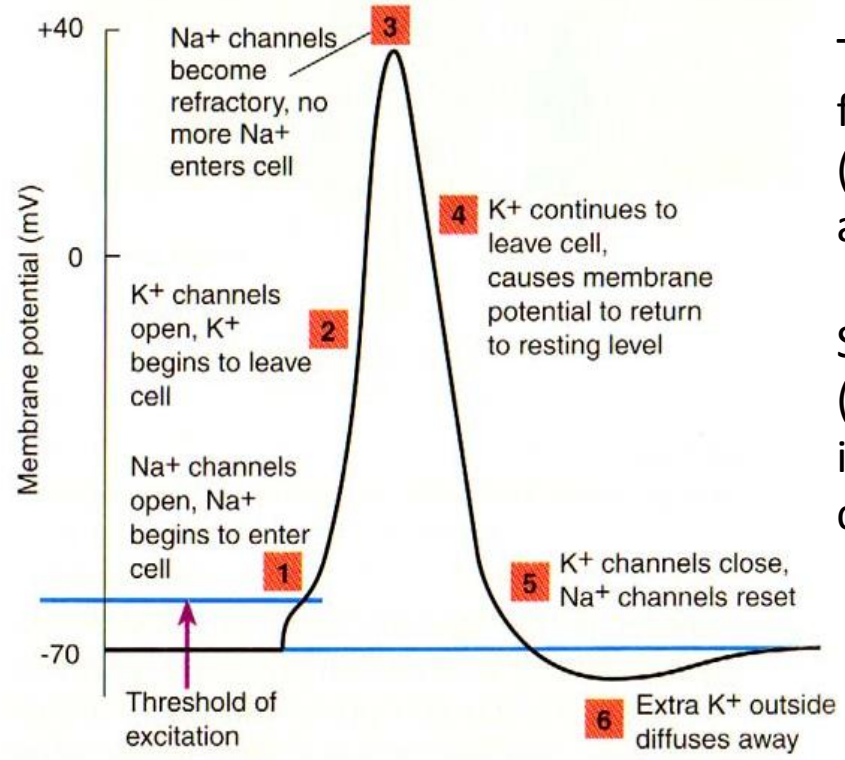


K^+ channels open with a delay, relative to Na^+

Voltage-gated channels

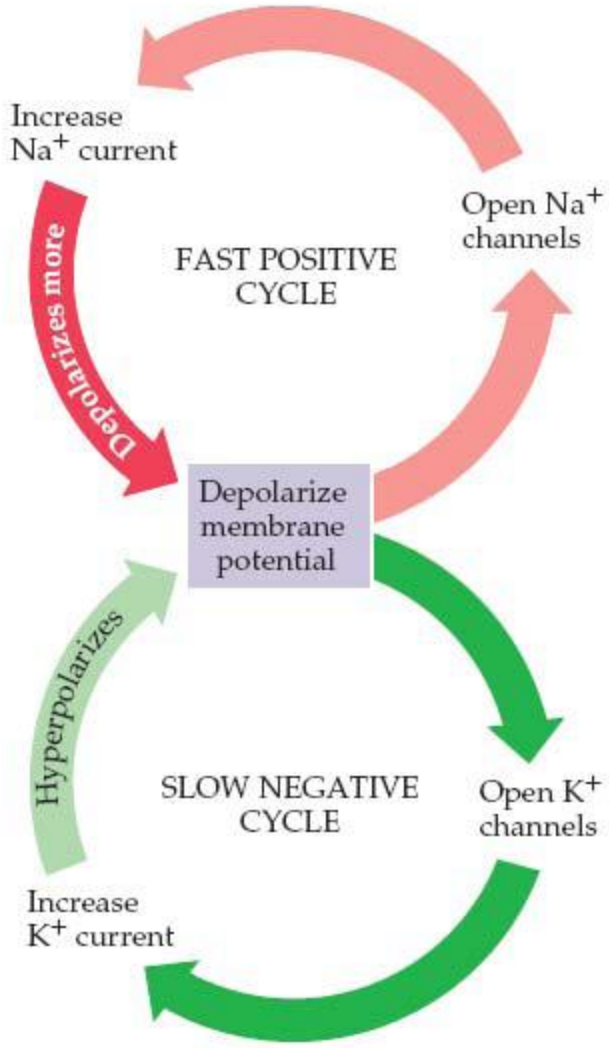
These facilitate the spread of information – the action potential





This means that there is fast positive feedback (sodium channel activation)

Slow negative feedback (sodium channel inactivation, potassium channel activation)


Increase
Na^+ current
Depolarizes more
FAST POSITIVE
CYCLE
Open Na^+
channels
Depolarize
membrane
potential
Hyperpolarizes
SLOW NEGATIVE
CYCLE
Open K^+
channels
Increase
K^+ current

Regulation and synchronization of Ion Channels

Ion channels influence their own behavior, such bas through self-excitation or self-deactivation

They also influence that of others through their influence on membrane potential, and sometimes through secondary messengers

There is a constant adjustment of their ratio, number and distribution to stabilize their behavior

How does a neuron become programmed to have a particular target of activity? Dynamic interplay between ion channels, receptors, secondary messengers that the neuron is born with, and changing gene expression over time of through modulation

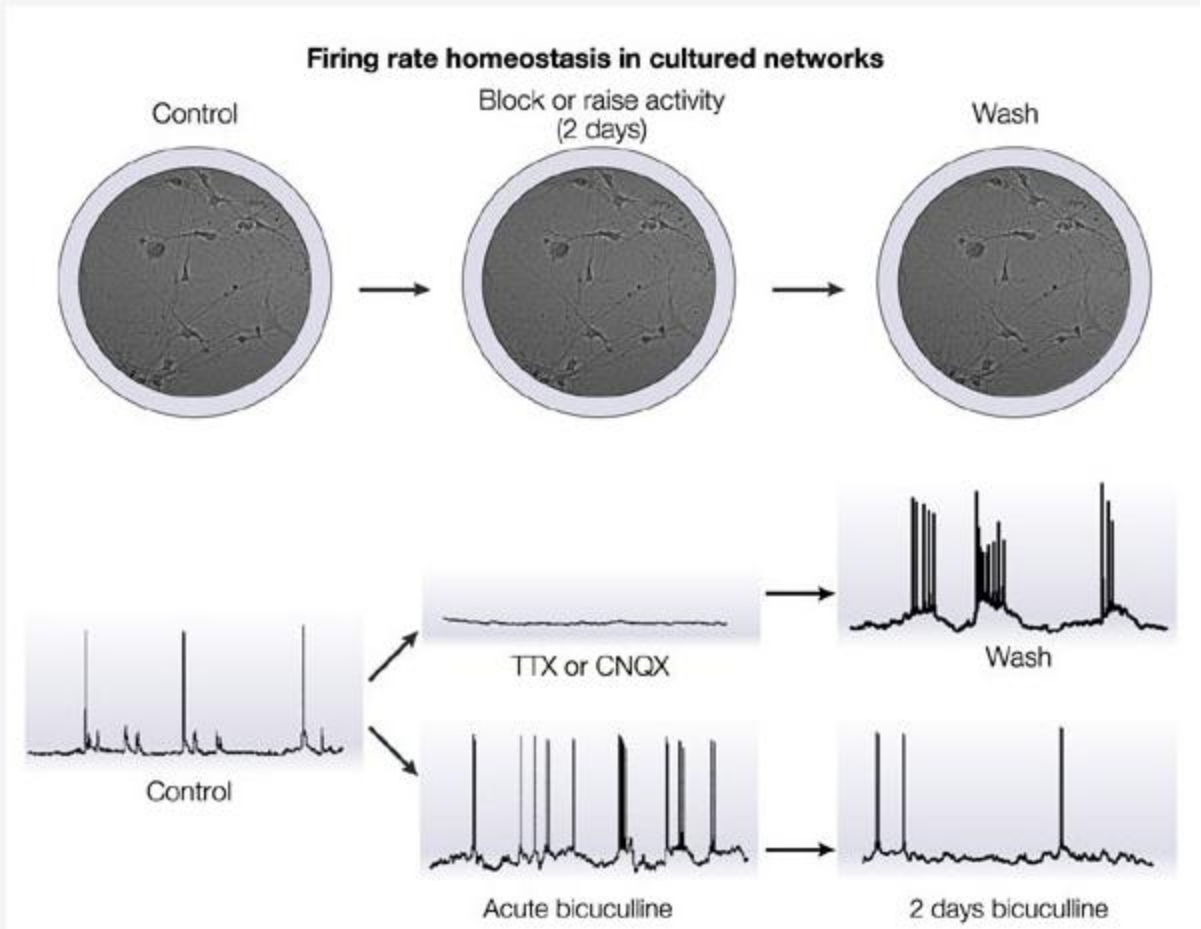
On the Network level

Homeostatic plasticity

The capacity of neurons to regulate their own excitability relative to the network activity

