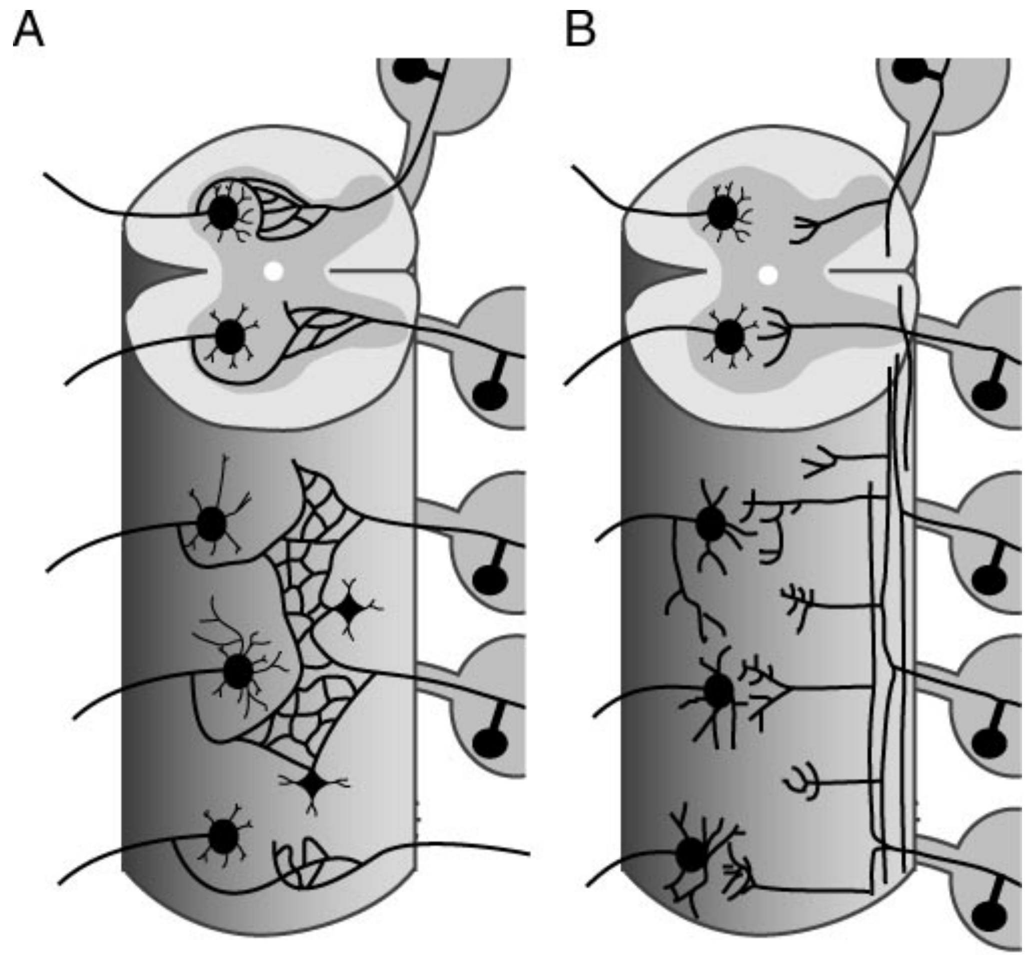
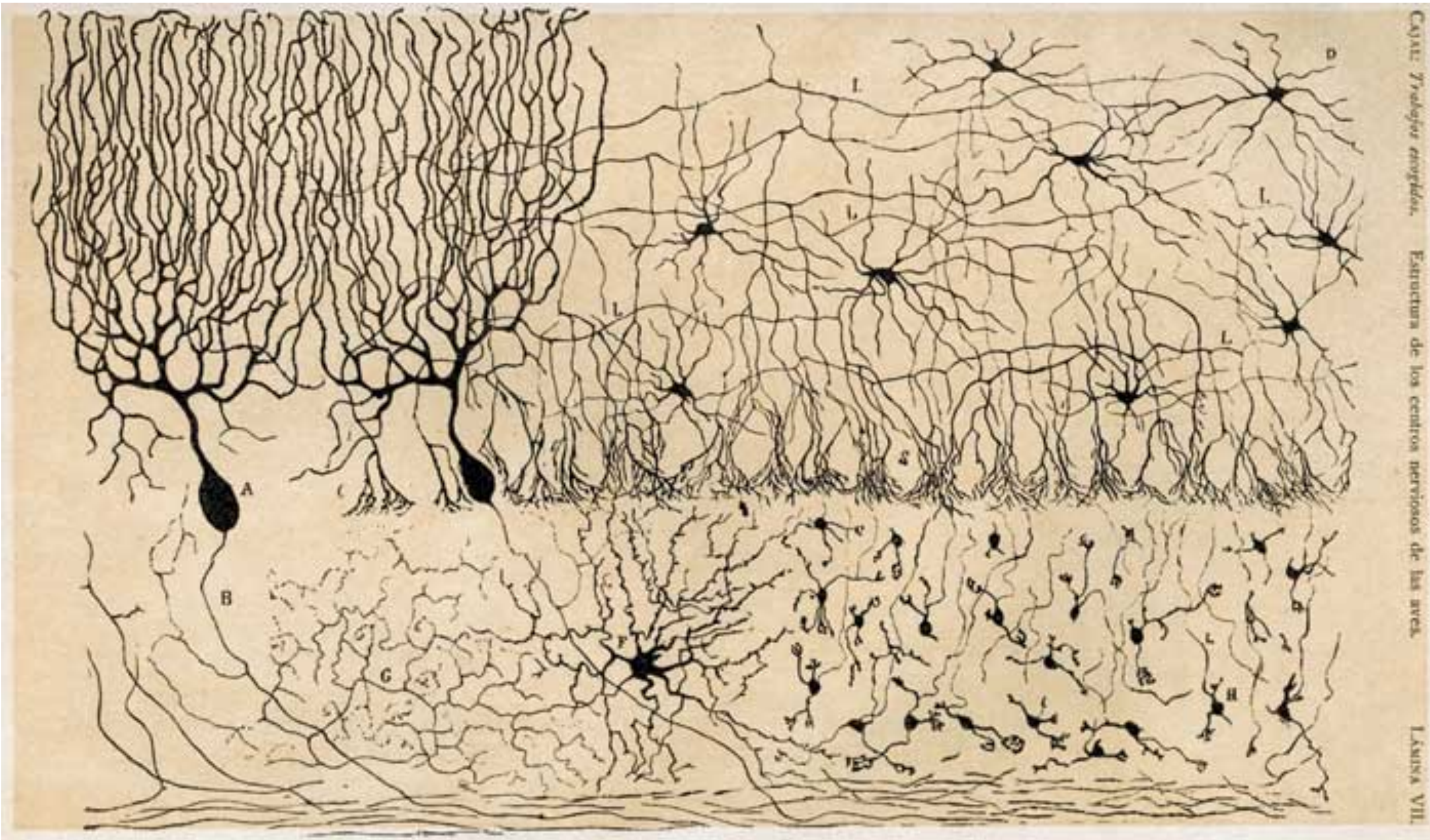
Vignette #1, Sweden 1906
Golgi and the Reticularists - vs. - Cajal and the Atomists



Cajal's Golgis



Embryonic chick cerebellum

Histology of the Nervous System of Man and Vertebrates

Santiago Ramon y Cajal, 1909

<u>Four tenets of the Neuron Doctrine:</u>

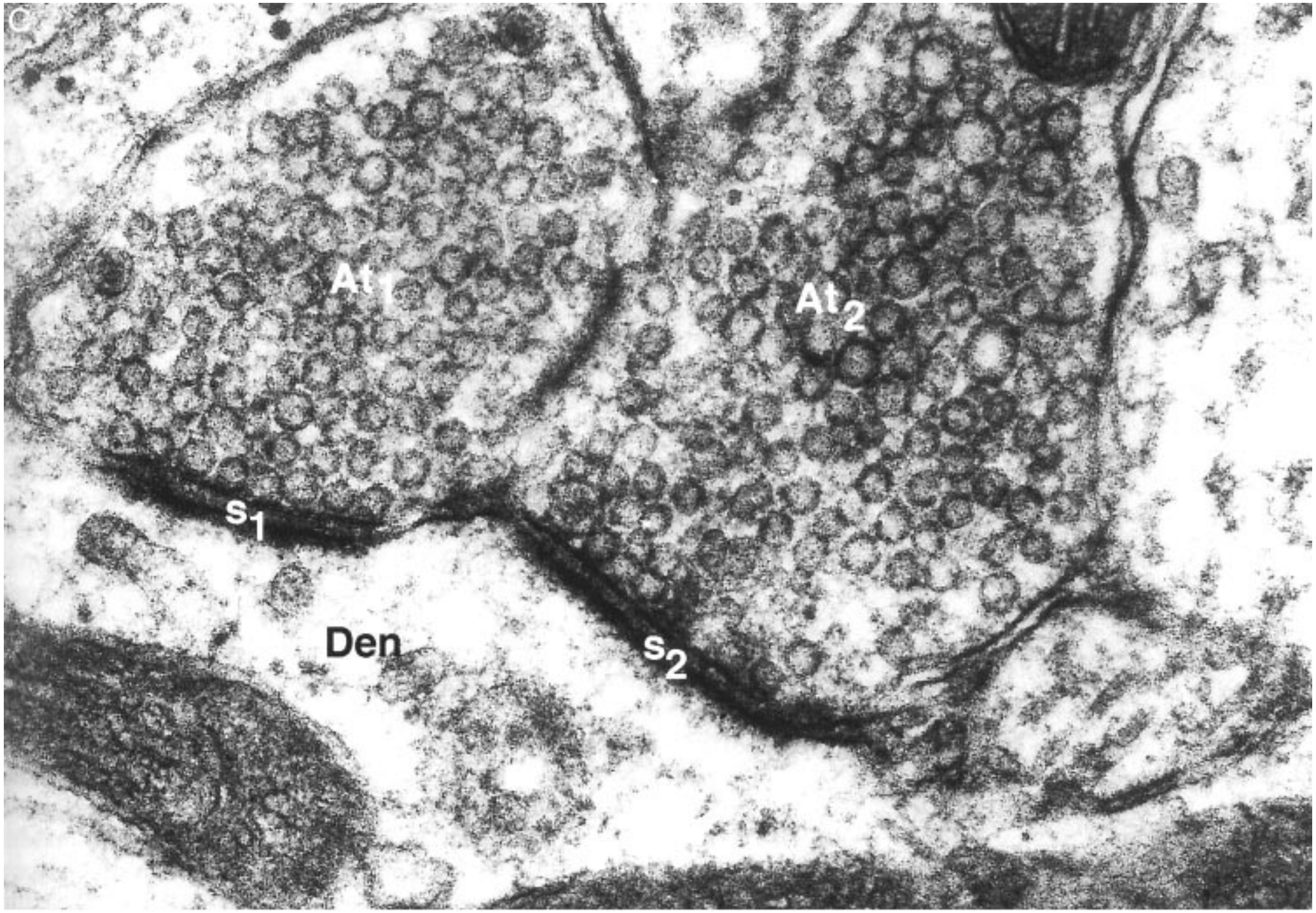
1. The fundamental structural and functional unit of the nervous system is the neuron.

2. Neurons are discrete cells that are not continuous with other cells.

3. The neuron is comprised of 3 parts - the dendrites, axon and cell body.

4. Information flows along the neuron in one direction (from the dendrites to the axon via the cell body).

Neurons communicate at adhesive junctions called synapses



Proposed by Sherrington 1897
Visualized in the 1950s by the electron microscope

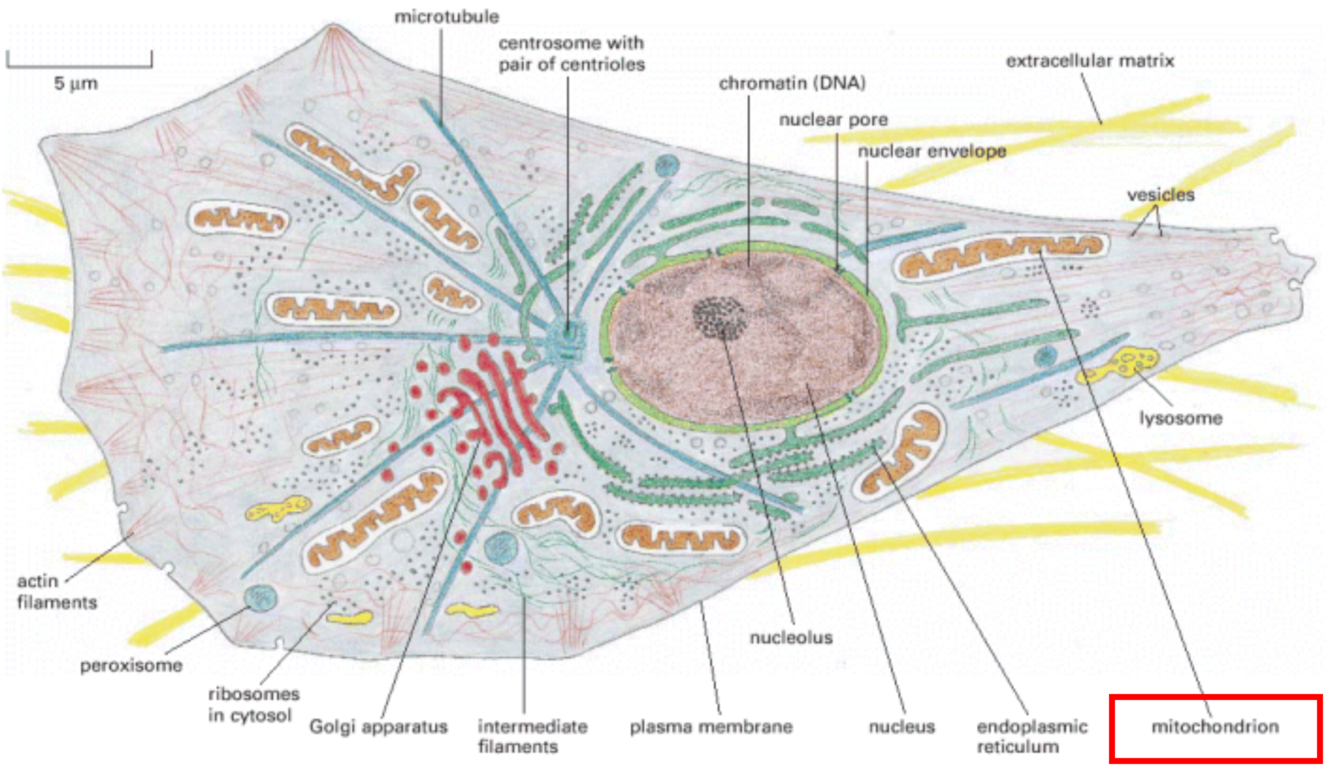
1. Neurons are CELLS

expect conservation of core mechanisms

2. Neurons have specialized problems to solve

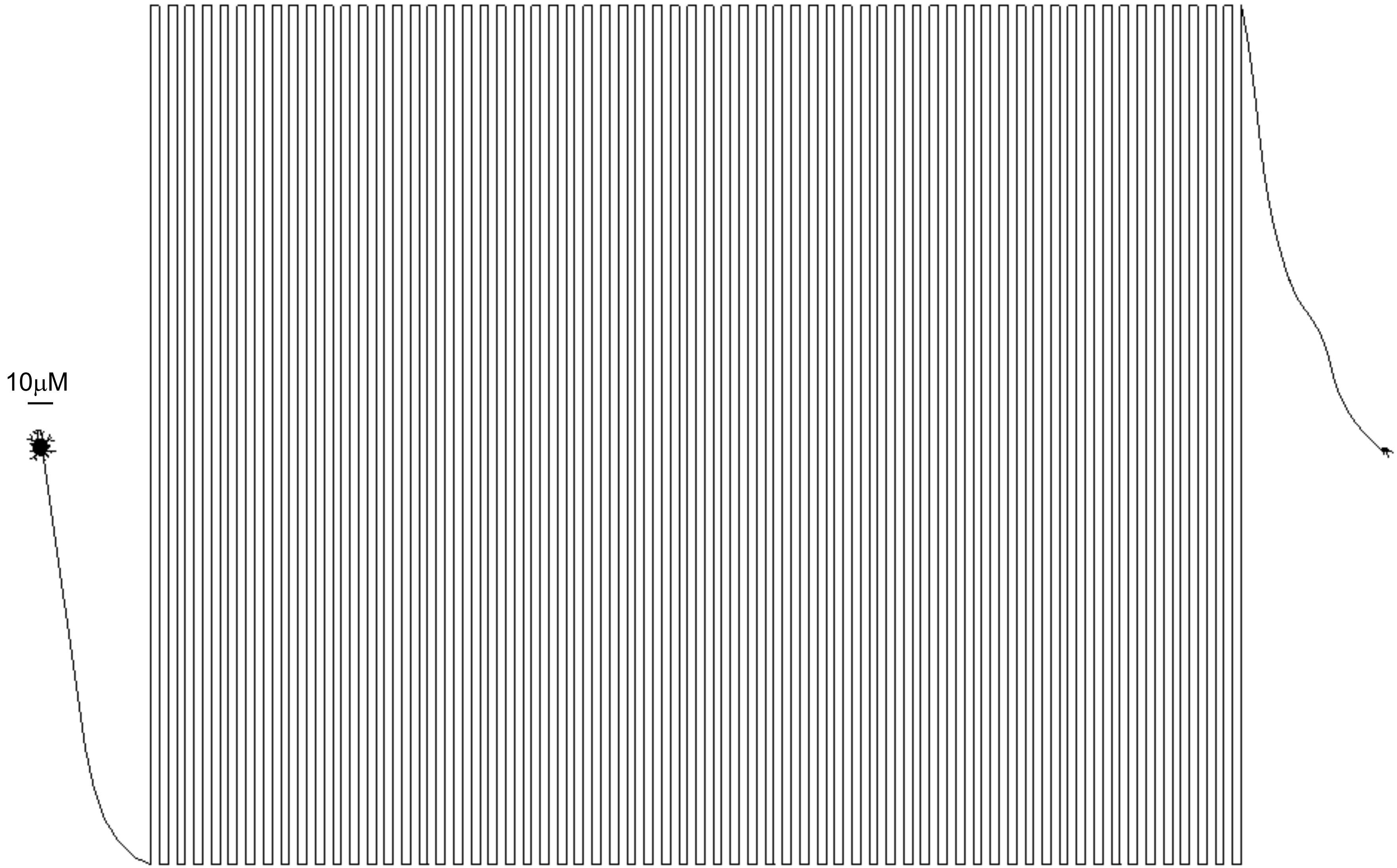
exhibit amplification of specific mechanisms

Major features of Eukaryotic cells



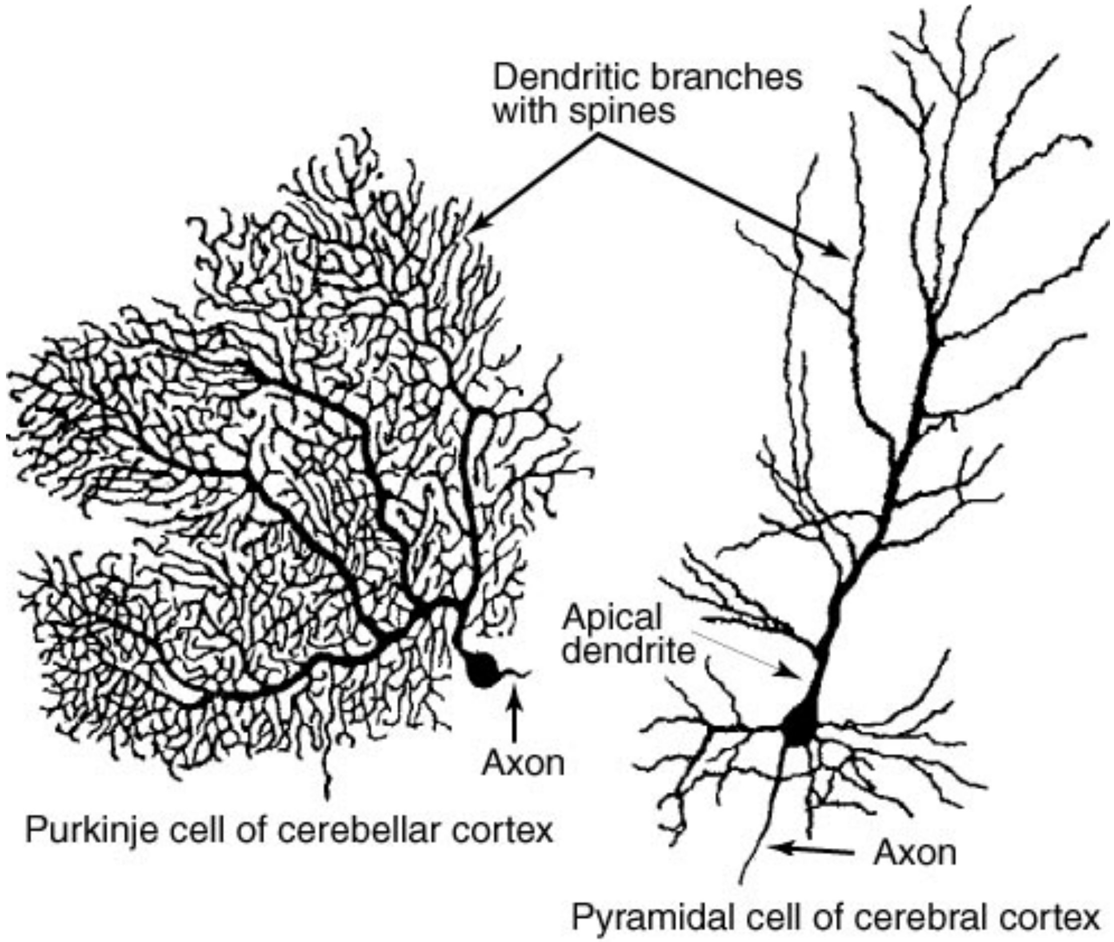
In a resting adult human, 40% of all mitochondrial energy is being used to pump ions in neurons.

Neurons are highly polarized cells



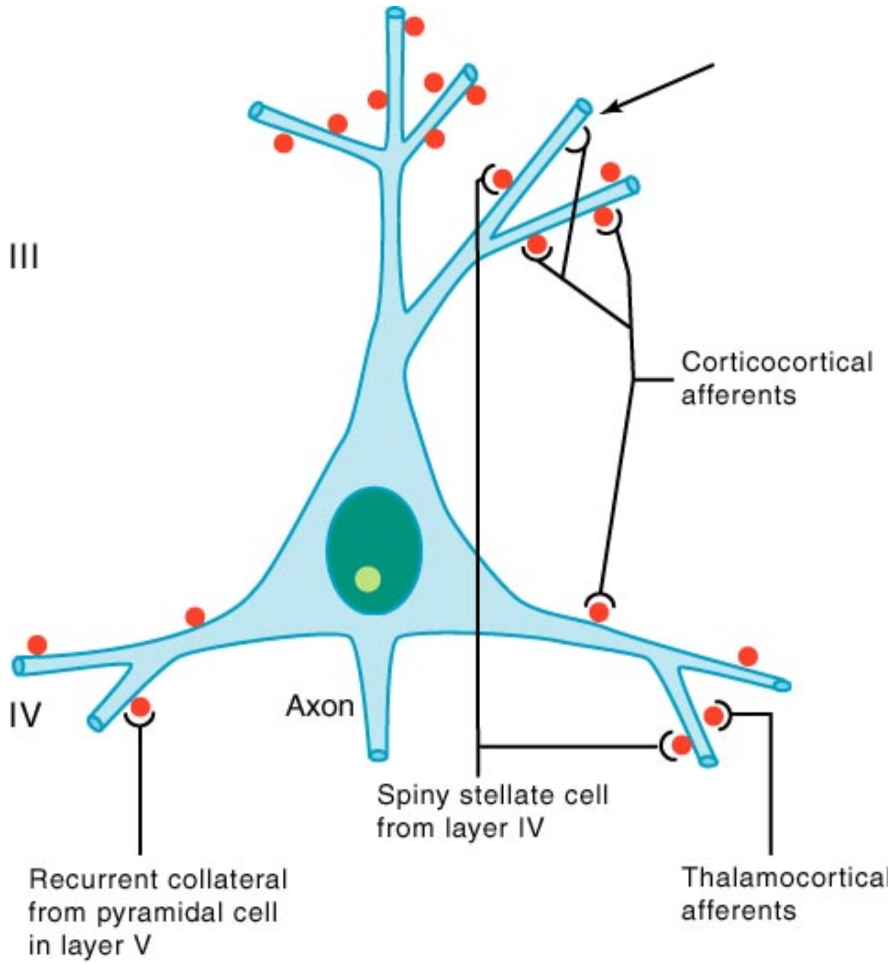
Courtesy of P. Hollenbeck, Purdue U.

