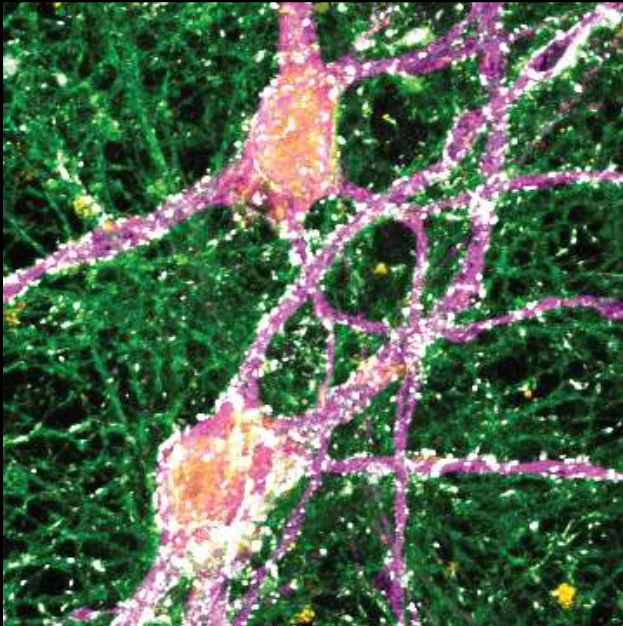
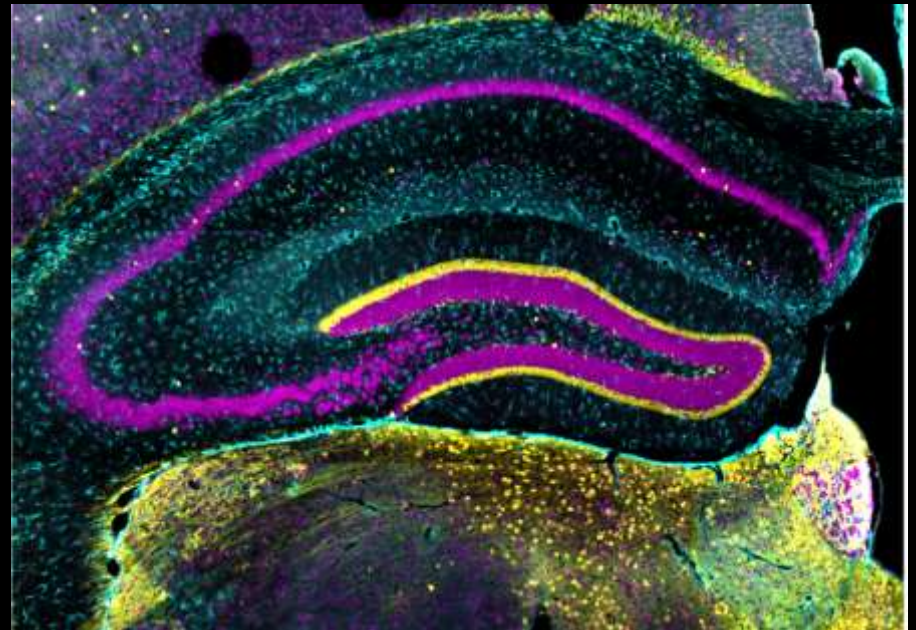


Neuroscience, synapses, and the most potent toxin known to man

Dr. Katie Hoffman
Lehigh University
Biological Sciences Department



Mouse stem cell derived neuron



Mouse hippocampal slice

What makes us who we are?



100 billion cells in our brains

400 billion stars in our galaxy



What makes us who we are?



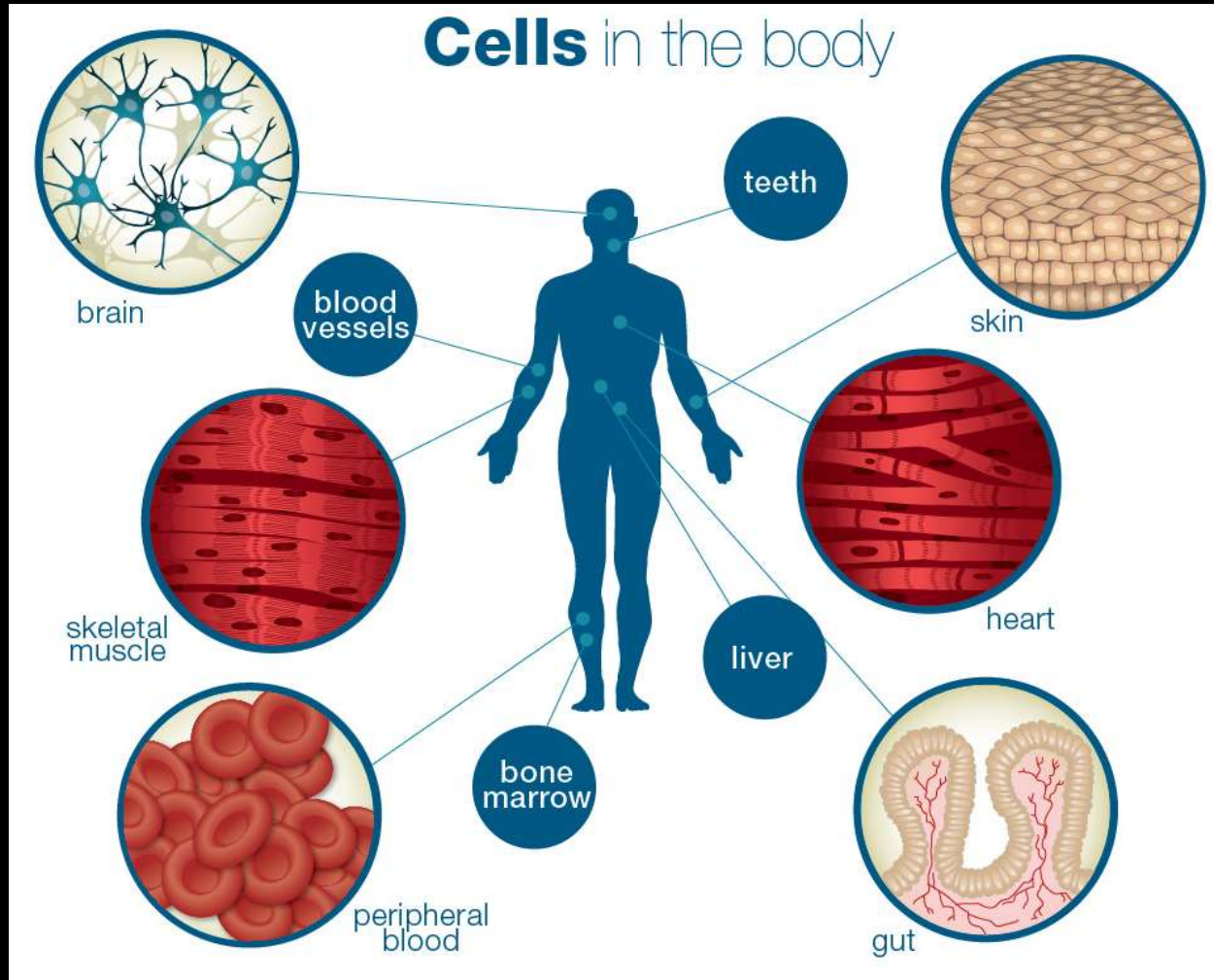
100 billion cells in our brains



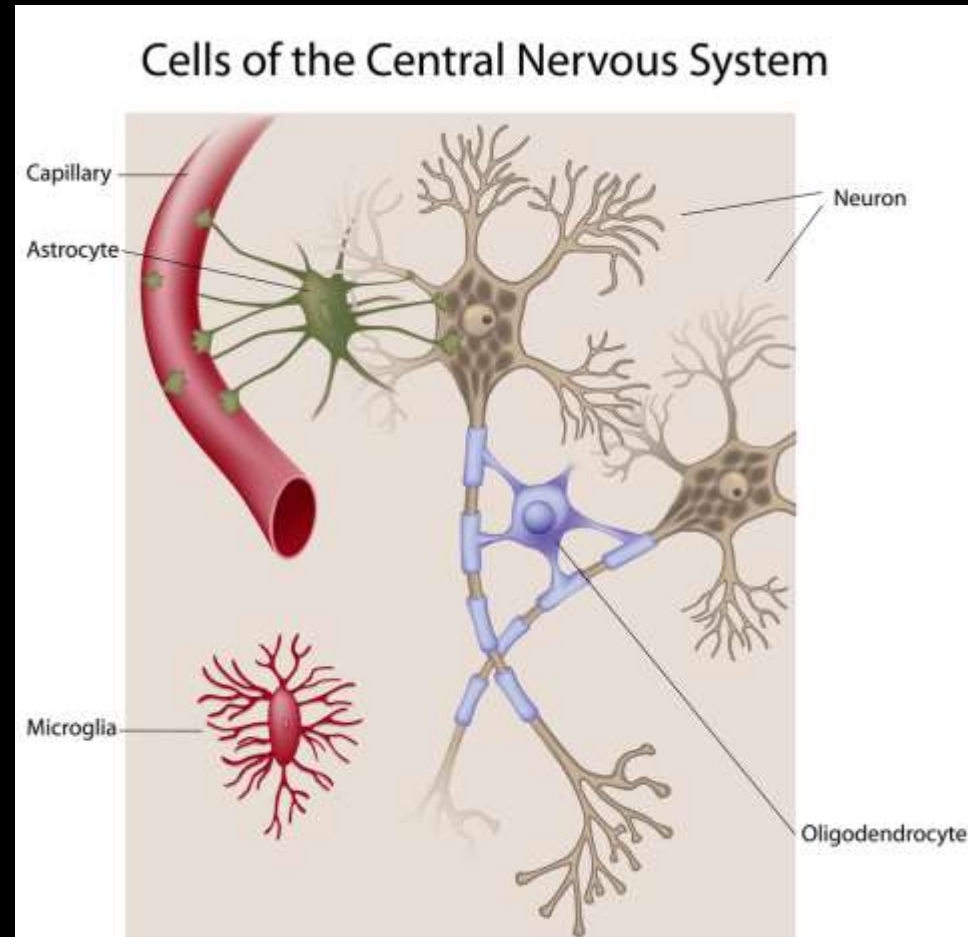
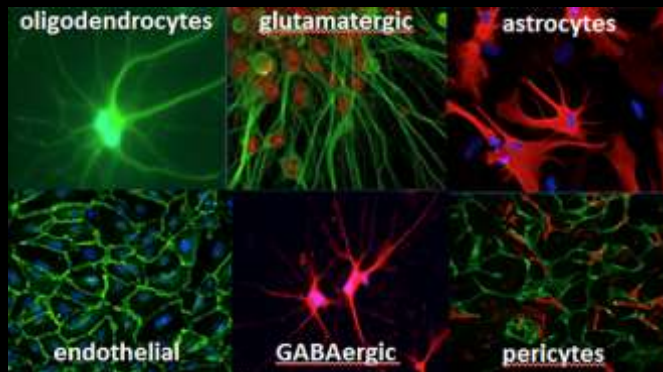
400 billion stars in our galaxy



What are we made of?