Fast positive feedback (Amplification) – Hebbian learning

Slow negative feedback (Regulation) – Homeostatic plasticity

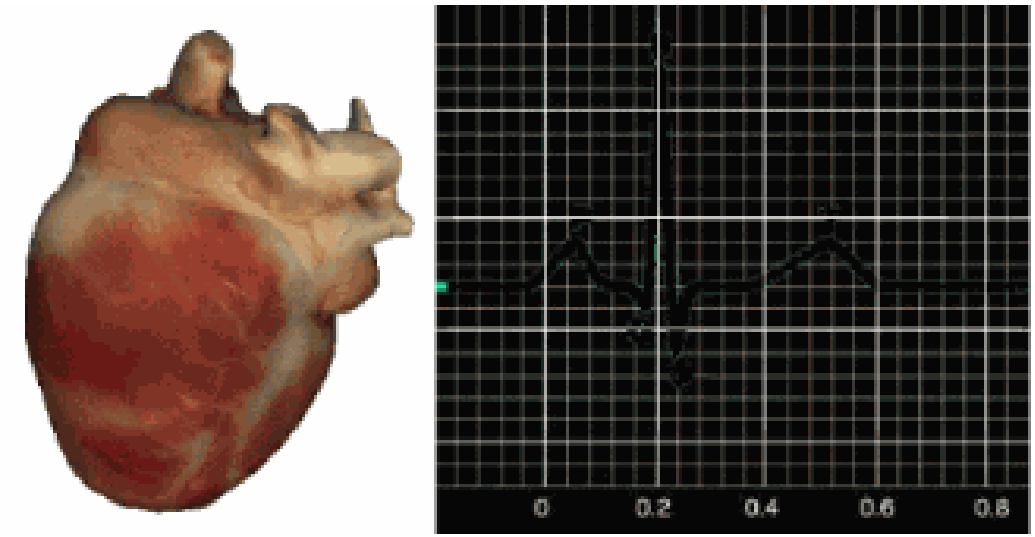


Another example, tissue level

Excitable heart cells

Propagation of electrical activity through the cells

Positive coupling (amplification) and negative (refractory/damping) feedback loops
Regulate the contraction delays between the atria and ventricles to allow efficient blood flow



An disruption of these leads to Cardiac arrhythmia

Interlinked fast and slow feedback loops drive reliable cell decisions (Brandman et al, Science 2005)

System	feedback loops	
Mitotic trigger	Cdc2 → Cdc25 → Cdc2 Cdc2 -| Wee1 -| Cdc2 Cdc2 -| Myt1 -| Cdc2	(*12, 13*)
p53 regulation	p53 → PTEN -| Akt → Mdm-2 -| p53 p53 → p21 -| CDK2 -| Rb -|Mdm-2 -| p53	(*14*)
Xenopus oocyte maturation	Cdc2 → Mos → Cdc2 Cdc2 → Cdc25 → Cdc2 Cdc2 → Myt1 → Cdc2	(*11*)
Budding yeast traversal of START	Cdc28 → Cln transcription → Cdc28 Cdc28 -| Sic1 -| Cdc28	(*15*)
Budding yeast polarization	Cdc42 → Cdc24 → Cdc42 Cdc42 → actin → Cdc42	(*6, 16, 17*)
Eukaryotic chemotaxis	PIP_3 → Rac/Cdc42 → PIP_3 PIP_3 → Rac/Cdc42 → actin → PIP_3	(*18*)
Muscle cell fate specification	MyoD → MyoD Myogenin → myogenin MyoD → CDO → MyoD MyoD → Akt2 → MyoD	(*19–21*)
B cell fate specification	IL-7 → EBF → IL-7 EBF -| Notch-1 -|E2A → EBF → Pax-5 -| Notch-1 -| E2A→ EBF	(*22, 23*)
Notch/delta signaling	Notch (cell A) -| Delta (cell A) -| Notch (cell A) Notch (cell A) -| Delta (cell A) → Notch (cell B) -| Delta (cell B) → Notch (cell A)	(*24*)
EGF receptor signaling	EGFR -| PTP -| EGFR Sos → Ras → Sos ERK2 → arachidonic acid → ERK2 EGFR → sheddases → EGFR	(*25–28*)
S. cerevisiae galactose regulation	Gal2 → galactose -| Gal80 -| Gal2 Gal3 -| Gal80 -| Gal3	(*29*)
Blood clotting	thrombin → Xa:Va → thrombin XIIa → XIIa IXa:VIIIa → Xa → IXa:VIIIa	(*30*)
Platelet activation	activation → ADP secretion → activation activation → 5-HT secretion → activation activation → TxA_2 secretion → activation activation → aggregation → activation	(*31*)
Ca^{2+} spikes/oscillations	Ca^{2+}_{cyt} → PLC → IP_3 → Ca^{2+}_{cyt} Ca^{2+}_{cyt} → IP_3R → Ca^{2+}_{cyt} Ca^{2+}_{cyt} → IP_3R -| Ca^{2+}_{ER} -| SOC → Ca^{2+}_{cyt}	(*7, 8*)

Synchronization and Oscillations

(classic definition of sync) adjustment of rhythms of self-sustained periodic oscillators due to their weak interaction
This was extended to include objects such as rotators and chaotic systems

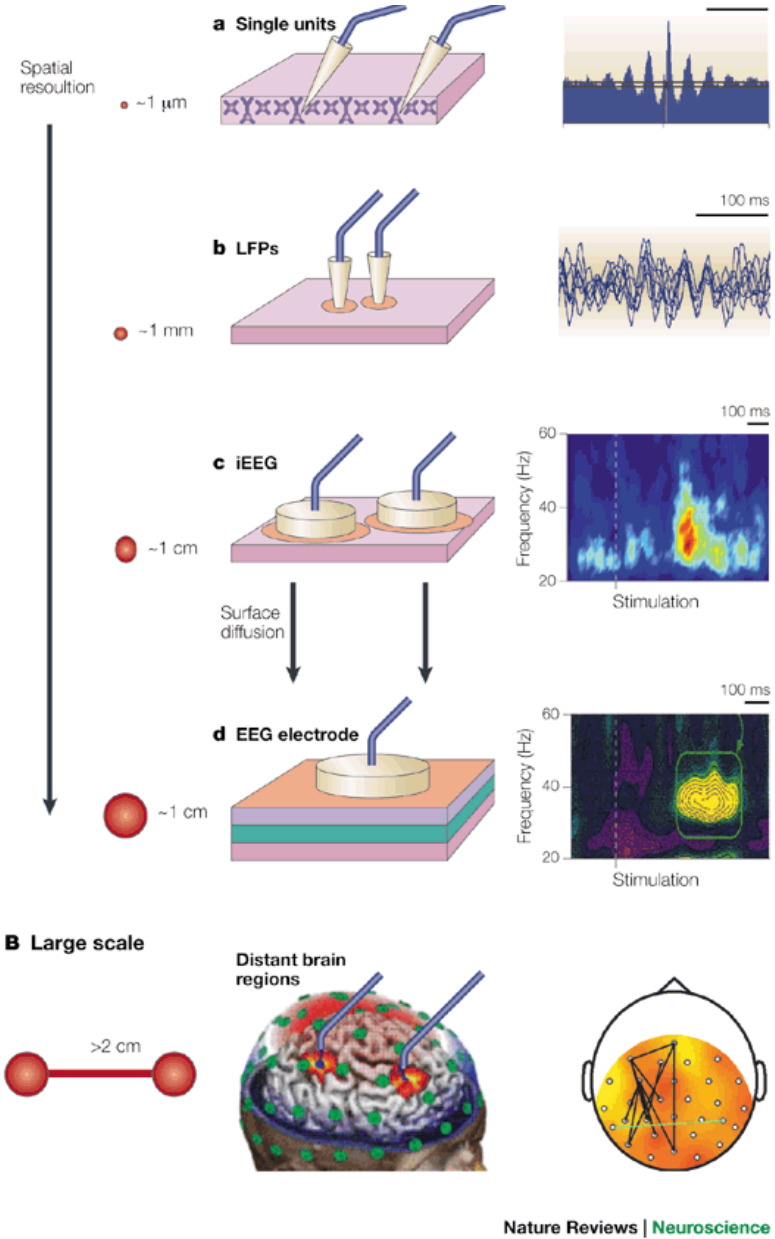
"One of the most pervasive drives in the Universe" Steven Strogatz

