IH & GABA

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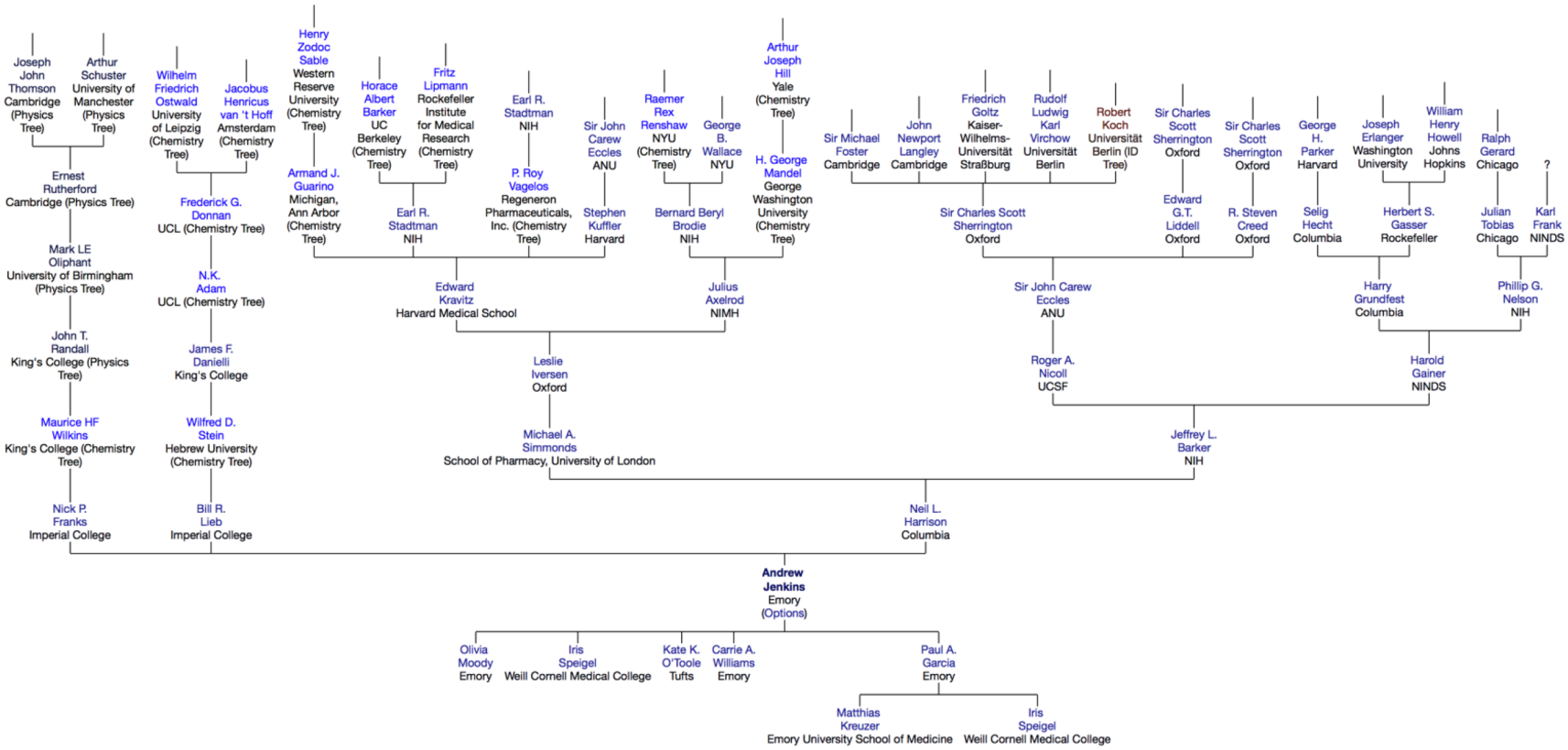
Personal/Professional Financial Relationships with Industry

External Industry Relationships *	Company Name	Role
Equity, stock, or options in biomedical industry companies or publishers	Balance Pharma / pending	IP license agreement
Board of Directors or officer	Somnolytics	Scientific Founder
Royalties from Emory or from external entity	Balance Pharma, $7,000 in 2016	IP license fee
Industry funds to Emory for my research	None	
Other	None	