Functional variations in neuronal morphology

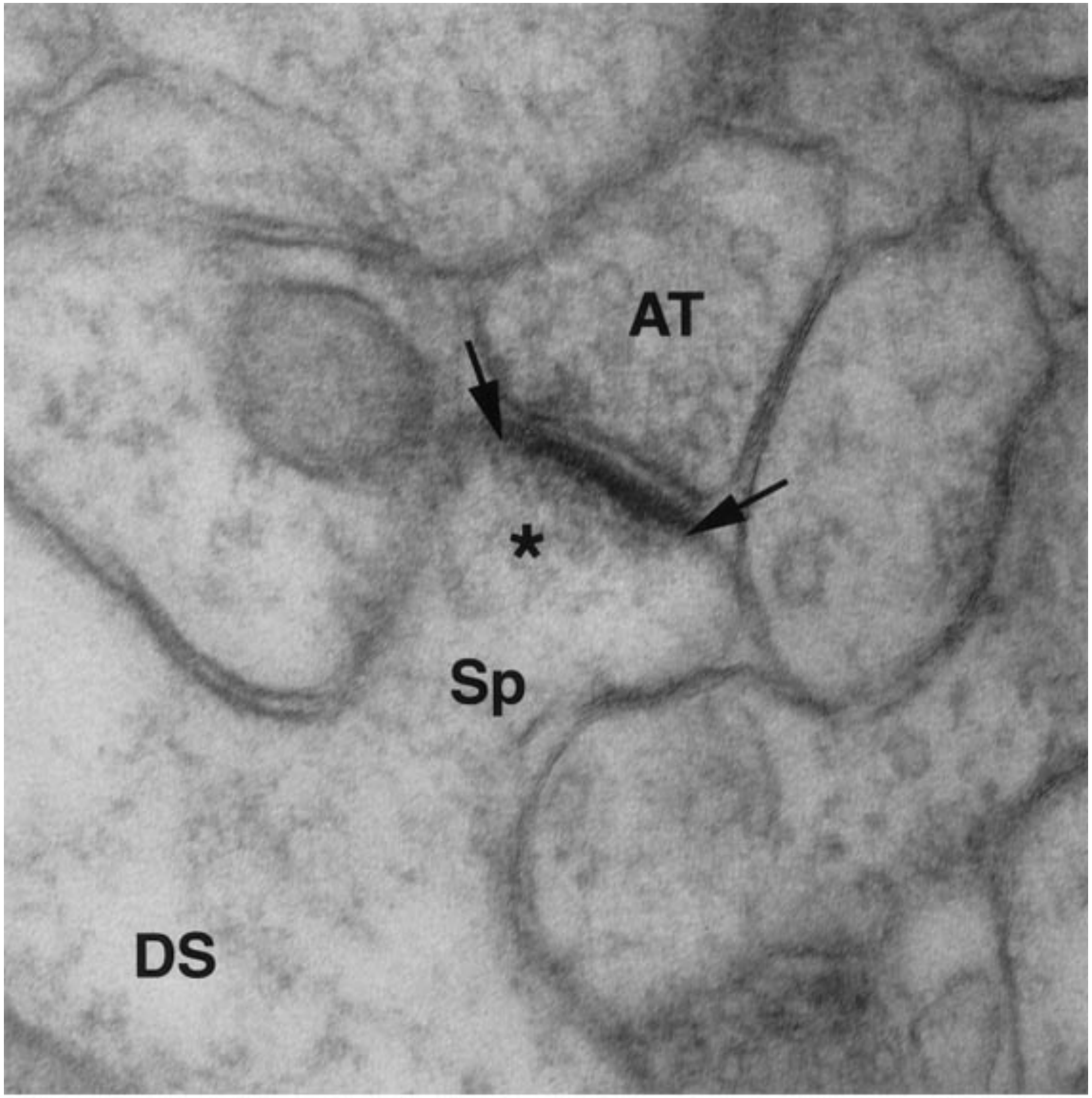


dendrites, cell soma, axon

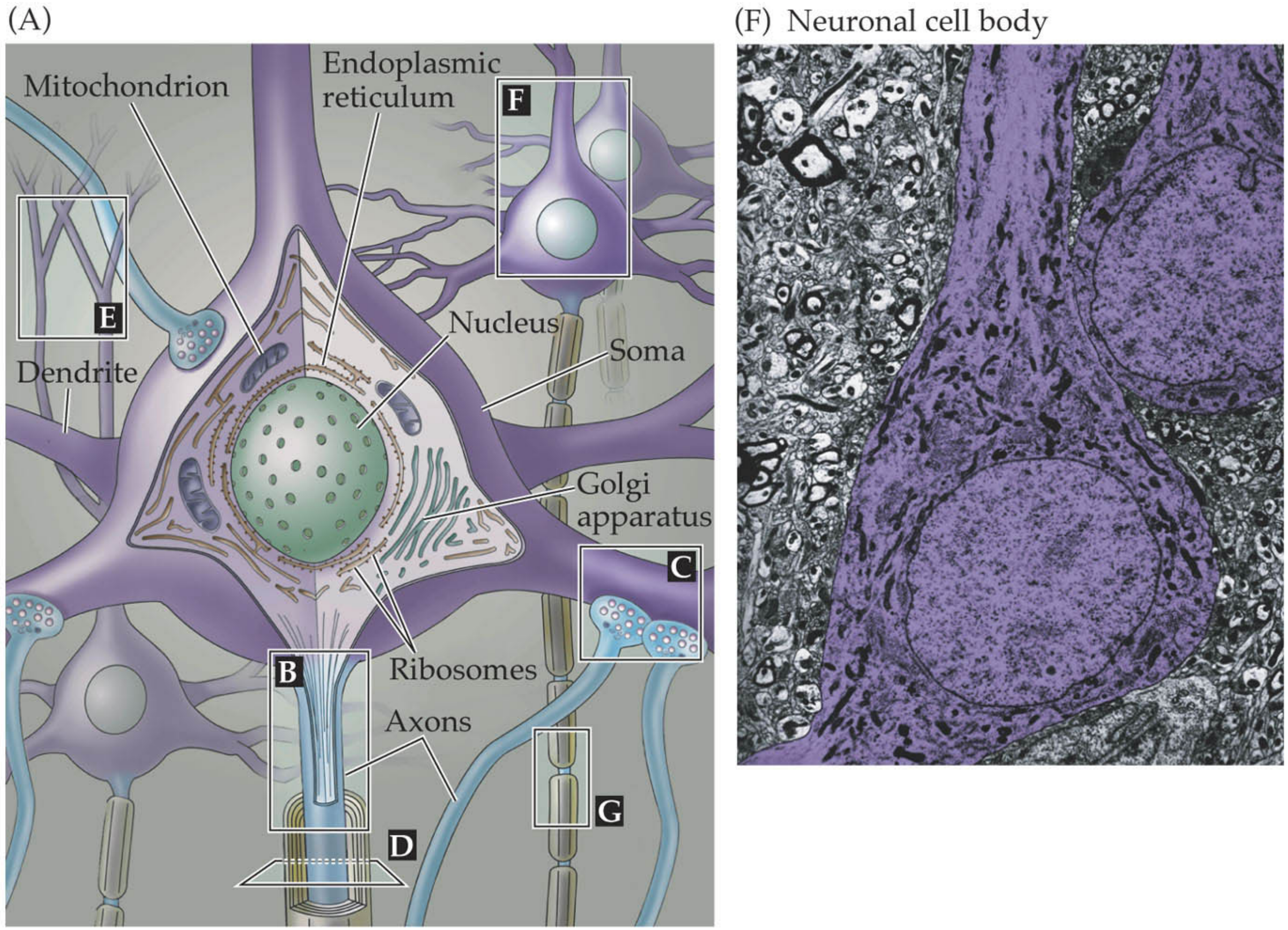
Most synapses are made onto dendrites and spines



Spines are specialized actin-rich dendritic protrusions

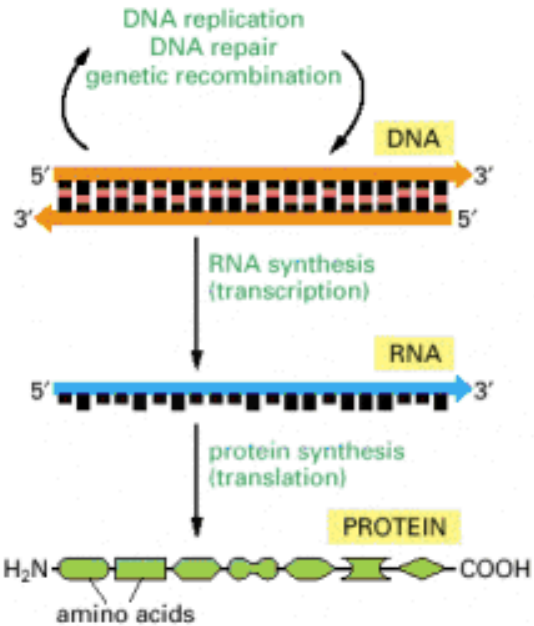


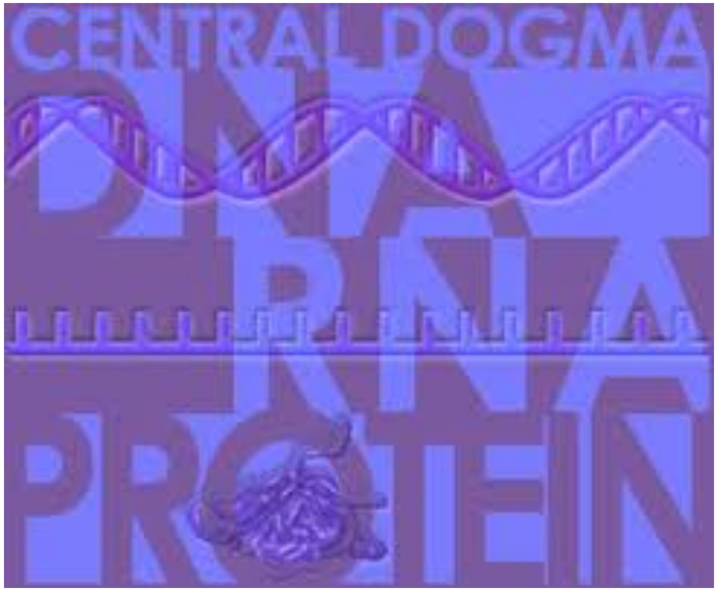
The soma contains the biosynthetic machinery of the cell

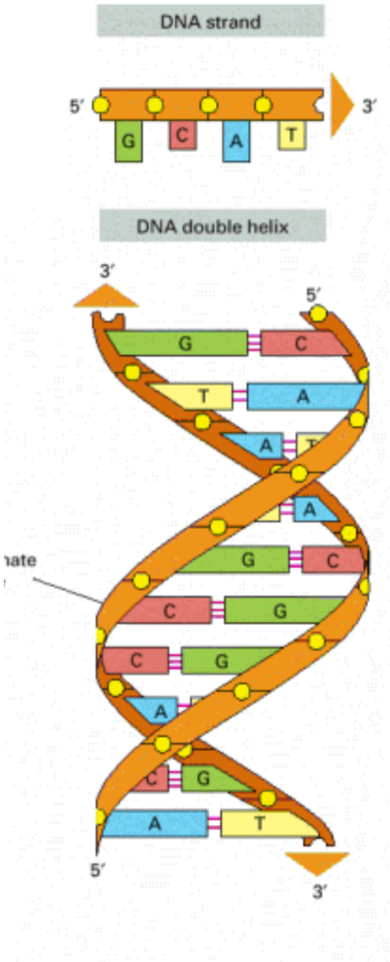


NEUROSCIENCE, Third Edition, **Figure 1.3 (Part 4)**

DNA encodes genetic information

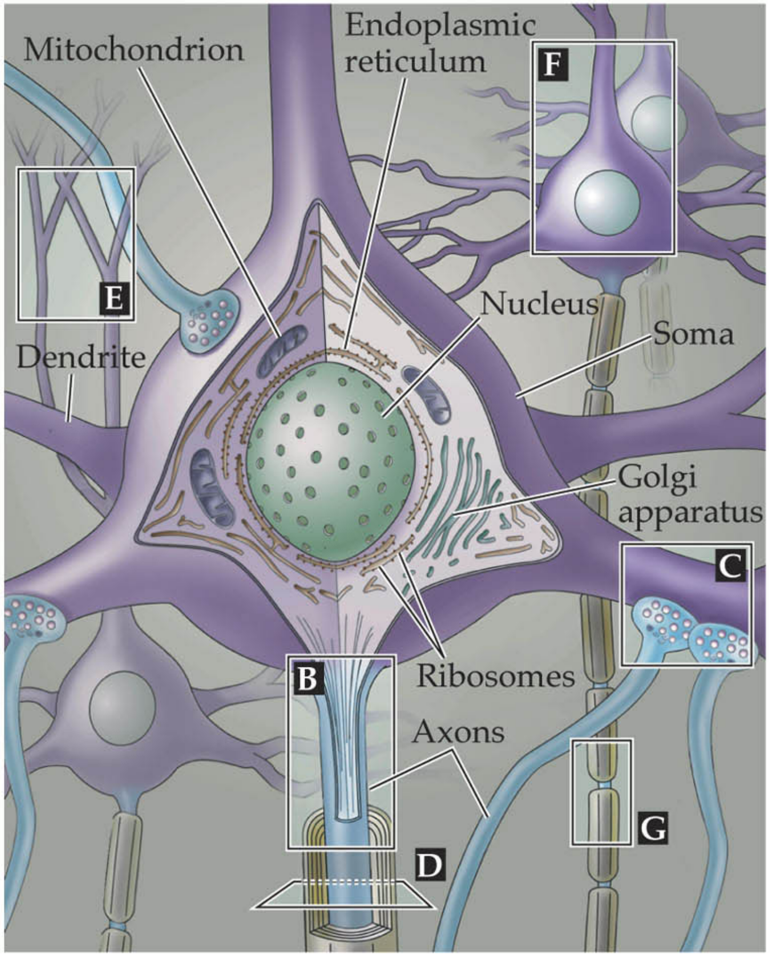


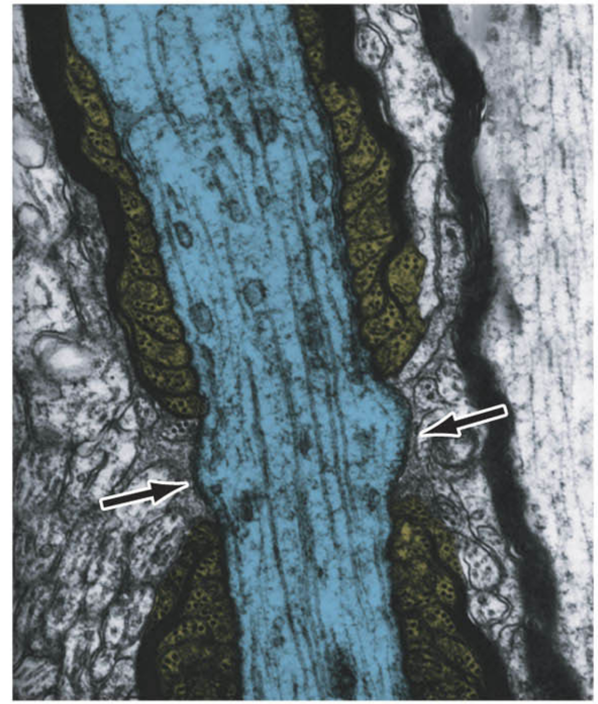




The same DNA is in every cell.
What makes cells different is which DNA is transcribed.

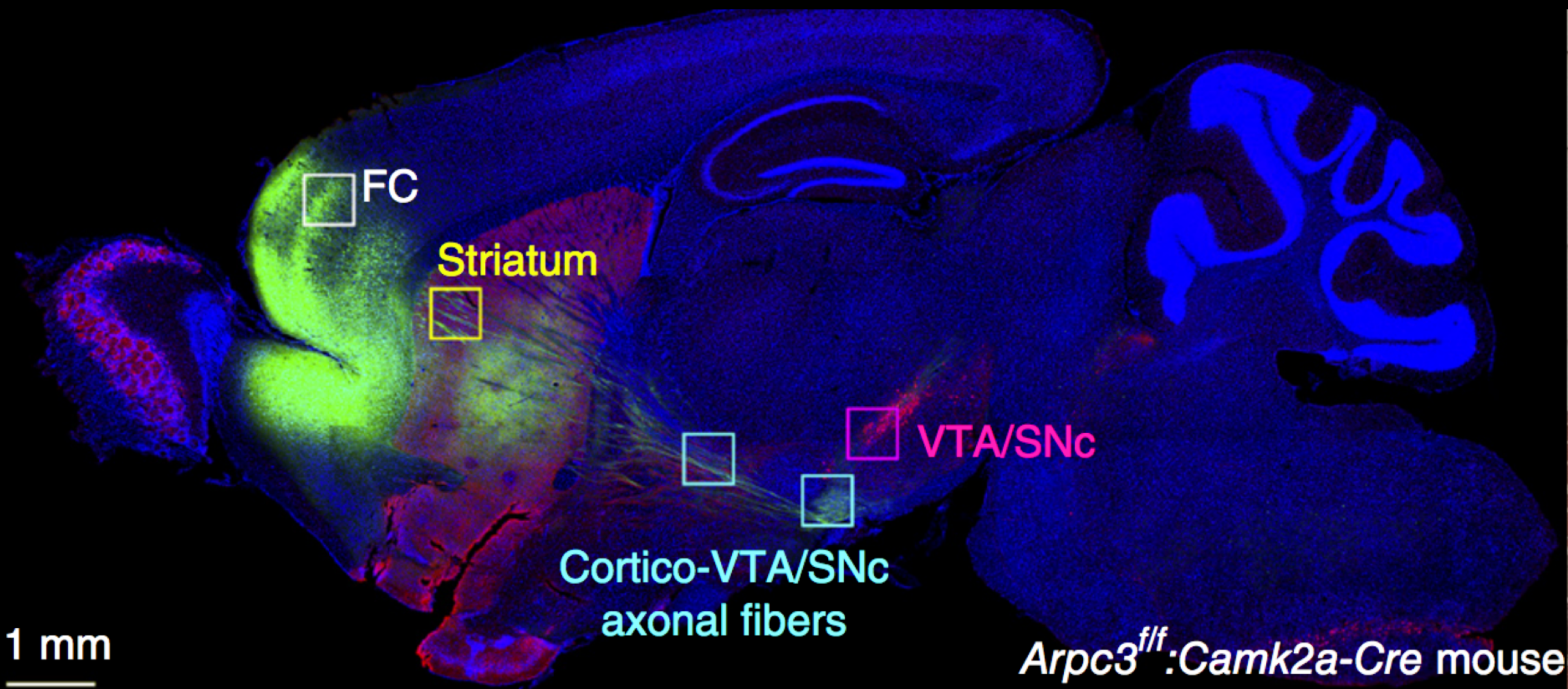
Neurons have a single axon that may be myelinated





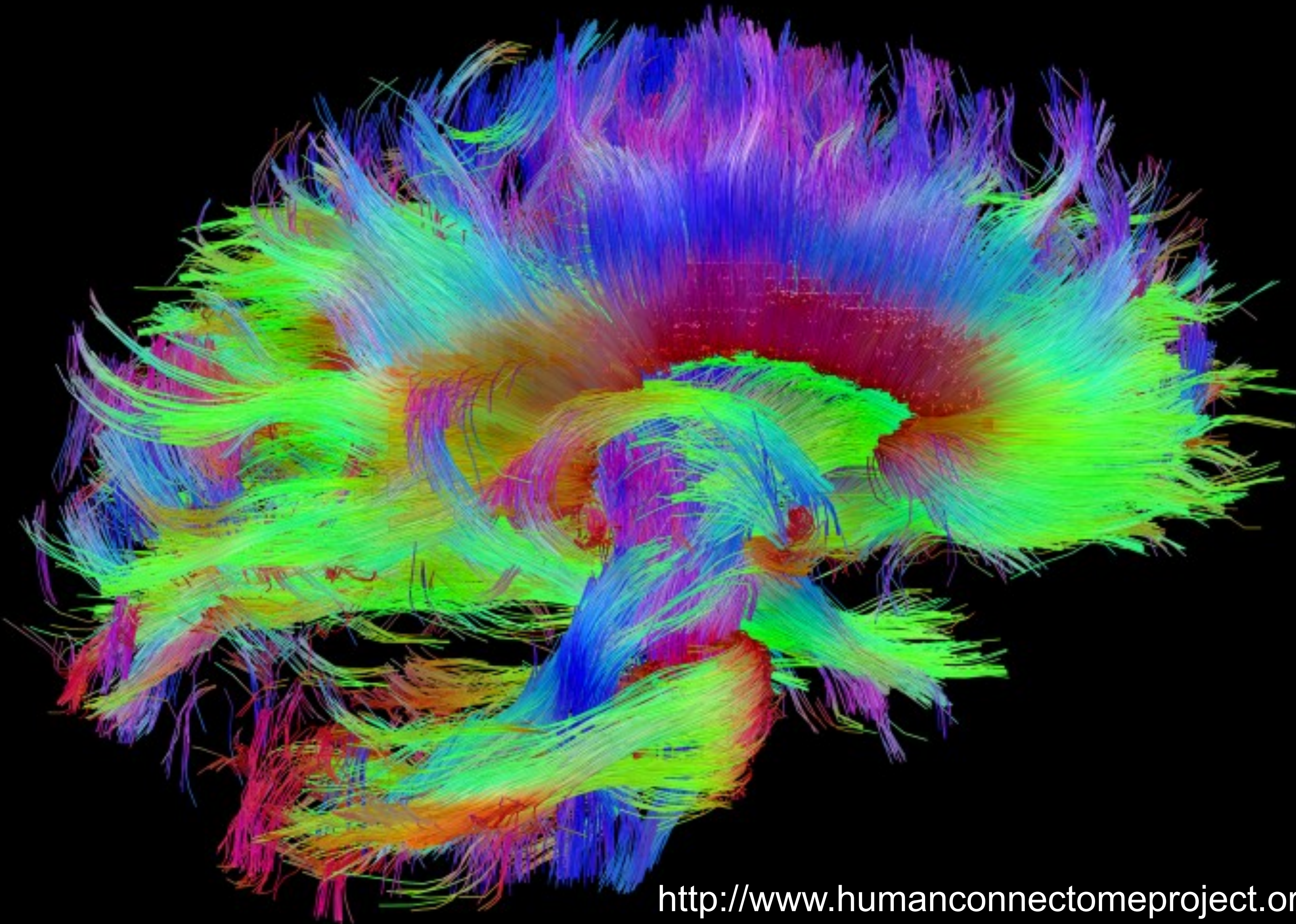
Action potential generated at the initial segment (hillock)
A single axon may ramify extensively to contact many target cells

Axons connect distant brain regions



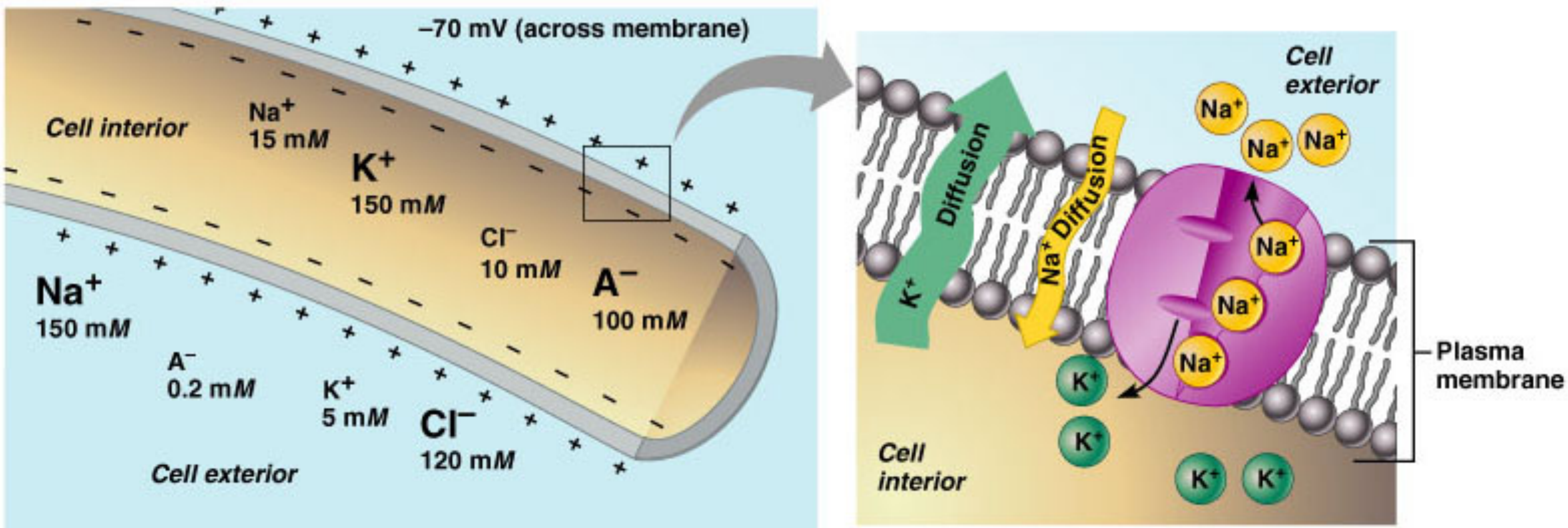
Kim, I et al (2015) *Nat Neurosci* 18: 883-91

