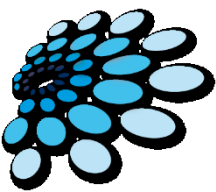


“ Techniques in Cellular and Molecular Neurobiology ”

International Master's Degree in Neuroscience

Lesson 8



DIPARTIMENTO DI
SCIENZE DELLA VITA

Gabriele Baj
gbaj@units.it



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FOR NEXT LESSON



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DOWNLOAD:

<https://imagej.net/Fiji/Downloads>

Plugin: NeuronJ

<https://imagej.net/NeuronJ>

Nissl Staining Method and Protocol on Sections for Brain & Spinal Cord



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Description: This method is used for the detection of Nissl body in the cytoplasm of neurons on paraformaldehyde or formalin-fixed, paraffin embedded tissue sections. The Nissl body will be stained purple-blue. This stain is commonly used for identifying the basic neuronal structure in brain and spinal cord tissue.

Fixation: 4% paraformaldehyde in 0.1M PB or 10% formalin.

Section: paraffin sections at 5~30 μ m

Solutions and Reagents:

0.1% Cresyl violet solution:

Cresyl echt violet (or cresyl violet acetate) --- 0.1 g

Distilled water ----- 100 ml

Add 10 drops (or 0.3 ml) of glacial acetic acid just before use and filter.

Procedure:



1. Hydrate in 95% alcohol 5 minutes, 70% 3 minutes.
2. Rinse in tap water and then in distilled water.
3. Stain in 0.1% cresyl violet solution for 3-10 minutes. **Notes:** Staining in warmed cresyl violet solution (warm up in 37-50 °C oven) can improve penetration and enhance even staining. It is particularly beneficial for thicker (20-50 um) sections.
4. Rinse quickly in distilled water.
5. Differentiate in 70% ethyl alcohol for 2-30 minutes and check microscopically for best result.
6. Dehydrate in 100% alcohol 5 min.
7. Clear in xylene 3 min.
8. Mount with permanent mounting medium.

Results:

Neuron (Nissl body) ----- pink-violet

Positive Controls:

Brain tissue.

Allen Brain Atlas



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<https://www.youtube.com/watch?v=C8yNe-Vtv0s>

<https://www.youtube.com/watch?v=hRJupoWlahw>

<https://www.youtube.com/watch?v=WnbOLuEeJsU>

IMAGE SELECTION

Click a thumbnail to select it; double-click to open image in resizable viewer.

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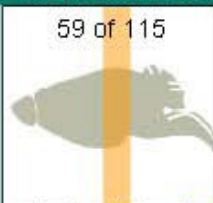


Allen Reference Atlas



Gpr12-Coronal-05-3258

59 of 115



Gpr12_249

Position: 6225



Gpr12_245

Position: 6125



Gpr12_237

Position: 5925



Gpr12_233

Position: 5825



Gpr12_229

Position: 5725

Allen Reference Atlas

[Show Legend](#)

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1



Reset



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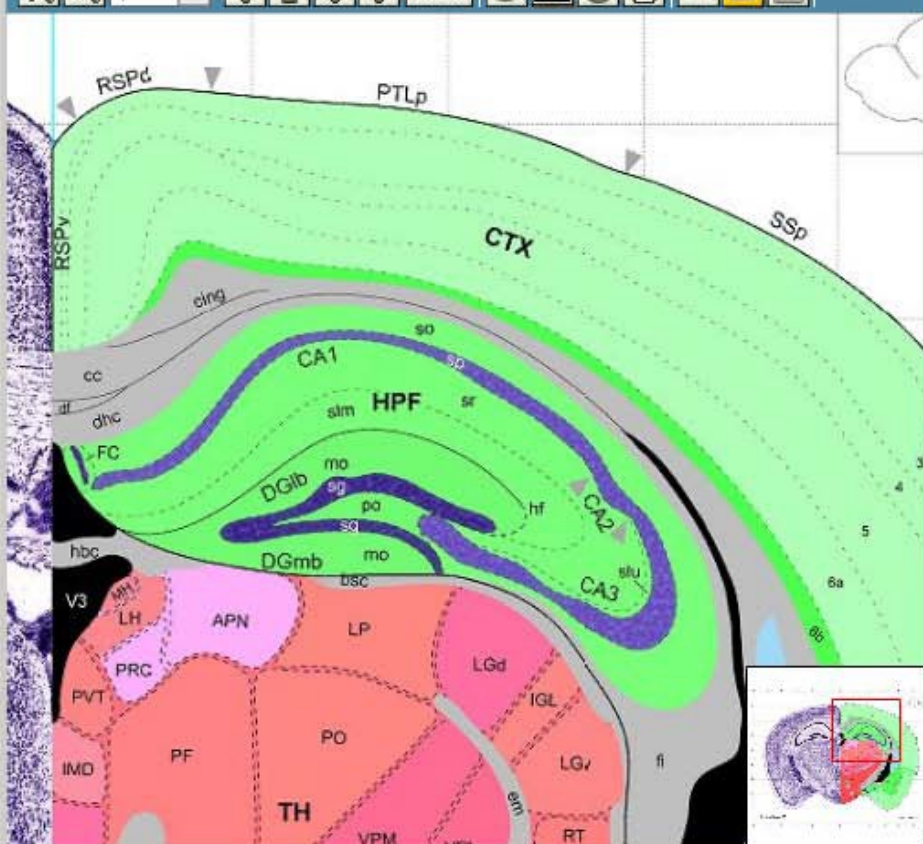
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View 1



Gpr12_233



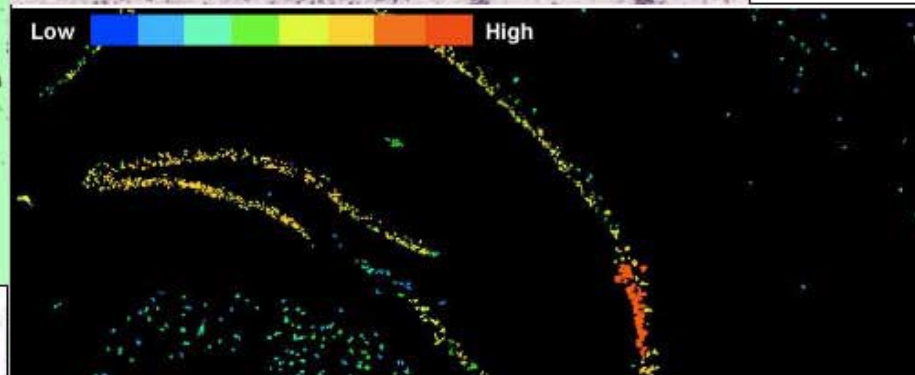
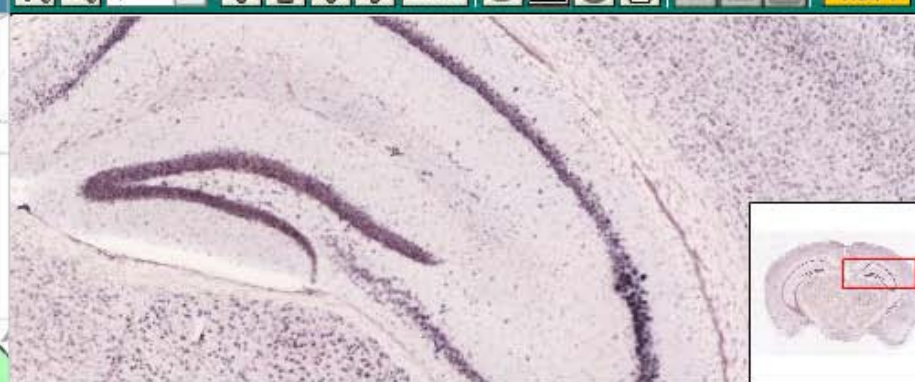
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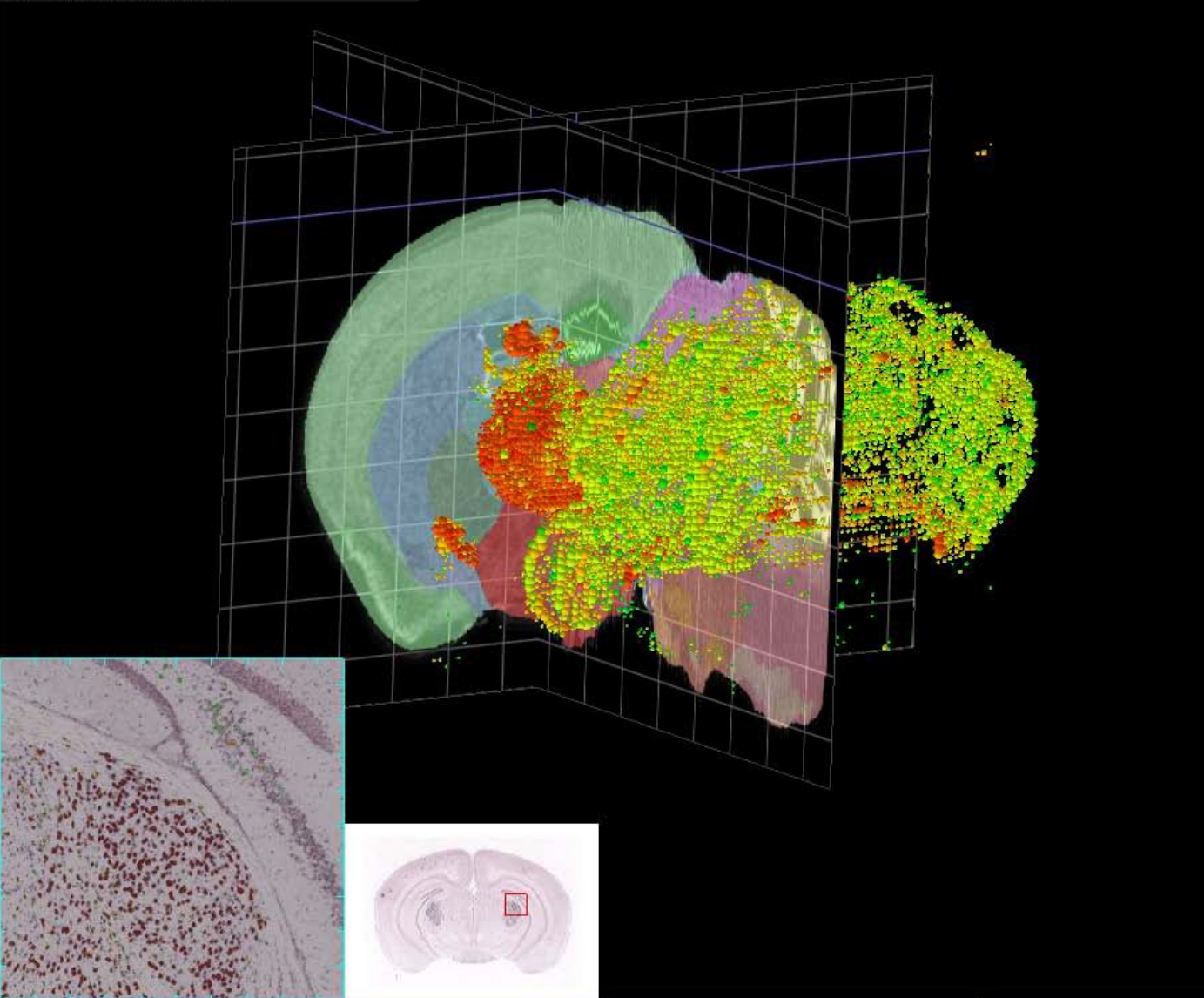
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View 1



Detail View prkcd
 Gene: Prkcd
 Annotation: Dorsal part of the lateral geniculate complex (LGd)
 #Expressors: 11
 Cell Diameter: 162.122 µm
 Grid Area Spanned: 21%
 Expression Level: 242.364
 Grid ID: 392964 (81, 38, 36)
 Section: 213 Location: 7240, 3240



Structure	A	G
Basic cell groups and regions (Brain)		
Cerebrum (CH)		
Cerebral cortex (CTX)		
Cortical plate (CTXpl)		
Olfactory areas (OLF)		
Main olfactory bulb (MOB)		
Accessory olfactory bulb (AOB)		
Anterior olfactory nucleus (AON)		
Taenia tecta (TT)		
Piriform area (PIR)		
Nucleus of the lateral olfactory tract (LOT)		
Piriform-amygdalar area (PAA)		
Cortical amygdalar area (COA)		
Postpiriform transition area (TR)		
Hippocampal formation (HPF)		
Hippocampal region (HIP)		
Ammon's Horn (CA)		
Dentate gyrus (DG)		
Retrohippocampal region (RHP)		
Subiculum (SUB)		
Cerebral nuclei (CNU)		
Striatum (STR)		
Striatum dorsal region (STRd)		
Caudoputamen (CP)		
Striatum ventral region (STRv)		
Nucleus accumbens (ACB)		
Fundus of striatum (FS)		
Olfactory tubercle (OT)		
Lateral septal complex (LSX)		
Lateral septal nucleus (LS)		
Septofimbrial nucleus (SF)		
Striatum-like amygdalar nuclei (sAMY)		
Central amygdalar nucleus (CEA)		
Anterior amygdalar area (AAA)		
Medial amygdalar nucleus (MEA)		
Pallidum (PAL)		
Pallidum dorsal region (PALd)		
Pallidum ventral region (PALv)		
Magnocellular nucleus (MA)		
Substantia innominata (SI)		
Pallidum caudal region (PALc)		
Bed nuclei of the stria terminalis (BST)		
Pallidum medial region (PALm)		
Brain stem (BS)		
Interbrain (IB)		
Thalamus (TH)		
Thalamus polymodal association cortex (PMAC)		

DI TRIESTE

The Central Dogma of Molecular Biology



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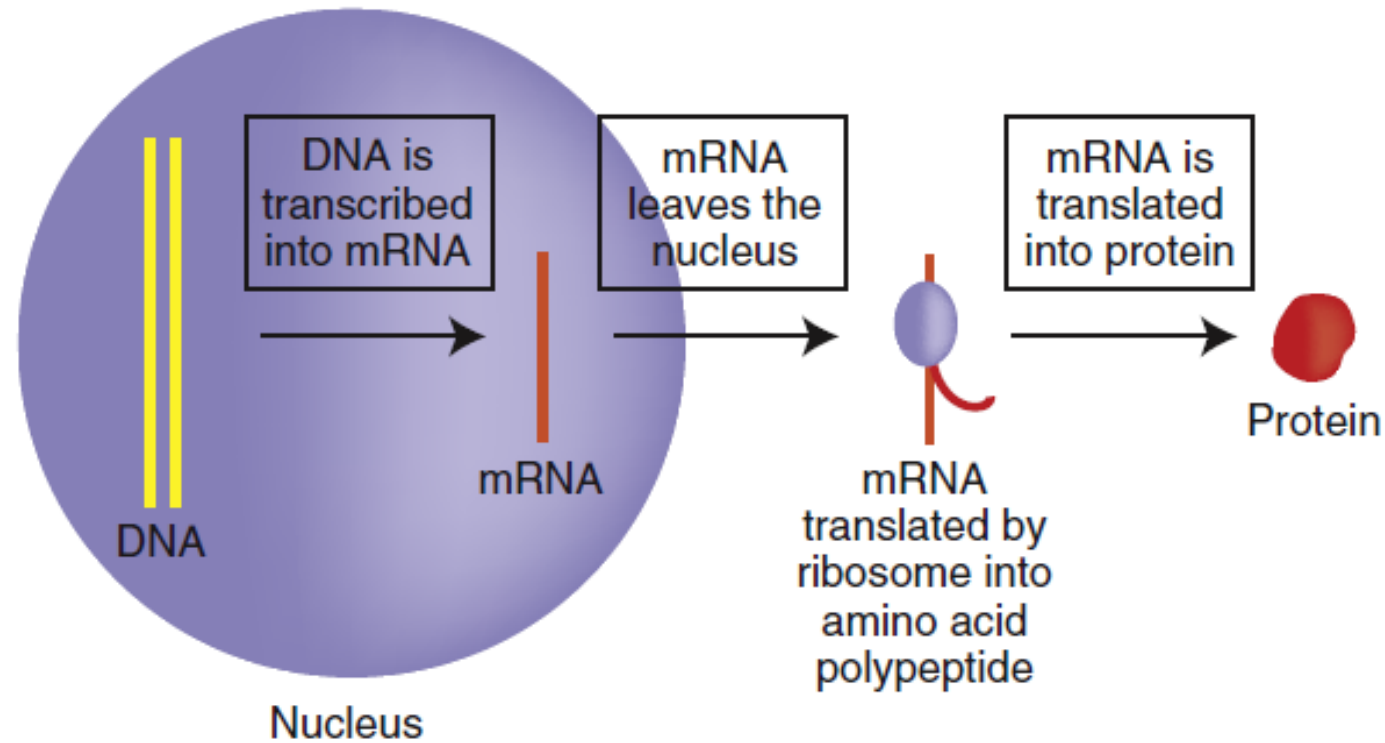


FIGURE 9.1 The flow of information within a cell. DNA resides in the nucleus and codes for relatively short sequences of mRNA. After transcription, an mRNA molecule leaves the nucleus for the cytoplasm, where it is translated by ribosomes into proteins. Thus, the molecular flow of information starts with nucleic acids and ends with amino acids.