Why is this important?

Our lives depend on it – e.g. calcium regulations, pacemaker cells, brain waves (memory storage), circadian rhythm, control of rhythmic behavior (breathing, chewing, walking) and in general to turn microscopic phenomena into macroscopic responses (ion channels and action potential)

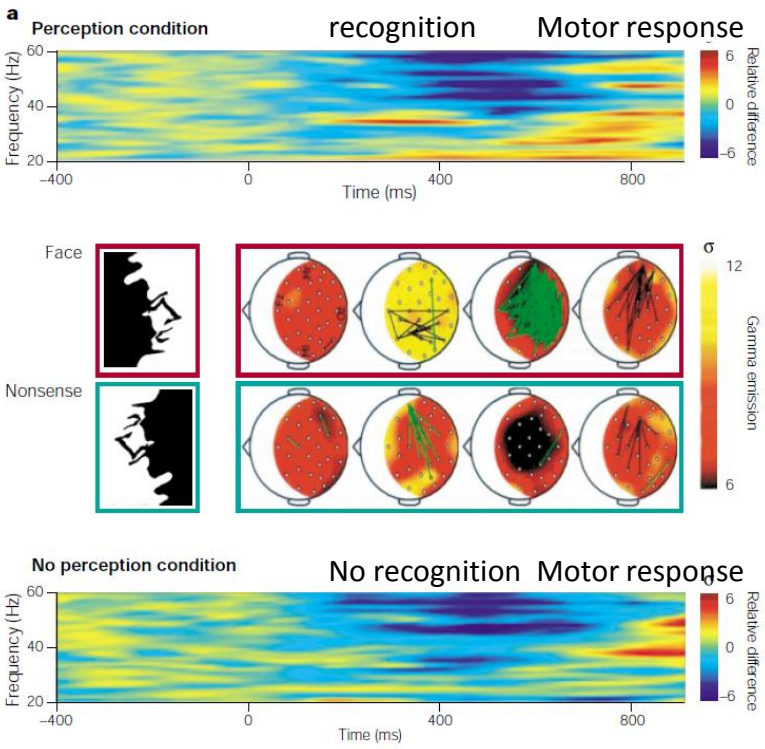
Synchronization

Local scale: within a small brain region



Large scale: through either cortico-cortical fibres or thalamocortical reciprocal pathways

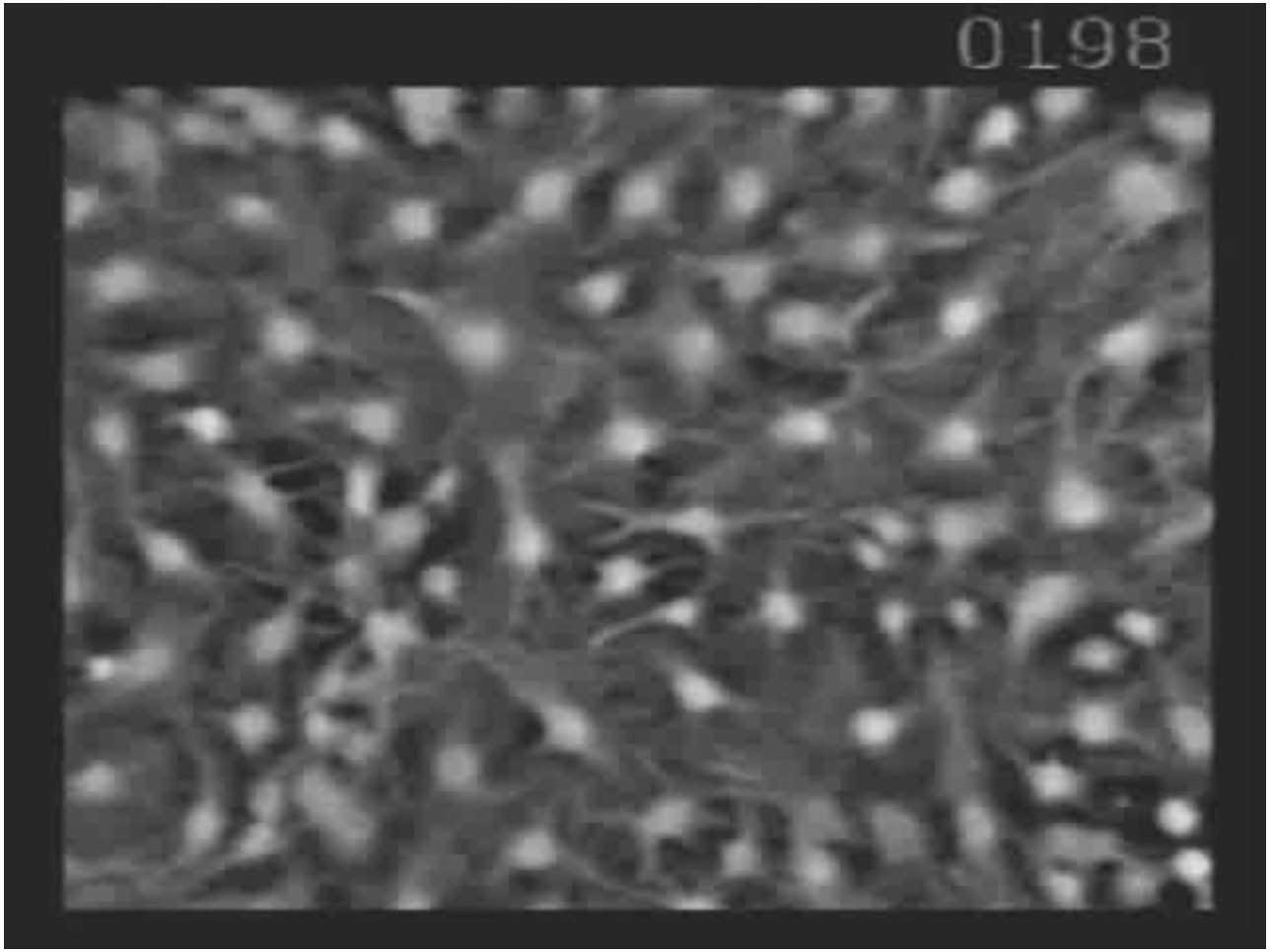
patches of local synchrony in distant brain sites can enter into synchrony during cognitive tasks.



