

CELLULAR NEUROBIOLOGY COURSE

Teacher Prof. Enrico Tongiorgi

HOW IS THE COURSE ORGANIZED ?

CELLULAR NEUROBIOLOGY COURSE 6 Credits (Tongiorgi)

40 hours frontal teaching = 20 lessons

2-3 class exercise (group work and presentations)

Time period = October – December

Evaluation = up to 27 by written exam + orala until 30 (Laude)/6 Credits

TECHNIQUES IN CELLULAR NEUROBIOLOGY COURSE 3 Credits (Baj)

12 hours frontal teaching = 4-6 lessons

4 Lab experiences

Time period = October –December

Evaluation = together with main course

HOW IS THE EXAM ORGANIZED ?

CELLULAR NEUROBIOLOGY

TRUE/FALSE test: one correct response is 0.45 points, each wrong response is - 0,2

LESS1. The cellular organization of the nervous system I: neurons

- | | T | F | |
|----|--------------------------|---|--|
| 1. | <input type="checkbox"/> | <input type="checkbox"/> | With the Golgi impregnation technique all neurons in a brain section are stained (no-one remains unstained) |
| 2. | <input type="checkbox"/> | <input type="checkbox"/> | According to Ramon y Cajal's neuron doctrine each neuron is an individual entity (a cell) anatomically, embryologically, and functionally distinct |
| 3. | <input type="checkbox"/> | <input type="checkbox"/> | The nervous system from an anatomical point of view is a diffuse reticular syncytium |
| 4. | <input type="checkbox"/> | <input type="checkbox"/> | Thanks to the presence of electrical synapses (gap junctions) neurons can work as a functional syncytium although neurons remain as separate entities |
| 5. | <input type="checkbox"/> | <input type="checkbox"/> | On the basis of the classical morphological classification made by Cajal only 3-4 general types of neurons are described. |
| 6. | <input type="checkbox"/> | <input type="checkbox"/> | Interneurons lie between sensory and motor pathways in CNS and represent 90% of our neurons |
| 7. | <input type="checkbox"/> | <input type="checkbox"/> | Highly connected neurons usually have a single, short and poorly ramified dendrite |
| 8. | | | What type of dendritic arborization should a neuron have to best serve a sensory system in which a very high level of signal discrimination is required? |
| | <input type="checkbox"/> | with a space filling arborization | |
| | <input type="checkbox"/> | with a biconical sampling arborization | |
| | <input type="checkbox"/> | with a selective, single target restricted arborization | |

**EXAM: Written (English-True/False) up to 27/30
+ Oral (English or Italian) to improve the grade of the written exam**

Lesson (1)

**The cellular organization
of the nervous system I
(intro + neurons)**

Vocabulary

Central nervous system: spinal cord and brain, including retina.

areas containing nerve cell bodies = **gray matter**

areas containing axons = **white matter**

areas in which axons and dendrites synapse = **neuropil**

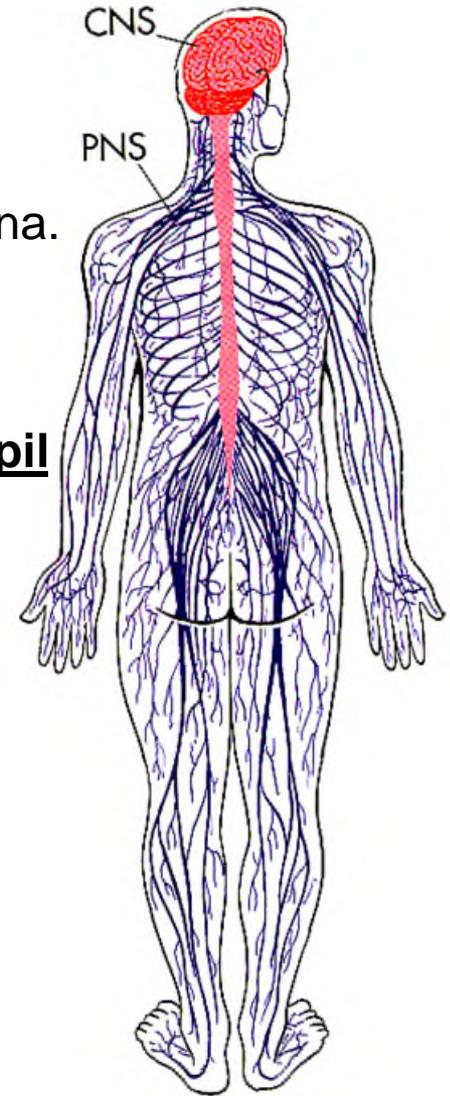
collections of nerve cell bodies = **nuclei** (nucleus)

axon fiber tracts: fasciculi (*fasciculus*), peduncle, commissure, lemnisci (*lemniscus*), tracts

Peripheral nervous system: sensory and autonomic ganglia, peripheral nerves.

collections of nerve cell bodies = **ganglia**

collections of axons = **nerves**



The numbers of the nervous system

- 180 billions of cells in an average human brain (see Suzanne Herculano-Houza lab)
- 2 principal cell types: Neurons & Glial cells (=glia or neuroglia)
- 86 billion neurons in human brain (+same number of glial cells)
- At least 3000 different types of neurons, 5 types of glial cells only.
- 3.000-160.000 synaptic contacts per neuron (retinal/Purkinje neuron)
- 3.000-6.000 different proteins are synthesized by a typical somatic cell while neurons produce 10.000-30.000 different ones.

NEWS

NEUROSCIENCE

What a look at more than 3,000 kinds of cells in the human brain tells us

Analyzing single cells leads to new insights about how brains grow and operate



S. Ament *et al.* [A single-cell genomic atlas for the postnatal maturation of the human cerebellum and the effects of early-childhood inflammation](#). *Science Translational Medicine*. October 12, 2023. doi: 10.1126/scitranslmed.ade1283.

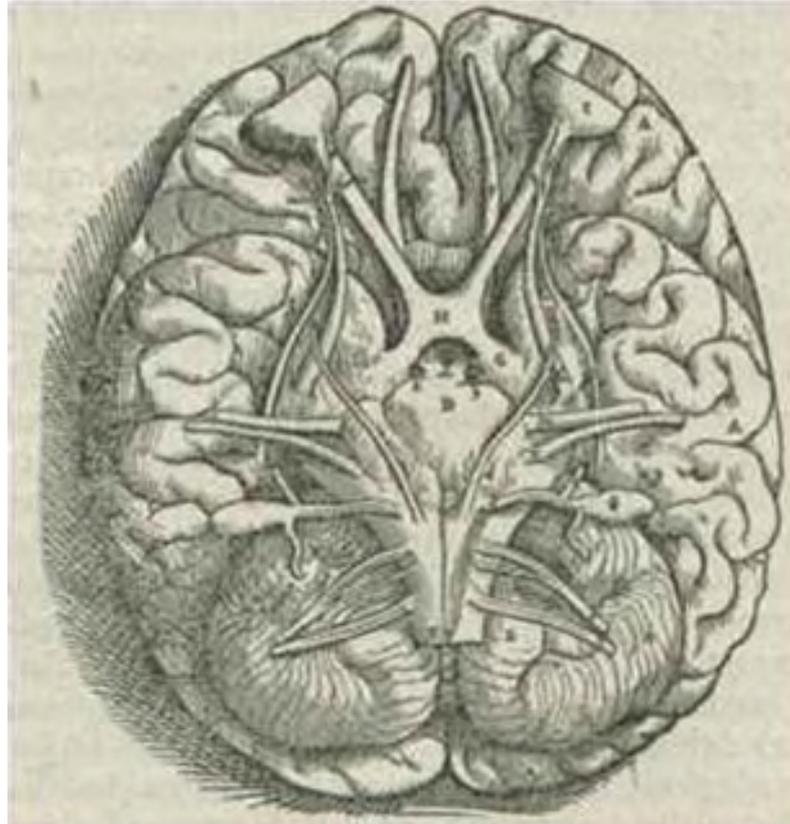
<https://www.sciencenews.org/article/human-brain-cells-new-census>

History of neurosciences



V century B.C

HIPPOCRATES
b. 450 b.C.
(brain)



ARISTOTLE
b. 383 b.C.
(heart)

VS

Andrea Vesalio (1543)

History of neurosciences



Hippocrates

four fluids or **humours**;

- ***sanguis*** or blood, produced by the heart;
- ***choler*** or yellow bile, produced by the liver;
- ***melancholia*** or black bile, produced by the spleen;
- ***phlegma*** or phlegm, produced by the brain,

History of neurosciences



Galen

- Elaborated on the Ideas of Hippocrates
 1. Four Organs
 2. Four Seasons
 3. Four Stages of Life



GALENI IN LIBRVM HIPPOCRATIS

History of neurosciences



Galen (131-201 AD) was born in the ancient Greek city of Pergamon, now Bergama in Turkey, which was part of the Roman Empire. He was a famous physician.

Galen's works covered a wide range of topics, from anatomy and physiology, and medicine to logic and philosophy. His writings pay homage to, amongst others, Plato, Aristotle and the Stoics, but above all to Hippocrates, whom he refers to as "divine".

Much of his explanation of pathology relies on Hippocrates' humoral theories.

Although Hippocrates is the one credited with applying this idea to medicine, **humoralism**, or the doctrine of the four temperaments, as a medical theory retained its popularity for centuries largely through the influence of the writings of Galen



History of neurosciences



Essentially, this theory held that the human body was filled with four basic substances, called **four humours**, which are in balance when a person is healthy.

The four humors were identified as black bile, yellow bile, phlegm, and blood.

Greeks and Romans, and the later Muslim and Western European medical establishments that adopted and adapted classical medical philosophy, believed that each of these humors would wax and wane in the body, depending on diet and activity.

When a patient was suffering from a surplus or imbalance of one fluid, then his or her personality and physical health would be affected.

This theory was closely related to the theory of the four elements: earth, fire, water and air - earth was predominantly present in the black bile, fire in the yellow bile, water in the phlegm, and all four elements were present in the blood.



Name (Arabic)	Name (Latin)	Life span (AD)	Specialty & contribution	Special books	City/Country
Jurjis ibn Bakhtishu Jibril Yuhanna ibn Masawayh		700-800	Translation from Greek and Syriac into Arabic	Translation of works of Hippocrates, Galen and Aristotle	Baghdad
Hunain Ibn Ishak		826-882			
Abu Zaid Ahmed ibn Sahl al-Balkhi		850-934	Muslim psychology, medicine, neuroscience	Masalih al-Abdan wa al-anfus	Shamistiyan, Persian province of Balkh (Afghanistan)
Al-Razi	Razes	841-926	Internal medicine, epidemiology, ophthalmology, chemistry, physics, philosophy	Kitab Al-Mansuri (The Liber Al-Mansuris), Al-Murshid, Al-Hawi (Continents), Al-Gudari wa, Al-Gudari (de Peste or de Pestilentia)	Kharasan, Baghdad
Abū Nasr Muhammad ibn al-Farakh al-Fārābi	Alpharabius	872-950	Political philosophy, Epistemology, medicine	Purposes of metaphysic Translations of works of Aristotle, Focus Al-Hekam, Kitab Mabda' ara'ahl Al-Madina Al-Fadhila	Central Asia, Iran, Iraq, Syria and Egypt
Al-Zahrawi (Abu-Al-Qasim Khalaf Ibn'Abbas Al-Zahrawi)	Abulcasis (Bucasis Alzahravius)	930-1013	Neurosurgery	al-Tasrif Liman Ajiz 'an al' Ta'lif	Al-Andalus (Cordova)
Abū Rayhān Muhammad ibn Ahmad Bīrunī	Abu-Rayhan Biruni	937-1048	Anthropology, pharmacology, psychology father of anthropology, geodesy and experimental mechanics, pioneer of experimental psychology	Ta'rikh al-Hind, The Mas'udi Canon, Understanding Astrology	Persia

Ibn-Sina	Avicenna	980-1037	Medical encyclopedia, philosophy, astronomy, poetry	100 books Al-Qanun (Canon Law) (over 1 million words)	Hamazan, Jurjan
Ibn-Rushd	Averroes	1126-1198	Philosophy, medicine, law	Kitab Al-Kulliat	Al-Andalus, Granada
Ibn-Maimon	Maimonides	1135-1208	Philosophy, translations Hebrew & Latin, poisons, hygiene and public health	Al-Tadbir El-Sihhi, Moushid El-Hairan	Cairo (Saladdin's physician)
Ibn-Al-Nafis		1208-1288	Pulmonary circulation, blood supply to the heart	Sharah Tashrih al Qanun, Al-Mujaz	Damascus, Cairo

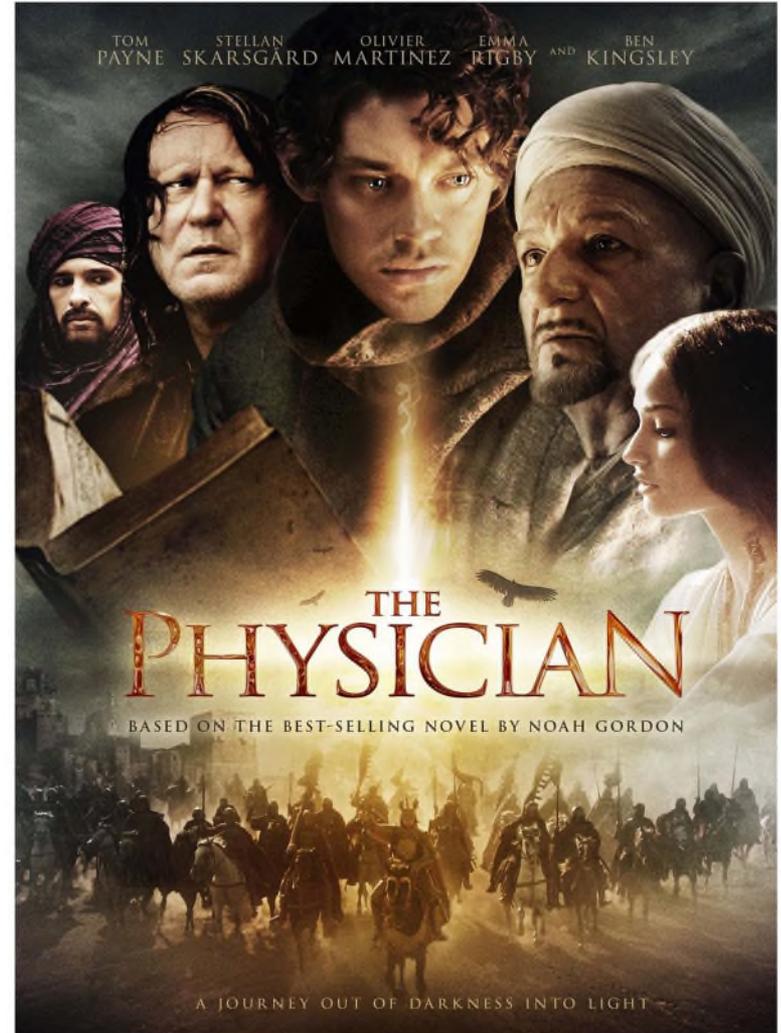
A father of modern neuroscience
ibn Sina (Avicenna) 980-1037

Ibn Sina (Persia 980-1037)

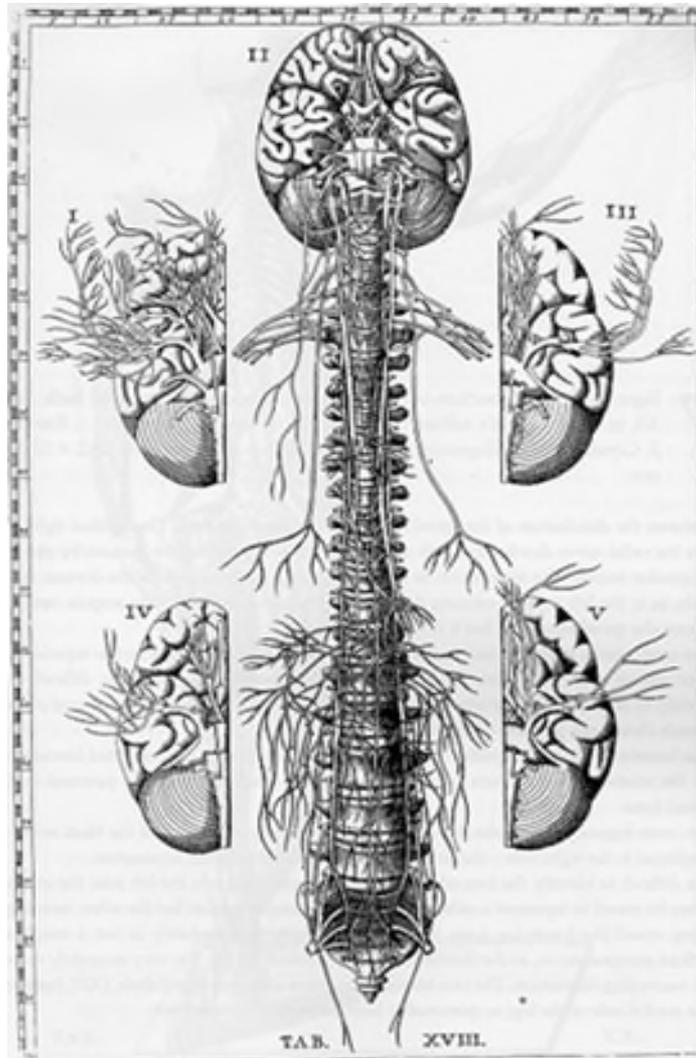
Ibn Sina is one of the most famous Medieval Hellenistic Muslim philosophers, who wrote approximately 99 books throughout his lifetime on philosophy, religion, poetry, and memory (Islamic Philosophy Online, 2011).

In the Golden Age of Islam, the validity of Galenic theory started being questioned by three notable scholars. While ibn Sina, ibn al-Nafis, and ibn al-Haytham began arguments against Galen's anatomic theory, widespread skepticism did not begin until the 16th century in Europe.