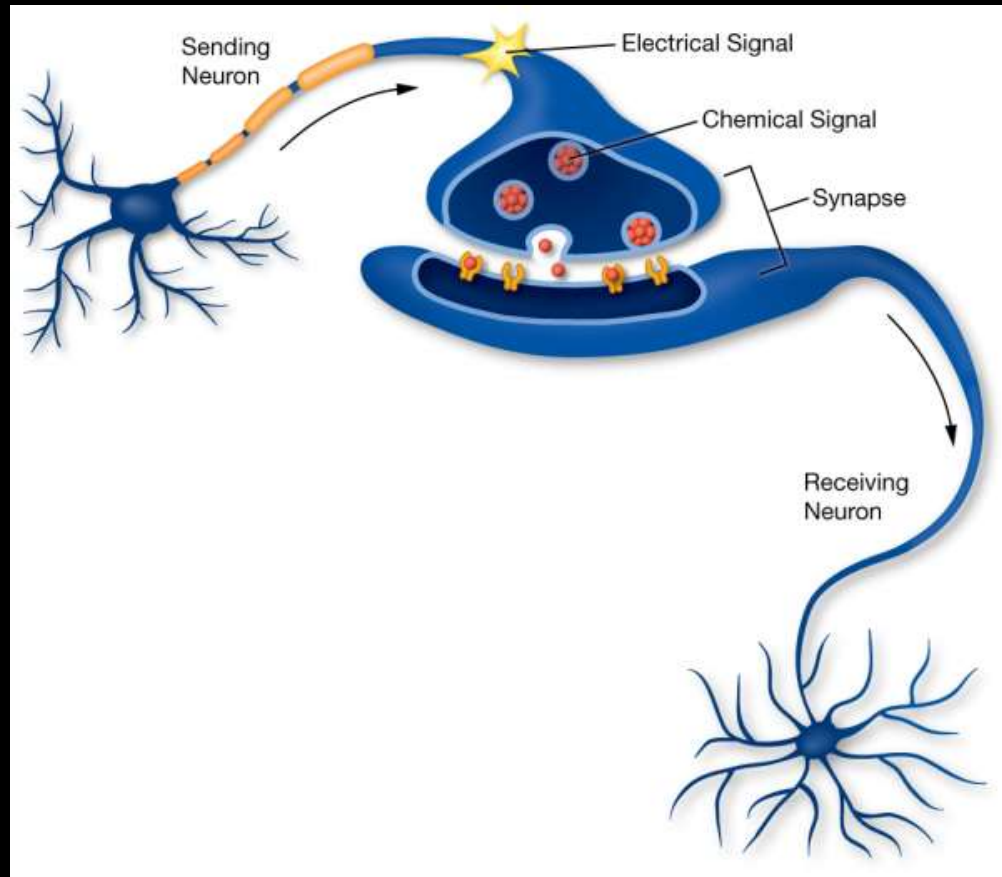
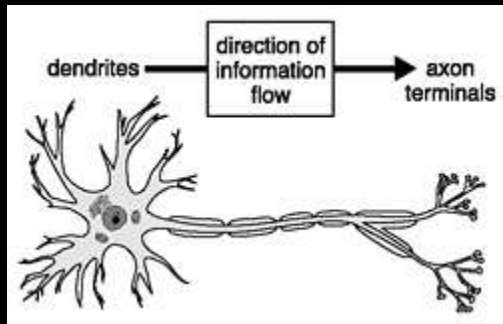


What types of cells make up our brain?



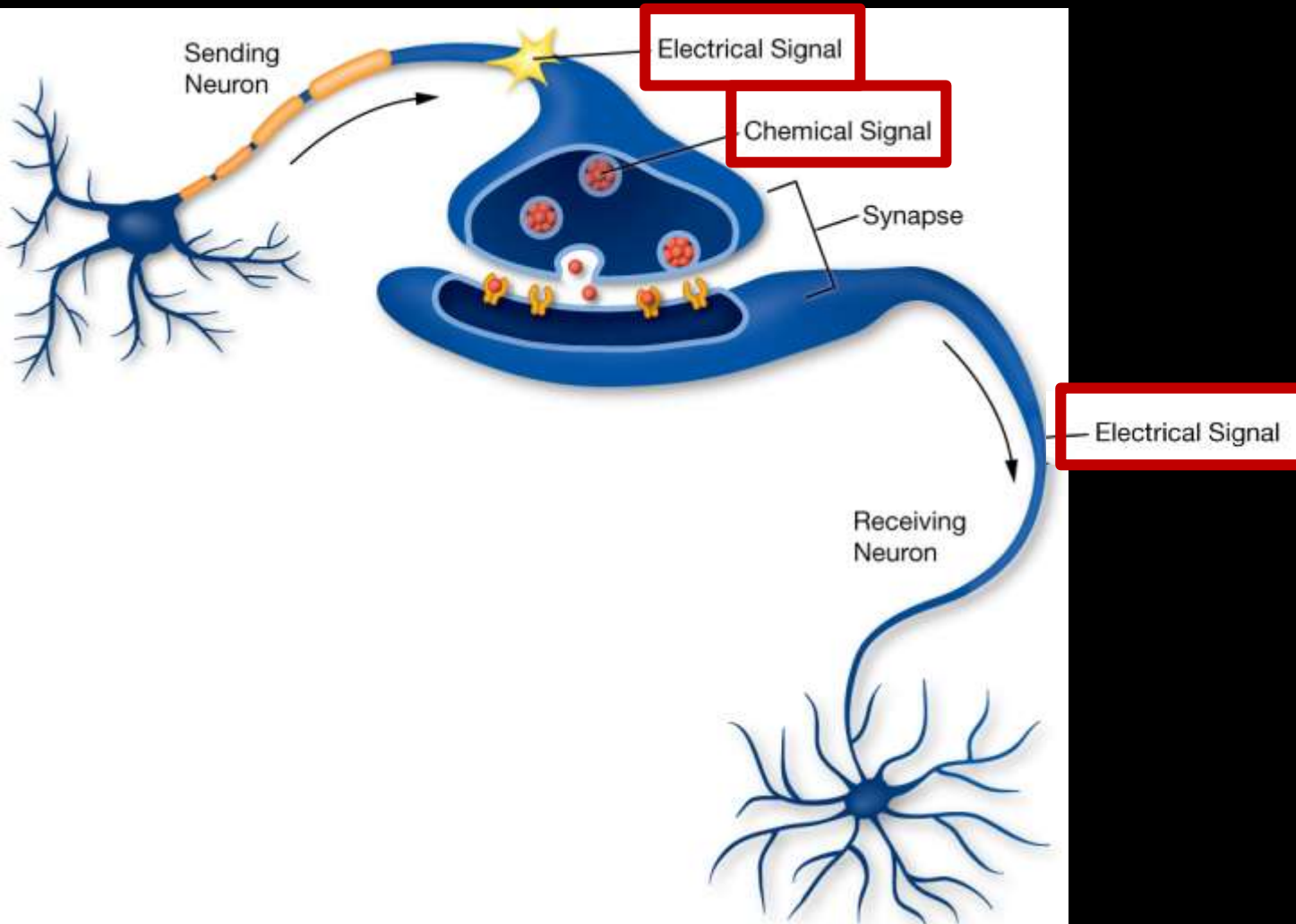
What do neurons do?

- Communicate!

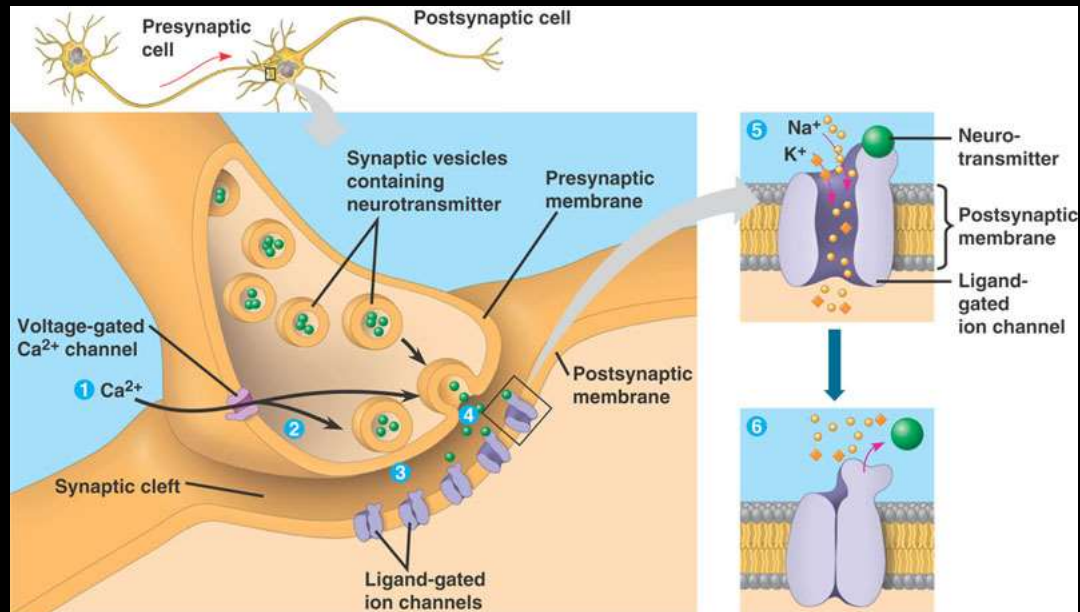


What does this communication look like?

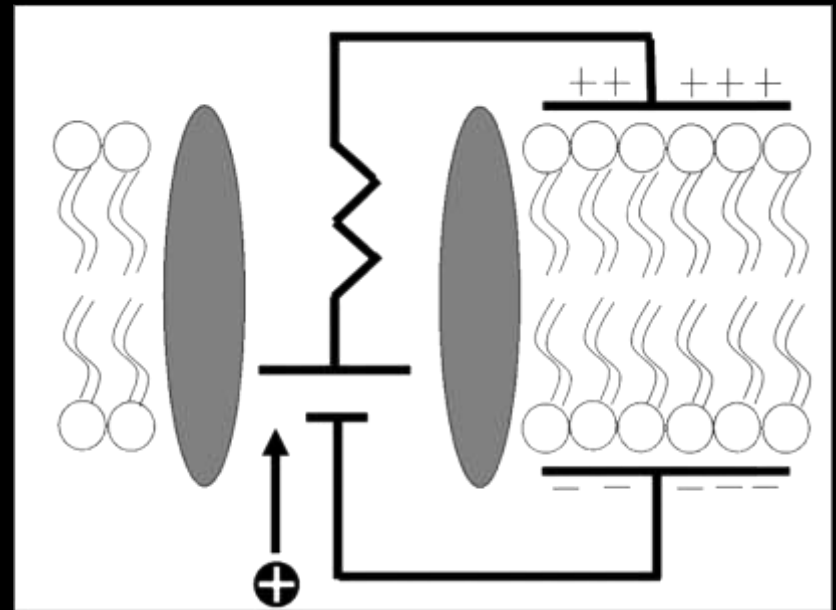
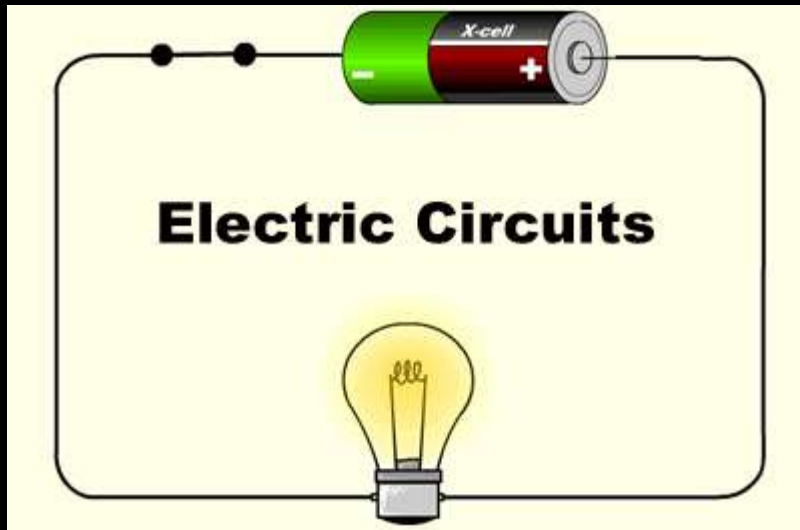
Electrical → Chemical → Electrical



