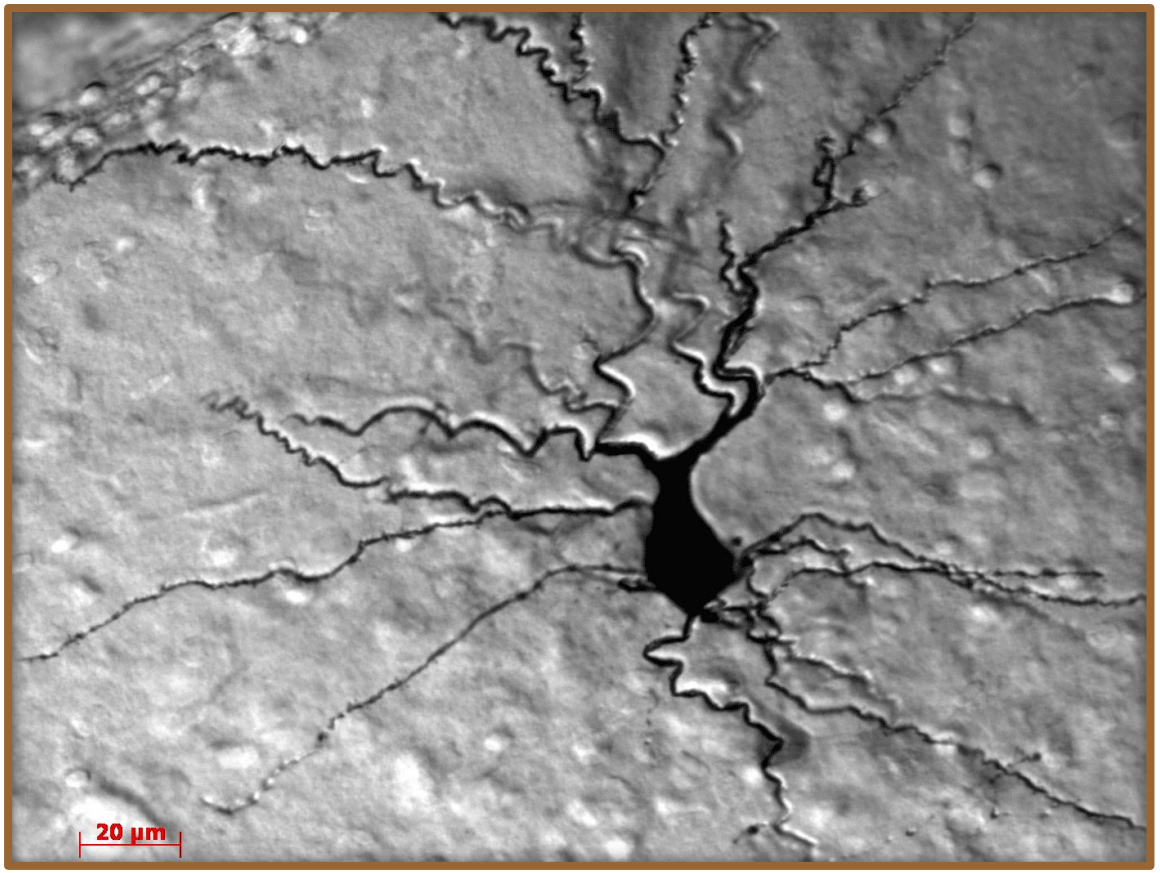
# The neuron




Biocytin labeled pyramidal neuron recorded in piriform cortex

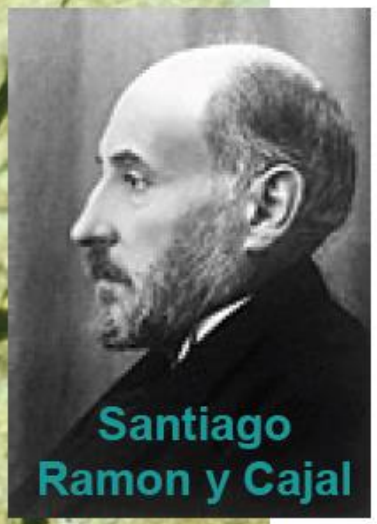
# Discovery of the neuron

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

(A) Reticularist Doctrine

(B) Neuron Doctrine

Exception……

….GAP JUNCTIONS between neurons

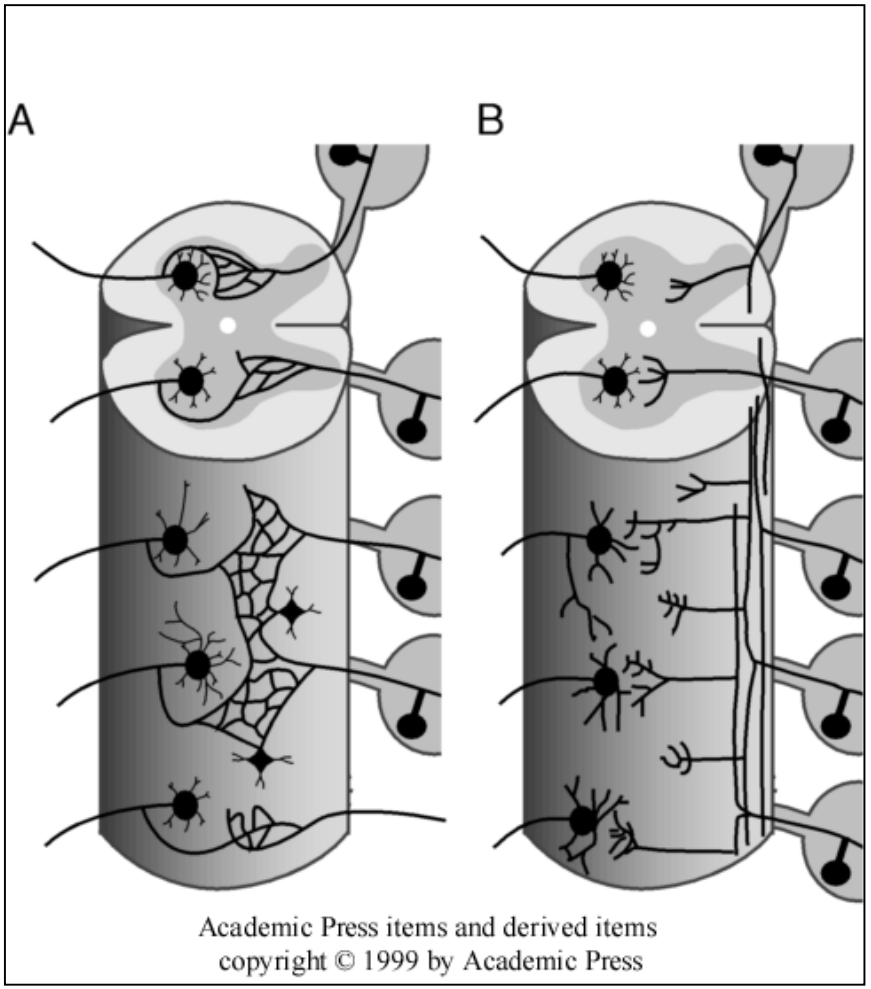
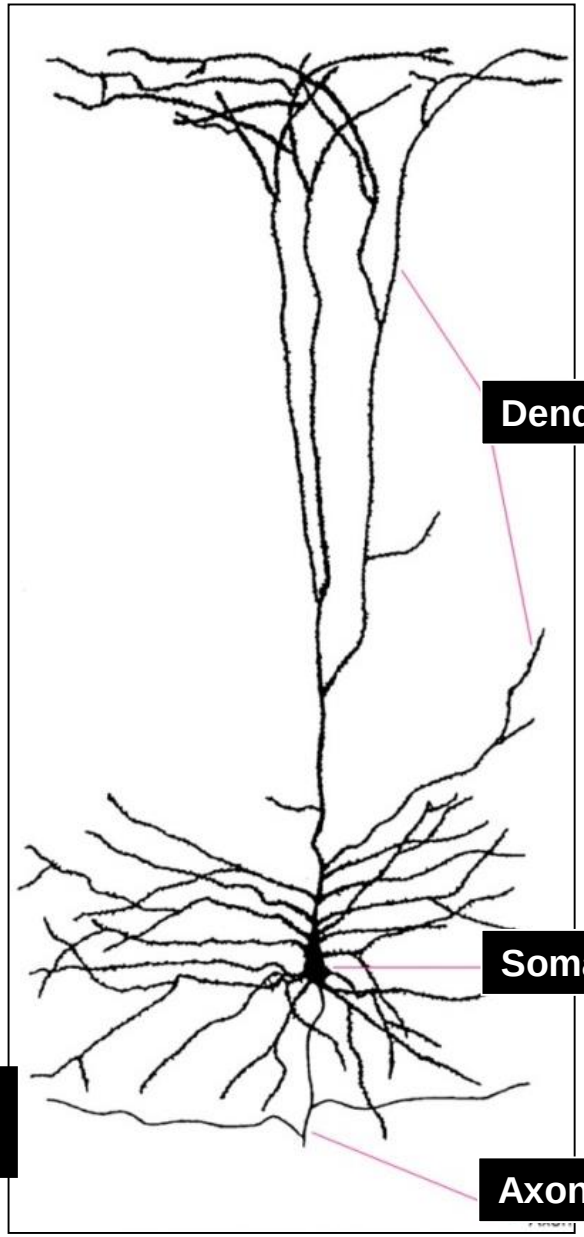





**FIGURE 2** The nervous system is a reticulum versus the neuron doctrine. (A) Proponents of the reticularist's view of the nervous system believed that neurons are physically connected to one another, forming an uninterrupted network. (B) The neuron doctrine, in contrast, considers each neuron an individual entity that communicates with target cells across an appropriate intercellular gap. Adapted from Cajal (1911–1913).

# General neuronal shape




Pyramidal neuron (multipolar)

# Questions on neuroanatomical techniques

https://www.jove.com/science-education/5204/an-introduction-to-neuroanatomy

1. **Who was the first person to publish a description of brain anatomy?**

A) Wilder Penfield
B) Korbinian Brodman
C) Thomas Willis
D) Andreas Vesalius

2. **Who developed a staining technique to visualize single neurons and in what year?**

A) Korbinian Brodman in 1909
B) Camillo Golgi in 1873
C) Andres Vesalius in 1906
D) Santiago Ramón y Cajal in 1888

4. **Which of the following terms most appropriately refers to the microscopic arrangement of neurons?**

A) Neurogenetics
B) Cytoarchitecture
C) Neuroplasticity
D) Neurodegeneration

3.

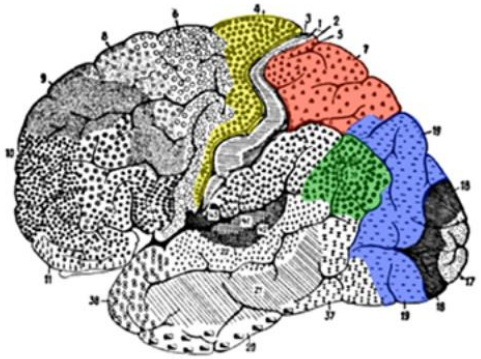


Figure 1 - Map of the Human Brain

**In <u>Figure 1</u>, how were the various highlighted regions of the brain first distinguished from each other?**

A) By their unique cellular architecture.
B) By their different motor functions.
C) By their different sensory functions.
D) By their sensitivity to neural stains.

5.