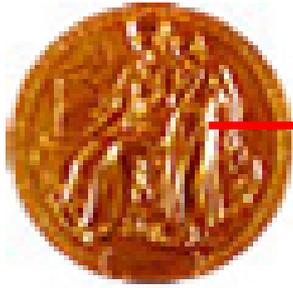
Ibn Sina places logic and reasoning abilities in the category of mental functioning. He believed that sensation and perception are factors of memory. Conceptualizing theories requires the use of manipulating memories in order to understand the concept. With this idea, Ibn Sina almost equates imagination to intellectual ability.



History of neurosciences



Can you see the date ?



History of neurosciences



The Nobel Prize in Physiology or Medicine 1906

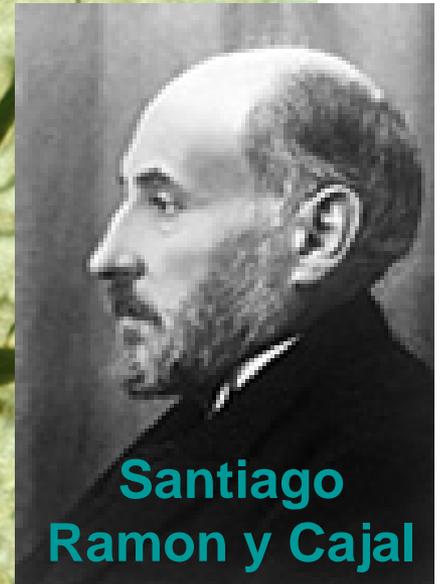


"in recognition of their work on the structure of the nervous system"



Camillo Golgi

Pavia University
Pavia, Italy



**Santiago
Ramon y Cajal**

Madrid University
Madrid, Spain

History of neuroanatomical techniques:

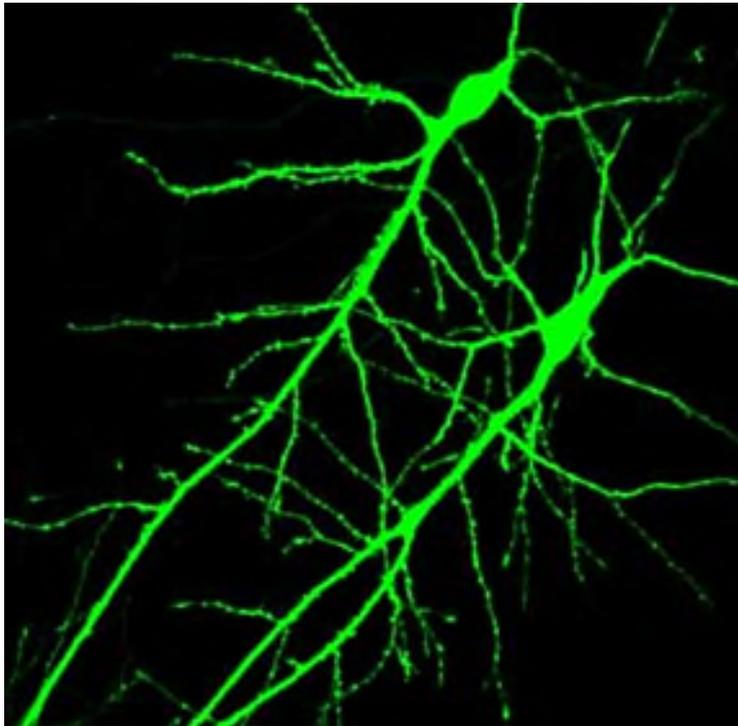
Before 1870: Nissl staining of cell bodies and blood vessel labeling were available

1873: Golgi impregnation (a major technical breakthrough !)

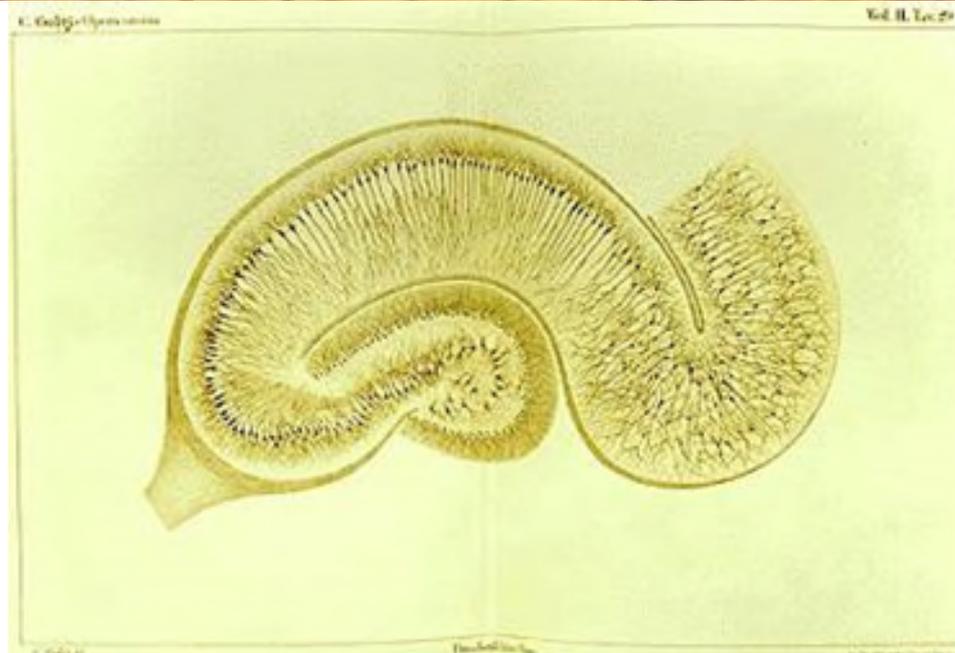
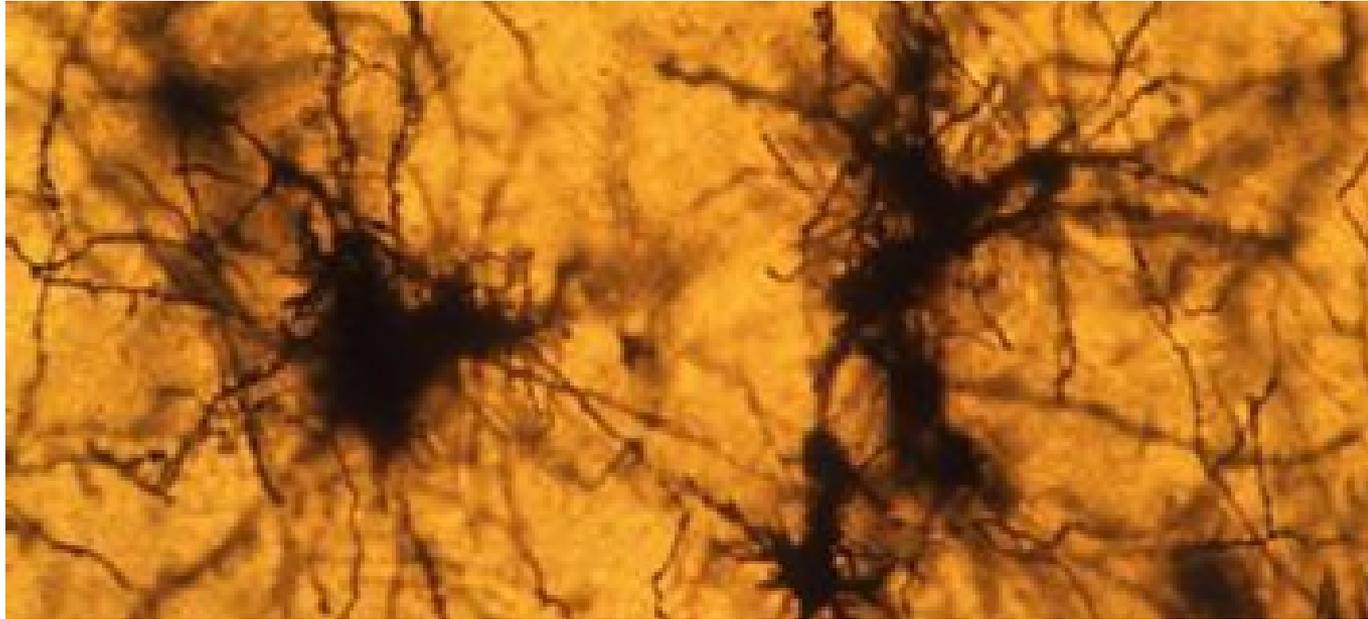
1885: Selective stains for degenerating myelinated fibers (Marchi and Algeri)

Mid 1950s: selective silver staining for all fibers (Nauta)

1970s: antibody staining, fluorescent markers, radiolabelled amino acids, retrograde tracers, *in situ* hybridization of nucleic acids



Golgi staining: potassium chromate and silver nitrate (1873)



Golgi's drawing of the hippocampus impregnated by his stain (from Golgi's *Opera Omnia*).

Nobel e-museum

The nervous system as a diffuse reticular syncytium?

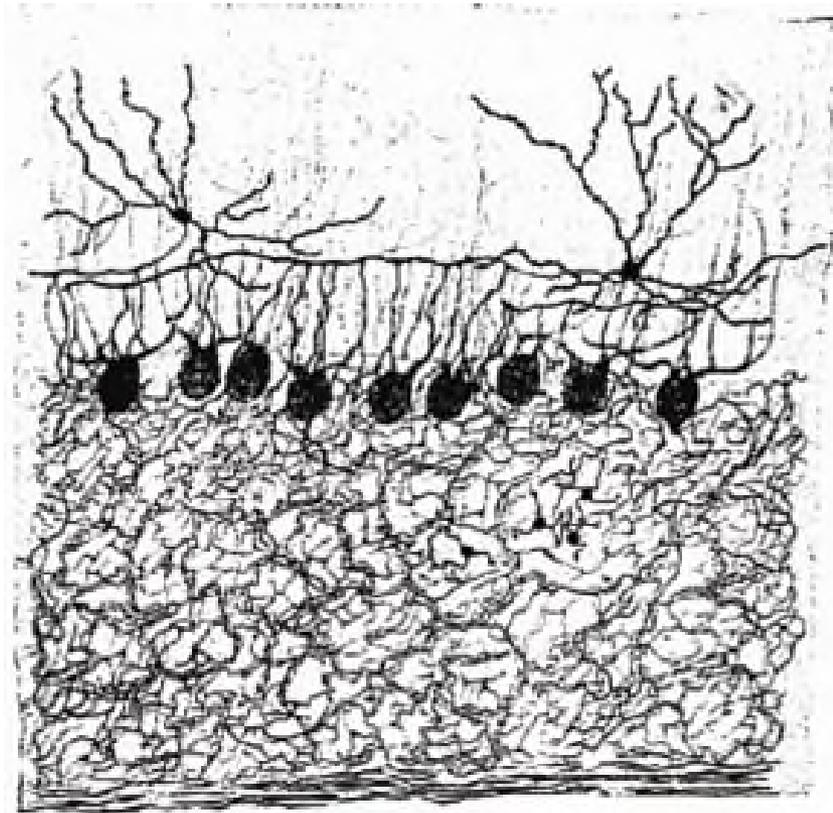
(i.e. a mass of cytoplasm with many nuclei but no internal cell boundaries)

Camillo Golgi

Nobel Lecture December 11, 1906

The Neuron Doctrine- theory and facts.

“..Far from being able to accept the idea of the individuality and independence of each nerve element, I have never had reason, up to now, to give up the concept which I have always stressed, that nerve cells, instead of working individually, act together, so that we must think that several groups of elements exercise a cumulative effect on the peripheral organs through whole bundles of fibers.”

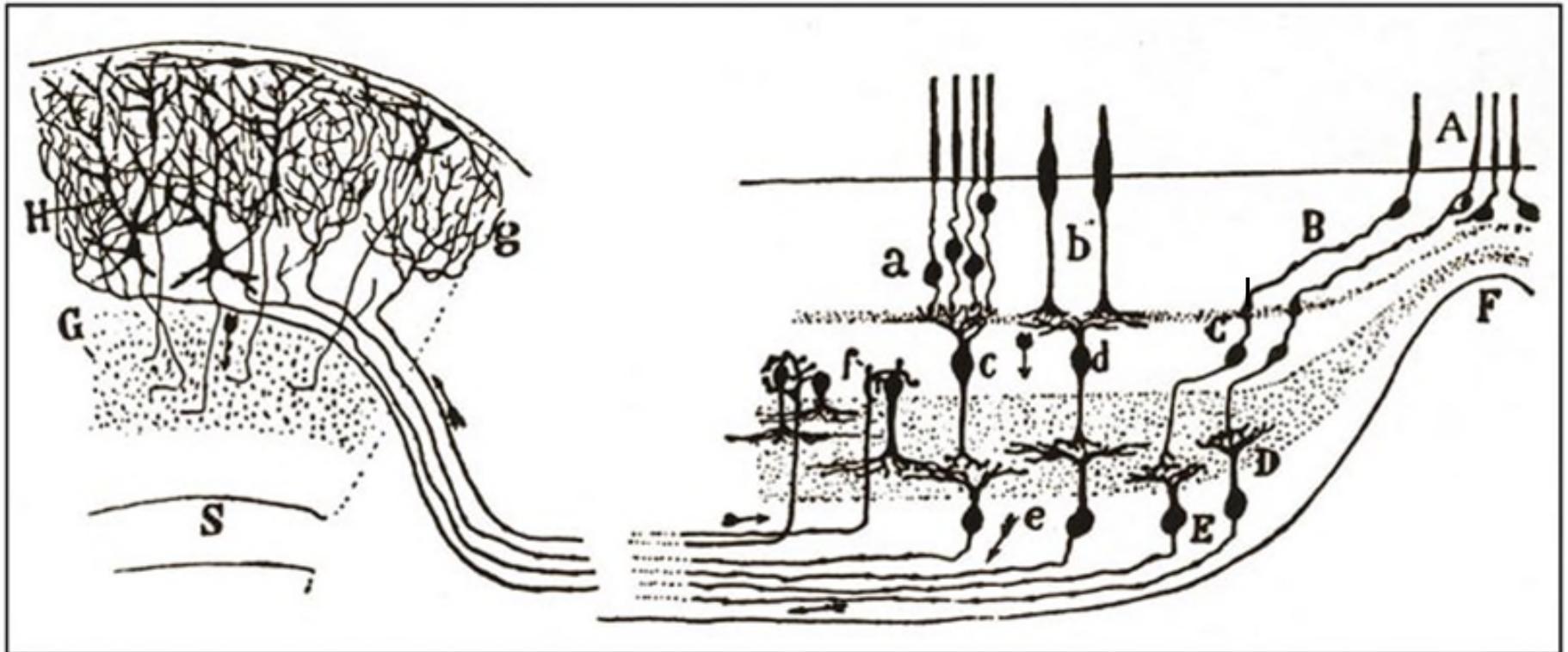


The Neuron Doctrine: (Santiago Ramon y Cajal)

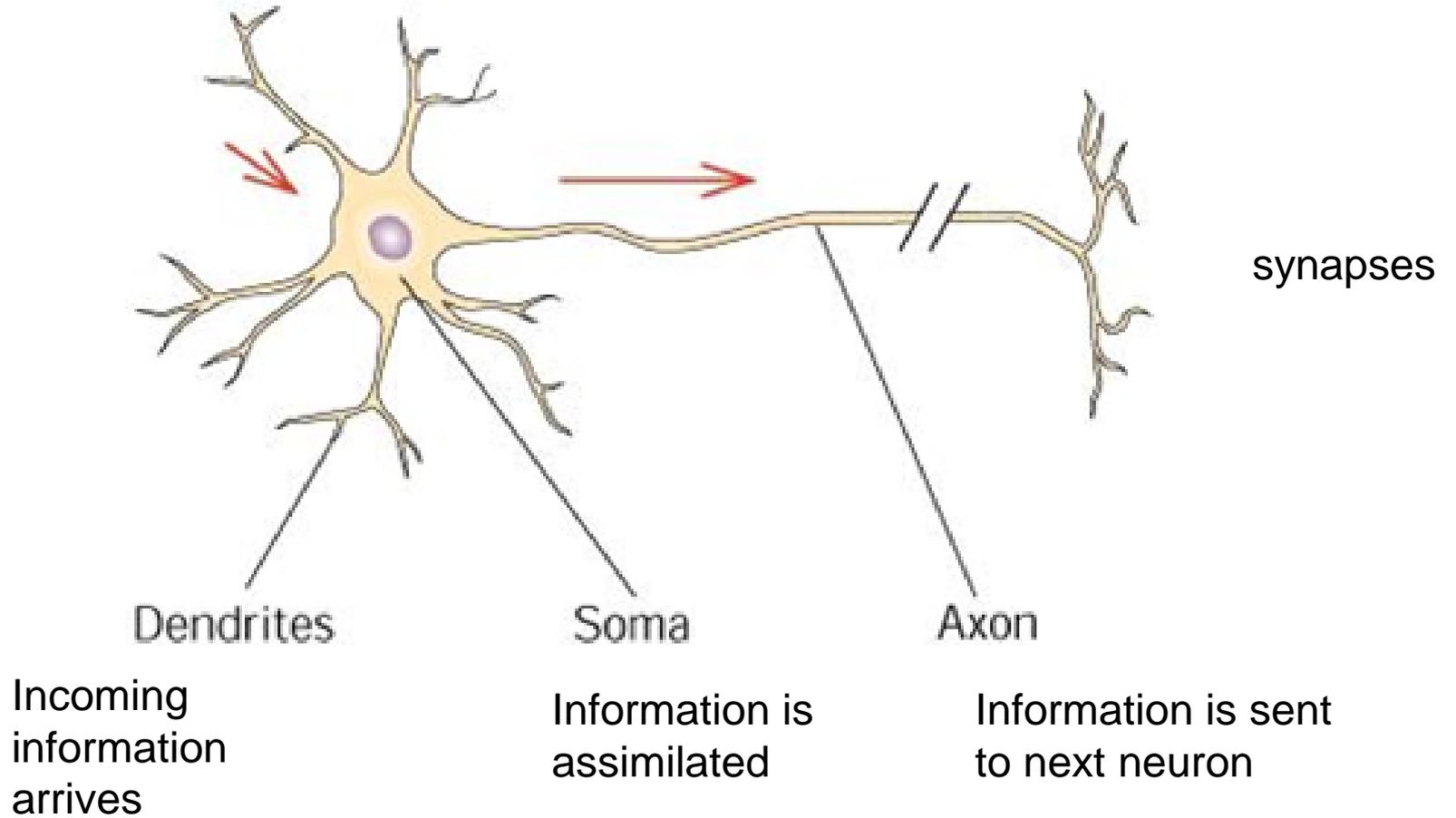
Neurons are cells.