TABLE 9.1 Relative Advantages and Disadvantages of Different Organisms Used for Genetic Studies

Species	Advantages	Disadvantages
Worm (<i>Caenorhabditis elegans</i>)	Simple, multicellular organism Genome sequenced Short lifespan Relatively easy to manipulate genes and screen for genes of interest through mutagenesis Can be frozen All neurons and their connections are known	Invertebrate Primitive nervous system
Fruit fly (<i>Drosophila melanogaster</i>)	Complex, multicellular organism Genome sequenced Short lifespan, rapid reproduction rate Relatively easy to manipulate genes Many mutant lines available	Invertebrate Primitive nervous system



Zebrafish (<i>Danio rerio</i>)	Genome sequenced Can use powerful invertebrate techniques like Gal4/UAS system Good for imaging studies during development because eggs are clear Large number of offspring	Not a mammal
Mouse (<i>Mus musculus</i>)	Complex, higher-order organisms Genome sequenced Possible to manipulate genes in whole organism or specific tissues Nervous system is homologous to humans	Relatively long lifespan and reproductive time Genetic manipulations are lengthy and costly Greater genetic redundancy than invertebrates
Human (<i>Homo sapiens</i>)	Complex, higher-order organism Genome sequenced Can observe naturally occurring genetic mutations and polymorphisms	Long lifespan Not tractable for experimental genetic manipulation

TABLE 6.2 Histochemical Stains for Studying Neuroanatomy

Stain	Use	Appearance	Comments
Cresyl violet	Cell nuclei, Nissl stain	Blue to purple	Useful for examining cytoarchitecture; stains each type of neuron slightly differently
Hematoxylin	Cell nuclei	Blue to blue-black	Often used in combination with eosin; known as H&E
Eosin Y	Cytoplasm	Pink to red	Counterstain with hematoxylin; acidophilic stain
Thionine	Cell nuclei, Nissl stain	Blue to purple	
Methylene blue	Cell nuclei	Blue	Can be perfused through the brain before fixation
Toluidine blue	Cell nuclei	Nucleus is stained blue; cytoplasm light blue	Often used to stain frozen sections

VISUALIZING CELLULAR ELEMENTS



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DAPI	Cell nuclei	Fluorescent blue	Fluorescent DNA intercalating agent; excited by UV illumination
Hoechst (bis-benzamide)	Cell nuclei	Fluorescent blue	Fluorescent DNA intercalating agent; excited by UV illumination
Propidium iodide (PI)	Cell nuclei	Fluorescent red	Fluorescent DNA intercalating agent; excited by green light illumination
Weigert	Myelin	Normal myelin is deep blue; degenerated myelin is light yellow	Combines hematoxylin with other chemicals to selectively stain myelin
Weil	Myelin	Black	Combines hematoxylin with other chemicals to selectively stain myelin
Luxol fast blue (LFB)	Myelin	Blue	
Golgi stain	Fills neuron cell bodies and processes	Black	Stains individual neurons at random

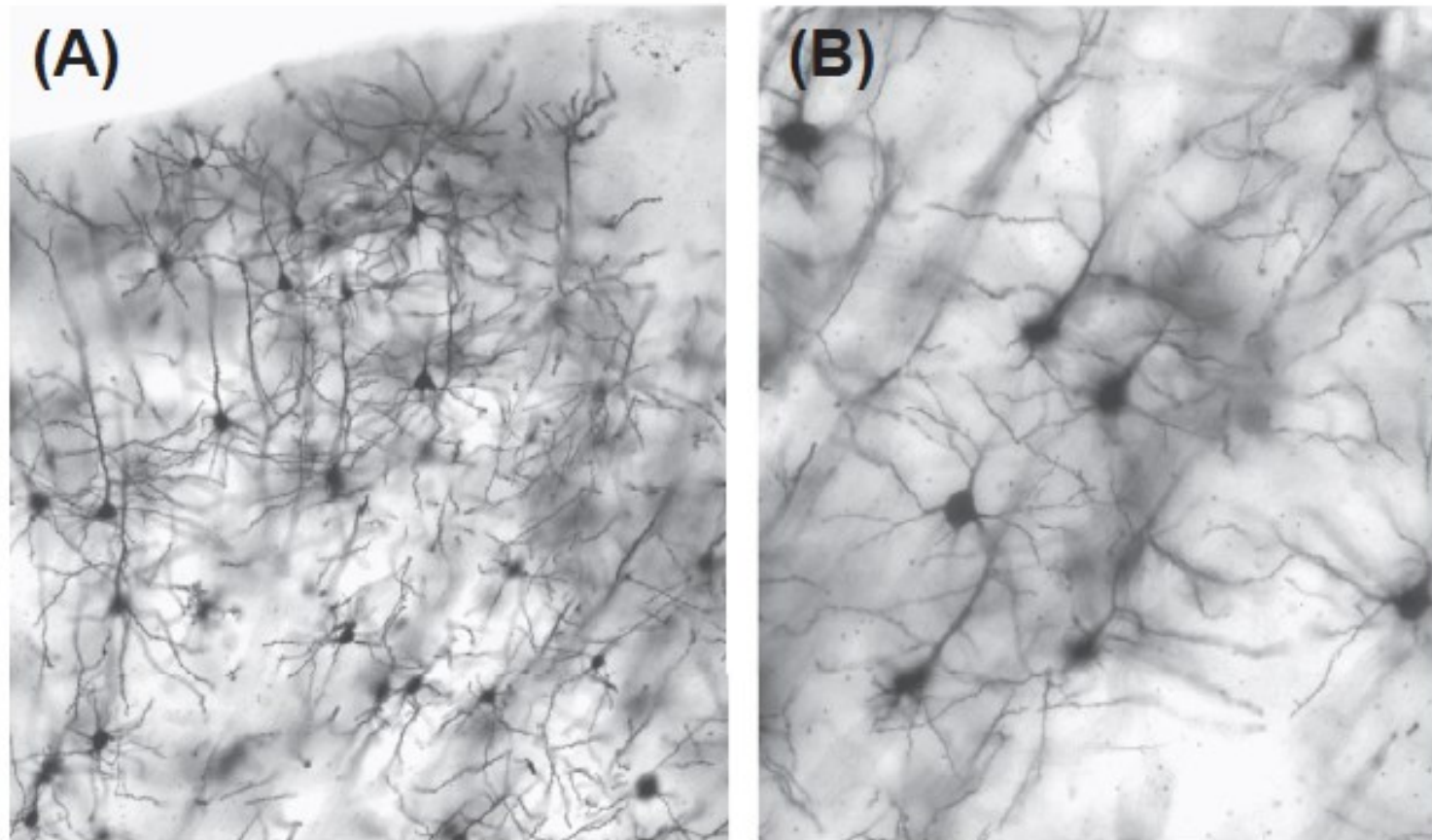


FIGURE 6.4 The Golgi stain reveals neuronal structure in fine detail. Random neurons are selectively stained in the (A) 100x view of a Golgi-stained mouse cortex and (B) a higher magnification. *Courtesy of Dr Jocelyn Krey.*

VISUALIZING GENE AND PROTEIN EXPRESSION



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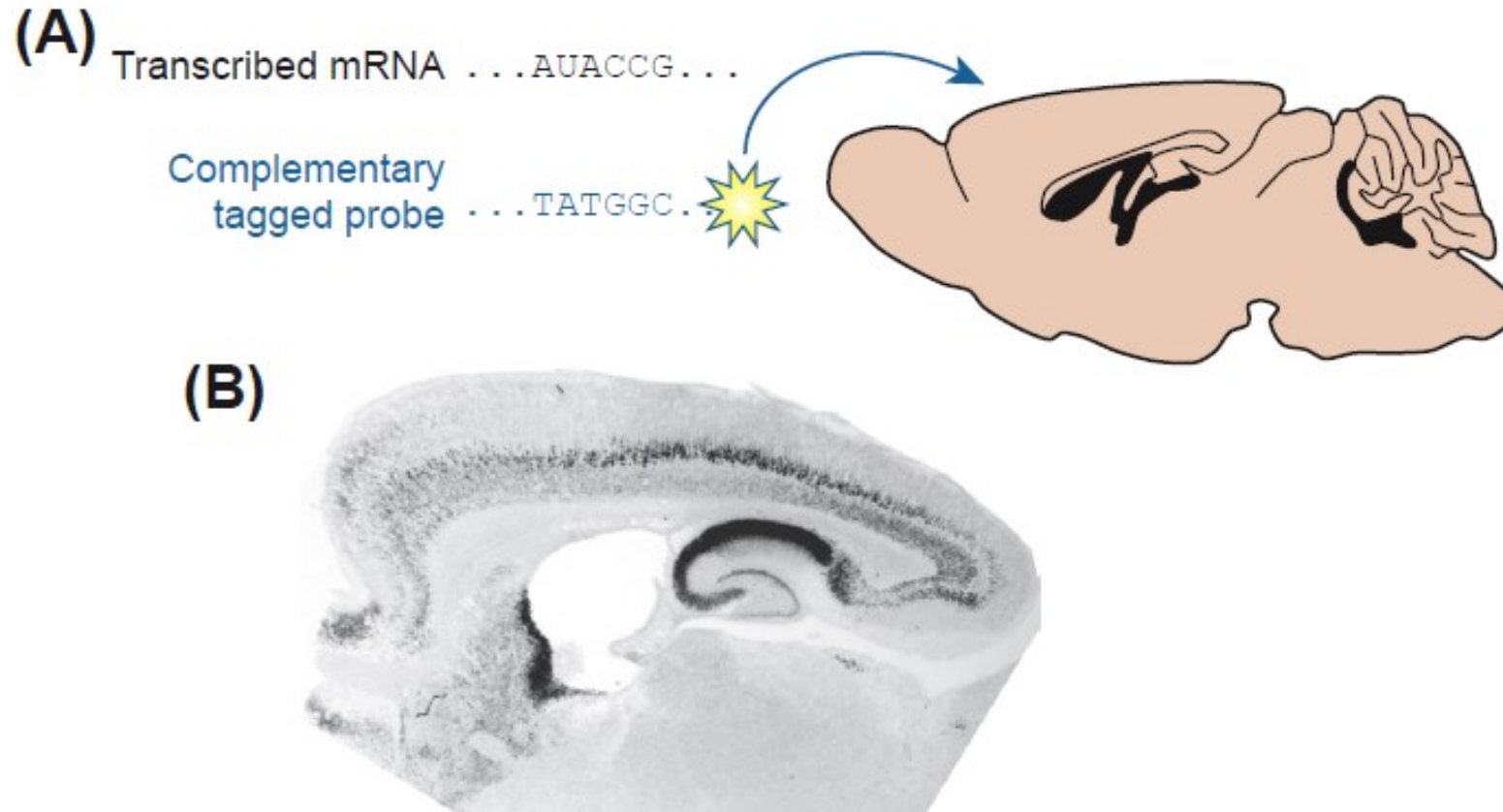


FIGURE 6.5 *In situ* hybridization shows where a gene is expressed. (A) Labeled oligonucleotide probes are synthesized with a complementary sequence to a gene of interest so they hybridize to the mRNA when applied to a brain section. (B) Example of an *in situ* hybridization reaction performed on a sagittal mouse brain section. The probe was conjugated to digoxigenin and visualized with a colorimetric reaction.

Immuno histo or cyto chemistry



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TABLE 6.3 Commonly Used Antibodies that Label Specific Neural Cell Types

Cell Type	Antibodies
Progenitors/radial glia	Nestin; Pax6; RC2; vimentin; NF (neurofilament)
Young neurons	Doublecortin (DCX); NeuroD
Neurons	Tuj1 (neuron-specific β -tubulin); NeuN
Dendrites	MAP2
Axons	Tau-1, L1, Tag-1
Synapses	PSD95, synapsin
Neuronal subtypes	GAD (GABAergic neurons); vGLUT (glutamatergic); TH (dopaminergic); 5-HT (serotonergic); AChE (cholinergic)
Glia	GFAP (astrocytes); MBP (oligodendrocytes, myelin); PLP
Oligodendrocyte progenitor cells (OPC)	NG2, A2B5, O4 (late progenitor)

Immuno histo or cyto chemistry



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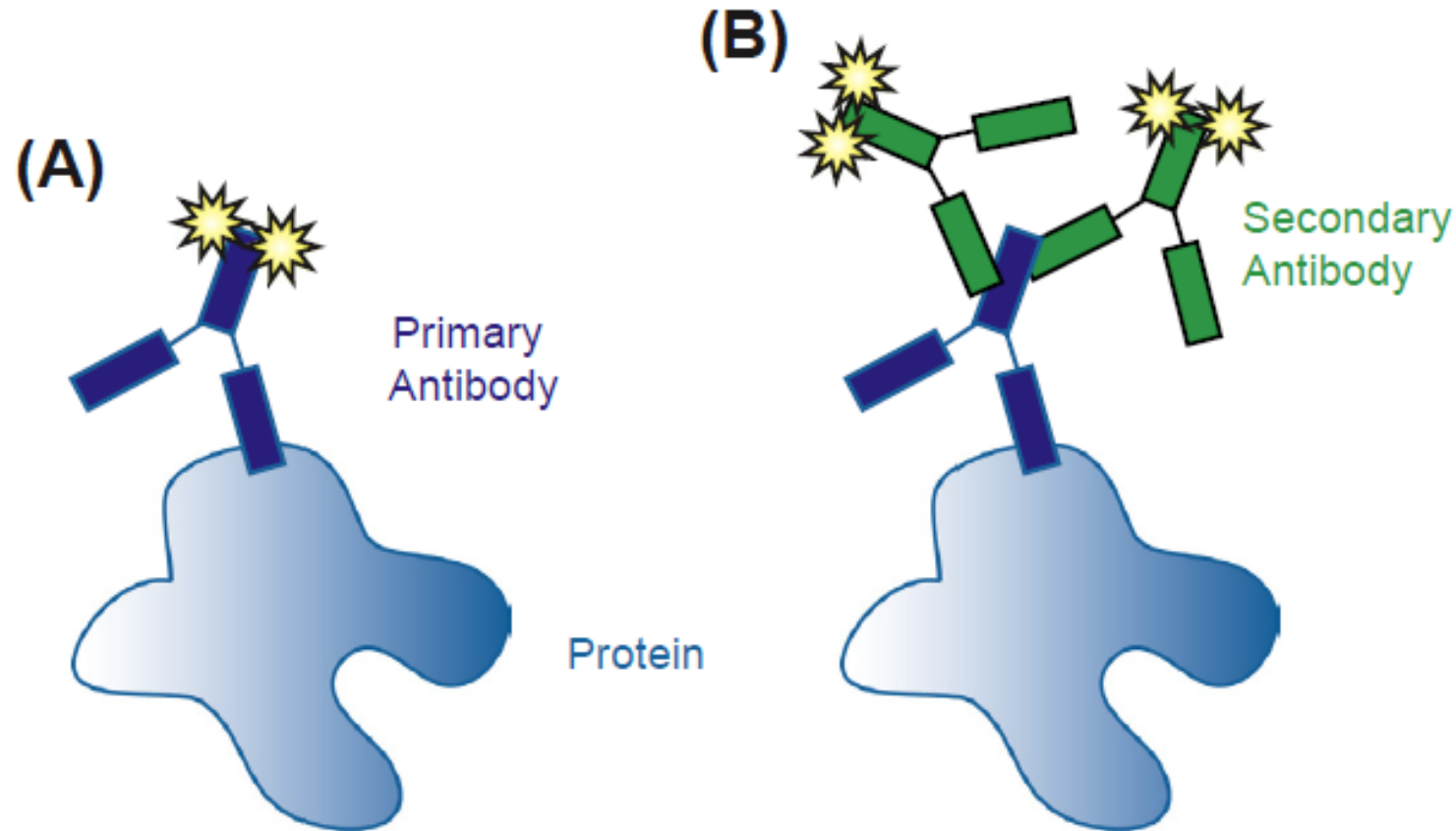


FIGURE 6.6 Immunohistochemistry (IHC). (A) In direct IHC, primary antibodies are conjugated directly to a label that can be visualized. (B) In indirect IHC, primary antibodies attract labeled secondary antibodies, further amplifying the signal.