The Chemical Synapse



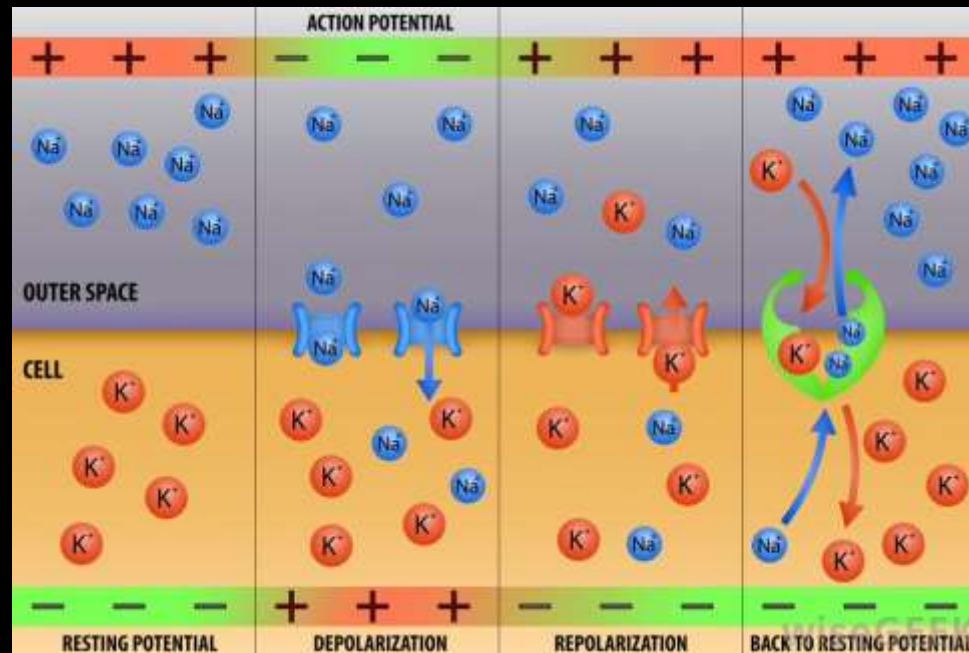
Resting Membrane Potential



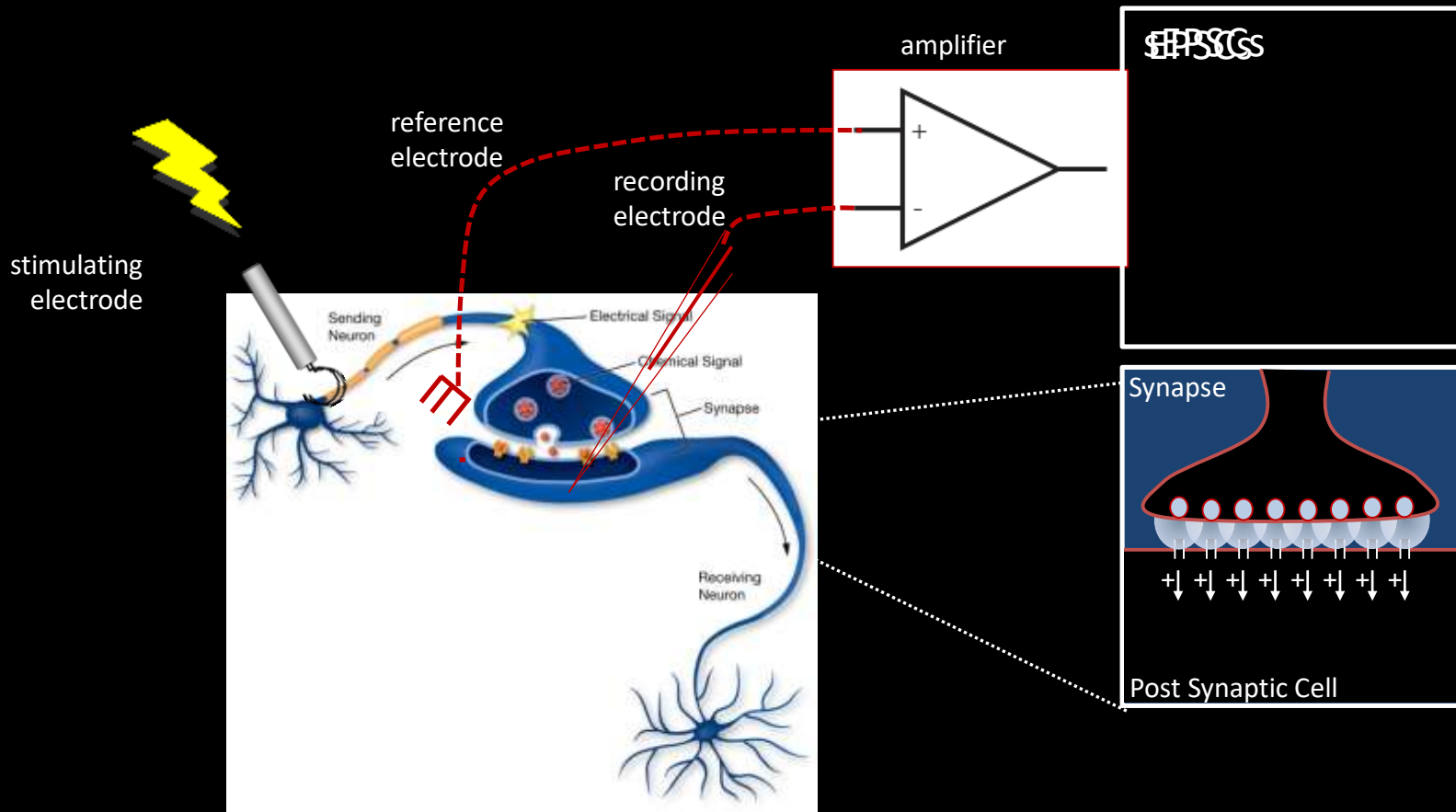
- Difference in ionic concentration creates an **Electro (charge) Chemical (concentration) gradient.**

Action Potential Players: the movement of two ions

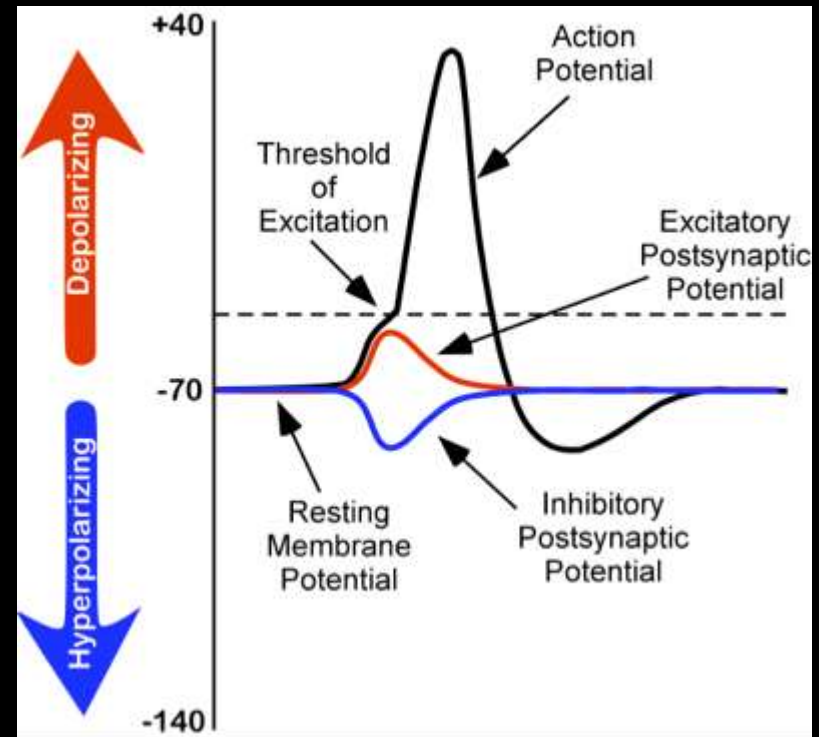
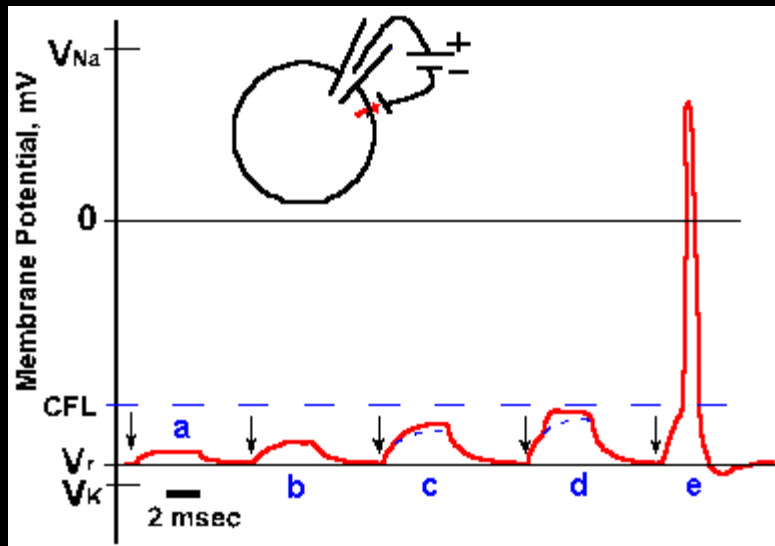
- Na^+ - a ton of sodium outside the cell, really wants to come in.
- K^+ - more potassium inside vs outside and really wants out.
- **Voltage-gated channels** – only open when they detect a voltage change.