Each is an individual entity anatomically, embryologically, and functionally.

Also: Neurons have a functional polarity.



Neurons have a functional polarity.

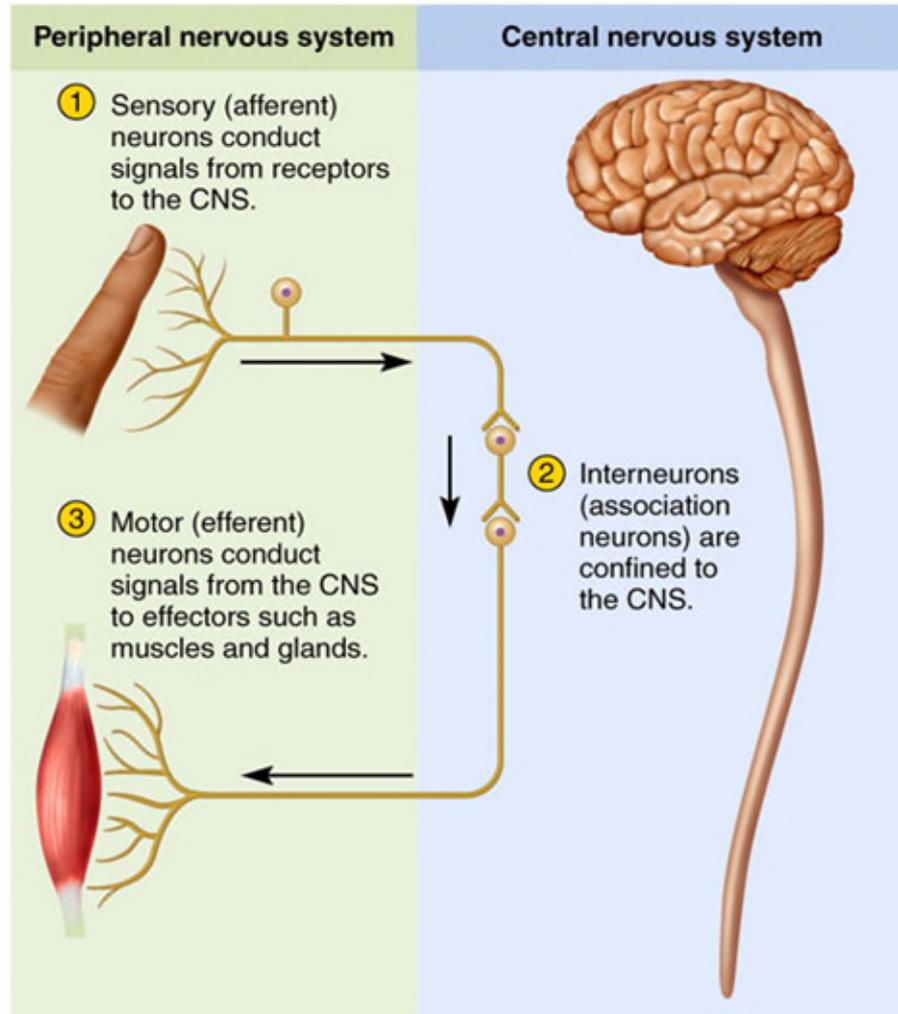


Fundamental Types of Neurons

- **Sensory (afferent) neurons**
 - detect changes in body and external environment
 - information transmitted into brain or spinal cord
- **Interneurons (association neurons)**
 - lie between sensory and motor pathways in CNS
 - 90% of our neurons are interneurons
 - process, store and retrieve information
- **Motor (efferent) neuron**
 - send signals out to muscles and gland cells
 - organs that carry out responses called effectors

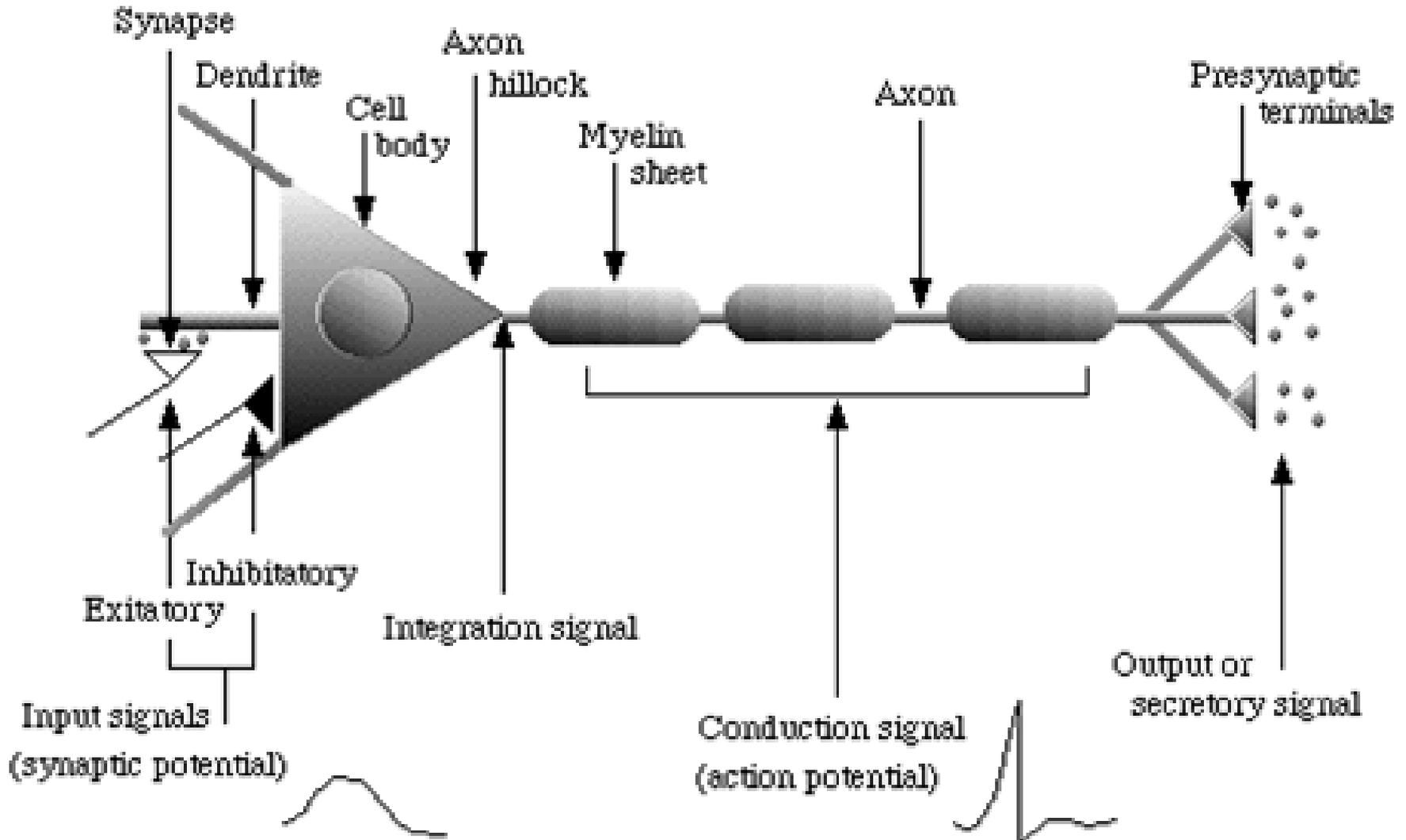
Fundamental Types of Neurons

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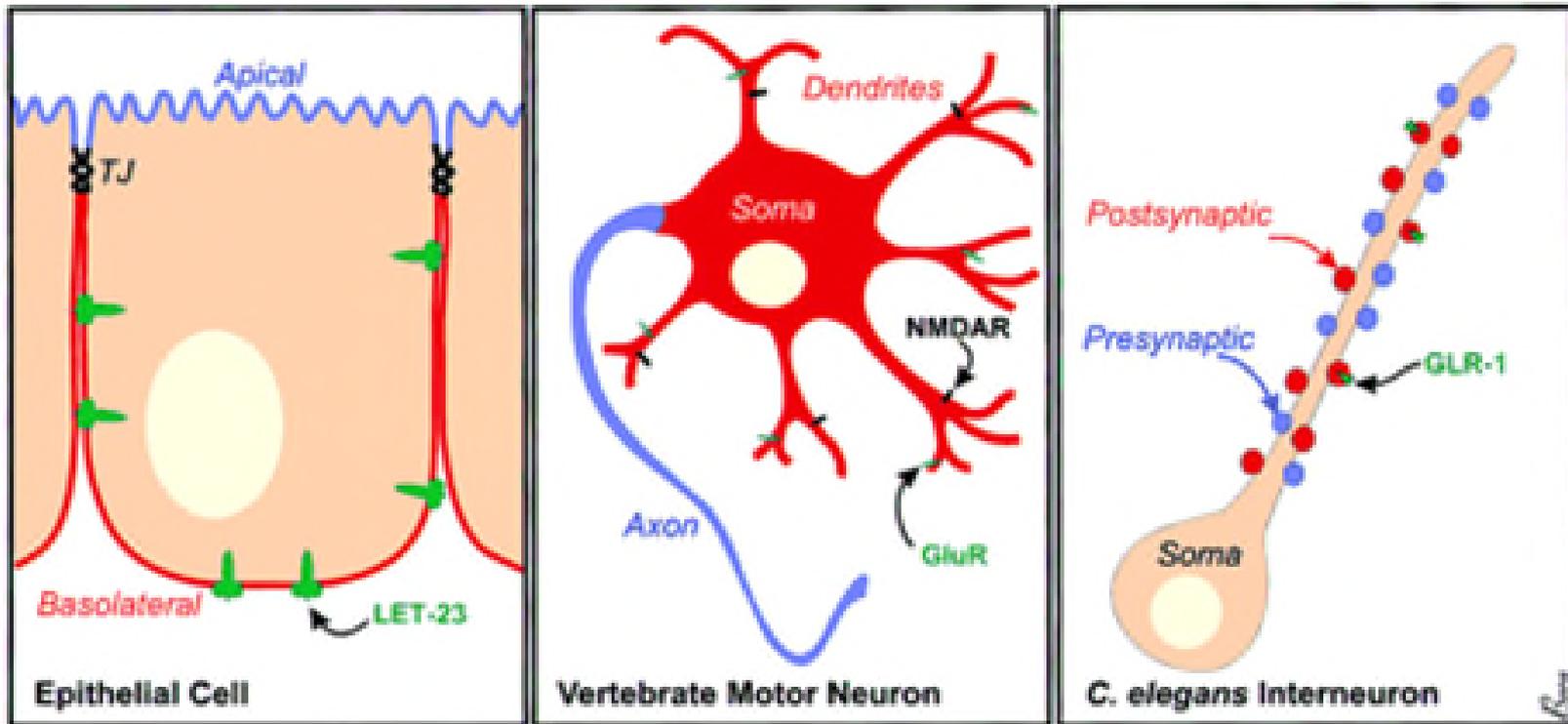
The generic cartoon neuron

(Ramon y Cajal: neurons are polarized cells)



THE (imperfect) EPITHELIAL METAPHOR

APICAL~AXON

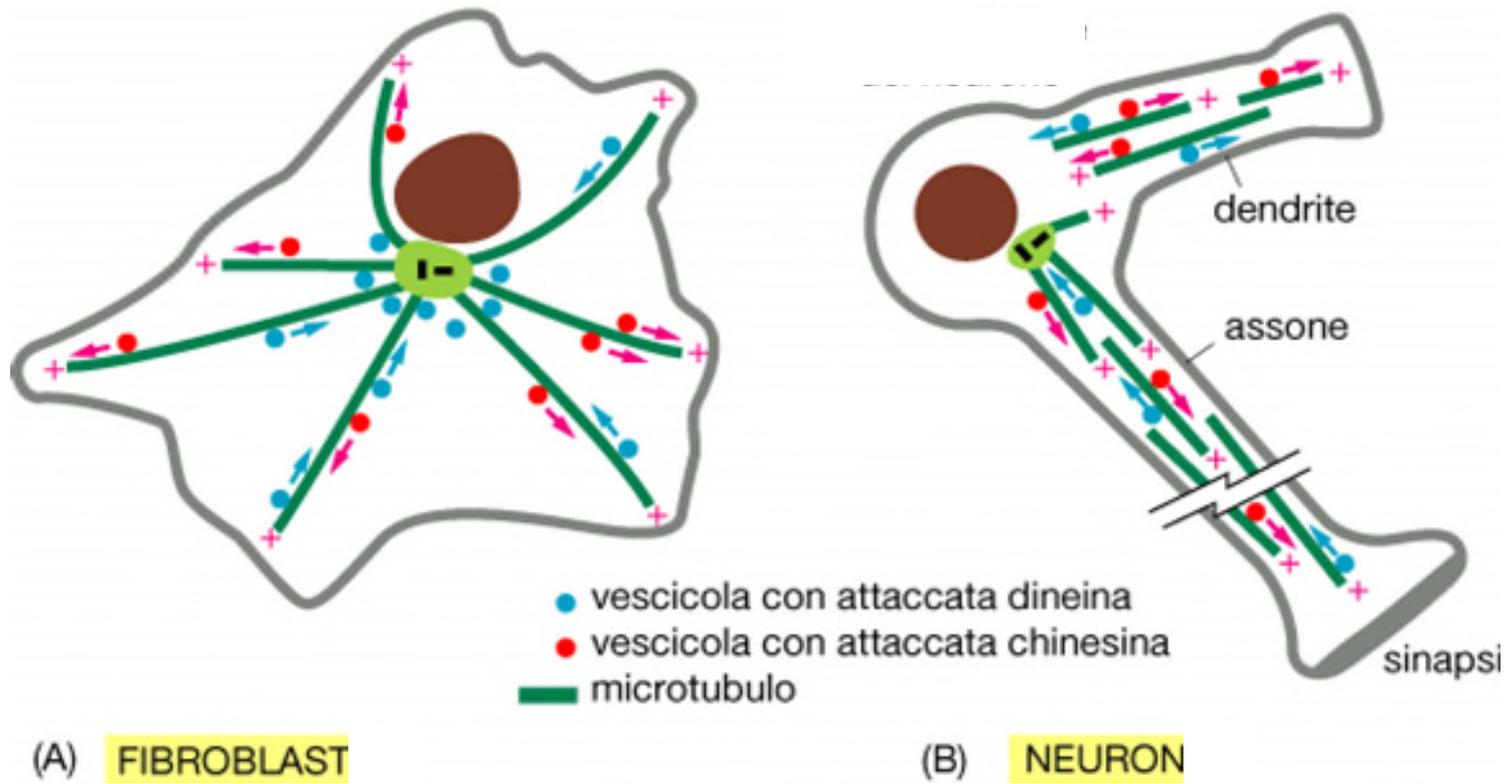


BASOLATERAL~DENDRITE

Polarized Sorting of Viral Glycoproteins to the Axon and Dendrites of Hippocampal Neurons In Culture

C. G. Dotti and K. Simons *Cell*, Vol. 62, 63-72, July, 1990

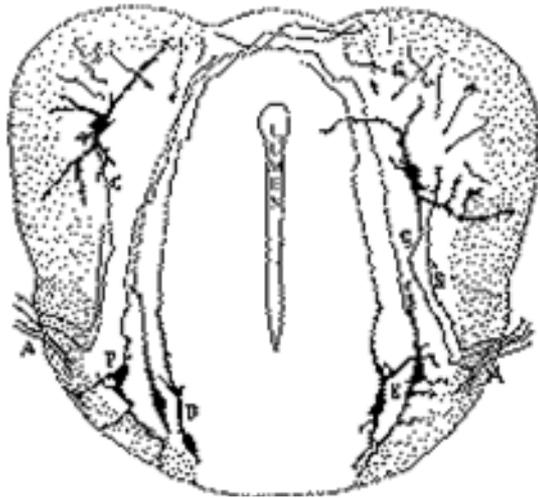
Microtubules orientation determines the cellular polarity in neural and non neural cells



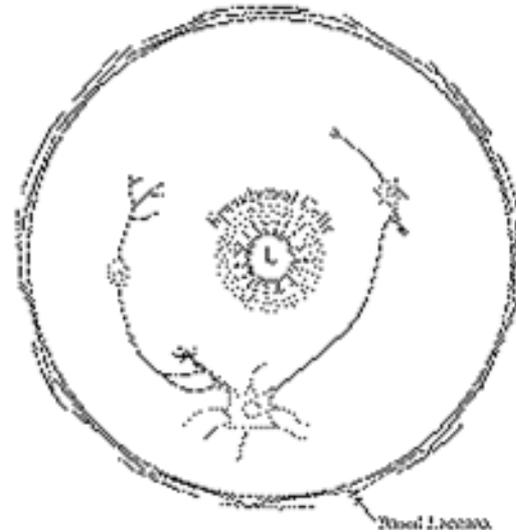
Failings of the metaphor

Colman, DR. (1999) Neuron.23, 649–651.