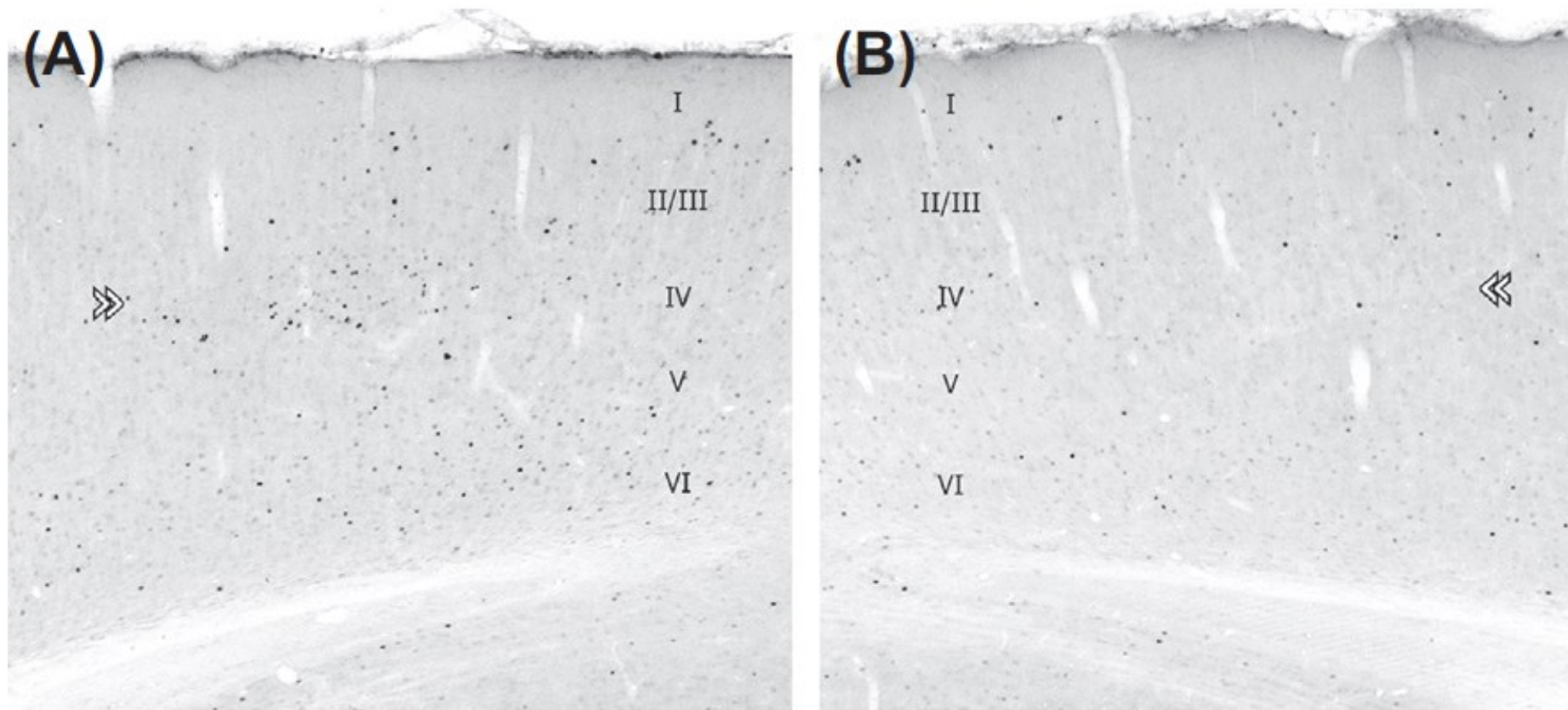


FIGURE 7.1 Measuring activity in a fixed brain sample. Overall activity levels can be detected in fixed tissue by labeling for IEGs using immunohistochemistry or *in situ* hybridization techniques. Here, Fos immunoreactivity demonstrates that patterned visual stimulation evokes more activity in the (A) contralateral visual cortex compared to the (B) ipsilateral visual cortex. *Reprinted from Dotigny, F., et al. (2008). Neuromodulatory role of acetylcholine in visually induced cortical activa-*

Imaging Voltage



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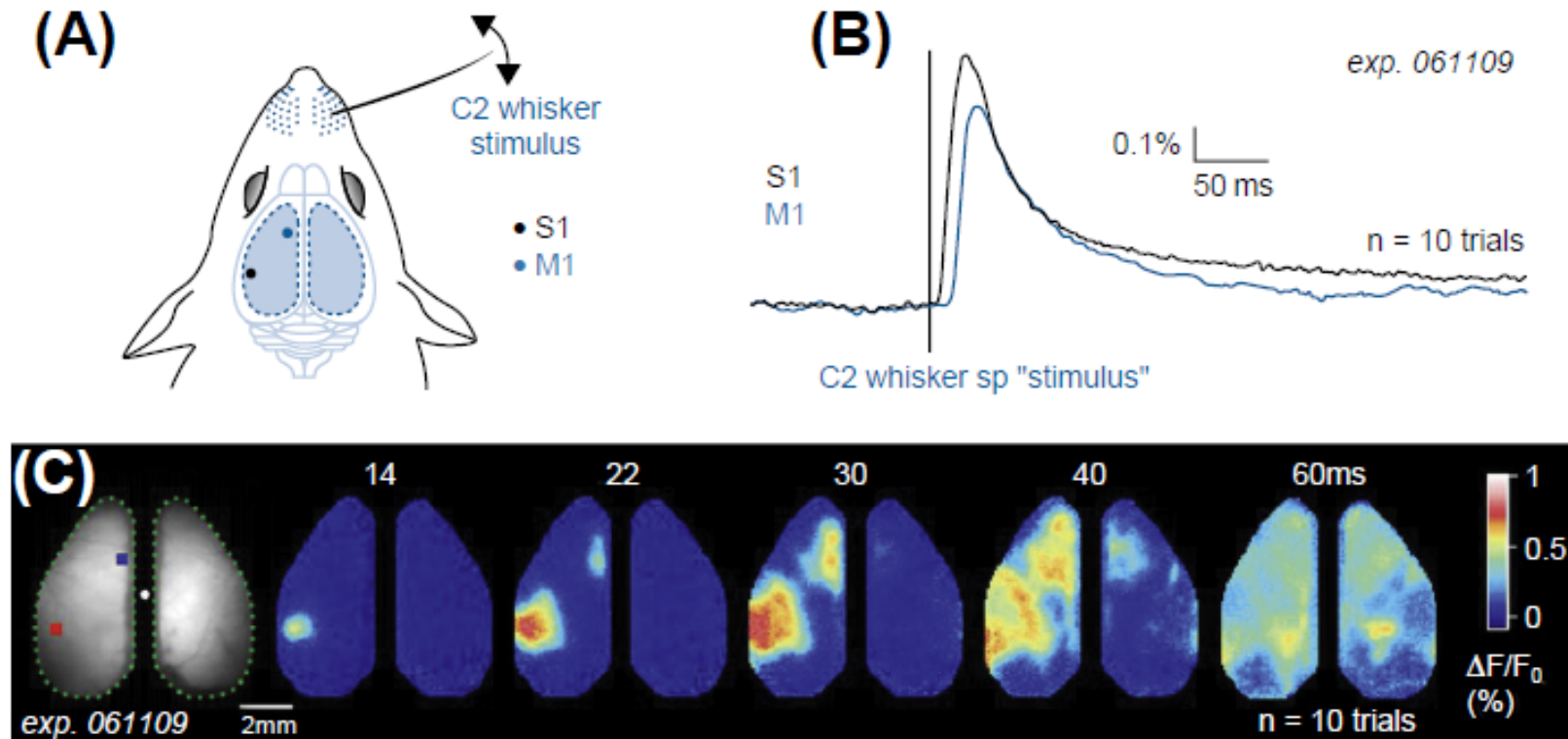


FIGURE 7.3 Example of a voltage-sensitive dye imaging experiment. (A) A craniotomy was performed over somatosensory cortex (S1) and motor cortex (M1) in anesthetized mice to deliver the voltage sensitive dye RH1691. (B) Moving the C2 whiskers evokes transient increases in fluorescence, first in S1 (black), then in M1 (blue). (C) The spatiotemporal dynamics of voltage changes across the brain are observed after deflection of the C2 whisker. *Reprinted from Ferezou, I., et al. (2007). Spatiotemporal dynamics of cortical sensorimotor integration in behaving mice. Neuron, 56, 907–923, with permission from Elsevier.*

TABLE 7.1 Commonly Used Neural Activity Sensors

Category	Examples	Advantages	Disadvantages
Voltage-sensitive dyes	Di-8-ANEPPS, RH 414	High temporal resolution (microsecond response time).	Low signal intensity changes (order of 0.1%). Promiscuous labeling.
Genetically encoded voltage sensors	ArcLight	Targeting specificity.	Lower temporal resolution than dyes (milliseconds). High background.
Nonratiometric calcium indicator dyes	Fluo-3, Fluo-4, calcium Green-2	Direct fluorescence intensity changes correlated with Ca^{2+} concentration.	Prone to artifacts from loading concentration, photobleaching, and other experiment-specific conditions. Cannot be calibrated. Buffering of intracellular Ca^{2+} can alter signaling pathways if present at high concentration.
Ratiometric calcium indicator dyes	Fura-2, Indo-1	Large signal changes (10%). Resistant to experiment-specific artifacts, such as loading concentration and photobleaching. Enables calibration.	More complicated data acquisition and measurement than nonratiometric dyes. Buffering of intracellular Ca^{2+} can alter signaling pathways if present at high concentrations.
Genetically encoded calcium sensors	GCaMP	Targeting specificity.	Can have long signal decay time, giving low temporal resolution. Buffering of intracellular Ca^{2+} can alter signaling pathways if present at high concentration.
Dye-based synaptic vesicle markers	FM dyes (FM1-43, AM4-64)	Can vary stimulation and exposure to dye to control number of vesicles labeled.	Labels all membrane surfaces so background can be high and difficult to wash out of brain slices.
pH-sensitive fluorescent proteins	SynaptopHluorin	Allows study of presynaptic release.	Many active release sites can make imaging of specific synapses difficult. Vesicle is only visible while fused for exocytosis, but becomes invisible after endocytosis.

VISUALIZING PROTEIN FUNCTION



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TABLE 7.2 Comparison of Techniques to Optically Investigate Protein Function

	Description
Reporter genes	Proteins or organelles can be fluorescently tagged and observed over time. Useful for observing behavior, movement and dynamics of tagged proteins.
Fluorescence/Förster resonance energy transfer (FRET)	A donor fluorophore with an emission spectra that overlaps with the excitation spectra of an acceptor fluorophore transfers energy to the acceptor when the donor and acceptor are in close proximity. Measures the shift in the ratio of donor emission intensity to acceptor emission intensity. Useful for observing interactions between two tagged proteins or two parts of a single protein.
Bimolecular fluorescence complementation (BiFC)	Two different proteins can be tagged with half a fluorophore. The fluorophore will fluoresce only if the two halves come near enough, reporting a close interaction between the tagged proteins. Measures the change in fluorescence intensity to indicate whether the two proteins are near each other.

VISUALIZING PROTEIN FUNCTION



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Fluorescence recovery after photobleaching (FRAP)

High-intensity light is used to photobleach a region of fluorescence.
Measures the kinetics of fluorescence intensity returning to photobleached region.
Useful for observing dynamics of trafficking, diffusion, or binding/dissociation of fluorescently tagged proteins.

Photoactivation/
Photoconversion

Photoactivation causes a dramatic increase in fluorescence intensity after stimulation by light in specialized fluorophores.
PA-GFP (photoactivatable GFP) becomes fluorescent after stimulation with UV light.
Photoconversion uses light to change the emission spectra of a fluorophore.
Useful for tracking localization changes of small populations or individual cells, organelles, or proteins.

Manipulating Neural Activity



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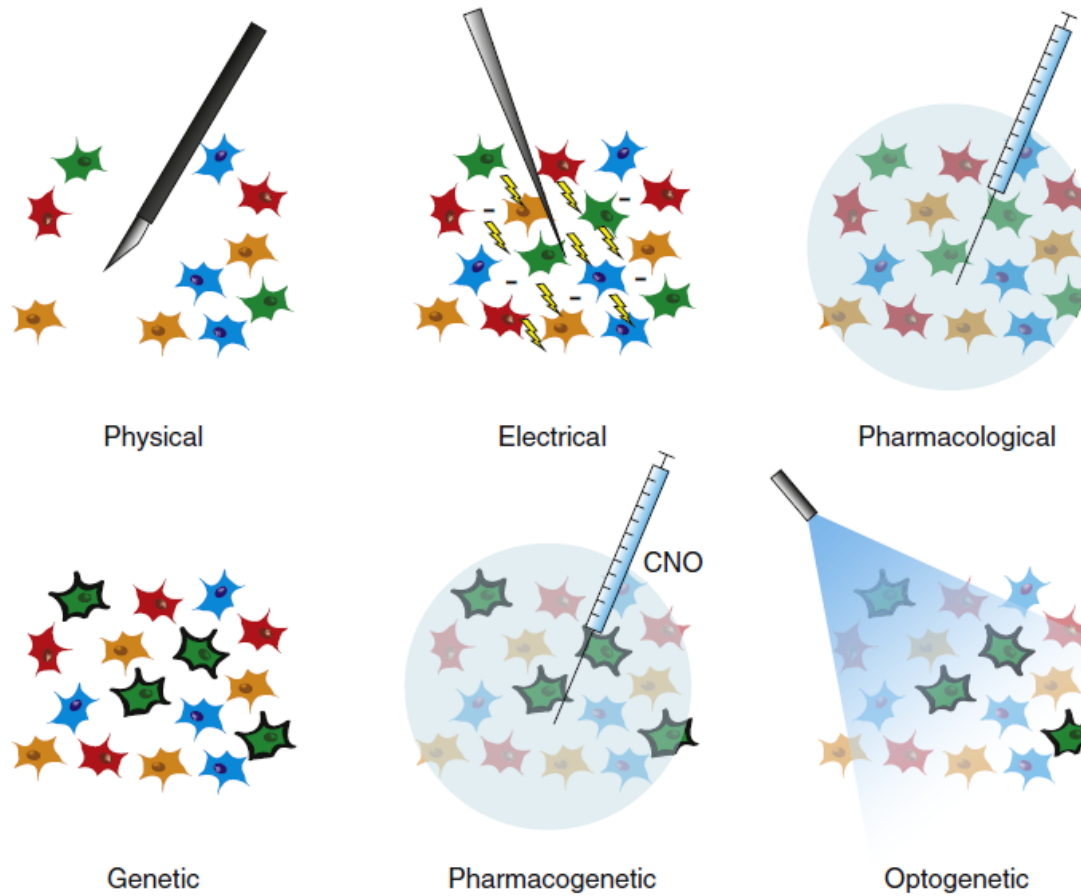


FIGURE 8.1 Methods of manipulating neural activity. Neurons in the brain typically reside within heterogeneous populations composed of many cell types (here denoted by different colors). Physical lesioning techniques can ablate cells indiscriminately. Electrical techniques can microstimulate brain regions or cause electrolytic lesions. Pharmacological techniques inject a psychoactive drug either centrally or systemically. Genetic methods are capable of affecting very specific cell types by taking advantage of endogenous promoter regions of genes. Pharmacogenetic techniques use genetic targeting methods to deliver receptors capable of affecting neural activity to specific cell types. Optogenetic techniques deliver light-activated neuronal actuators to specific cell types.