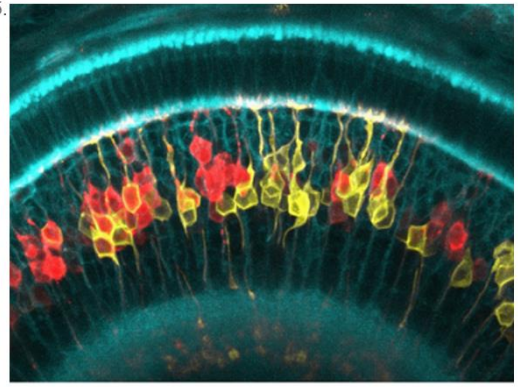


Figure 3 - Different Neurons Stained by Different Tracers

**Which methodology made it possible to capture the image in <u>Figure 3</u>?**

A) The Golgi stain

B) Dye injection

C) Fluorescence microscopy

D) Magnetic resonance imaging

6.

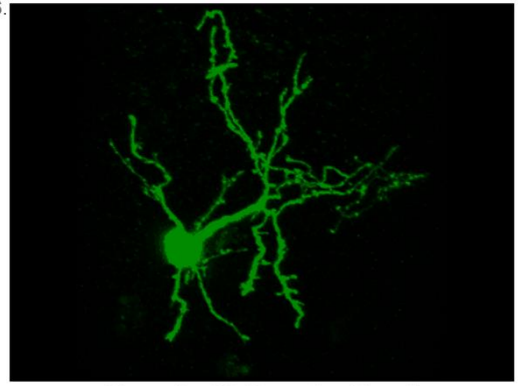


Figure 4 - 3D Reconstruction of Fluorescently Labeled Neuron

**_____ microscopy allows for the creation of images seen in <u>Figure 4</u>.**

A) Confocal

B) 2-Photon

C) Fluorescence

D) Electron

7.

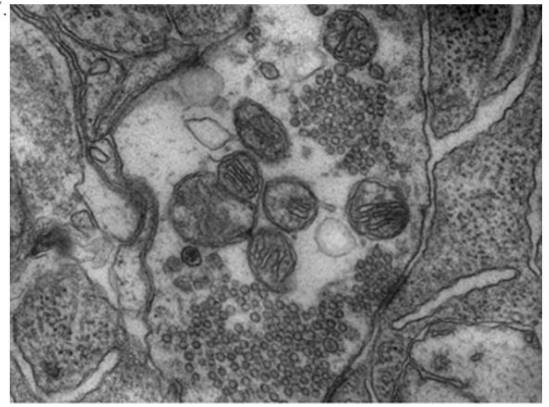


Figure 5 - Organelles in a Synaptic Terminal

**The image in <u>Figure 5</u> was generated using _____ microscopy.**

A) confocal

B) electron

C) 2-photon

D) atomic force

8. **Which of the following techniques is NOT a potential application of stereotaxic surgery?**

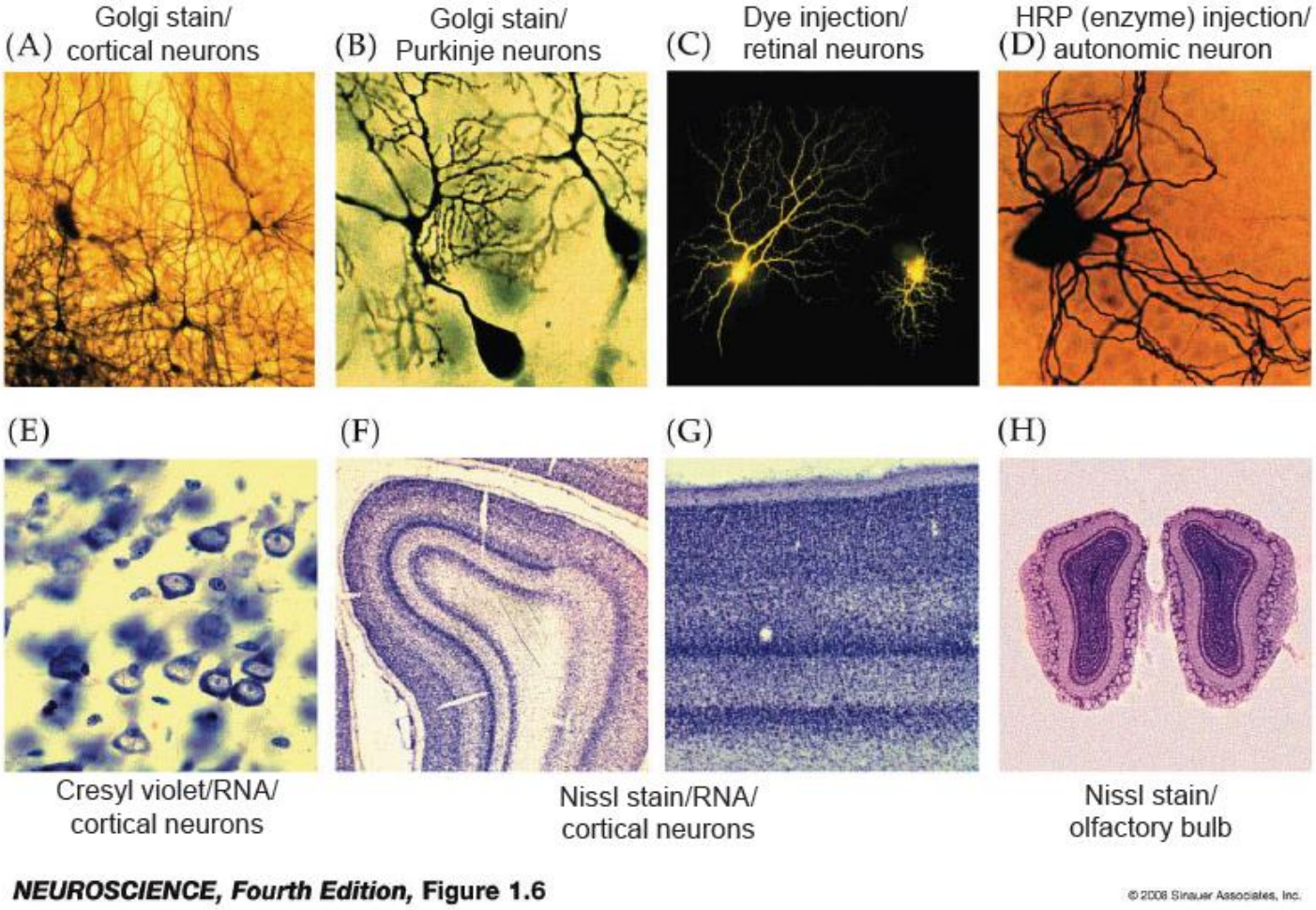
A) Injection of drugs

B) Generation of lesions

C) Neuronal cell culture

D) Delivery of electrical stimulation

# Studying structure




***NEUROSCIENCE, Fourth Edition,* Figure 1.6**



**Which other types of neural staining can be used? For what purpose?**


https://www.jove.com/science-education/5206/histological-staining-of-neural-tissue

# Questions on neural stainings

**2. What step in immunohistochemistry minimizes undesirable background staining?**

- blocking excess primary antibodies
- dewaxing and rehydrating tissue sections
- multiple applications of primary antibodies
- serum proteins binding to nonspecific sites

**4. Primary antibodies in an immunohistochemical procedure, as shown in Figure 1, function by…**

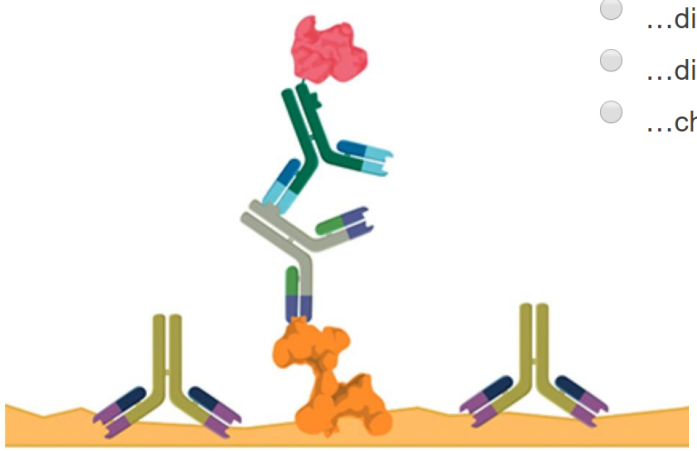


Figure 1 - Antibodies During Immunohistochemistry

- …enzymatically enhancing contrast.
- …directly binding to nonspecific sites.
- …directly attaching to targets in neuronal tissue.
- …changing color.

**5. Which of the following best describes the order in which the antibodies pictured in Figure 1 are applied during immunohistochemistry?**

- Blocking serum buffer → primary antibodies → secondary antibodies
- Primary antibodies → secondary antibodies → blocking serum buffer
- Blocking serum buffer → secondary antibodies → primary antibodies
- Secondary antibodies → primary antibodies → blocking serum buffer

**3. A chromagenic substrate is necessary to…**

- …excite fluorophores to help visualize staining.
- …penetrate the cell membrane.
- …provide a change in color to visualize staining.
- …minimize background staining.

**8. Replacing the blood in the brain with fixative is referred to as what?**

- perfusion
- blocking
- reconstitution
- dewaxing

**9. Which of the following is NOT an application of neuronal staining?**

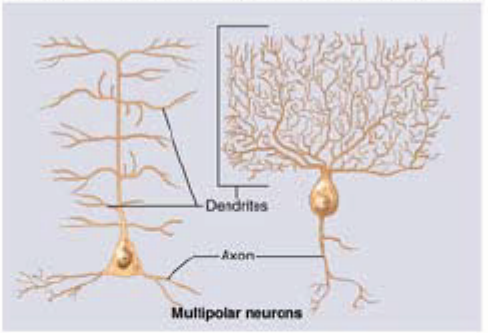
- Investigating neuron-specific gene expression.
- Functional characterization of neurons.
- Identifying the location of specialized neurons known as Purkinje cells.
- Revealing that dendrites undergo multiple changes in response to activating stimuli.