Neurons Do Not Have Contact with a Lumen

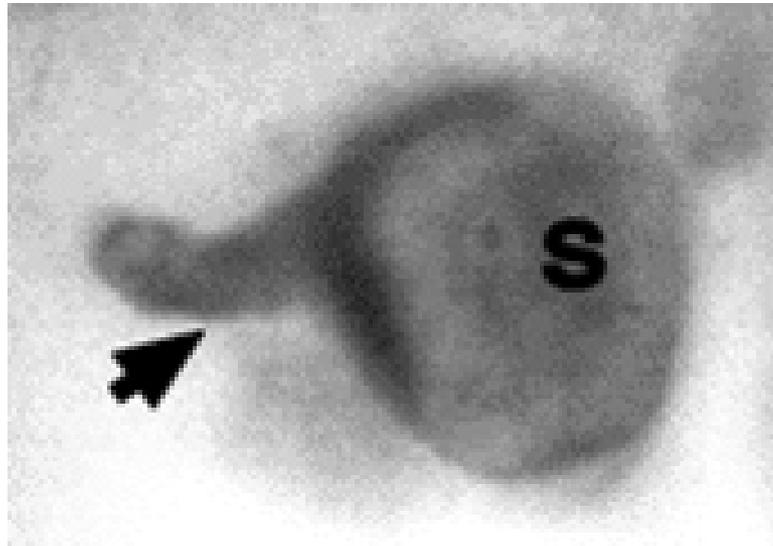


**The Synaptic Junctional Complex in the CNS,
an Adherens Junction Derivative, Is Found All
Over the Neuronal Surface**



“ the neuron has over the course of evolution developed some highly unique subpartitioning mechanisms of the basolateral domain that we just do not understand yet.”

Axon Hillock is molecularly distinct domain



Ji et al., 2001. Molecular identification and sequence analysis of Hillarin, a novel protein localized at the axon hillock. Biochimica et Biophysica Acta 28:246-249

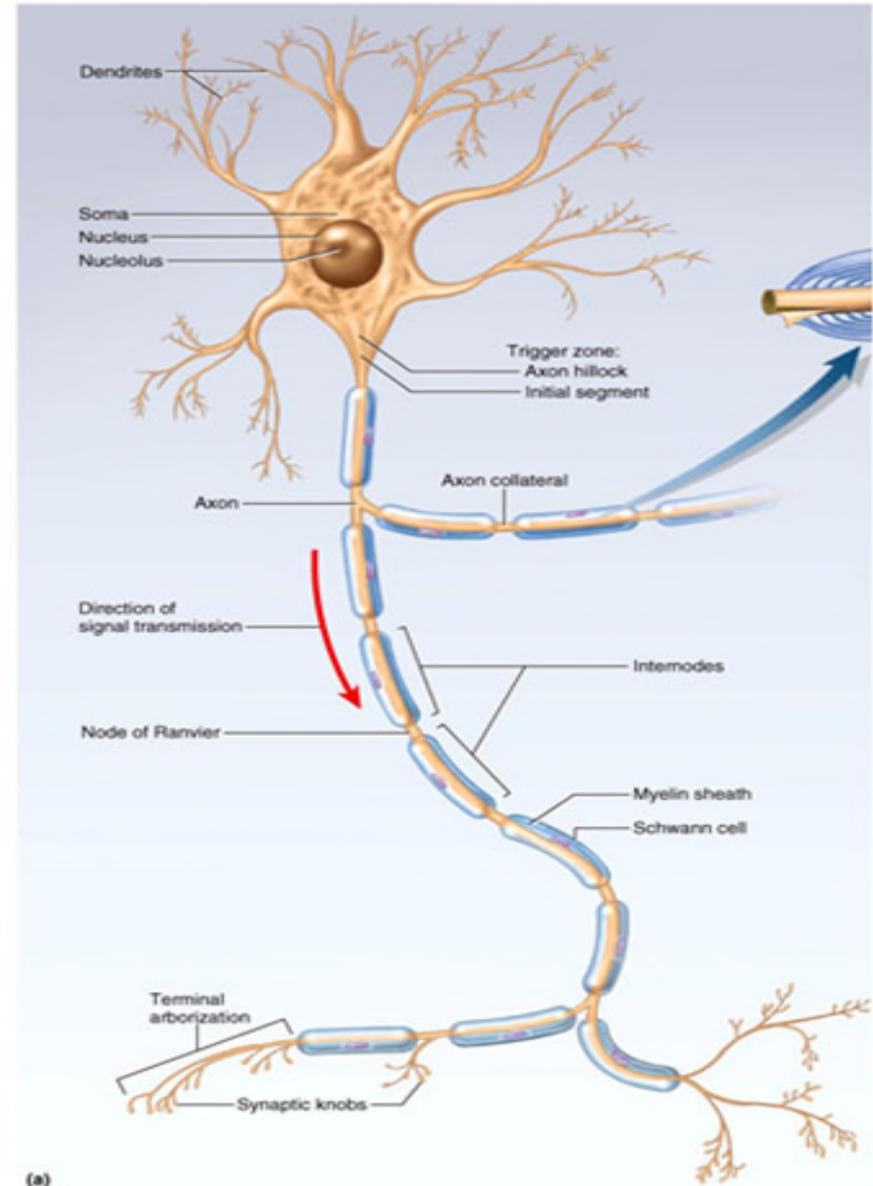
Diversity of neurons

Structure of a Neuron

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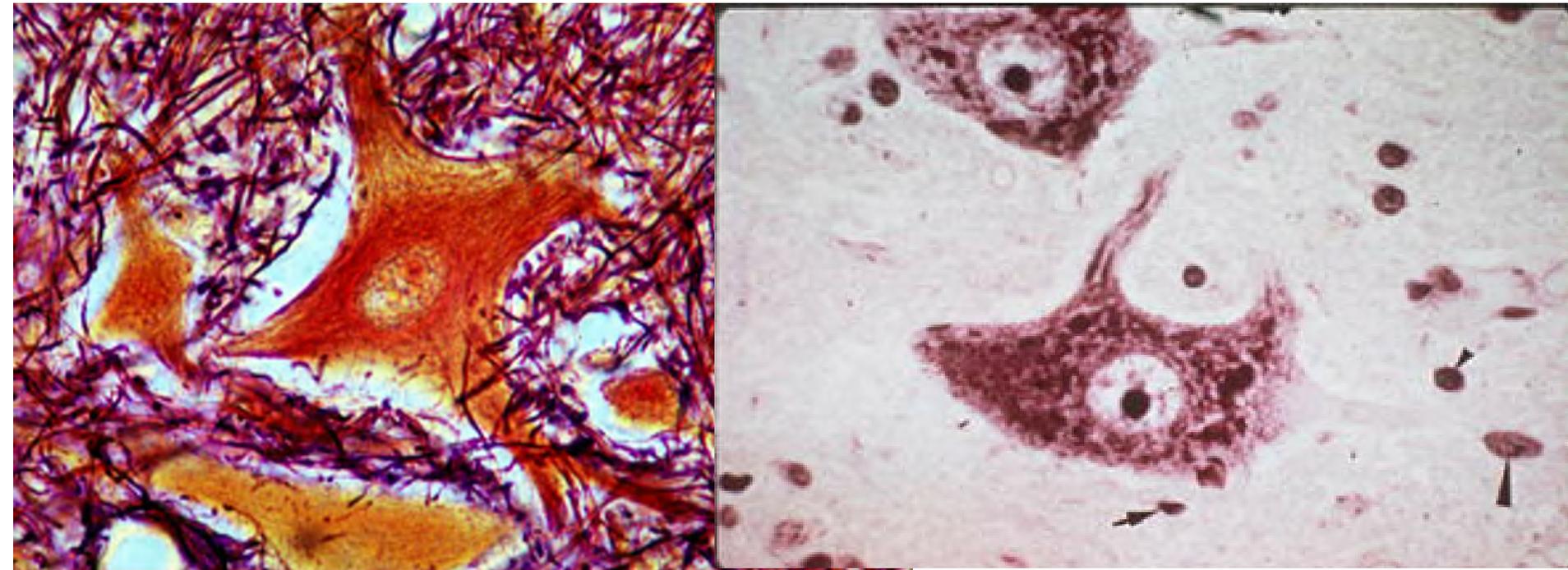
- **Cell body = perikaryon = soma**
(5-150 microns diameter)
 - single, central nucleus with large nucleolus
 - cytoskeleton of microtubules and neurofibrils (bundles of actin filaments)
 - compartmentalizes RER into Nissl bodies
 - lipofuscin product of breakdown of worn-out organelles -- more with age
- **Vast number of short dendrites**
(1-10, short and branched)
 - for receiving signals (large area for a small volume: $370.000\mu\text{m}^2$ for $300.000\mu\text{m}^3$)
- **Single axon (nerve fiber) arising from axon hillock for rapid conduction**
 - axoplasm and axolemma and synaptic vesicles

(a main axon even meters long, sometimes with secondary branchings = collaterals with possible terminal arborization)





Anterior Horn Motor Neuron



SILVER STAIN
(BIELSCHOWSKY)

NISSL STAIN

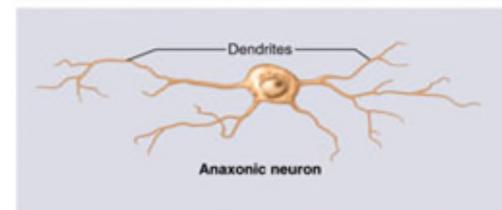
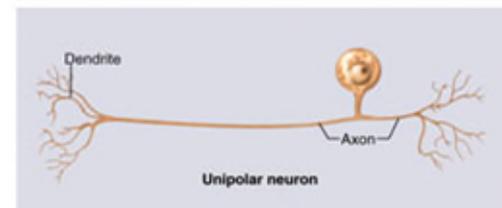
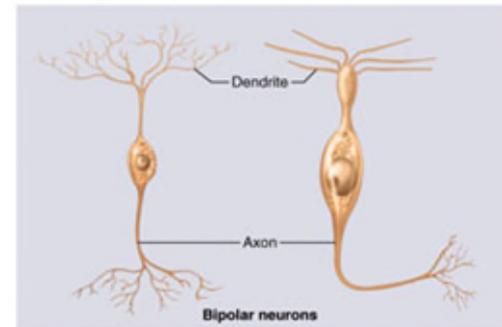
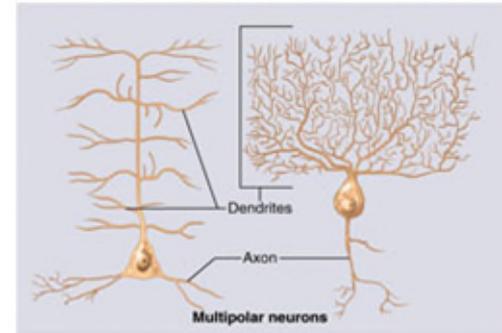
Even classical staining methods hint at molecular polarity!

Variation in Neural Structure

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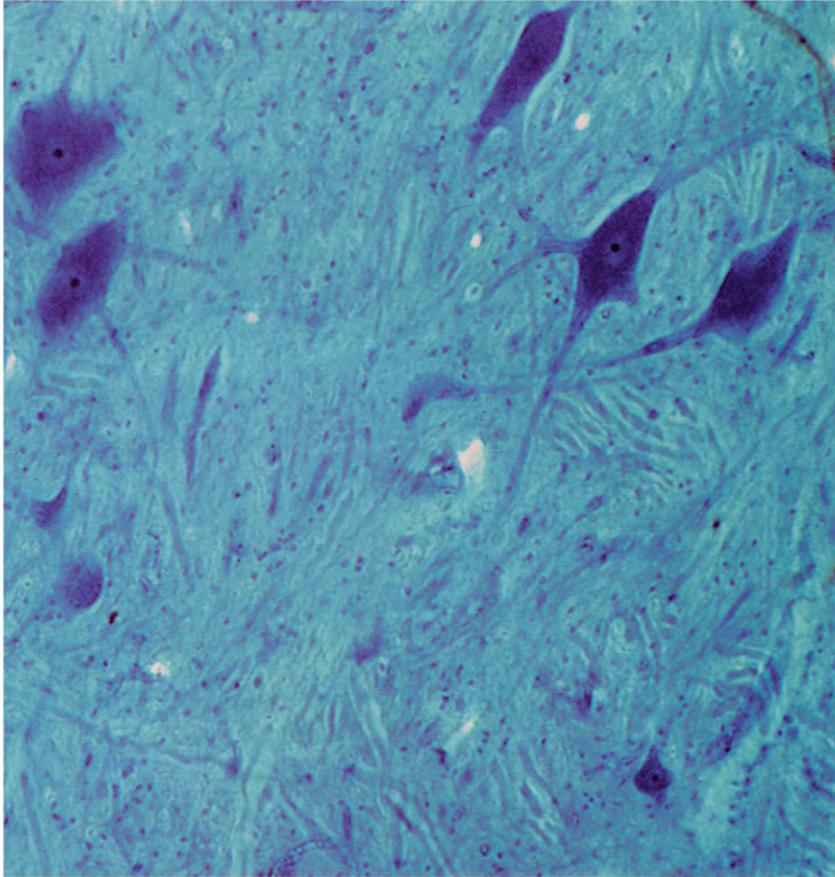
Basic classification of neurons

- **Multipolar neuron**
 - most common
 - many dendrites/one axon
- **Bipolar neuron**
 - one dendrite/one axon
 - olfactory, retina, ear
- **Unipolar neuron (pseudounipolar)**
 - sensory from skin and organs to spinal cord
- **Anaxonic neuron**
 - many dendrites/no axon
 - help in visual processes



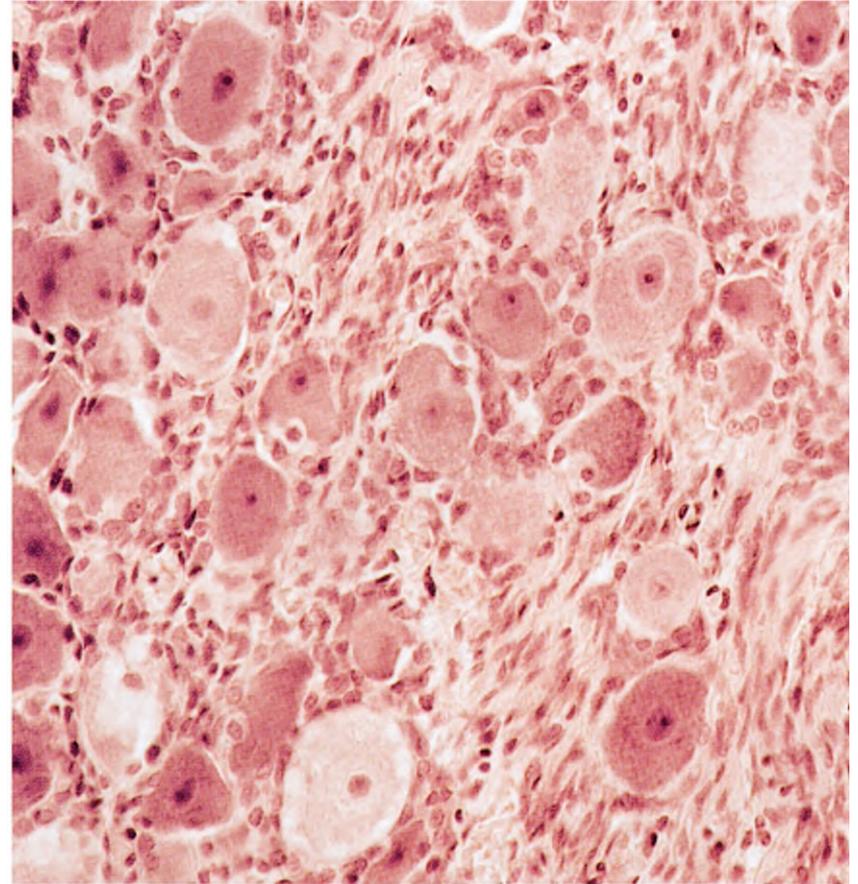
Mammalian neurons as you may visualize them in reality

Motorneurons (Nissl staining)



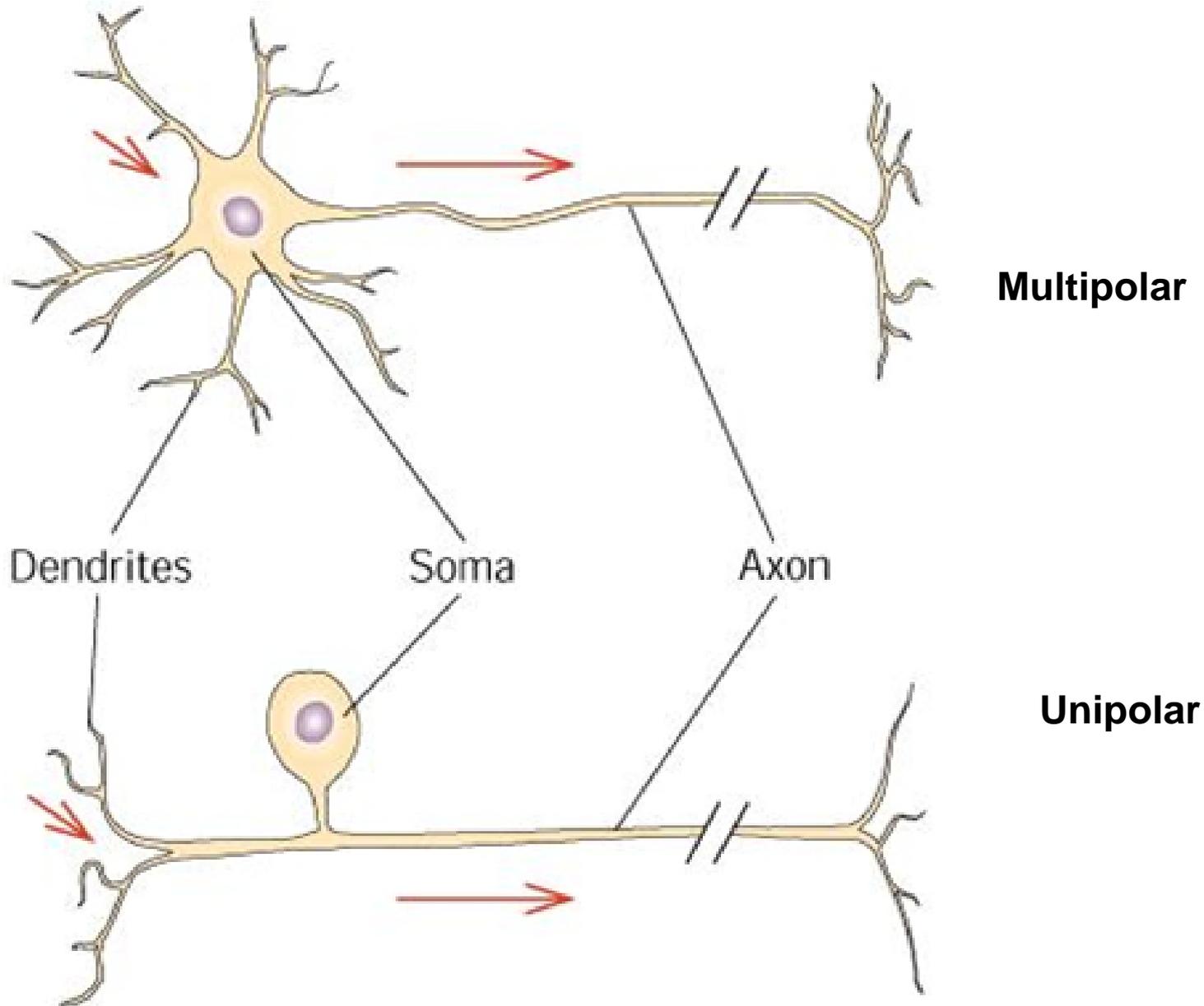
Multipolar

Sensory neurons (Erattox&Eosin)