TABLE 8.1 Methods of Lesioning the Nervous System

Category	Tool	Precision
Physical	Surgical blade	Not very precise. Cannot discriminate between cell types or fibers of passage
Electrical	Microelectrode	More spatially precise than surgical (physical) lesions, but cannot discriminate between cell types or fibers of passage
Pharmacological	Toxins (e.g., ibotenic acid)	Spatial precision determined by quality and specificity of injection. Fibers of passage are unaffected
Genetic	Genetically encoded proteins (e.g., ataxin, diphtheria toxin receptor)	Cell-type specificity

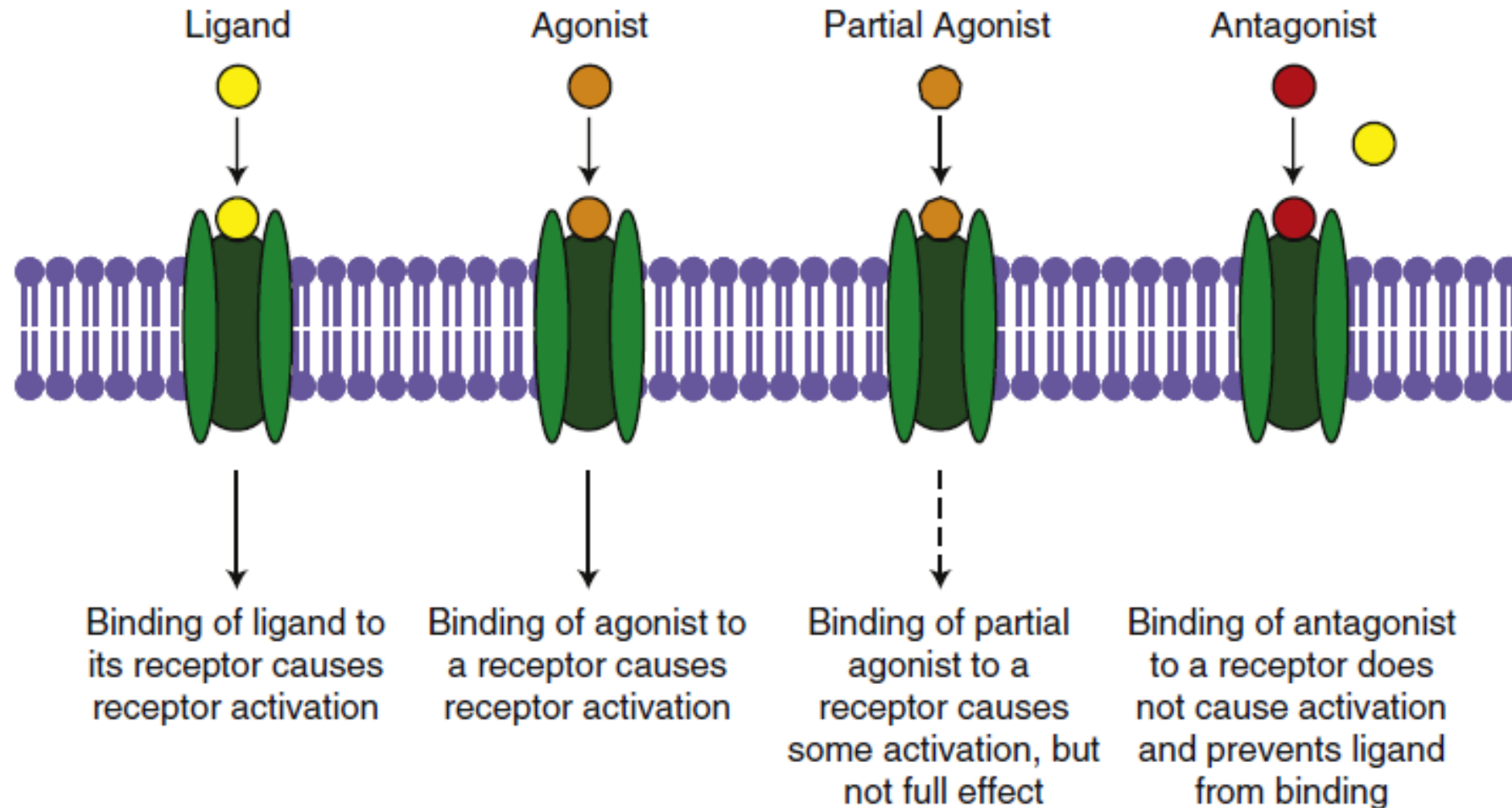


FIGURE 8.2 Compounds used in pharmacology: ligands, agonists, partial agonists, and antagonists.

Manipulating Endogenous Genes



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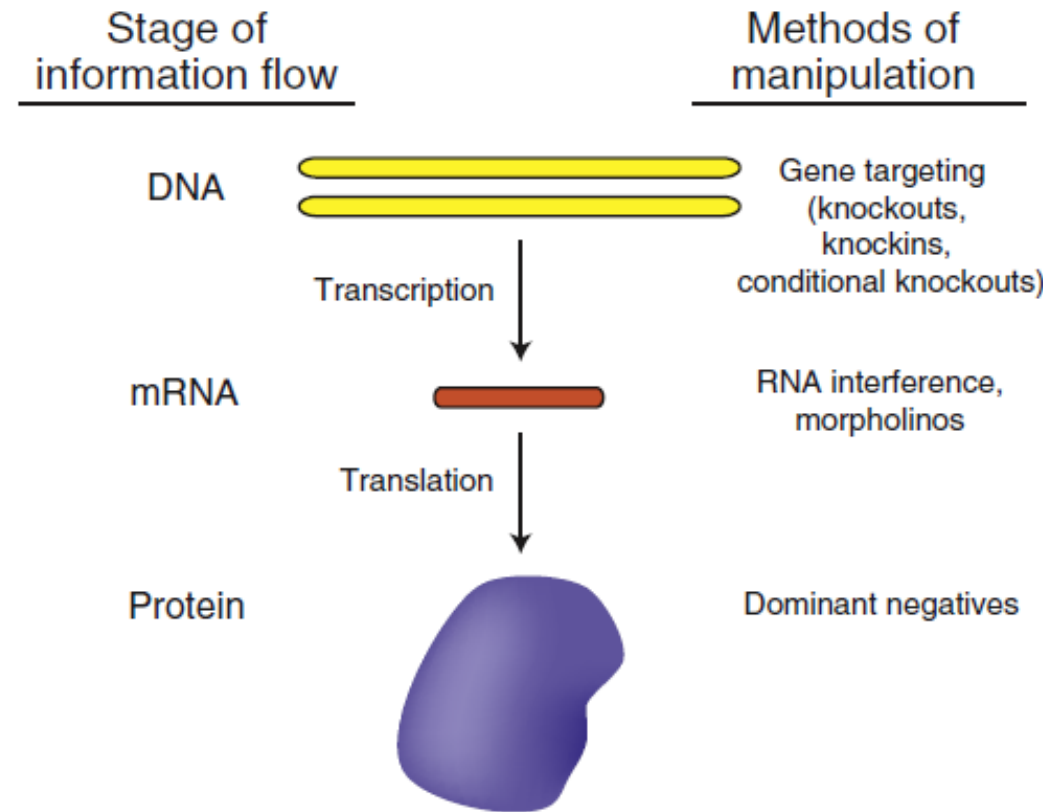


FIGURE 13.1 Methods of genetically perturbing endogenous genes and proteins. A scientist can manipulate gene and protein function at each stage of information processing. Gene-targeting techniques can be used to alter the genome; RNA interference and morpholino techniques can be used to functionally block mRNA transcripts; and other techniques, such as expression of dominant negative constructs, can block protein function.

Gene Delivery Strategies



TABLE 11.1 Categories of Gene Delivery Strategies

Method	Advantages	Disadvantages
Physical	High-efficiency gene transfer No limitations on construct size No cell type dependency	Low throughput Requires specialized equipment Can physically harm cells
Chemical	High efficiency <i>in vitro</i> No limitations on construct size Relatively easy to perform Rapid High throughput Low immunogenicity	Limited <i>in vivo</i> applications Efficiency depends on cell type Can be toxic to cells Transient expression
Viral	High-efficiency gene transfer Cell-specific targeting is possible Long-term expression Can be used <i>in vitro</i> and <i>in vivo</i>	Complex cloning required More expensive than transfection methods Safety concerns regarding production of infectious viruses in humans May provoke immune response Laborious preparation Limited construct size

TABLE 11.3 Choosing a Gene Delivery Strategy

Environment	Considerations	Commonly used gene delivery methods
Cells in the intact nervous system of living animals	Cells within the brain are notoriously resistant to most transfection methods.	Electroporation, virus
Cells in brain slices	Gene delivery must be fast and efficient and may need to go through thick tissue.	Electroporation, biolistics
Cells in dissociated cultures	Gene delivery must be highly efficient and high throughput to transfect/transduce thousands or millions of cells.	Electroporation, biolistics, chemical transfection, virus
Individual cells	Cells are often valuable, such as extracted embryos or newly fertilized eggs, so care is taken to efficiently deliver DNA to each cell.	Microinjection, electroporation

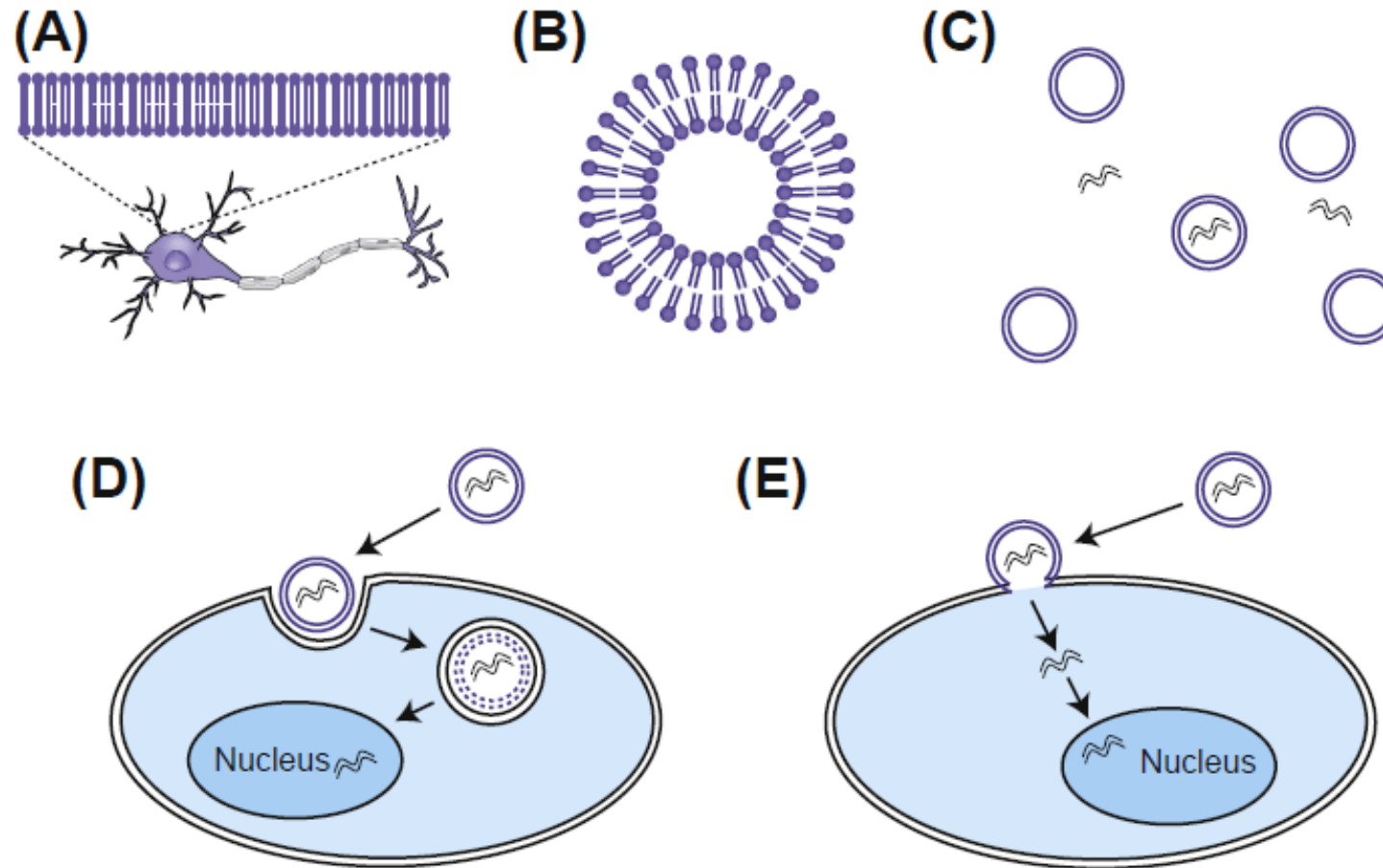


FIGURE 11.6 Lipofection. (A) The cell membrane is composed of a lipid bilayer, with a hydrophobic interior and hydrophilic exterior. (B) Liposomes are also composed of a lipid bilayer arranged as a spherical shell. (C) A scientist performs a brief reaction that allows liposomes to form around DNA. (D) Cells in culture can endocytose the liposome, digesting it within vesicles to release DNA. (E) Alternatively, liposomes can directly fuse with the plasma membrane, directly releasing DNA into cells.

Image J



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<http://rsbweb.nih.gov/ij/>

or simply type : imagej or



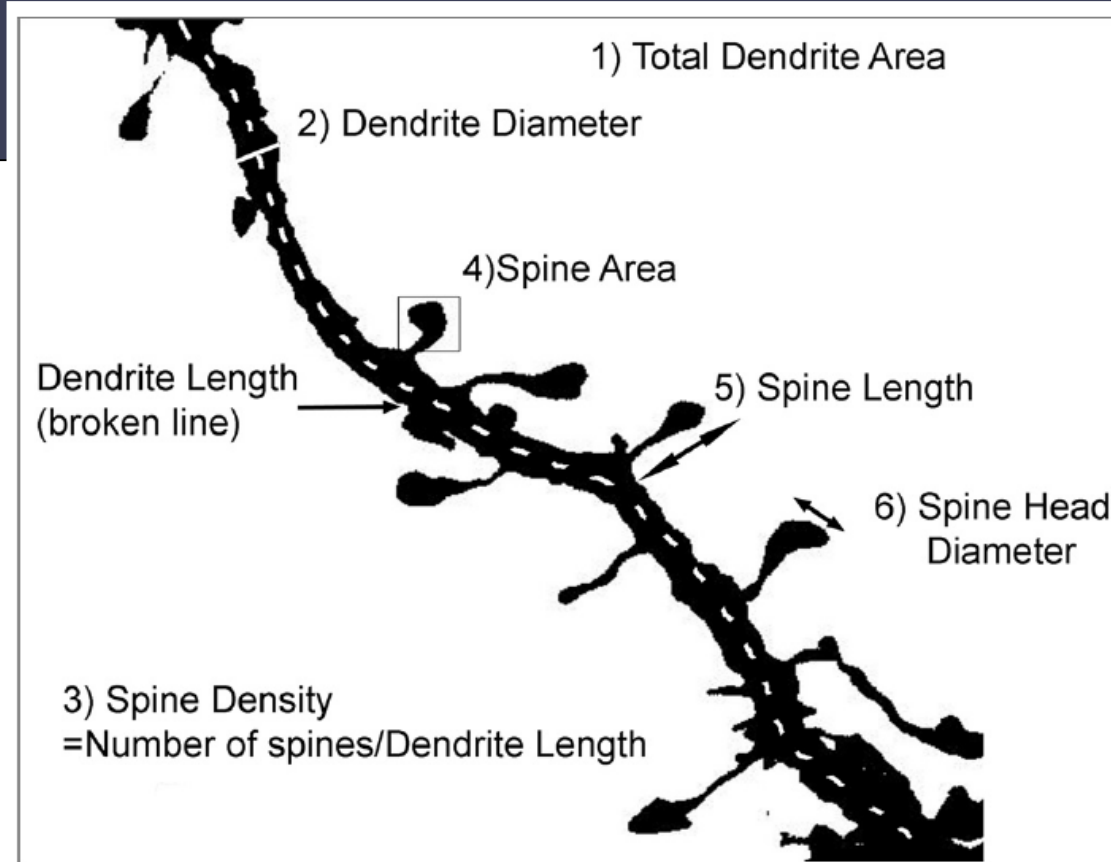


Fig. 1. Neuronal dendrite and spine measurement by Image J analysis. A typical dendrite segment from a pyramidal neuron is shown, and the six quantification parameters labeled as follows. 1) Total dendrite area is measured by drawing a box around the whole image; 2) dendrite diameter is obtained by drawing a line across the dendrite thickness at a place of average width; 3) spine density is the total number of spines divided by the dendrite length; 4) spine area is measured by drawing a box around the whole spine; 5) spine length uses the broken line tool to measure the length; and 6) spine head diameter again uses the broken line tool in Image J to measure the diameter across the head of the spine.