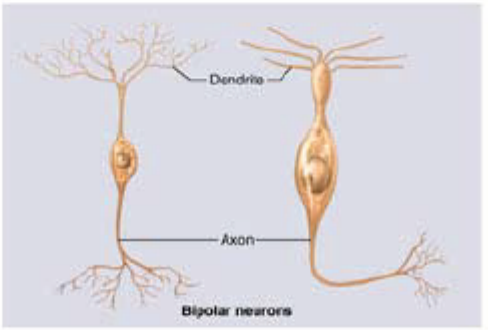
# Variations in neuronal shape

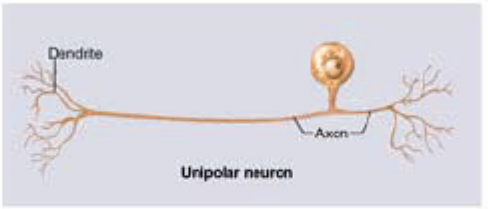


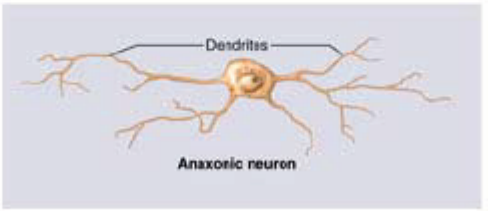
## Basic classification of neurons

- 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

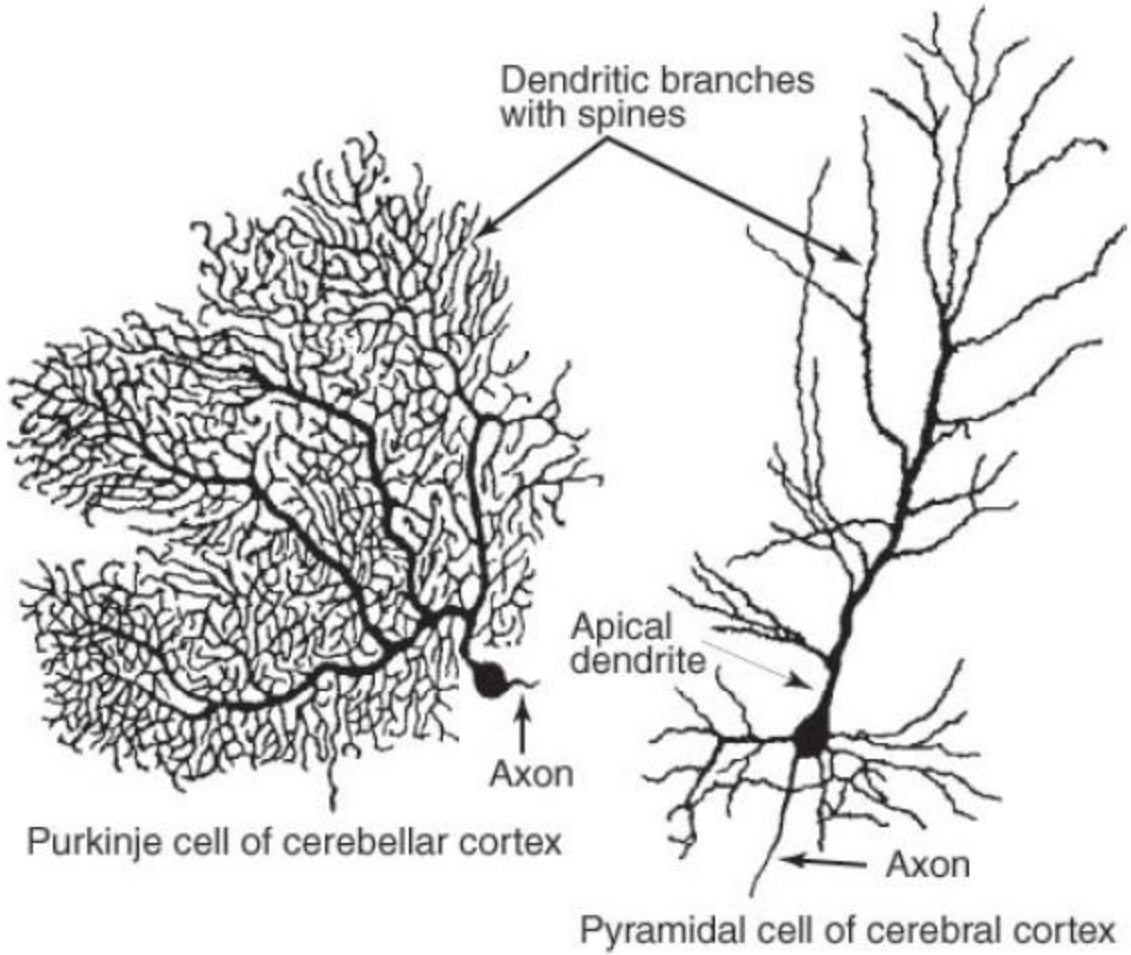


# Morphology of vertebrate multipolar neurons is highly variable



# Differences in arbor density reflect differences in connectivity

Fiala & Harris, 1999
Dendrite structure; in "Dendrites", Oxford Univ Press

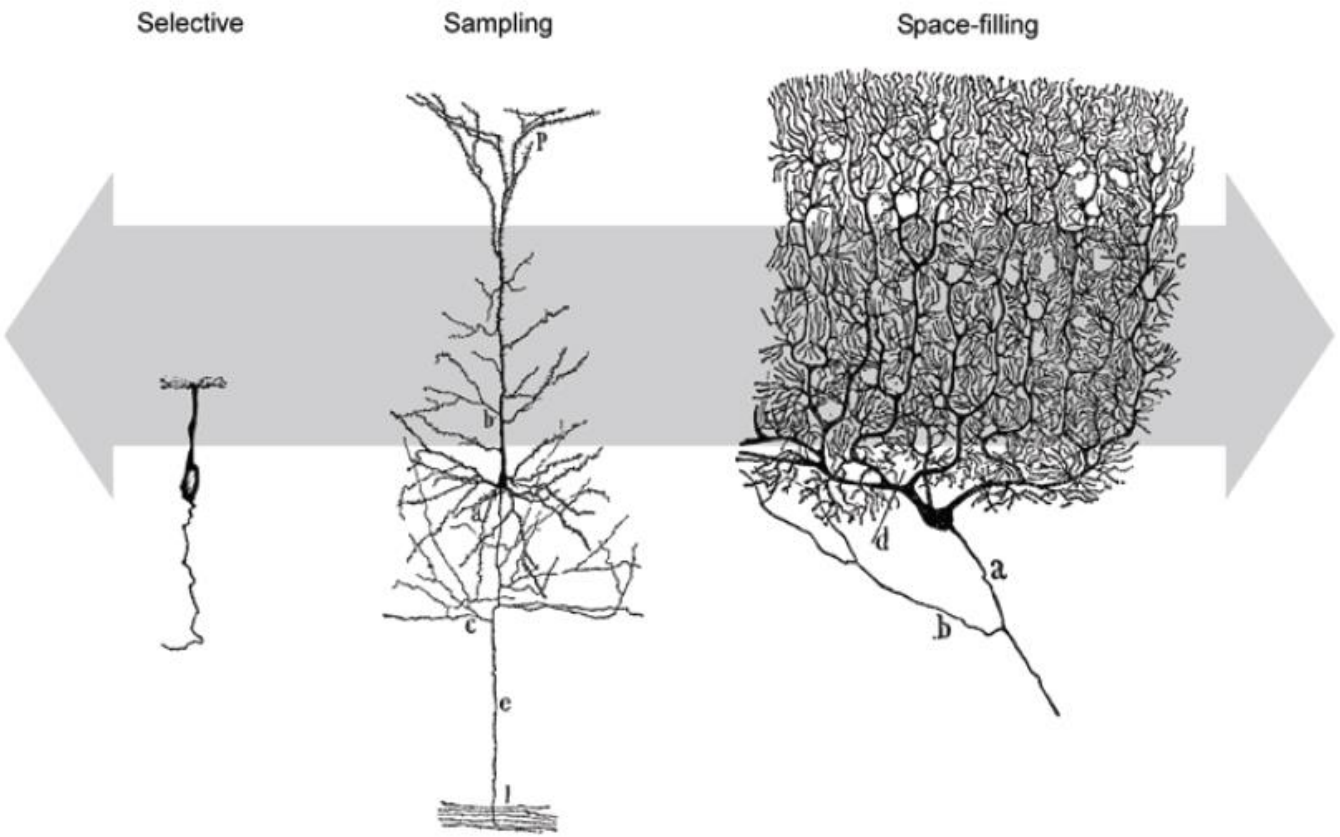
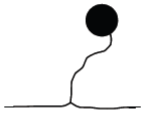
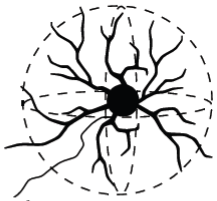
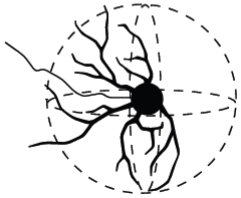
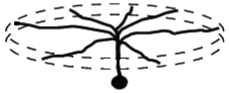
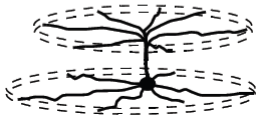


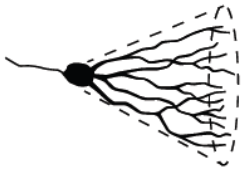
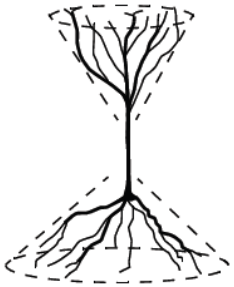

**Fig. 1.5** The densities of dendritic arbors lie on a continuum of values. Differences in arbor density reflect differences in connectivity. At one extreme are selective arborizations in which each dendrite connects the cell body to a single remote target. An olfactory sensory cell is used to illustrate this. At the other extreme lie space-filling arborizations in which the dendrites cover a region, as with the cerebellar Purkinje cell. Intermediate arbor densities are referred to as sampling arborizations, as demonstrated by a pyramidal cell from cerebral cortex. (Drawings of neurons from Ramón y Cajal, 1995.)

# Characteristic arborization patterns

| Pattern | Characteristics | Examples |
|---|---|---|
| **Adendritic**  | Cell body lacks dendrites | Dorsal root ganglion cells<br>Sympathetic ganglion cells |
| **Spindle radiation**  | Two dendrites emerge from opposite poles of the cell body and have few branches | Lugaro cells<br>Bipolar cells of cortex |
| **Spherical radiation**<br>Stellate  | Dendrites radiate in all directions from cell body | Spinal neurons<br>Neurons of subcortical nuclei (e.g. inferior olive, pons, thalamus, striatum)<br>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**<br>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 |

Fiala & Harris, 1999
Dendrite structure; in "Dendrites", Oxford Univ Press

# 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<br>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<br>Primary dendrites of mitral cells of olfactory bulb<br>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<br>Vertical cells of superior colliculus |
| **Fan radiation**  | One or a few dendrites radiate from cell body in a flat fan shape | Cerebellar Purkinje cells |

# 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 | | | | | | 11 900 |
| 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 | | | | | | 15 400 |
| basal dendrites | | 5 | 3 | — | — | 250 | 10 200 |
| apical dendrites | | 1 | 4 | 15 | 2-3 | 1800 | 5200 |
| Spinal a-motoneuron (cat) | 58 | 11 | 8 | 120 | 0.5-1.5 | 1100 | 52 000 |

* 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)

Fiala & Harris, 1999 Dendrite structure; in “Dendrites”, Oxford Univ Press

# Methods for measuring dendritic complexity

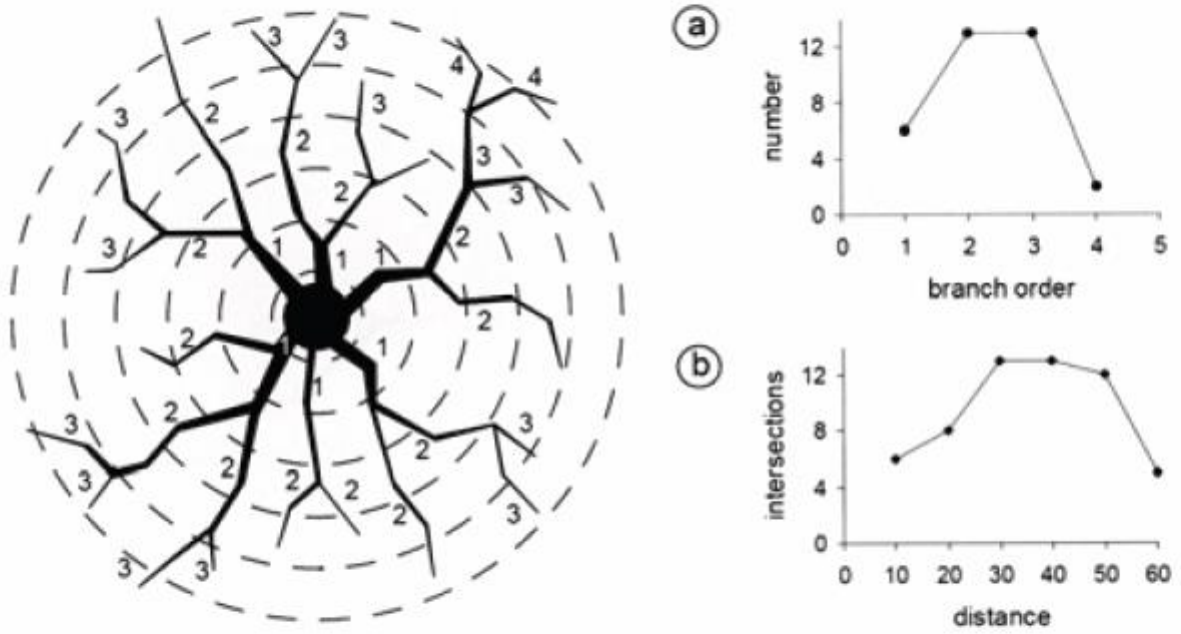



**Fig. 1.4** Methods for characterizing dendritic branching. **(a)** A plot of the number of branches of each order using the centrifugal method of branch ordering. The *Strahler method* is similar but the dendritic tips are order 1 and branch numbers increase sequentially toward the soma. **(b)** A *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.