Synchronization and Oscillations

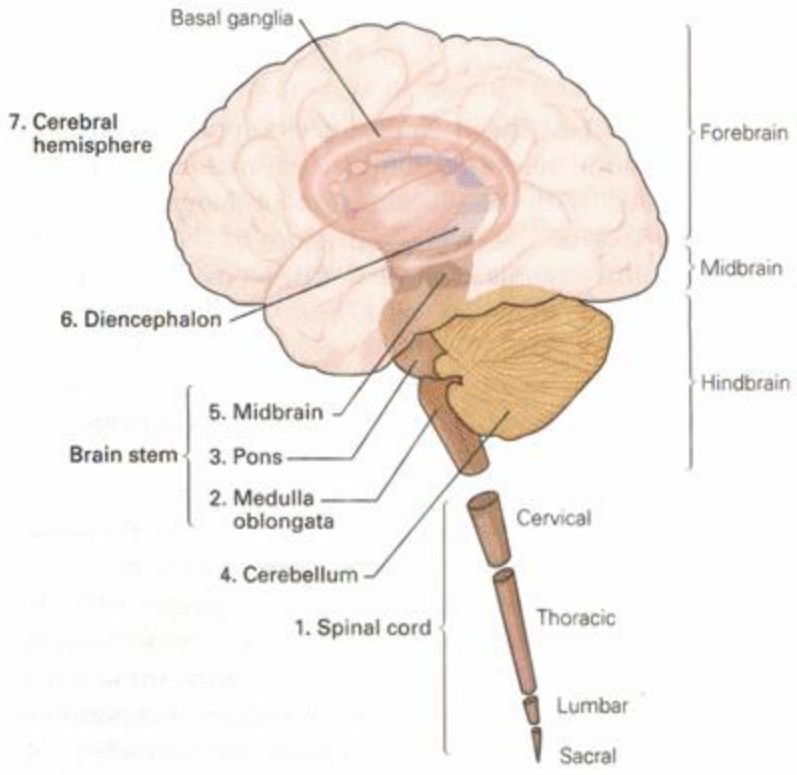
Derived from self-organization

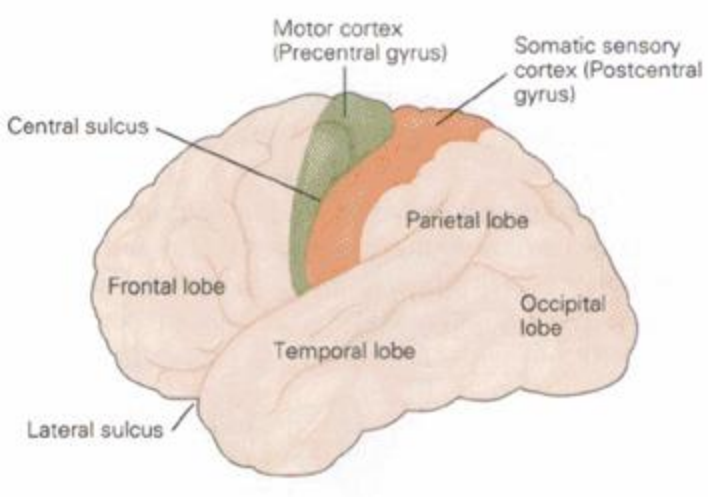
Provides deep insight into how spontaneous order emerges in biological systems



Studying brain's Spatial organization:

Brain areas differ in structure – and have different functions



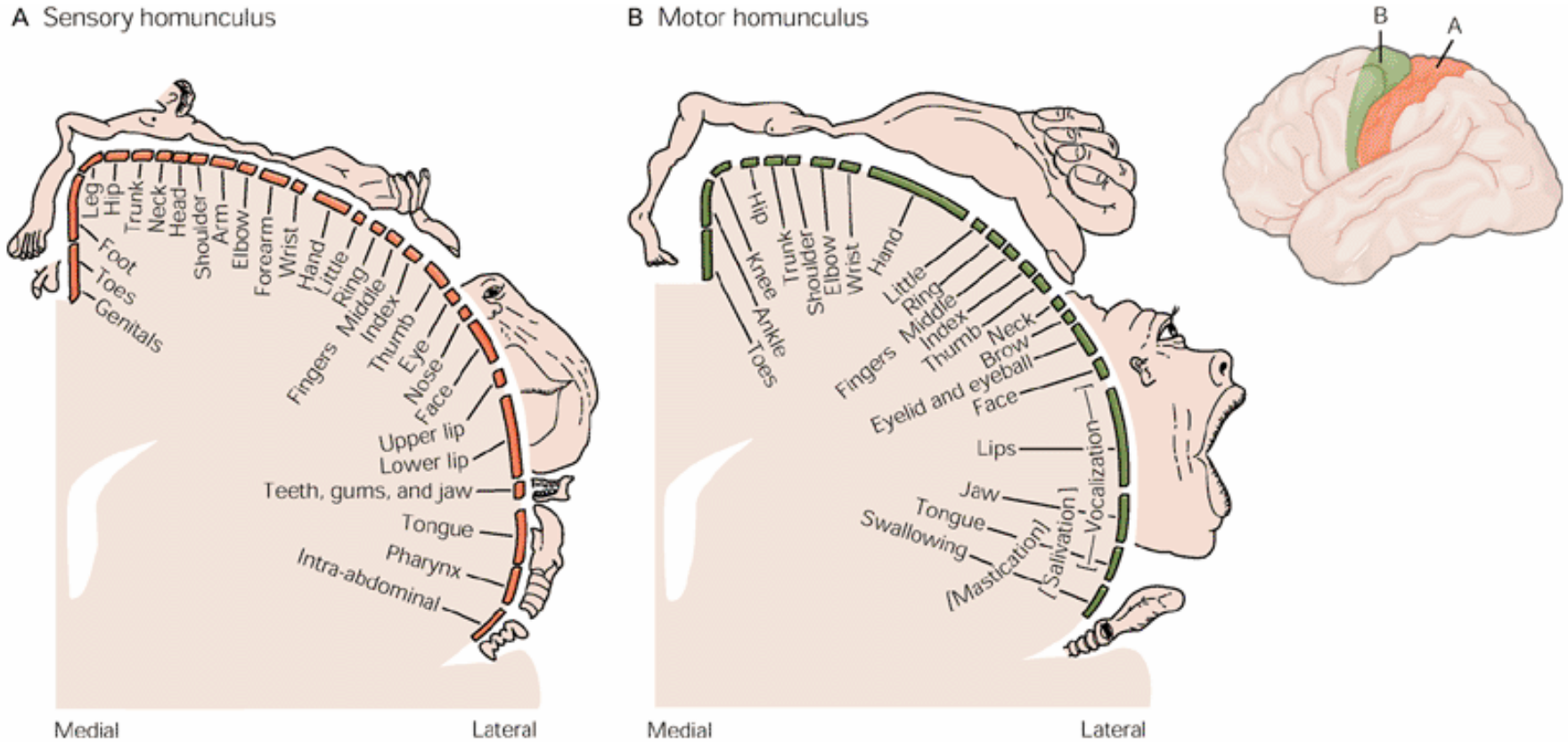


i.e Motor cortex and somatosensory cortex are located on different gyri, and are separated by the central sulcus

There is a strong spatial organization in which nearby neurons often have similar connectivity (and function).