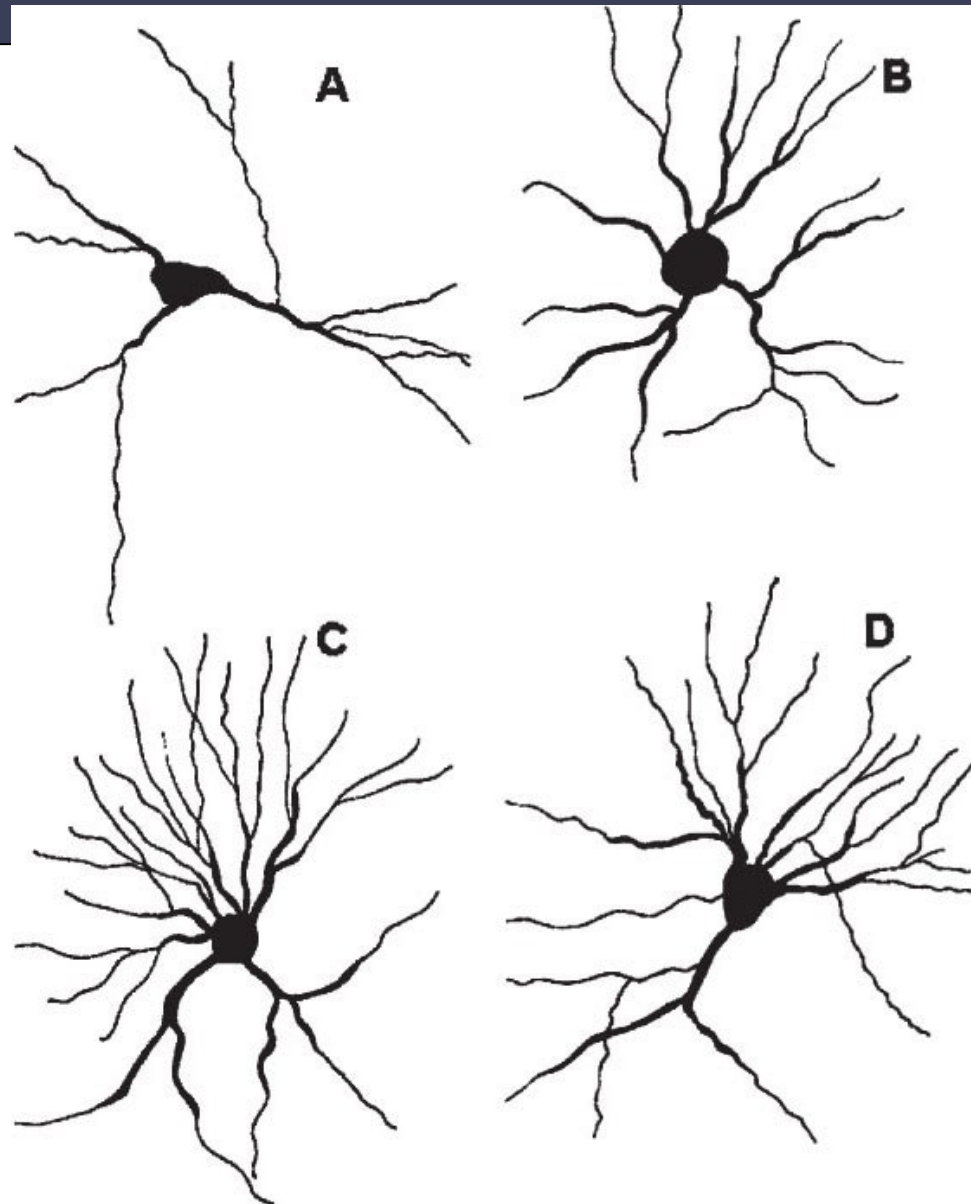
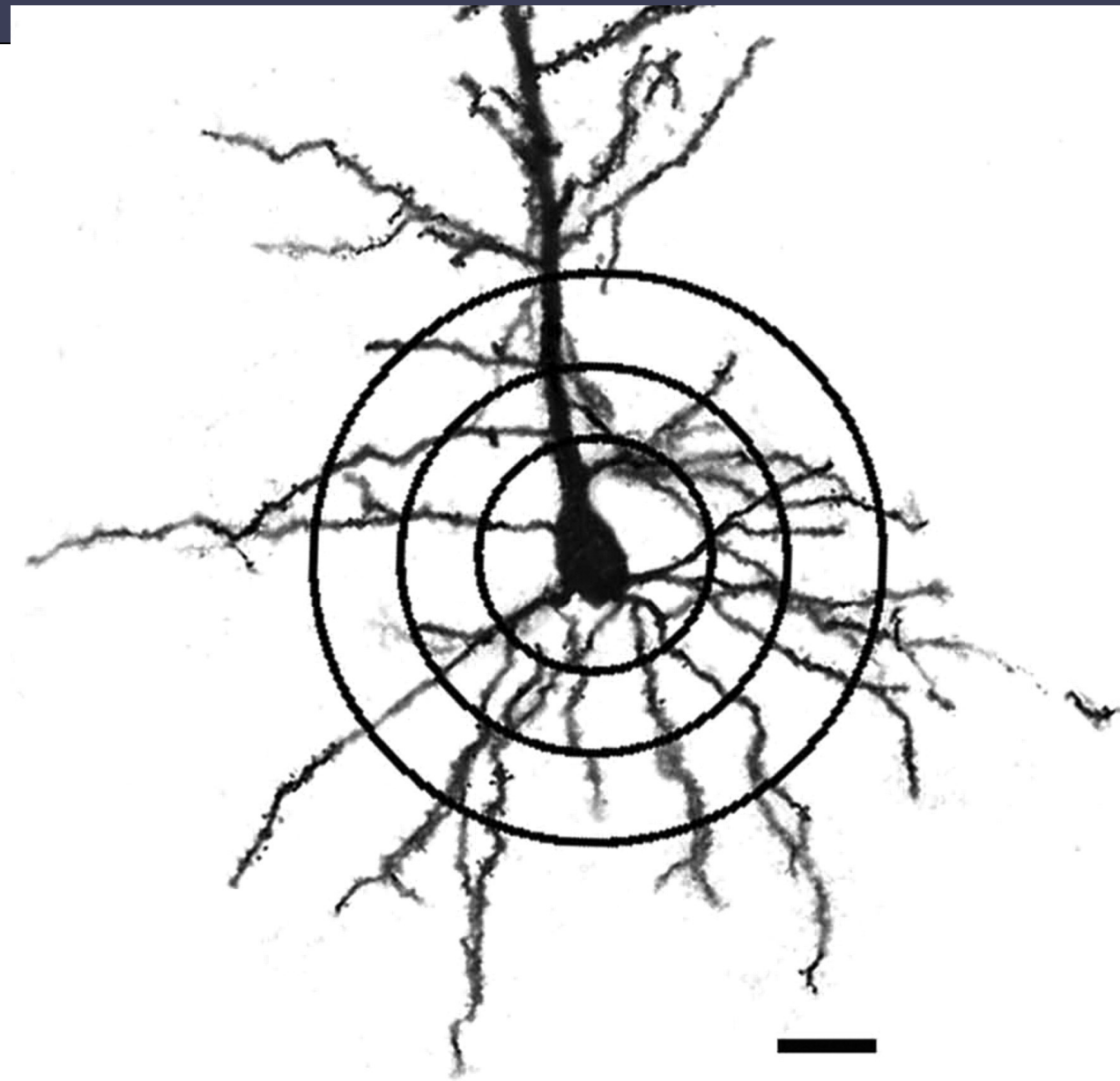


Figure 5. General histological types of neurons and neuroglia in the mammalian brain. A) multipolar neuron; B) granule cell; C) pyramidal neuron; D) Golgi cell.



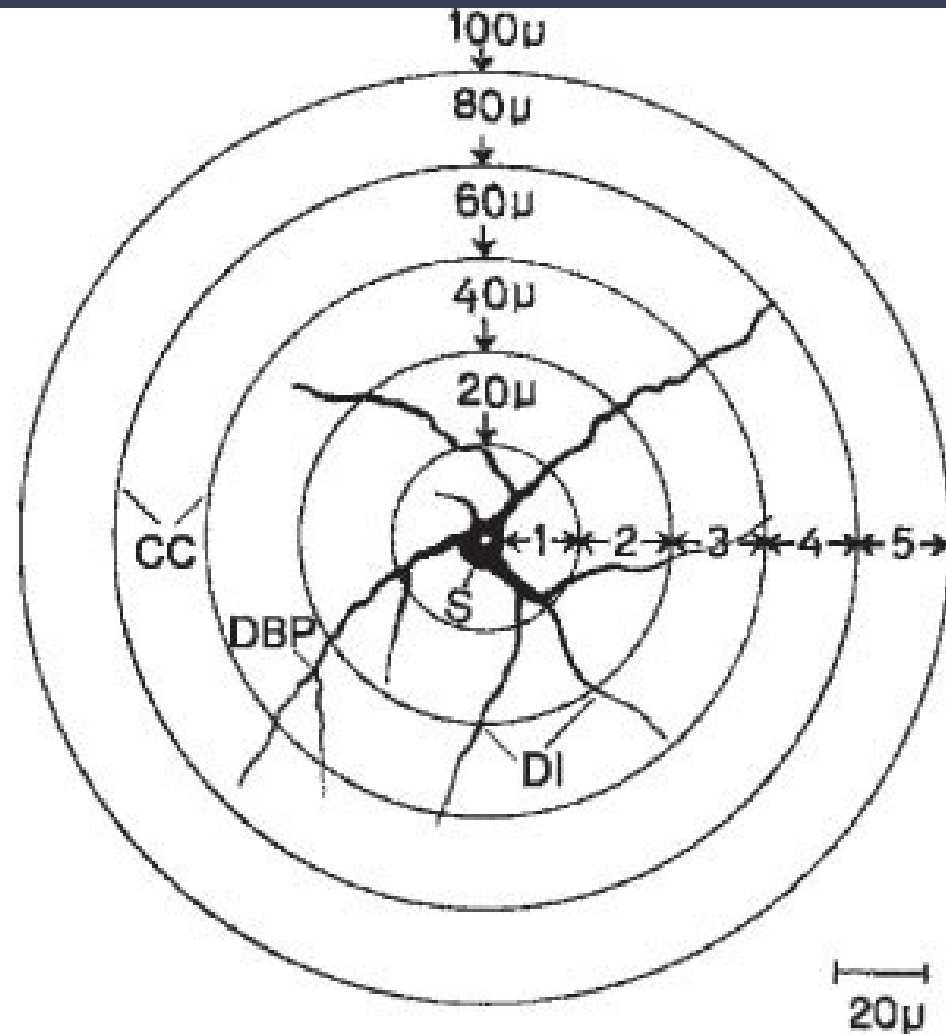
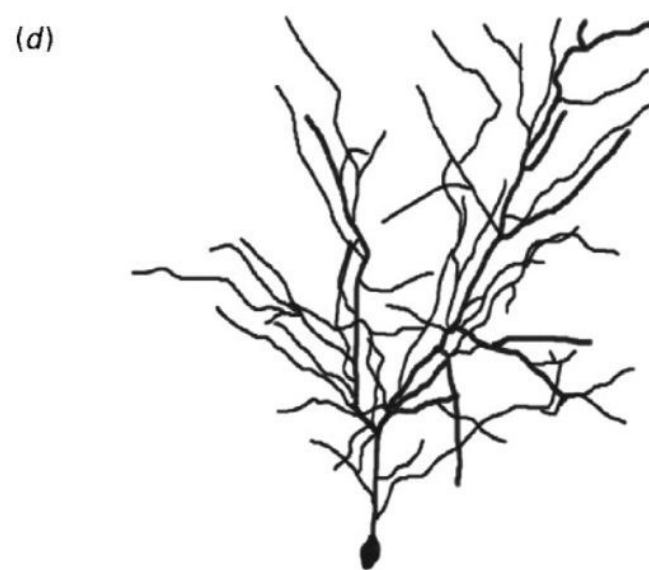
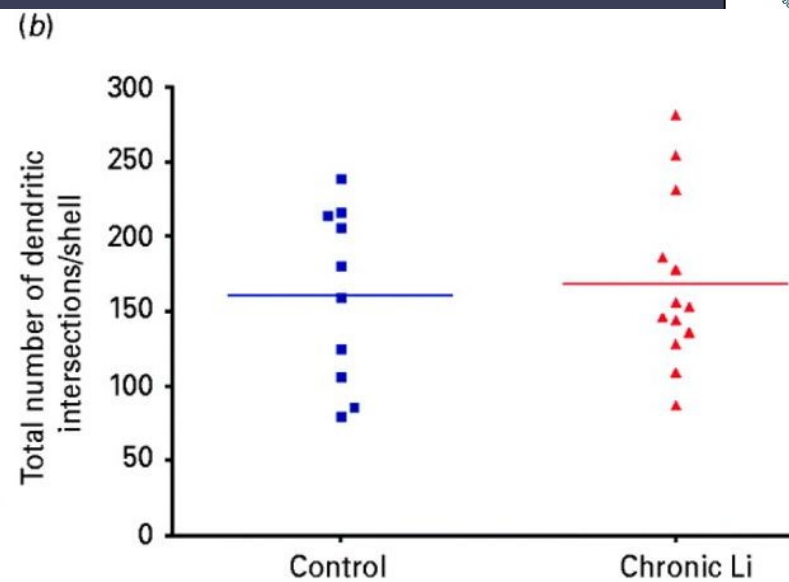
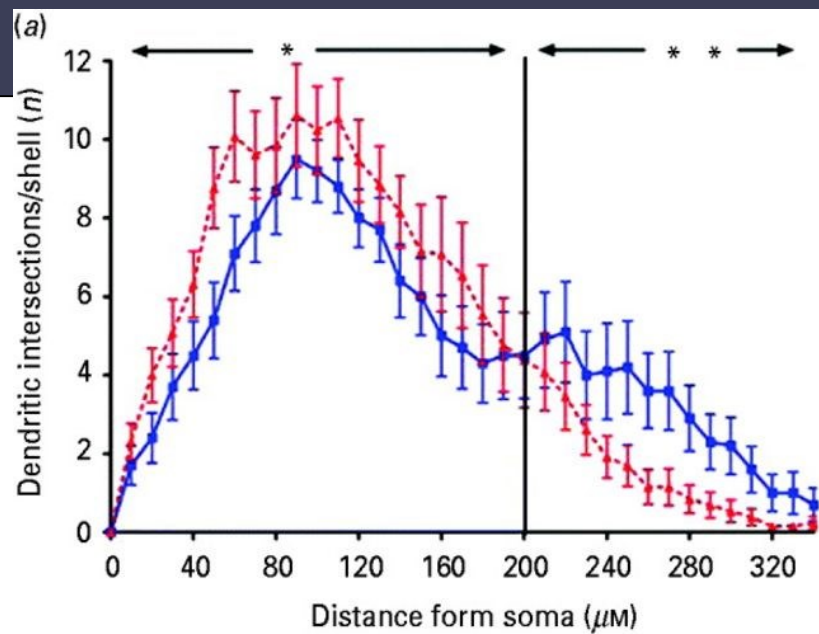
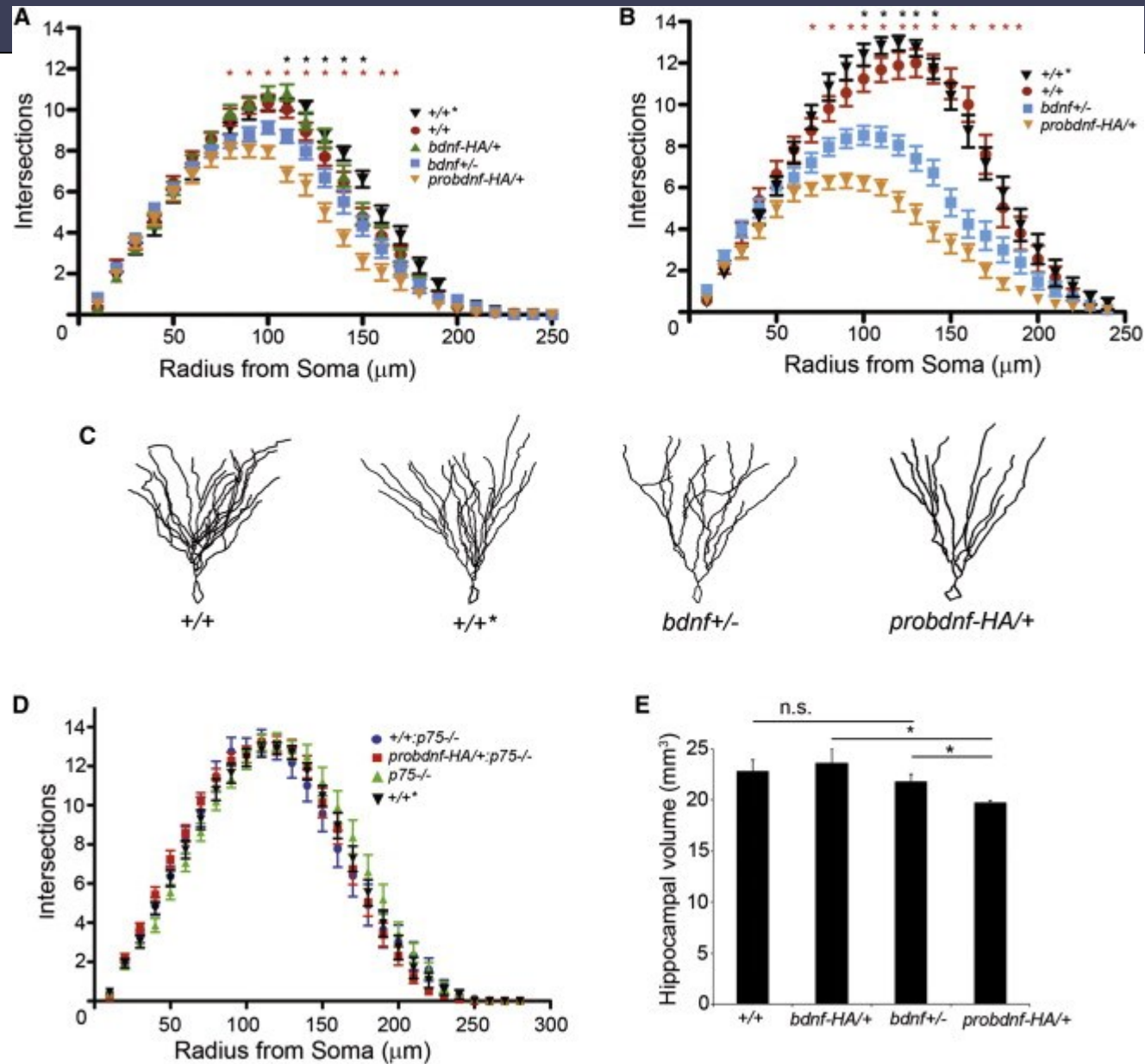
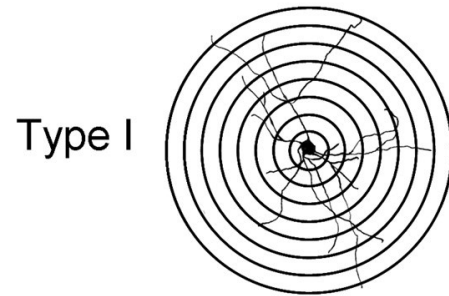


Figure 1 - Diagram of an amygdaloid neuron and the scheme of dendritic quantification. DBP – Dendritic branching points; DI – Dendritic intersections; CC – Concentric circles; S – Soma; and 1 to 5 – Concentric zones.