# Neuronal classification

**Structural classification**:

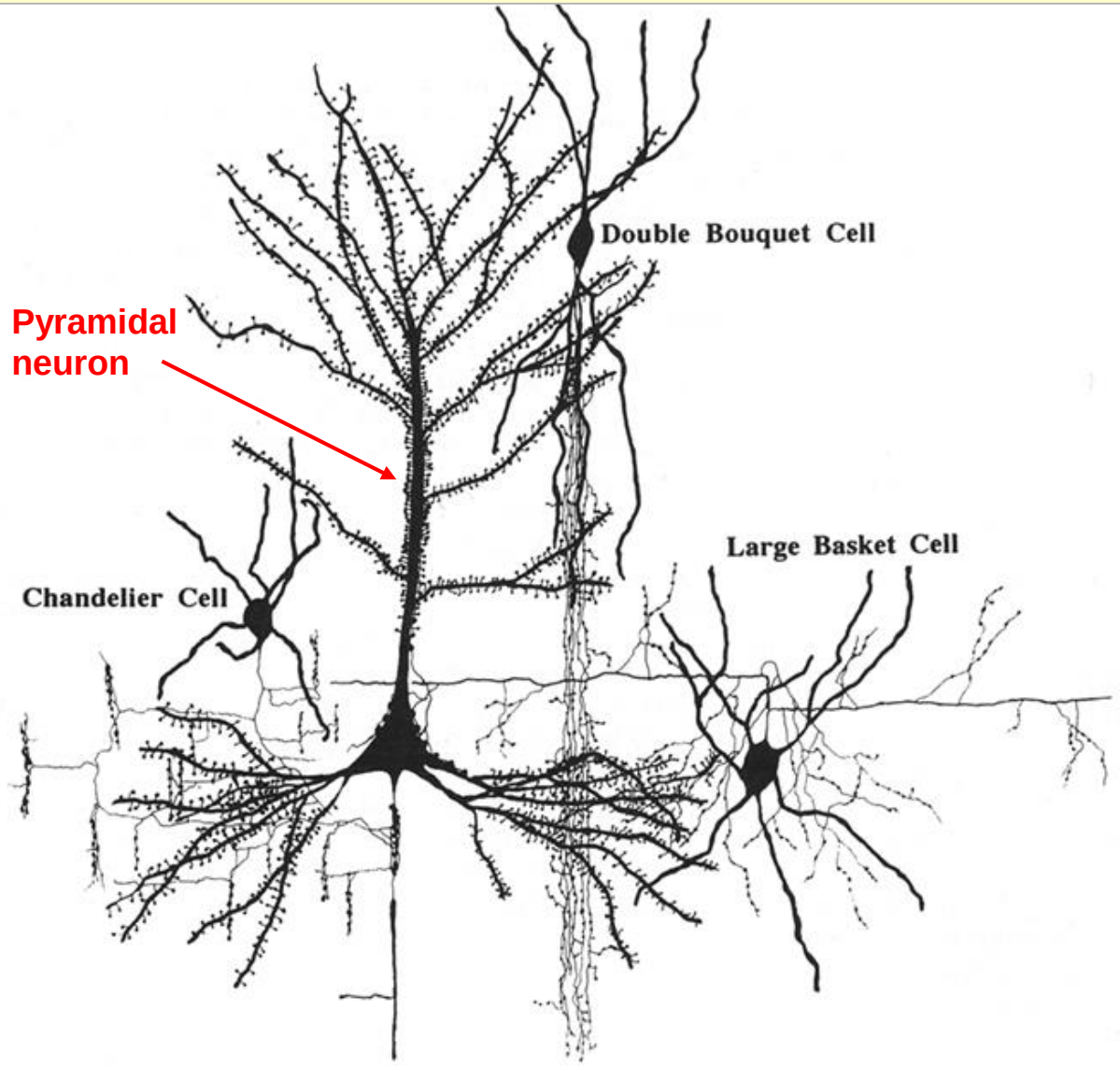
Unipolar, bipolar, multipolar, more …

**Functional classification:**

- projection (inter)neurons
- local circuit (inter)neurons

- excytatory (neurotransmitters: Glutamate, etc.)
- inhibitory (neurotransm.: GABA, glycine, etc.)

# Projection neurons (pyramidal) and local circuit neurons (interneurons) in cerebral cortex




Interneurons comprise 20–30% of the cortical neuronal population and are locally projecting cells that control and synchronize the output of pyramidal neurons. Interestingly, the influence of GABAergic interneurons on pyramidal cells is largely dependent on the subcellular location of their inputs, which varies among different interneuron subtypes.

# Neocortical interneurons diversity

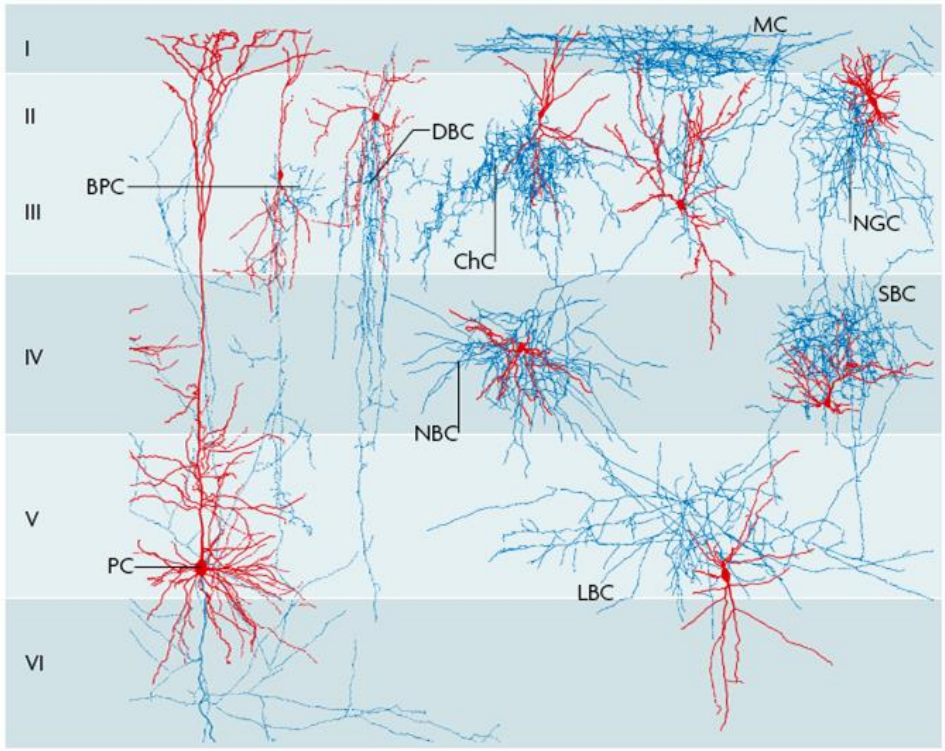



Figure 1 | **Axon arbors and innervation patterns of neocortical interneurons.** Interneuron axon arbors distribute inhibitory outputs to discrete spatial domains in the neural network. The figure shows a reconstruction of several classes of neocortical interneurons and, for comparison, a layer five pyramidal neuron (PC; left most). Axons are shown in blue and dendrites are shown in red. The geometry of interneuron axon arbors can be vertical, horizontal, or laminated, suggesting that their output can be distributed to the same or multiple cortical columns, and same or multiple cortical layers. Interneuron axons often elaborate highly exuberant local branches, innervate specific cell types, and impose strong control over local neural populations. Cortical layers are indicated on the left of the figure. BPC, bipolar cell; ChC, chandelier cell; DBC, double bouquet cell; LBC, large basket cell; MC, Martinotti cell; NBC, nested basket cell; NGC, neurogliaform cell; SBC, small basket cell. Images courtesy of Dr Henry Markram (EPFL, Switzerland).

Huang et al., 2007
Nature Rev Neurosci
doi:10.1038/nrn2188

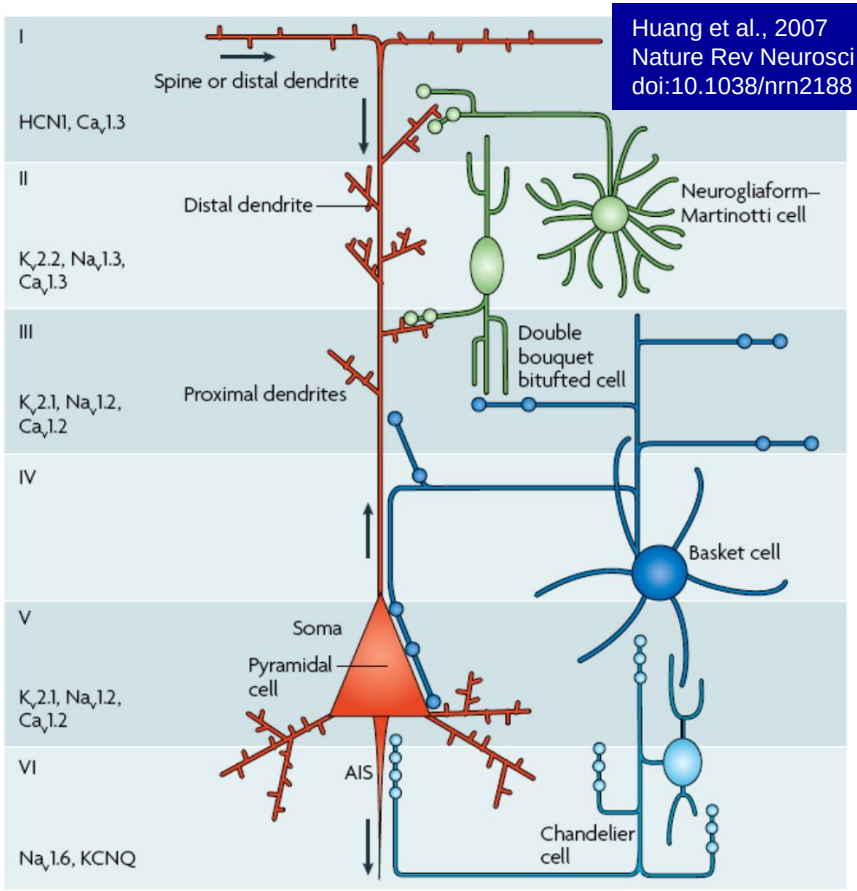



Figure 2 | **The subcellular organization of GABAergic inputs.** Pyramidal neurons (shown in red) in the neocortex are characterized by their large size, striking polarity and distinct subcellular domains. The compartmentalized forward and backward electrical signalling (depicted by arrows) arises from the targeted distribution of signalling mechanisms, receptors and ion channels. The distributions of several voltage-gated sodium ($Na_v$), potassium ($K_v$; KCNQ) and calcium ($Ca_v$) channels, and of a hyperpolarization-activated cyclic nucleotide-gated (HCN) cation channel are highlighted to the left of the pyramidal neuron. The subcellular organization of different classes of GABAergic inhibitory inputs is superimposed on the anatomical and physiological compartments of pyramidal neurons, allowing effective regulation of synaptic integration, spike generation, back propagation and plasticity. The stereotyped position and geometry of pyramidal neurons within a cortical column (cortical layers are indicated on the left) suggests that their subcellular architecture significantly affects neuronal signalling in cortical circuits. AIS, axon initial segment; GABA, γ-aminobutyric acid. Modified, with permission, from *Nature Rev. Neurosci.* REF. 27 © (2005) Macmillan Publishers Ltd.

systematic categorization of cell types is an essential prerequisite for understanding mechanisms

# "Easy" neuronal classification

**Structural classification**:

Unipolar, bipolar, multipolar, more … (Cajal's classification)

**Functional classification:**

- projection (inter)neurons
- local circuit (inter)neurons

- excytatory (neurotransmitters: Glutamate, etc.)
- inhibitory (neurotransm.: GABA, glycine, etc.)