Off label use of flumazenil (Romazicon)

www.neurotree.org

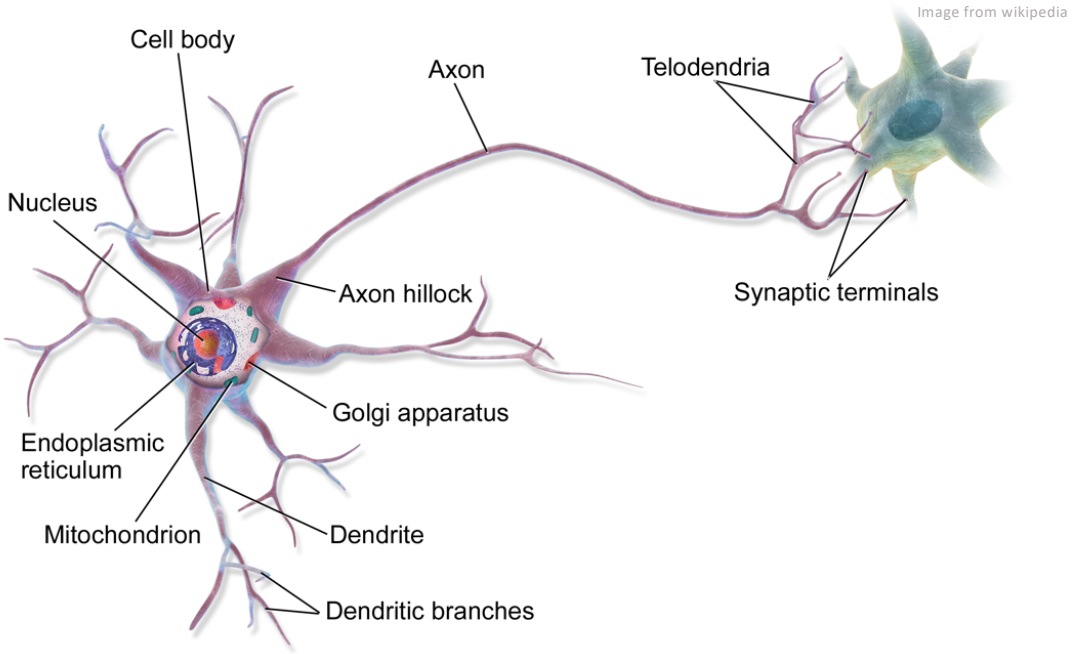


Talk Overview

- How do our brain cells communicate?
- How do drugs and disease change this?
- What is GABA?
- Why is it important in IH?
- ***How can we relieve hypersomnia symptoms with drugs working on GABA?***
- ***What does the future hold for IH-GABA research?***

Take Home Messages

- Our brain contains cells called **neurons**
- Neurons are responsible for sending and receiving chemical messages in the brain
- Neurons use **synapses** to send and receive these chemical messages
- Synapses use receptors to capture the chemical messages
- We call these messages **neurotransmitters**



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Take Home Messages

- We call these messages **neurotransmitters**
- These messages can be “excitatory”
  - They tell other neurons to GO!
- These messages can be “inhibitory”
  - They tell other neurons to STOP!
- Neurons use several different neurotransmitter chemicals
- GABA is an inhibitory neurotransmitter

Take Home Messages

- GABA is an inhibitory neurotransmitter
- GABA binds to $GABA_A$ **receptors**
- Receptors are proteins that:
  - Bind chemical messages
  - After binding, the receptor changes shape
  - It initiates changes in the neuron
- $GABA_A$ receptors are ion channels
  - They let negative charge into neurons
  - This inhibits neuronal activity

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Take Home Messages

- $GABA_A$ receptors are ion channels
- $GABA_A$ receptors also are drug targets
  - Anxiolytics (valium)
  - Sedatives (ambien)
  - General Anesthetics (propofol, isoflurane)
- These drugs are all PAMs
  - Positive Allosteric Modulators
  - They make the receptor “work harder”
  - This generates more inhibition