Measuring synaptic function

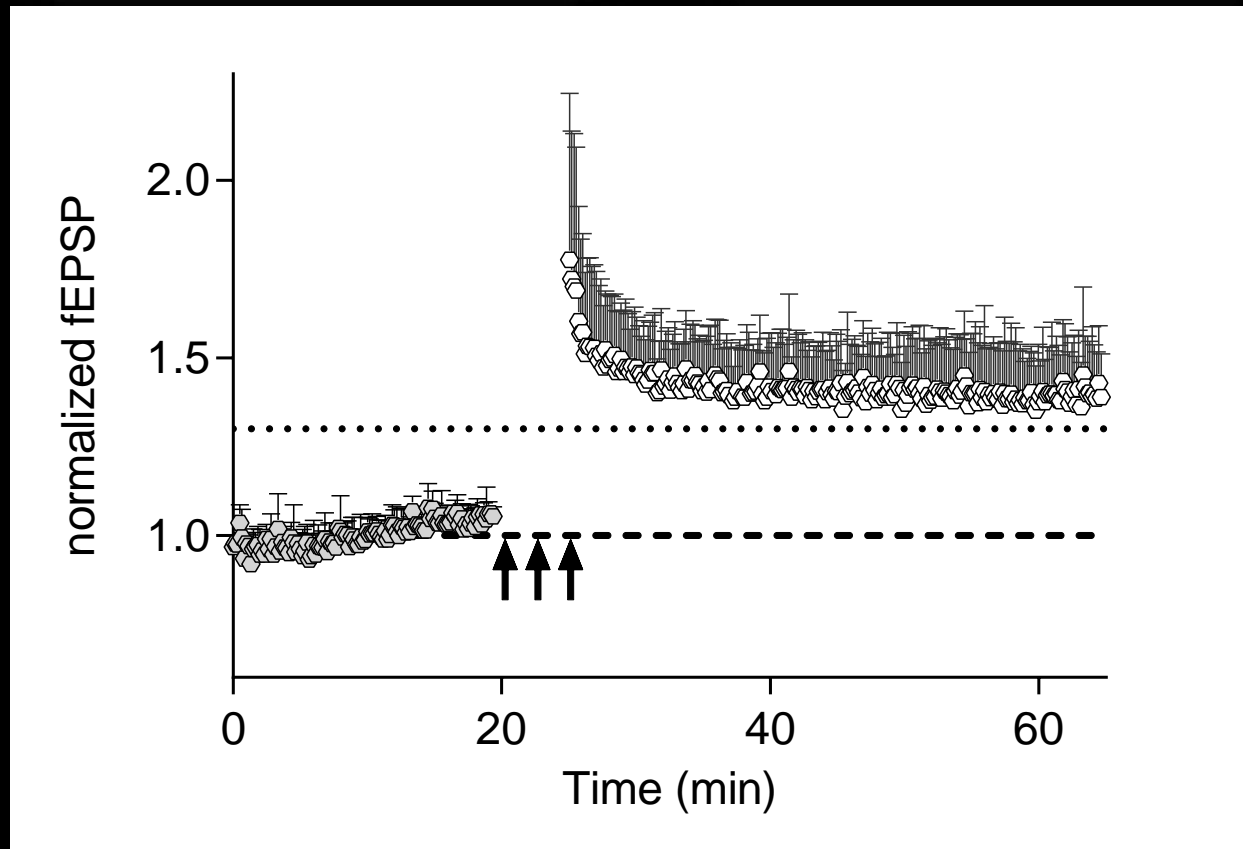
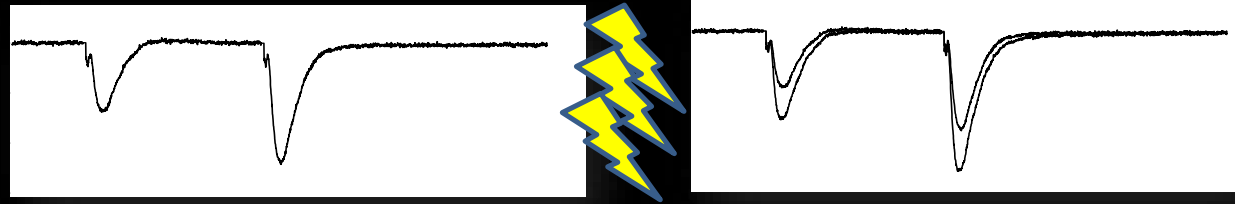


How to generate an Action Potential:



How do synapses wire together or how
to they re-wire?

How do synapses wire together or how to they re-wire?



My Big Question

Use-dependent activation of voltage-gated calcium channels restores evoked neurotransmission to synapses comprehensively intoxicated by botulinum neurotoxin serotype A

AKA

How do toxins/toxicants change how neurons “talk” to one other?

How can we rescue them?

Neurotoxicology

What can harm or kill our neurons (brain cells)?



Diseases

(Parkinson's, Alzheimer's, MS, ALS)

Bacteria

(Meningitis, botulism, tetanus)

Environmental
(Lead, Methylmercury, PCBs)

Drugs
(Alcohol, cocaine, nicotine)

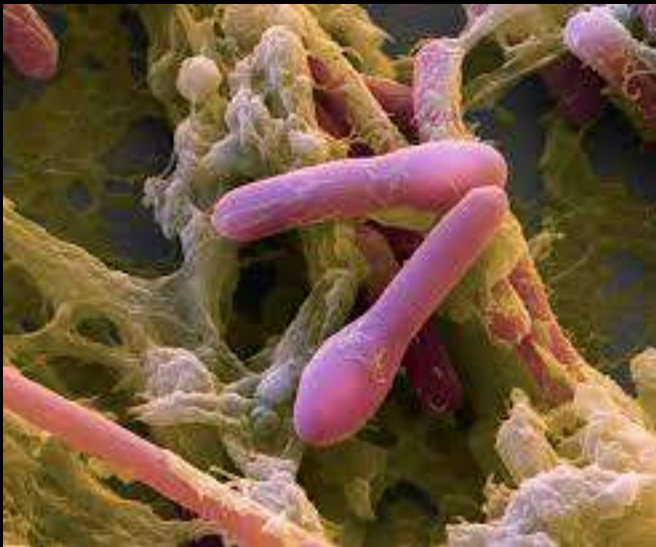
Occupational
(Solvents, Pesticides)

Neurotoxicants

- General mechanisms by which these agents disrupt presynaptic processes associated with transmission include:
 - Disruption of axonal excitability (pyrethroid **insecticides**)
 - Disruption of intracellular buffering of calcium (**heavy metals**).
 - Mechanisms by which these agents may disrupt postsynaptic processes include effects on transmitter degradation (organophosphates) or effects on the postsynaptic membrane receptors or associated ionic channels (organophosphates, antibiotics, and perhaps pyrethroids).
 - Disruption of calcium-dependent neurotransmitter release (heavy metals, antibiotics, certain snake and spider venom toxins, **botulinum toxin**)

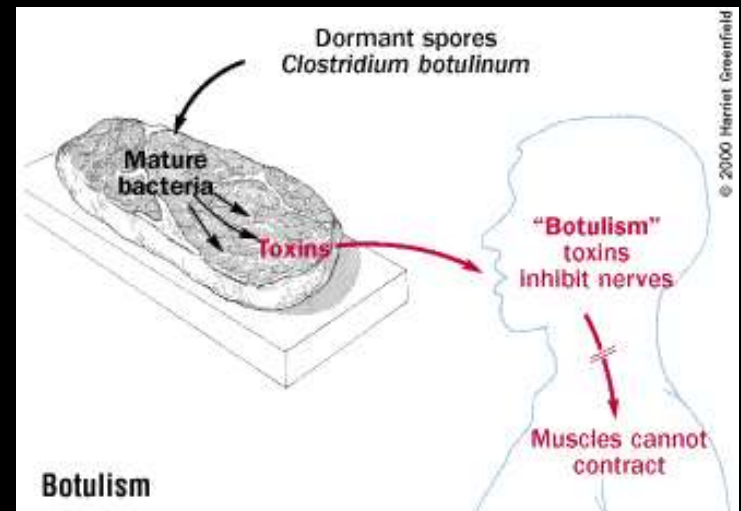
Clostridium botulinum

- Gram + rod, located everywhere (soil, vegetables, water)
- Anaerobic
- Spore forming (resistant to harsh environments)
- Seven subtypes of toxin produced (A-G)



How toxic is it?

- **Botulinum toxin** is the most toxic substance known.
- Botulism, a rare disease, occurs naturally as **four syndromes**:
 - (1) **food-borne illness** due to ingestion of toxin in contaminated food;
 - (2) **wound infection** due to wound colonization by toxigenic clostridia with in situ toxin production;
 - (3) **infant botulism** due to colonization of the infant intestine by toxigenic clostridia with in situ toxin production; and
 - (4) **adult intestinal toxemia**, a rare form of colonization with similarities to infant botulism.



Poison is in the dose

Botox treatment uses

- Migraines**
Minimize the frequency and severity of migraines
- Frown Lines**
Relax lines caused by frowning
- Crows Feet**
Reduce lines from the outer corner of the eye
- Under Eye lines**
Reduce tiny lines under eyes
- Bunny Lines**
Smooth horizontal lines on the nose
- Bruxism**
Prevent teeth grinding and make face more oval
- Pitted/Orange Skin**
Reduce the appearance of a dimpled chin
- Platysmal Bands**
Tightens neck, decreasing banding
- Neck Rings**
Diminish the appearance of neck rings
- Forehead lines**
Diminish the appearance of forehead lines.
- Glabellar lines**
Smooth the vertical lines (the 11's)
- Eyebrow lifts**
Relax the muscles that pull the brow line down
- Gummy smile**
Relax the top lip to reduce a gummy smile
- Lip Lines**
Smooth lines on the upper and lower lip
- Marionette Lines/Prejowl**
Turn corners of mouth up to improve prejowl
- Hyperhidrosis**
Dramatically reduce sweating in the armpits, palms



EATING AND HEALTH

Nacho Cheese Sauce Tainted With Botulism Kills California Man

May 23, 2017 - 12:45 AM ET



A rare outbreak of botulism has hospitalized nine people and killed one man in northern California, health officials say.

The outbreak began early last month when several people fell ill after eating nacho cheese sauce bought at a gas station in Walnut Grove, Calif., just outside Sacramento.

Health officials removed four batches of nacho cheese sauce from the Valley Oak Food and Fuel gas station on May 5. Now, that cheese has tested positive for the botulinum toxin, the California Department of Public Health says.

In a statement issued Monday, the department said it "believes there is no continuing risk to the public."

The man who died, Martin Galindo, had been in the hospital for several weeks before his death, according to a

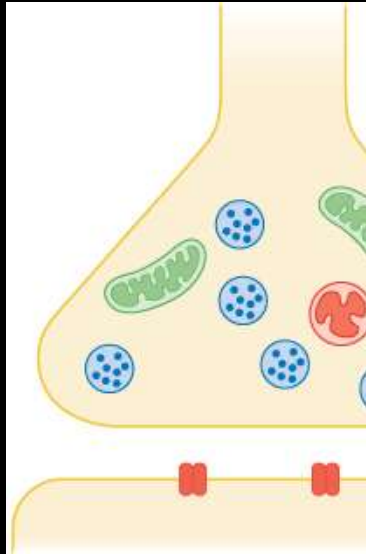


A depiction of *Clostridium botulinum*, the bacteria that create a deadly toxin. The preferred toxin can be found in home-canned foods and some retail products, such as canned chesee, chili sauce and oil infused with garlic.