Is it enough?

# The problem of neuronal classification and subtype identification…….

We need to classify different neuronal types in order to speak a "common language" with other neuroscientists and in order to understand the complexity of brain function…

HOW should we classify neurons?

**By morphology?**

**By functional features?**

**By expression markers?**

How do we put together information from different approaches?

for discussion see Yuste, 2005

# The problem of neuronal classification…

The obvious (but not the easiest) solution would be COMBINING different approaches in the same experimental model. An example:

## Localization of Calcium-binding Proteins in Physiologically and Morphologically Characterized Interneurons of Monkey Dorsolateral Prefrontal Cortex

A.V. Zaitsev[1], G. Gonzalez-Burgos[1], N.V. Povysheva[1], S. Kröner[2,3], D.A. Lewis[1,2] and L.S. Krimer[1]

Cerebral Cortex August 2005;15:1178-1186

(1) Whole-cell electrophysiological recording on cortical slices

+

(2) intracellular injection of byocytin in recorded neurons (for later recognition and morphological analysis)

+

(3) fluorescence immunocytochemistry for selected markers (calcium-binding proteins: parvalbumin, calbindin and calretinin)

ABSTRACT

In the primate neocortex, little is known about the possible associations between functional subclasses of GABA neurons, their morphological properties and calcium-binding protein (CaBP) content. We used whole-cell current clamp recordings, combined with intracellular labeling and fluorescence immunohistochemistry, to determine these relationships for interneurons in layers 2–3 of monkey prefrontal cortex (PFC). Eighty-one interneurons were included in the analysis. Thirty-eight of these cells showed immunoreactivity for one of the three CaBPs tested. Co-localization of more than one CaBP was not observed in any of the interneurons examined. Interneurons with different CaBPs formed distinct populations with specific physiological membrane properties and morphological features. Parvalbumin (PV)-positive cells had the physiological properties characteristic of fast-spiking interneurons (FS) and the morphology of basket or chandelier neurons. Most calretinin (CR)-containing cells had the physiological properties ascribed to non-fast-spiking cells (non-FS) and a vertically oriented axonal morphology, similar to that of double bouquet cells. Calbindin (CB)-positive interneurons also had non-FS properties and included cells with double bouquet morphology or with a characteristic dense web of axonal collaterals in layer 1. Classification of the interneurons based on cluster analysis of multiple electrophysiological properties suggested the existence of at least two distinct groups of interneurons. The first group contained mainly PV-positive FS cells and the second group consisted predominantly of CR- and CB-positive non-FS interneurons. These findings may help to illuminate the functional roles of different groups of interneurons in primate PFC circuitry.

# The phenotype of BC-injected/electrophysiologically-recorded interneurons is determined by immunocytochemistry

Zaitsev et al., 2005
Cerebral Cortex
doi:10.1093/cercor/bhh218

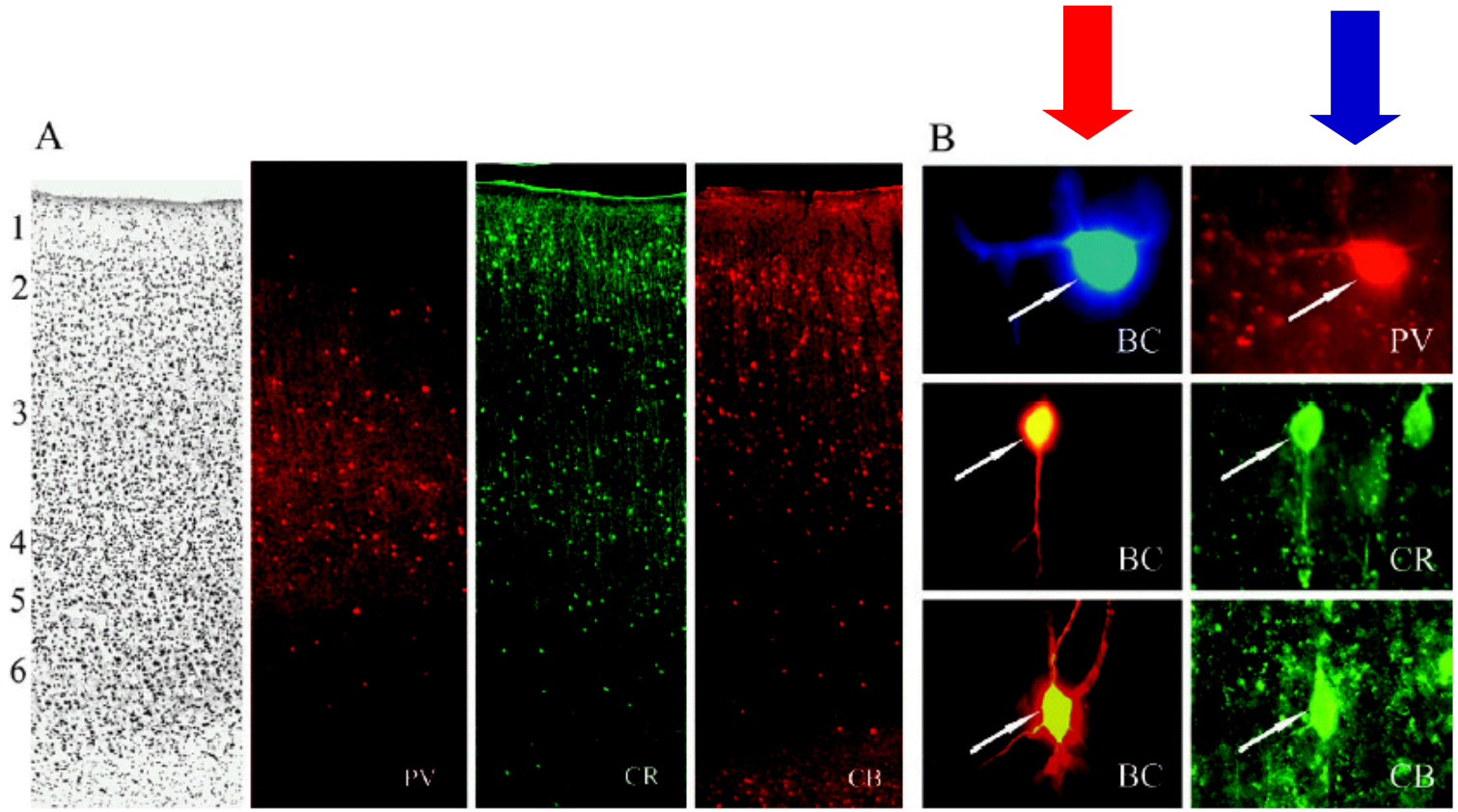



**Figure 1.** Fluorescence-labeling of CaBPs in monkey DLPFC interneurons. (A) Photomicrographs of adjacent coronal sections (area 46) with laminar boundaries; from left Nissl stain, PV-, CR- and CB-IR structures. Note the substantial differences in the laminar distribution of the neurons labeled for each CaBP. (B) Dual-label photomicrographs from the same microscopic field, showing immunohistochemical identification of physiologically characterized biocytin (BC)-injected interneurons as positive for PV, CR or CB. Arrows show the cell bodies. Top: BC visualized by streptavidin-Alexa FluorÒ 350 conjugate (blue), PV-IR visualized by Alexa FluorÒ 594 conjugated secondary antibody. Middle: BC visualized by streptavidin-Alexa FluorÒ 568 conjugate, CR-IR visualized by Alexa FluorÒ 488 conjugated secondary antibody. Bottom: BC visualized by streptavidin-Alexa FluorÒ 568 conjugate, CB-IR visualized by Alexa FluorÒ 488 conjugated secondary antibody.


https://www.jove.com/science-education/5040/introduction-to-fluorescence-microscopy

The combination of intracellular-injection techniques and immunocytochemistry suggests that the same phenotypic marker is expressed by interneurons with different morphologies

Do different morphologies indicate different functional features?

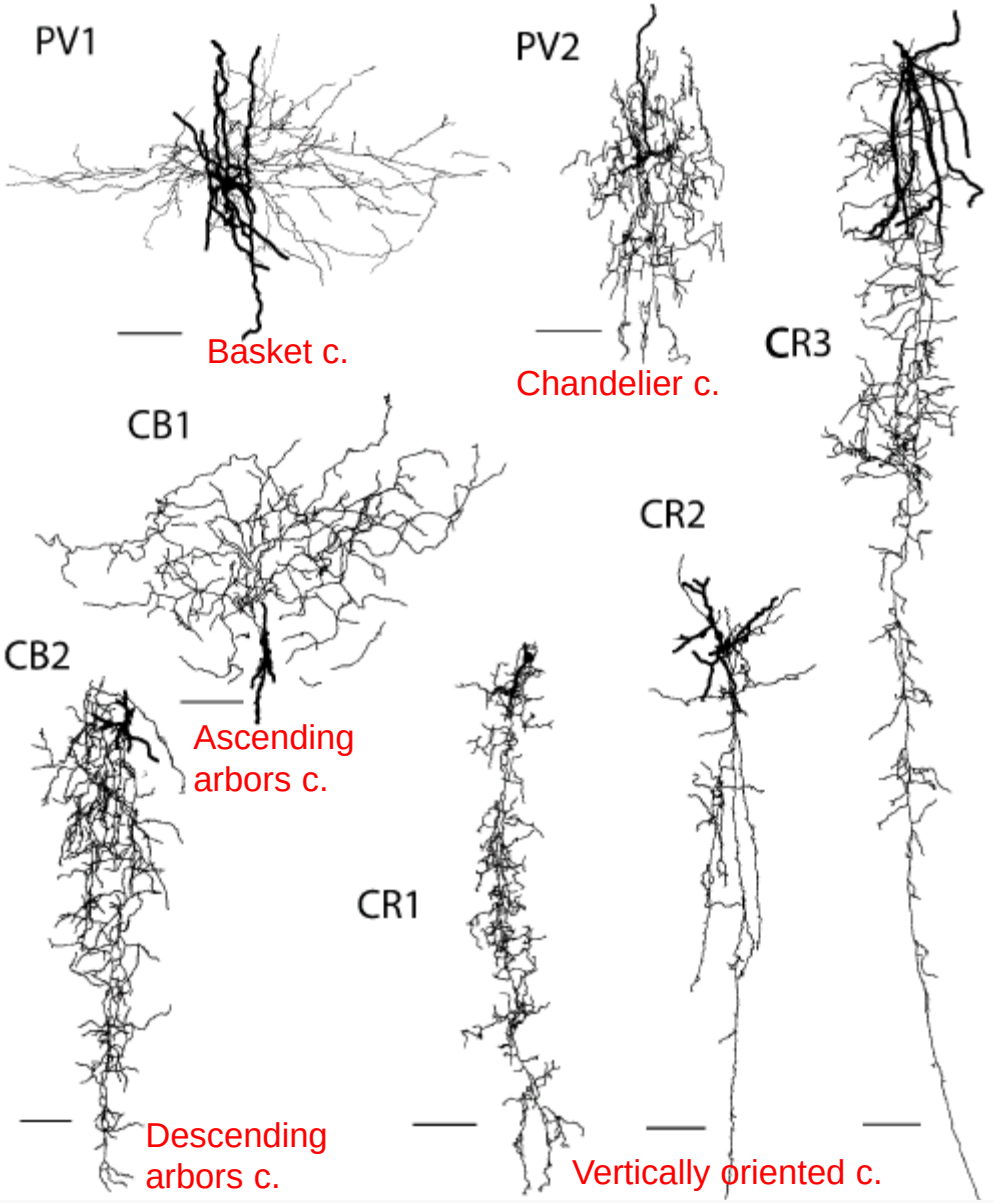



**Figure 2.** Three-dimensional reconstructions of biocytin-labeled interneurons from monkey DLPFC. PV1, PV-IR spreading arbor (basket) cell; PV2, PV-IR chandelier cell; CR1-CR3, examples of CR-IR vertically oriented cells; CB1, CB-IR cell with ascending arbors; CB2, CB-IR cell with descending arbors. Calibration bars = 100 μm.