A complex, yet specific, connectome



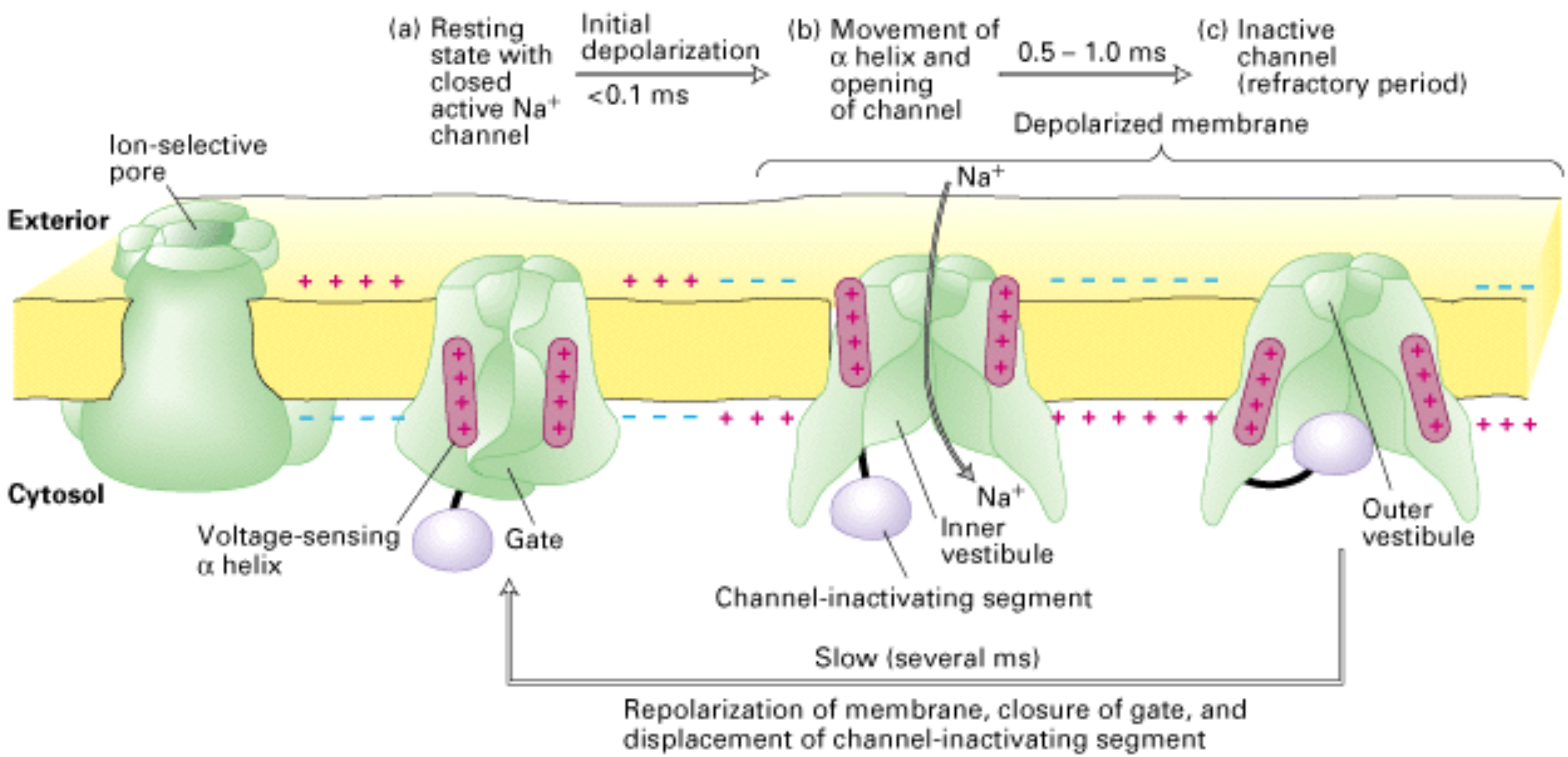
http://www.humanconnectomeproject.org

Neurons carry electrical charge through ionic disequilibrium

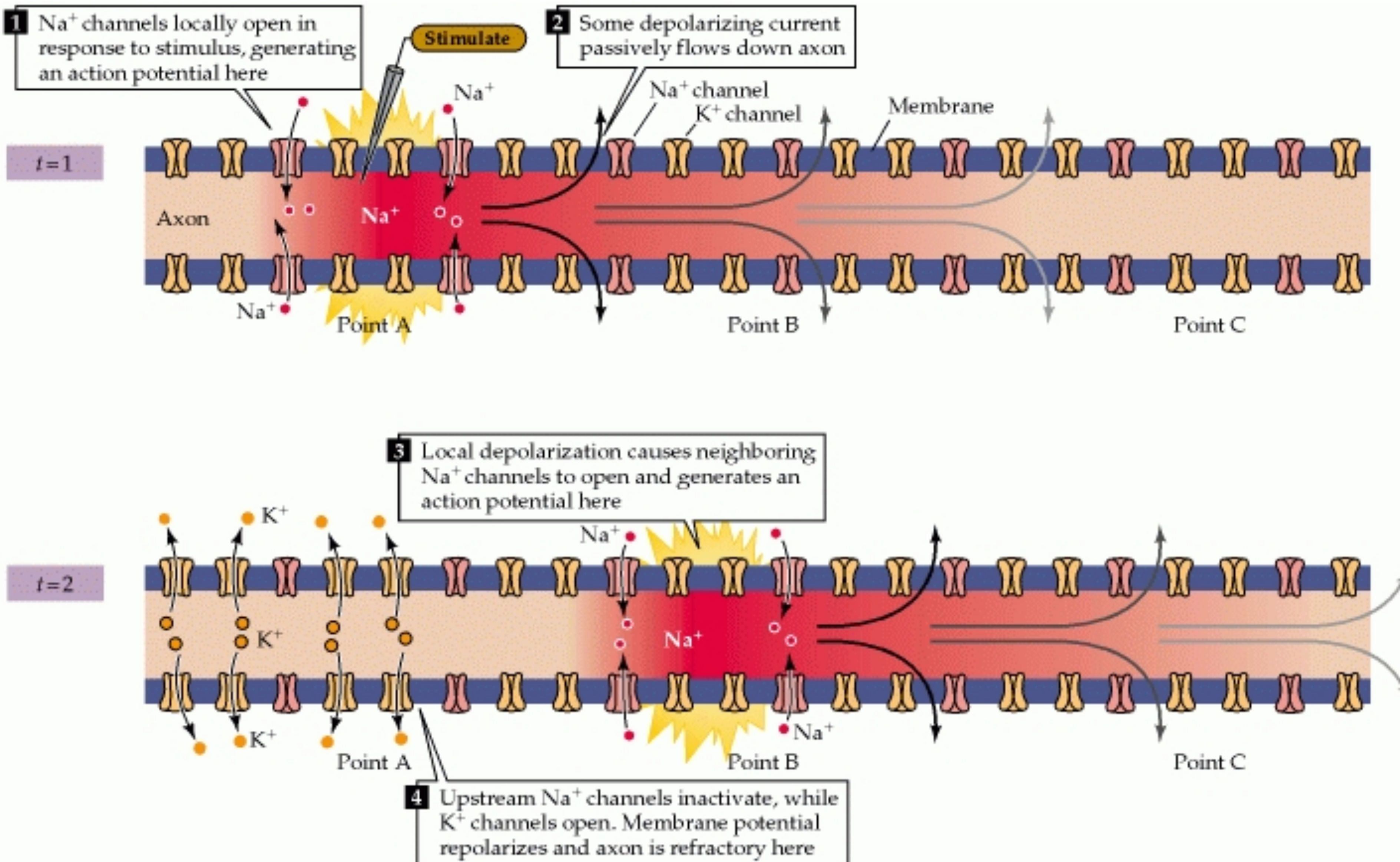


Ion <u>transporters</u> establish and maintain disequilibrium
Ion <u>channels</u> permit ionic equilibration across the membrane

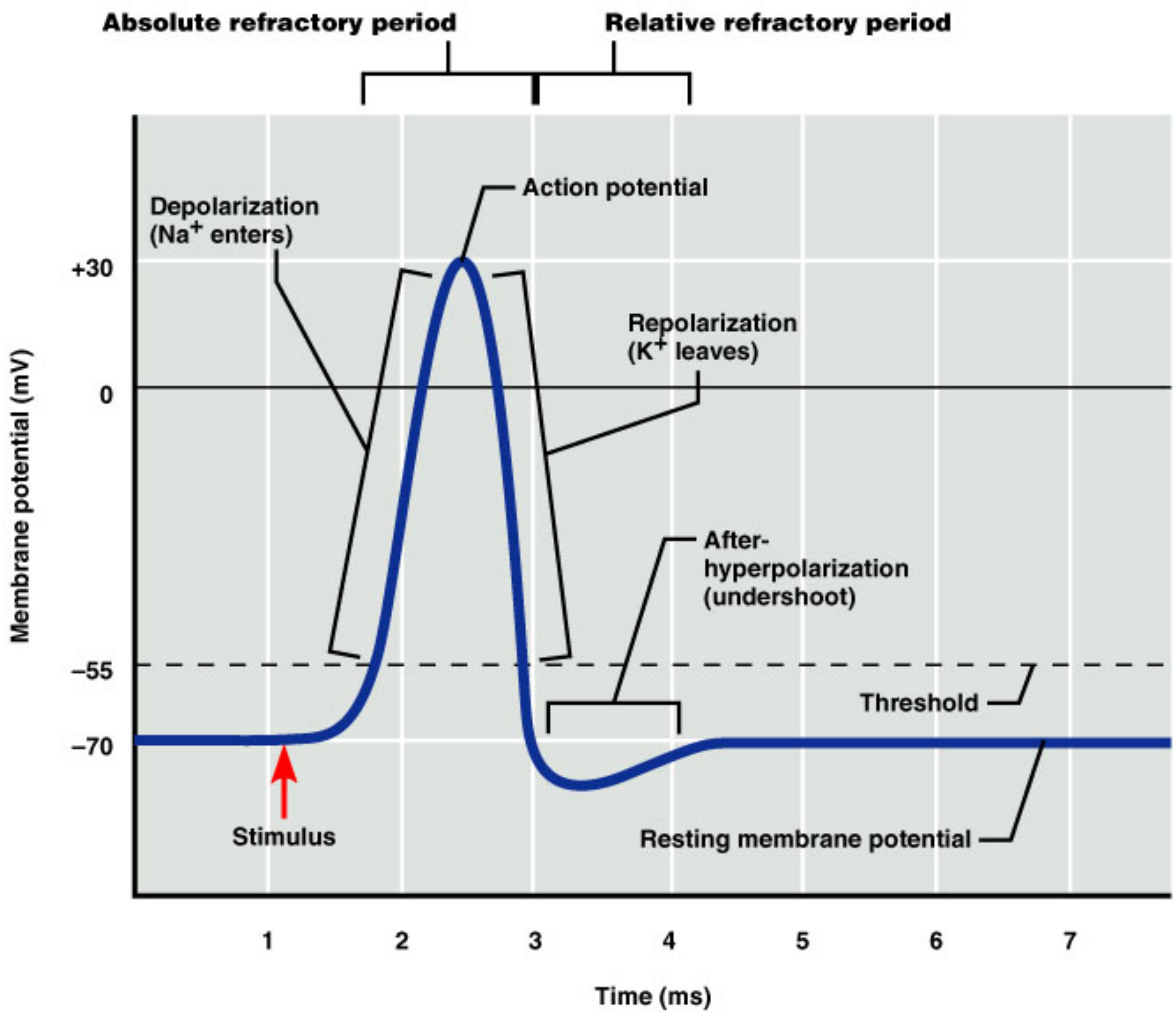
Voltage-gated ion channels are time keepers



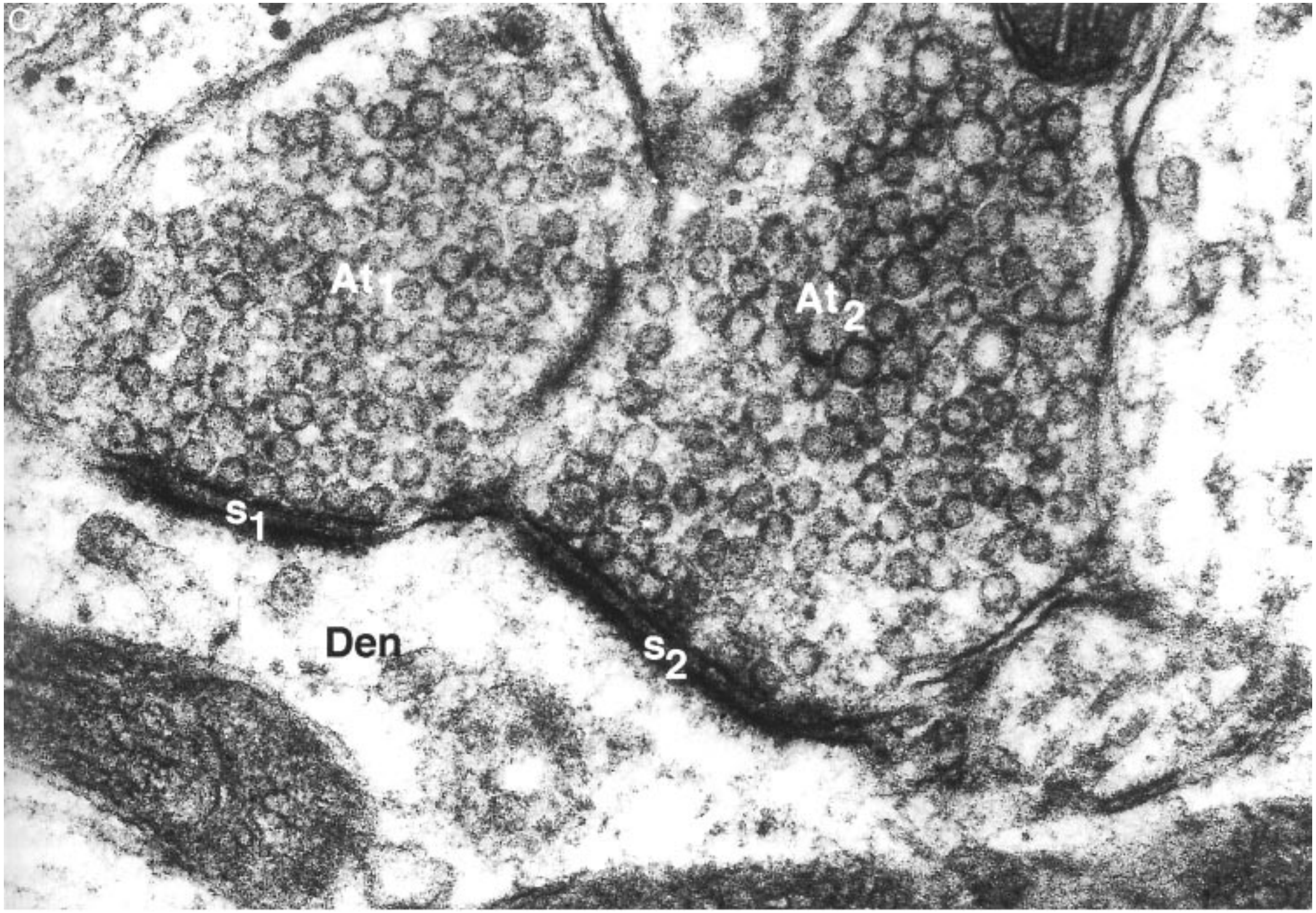
Voltage-sensitive ion channels confer temporal and spatial structure upon electrical signals in neurons



The action potential



Back to the Synapse



Summary #1

- Cajal was right – the brain is comprised of discrete cells called neurons
- Information travels directionally in neurons from dendrites to axons
- Information travels electrically within neurons and chemically between them
- The morphology and connectivity of neurons encodes information

Three main cell types in the nervous system

1. Neurons
2. Glia
3. Vasculature

Major Classes of Neurons

Cortex versus non-cortical neurons

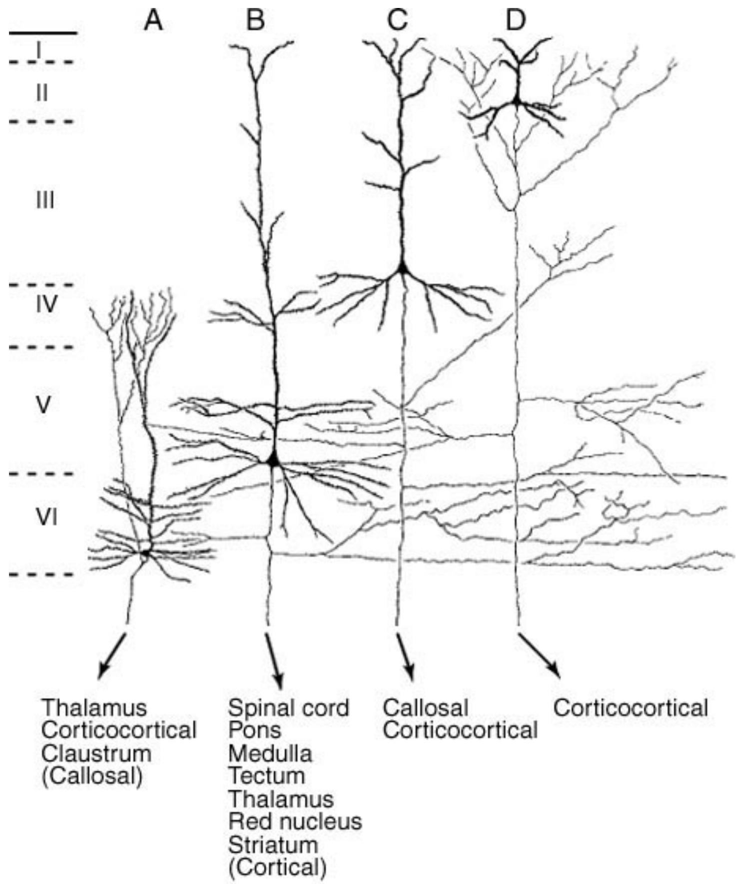
Axonal targets

- Projection neurons
- Local circuit interneurons

Neurotransmitter phenotype

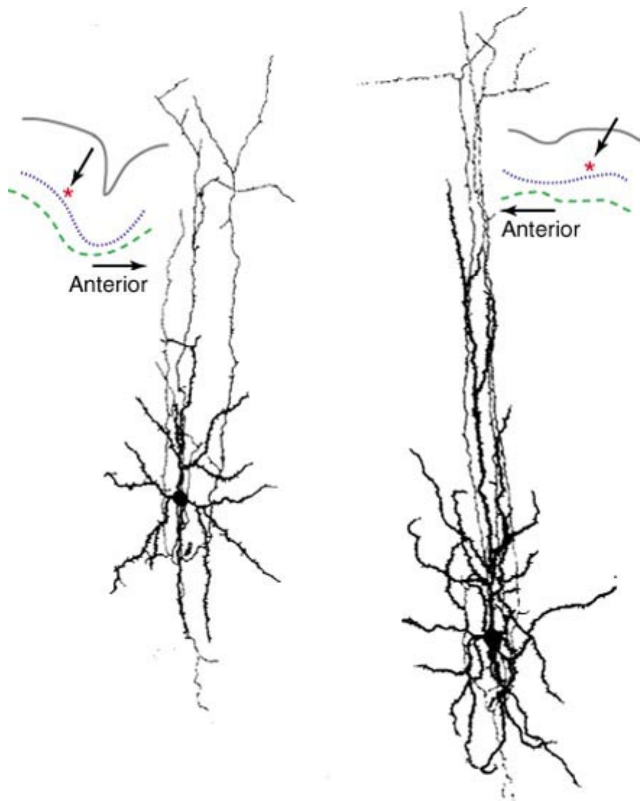
- Glutamate, GABA, Glycine, ACh, monoamines

Pyramidal neurons are the major excitatory projection neurons in the cerebral cortex



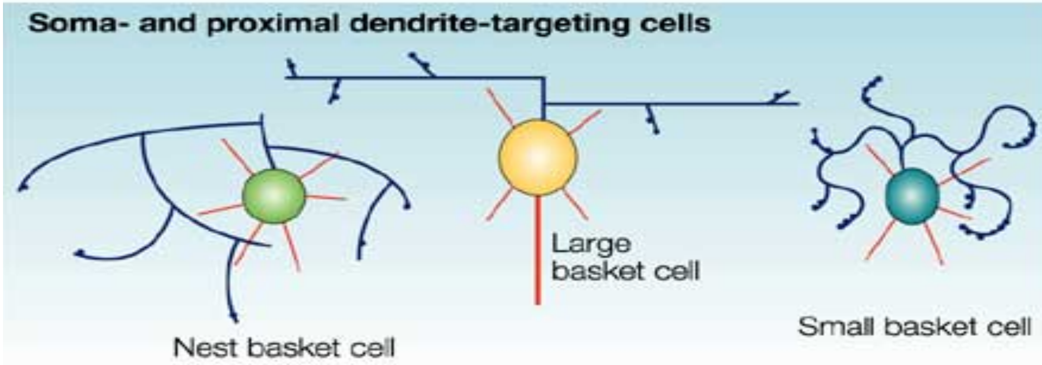
Pyramidal neurons with different morphology and terminal projection are localized to distinct cortical laminae

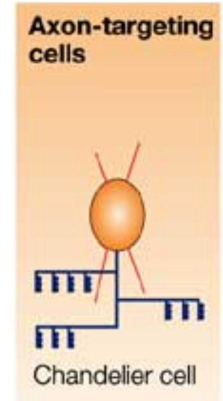
Spiny stellate cells are excitatory glutamatergic interneurons



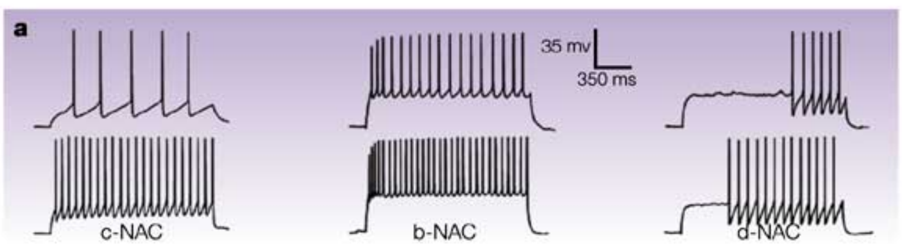
Locally ramifying axonal arbors
Most common in Layer IV (esp. visual cortex)
Serve as high fidelity translators of thalamic input

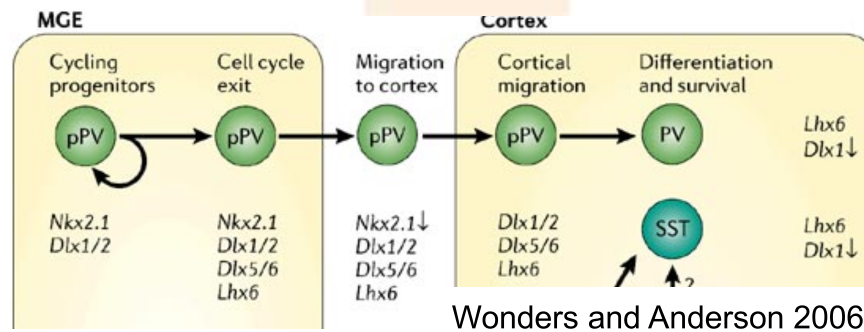
Inhibitory GABAergic cortical interneurons are diverse





Markram et al 2004





Wonders and Anderson 2006

1. Morphology (chandelier, double bouquet, basket)
2. Physiology (fast spiking, etc)
3. Neurochemical phenotype (Parvalbumin, Somatostatin, CCK, etc.)
4. Connectivity (region of cell innervated)
5. Location (cortical layer)
6. Genetic origin (Transcription factor expression)

Some of the Noncortical Neurons

1. Medium spiny neuron - GABAergic projection neurons of the striatum
   Huntington's
2. Purkinje cells - GABAergic projection neurons of the cerebellum
   Ataxia
3. Dopaminergic neurons - Substantia nigra and ventral tegmental area of brainstem
   Parkinson's
4. Spinal motor neurons - Acetyl choline
   ALS
5. Specialized sensory receptor - Photoreceptors and Cochlear hair cells
   Blindness/Deafness
6. Enteric motor neurons
   Hirschprung's Disease

The neuroglia are more than mere “nerve glue”

Morphology, gene expression, and function define the classes of glia

CNS:
Oligodendrocytes
Astrocytes
Microglia

PNS:
Schwann cells

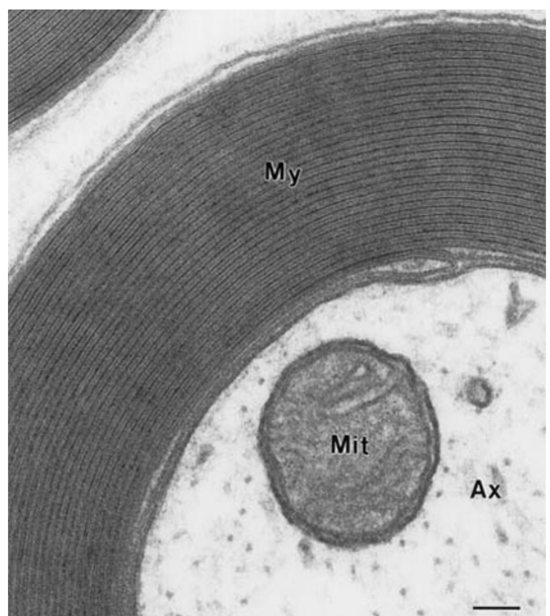
Oligodendrocytes and Schwann cells synthesize myelin

How do you make an action potential conduct faster?

1. Increase axon diameter (invertebrates)
2. Insulate the axon with myelin (vertebrates)

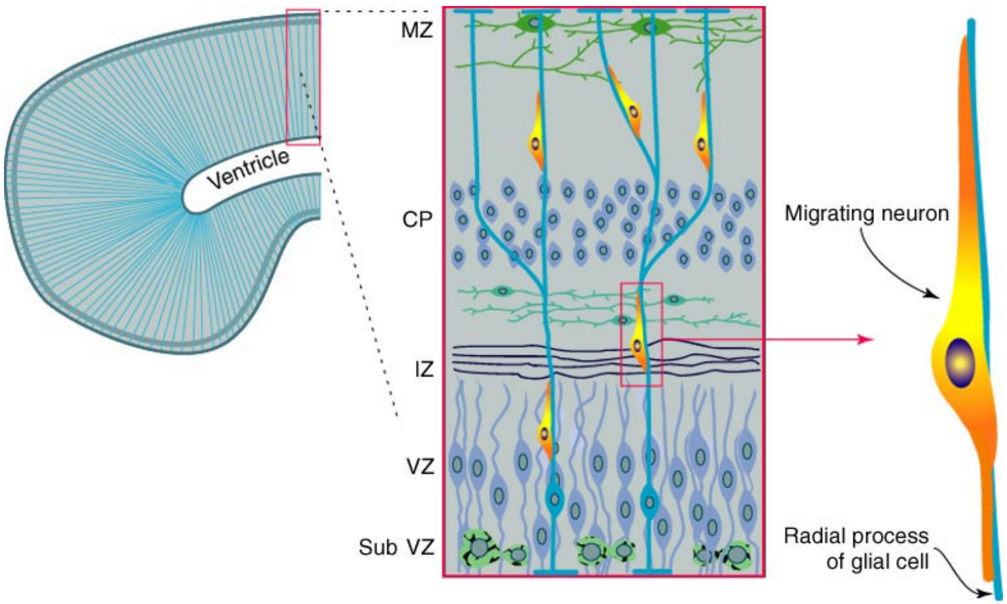
The tight wrapping of myelin requires adhesion between the membrane layers

PNS:
Protein Zero (P0)
IgG domain family



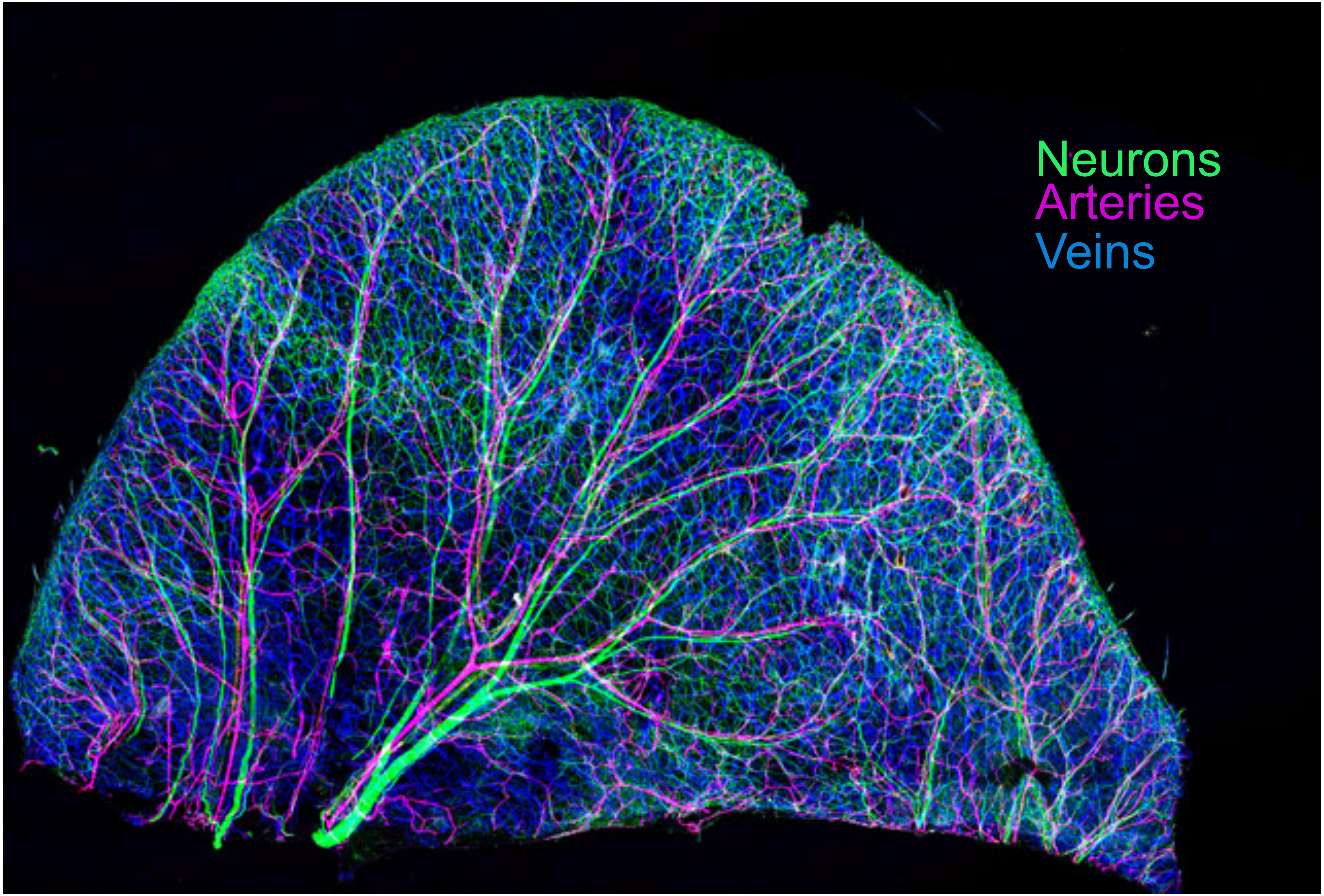
CNS:
PLP and DM-20
4TM domains like
connexins of gap junctions