The homunculi

The description of which parts of the cortex directly responsible for which parts of the body movement and sensation

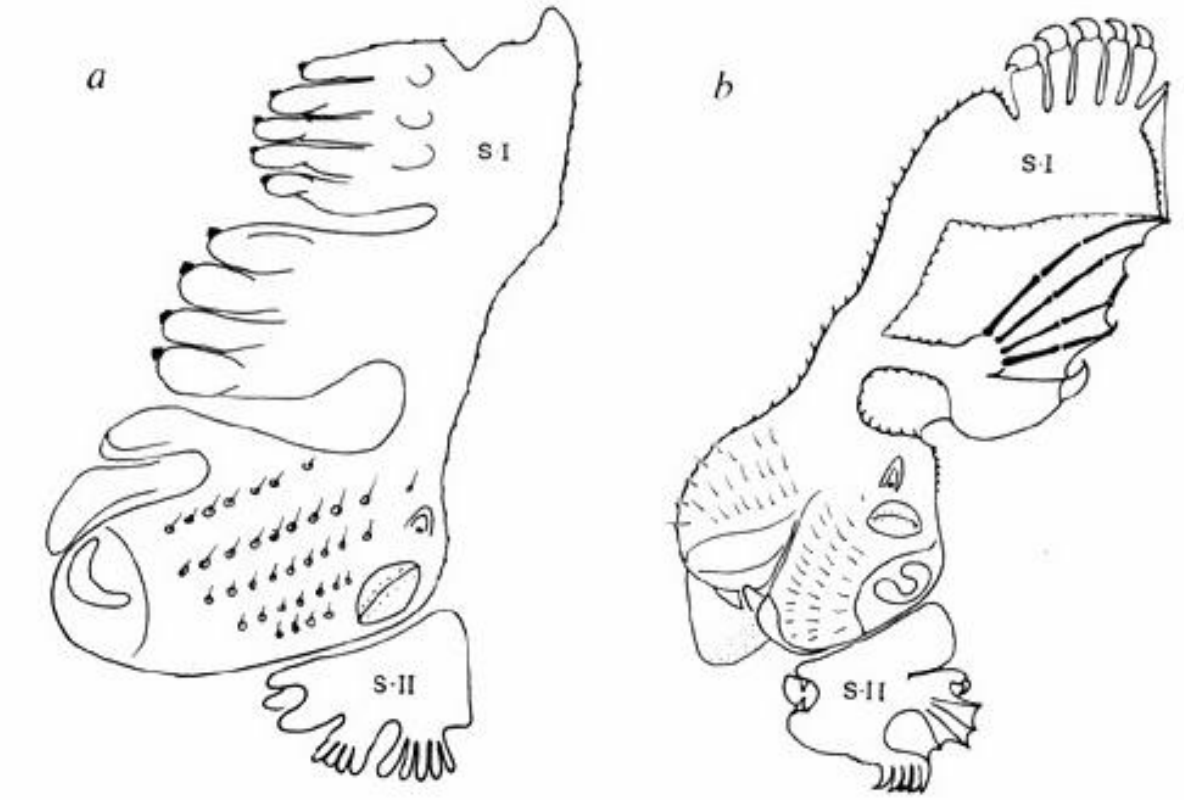


The homunculi were discovered by Wilder Penfield (pub in 1951), by stimulating different cortical regions in human patients undergoing brain surgery (epileptic patients).

Analogs to the homunculus were found in numerous species

Rat-unculus

Bat-unculus



The maps order are usually consistent across species, however larger areas of cortex are devoted to body parts that are important for the specific species (e.g. face and fingers in humans ; face, wings and thumb in bats).

Note that there are multiple maps of the body (S-I, S-II...).
This multiplicity of maps generally applies to other senses (Calford et al. *Nature 1985)*.

However:

• Not all brain regions have columns or maps. *Example: Hippocampus (no* columns – nearby neurons have different place coding).

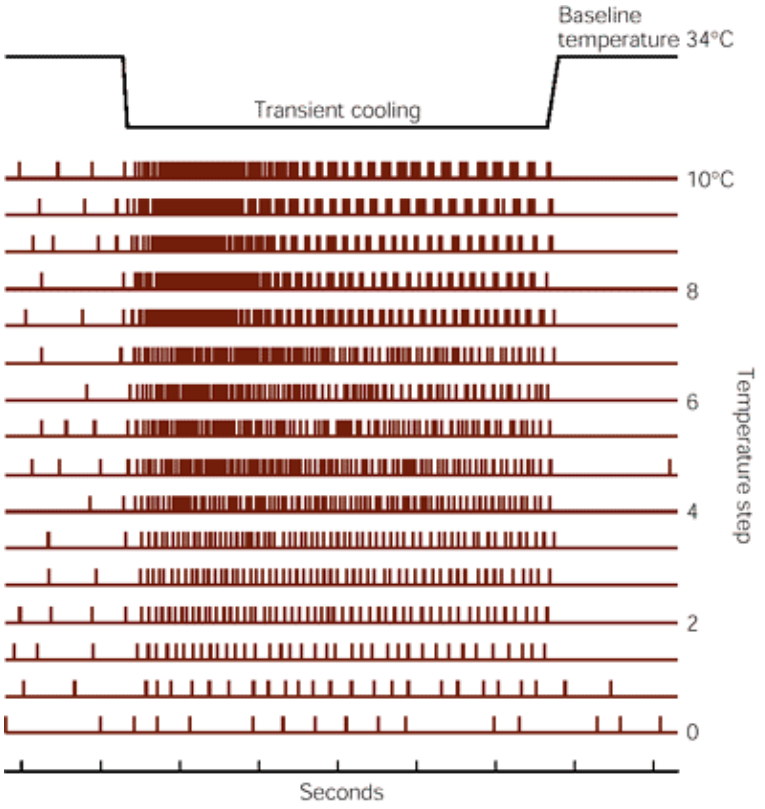
• Even in the cortex, there are stimulus properties that are arranged in columns (nearby neurons do similar things) but *not in maps (no large-scale* organization of the columns).

• In addition to understanding brain structures and topology it is important to learn the temporal dynamics (the neural coding), which constitutes the 'functional connectivity' maps

Temporal information according to neural dynamics

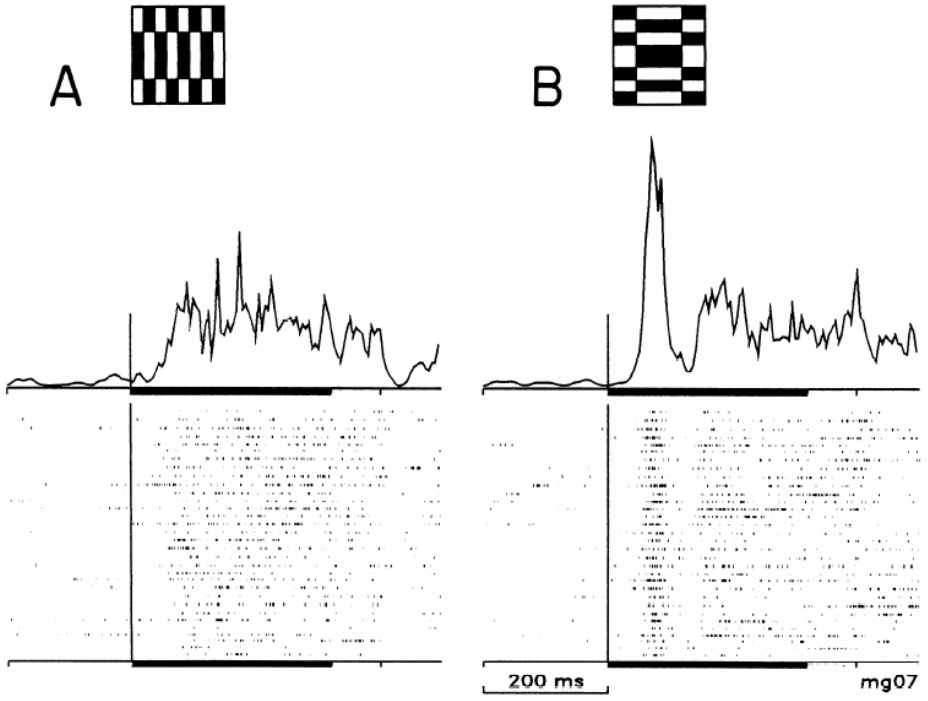
Rate Coding:

Example: a cold receptor encoding temperature cooling by changes in its firing rate



Temporal Coding:

Example: Visual cortex neuron responding with the same firing-rate, but with different temporal patterns to two stimuli



Richmond et al. (1990)

Another type of information could be encoded by the firing-rate adaptation (Gradual *decrease in the neuron's* firing rate during the presentation of a *constant stimulus).*

Neural Coding

- **Rate coding: Stimulus identity is encoded by the neuron's firing-rate** (while the temporal dynamics of the firing is irrelevant.
- **Temporal coding: Stimulus identity is encoded by fine temporal dynamics of the** neuron's response, or even by the precise timing of spikes at the millisecond level.
- **Labeled-line coding: Stimulus identity is encoded by the identity of the active** neuron (active / non-active)
- **Oscillation coding: Example of temporal coding, where information is carried by** neural oscillations, or by the firing phase of neurons relative to ongoing oscillations.
- **Population coding: Stimulus identity is encoded by groups of neurons.**
- **Synchrony coding: Example of population temporal coding, where information is** carried by synchronization between groups of neurons (cell assemblies), even without changes in firing-rate or temporal dynamics of individual neurons.