Zaitsev et al., 2005
Cerebral Cortex
doi:10.1093/cercor/bhh218

Data were processed using **CLUSTER ANALYSIS:** correlation between **electrophysiological properties** and **expression of specific $Ca^{+}$-binding proteins**

When cells are grouped based only on electrophysiological properties, two main groups (= clusters) of interneurons are obtained: FS (Fast Spiking) and non-FS.

These two clusters do show significant differences in $Ca^{+}$-binding protein content

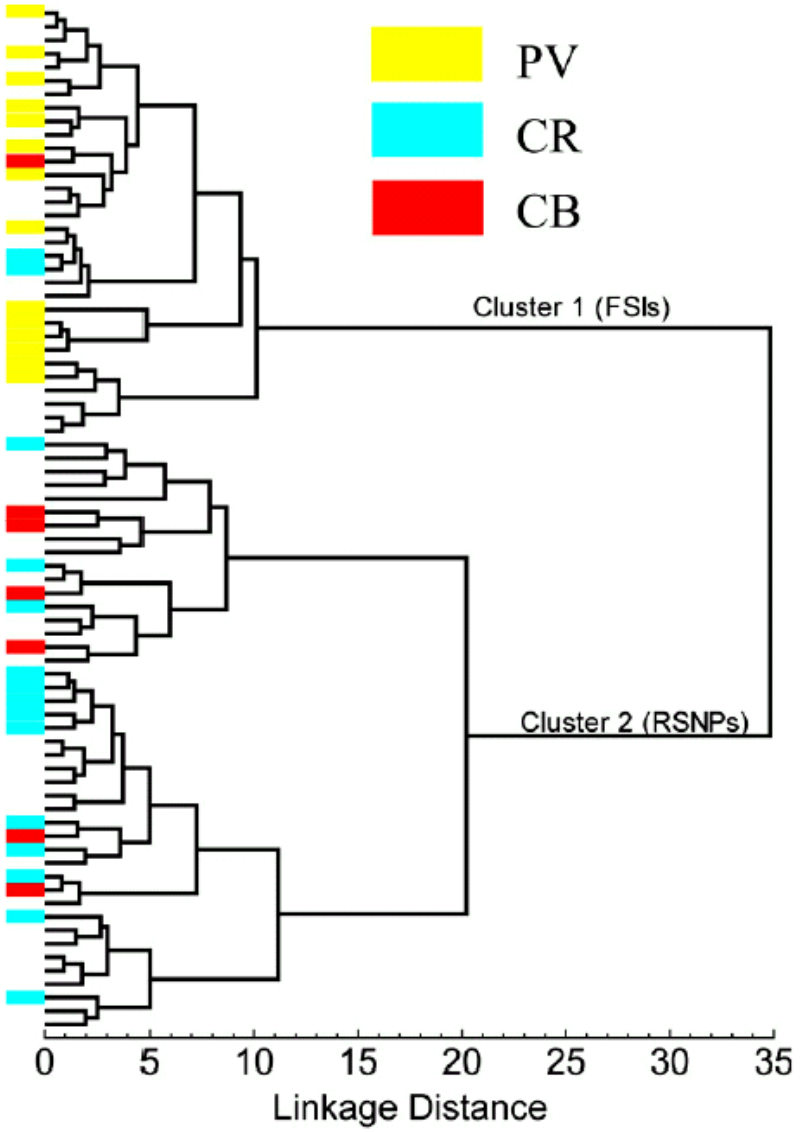
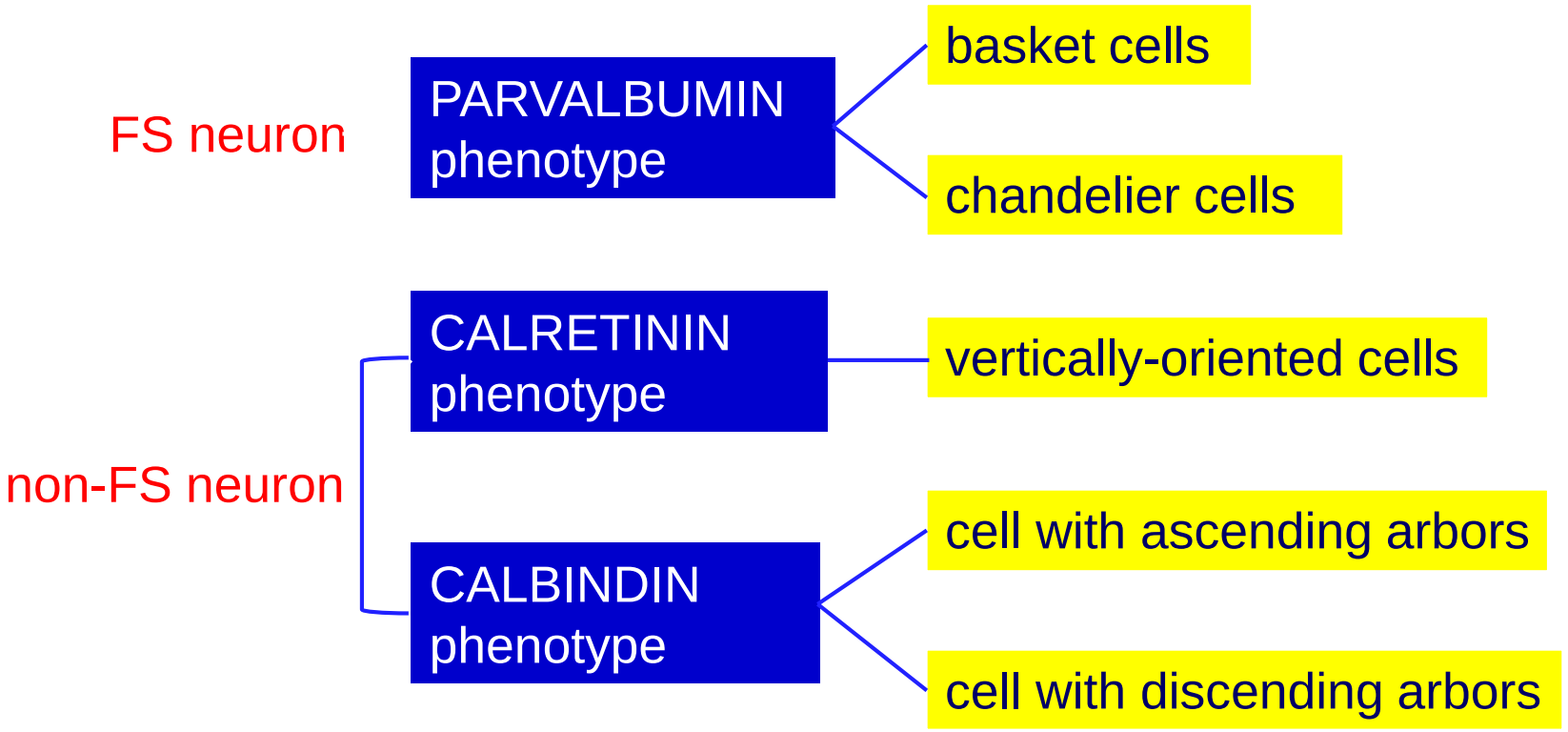



**Figure 4.** Hierarchical tree plot illustrating the results of cluster analysis. There are two main branches corresponding to FS interneurons (FSI) and non-FS cells. The first cluster mainly contained PV-positive interneurons and the second one consisted exclusively of CB- and CR-positive cells.

Zaitsev et al., 2005
Cerebral Cortex
doi:10.1093/cercor/bhh218

**CONCLUSIONS:**

- parvalbumin-expressing interneurons are exclusively FS
- calretinin- and calbindin-expressing interneurons are mainly non-FS
- multiple morphologies can correspond to a single functionally-defined phenotype