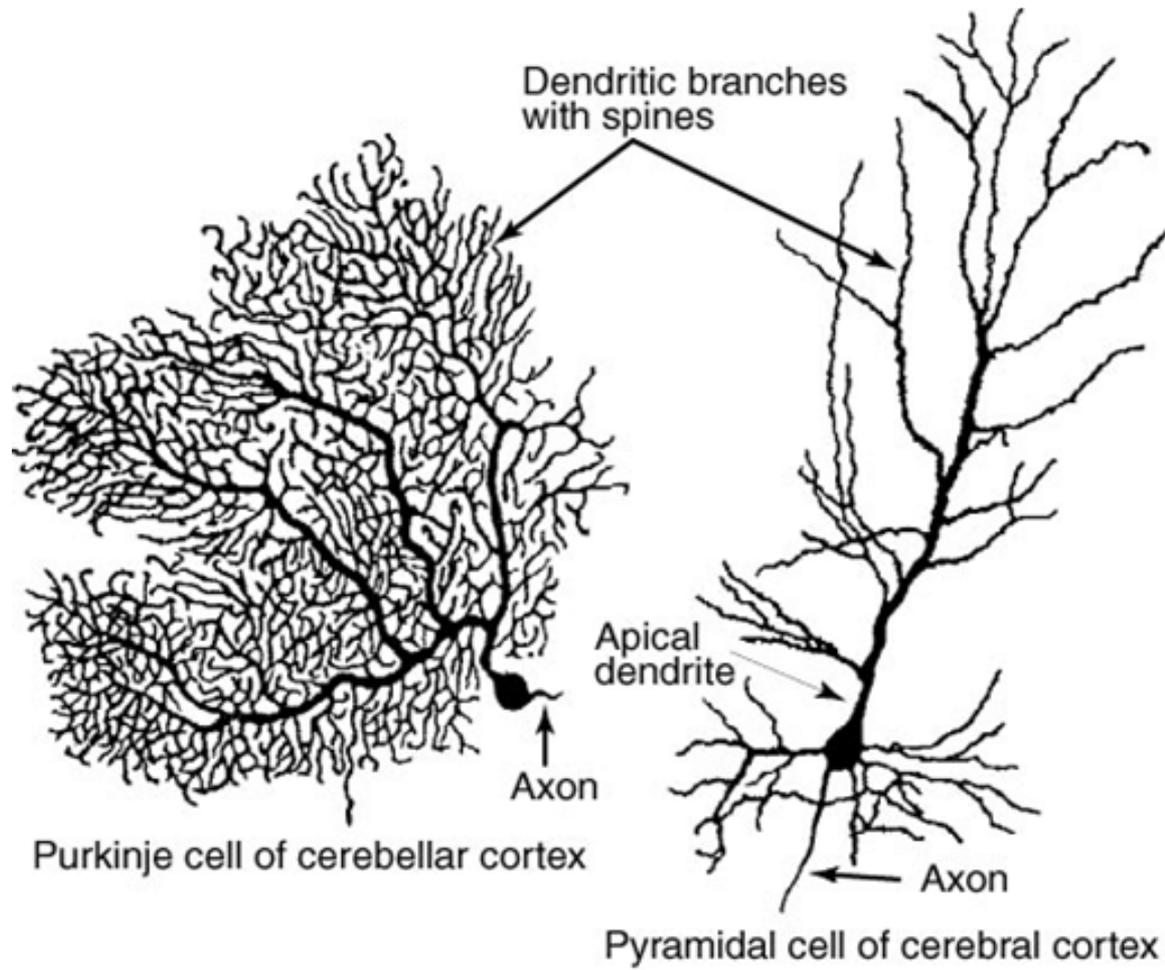


Unipolar

What is the relationship between multipolar and unipolar neurons?



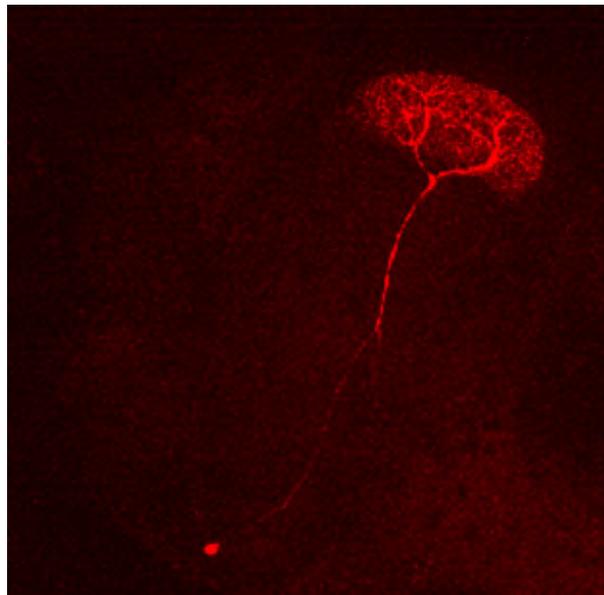
Morphology of vertebrate multipolar neurons is highly variable



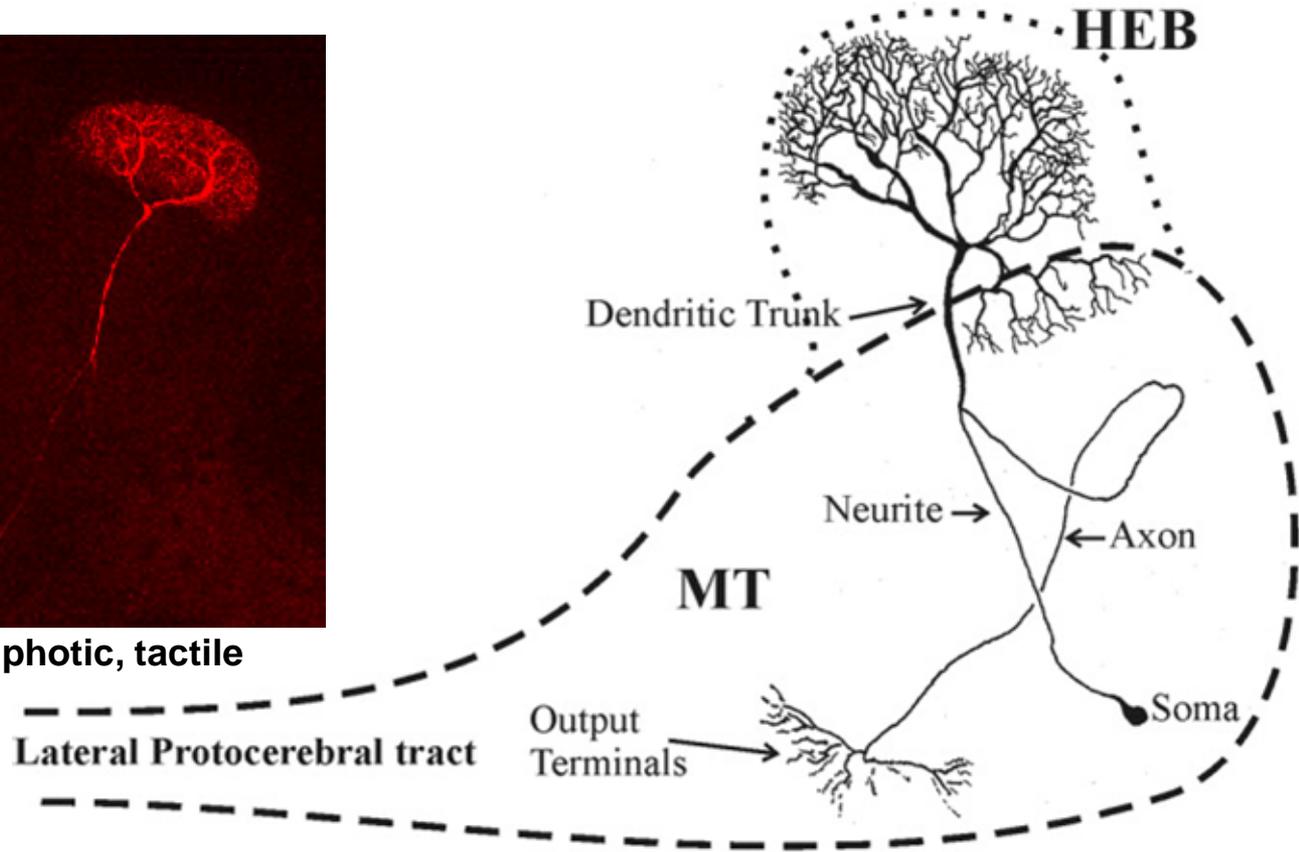
Example of morphology of an invertebrate neuron:

the parasol cell of the crayfish brain.

(Mike Mellon, UVA Dept of Biology)



Olfactory, photic, tactile



Similarities and differences between the nervous systems of invertebrates and vertebrates

Invertebrate nervous systems

Motor neurons and interneurons typically unipolar

Neuronal somata in rind or ganglia

Dendritic processes arise directly from axons in most cases

Synapses in neuropil

Few types glia

Lack myelin

Large cells in many instances

Individually identifiable in many instances

Neural circuits have relatively few neurons

Vertebrate nervous systems

Motor neurons and interneurons typically multipolar

Neuronal somata typically grouped in nuclei, cortical lamina or throughout ganglia

Dendritic processes arise from soma

Several distinct types of glia

Have myelin and thus saltatory conduction

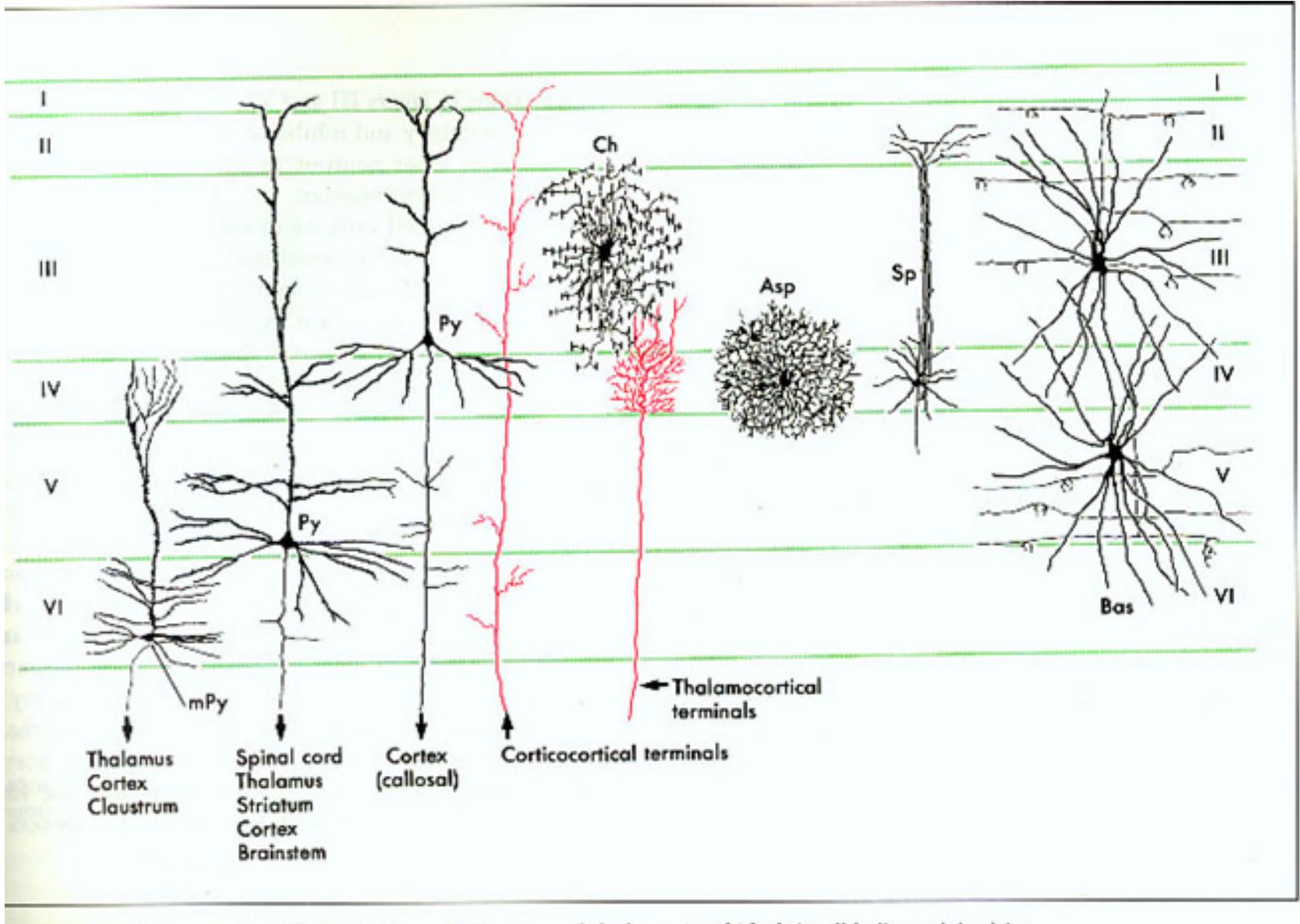
Few cells that are very large

Few individually identified neurons

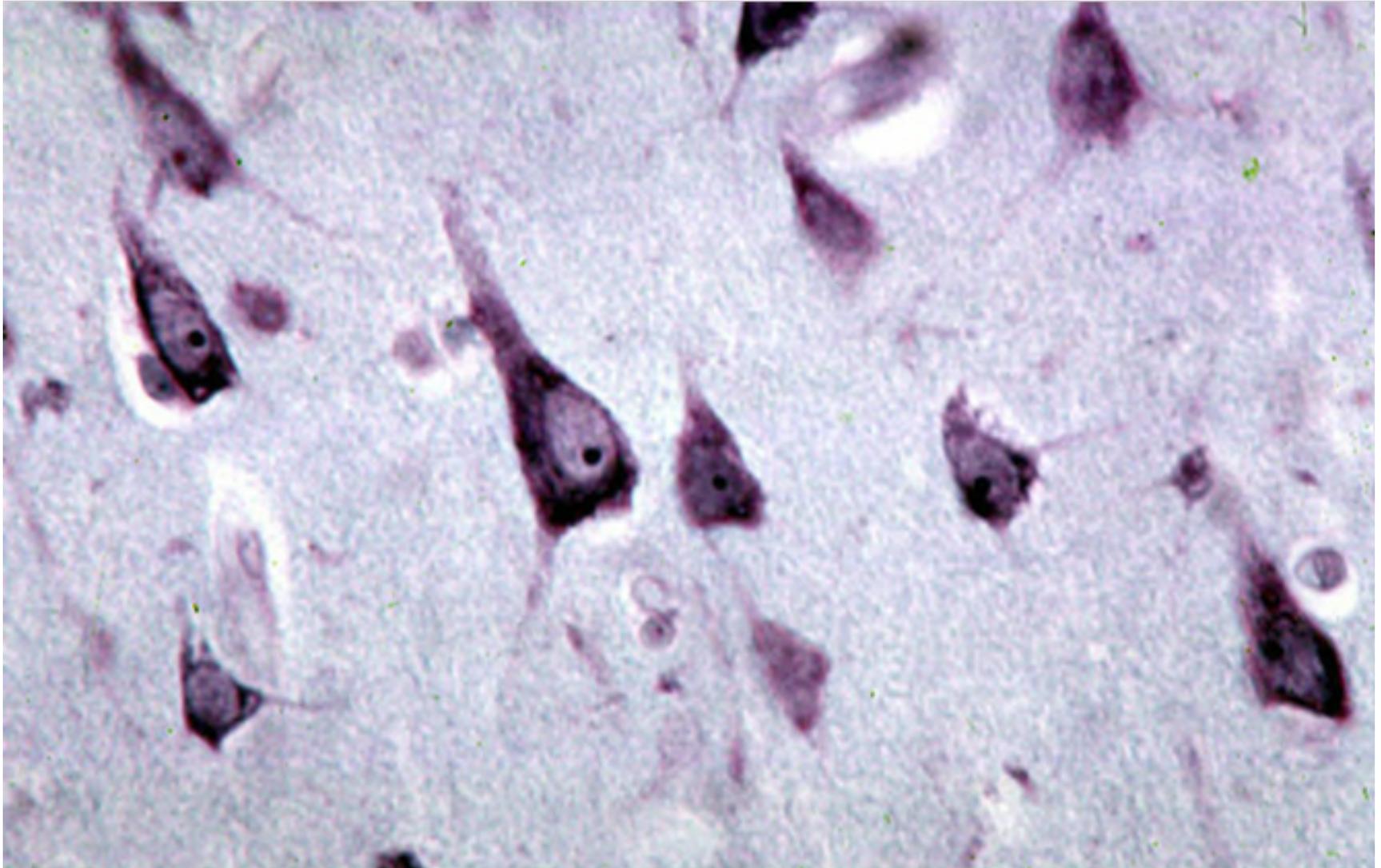
Neural circuits have many components

Diversity of cortical neurons

Cerebral Cortex



What general class of neuron?