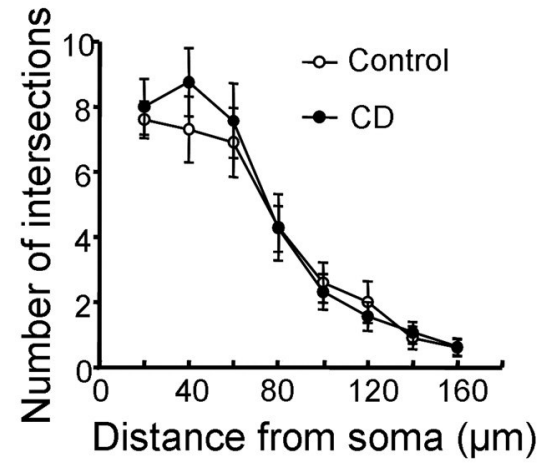




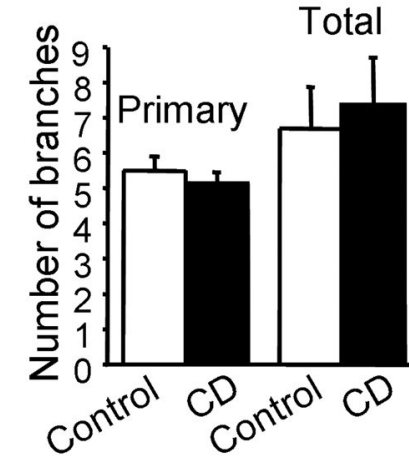
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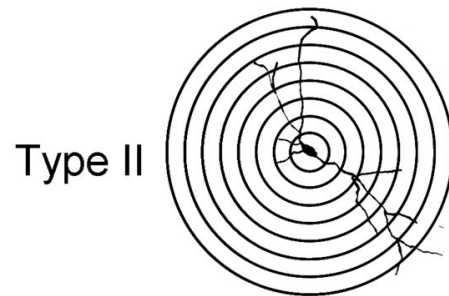
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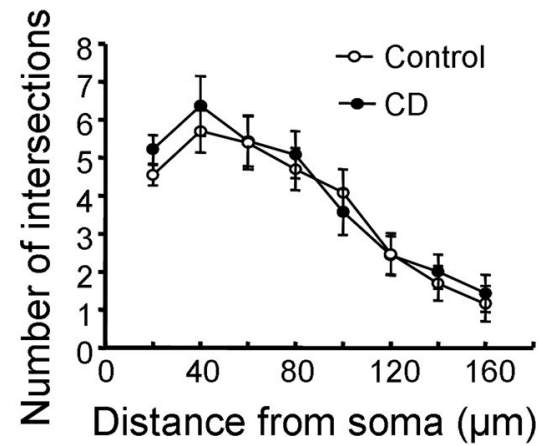
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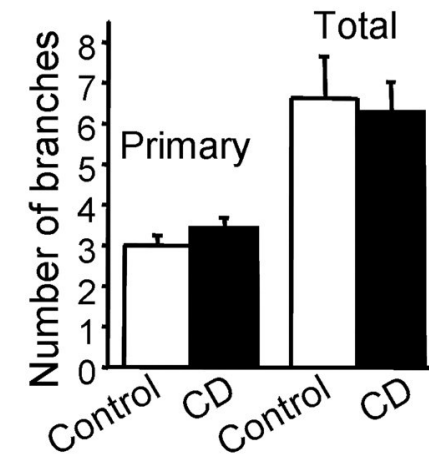
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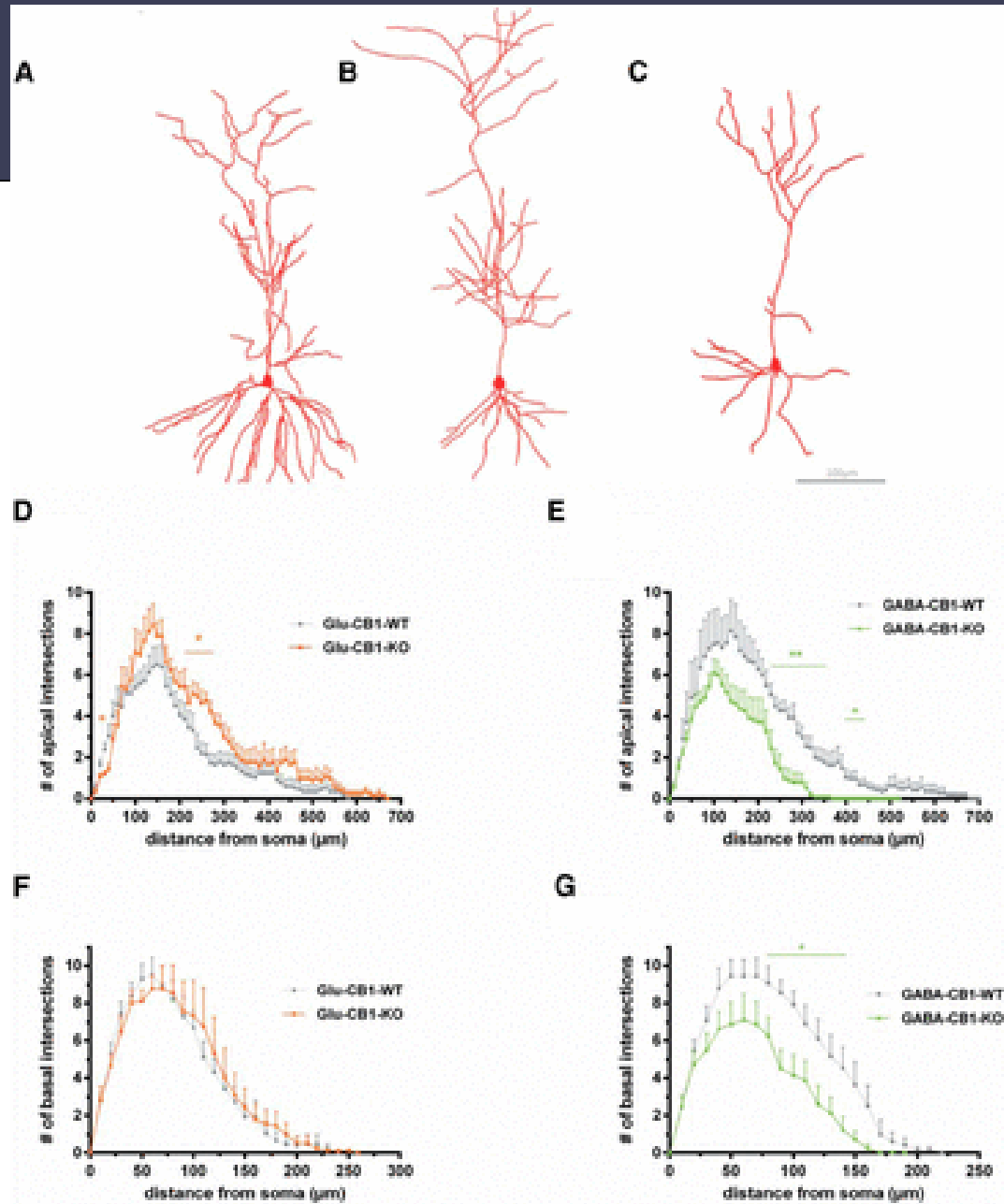


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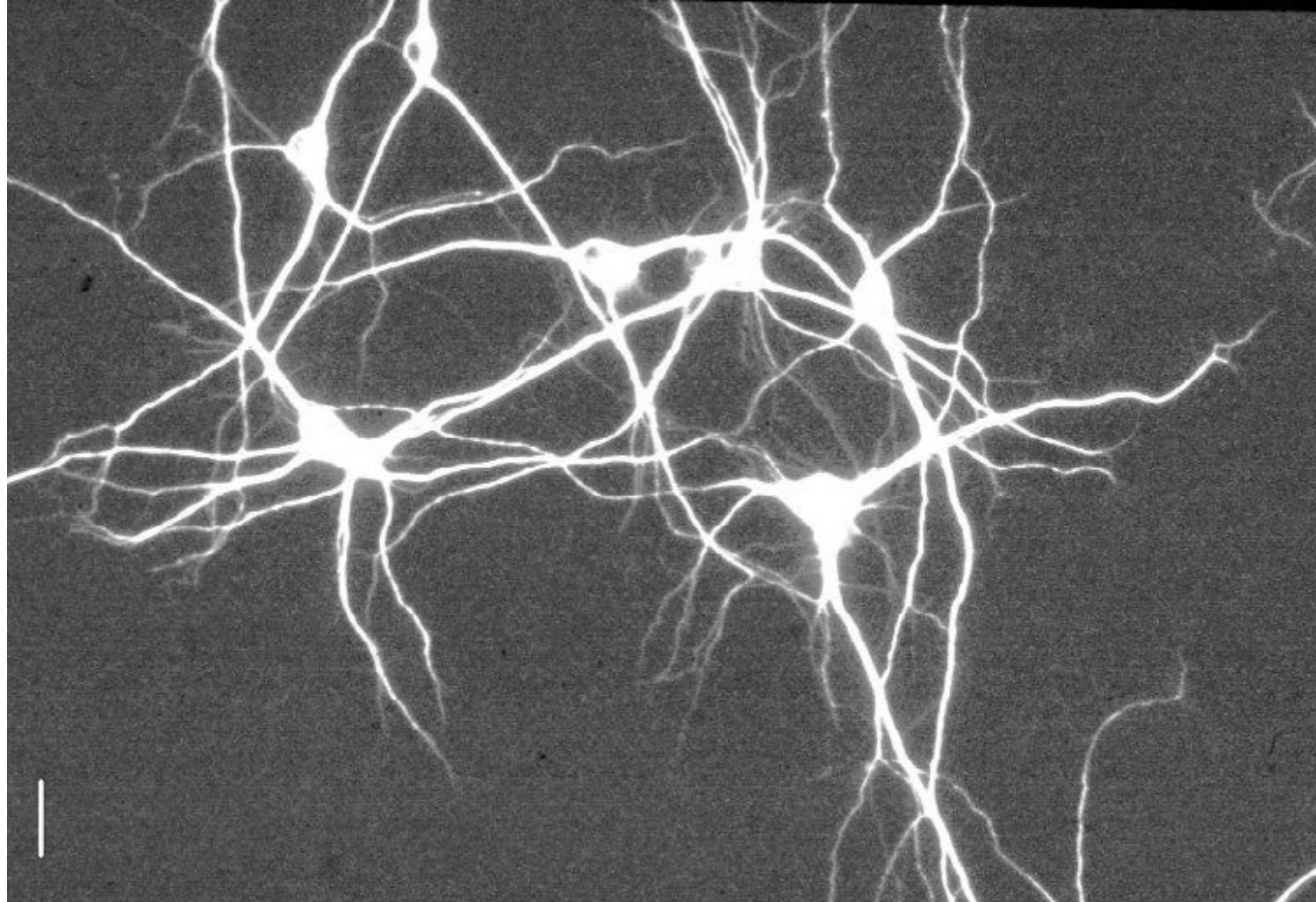
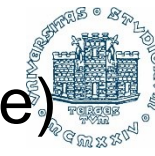


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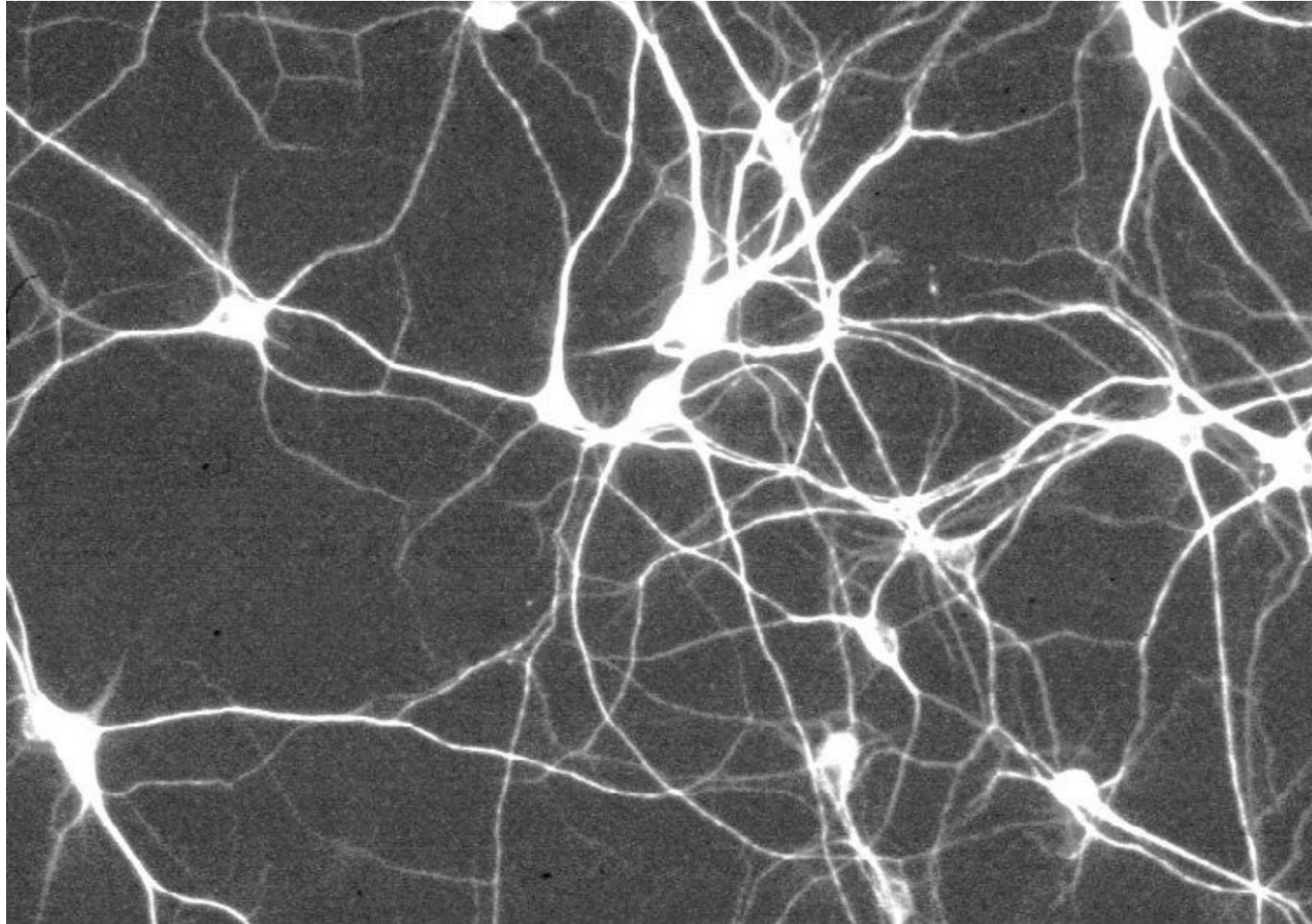
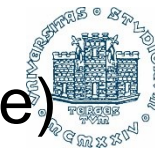