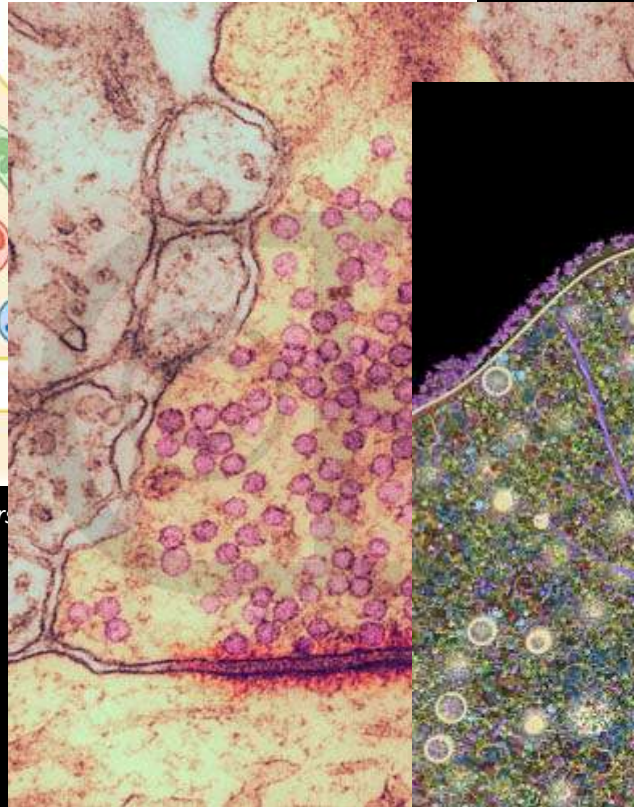


What make Botulinum toxin a neurotoxin?

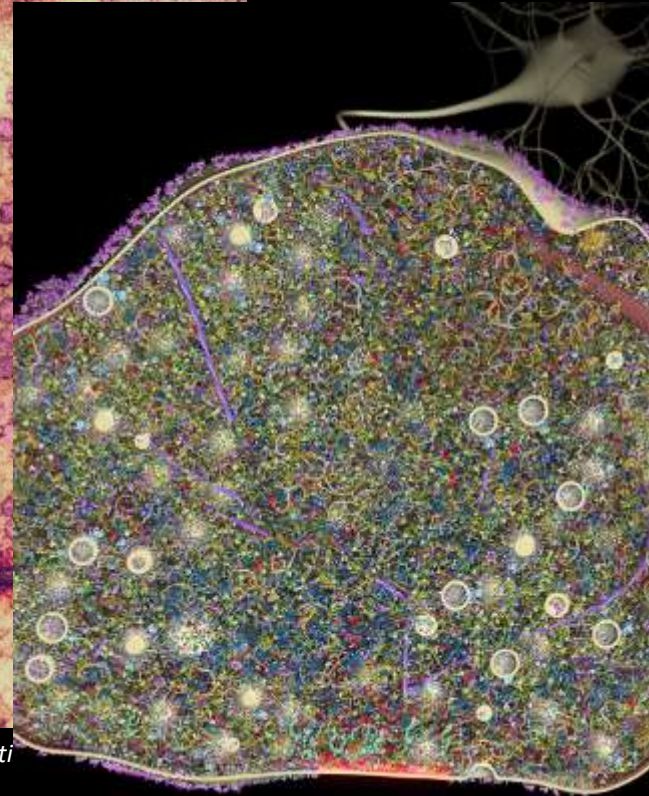
Botulinum Neurotoxin (BoNT) and the presynaptic compartment



<http://www.r>



<http://www.scienceti>



Wilhelm *et al. Science* 2014, **344**(6187): 1023-1028

Botulinum Neurotoxin (BoNT)

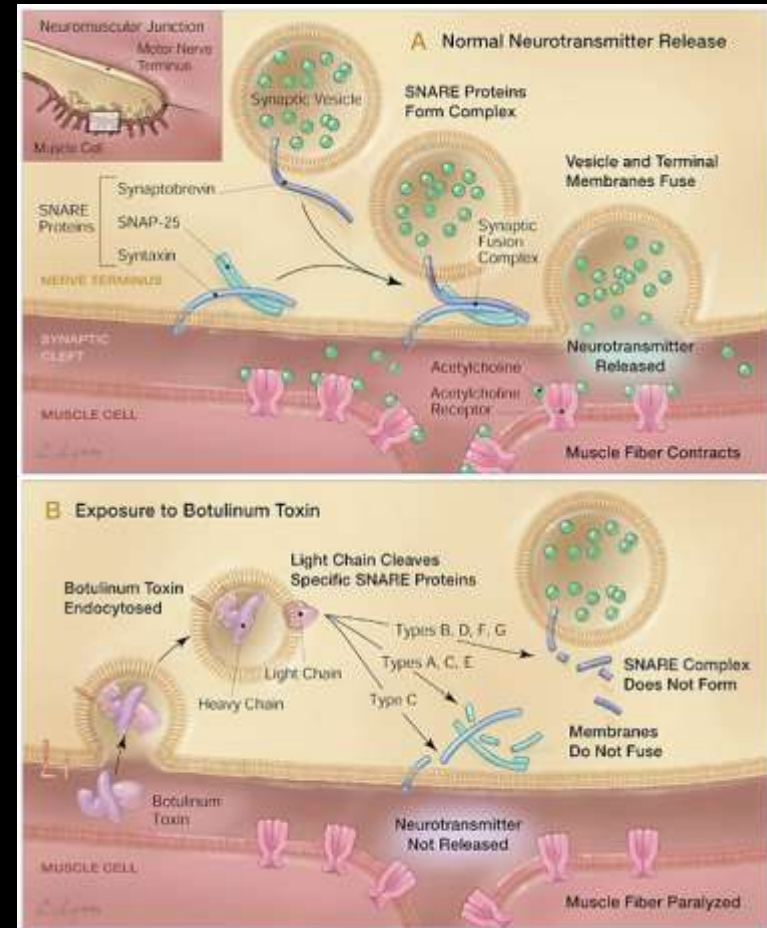
- Most potent substance known: human LD₅₀ of 0.1-1.0 ng/kg
- Cleave presynaptic SNARE proteins, preventing neurotransmitter release
- Paralysis can last months; there is no therapeutic treatment

Clostridium botulinum



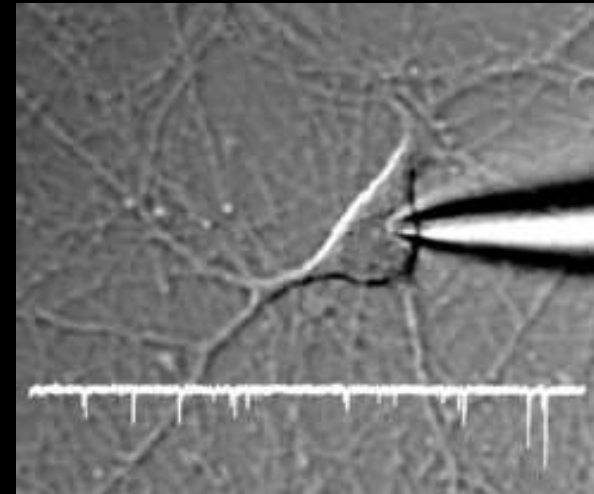
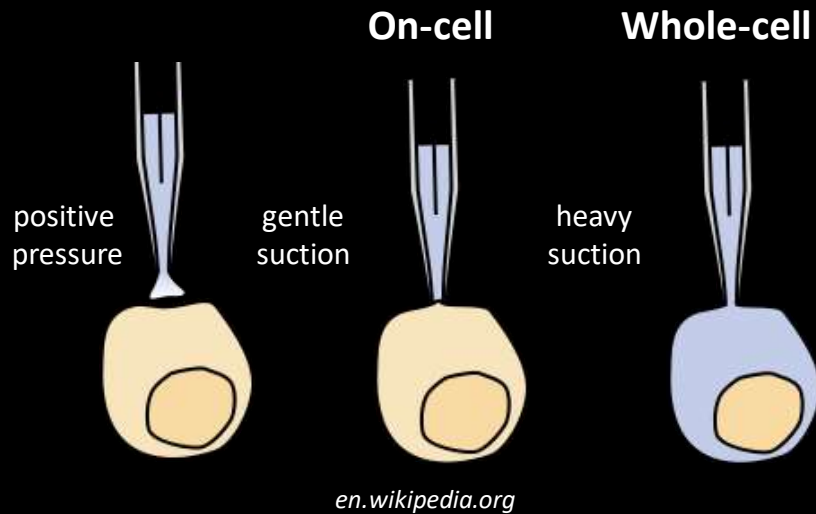
https://en.wikipedia.org/wiki/Clostridium_botulinum

BoNT mechanism of action



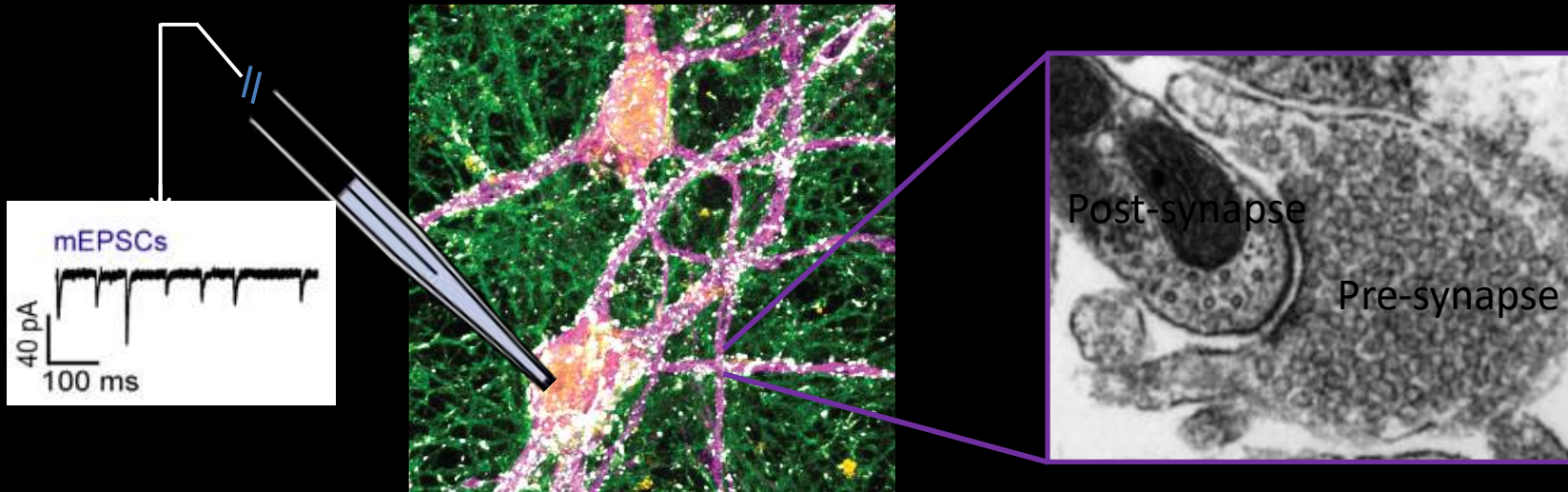
Arnon SS *et al.* (2001). JAMA, 285(8): 1059-1070

Measuring synaptic activity *in vitro* with whole-cell patch-clamp electrophysiology



The continuous electrical connection between the recording electrode and the cytoplasm of a neuron allows the detection of small ion currents resulting from synaptic neurotransmission

Miniature excitatory post-synaptic currents (mEPSCs) as a measure of presynaptic neurotransmitter release