Astrocytes play key roles in CNS development, synaptogenesis and homeostasis



Newborn neurons migrate along radial glia (cortex)
And Bergmann glia (cerebellum) to reach their final positions

Blood vessels and nerves travel together



Tomoko Yamazaki, NIH Nikon small world

Summary #2

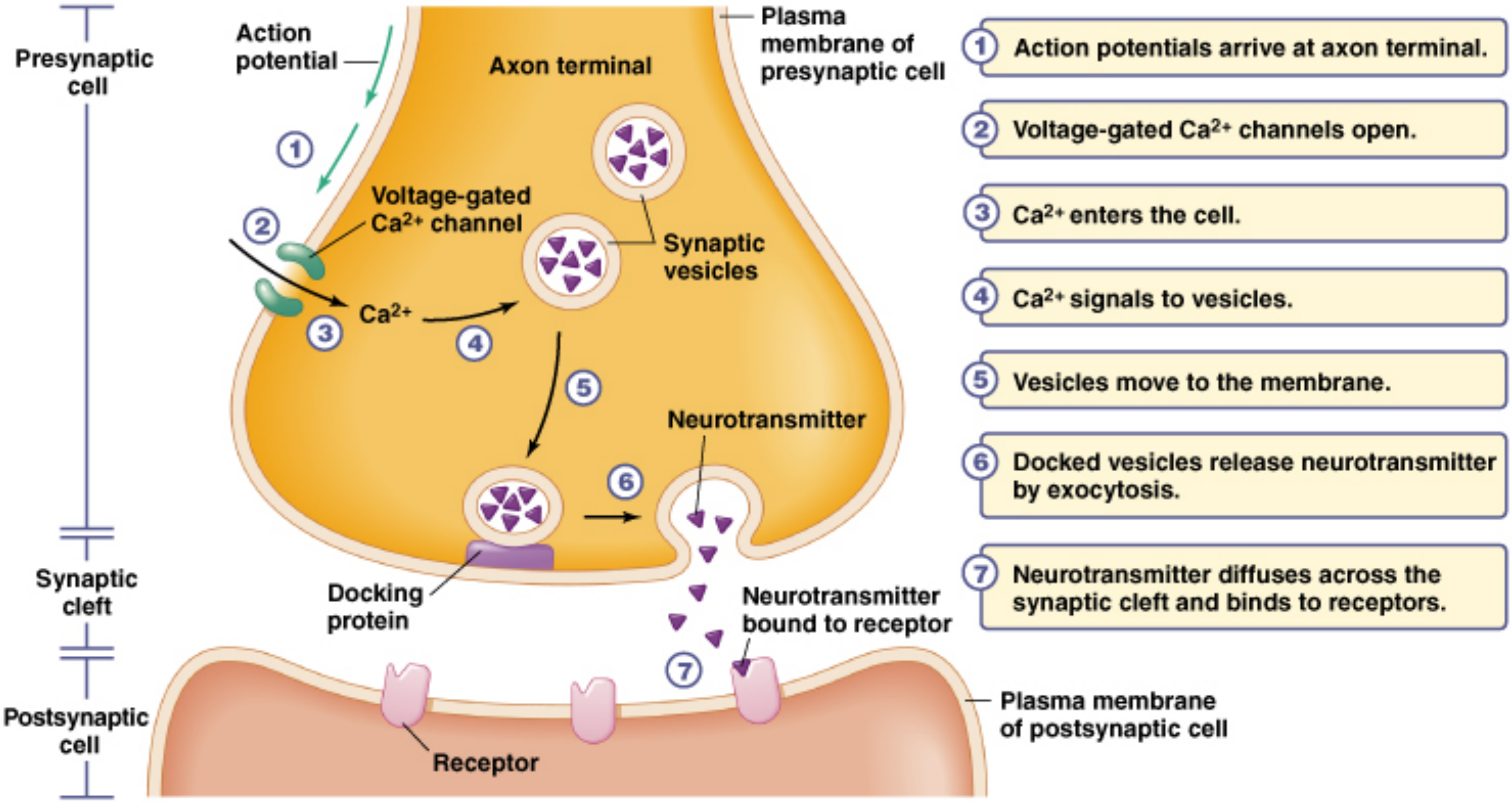
- There are many different types of neurons that perform different functions
- The brain contains additional cell types that critically influence neurons

Vignette #2, Boston 1994
A Brief Case Full of Clues

Mr. Lester Parker
65 yo Male
referred for “muscle weakness”

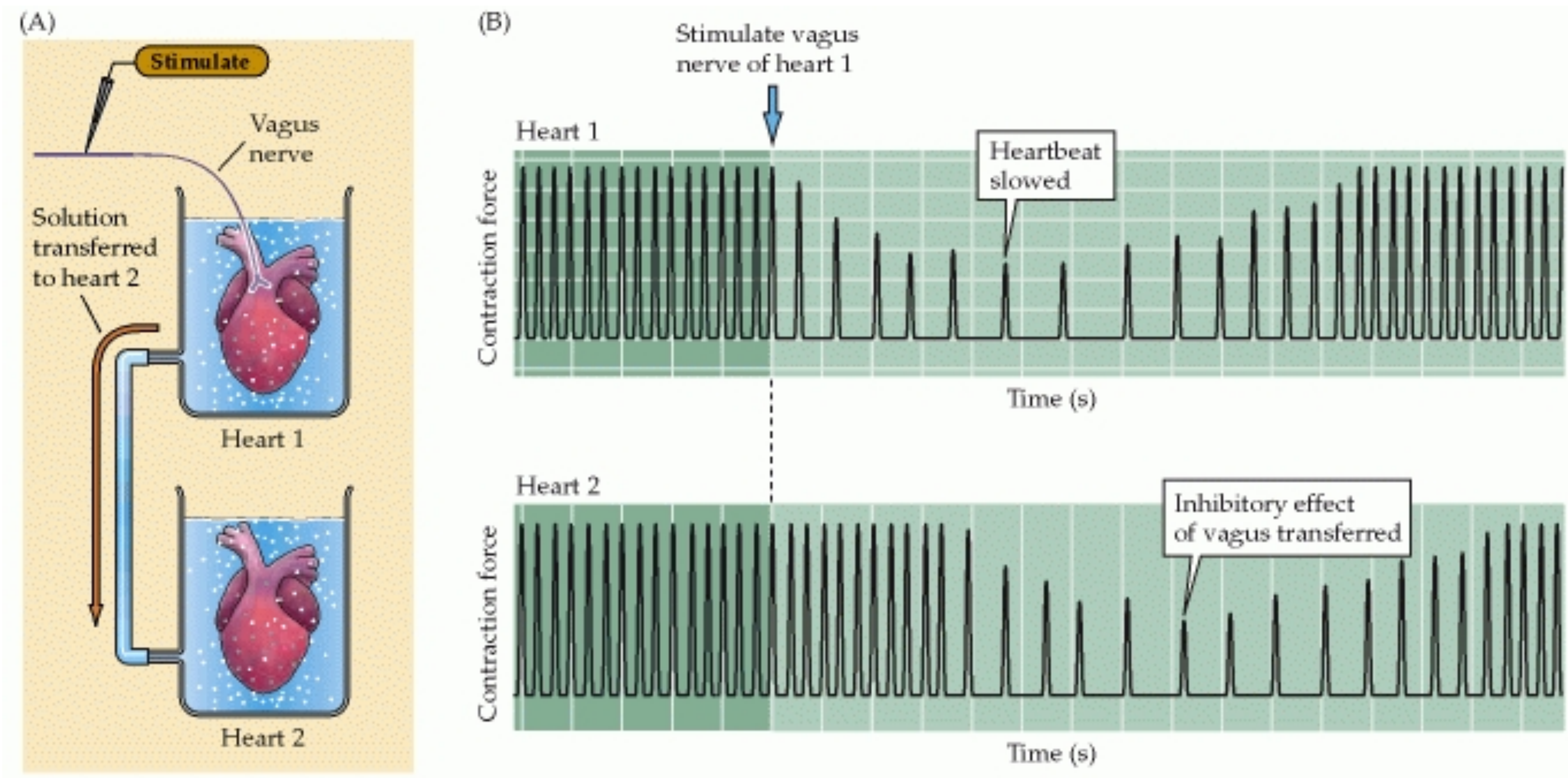
Reports reduction in physical activity
Physical exam c/w low muscle strength
No abnormalities on CAT scan of the brain

The Synapse

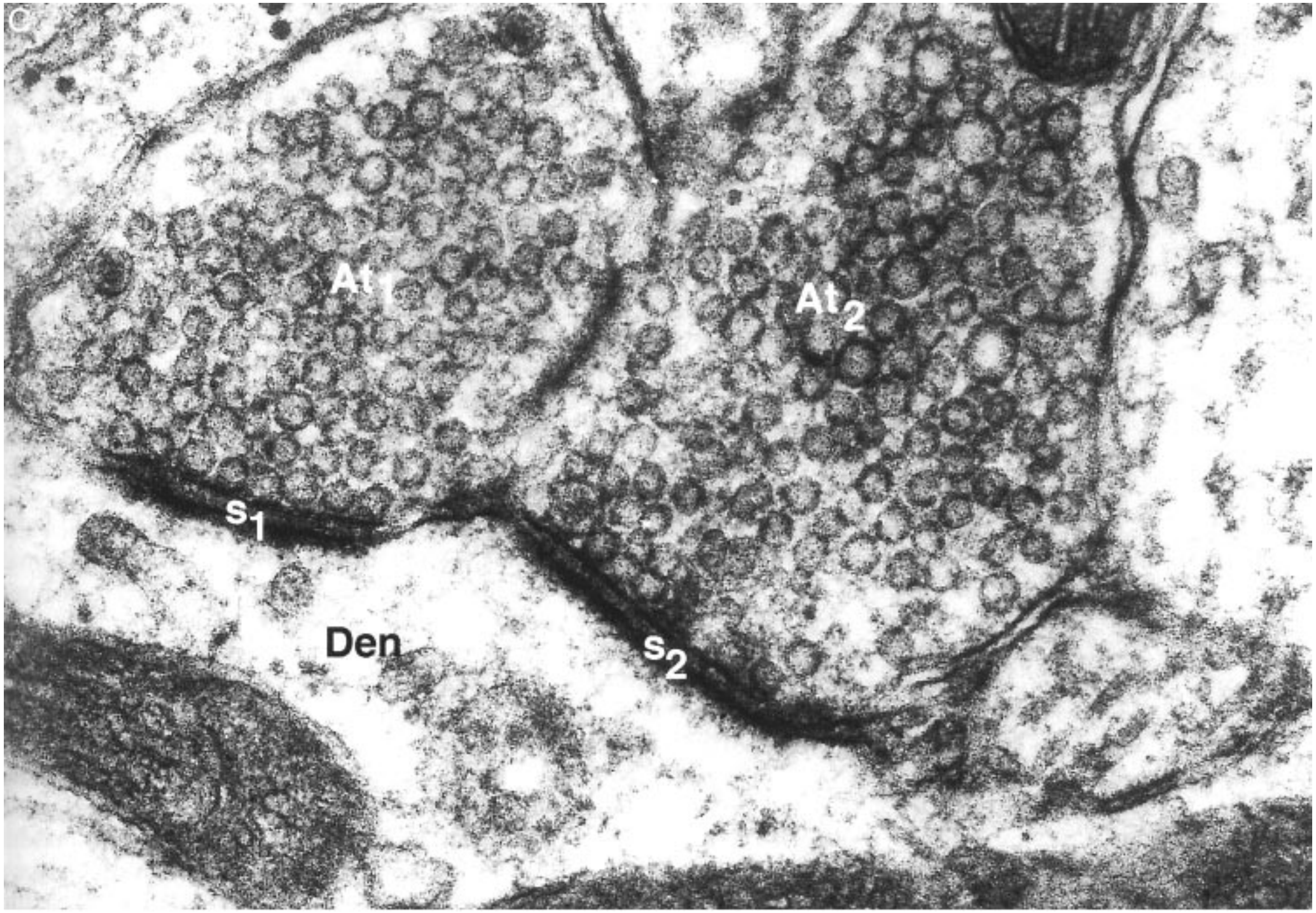


Presynaptic cell
Synaptic cleft
Postsynaptic cell
Action potential
Axon terminal
Plasma membrane of presynaptic cell
1
2
3
4
5
6
7
Voltage-gated Ca^{2+} channel
Ca^{2+}
Synaptic vesicles
Neurotransmitter
Docking protein
Neurotransmitter bound to receptor
Receptor
Plasma membrane of postsynaptic cell
1 Action potentials arrive at axon terminal.
2 Voltage-gated Ca^{2+} channels open.
3 Ca^{2+} enters the cell.
4 Ca^{2+} signals to vesicles.
5 Vesicles move to the membrane.
6 Docked vesicles release neurotransmitter by exocytosis.
7 Neurotransmitter diffuses across the synaptic cleft and binds to receptors.

Otto Loewi and the discovery of chemical neurotransmission

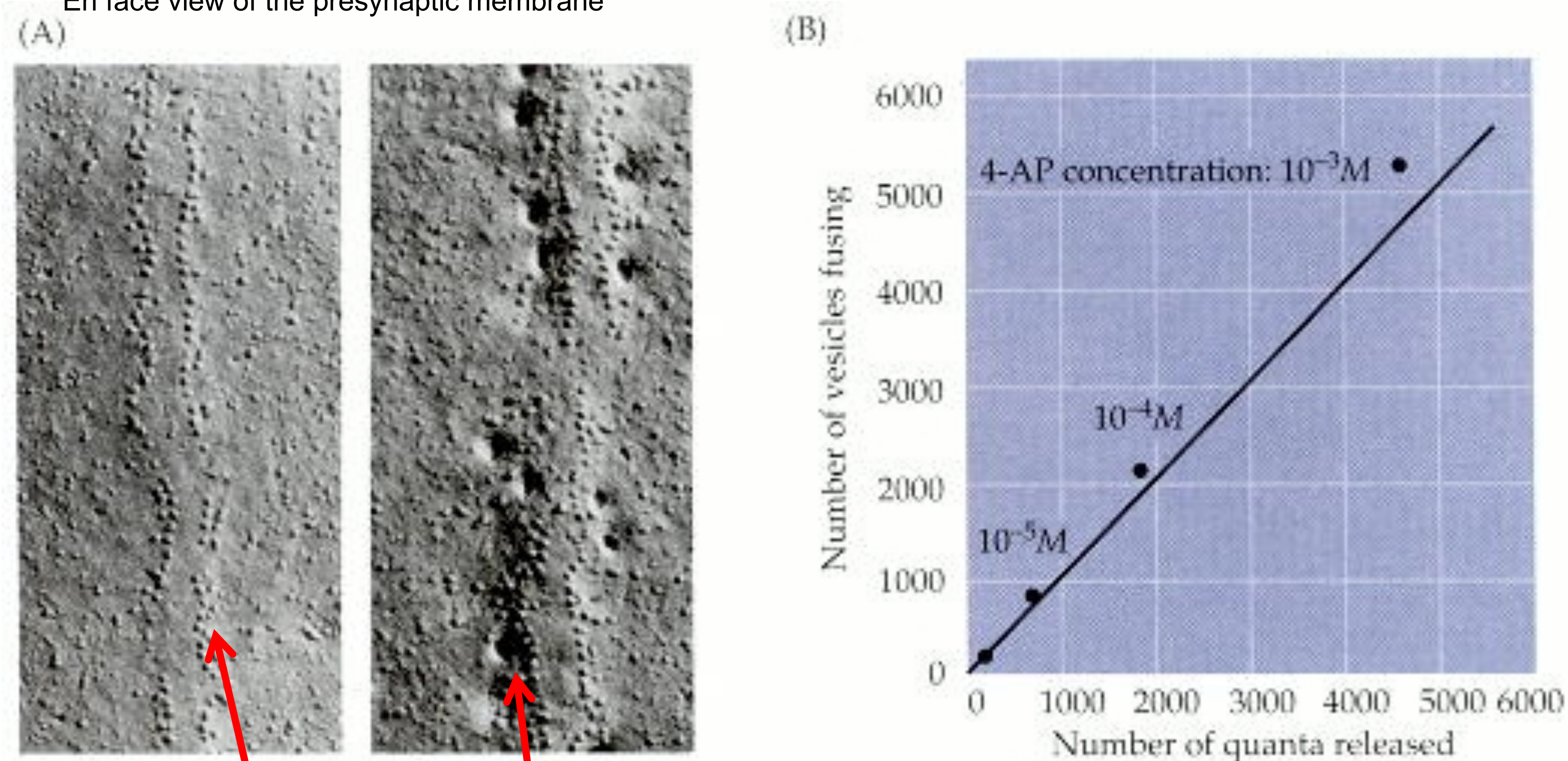


Electron microscopy shows synapses filled with vesicles



“Ja Kalzium, das is alles!” –Otto Loewi, 1959

Freeze fracture
En face view of the presynaptic membrane

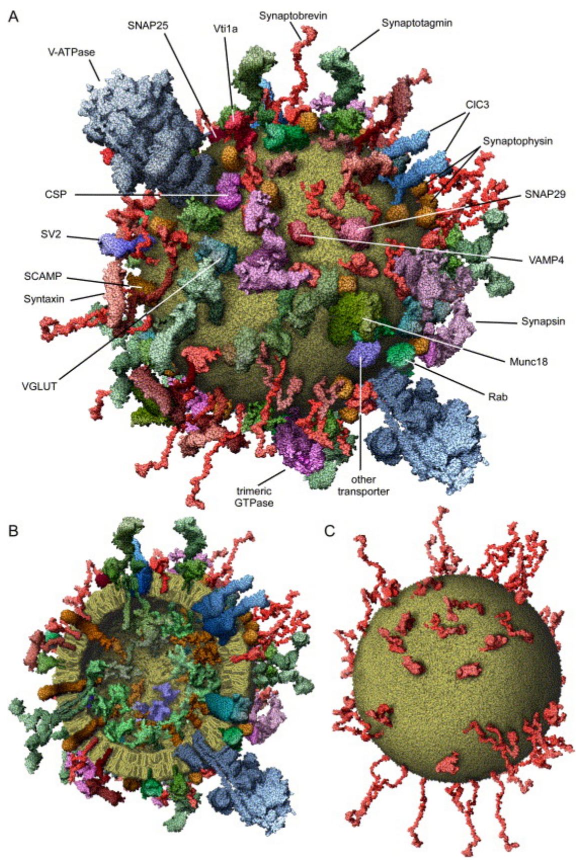


Calcium channels

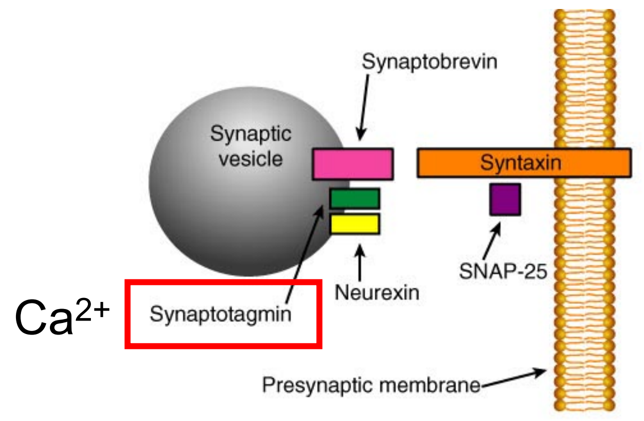
“Omega figures”
Vesicles in the act of fusion

4-AP increases calcium and
increases vesicle release

Synaptic vesicles are highly specialized for rapid exocyosis

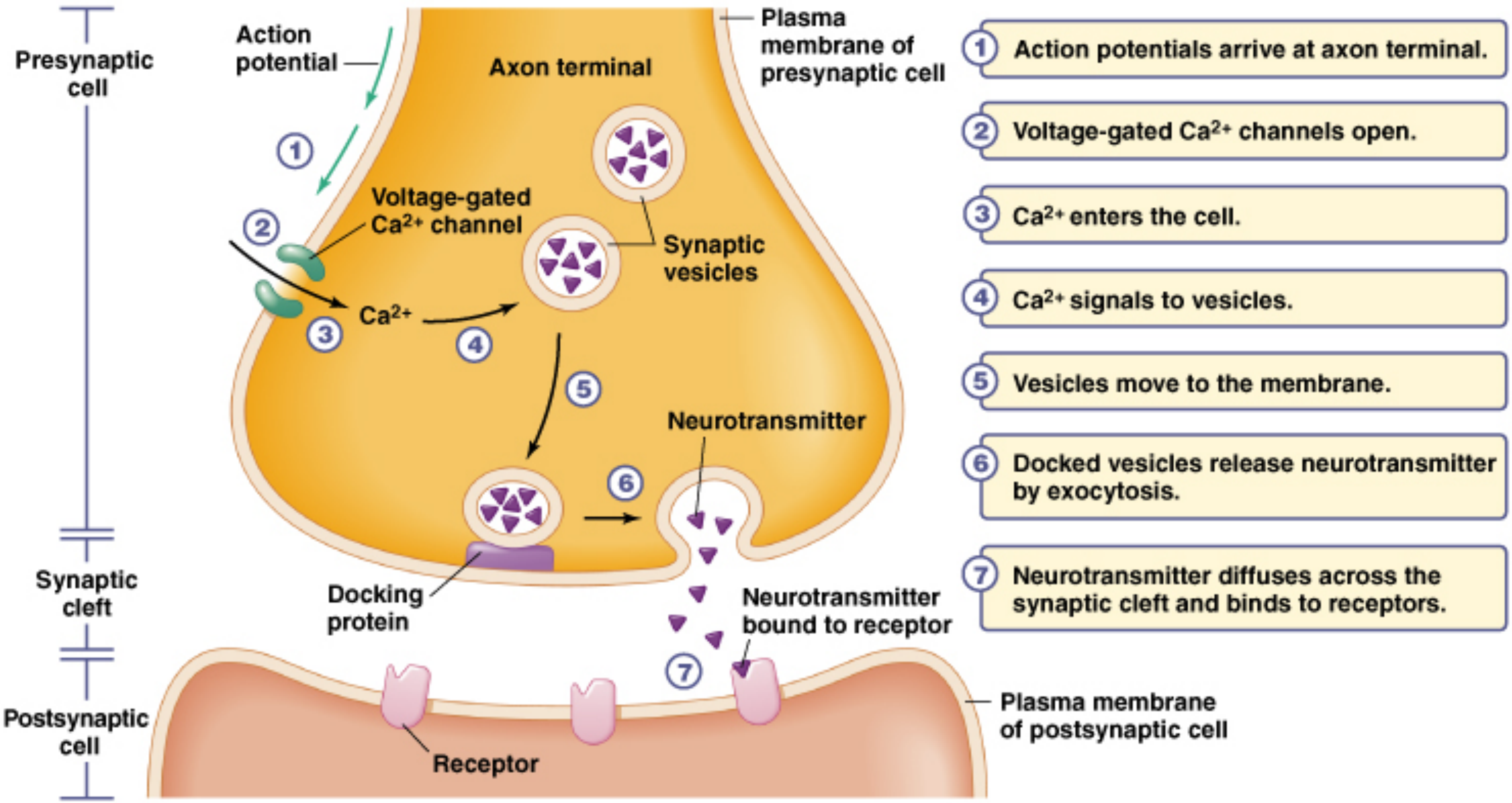


Takamori Cell 2006



SV release 100X faster than insulin secretion

The Synapse



Presynaptic cell
Synaptic cleft
Postsynaptic cell
Action potential
Axon terminal
Plasma membrane of presynaptic cell
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Voltage-gated Ca2+ channel
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Ca2+
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Docking protein
Neurotransmitter bound to receptor
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5 Vesicles move to the membrane.
6 Docked vesicles release neurotransmitter by exocytosis.
7 Neurotransmitter diffuses across the synaptic cleft and binds to receptors.

William James disses the “phosphorus-philosophers”

Psychology, *Briefer Course* (AKA “Jimmy”) by William James, 1892, Henry Holt and Co. NY, NY p132-133

Phosphorus and thought: Considering the large amount of popular nonsense which passes current on this subject I may be pardoned for a brief mention of it here. *‘Ohne phosphor, kein Gedanke’* was a noted war-cry of the materialists during the excitement on that subject which filled Germany in the ’60s.

The phosphorus-philosophers have often compared thought to a secretion. “The brain secretes thought as the kidneys secrete urine or the liver secretes bile,” are phrases one sometimes hears. The lame analogy need hardly be pointed out.