# Electrophysiological and gene expression profiling of neuronal cell types

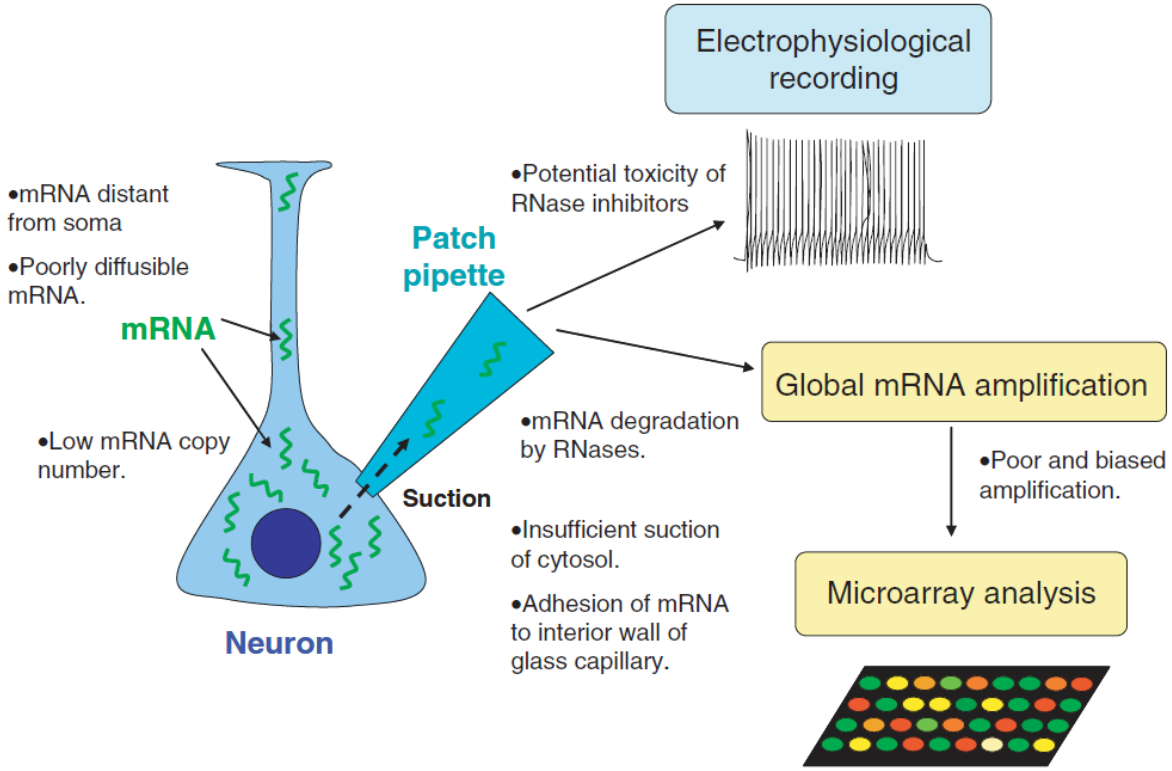
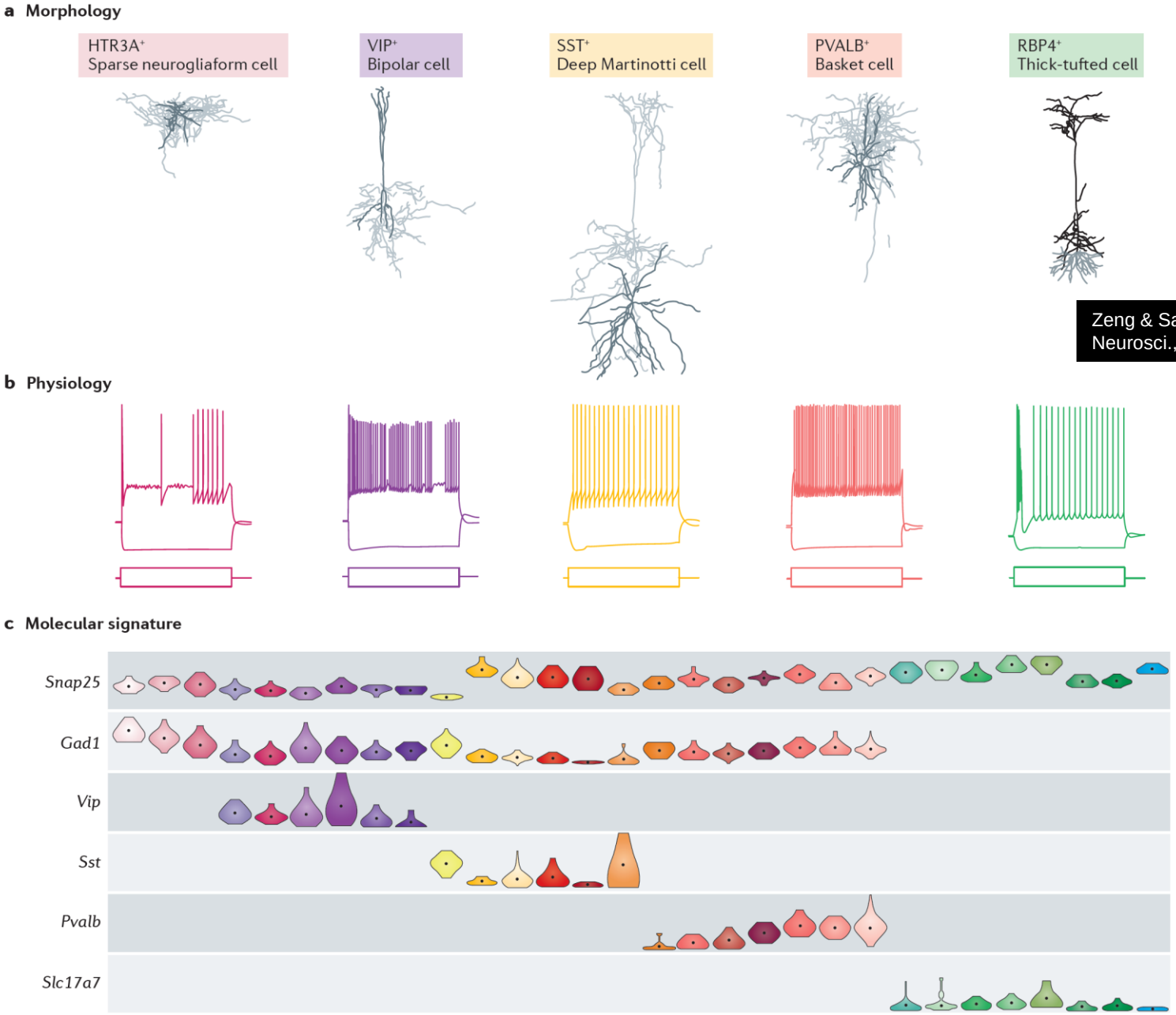



**Figure 1. Potential problems with single-cell gene expression profiling by cytoplasmic harvesting via patch-pipette**

Collecting extremely low amounts of mRNA from single cells is the biggest challenge of this technique. Only a small proportion of cytosol can be obtained by suction via patch-pipette, and poorly diffusible mRNA or dendritic mRNA are particularly hard to collect. The yield of mRNA can be improved by including inhibitors of RNases in the pipette, but these are often cytotoxic and can be detrimental to electrophysiological recording. It is also possible that some mRNA adheres to the interior wall of the glass capillary and evades expulsion from the patch-pipette.

Yano et al., 2006 J Physiol
DOI: 10.1113/jphysiol.2006.113712

# Neurons can be classified using morphological, physiological and molecular criteria




Zeng & Sanes, 2017 Nature Rev. Neurosci., doi:10.1038/nrn.2017.85

# Integration of electrophysiological recordings with single-cell RNA-seq data identifies neuronal subtypes

nature biotechnology
doi:10.1038/nbt.3443

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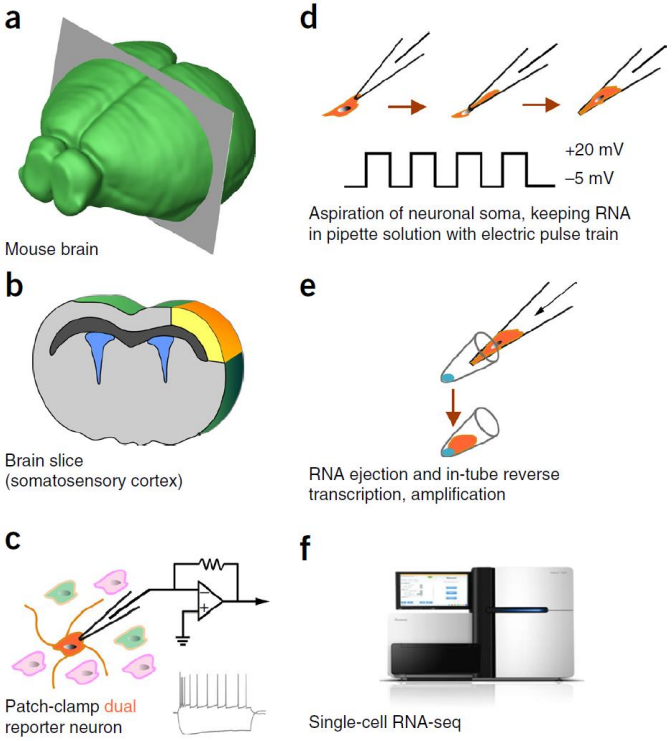
Traditionally, neuroscientists have defined the identity of neurons by the cells' location, morphology, connectivity and excitability. However, the direct relationship between these parameters and the molecular phenotypes has remained largely unexplored. Here, we present a method for obtaining full transcriptome data from single neocortical pyramidal cells and interneurons after whole-cell patch-clamp recordings in mouse brain slices. In our approach, termed Patch-seq, a patch-clamp stimulus protocol is followed by the aspiration of the entire somatic compartment into the recording pipette, reverse transcription of RNA including addition of unique molecular identifiers, cDNA amplification, Illumina library preparation and sequencing. We show that Patch-seq reveals a close link between electrophysiological characteristics, responses to acute chemical challenges and RNA expression of neurotransmitter receptors and channels. Moreover, it distinguishes neuronal subpopulations that correspond to both well-established and, to our knowledge, hitherto undescribed neuronal subtypes. Our findings demonstrate the ability of Patch-seq to precisely map neuronal subtypes and predict their network contributions in the brain.



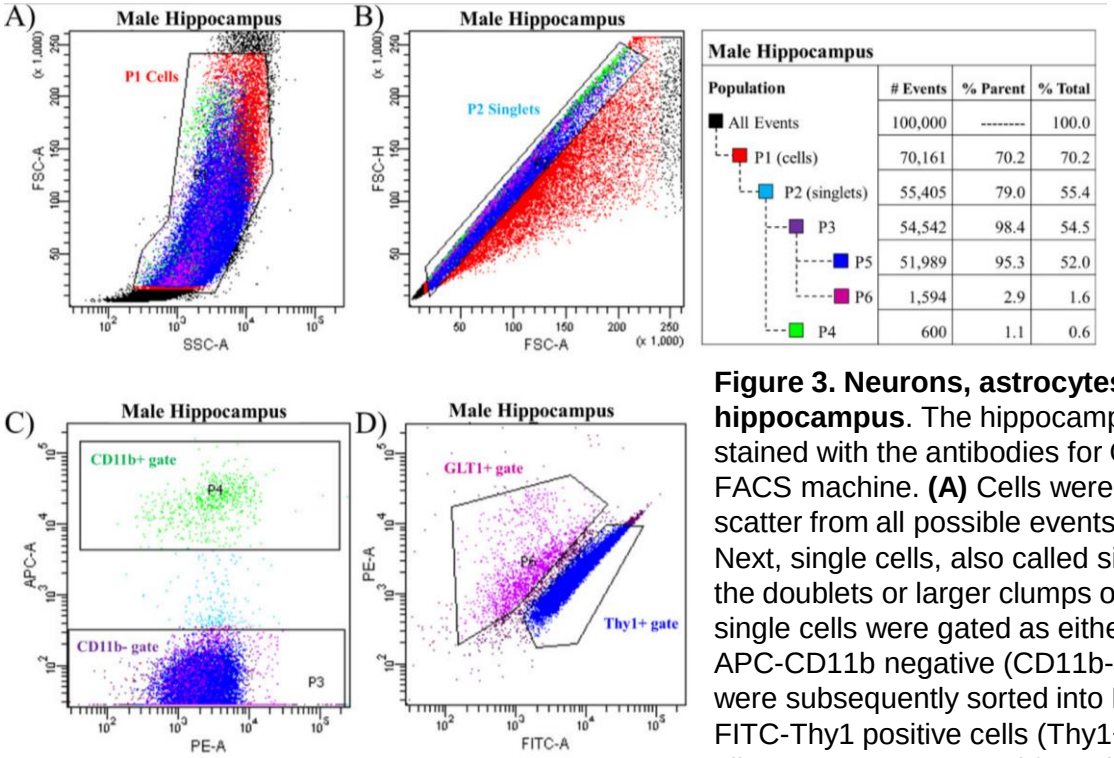

## A new way to identify neuronal subtypes with transcriptomics: Patch-seq = patch-clamp + Next Generation Sequencing

focusing on cholecystokinin (CCK)-containing(+) GABAergic interneurons by using **dual-labeled CCKBAC/dsRed::GAD67gfp/+ mouse reporter**

**Figure 2** Workflow diagram of Patch-seq procedures. (**a**) Coronal cutting plane of a mouse brain to access the somatosensory cortex. (**b**) *Ex vivo* brain slice anatomy with the somatosensory cortex highlighted in yellow and orange. (**c**) Whole-cell patch-clamp recording of DsRed$^{+}$/GFP$^{+}$ dual-tagged interneurons. (**d**) Aspiration of neuronal somata was followed by square voltage pulses from –5 mV (holding potential) to +20 mV, while maintaining negative pressure. (**e**) The sample was expelled into lysis buffer, which allowed for in-tube reverse transcription by PCR. (**f**) Single-cell RNA sequencing performed on an Illumina Hiseq2000 instrument.

http://www.jove.com/video/52537/using-fluorescence-activated-cell-sorting-to-examine-cell-type

# Using Fluorescence Activated Cell Sorting to Examine Cell-Type-Specific Gene Expression in Rat Brain Tissue




Male Hippocampus

| Population | # Events | % Parent | % Total |
|---|---|---|---|
| All Events | 100,000 | -------- | 100.0 |
| P1 (cells) | 70,161 | 70.2 | 70.2 |
| P2 (singlets) | 55,405 | 79.0 | 55.4 |
| P3 | 54,542 | 98.4 | 54.5 |
| P5 | 51,989 | 95.3 | 52.0 |
| P6 | 1,594 | 2.9 | 1.6 |
| P4 | 600 | 1.1 | 0.6 |

**Figure 3. Neurons, astrocytes, and microglia sorted from a male hippocampus**. The hippocampus from one male rat was dissociated and stained with the antibodies for CD11b, GLT1 and Thy1 and sorted using a FACS machine. **(A)** Cells were first sorted based on their forward and side scatter from all possible events. This gate is called P1 (population 1). **(B)** Next, single cells, also called singlets, were sorted based on their size from the doublets or larger clumps of cells. This gate is called P2. **(C)** Third, the single cells were gated as either APC-CD11b positive (CD11b+ gate, P4) or APC-CD11b negative (CD11b- gate, P3). **(D)** APC-CD11b negative cells were subsequently sorted into PE-GLT1 positive cells (GLT1+ gate, P6) and FITC-Thy1 positive cells (Thy1+ gate, P5). The breakdown of all events and all gates was generated from the FACS software depicted in a table which is presented on the right.