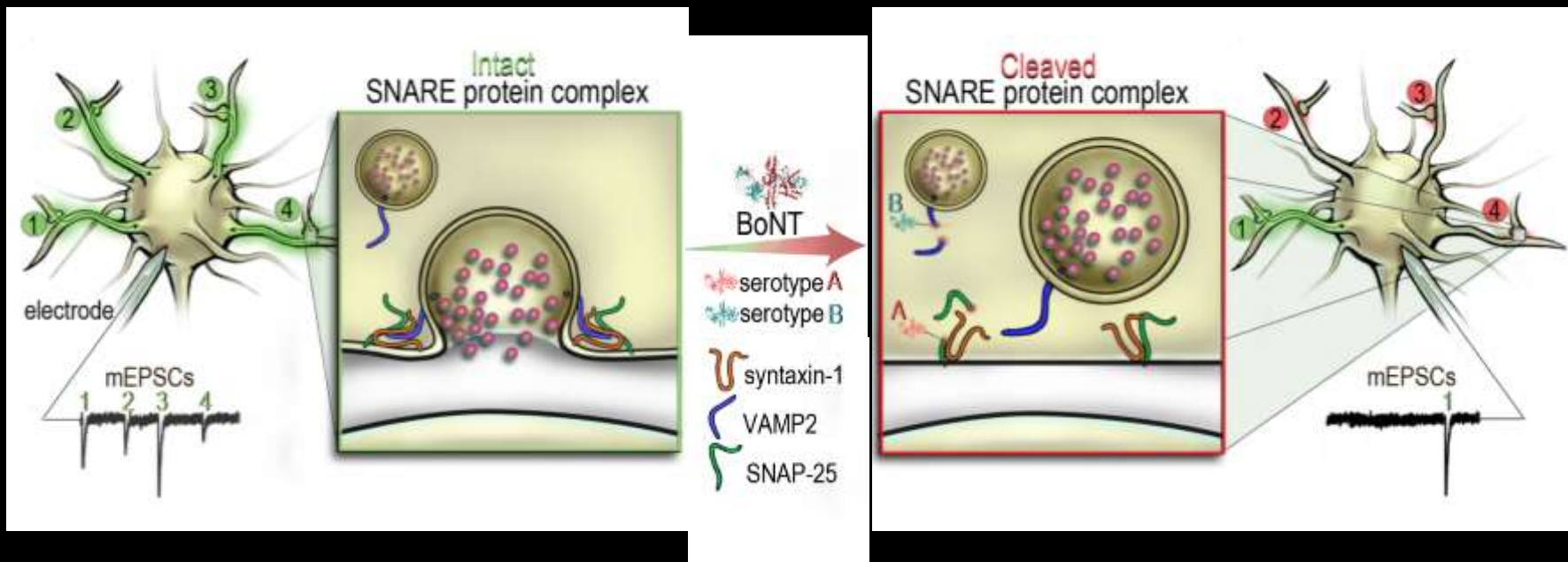
Spontaneous release of neurotransmitter in the absence of an action potential is a salient feature of all active synapses

Release occurs in a probabilistic manner at a given synapse at low frequency (once every 10-100 minutes)

In CNS neurons, the large number of synapses per neuron (1,000 to 10,000) results in post-synaptic events with aggregate frequencies at high frequency (1-10 times per second)

Thus, whole-cell recordings allows simultaneous evaluation of release from hundreds to thousands of pre-synaptic compartments with single synapse-level resolution

Measured Inhibition of Synaptic Transmission (MIST) assay for neurotoxin studies



Step 1: Measure frequency (Hz) of spontaneous synaptic activity (mEPSCs) in vehicle-treated neurons (n = 8 - 12 recordings)

Step 2: Add neurotoxin at desired concentration for 20 h

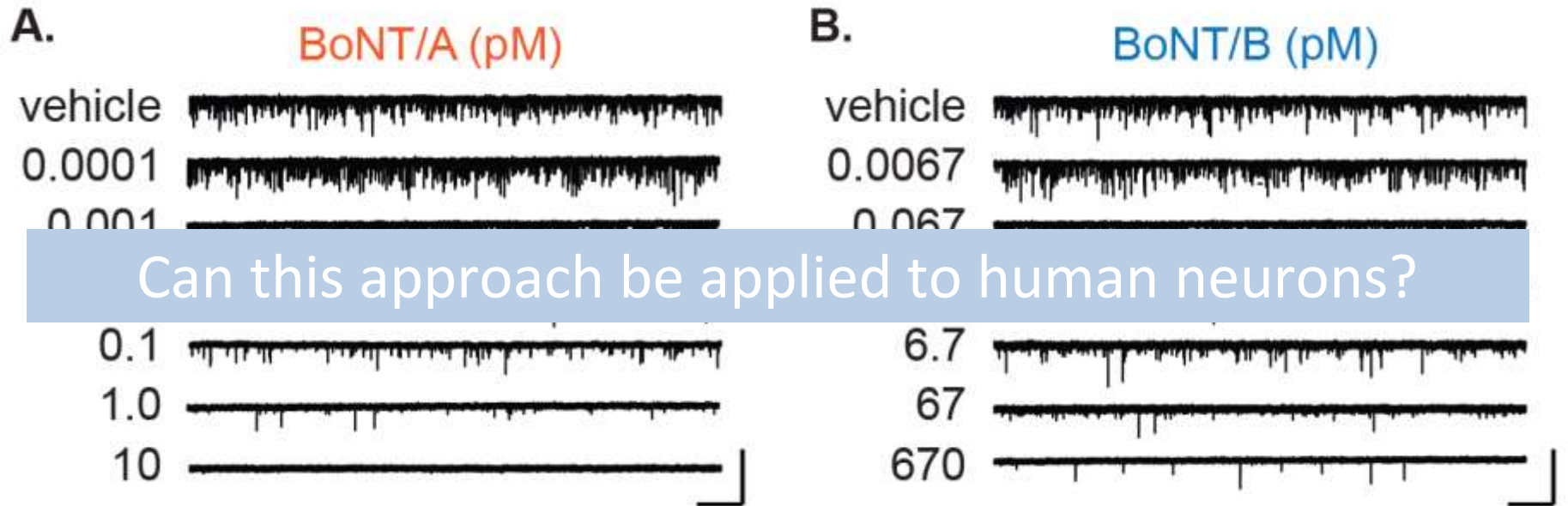
Step 3: Measure rate of spontaneous synaptic activity in toxin-treated neurons (n= 8-12 recordings), normalize data to vehicle-treated control and determine % inhibition

Synaptic function-based assays

Impaired synaptic activity can be used as a functional correlate of intoxication

Function-based assays thus allow us to directly evaluate the pathophysiological responses to intoxication that are responsible for the clinical manifestation of botulism

Not only does this avail us of a highly sensitive detection platform, but it also facilitates the function-based evaluation of therapeutic treatments *in vitro*



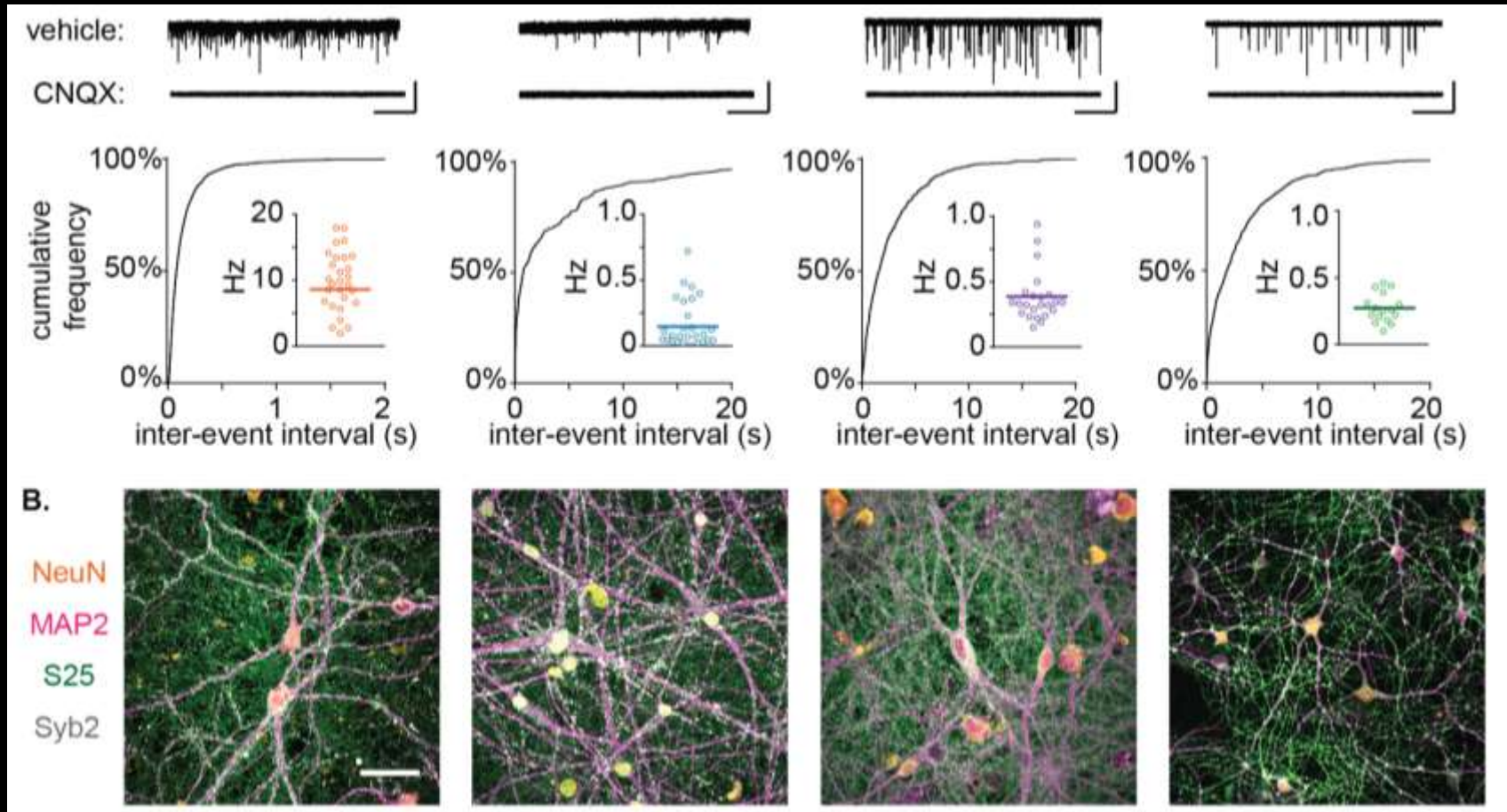
Comparison of synaptic activity in diverse *in vitro* neuron cultures