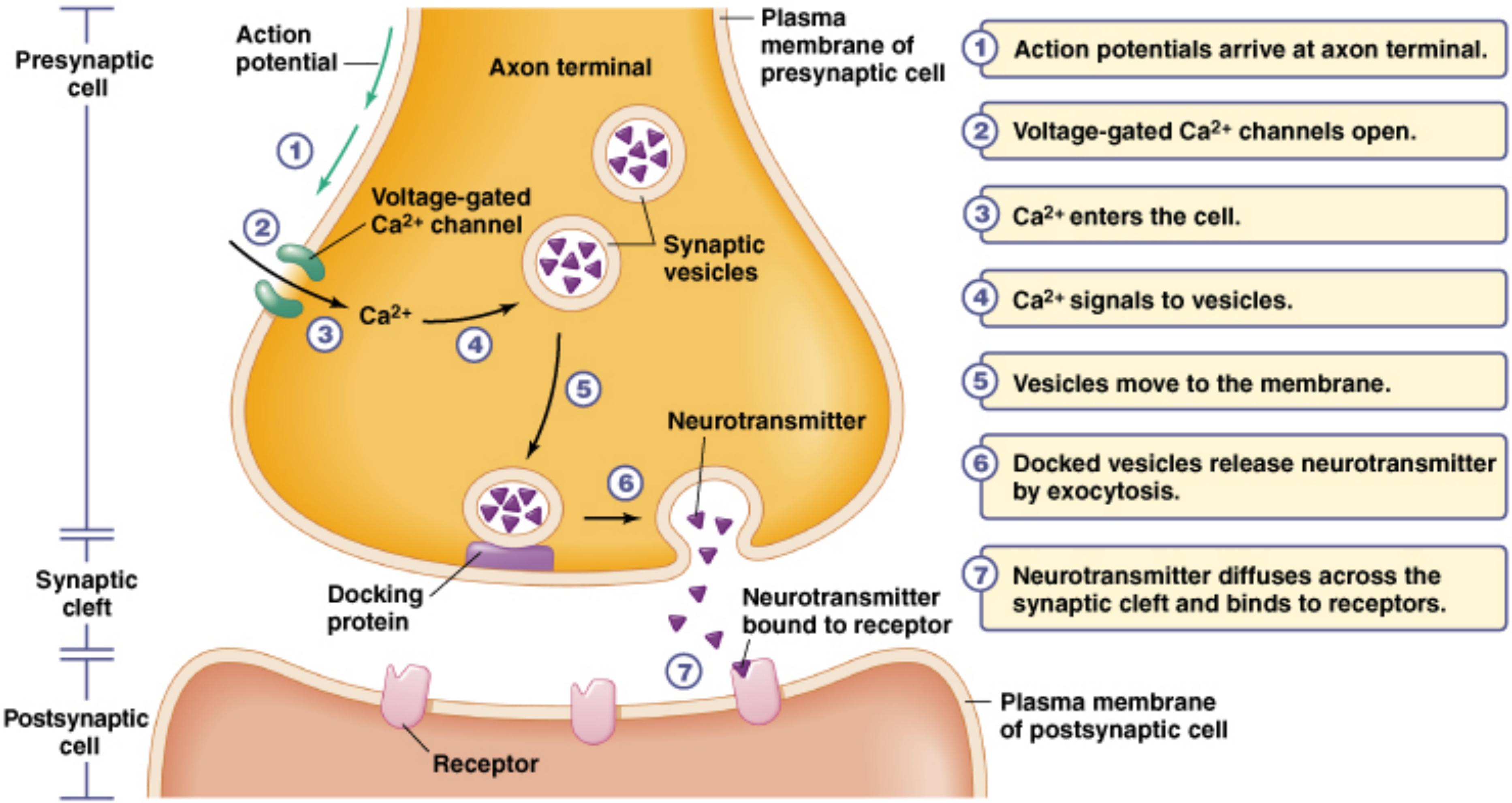
Though it turns out they were right after all…

Calcium Signaling by David A Clapham*, Cell* 2007 131:1047-*1058*

In the furnaces of the stars the elements evolved from hydrogen. When oxygen and neon captured successive α particles, the element calcium was born. Roughly 10 billion years later, cell membranes began to parse the world by charge, temporarily and locally defying relentless entropy. To adapt to changing environments, cells must signal, and signaling requires messengers whose concentration varies with time. Filling this role, calcium ions (Ca2+) and phosphate ions have come to rule cell signaling.

Protein function is governed by shape and charge. Ca2+ binding triggers changes in protein shape and charge. Similarly, phosphorylation imparts a negative charge, altering protein conformations and their interactions. The abilities of Ca2+ and phosphate ions to alter local electrostatic fields and protein conformations are the two universal tools of signal transduction.

The Synapse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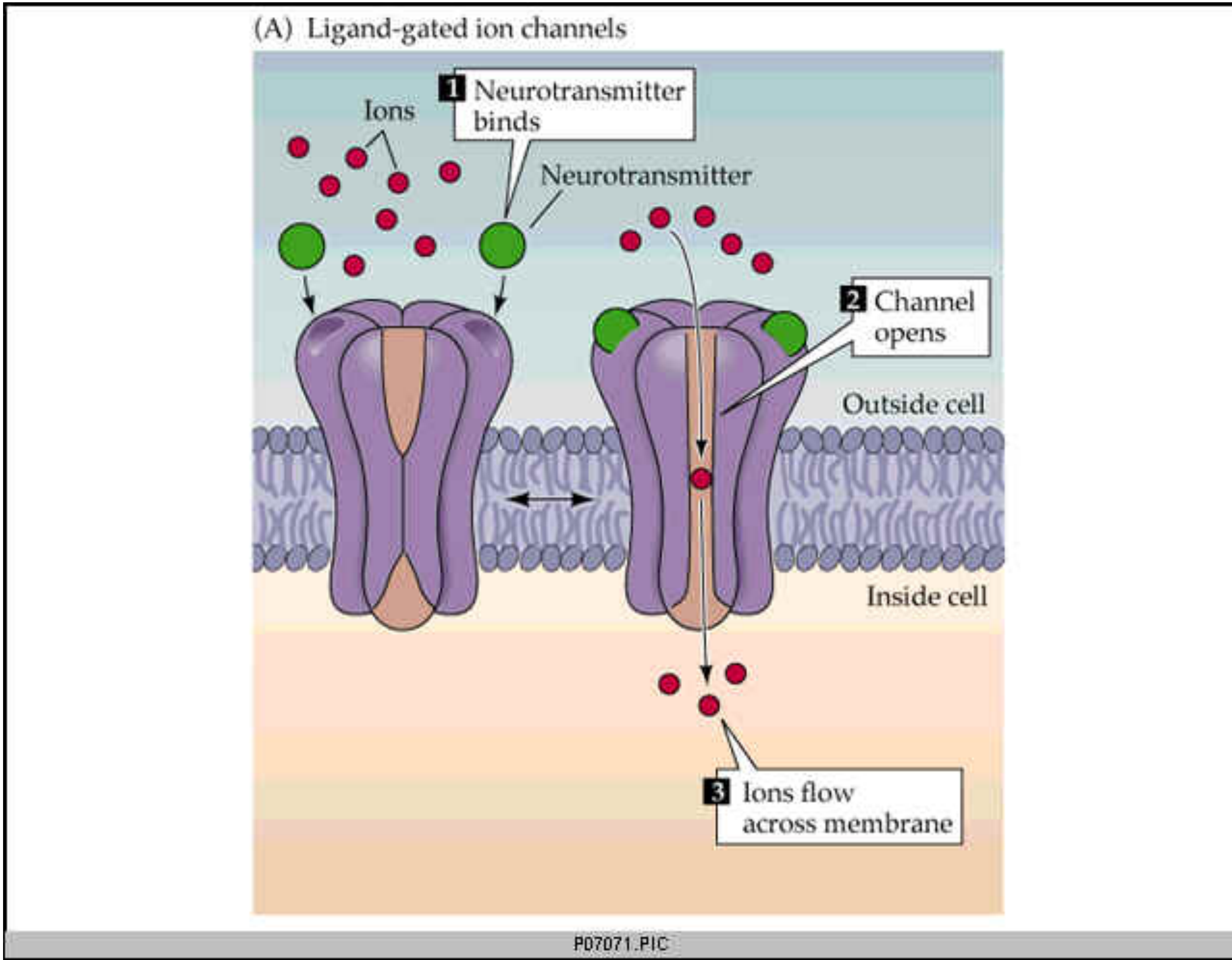
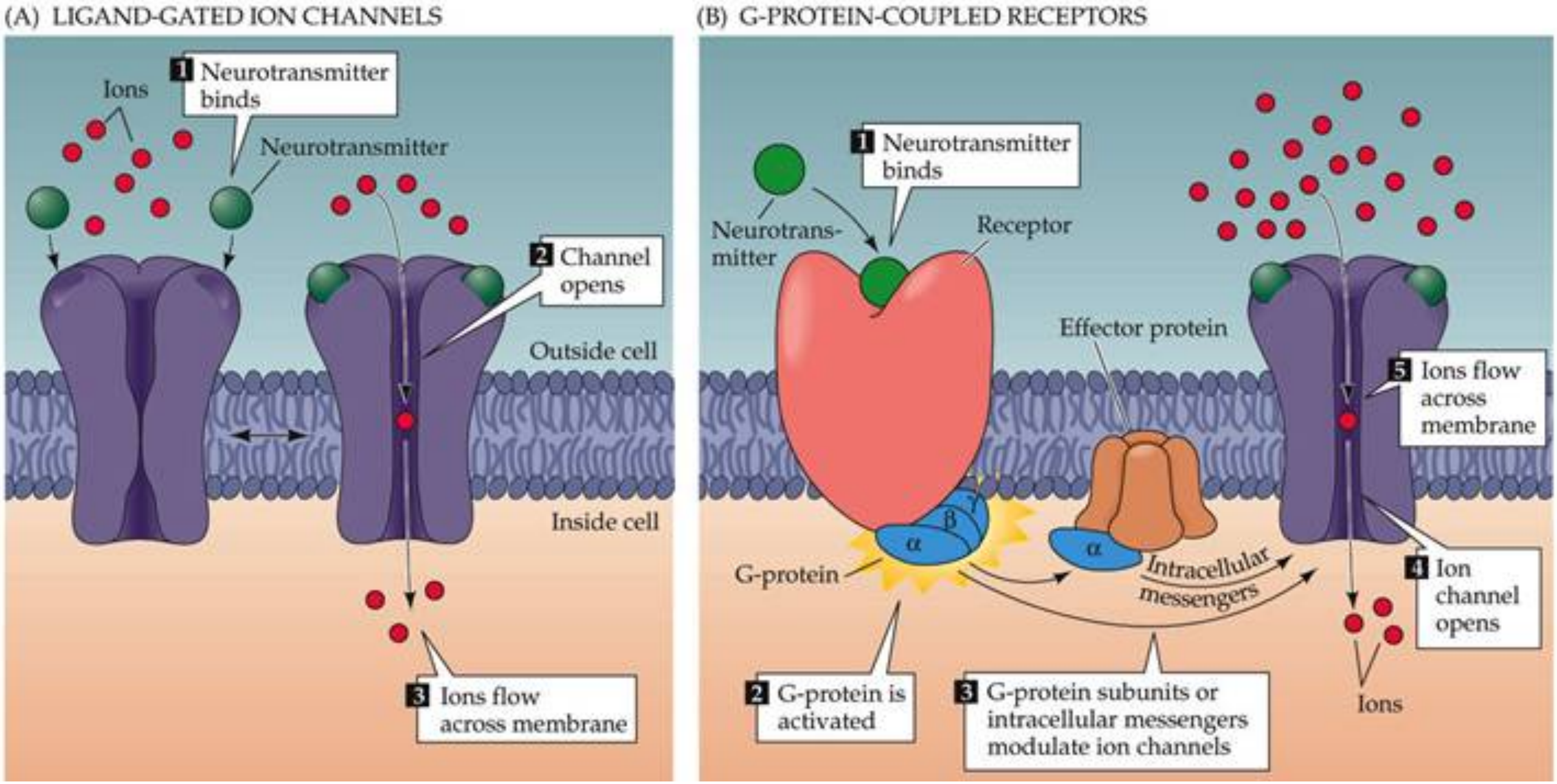
Chemical Neurotransmission



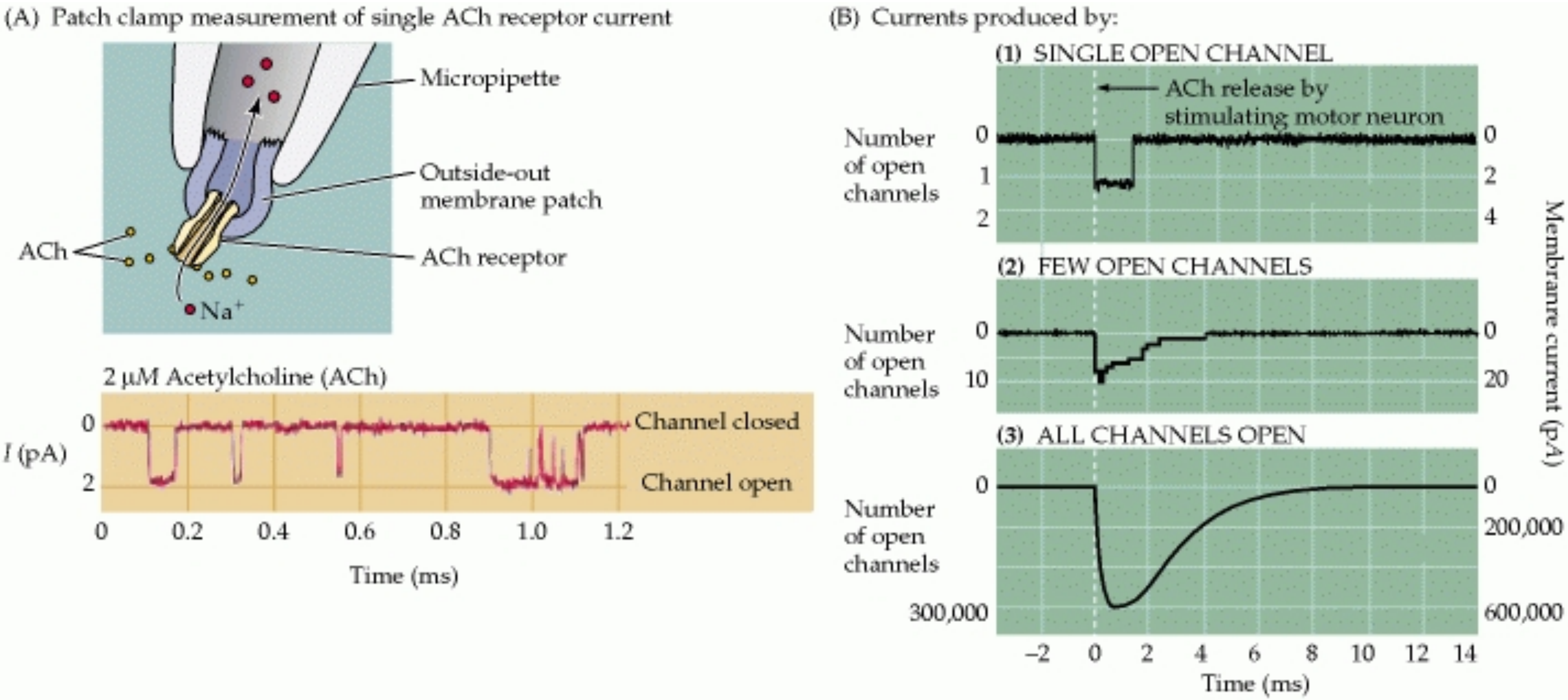
The nature of the postsynaptic response depends on the neurotransmitter receptor



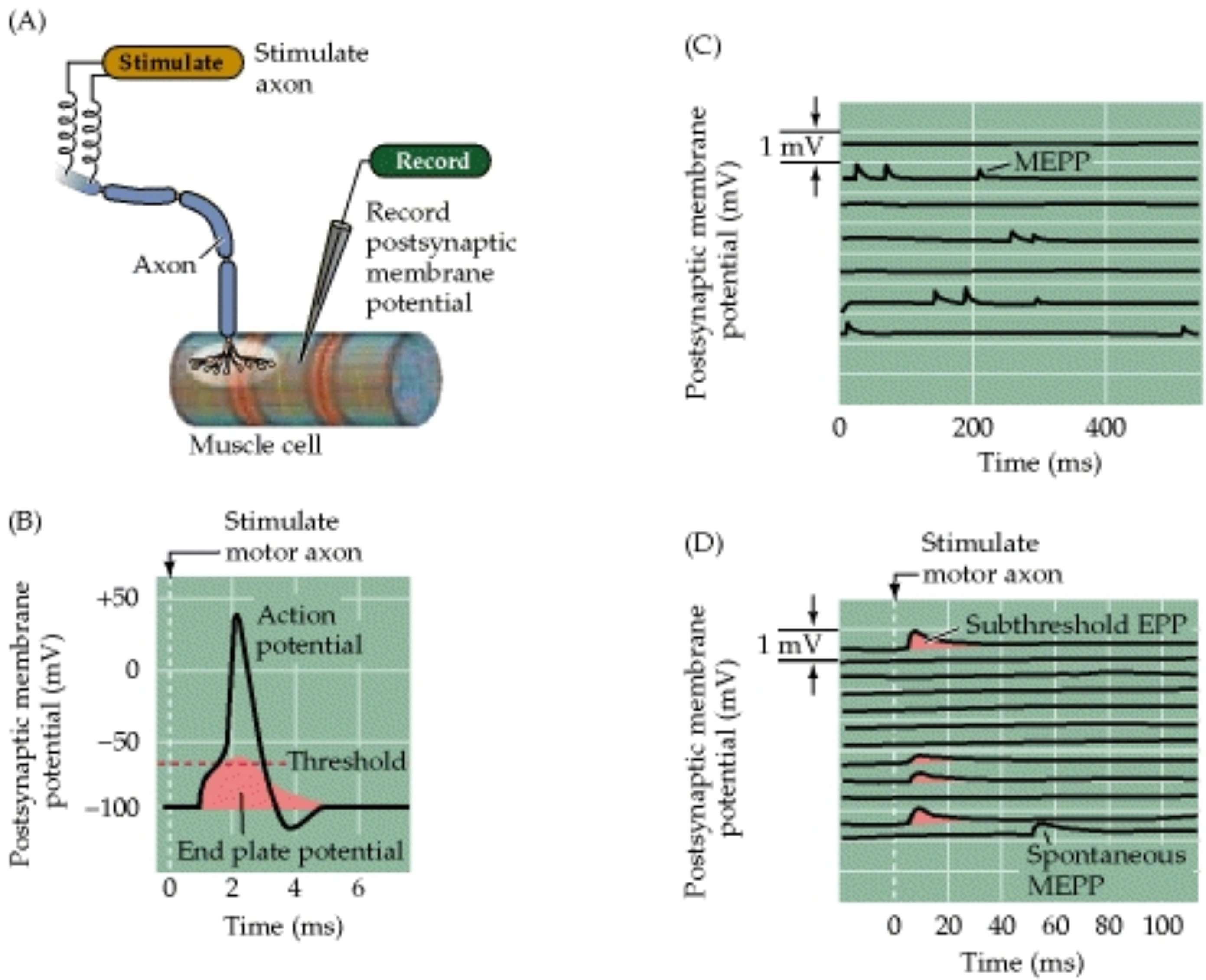
Neurotransmitter

Neuromodulator

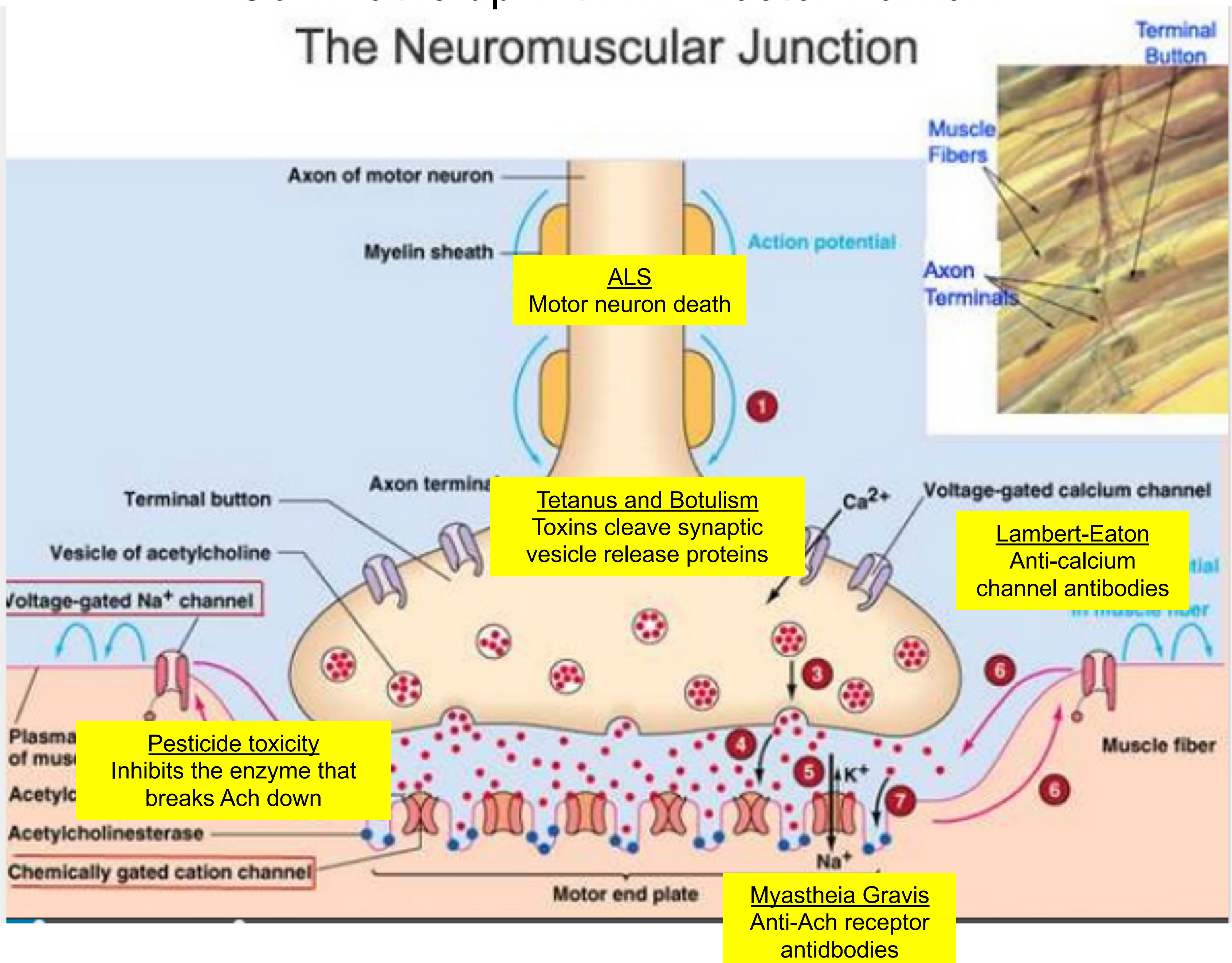
ACh opens ion channels generating electrical signals



Synaptic potential are integrated to trigger action potentials



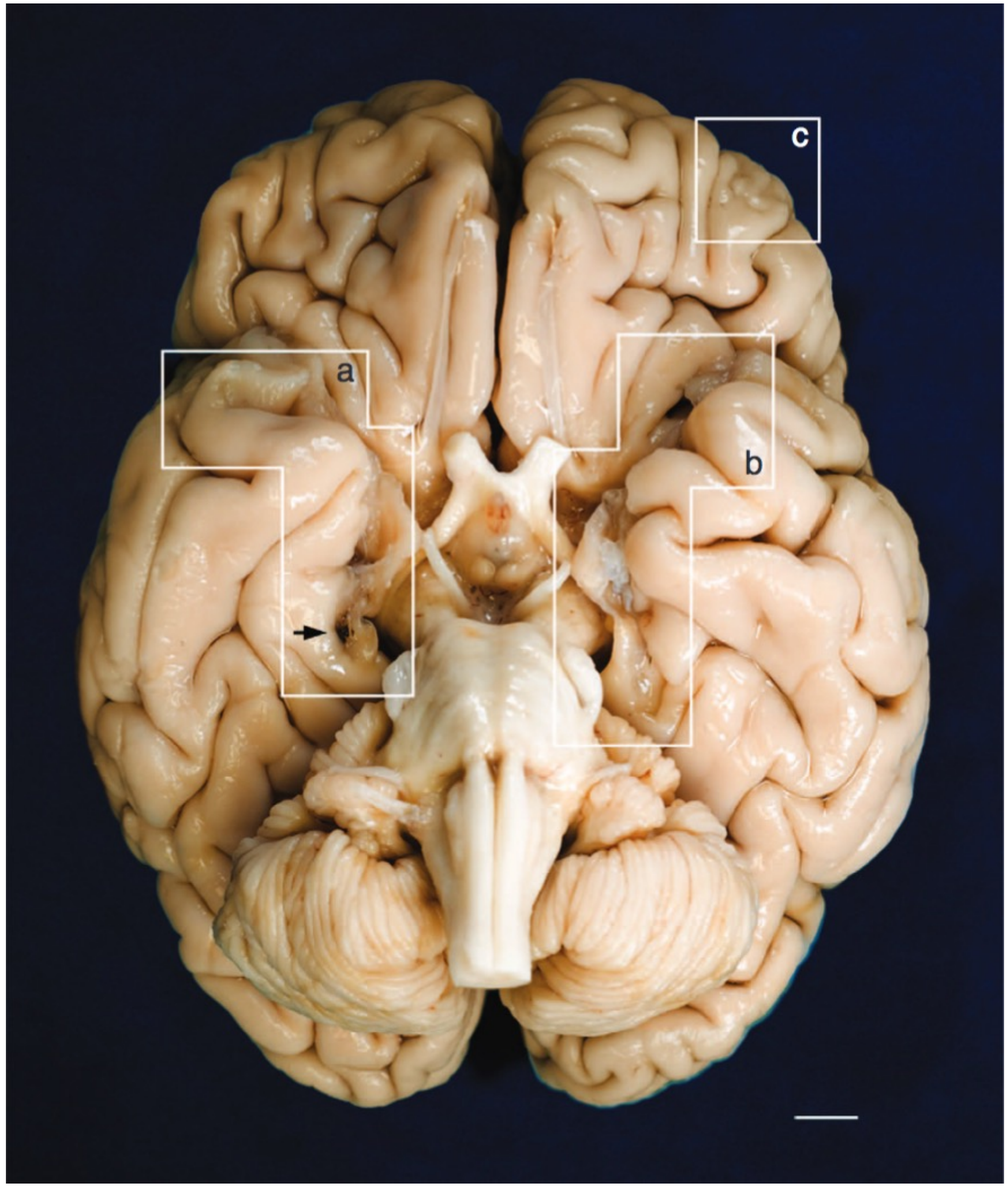
So what is up with Mr. Lester Parker?



Summary #3

- Neurotransmitters packaged in synaptic vesicles carry information between neurons
- Calcium is a key ion for inducing neurotransmitter release
- Neurotransmitters bind receptors on the postsynaptic cell
- Ligation of receptors induces ionic currents that can sum to trigger an action potential
- Even William James got a few things wrong along the way so there is room in the world for the rest of us☺

Vignette #3: Hartford CT, 1953
"Look, a castle!" The story of H.M.



Annesse et al, 2014 *Nat Comm* DOI: 10.1038/ncomms4122

Distributed memory

"…this series of experiments has yielded a good bit of information about what and where the memory trace is not. It has discovered nothing directly of the real nature of the memory trace. I sometimes feel, in reviewing the evidence of the localization of the memory trace, that the necessary conclusion is that learning is just not possible. It is difficult to conceive of a mechanism that can satisfy the conditions set for it. Nevertheless, in spite of such evidence against it, learning sometimes does occur."