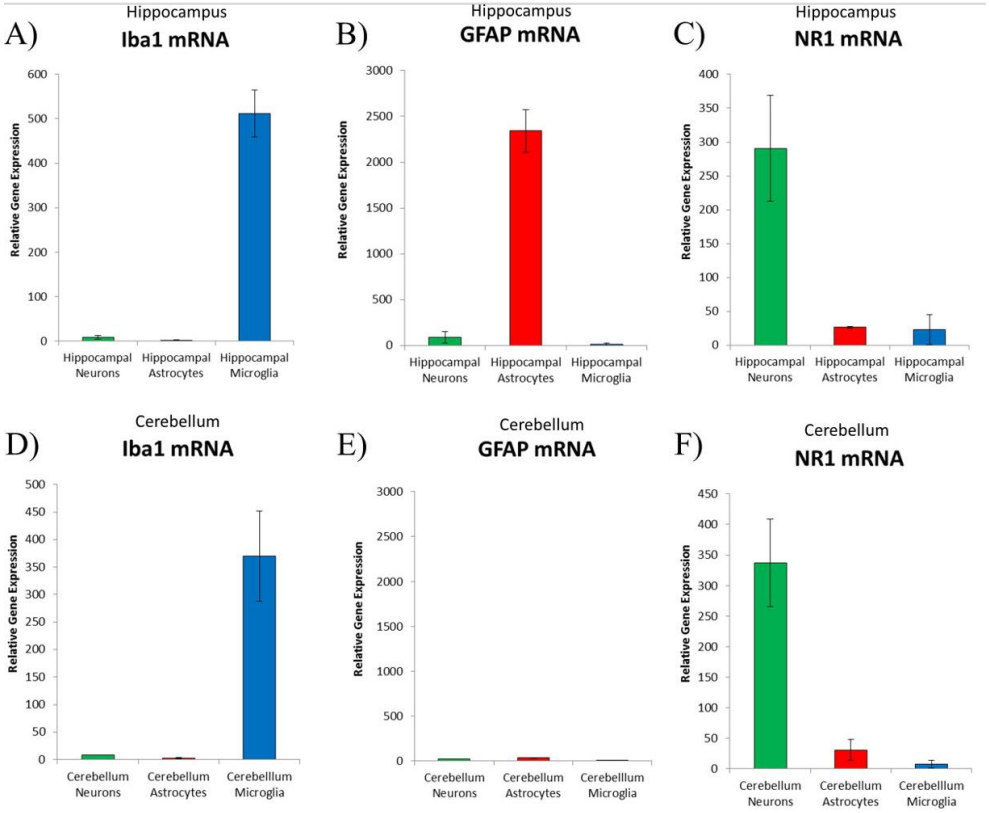



**Figure 5. Real-time PCR analysis of cell-type-specific genes from sorted cells.** Neurons (green bars), astrocytes (red bars) and microglia (blue bars) were sorted based on the protocol described above and mRNA was extracted for confirmation of cell-type-specific gene expression. **(A)** **Iba1** is a calcium binding protein expressed exclusively in **microglia** sorted from the male hippocampus. **(B)** **GFAP** is a filament protein expressed predominantly in **astrocytes** sorted from the male hippocampus **(C)** **NR1** is a ubiquitous subunit of the NMDA glutamatergic receptor that was expressed predominantly on **neurons** sorted from the male hippocampus. **(D)** Iba1 was also expressed exclusively on microglia sorted from the male cerebellum. **(E)** Interestingly, GFAP was not expressed in any of the cell types sorted from the male cerebellum. **(F)** The NR1 subunit of the NMDA receptor was also expressed predominantly on neurons sorted from the male cerebellum.

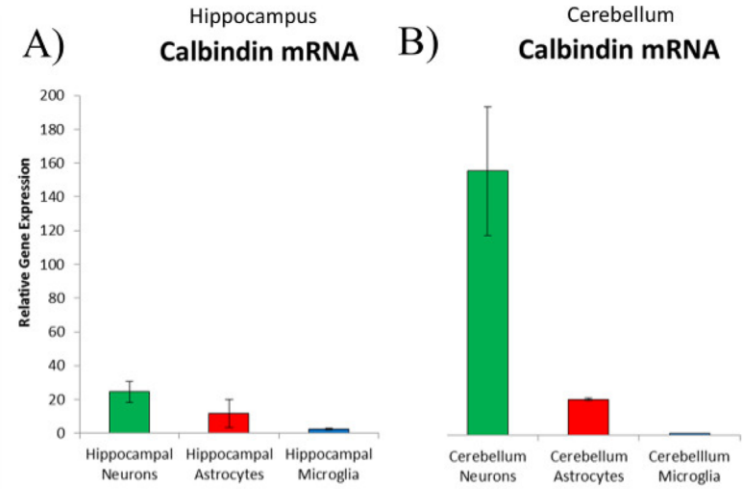



**Figure 6. Real-time PCR analysis of calbindin expressed in sorted neural cells.** Cells sorted using FACS can be used to analyze cell-type specific gene expression. **(A)** **Neurons** (green bars) expressed significantly more **Calbindin** than either astrocytes (red bars) or microglia (blue bars) sorted from the male hippocampus. **(B)** Neurons sorted from the male cerebellum expressed significantly higher levels of Calbindin than either astrocytes or microglia sorted from the cerebellum, but also significantly higher levels than the neurons sorted from the hippocampus.

NATURE NEUROSCIENCE VOLUME 19 | NUMBER 2 | FEBRUARY 2016

# Adult mouse cortical cell taxonomy revealed by single cell transcriptomics

Bosiljka Tasic[1,2], Vilas Menon[1,2], Thuc Nghi Nguyen[1], Tae Kyung Kim[1], Tim Jarsky[1], Zizhen Yao[1], Boaz Levi[1], Lucas T Gray[1], Staci A Sorensen[1], Tim Dolbeare[1], Darren Bertagnolli[1], Jeff Goldy[1], Nadiya Shapovalova[1], Sheana Parry[1], Changkyu Lee[1], Kimberly Smith[1], Amy Bernard[1], Linda Madisen[1], Susan M Sunkin[1], Michael Hawrylycz[1], Christof Koch[1] & Hongkui Zeng[1]

**The most complete single-neuron transcriptome database of the mouse visual cortex was performed using a large collection of reporter mouse lines. Results highlight the unmatched neuronal diversity of the cerebral cortex.**

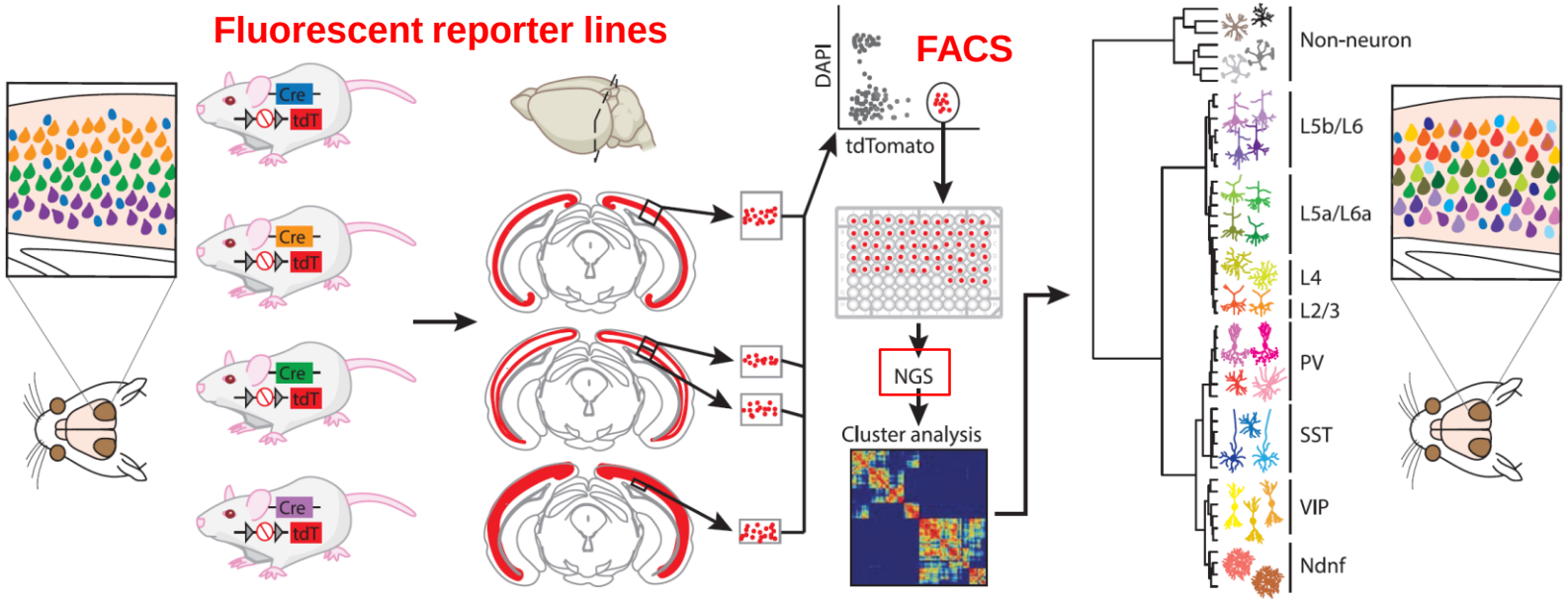



**Figure 1** Single-neuron RNA-seq analysis of the adult mouse visual cortex. A large repertoire of Cre driver lines crossed to *loxP* tdTomato (tdT) reporter lines was used to label distinct neuronal populations in the mouse visual cortex. Specific layers of the primary visual cortex were microdissected from freshly sectioned adult mouse brains, and single neurons from these samples were purified by fluorescence-activated cell sorting for use in single-cell RNA-seq. Cluster analysis was conducted agnostic to the reporter line of origin. The resulting clusters were assigned to 49 transcriptionally defined cell types, 42 of them neuronal, highlighting the molecular diversity in classes of cortical excitatory and inhibitory neurons. PV, parvalbumin; SST, somatostatin; VIP, vasoactive intestinal polypeptide; Ndnf, neuron derived neurotrophic factor; NGS, next generation sequencing.

http://casestudies.brain-map.org/celltax#section_introa

# The Allen Brain Atlas cell taxonomy project