DAP 24 mouse embryonic stem cell-derived neurons

DAP 85 HIPTM neurons

DAP 16 primary rat cortical neurons

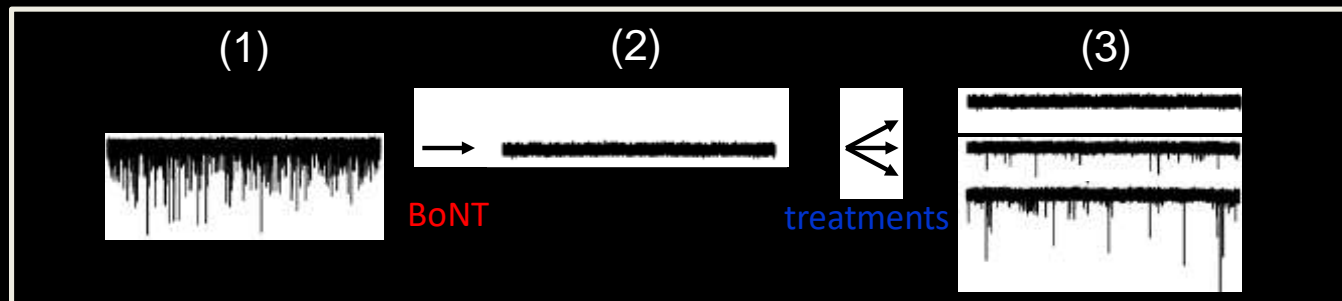
DAP 12 primary rat cerebellar neurons



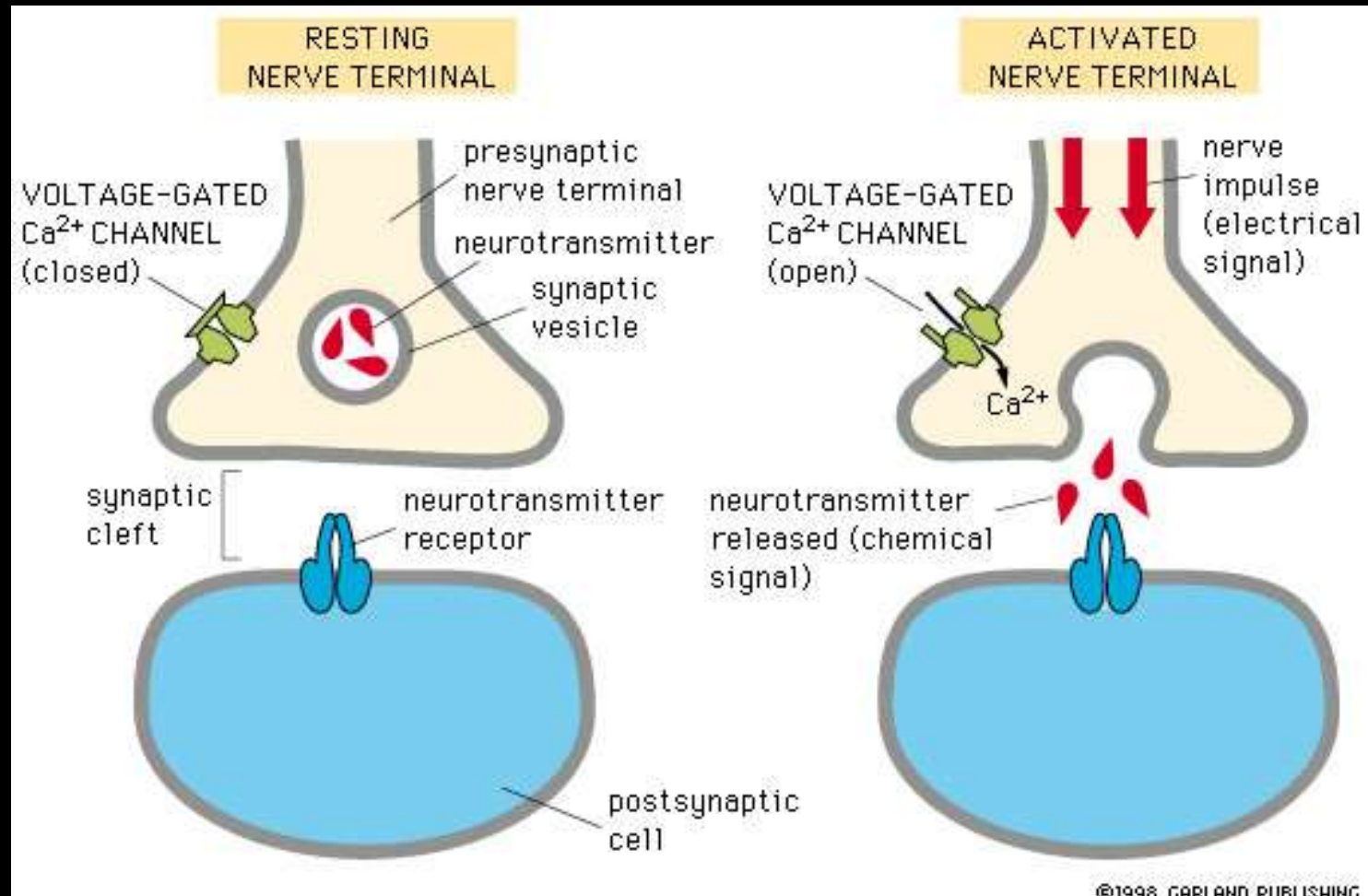
Therapeutic screening strategy
“Restoration of Quantal Exocytosis” (ResQuE) assay

Steps

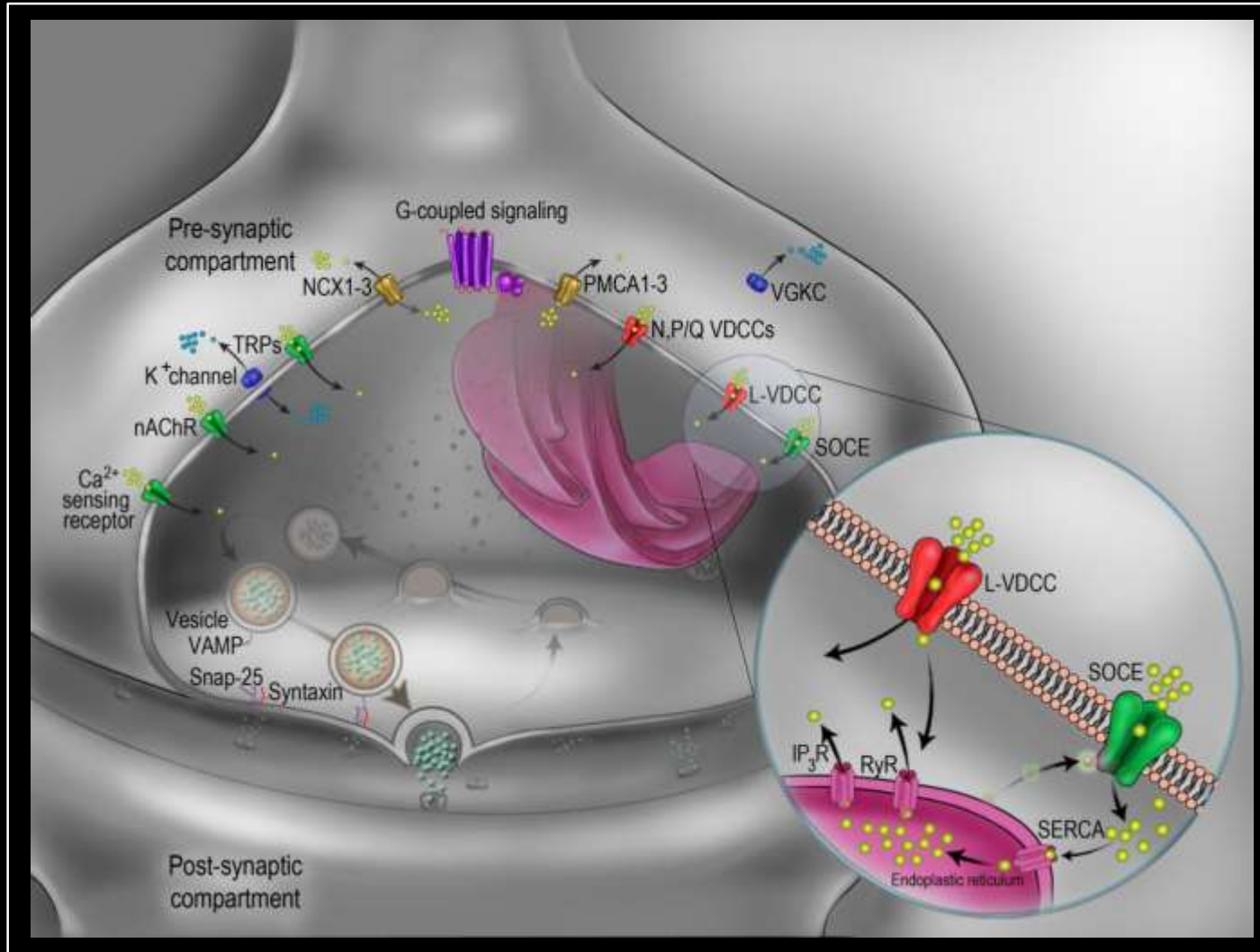
- (1) Confirm spontaneous synaptic activity using whole-cell patch-clamp electrophysiology
- (2) Thoroughly intoxicate with BoNT ($10e^5 \times IC_{50}$ values) for 20 h and functionally confirm synaptic blockade
- (3) Measure spontaneous rates of monosynaptic activity in presence of candidate treatments



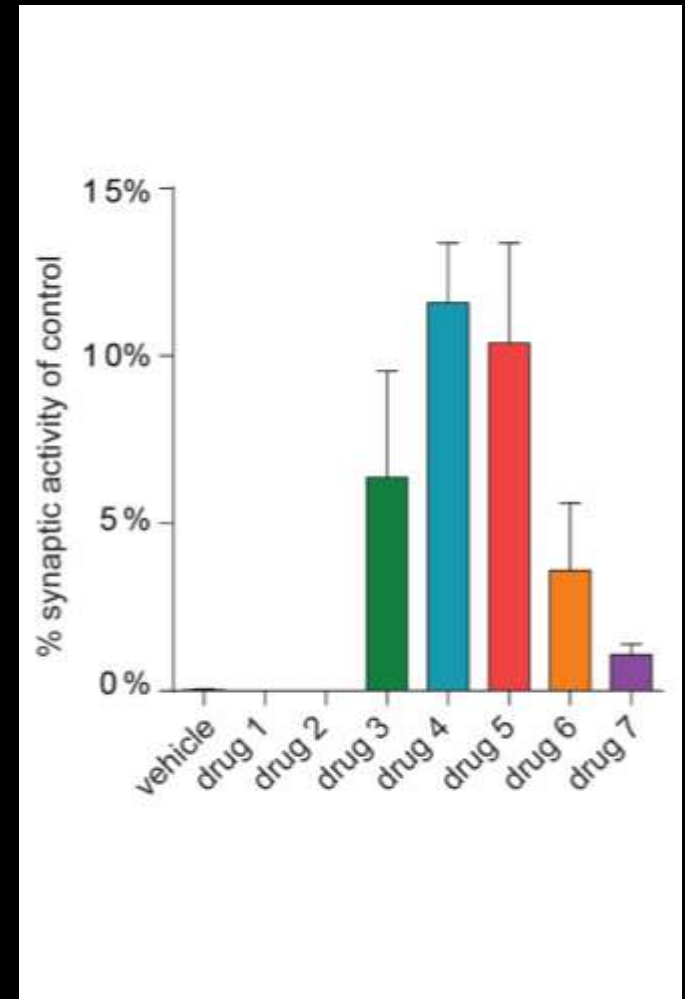
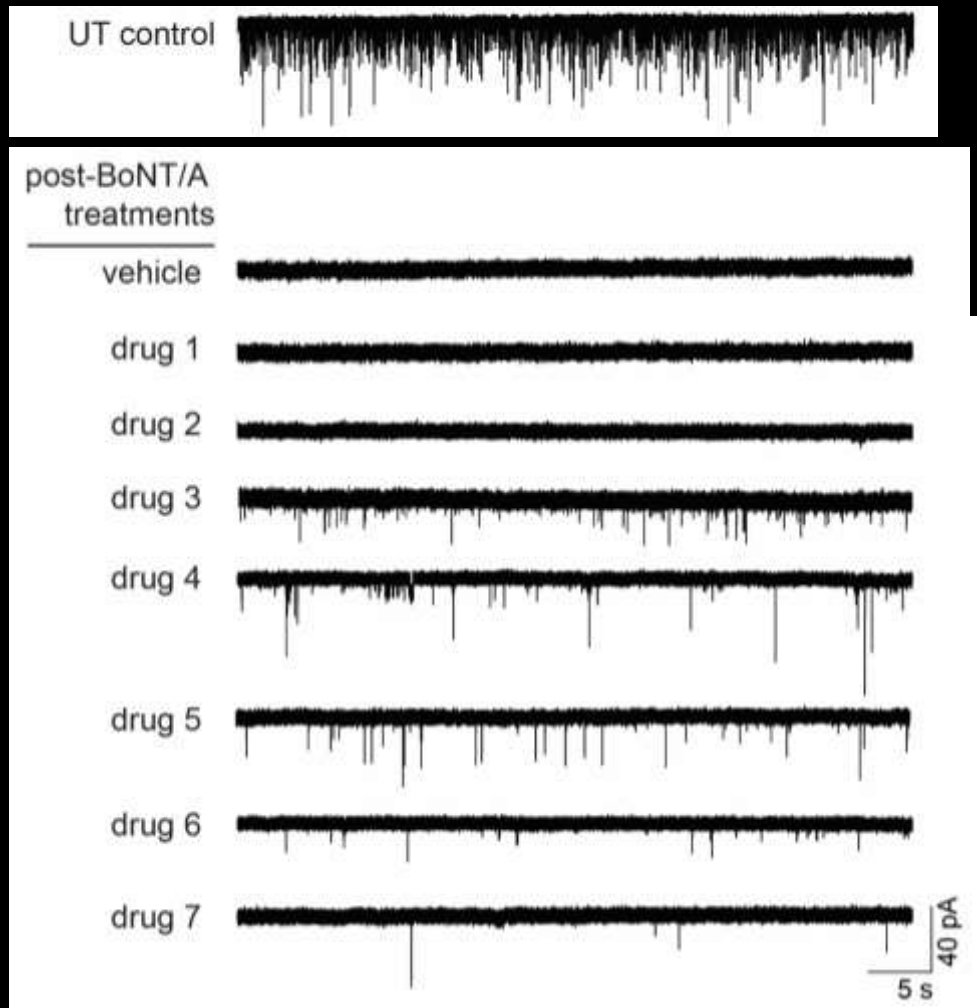
What do you need to make a synapse fire?