-Karl Lashley
Symp Soc Exp Biol. 1950;4:454–82

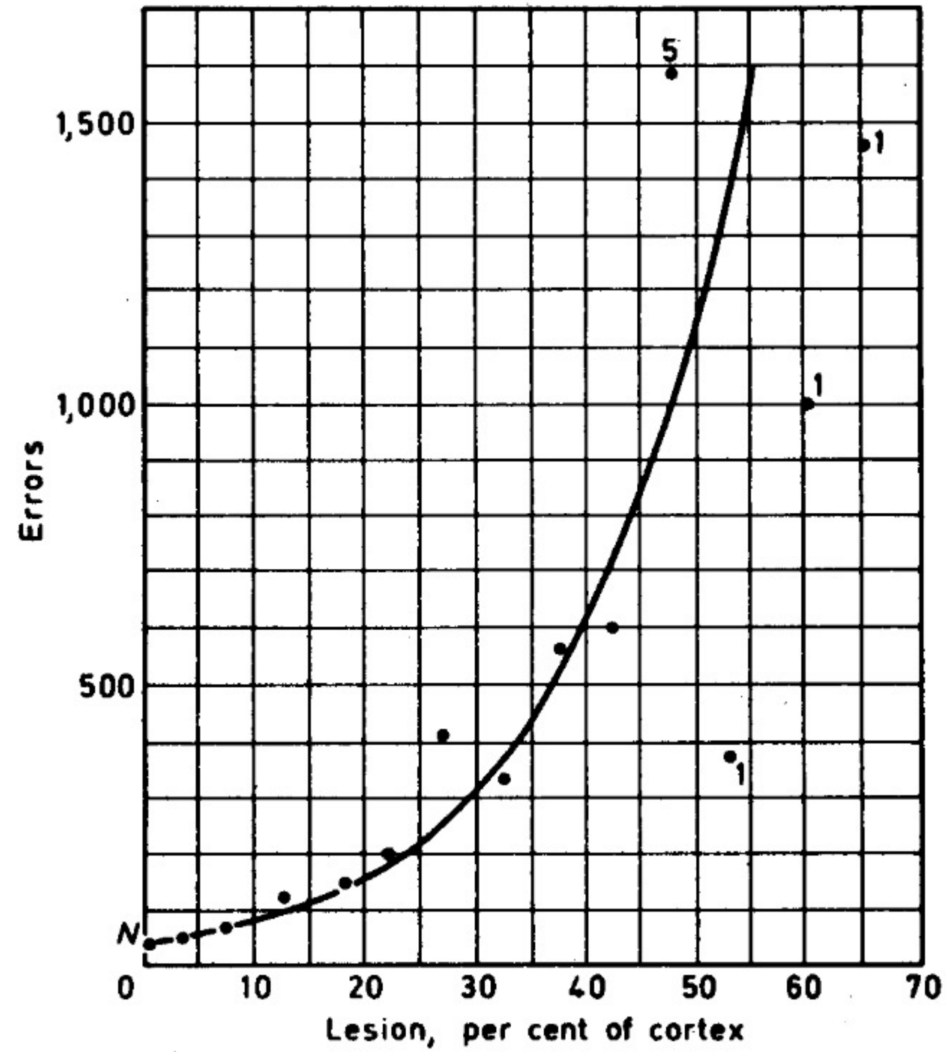
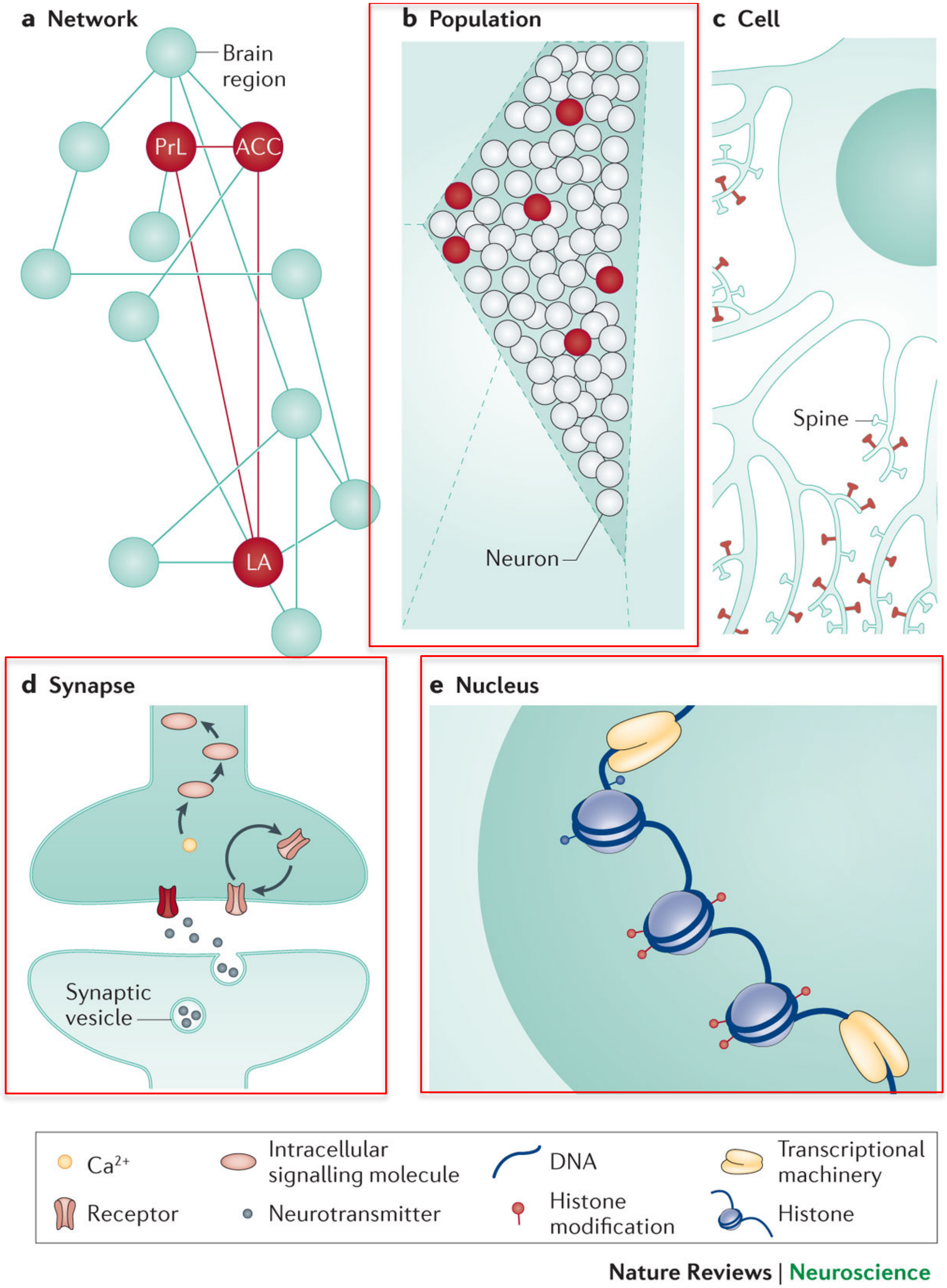
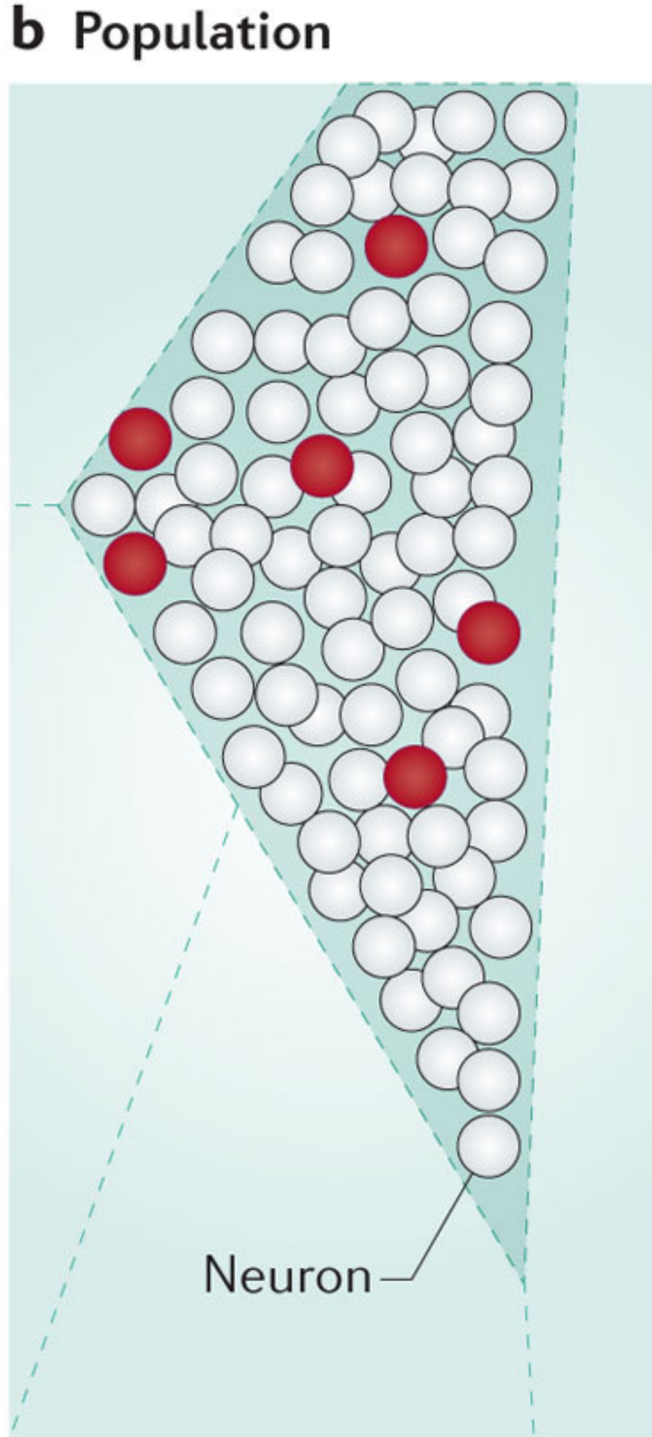


Figure 7. The relation of errors in maze learning to extent of cerebral damage in the rat. The extent of brain injury is expressed as the percentage of the surface area of the isocortex destroyed. Data from 60 normal and 127 brain-operated animals are averaged by class intervals of 5 per cent destruction. The curve is the best fitting one of logarithmic form. For lesions above 45 per cent the number of cases (indicated by numerals on the graph) is too small for reliability (after Lashley and Wiley, 1933)

Scales of the Memory Engram

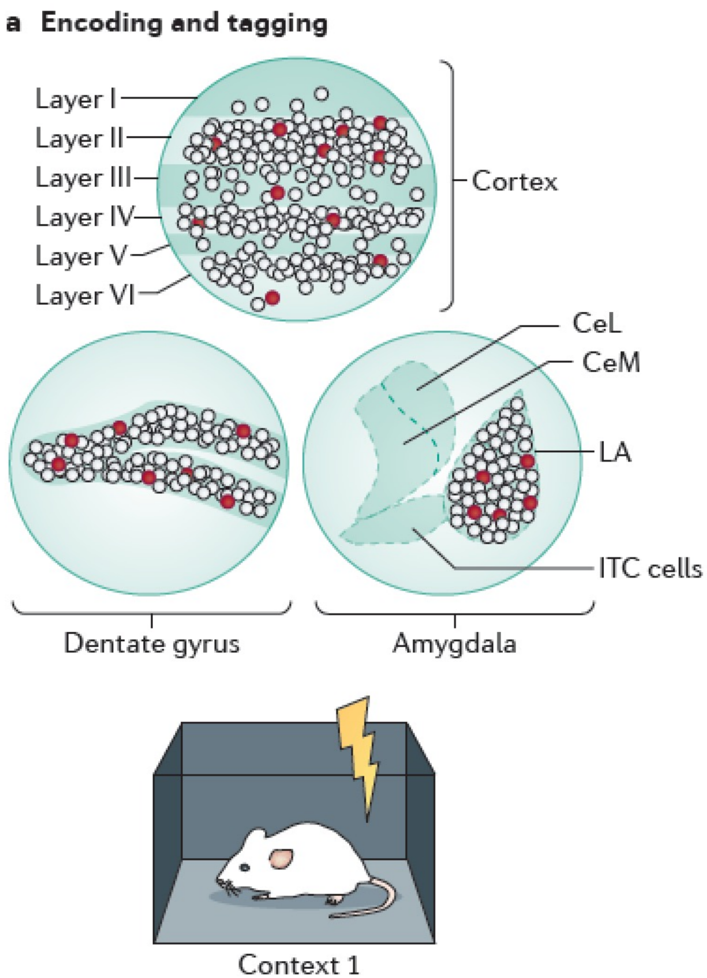


Josselyn et al (2015) *Nat Rev Neurosci* **16**: 521.



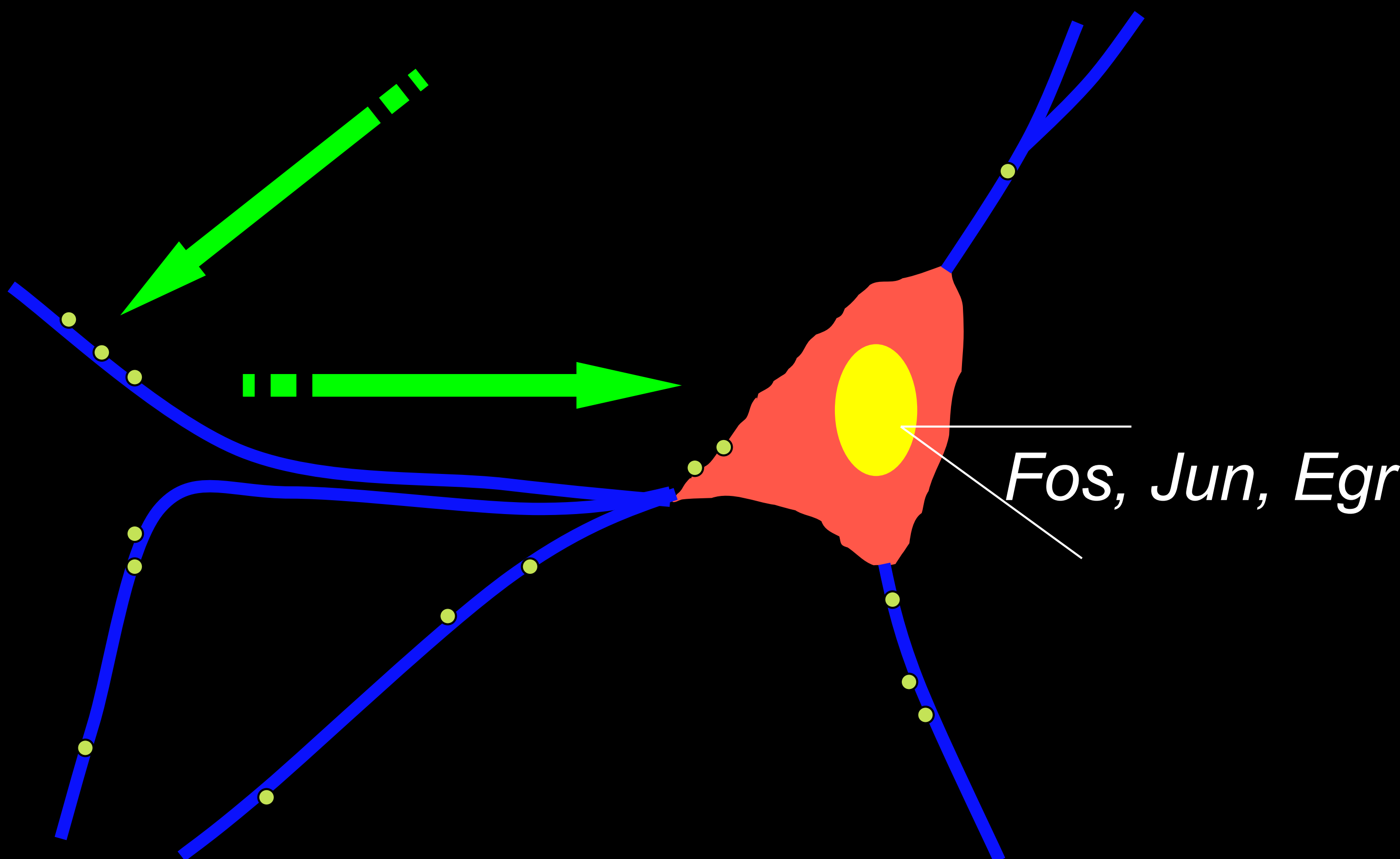
Josselyn et al (2015) *Nat Rev Neurosci* **16**: 521.

The population Memory Engram – spatial neural activity



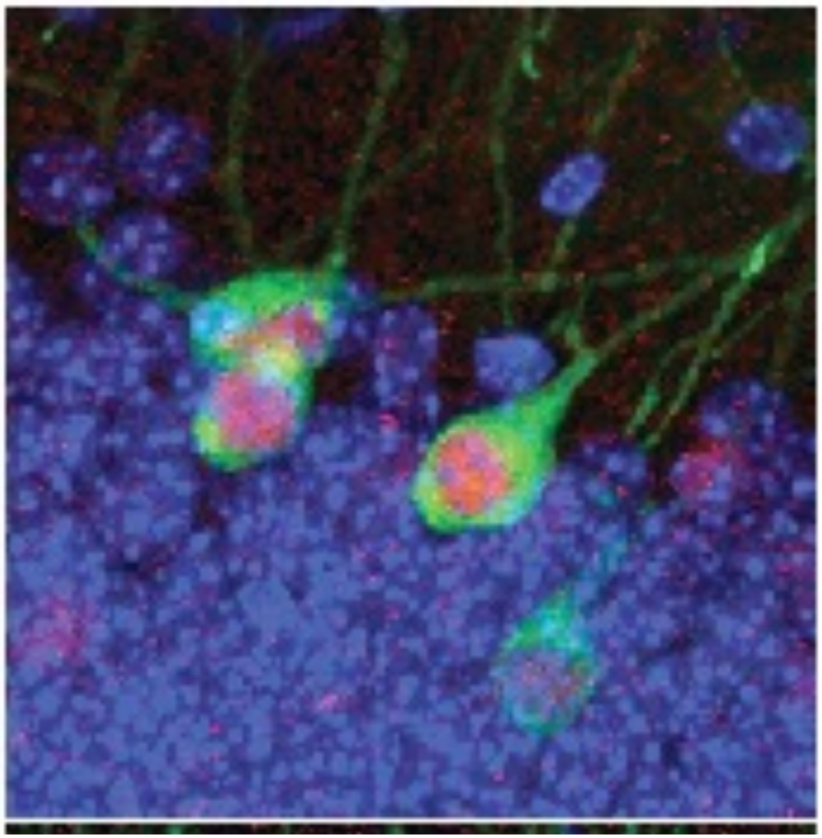
Josselyn et al (2015) *Nat Rev Neurosci* **16**: 521.

Activity-regulated transcription transforms brief synaptic stimuli into persistent changes in neuronal gene expression



Fos allows you to "see" the population Memory Engram

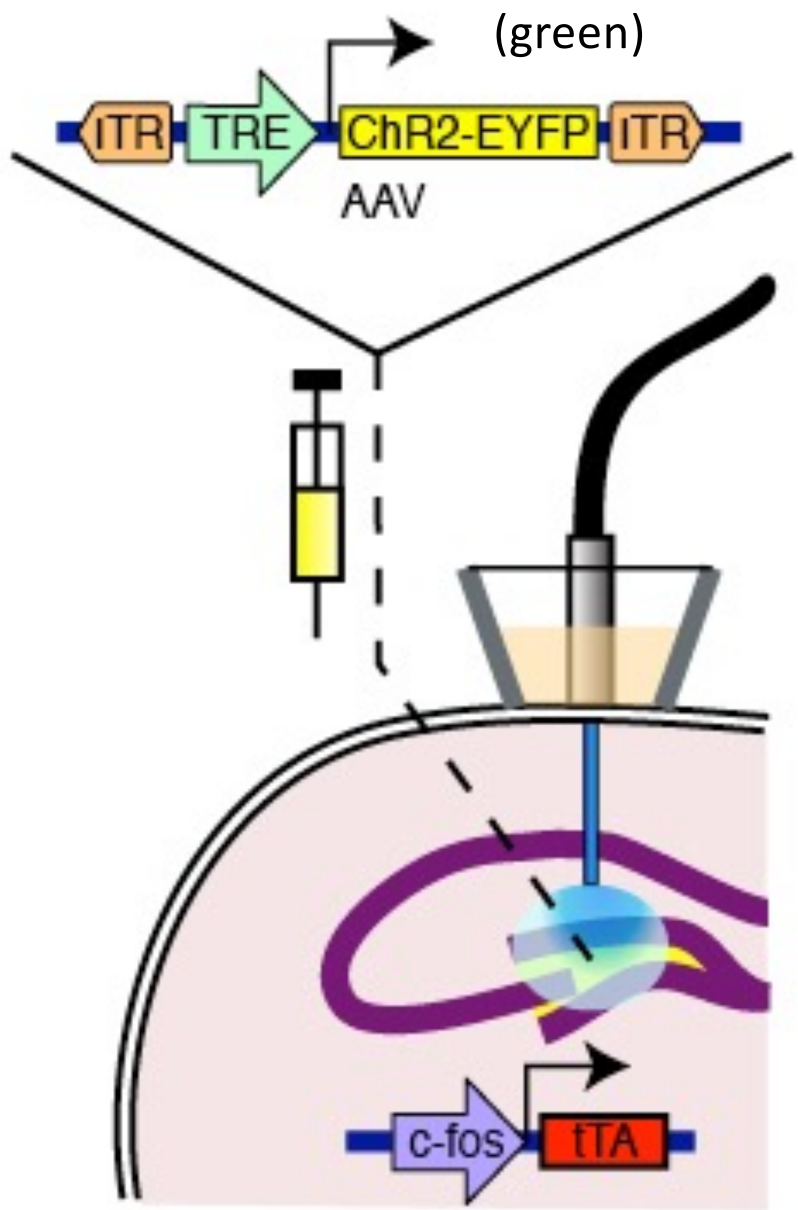
<u>Fos (red)</u>: a transcription factor whose expression is induced by neuronal activity



Only a subset of cells (blue) in the hippocampus are activated upon exposure to a context

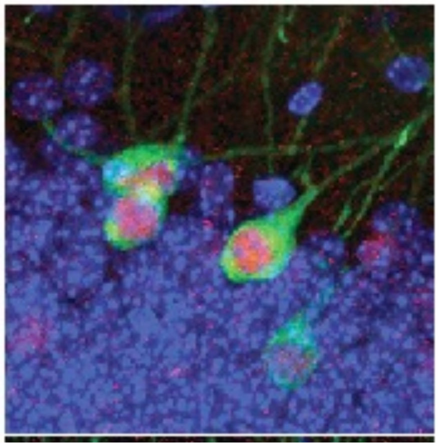
Liu X.et al. Nature 484: 381–385 (2012).

Tagging the population Memory Engram so it can be seen over time



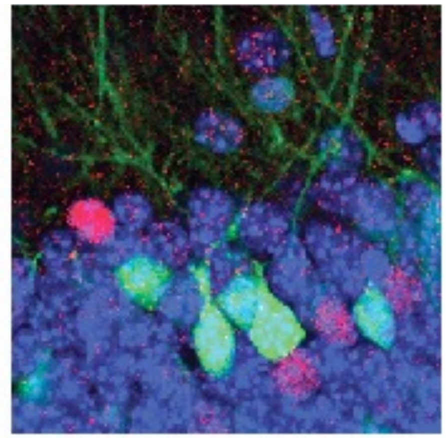
Liu X.et al. Nature 484: 381–385 (2012).

Different exposures generate spatially distinct engrams



Same context

Fos protein (red) induced by exposure to the same context overlaps the tag (green) induced by Fos transcription during the first exposure to the context.

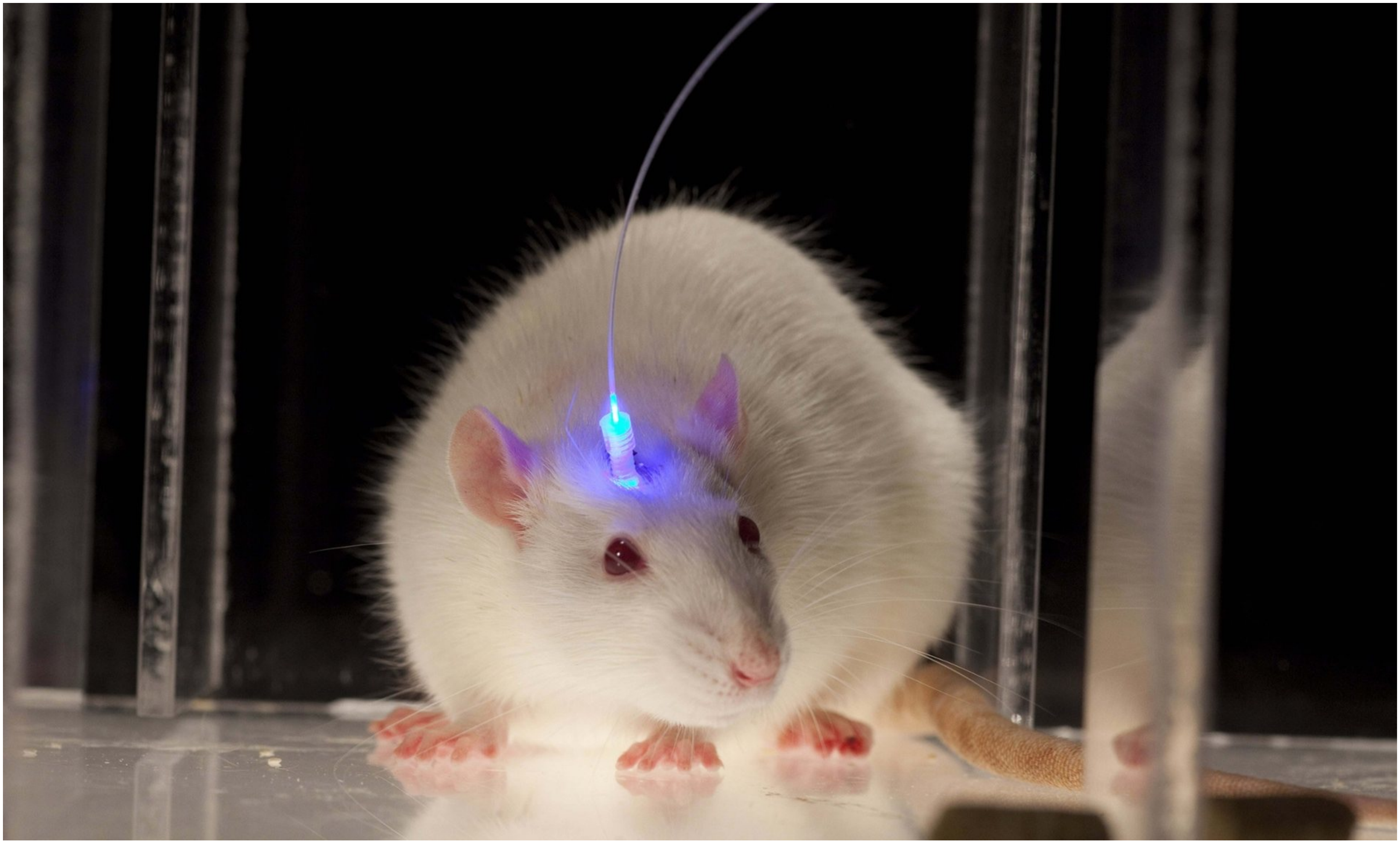


New context

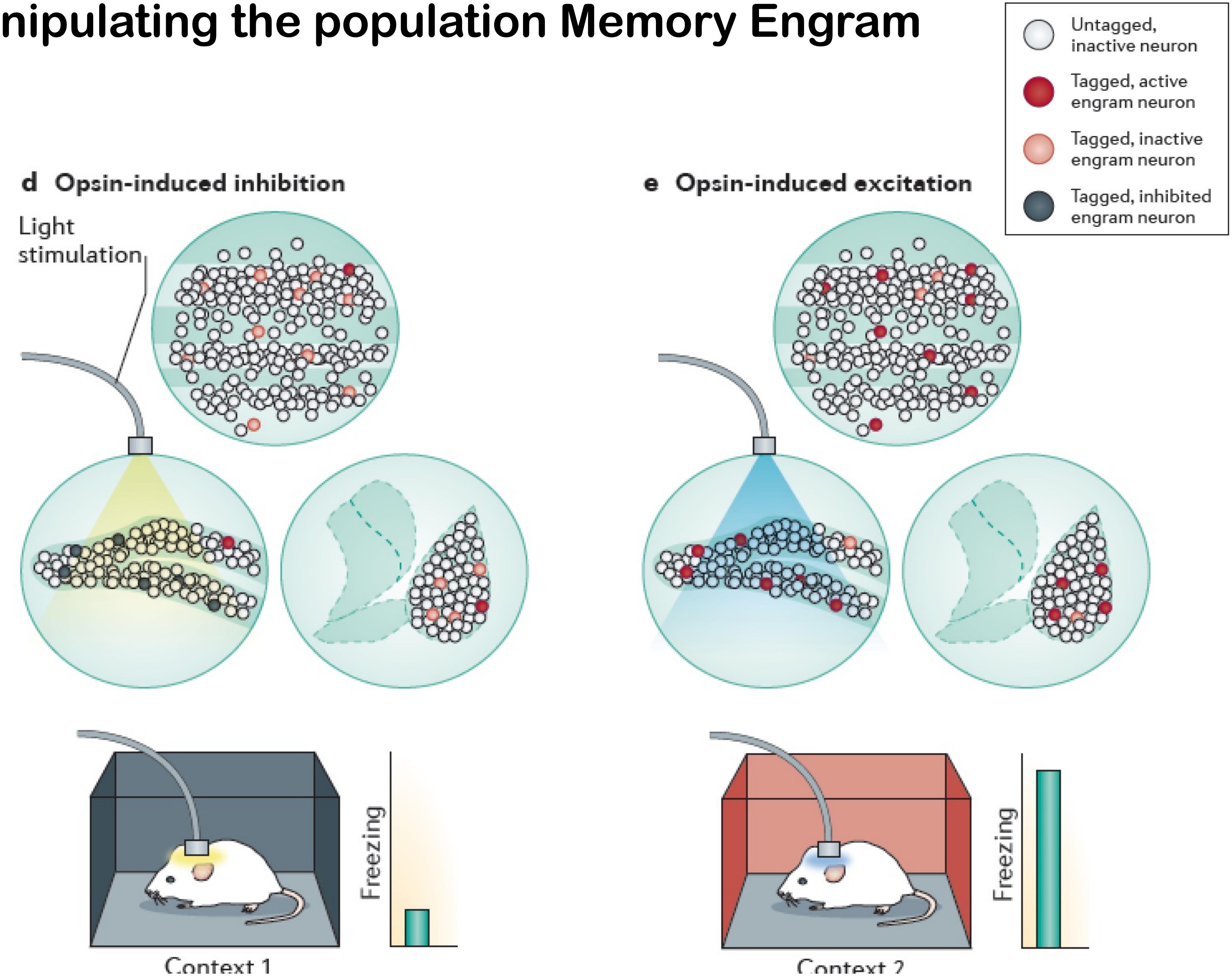
Fos protein (red) induced by exposure to a new context does not overlap the tag (green) induced by Fos transcription during exposure to the first context

Liu X.et al. Nature 484: 381–385 (2012).