Neural Coding

- Neural responses depend on stimulus history (i.e adaptation) and stimulus probability:

p1 p1 p1 p2 p1 p1 p1 p1 p2 p1 versus p2 p2 p2 p2 p1 p2 p2 p2 p1 p2 p2 p2

Responses to the same physical stimulus (p1 vs. p2) differ depending on its probability –
sensory neurons can perform *novelty detection (Ulanovsky et al., Nature Neurosci 2003)*

This is energetically efficient (Adaptation to stimulus statistics optimizes neural coding)

Methods in Neuroscience - Measuring neural activity

	Spatial Resolution		Temporal Resolution	
	signal	**neurons**	**signal**	**spikes**
behavior	brain	$>10^{11}$	10ms	10
fMRI	>200µm	$>10^{5}$	>10s	1000
EEG	100 mm	$>10^{9}$	50ms	50
MEG	100mm	$>10^{9}$	50ms	50
ECoG	1-10mm	$>10^{4-6}$	10ms	10
VSD Imaging	30µm	<100	1ms	<1
µElec	10µm	1	1ms	1
Intracellular elec	10µm	<1	<1ms	1
Ca imaging	1µm	<1	1ms	>100

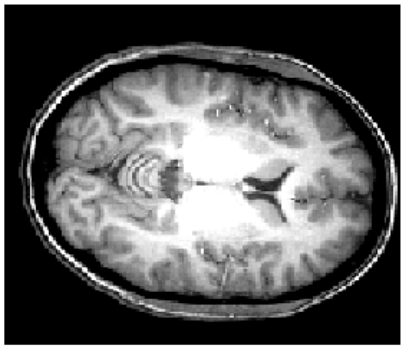
Tradeoffs between spatial-temporal resolution and the sampling size

MRI vs. fMRI

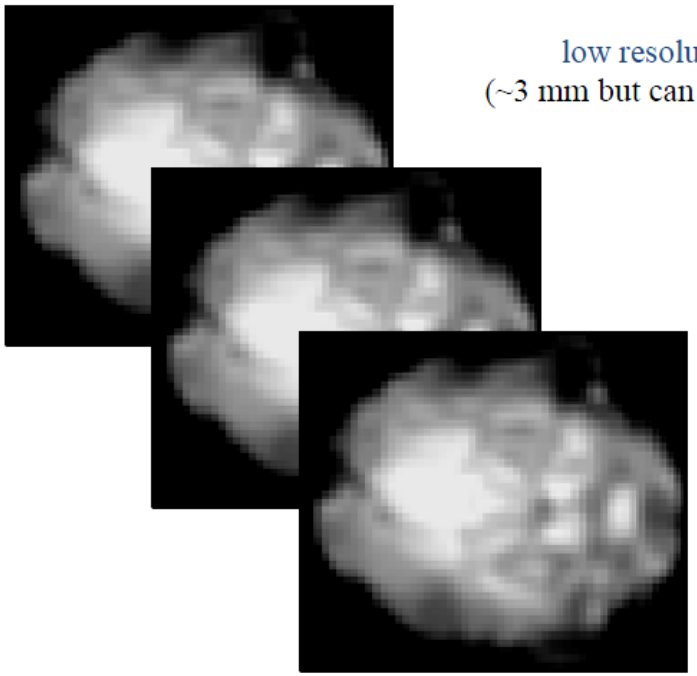
MRI

fMRI

high resolution (1 mm)



one image



low resolution (~3 mm but can be better)

many images (e.g., every 2 sec for 5 mins)

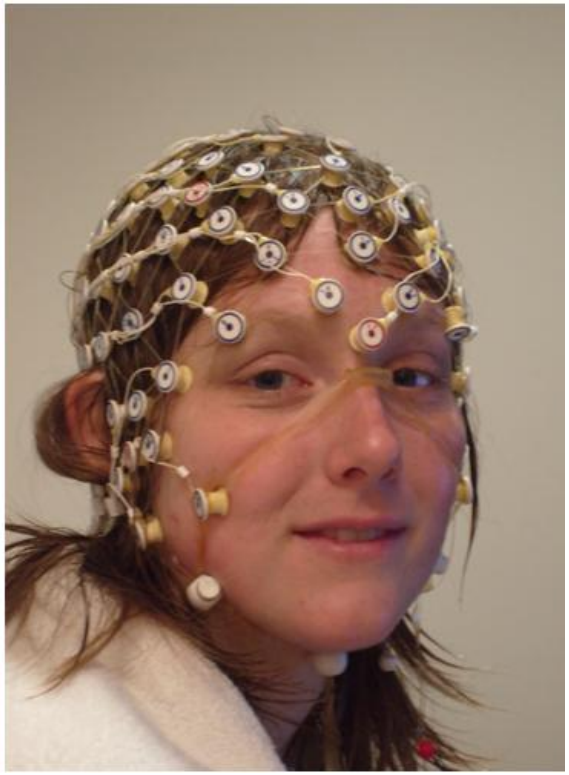
fMRI – Blood Oxygenation Level Dependent. An indirect measure of neural activity

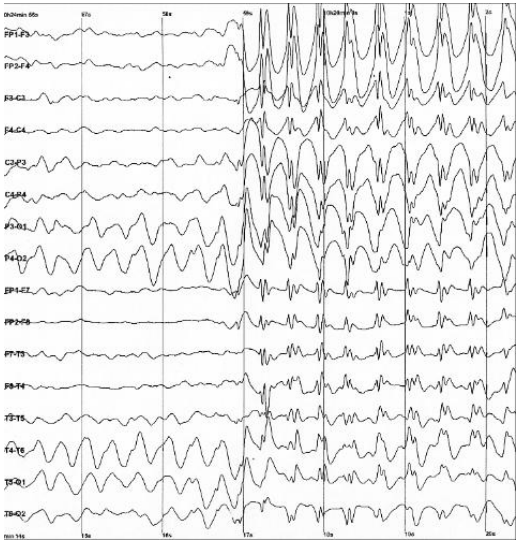
↑ Neural activity -> ↑ blood oxygen -> ↑ fMRI signal

Similar principles are for PET imagining that relies on increased delivery of injected radioactive matter when blood flow increases

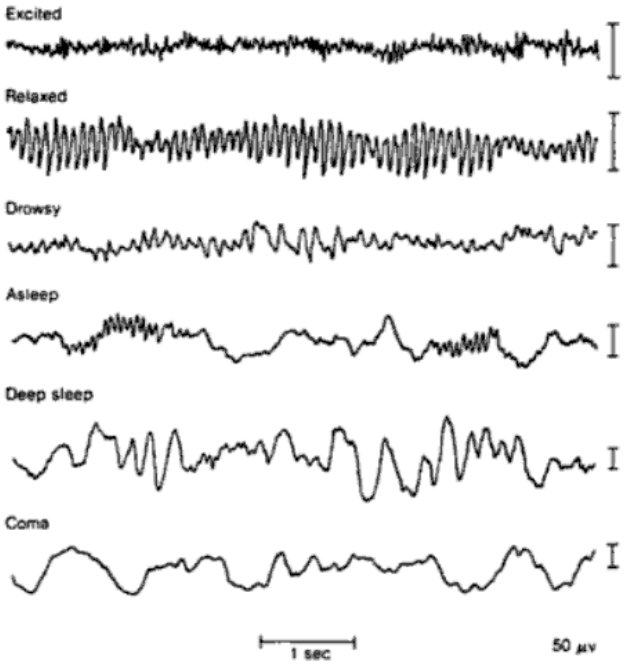
Electroencephalography (EEG)

Scalp electrodes measure the summed electrical activity of large populations of synchronously active neurons





Epileptic spike and wave discharges monitored with EEG.