PYRAMIDAL NEURON

(HIPPOCAMPAL)

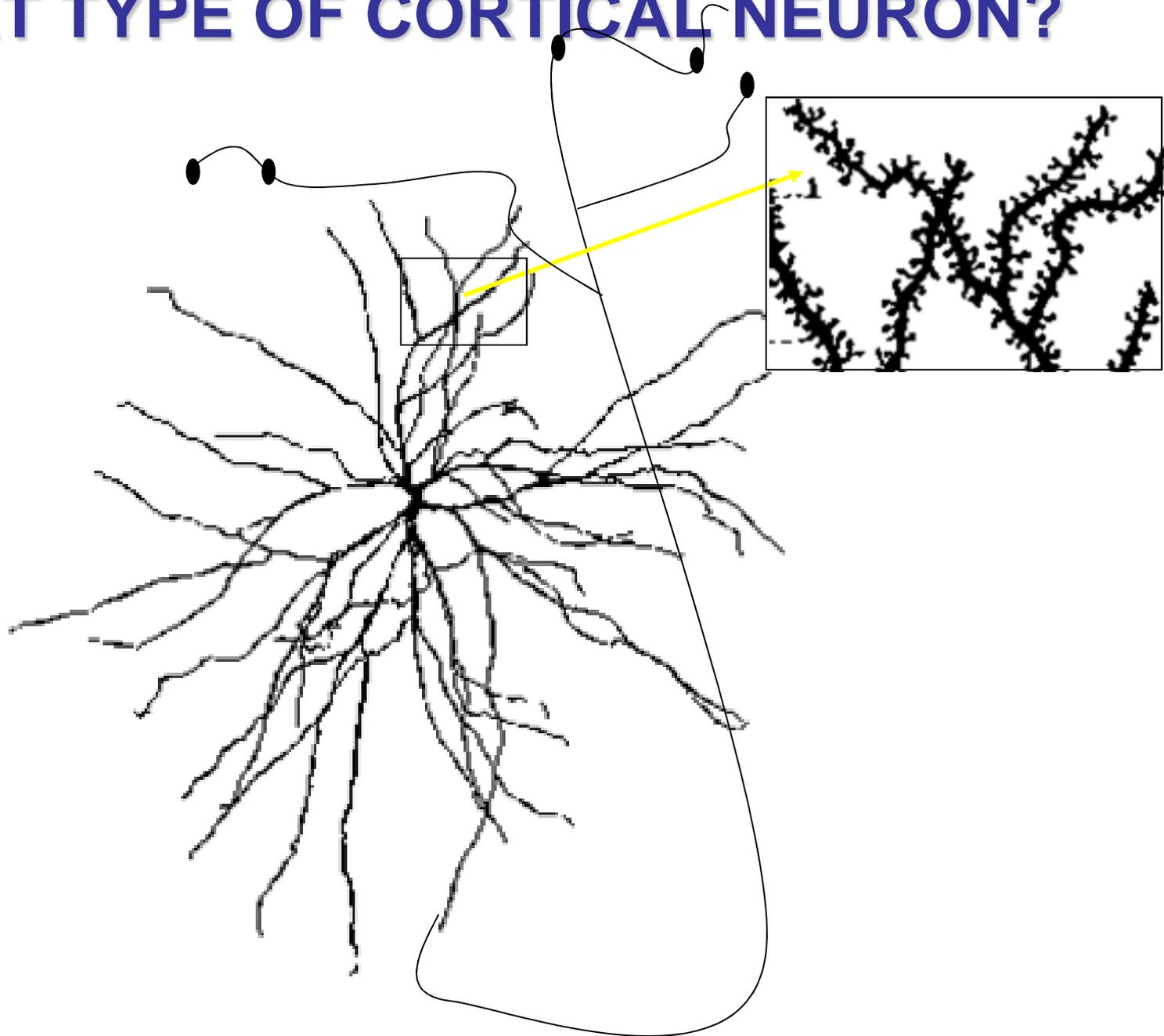


GOLGI STAIN: *potassium dichromate*
+ *silver nitrate*=*silver chromate*
precipitates within the matrix of the
cytoplasm

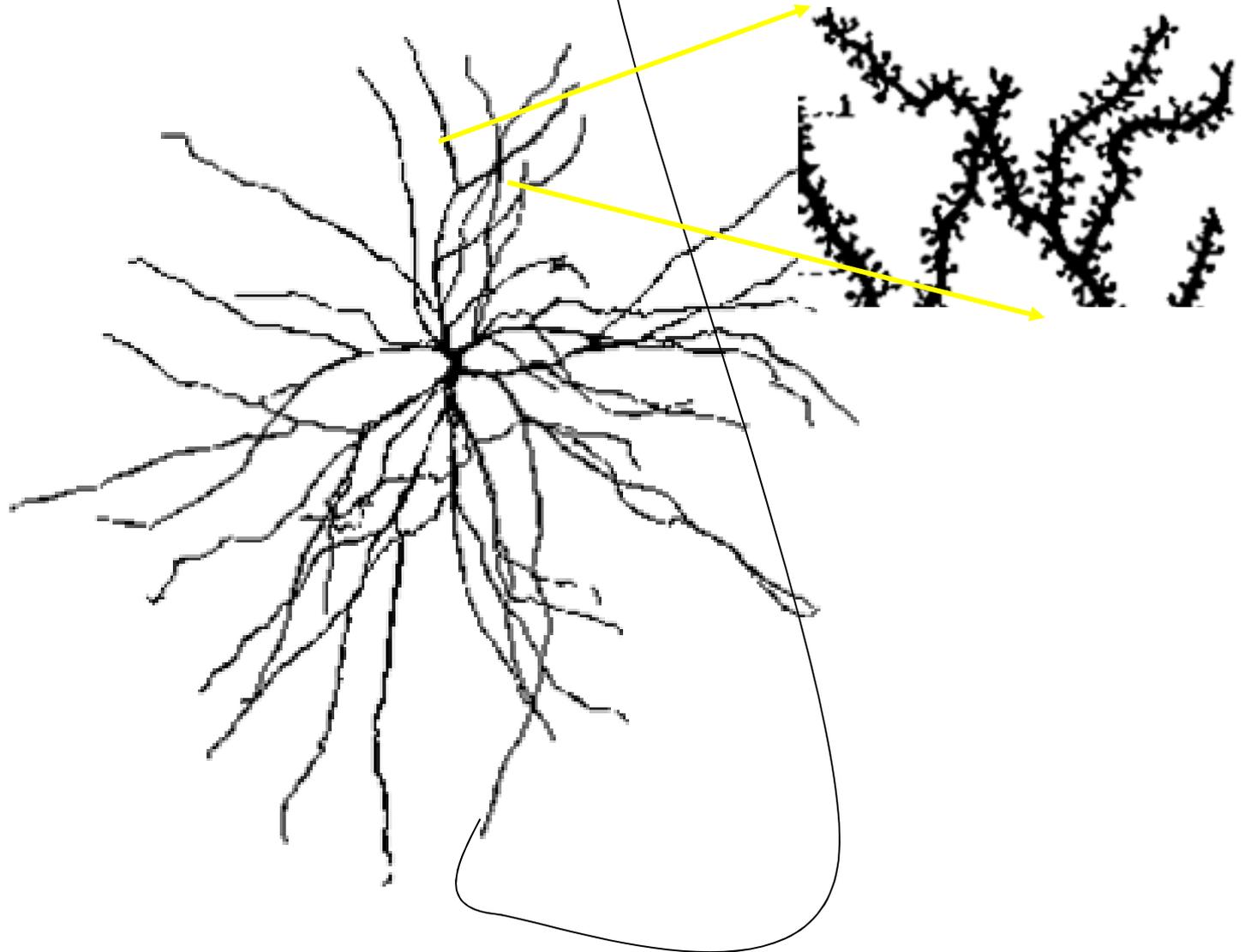
SERENDIPITOUSLY RANDOM!!

**USED BY RAMON Y CAJAL TO
FORMULATE THE NEURON
DOCTRINE**

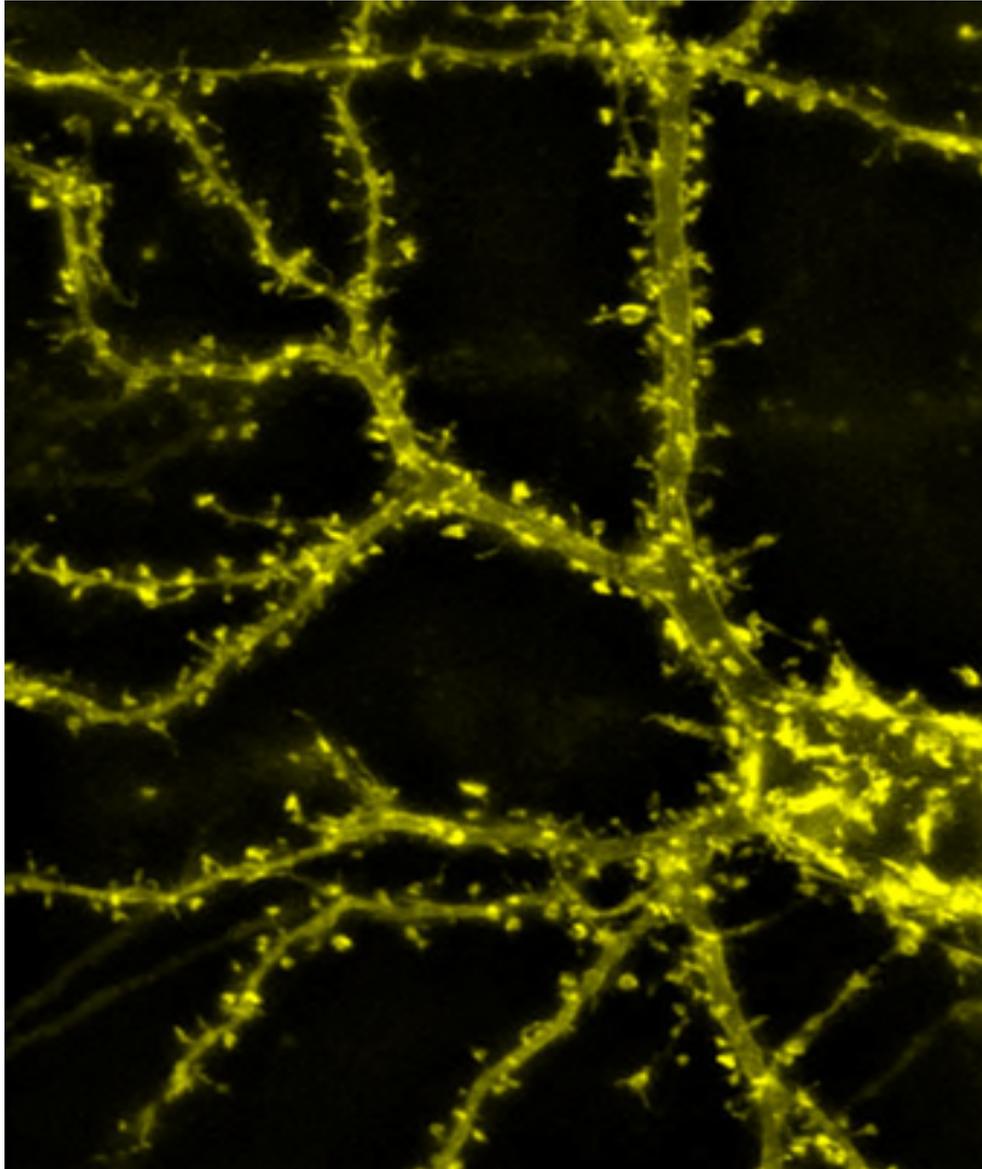
WHAT TYPE OF CORTICAL NEURON?



SPINY STELLATE CELL EXCITATORY INTERNEURON

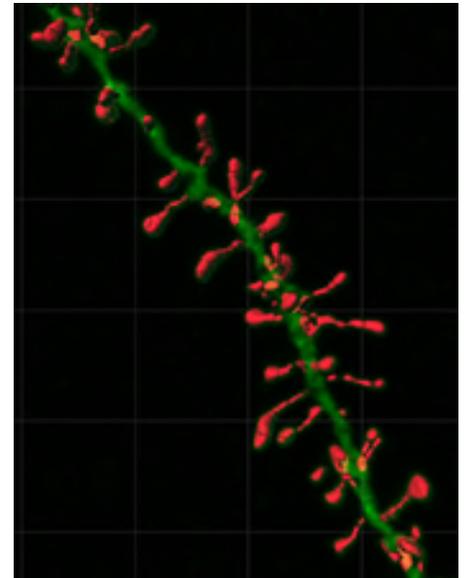


Dendritic Spines

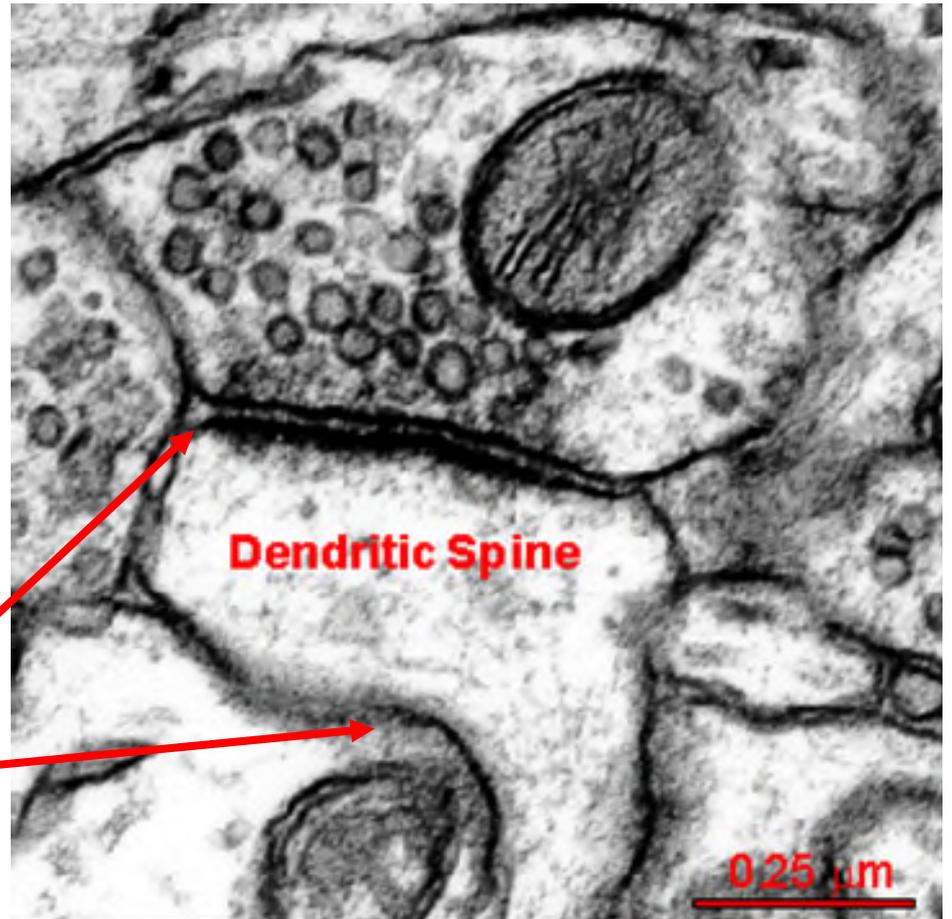
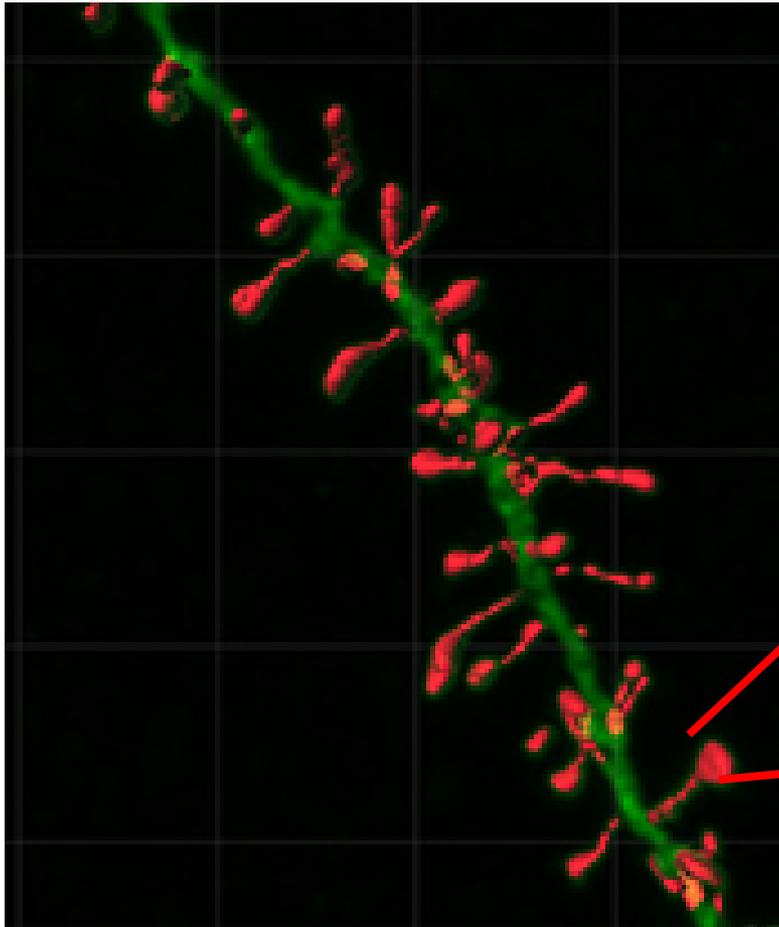


20-40,000 spines on
one neuron!