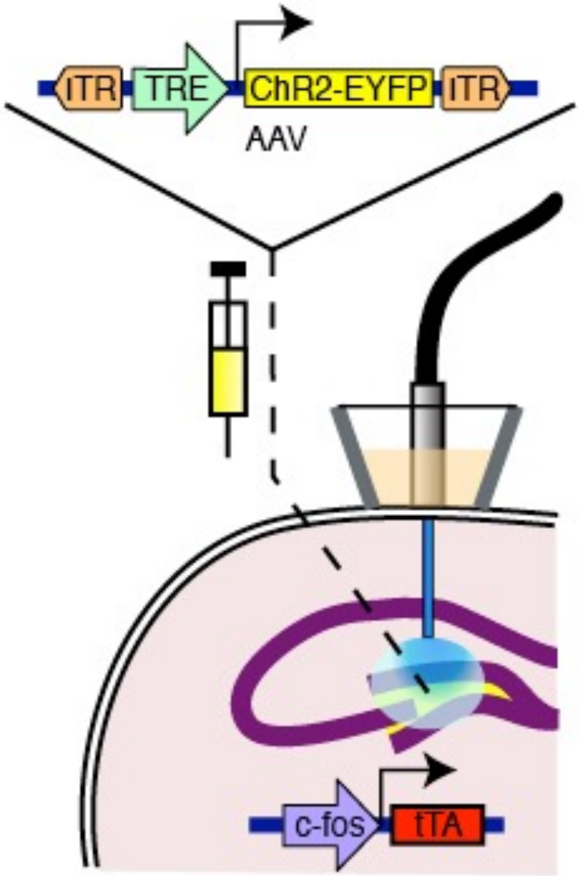
Optogenetics: Light-dependent, remote-control regulation of neuronal activity

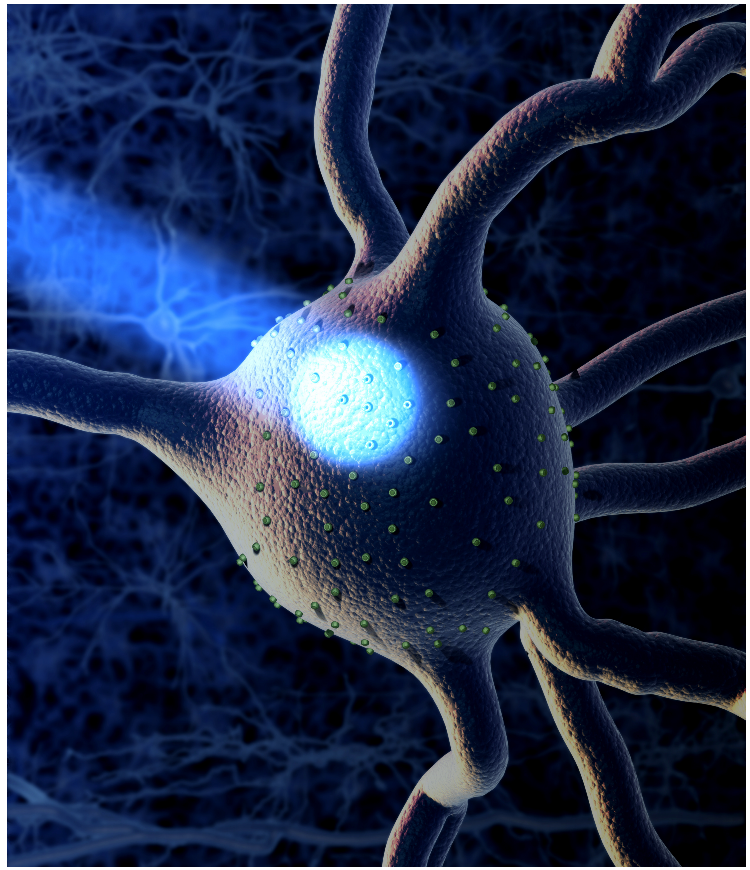


Manipulating the population Memory Engram

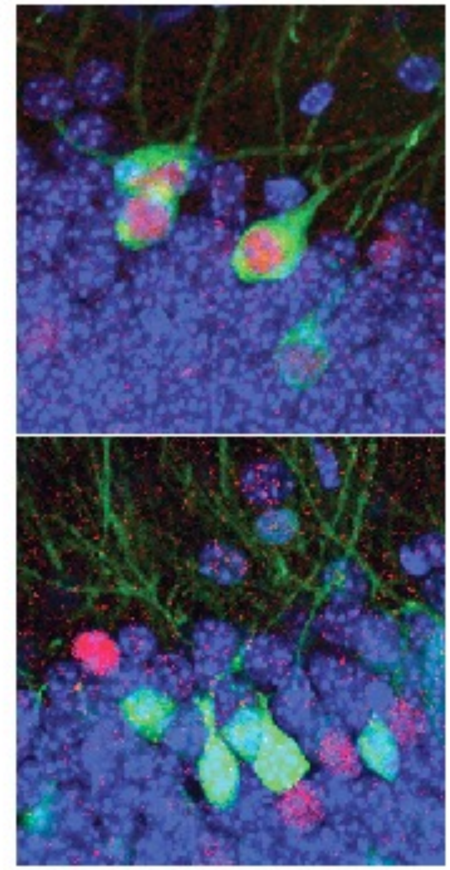


Manipulating the population Memory Engram





Context A

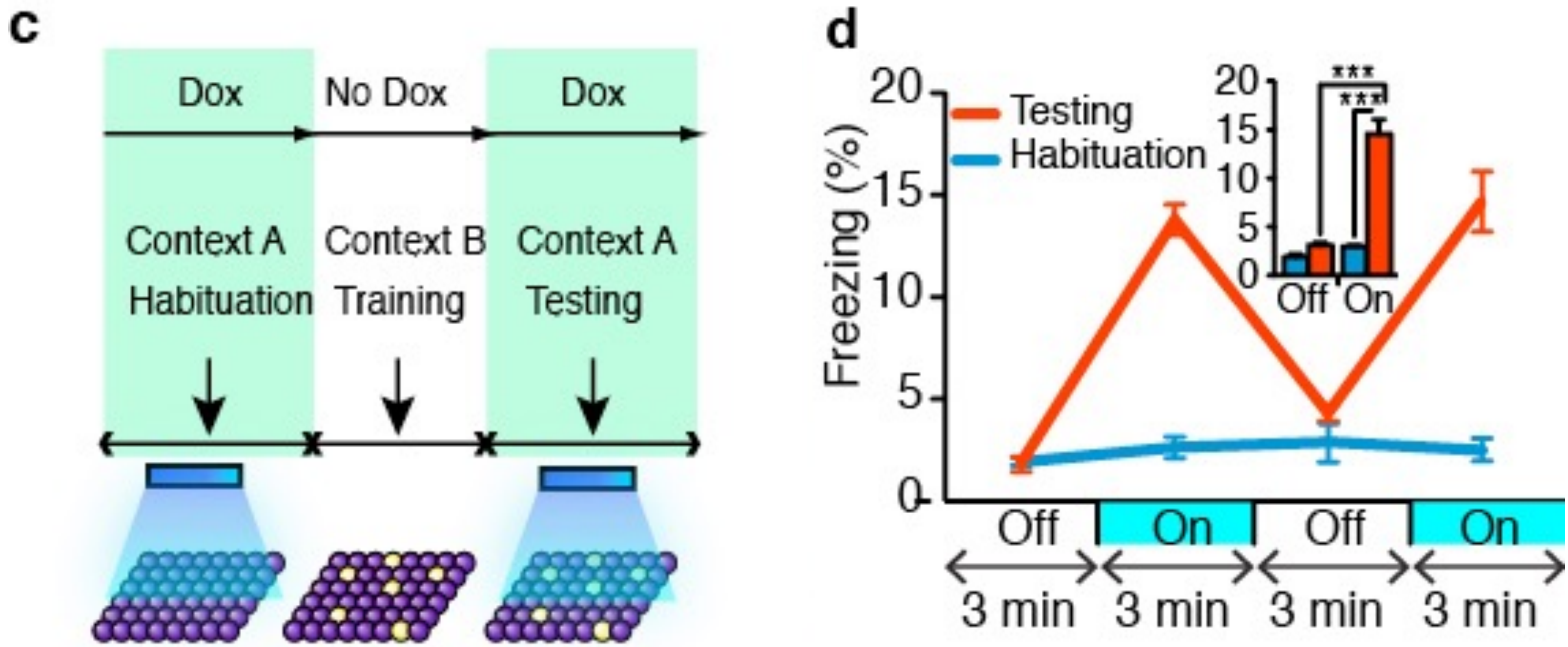


Context B

Liu X.et al. Nature 484: 381–385 (2012).

Transfer “memory” from context A to B by firing context A tagged ensemble in context B?

Activating the population Memory Engram activates behavior learned by past experience



Shock
induces
freezing

Liu X.et al. Nature 484: 381–385 (2012).

Fun with philosophers 1

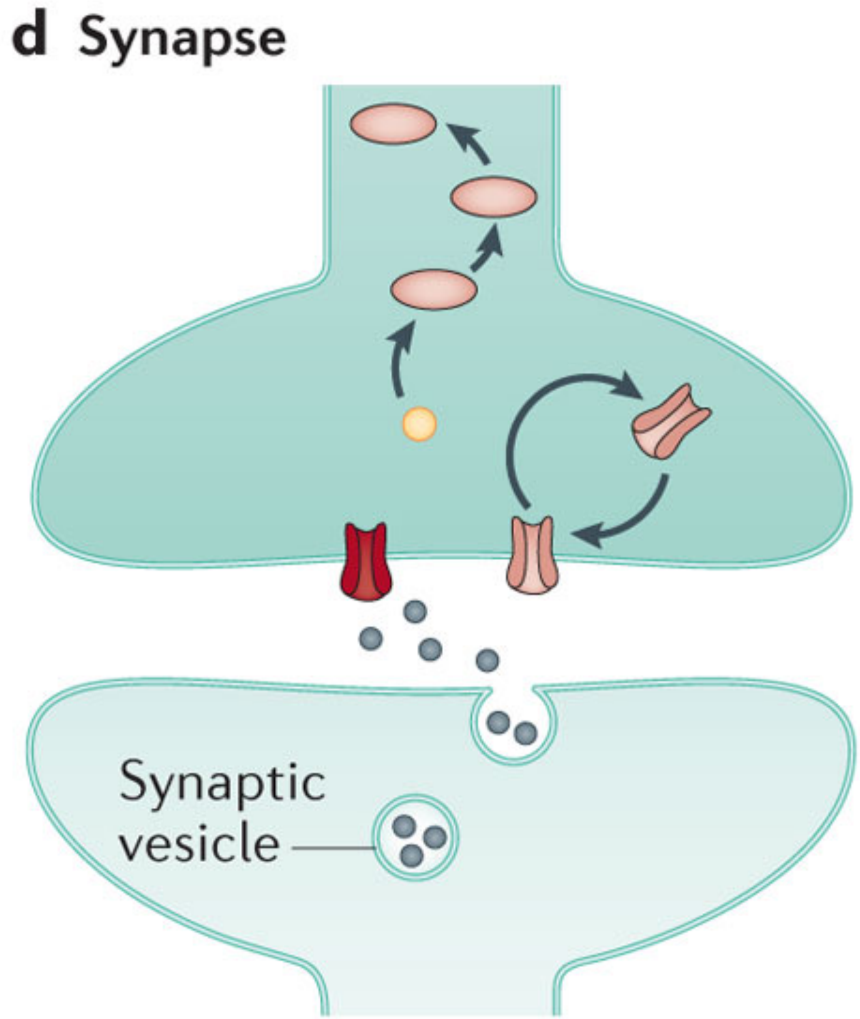
Is this “memory”?

What is the mouse ”thinking”?

Summary 1: Populations

The firing of populations of neurons appears to encode memories.

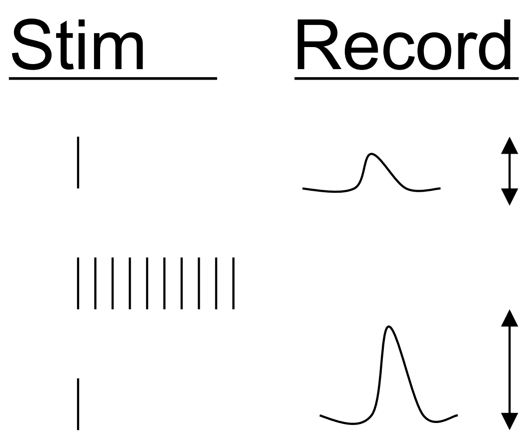
What makes those populations of neurons fire together?

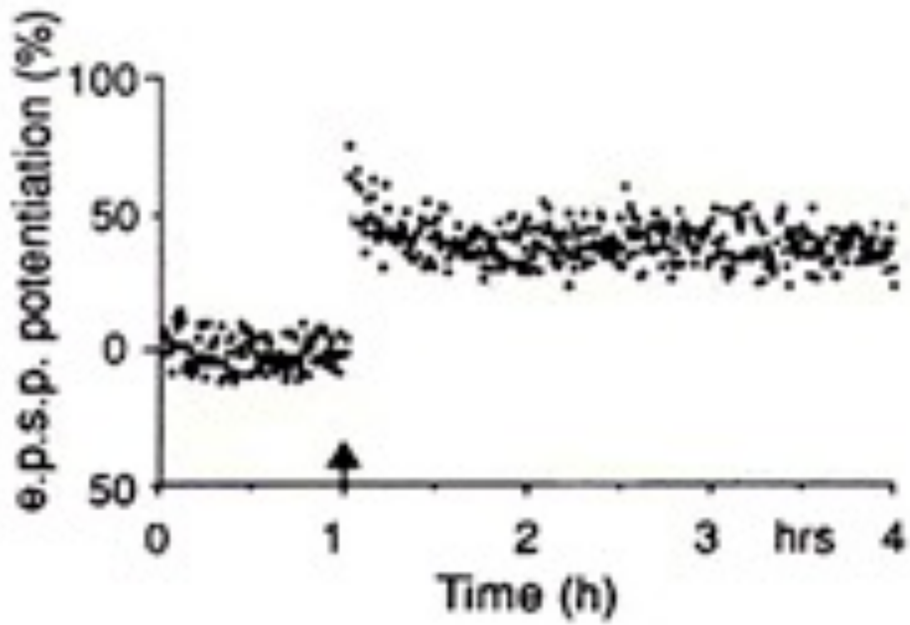


Josselyn et al (2015) *Nat Rev Neurosci* **16**: 521.

Long-term potentiation (LTP) is the canonical cellular model for learning and memory

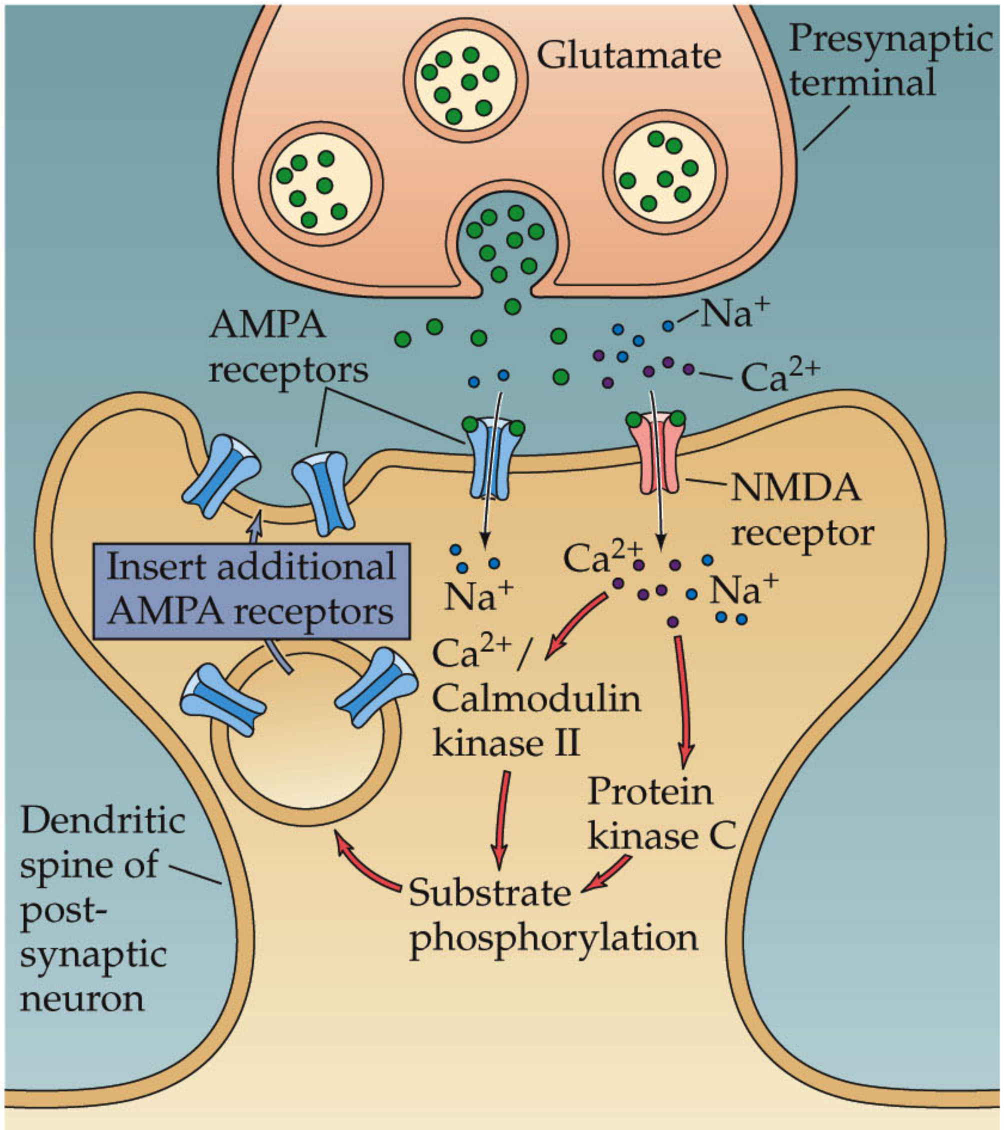
Bliss and Lomo (1973) *J Physiol*
"Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path."





Proposed: Persistent alterations in synaptic strength are the cellular substrate of learning and memory.

The mechanism of Long-Term Potentiation (LTP)



Inducers – NMDARs
Mediators – AMPARs
Modulators – many

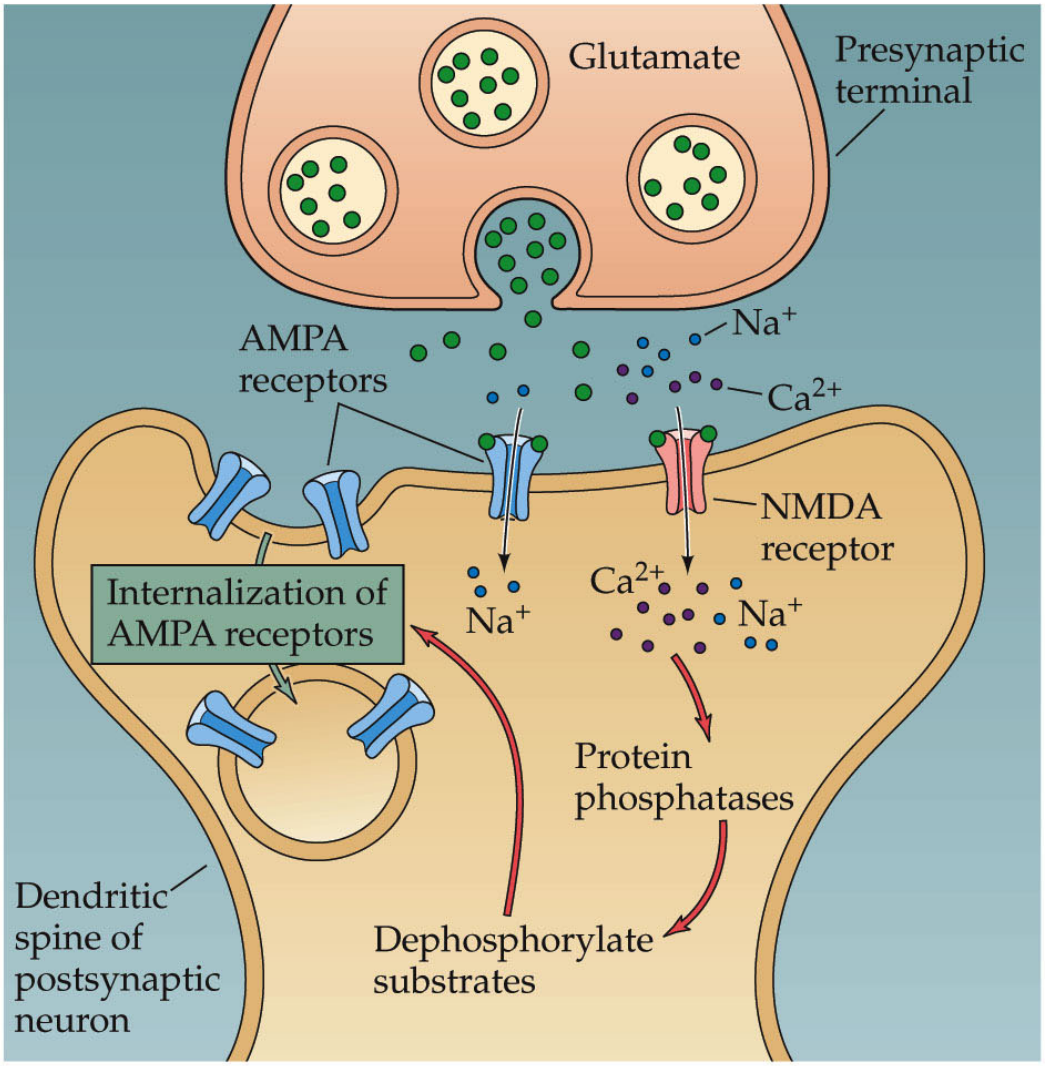
<u>Associative</u> process (Hebbian)

Induced by **high frequency** paired stimulation

NEUROSCIENCE, Fourth Edition, Figure 8.11

The mechanism of Long-Term Depression (LTD)

(C)



NEUROSCIENCE, Fourth Edition, Figure 8.15 (Part 2)

Inducers – NMDARs, mGluRs
Mediators – AMPARs
Modulators – many

Associative process (Hebbian)

Induced by **low frequency** paired stimulation

Fun with philosophers 2

Is this "addiction"?