Take Home Messages

- In IH, the brain contains an additional signal
- It is a $GABA_A$ receptor PAM
- During IH, your brain is being inhibited
- Brain fog, excessive sleepiness…
- These are similar to the effects of valium ambien, and propofol
- ***We are identifying the additional signal***
- ***We are developing strategies for blocking it***

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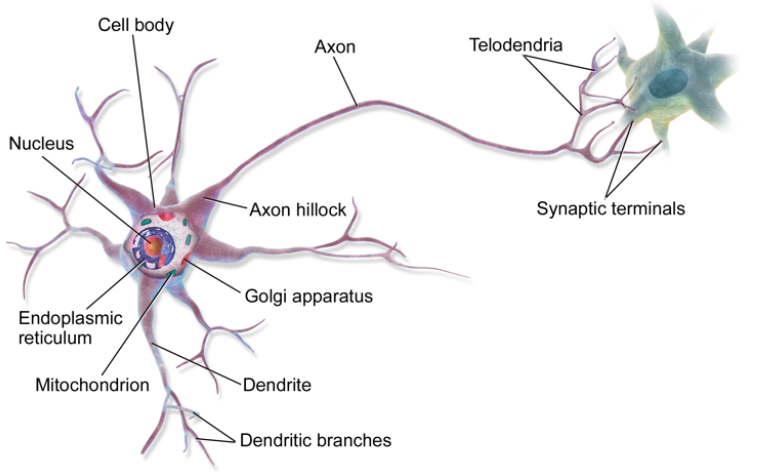
That was a LOT of information!

Take a deep breath – Let's go over those definitions again…

- **neuron**
- neurotransmitter
- synapse
- Receptor
- Neurons transmit and receive messages in the brain….