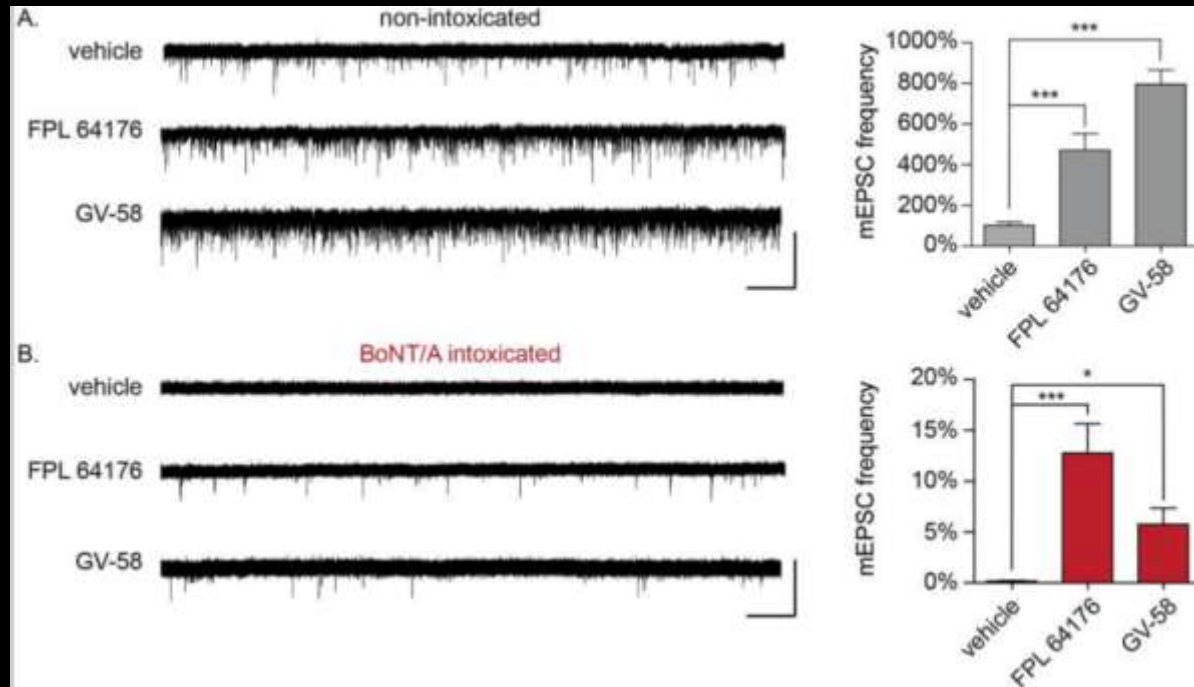
Potential targets to modulate presynaptic Ca^{2+} levels



In vitro screening of candidate therapeutic drugs to restore neurotransmission in BoNT/A-intoxicated synapses



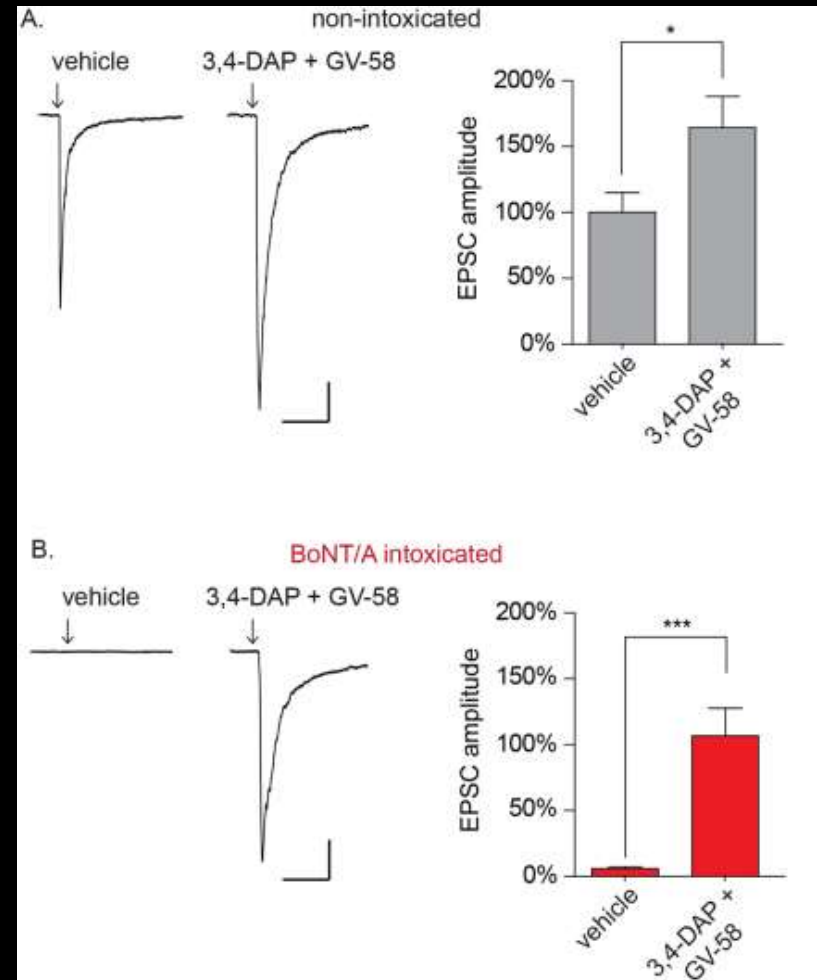
Drugs that rescue synapse function



Hoffman *et al.*, Scientific Reports 2017, 7:15862

...and completely restore function

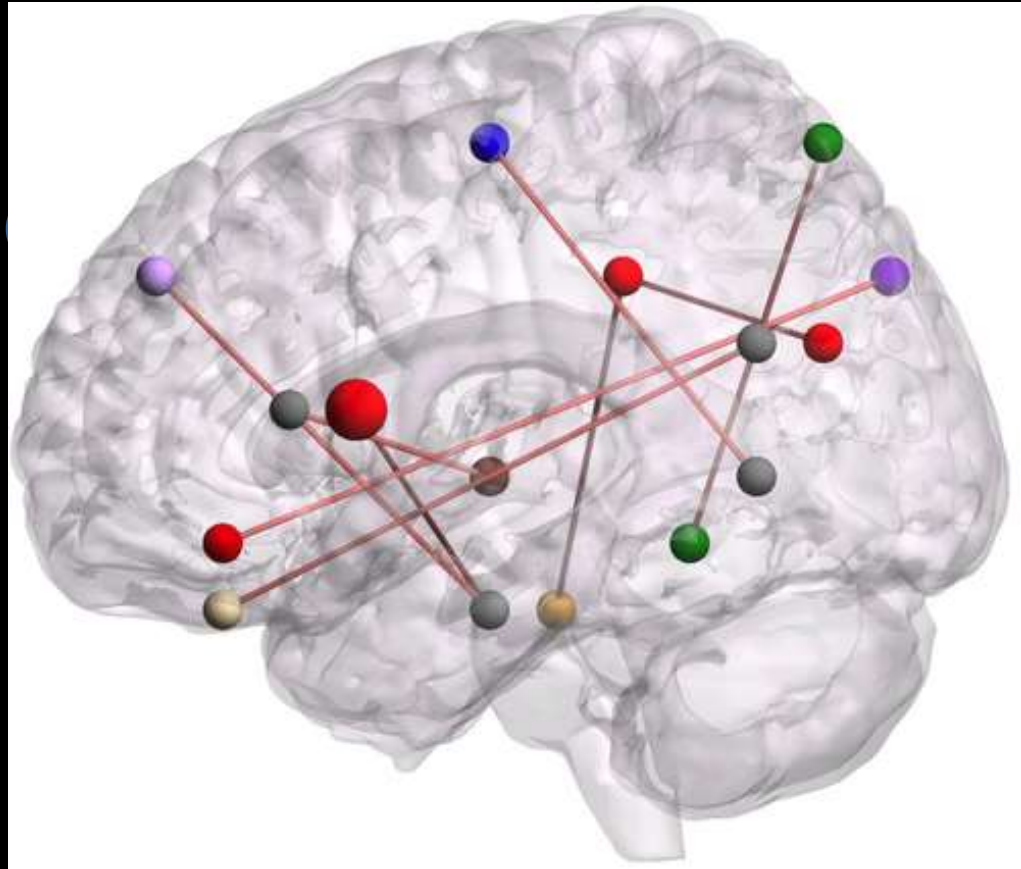
By stimulating release of neurotransmitter and applying two drugs at the same time we can completely restore synaptic function.



Take Away

- We have a lot of neurons
- Those neurons uses electrical-chemical-electrical signaling
- Neurons that fire together wire together
- Neurotoxins vary widely
- Botulinum neurotoxin is incredibly potent
 - Effects pre-synaptic release of neurotransmitter
 - Can rescue this by increasing the amount of pre-synaptic Ca^{+2}

Why does it matter?



Thank you

Questions?