Neuron

Persistent LTP in VTA after Cocaine Administration

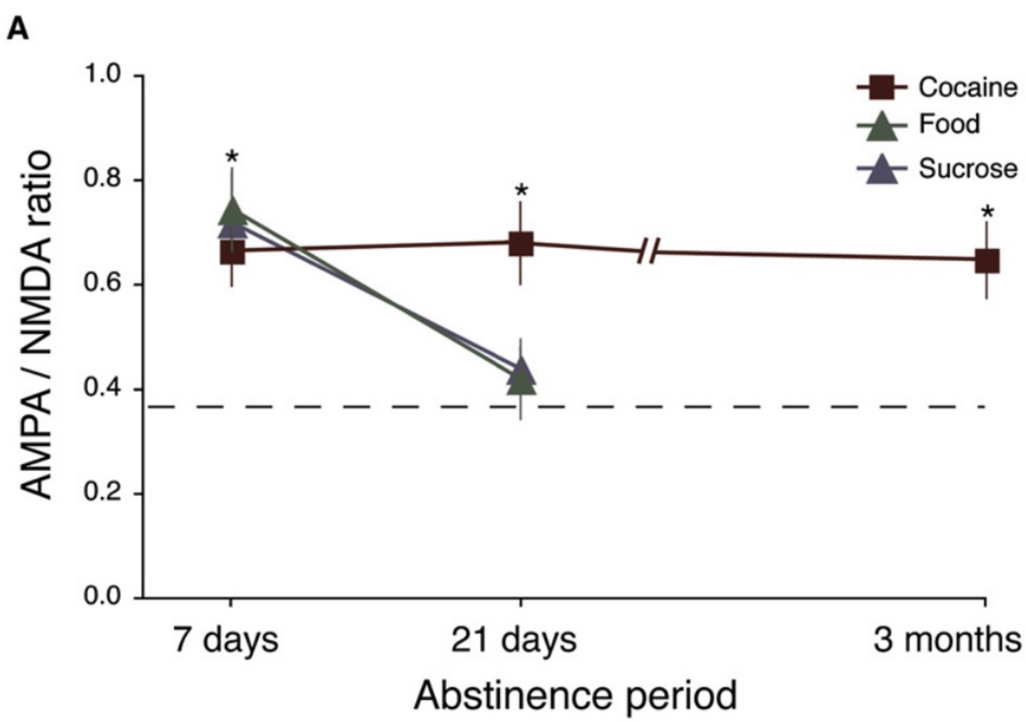


Figure 3. Cocaine Self-Administration Produced a Significantly Longer Potentiation of Glutamatergic Transmission than Food or Sucrose Self-Administration
(A) AMPAR/NMDAR ratio in cocaine self-administering rats remained enhanced after 90 days of abstinence. However, in food and sucrose self-administering rats, increased AMPAR/NMDAR ratio was observed following 7 days of abstinence but returned to Naive levels by 21 days of abstinence.
(B and C) Following 7 day and 21 day abstinence, mEPSC frequencies from Cocaine and Sucrose groups remained significantly enhanced relative to Naive. In Food rats (B), the mEPSC frequency was not significantly different than Naive rats by 7 days of abstinence and remained unchanged at 21 days of abstinence (C). mEPSC frequencies from Cocaine continued to be significantly enhanced relative to Naive at 3 months abstinence. Dotted line represents averages from Naive rats. *$p < 0.05$ versus Naive.

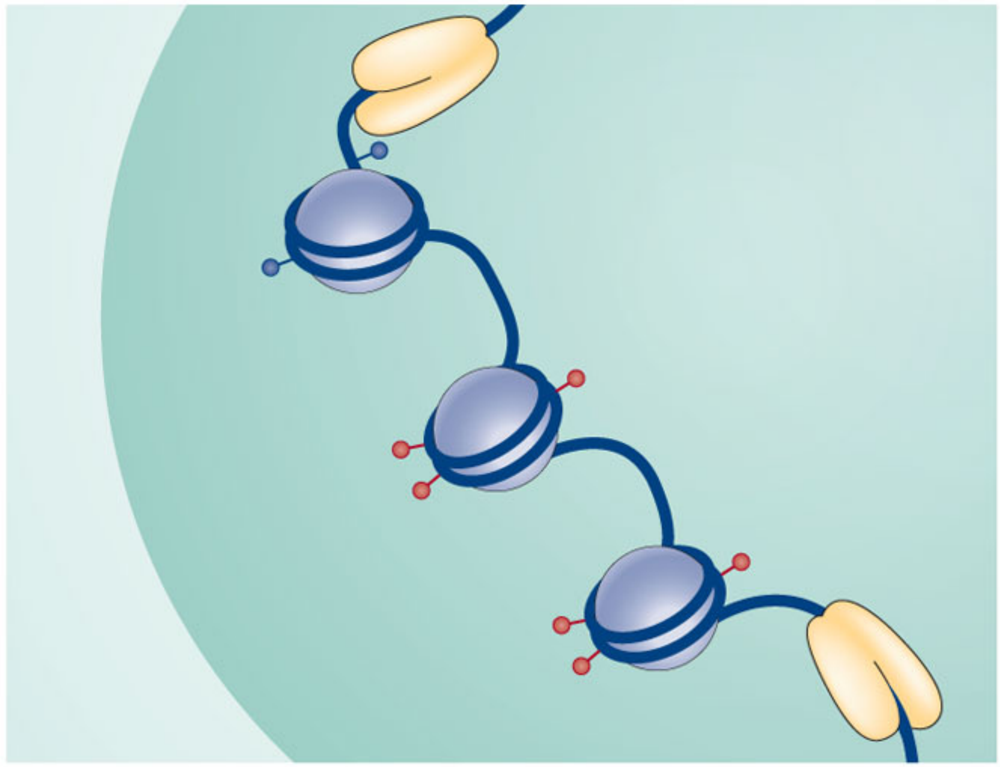
Chen BT et al, Neuron 59, 288–297, July 31, 2008

Summary 2: Synapses

Experience dependent changes in synaptic strength couple neurons into ensembles.

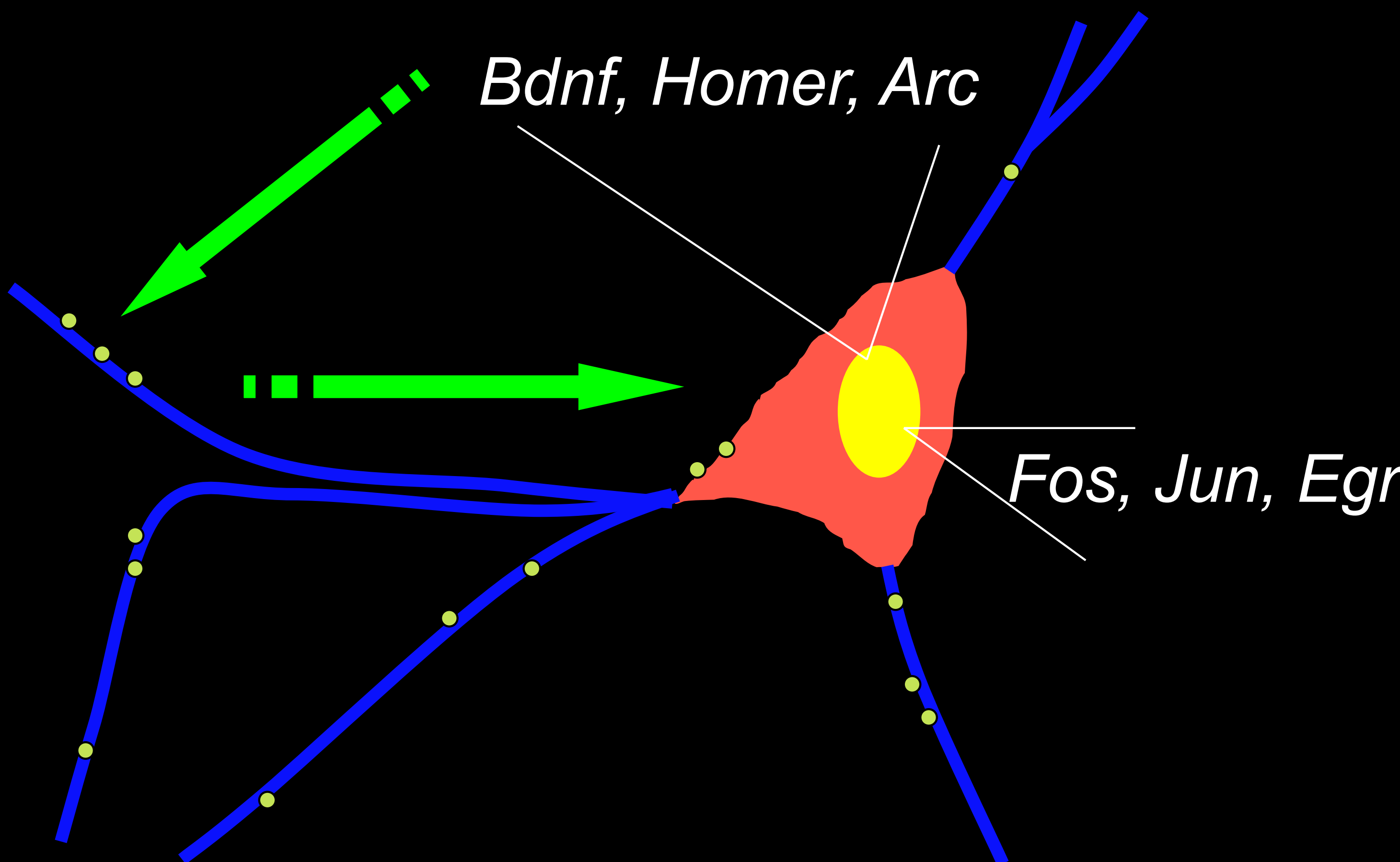
What mechanisms underlie persistent changes in synaptic strength?

e Nucleus

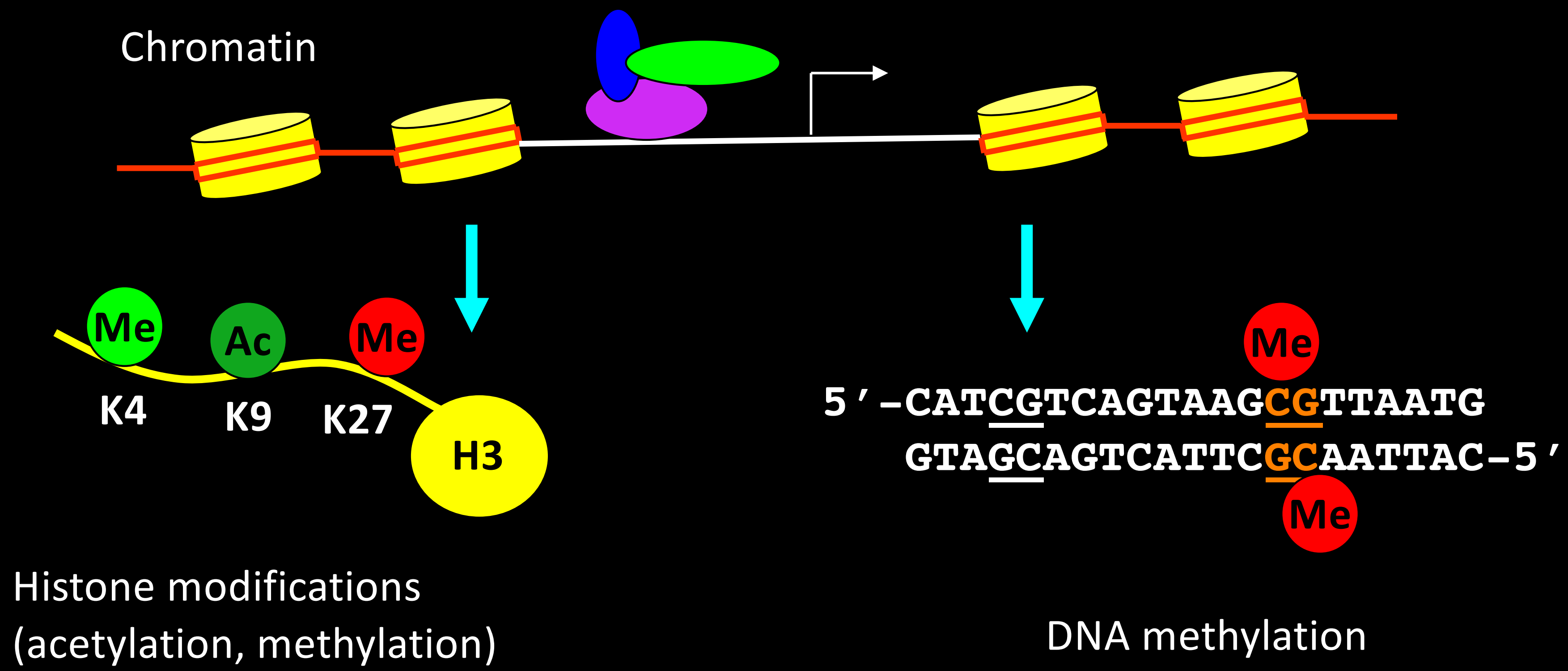


Josselyn et al (2015) *Nat Rev Neurosci* **16**: 521.

Activity-regulated transcription modulates synaptic plasticity

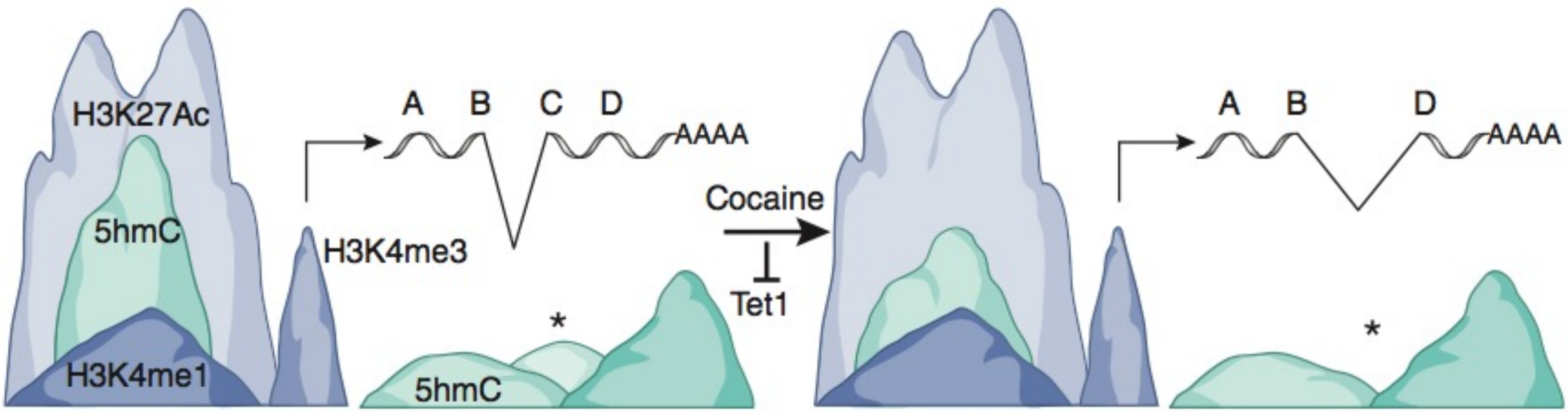


Chromatin regulation:
A mechanism of cellular memory?



Persistent – like memory
Stimulus-regulated – like learning

Cocaine Remodels the Chromatin Landscape



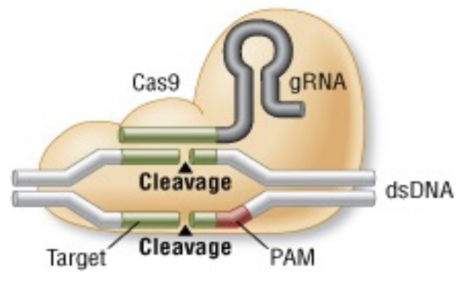
Feng et al (2015) *Nat Neurosci* **18**: 536-44

The "breadcrumb" hypothesis:
Epigenetic marks on the genome are "memories" of past exposures that changes the transcriptional response when the exposure occurs again.

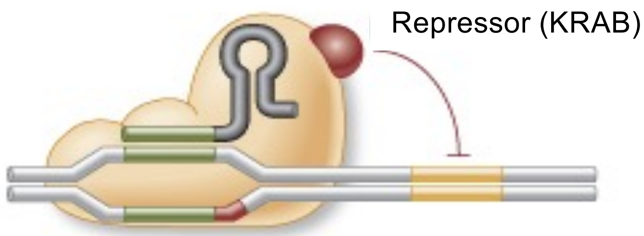
Precision Epigenome Editing

Versatility of the Cas9/CRISPR system

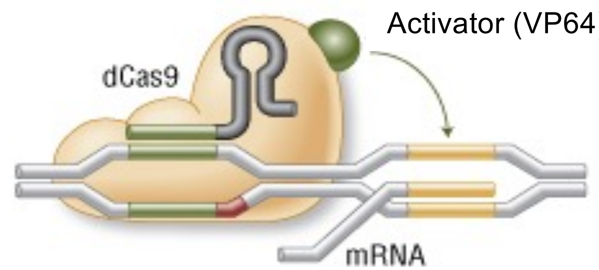
1. Genome Engineering



2. Functional Genomics

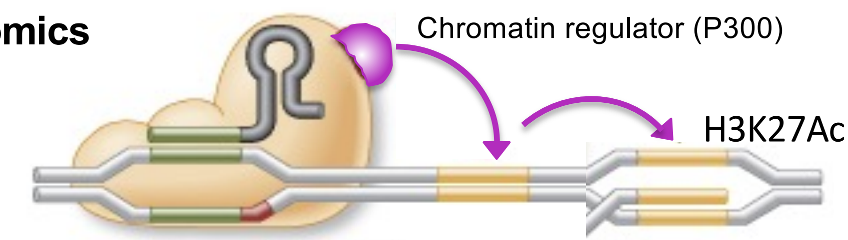


Inhibition (CRISPRi)



Activation (CRISPRa)

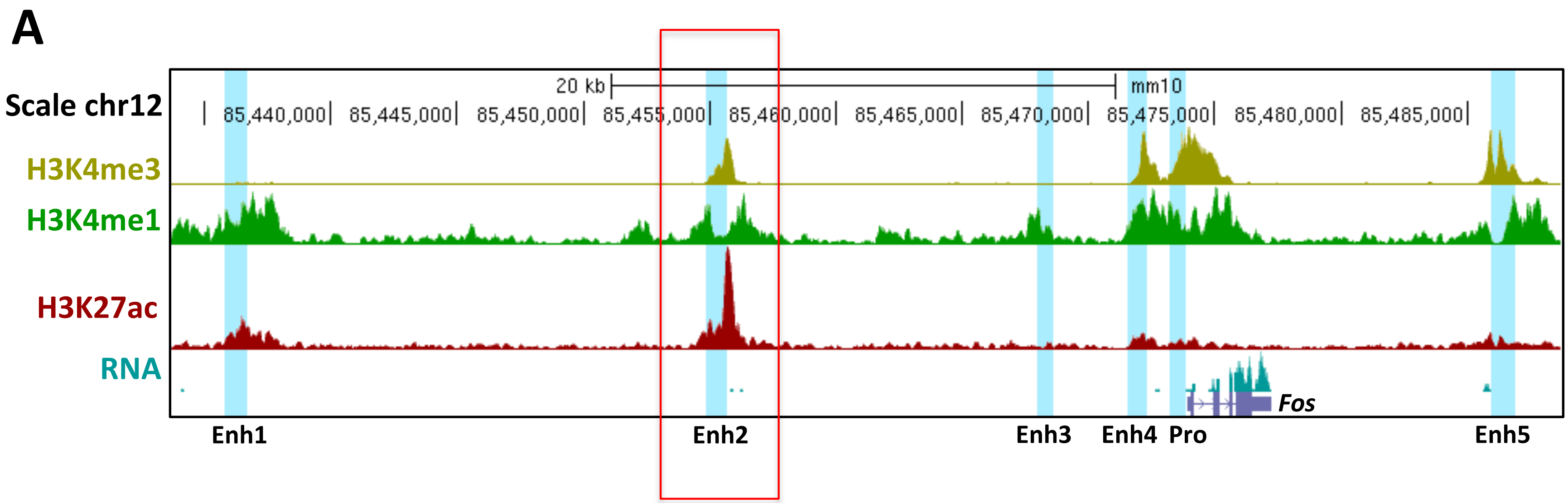
3. Functional Epigenomics



Modulation (CRISPRm)

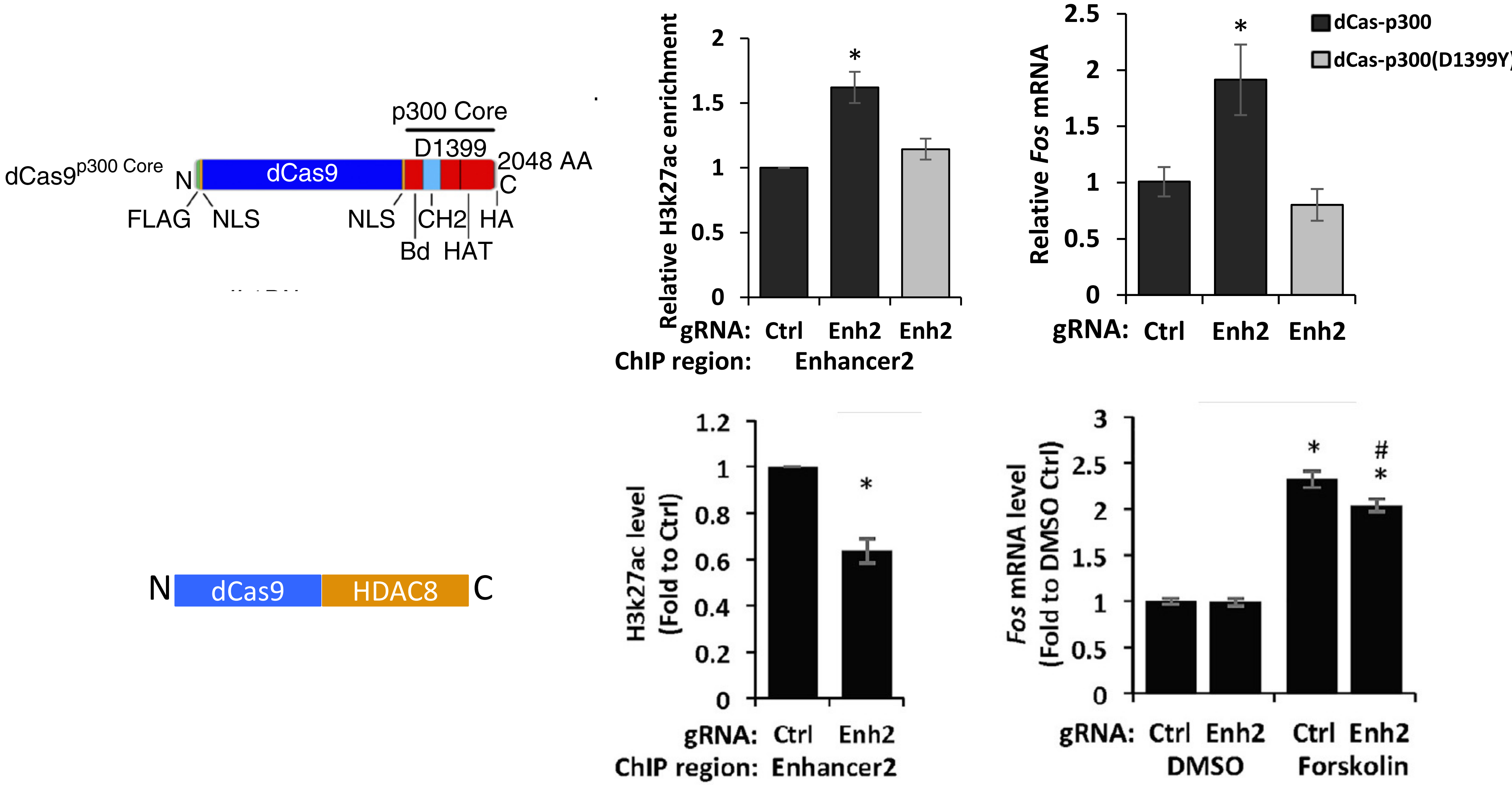
Modified from NEB.com

Targeted epigenome editing at *Fos* enhancers



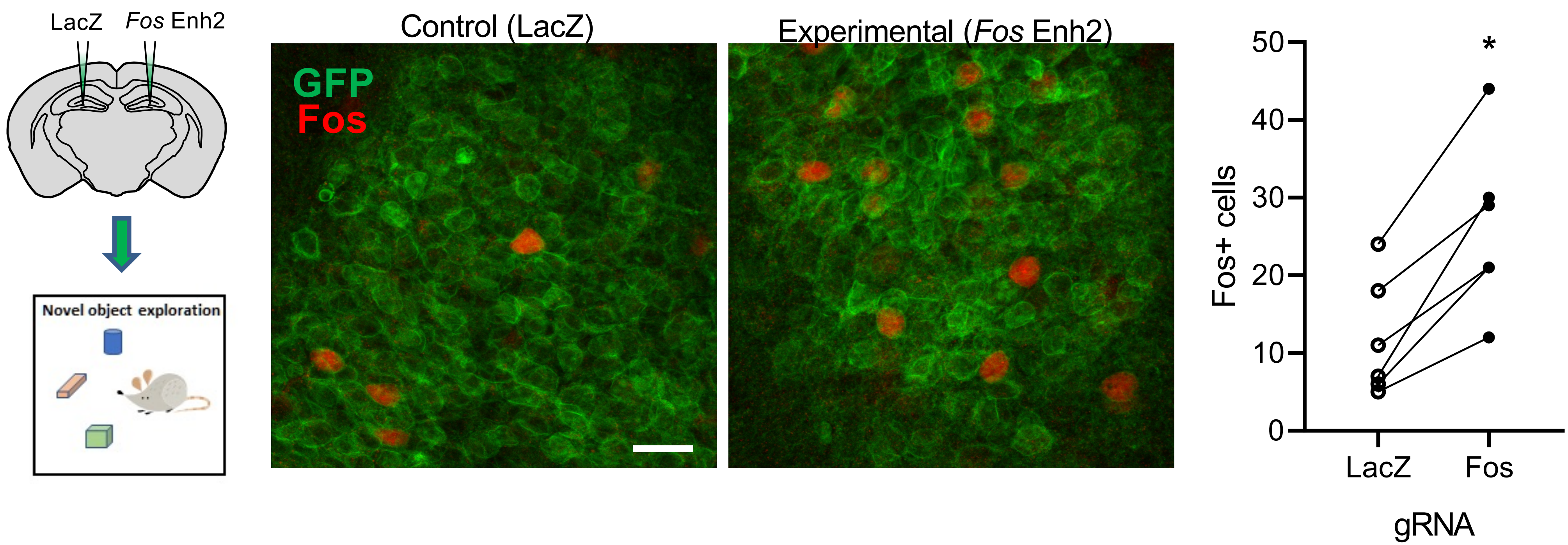
Based on Kim, T.K. et al (2010) *Nature* **465**: 182-187.

Bidirectional regulation of H3K27ac and *Fos* transcription by dCas9-mediated enhancer epigenome editing



Chen, L-F. et al (2019) *Cell Rep* 26:1174-1188

Enhancer epigenome editing modulates Fos induction in hippocampal neurons



Gemberling (2021) *Nat Methods* in press

Fun with Philosophers 3

Can memory be reduced to epigenetics? (“written on the genome”)

Does the “epi” make genetics less deterministic?

Summary 3: Nucleus

Neuronal firing changes the epigenome in neurons, altering the expression of genes that function at synapses.

How are these transcriptional and synaptic networks integrated to produce the psychological construct of memory?

Final Thoughts

- Experimental neurobiology is really fun right now!
- When you learn something the physical substrate of your brain changes
- Memory is encoded in the activation of sparse neural ensembles, the physical changes in structure and function at specific subsets of synapses, and in the biochemical nature of the neuron itself
- Protein engineering has provided innovative ways to probe neural function