- agonist
- antagonist
- modulator
- **ion channel**

Image from wikipedia



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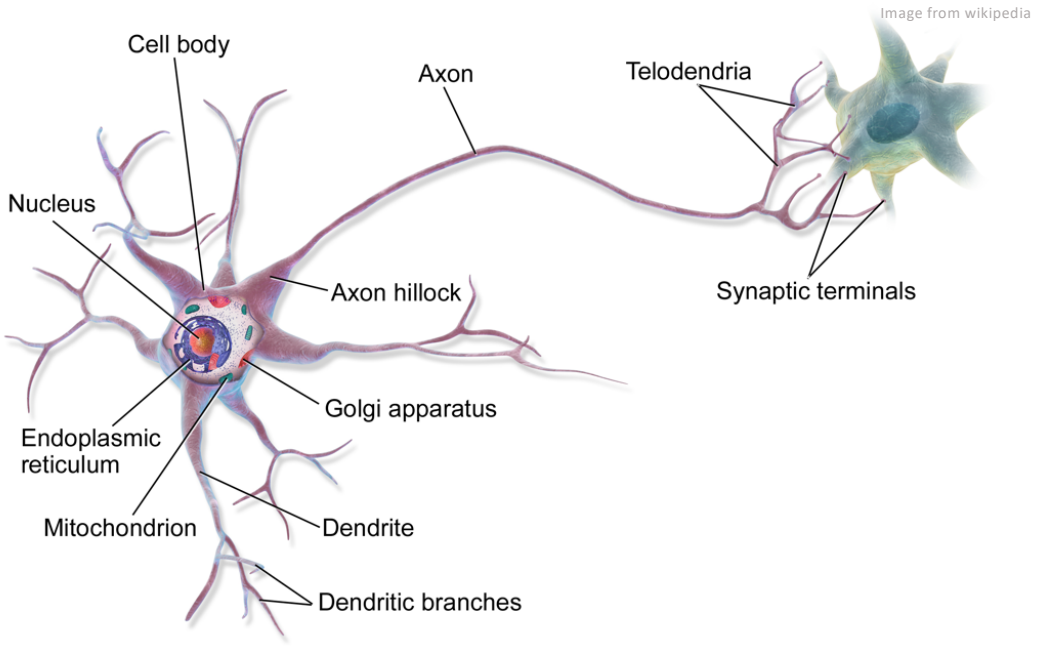
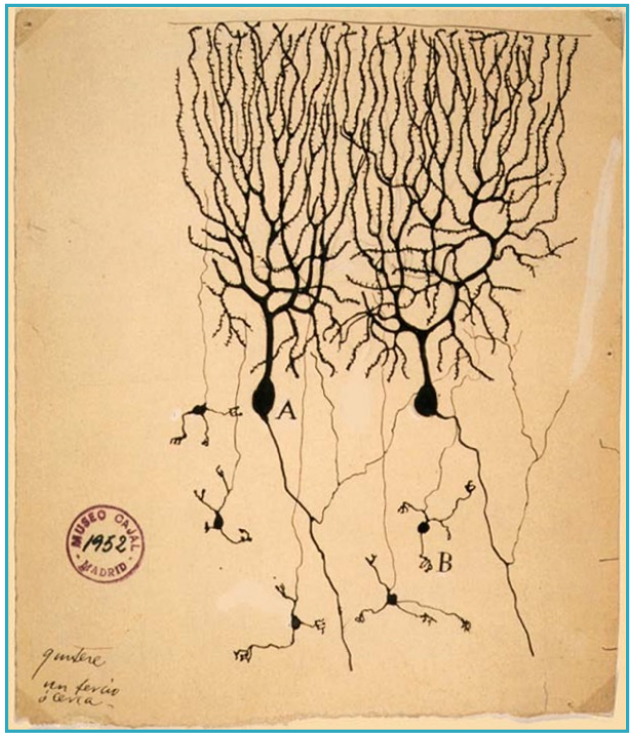
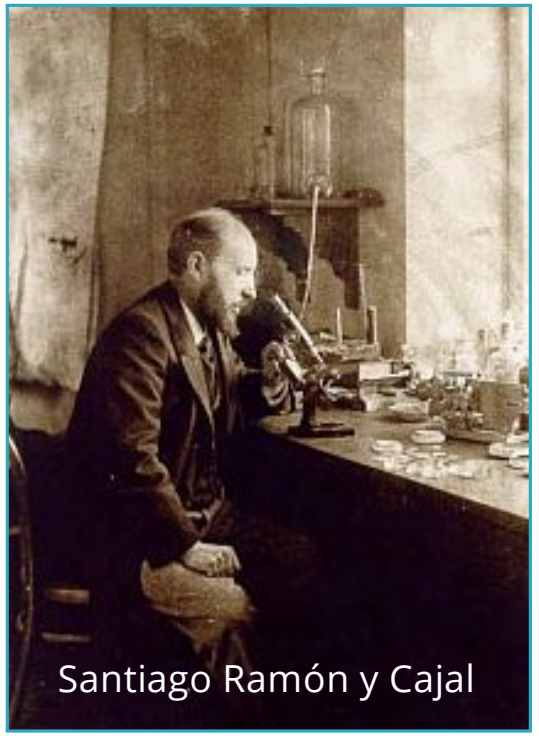