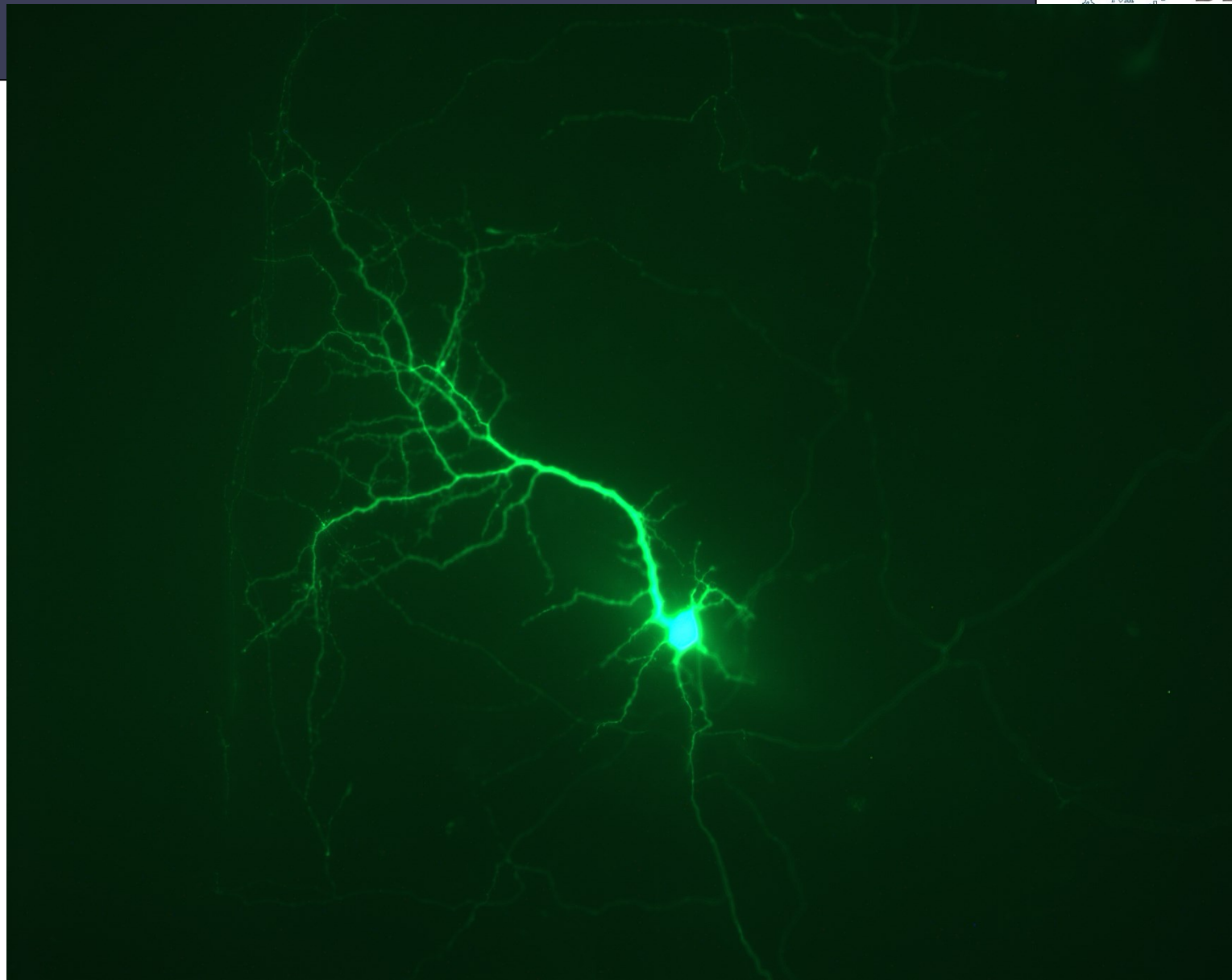


- Primary culture (directly from animal tissue)



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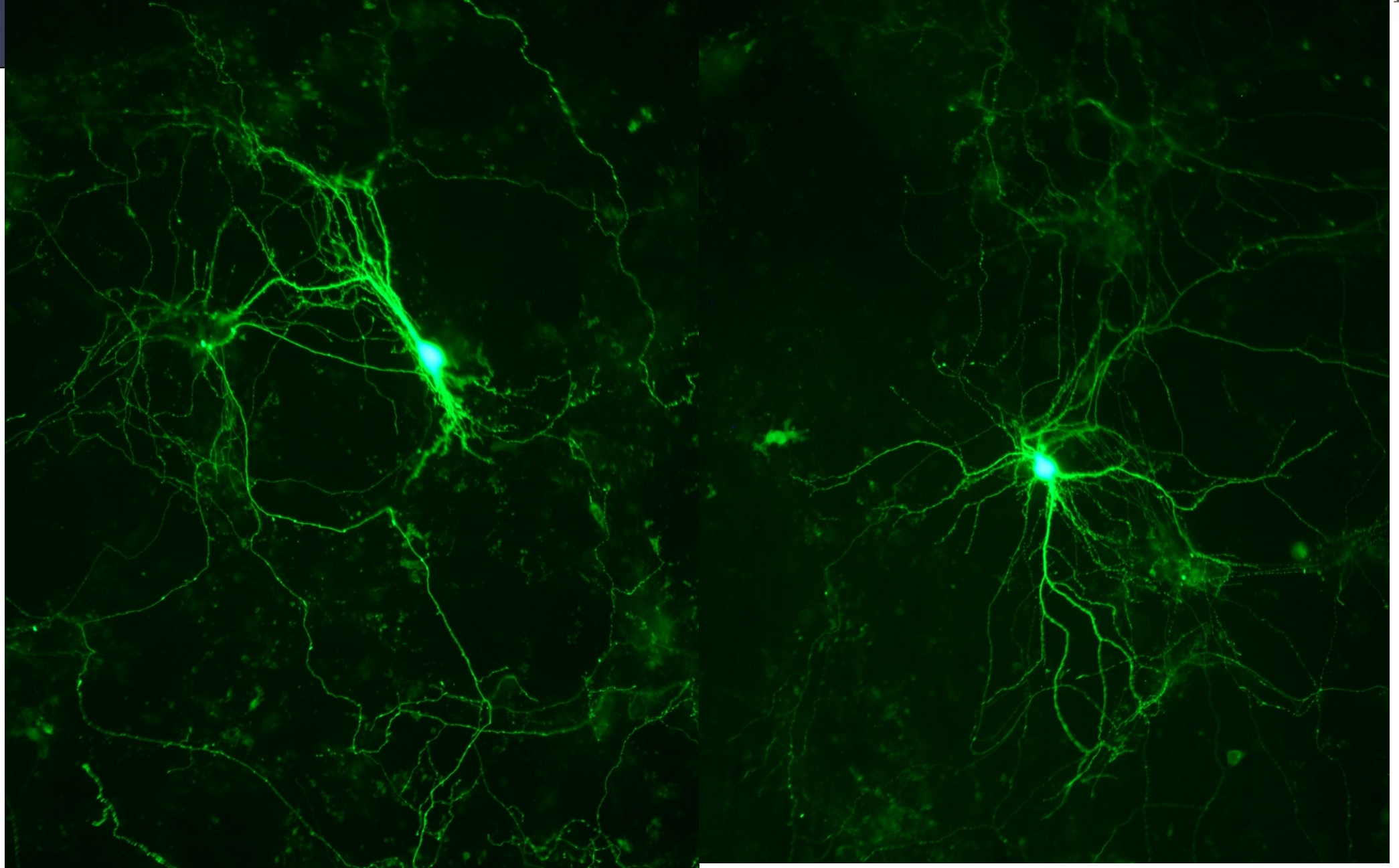




Neurons



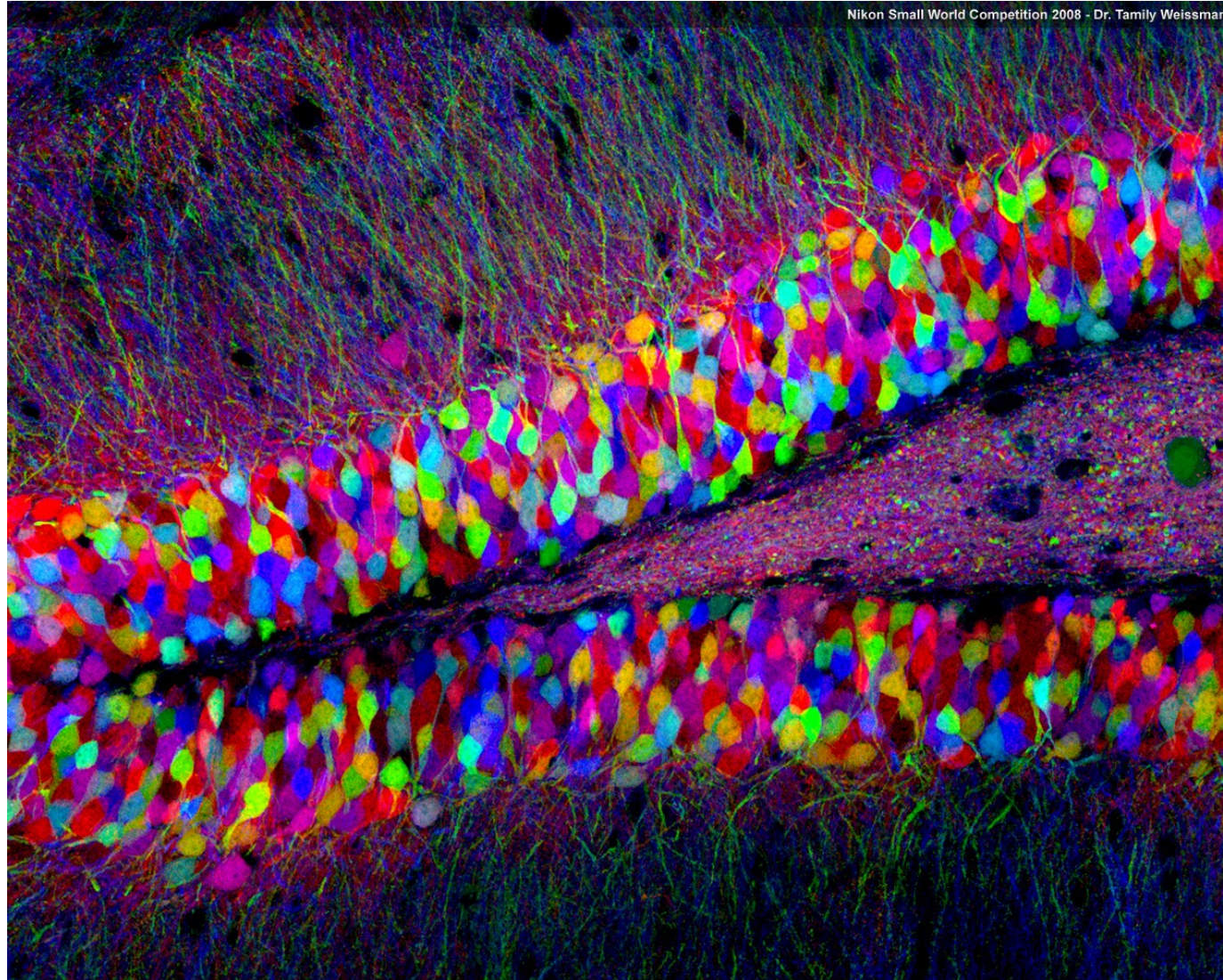
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Brainbow MODEL



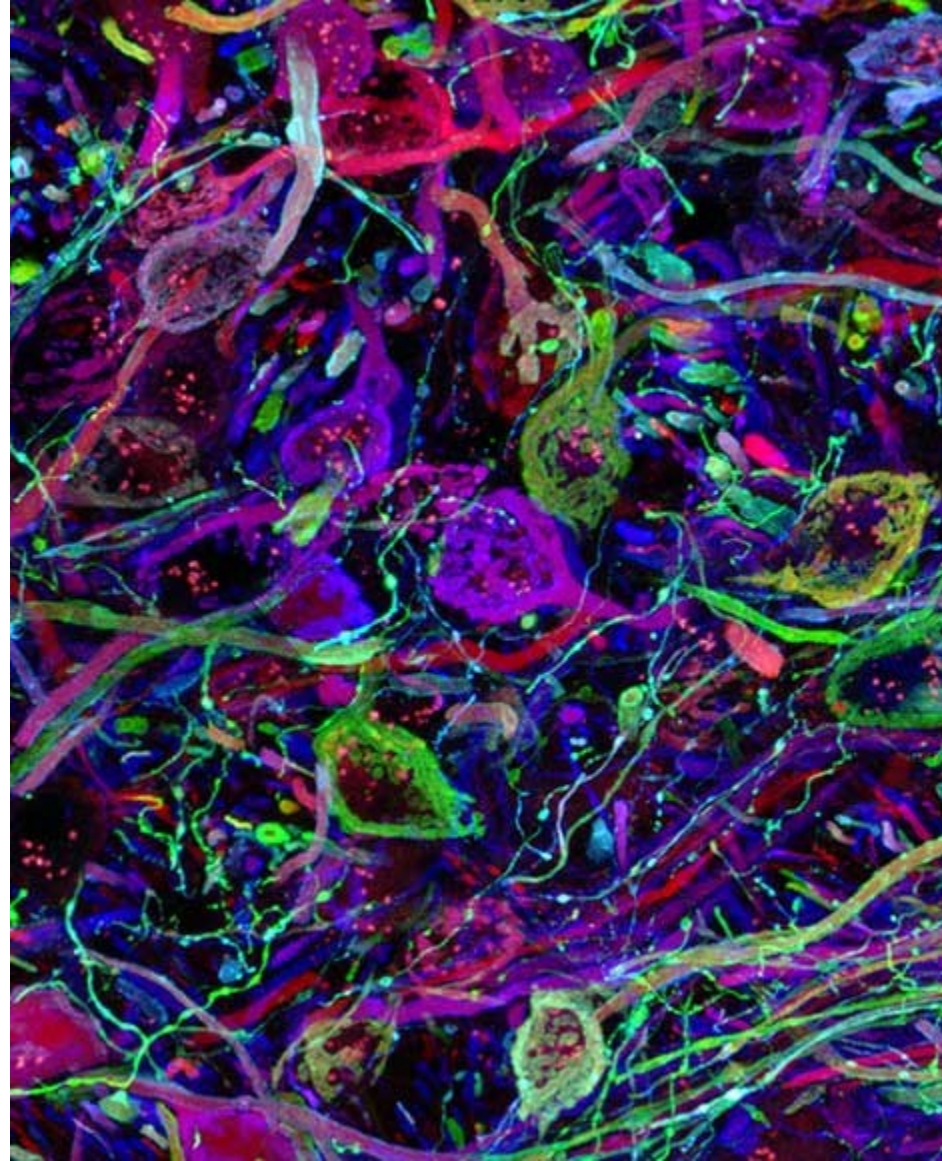
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Brainbow MODEL



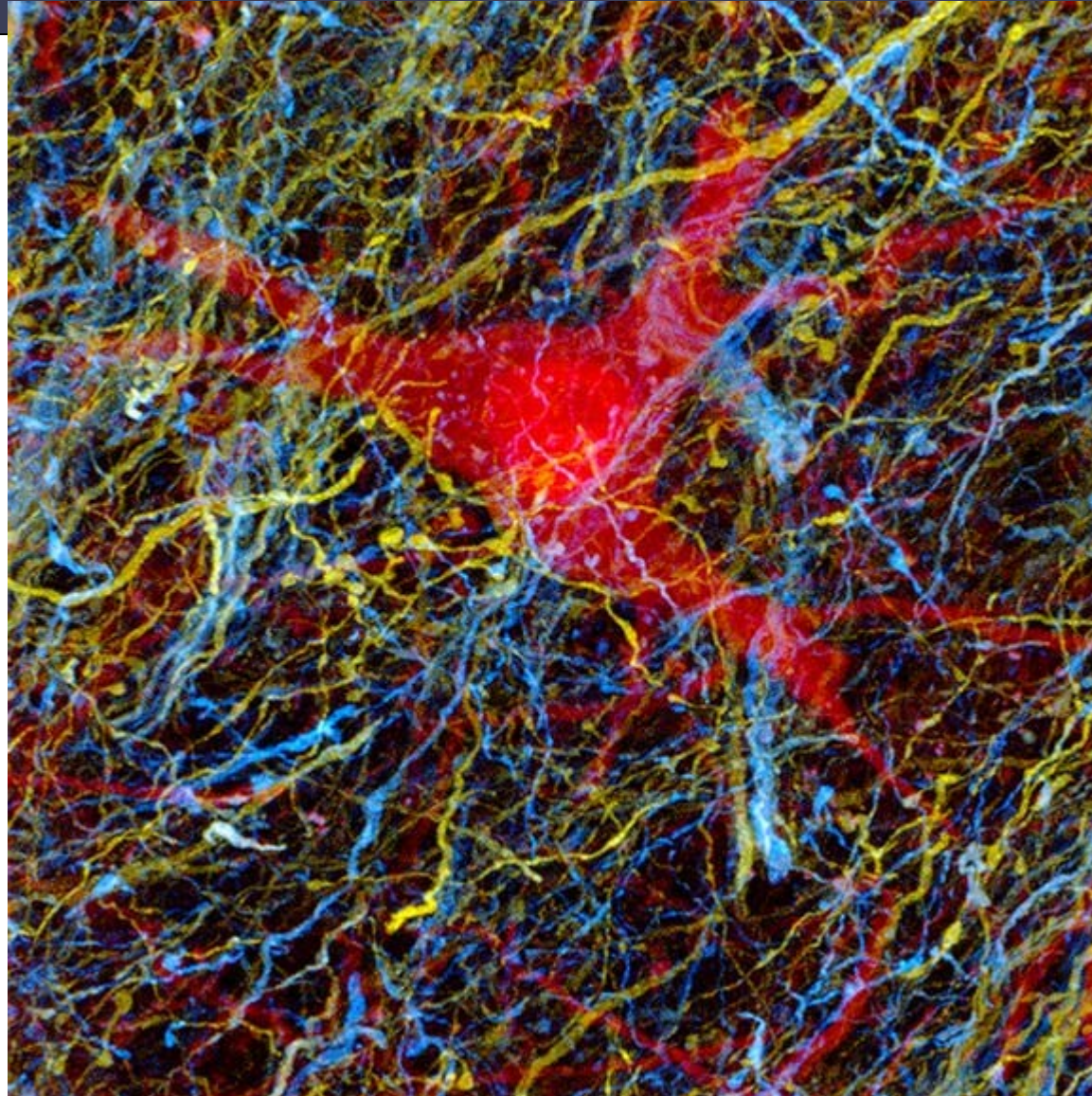
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Brainbow MODEL