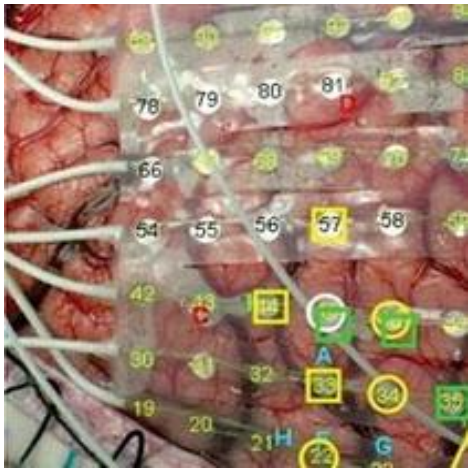
Characteristic EEG recorded during various behavioral states in man. Penfield and Jasper 1954

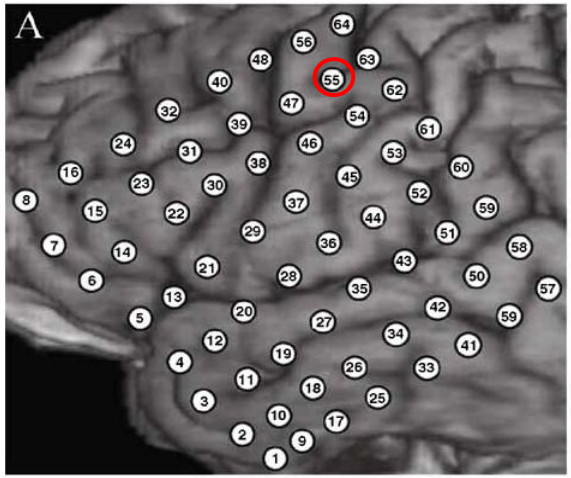
MEG detects magnetic fields generated by ionic currents. Has similar spatial (poor)/temporal (not bad) resolutions and only show synchronized and coordinated currents, mainly from cortical pyramidal cells

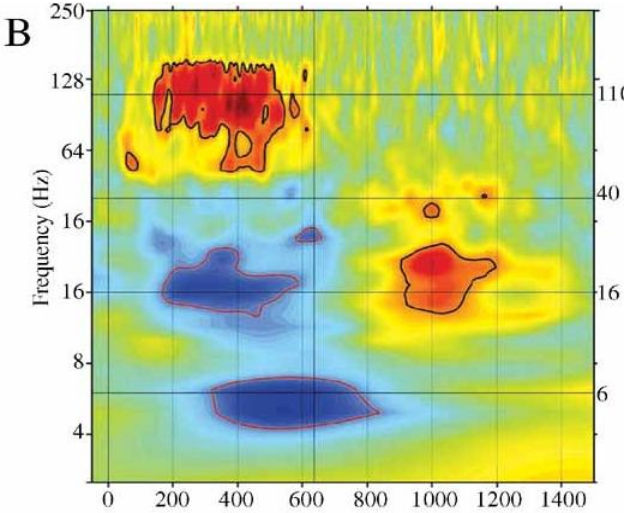
Electro-cortico-graphy(ECoG)

An invasive procedure in which electrodes are placed on the surface of the brain.
The method is used mainly in the treatment of epilepsy (currently the standard method for defining epileptogenic zones in clinical practice), but also used to collect experimental data

There is now extensive study to develop non-invasive methods with similar prediction levels by combining MRI and EEG methods to image the locations and estimate the extents of current sources from the scalp EEG.







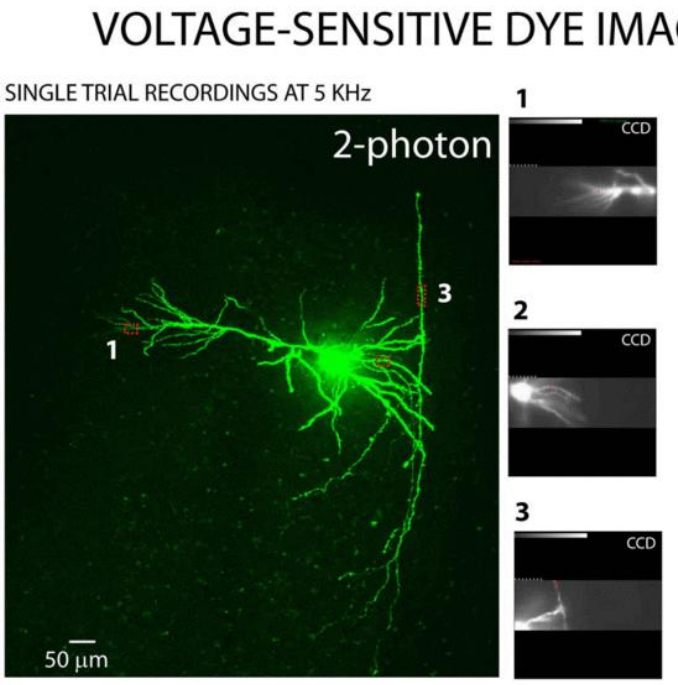
Event-related time–frequency plot for ECoG response at electrode 55. Canolty et al 2011

Voltage Sensitive Dye (VSD)

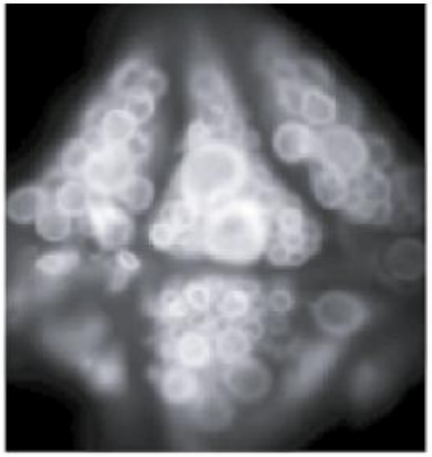
Dyes which change their spectral properties in response to voltage changes.

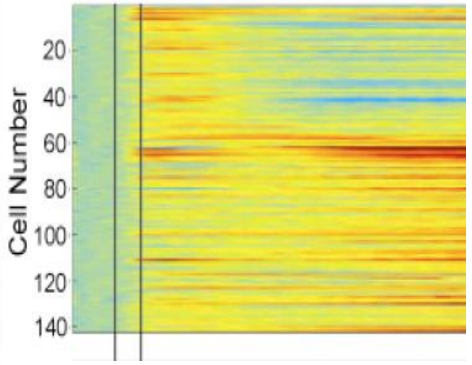
Measures the weighted sum of membrane-potential changes in neuronal somata, dendritic and axonal arbors, and often glia

The dye signal has good spatial resolution as it is restricted to the site of the electrical activity



optical recordings of an action potential detected from different sites on axonal and dendritic processes of a neuron stained with a styryl dye (Zecevic et al. (2009))



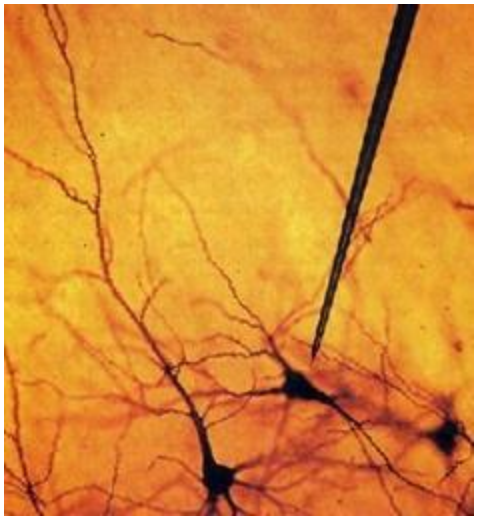


Activity can be recorded from a large fraction of the cells in an invertebrate action potentials ganglion