Your Brain Contains 85 Billion Neurons

Neuron

85 billion (85 x 10^9) per human brain



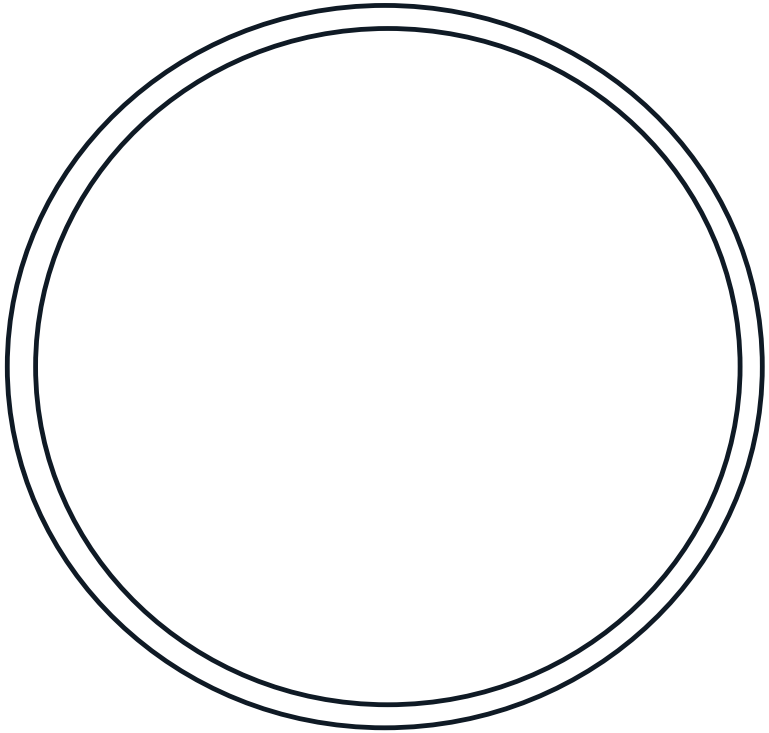


Images from wikipedia

What Makes Neurons so Special?

Neuroanatomy:

- Every neuron has a cell wall – also known as a membrane.
- This is a barrier to water and salts.



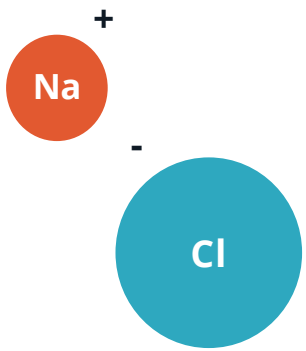
Electrochemistry

Salt: Sodium Chloride (NaCl)
When dissolved in water separates into:

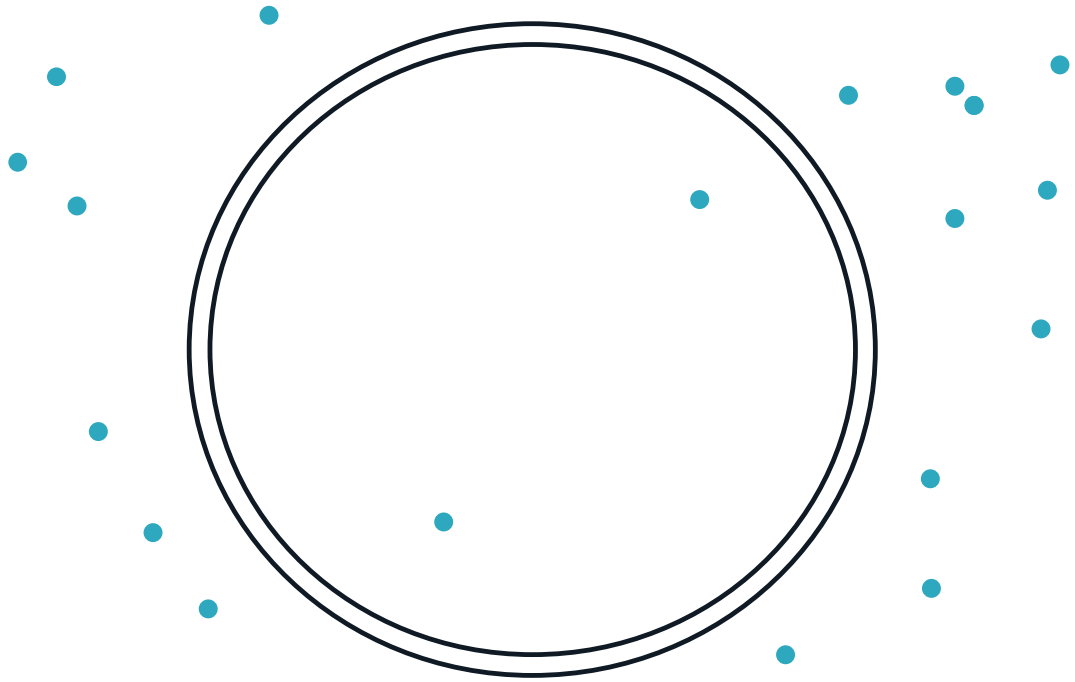
Na+ ions

&

Cl- ions



Every Neuron Has a Cell Wall.
This is a Barrier to Water and Salts.