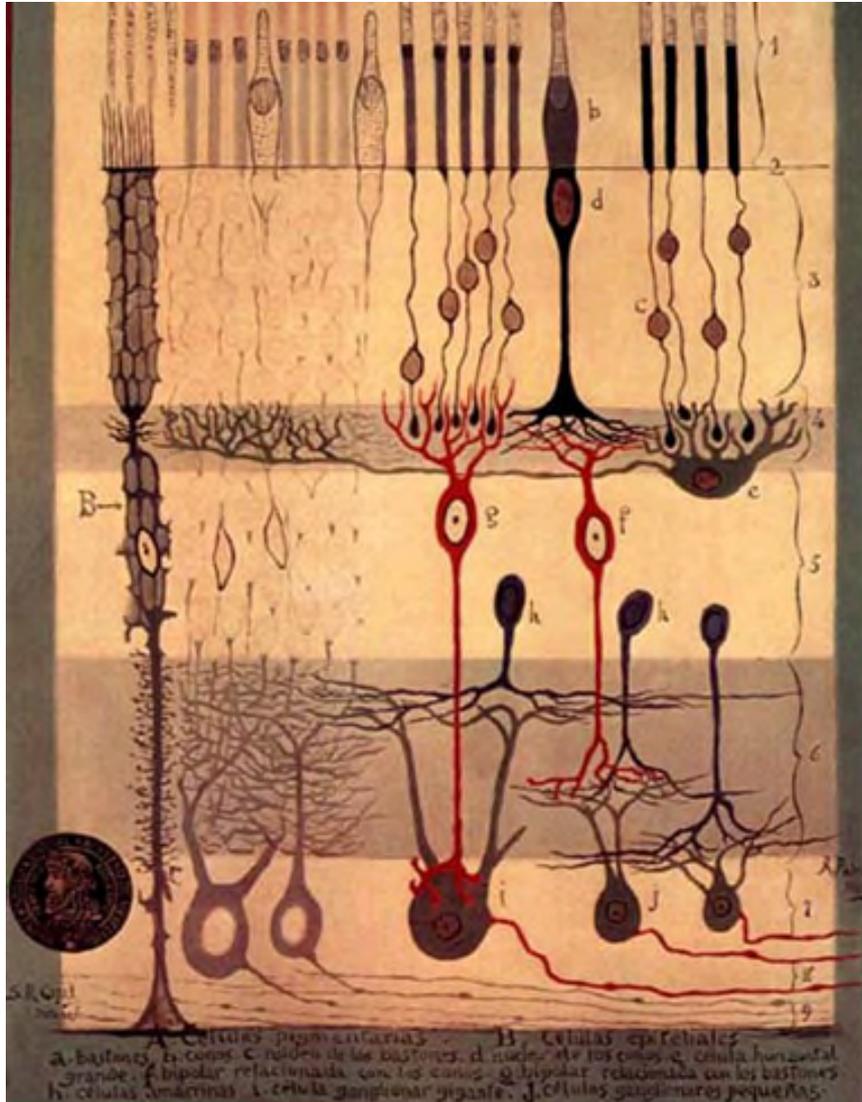
Each spine contains
at least one
Excitatory Synapse



Dendritic Spines



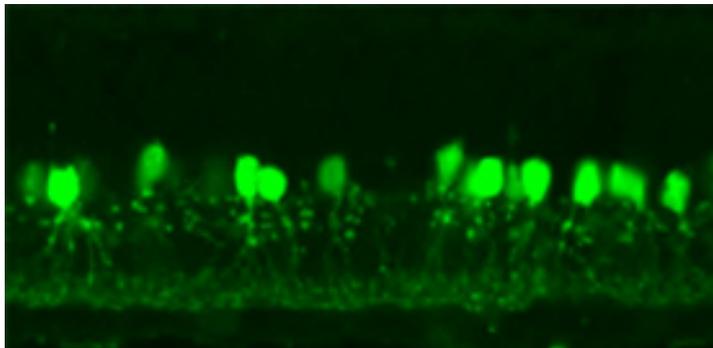
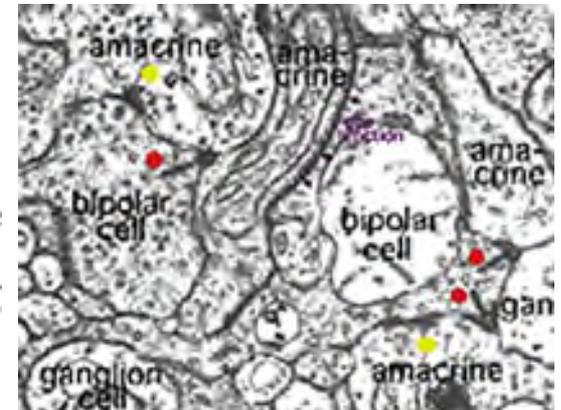
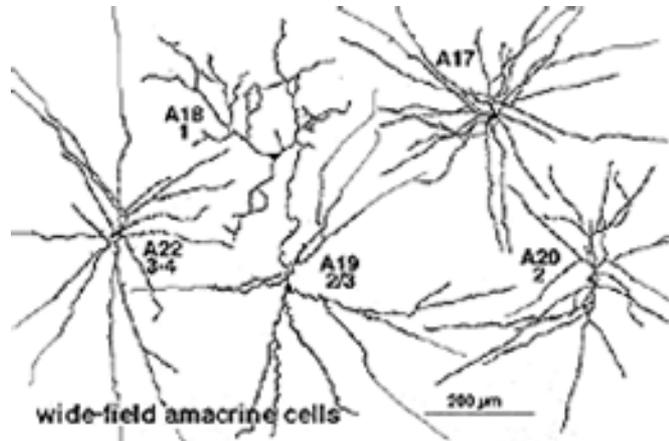
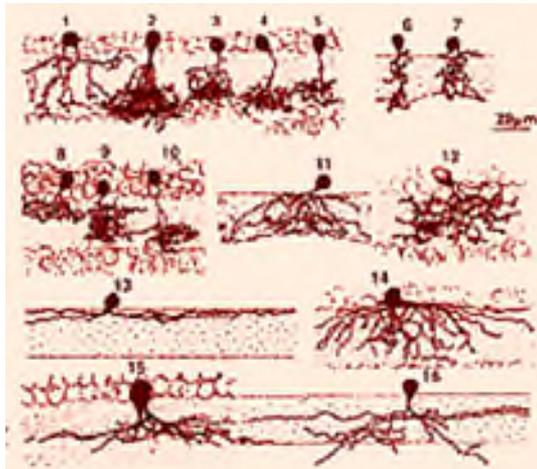
RETINAL CELLS AND WIRING



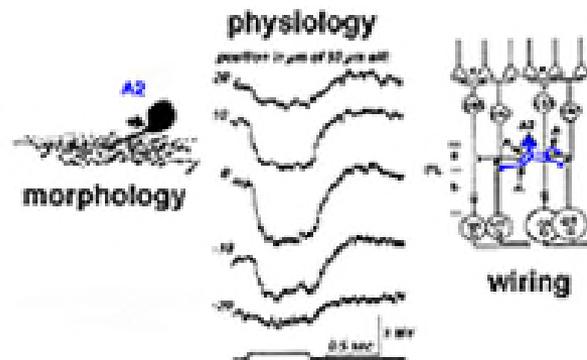
Ramon y Cajal, 1892

RETINA NOT SO SIMPLE

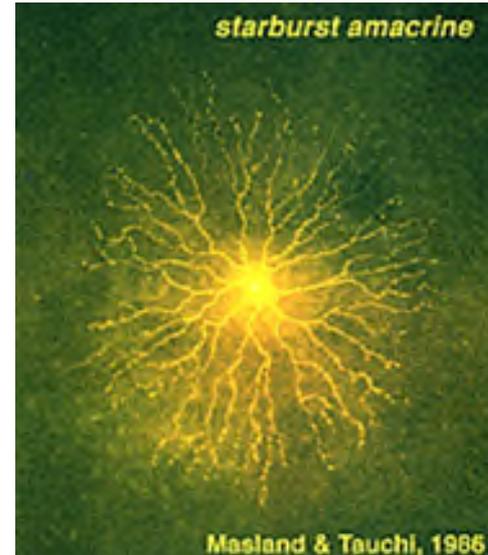
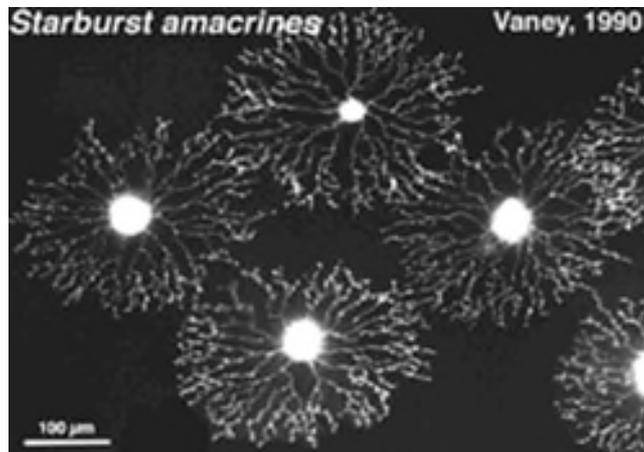
More than 40 subtypes of amacrine cell,
e.g.



Parvalbumin immunofluor.

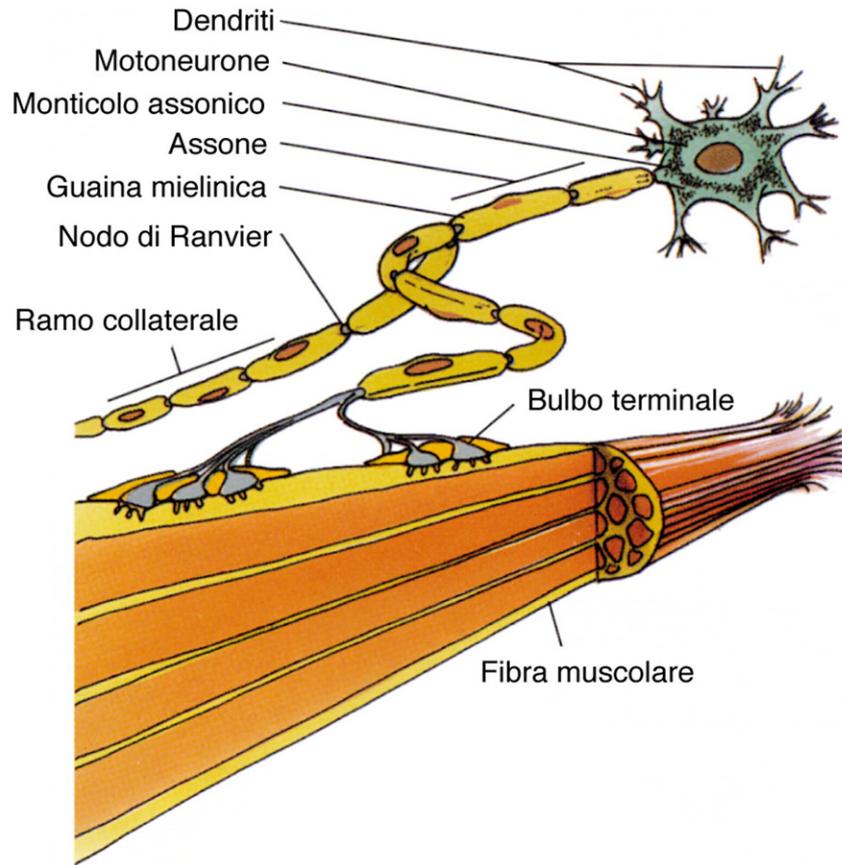


The most beautiful amacrine cell: “Starburst”



Can we better classify neurons??

First, axons can be myelinated...



Dove c'è mielina, il tessuto nervoso appare biancastro = sostanza bianca

Dove non c'è mielina, il tessuto nervoso appare grigiastro = sostanza grigia

A



B

axons can be myelinated... or not

Nervous fibre unmyelinated

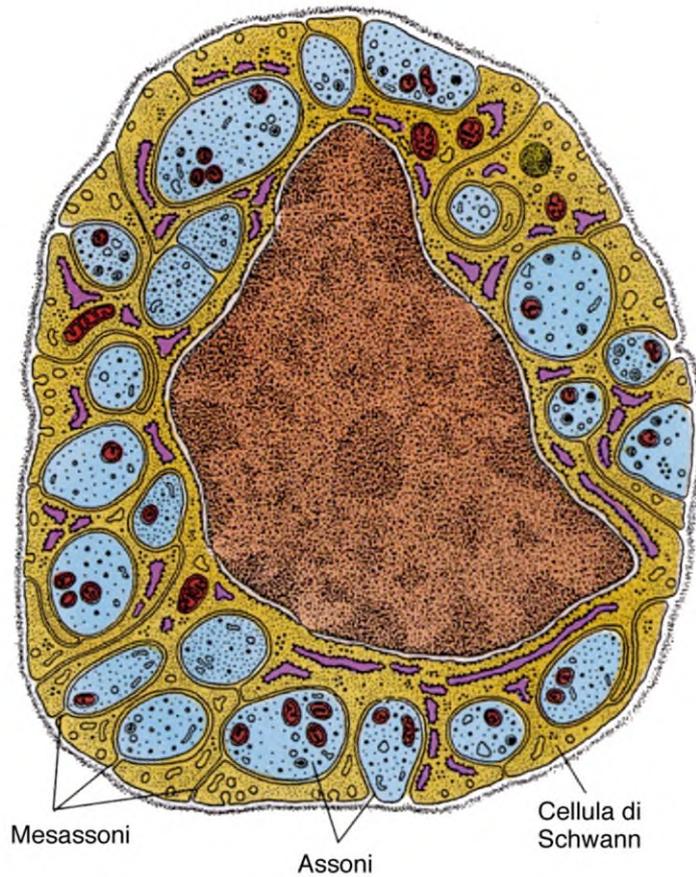


Figure 9-8

Myelinated nervous fibre

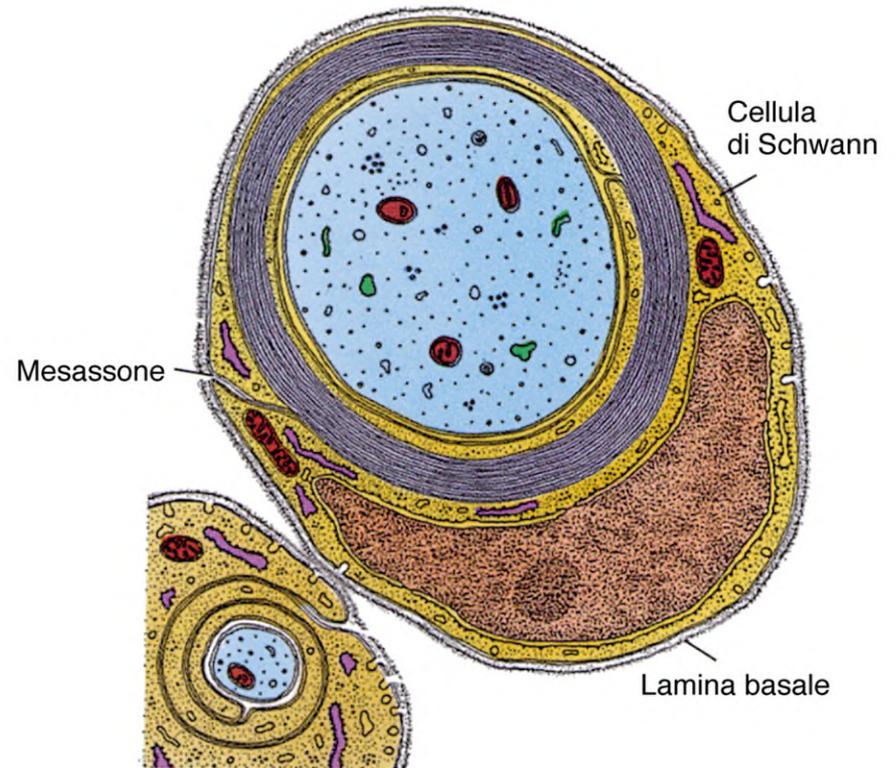
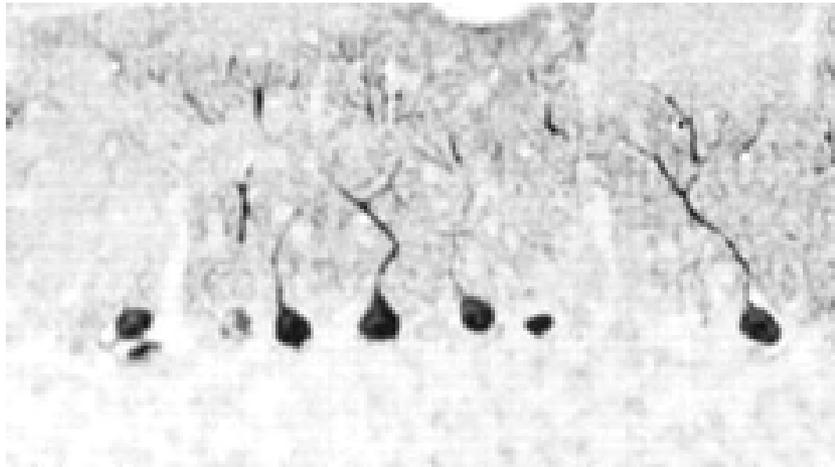
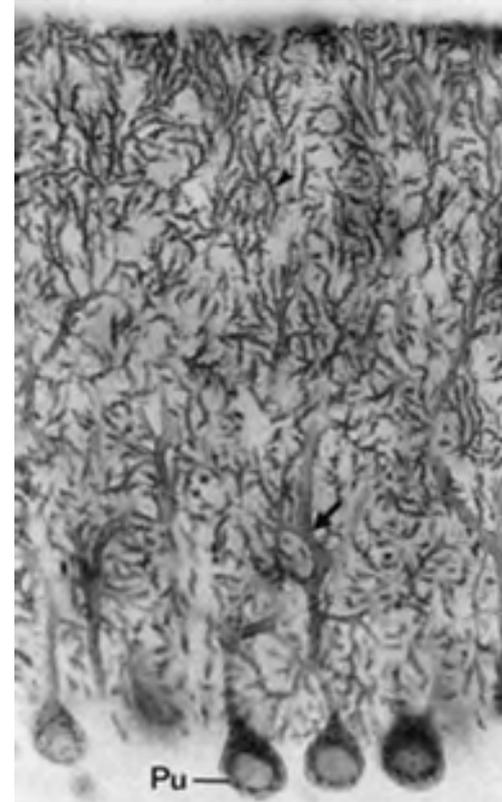