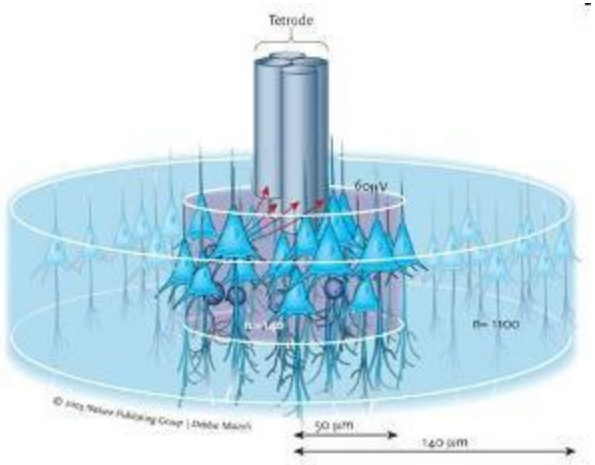
Microelectrode recordings

Exctracellular recording of local field potential and single-unit activity (using spike sorting)

Used extensively in animal studies
Great spatio/temporal resolution but very limited sampling



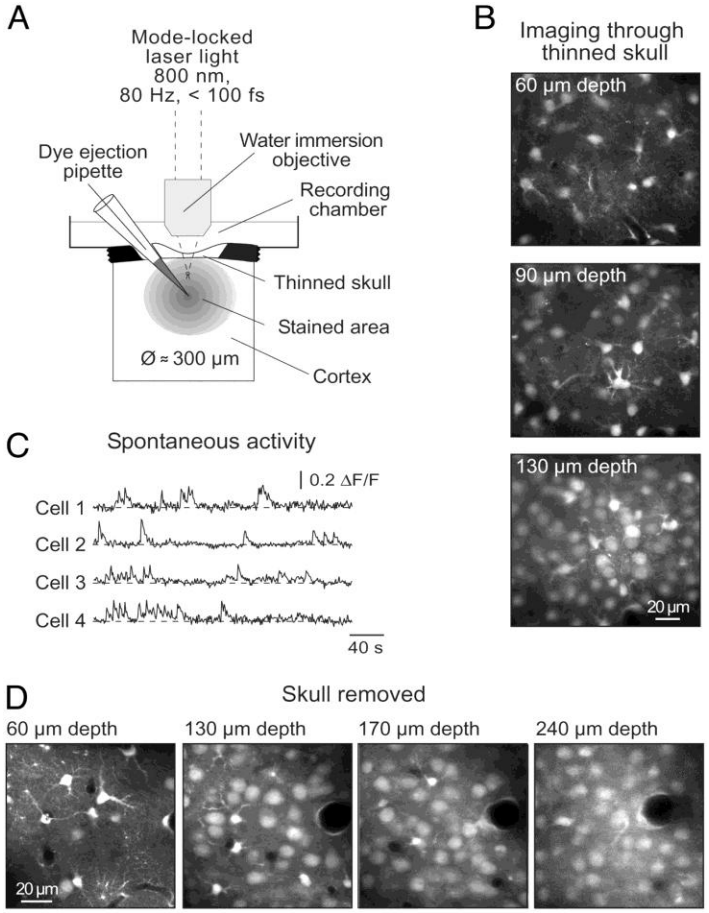
Multiple single unit recording using tetrodes



Calcium imaging

Imaging level of activity using calcium indicators (chemical or genetically encoded indicators), molecules that can respond to the binding of Ca^{2+} ions by changing their spectral properties.

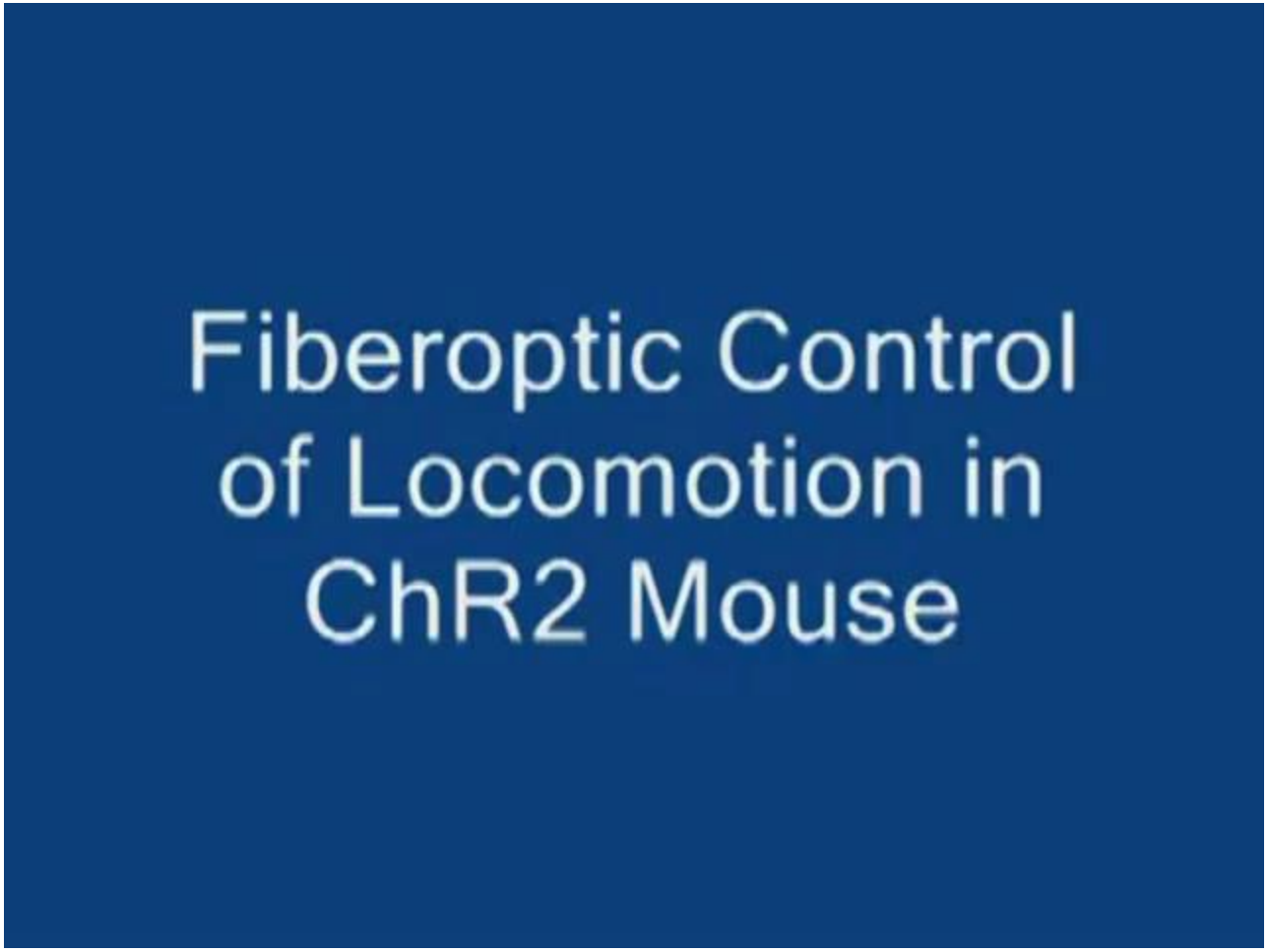
In vivo calcium imaging of neuronal populations.



Genetically encoded indicators do not need to be loaded onto cells, instead the genes encoding for these proteins (i.e GFP) can be transfected to cell lines. It is also possible to create transgenic animals expressing the dye in all cells or selectively in certain cellular subtypes.

Stosiek C et al. PNAS 2003

This lead to a more recent technique OPTOGENETICS developed to probe genetically targeted neurons within within intact animals for optically stimulating specific cells at the high speeds (milliseconds)



Many of the Brain's Circuits Are Crossed