Chloride (-)

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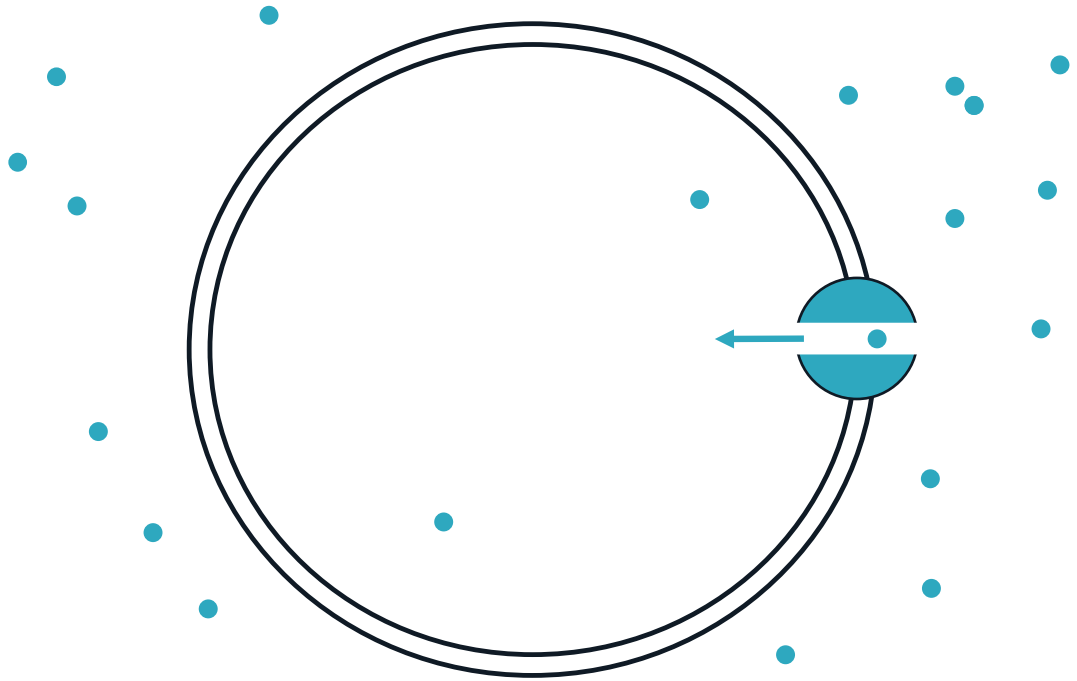
Ion Channels Let the Salts Cross the Cell Wall



Chloride (-)