Figura 9-7

Neurons can also be identified by immunohistochemistry

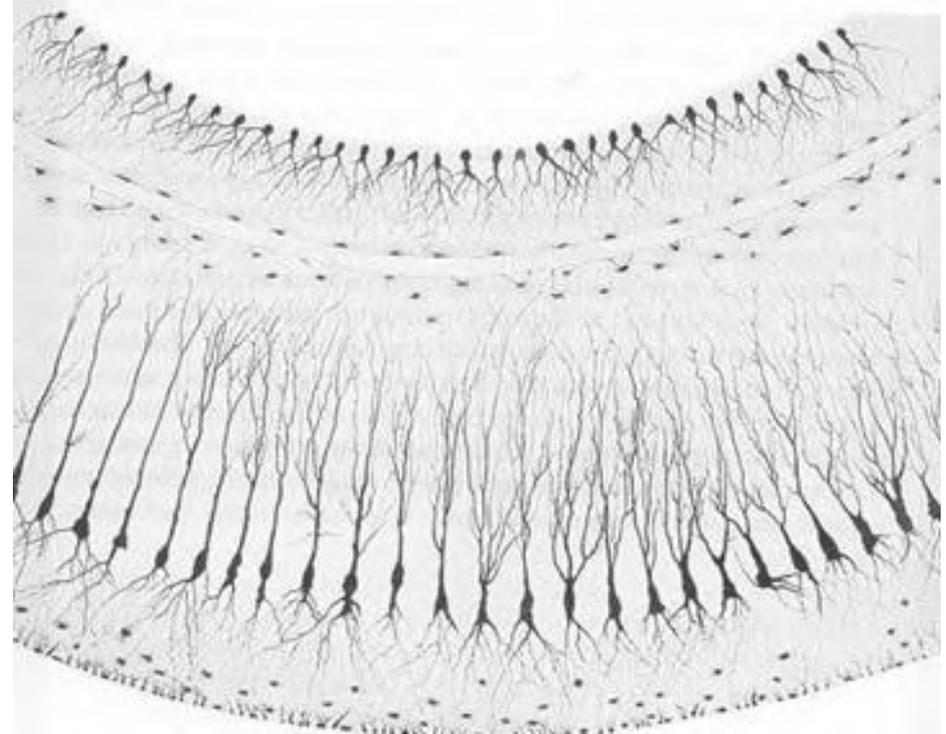


calbindin



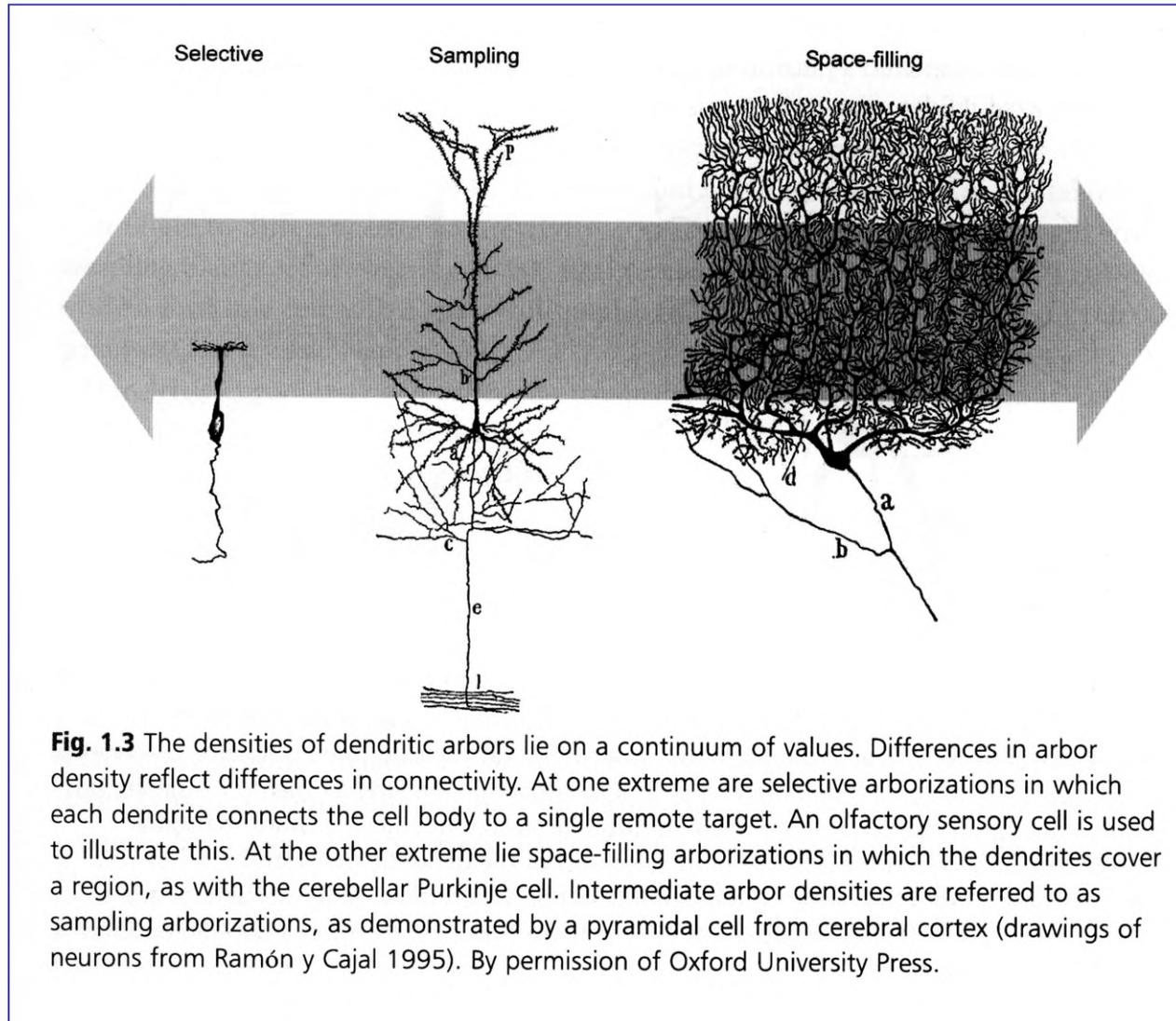
Glutamate transporter
EAAT4

Indeed, no two trees...oops neurons look the same !!



Dendrites: from the greek *dendros*= **tree**

Differences in arbor density reflect differences in connectivity



What changes among the dendrites ?

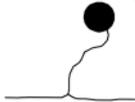
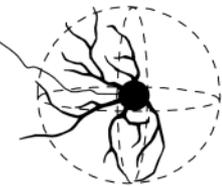
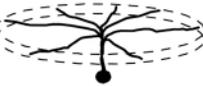
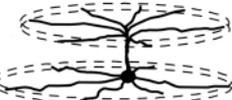
Table 1.1 Typical dimensions of dendrites for a few types of neurons

Neuron	Average soma diameter (μm)	Number of dendrites at soma	Proximal dendrite diameter (μm)	Number of branch points	Distal dendrite diameter (μm)	Dendrite extent* (μm)	Total dendritic length (μm)
Cerebellar granule cell (cat)	7	4	1	0	0.2–2	15	60
Starburst amacrine cell (rhesus)	9	1	1	40	0.2–2	120	—
Dentate gyrus granule cell (rat)	14	2	3	14	0.5–1	300	3200
CA1 pyramidal cell (rat)	21						11900
basal dendrites		5	1	30	0.5–1	130	5500
stratum radiatum		1	3	30	0.25–1	110	4100
stratum lacunosum-moleculare				15	0.25–1	500	2300
Cerebellar Purkinje cell (guinea pig)	25	1	3	440	0.8–2.2	200	9100
Principal cell of globus pallidus (human)	33	4	4	12	0.3–0.5	1000	7600
Meynert cell of visual cortex (macaque)	35						15400
basal dendrites		5	3	—	—	250	10200
apical dendrites		1	4	15	2–3	1800	5200
Spinal α -motoneuron (cat)	58	11	8	120	0.5–1.5	1100	52000

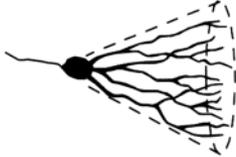
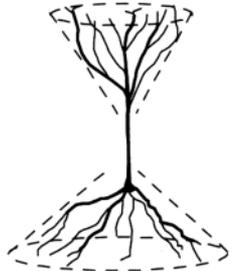
*The average distance from the cell body to the tips of the longest dendrites.

Sources: Ito (1984); Mariani (1990); Claiborne *et al.* (1990); Bannister and Larkman (1995a); Rapp *et al.* (1994); Palay (1978); Yelnik *et al.* (1984); Ulfhake and Kellerth (1981).

Characteristic arborization patterns

Pattern	Characteristics	Examples
Adendritic 	Cell body lacks dendrites	Dorsal root ganglion cells Sympathetic ganglion cells
Spindle radiation 	Two dendrites emerge from opposite poles of the cell body and have few branches	Lugaro cells Bipolar cells of cortex
Spherical radiation Stellate 	Dendrites radiate in all directions from cell body	Spinal neurons Neurons of subcortical nuclei (e.g. inferior olive, pons, thalamus, striatum) Cerebellar granule cells
Partial 	Dendrites radiate from cell body in directions restricted to a part of a sphere	Neurons at edges of 'closed' nuclei (e.g. Clarke's column, inferior olive, vestibular nuclei)
Laminar radiation Planar 	Dendrites radiate from cell body in all directions within a thin domain	Retinal horizontal cells
Offset 	Plane of radial dendrites offset from cell body by one or more stems	Retinal ganglion cells
Multi 	Cell has multiple layers of radial dendrites	Retinal amacrine cells

Characteristic arborization patterns

Pattern	Characteristics	Examples
Cylindrical radiation 	Dendrites ramify from a central soma or dendrite in a thick cylindrical (disk-shaped) domain	Pallidal neurons Reticular neurons
Conical radiation 	Dendrites radiate from cell body or apical stem within a cone or paraboloid	Granule cells of dentate gyrus and olfactory bulb Primary dendrites of mitral cells of olfactory bulb Semilunar cells of piriform cortex
Biconical radiation 	Dendrites radiate in opposite directions from the cell body	Bitufted, double bouquet, and pyramidal cells of cerebral cortex Vertical cells of superior colliculus
Fan radiation 	One or a few dendrites radiate from cell body in a flat fan shape	Cerebellar Purkinje cells

Methods for measuring dendritic complexity

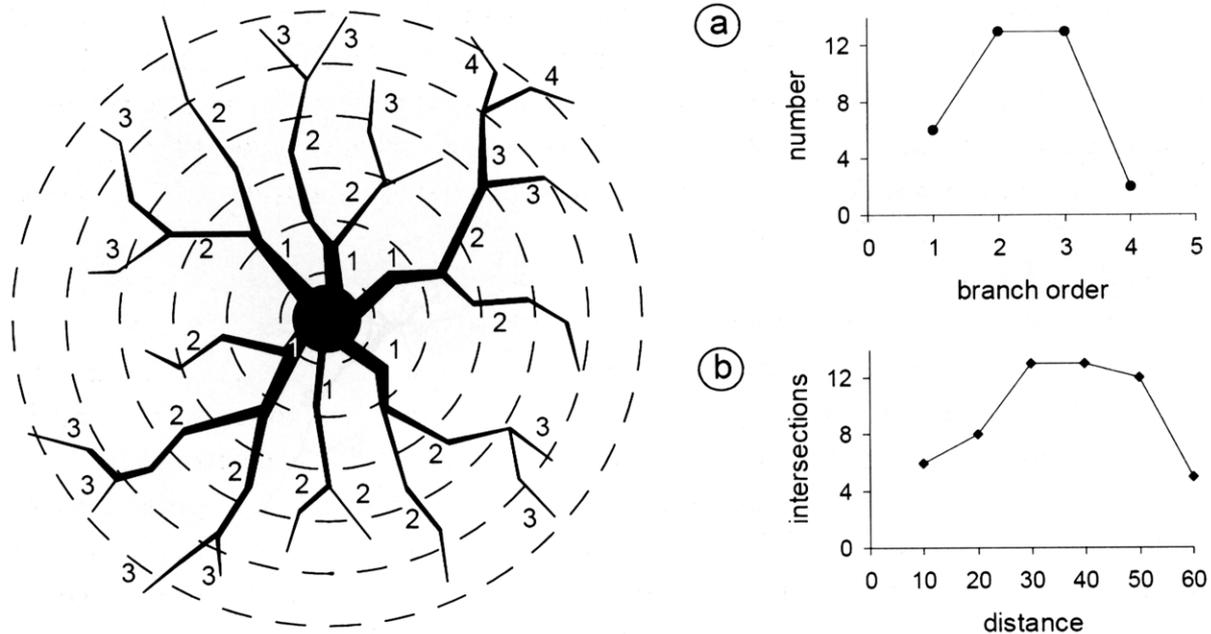


Fig. 1.4 Methods for characterizing dendritic branching. (a) A plot of the number of branches of each order using the centrifugal method of branch ordering. The *Strahler method* is similar but the dendritic tips are order 1 and branch numbers increase sequentially toward the soma. (b) A *Sholl plot* showing the number of intersections of the dendritic tree with circles of increasing radius from the center of the dendritic arbor. When three-dimensional data are available, concentric spheres are used rather than these circles centered on a two-dimensional projection of the neuron.

Comparative neuroanatomy: scaling neurons with brain size

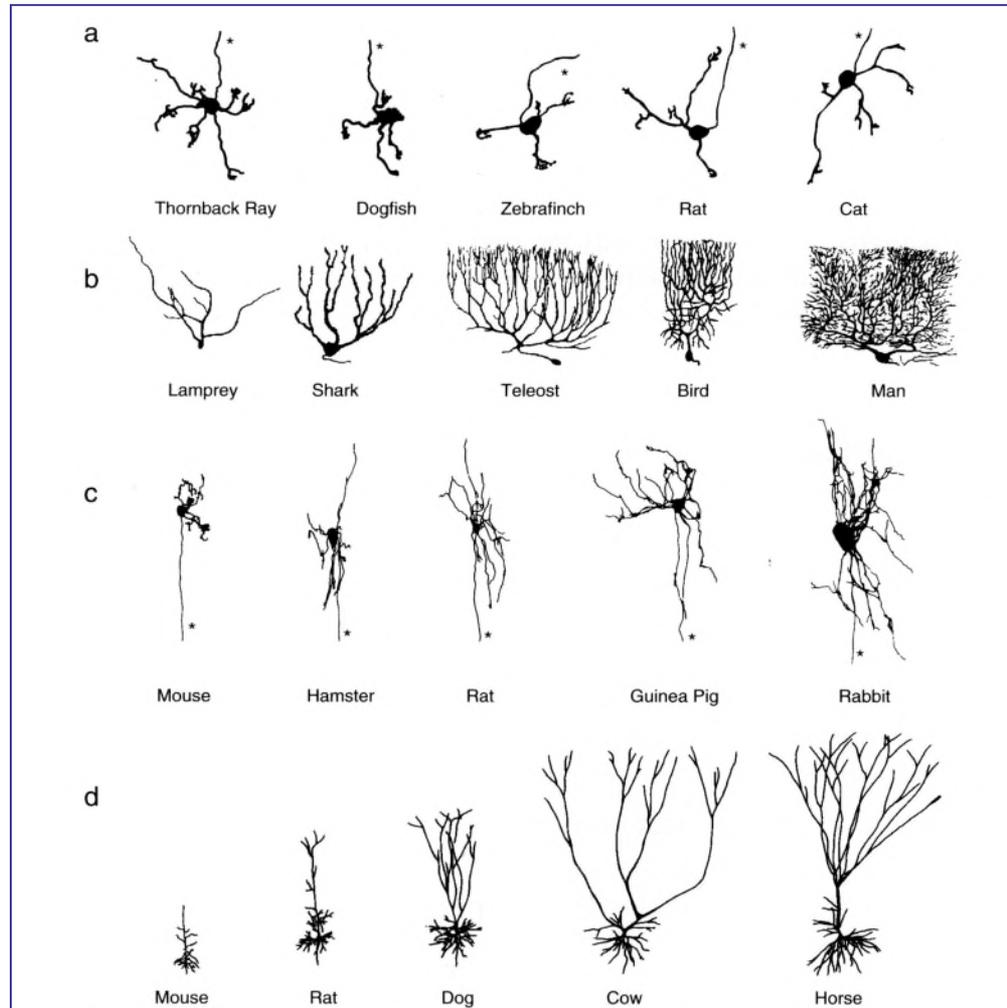


Fig. 2.1 Comparison of vertebrate dendrites across phylogeny. (a) Cerebellar granule cells. Thornback ray from Nicholson *et al.* 1969; dogfish from Schaper 1898; zebra finch, rat, and cat from our own laboratory. (b) Cerebellar Purkinje neurons (Nieuwenhuys 1967). Neurons in panels a and b are scaled to fit page. (c) Sympathetic neurons (Purves 1988). (d) Neocortical pyramidal neurons (Purves 1988, from Barasa 1960). Neurons in panels c and d are scaled to reflect their true size relative to one another. Where appropriate, axons are indicated with asterisks (*)

Summary

- Historical background of the neuronal theory
- Neuronal theory (Golgi & Cajal)
- Polarity of neurons (imperfect epithelial similarity)
- Comparison between unipolar, multipolar neurons
- Diversity of neurons: dimensions, myelin, spines
- Diversity of neurons: dendrites, different shape reflect different function,
- Evolution: not all neurons show scaling with increasing brain size