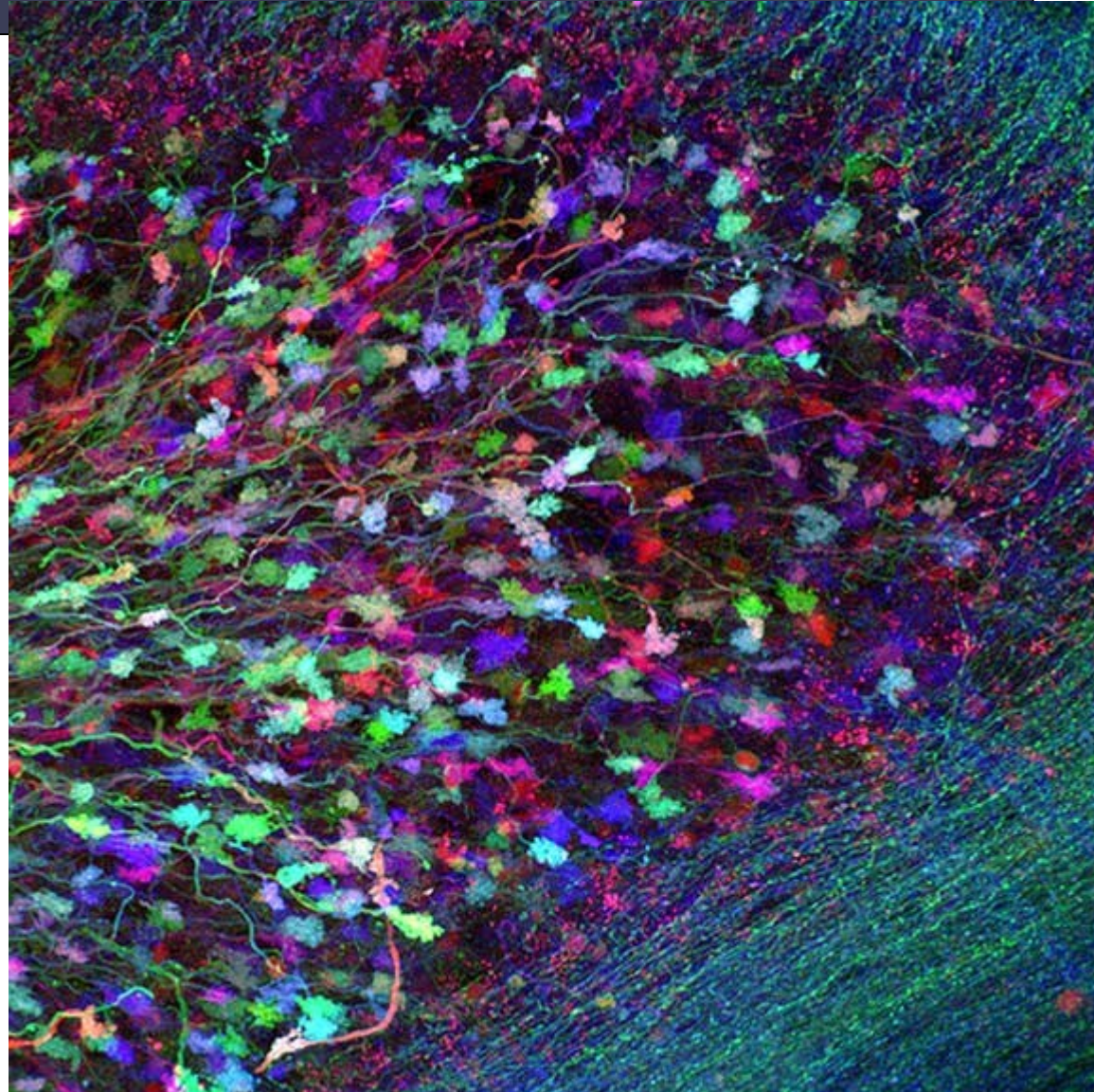
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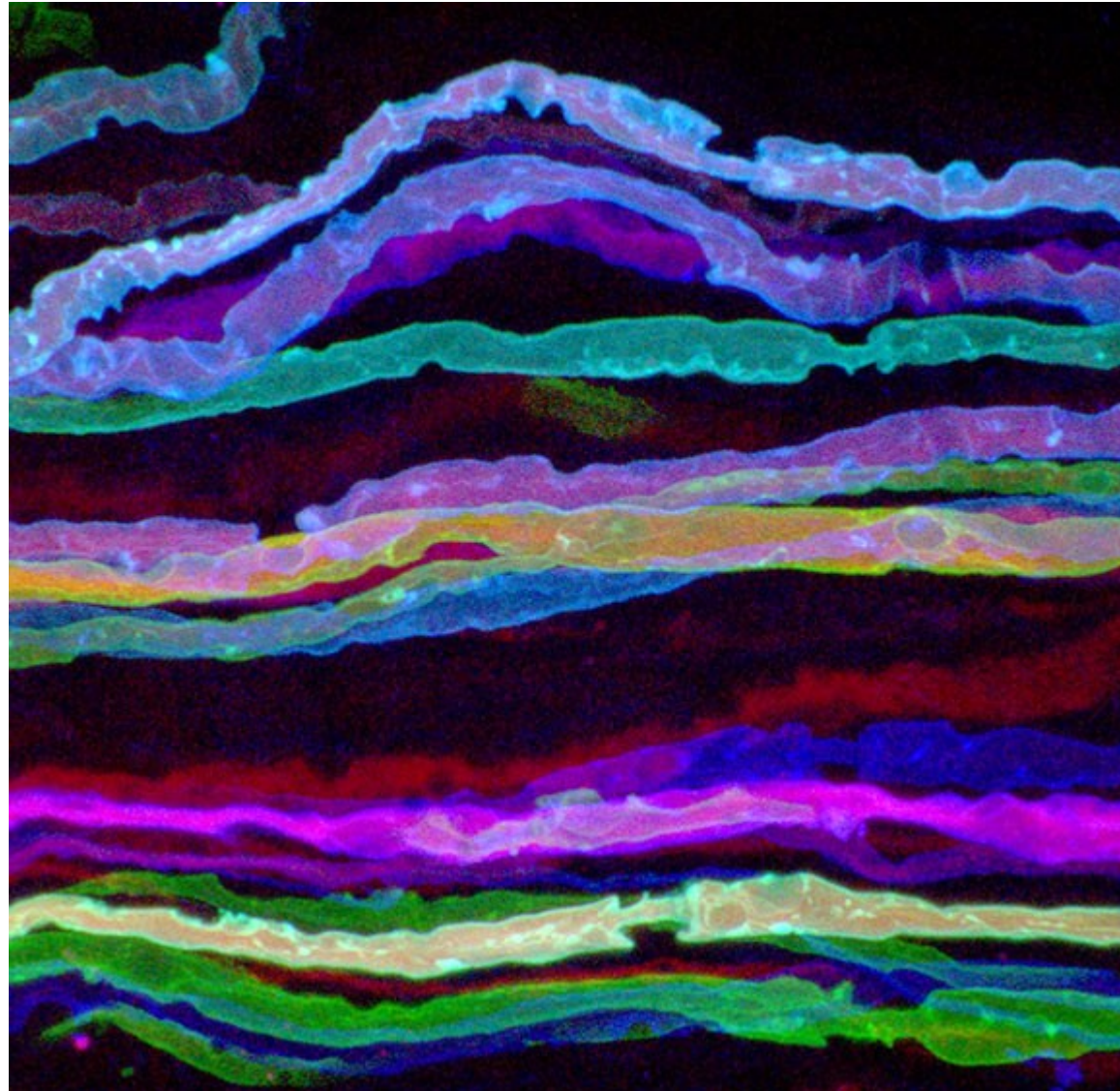
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Brainbow MODEL



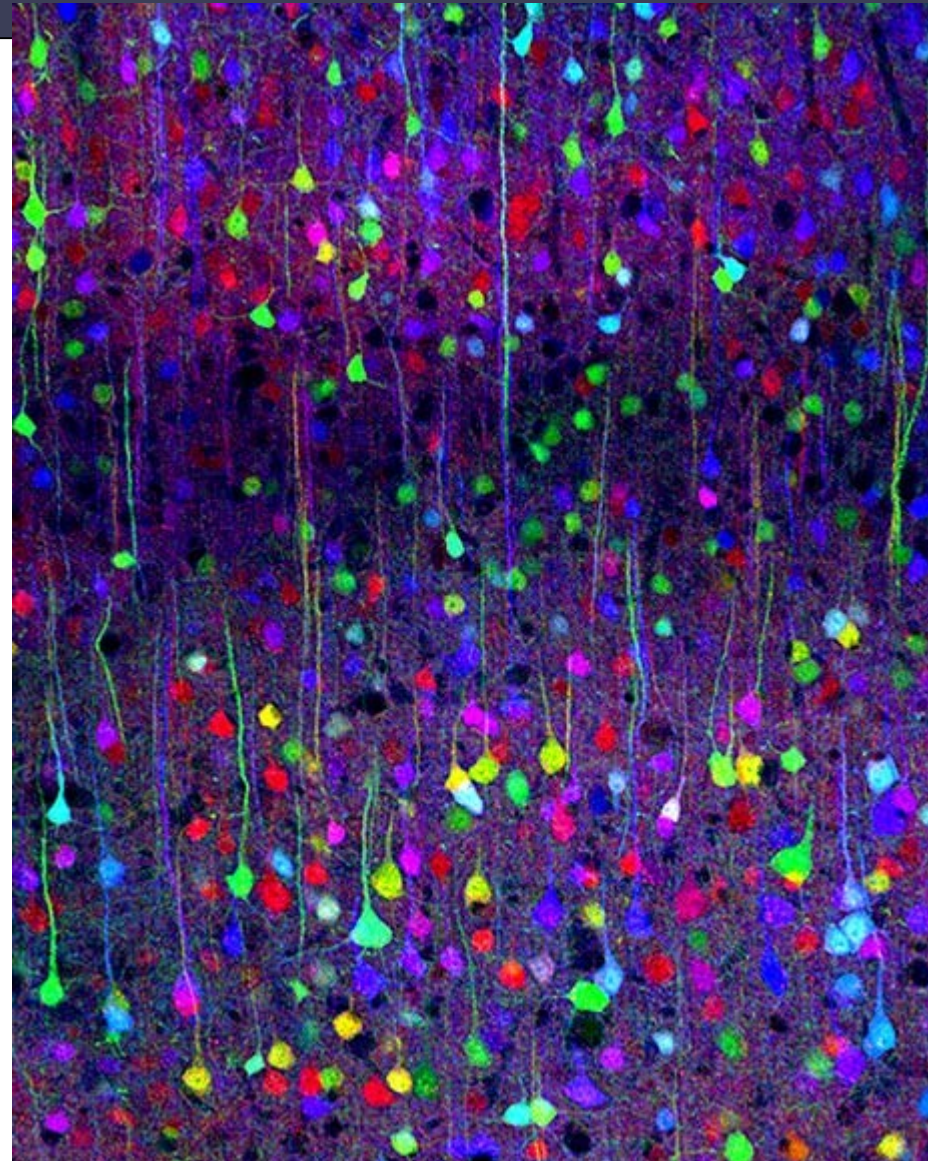
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Brainbow MODEL



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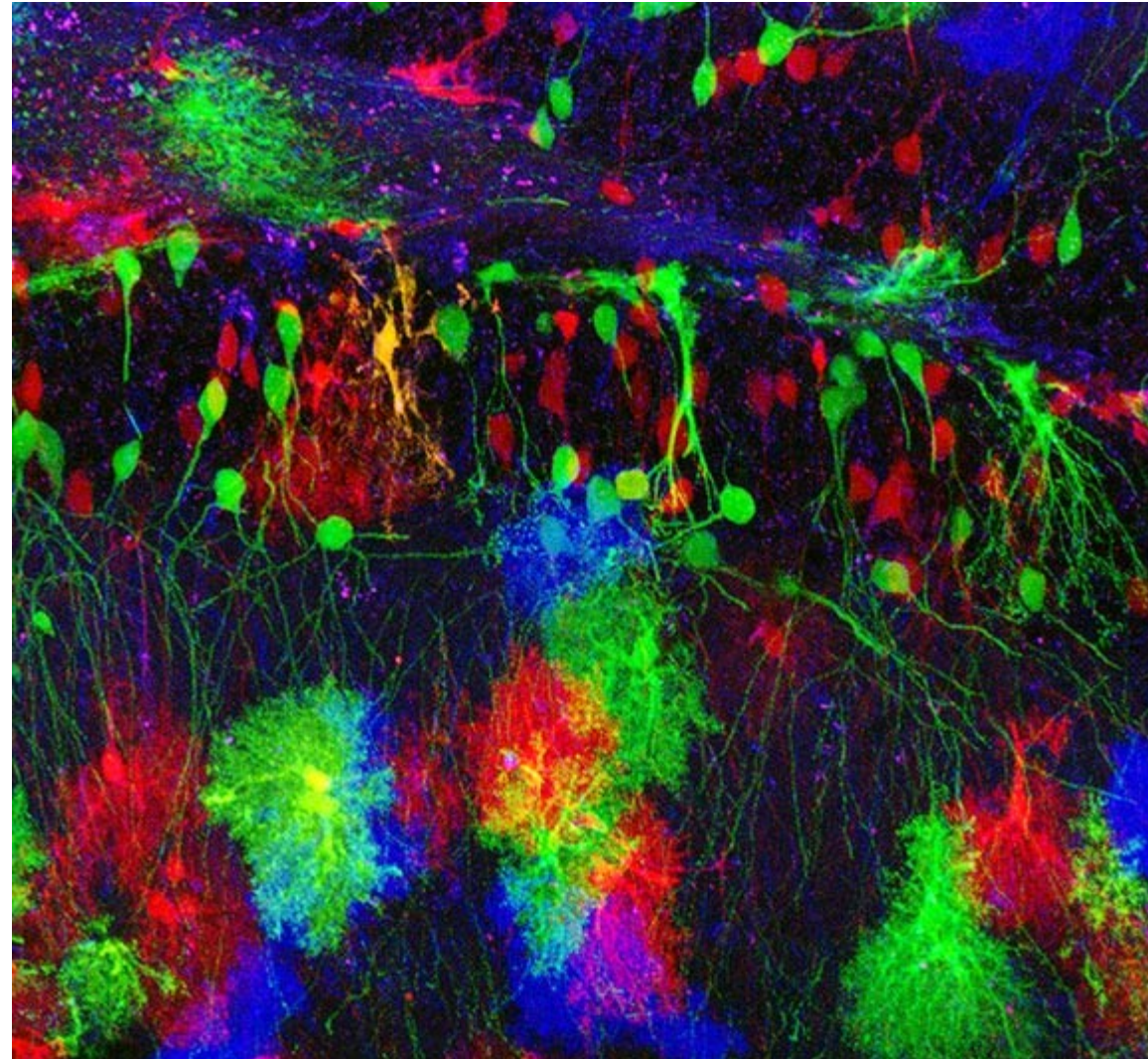


Image J



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<http://rsbweb.nih.gov/ij/>

or simply type : imagej or





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