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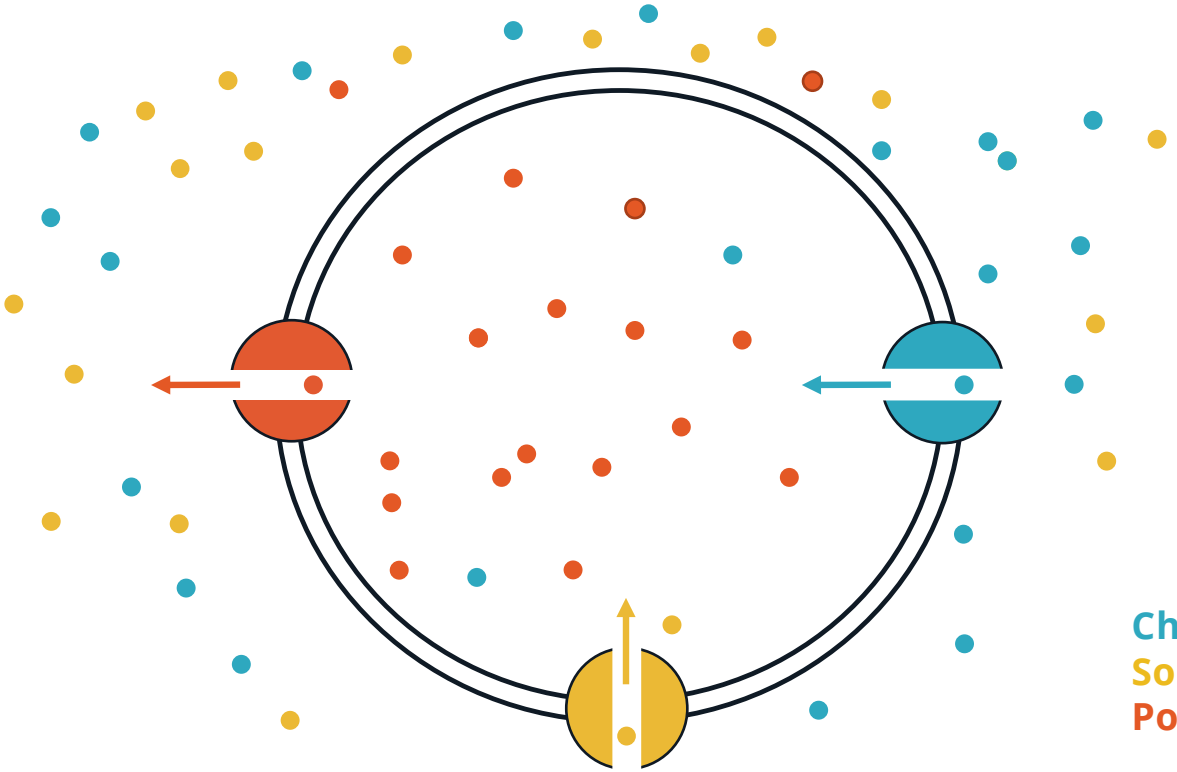
Neurons Have Many Different Ion Channels



Chloride (-)
Sodium (+)
Potassium (+)

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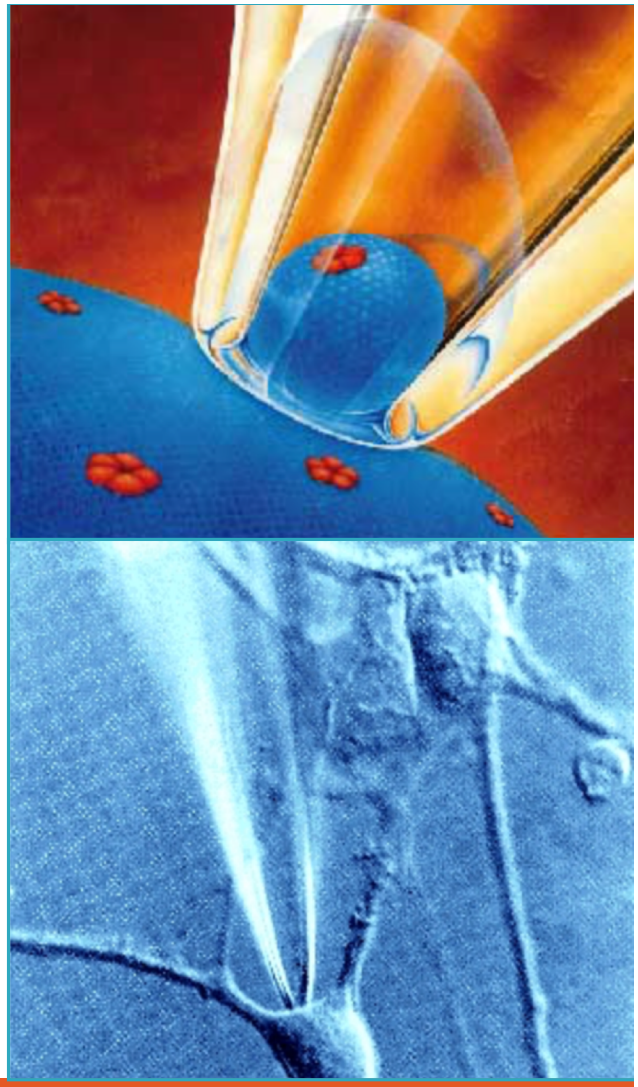
Electrochemistry

Moving ions = same as electrons moving in a wire current

Differences in number of ions inside vs outside the cell = **Voltage**

How does an electrophysiologist measure this?



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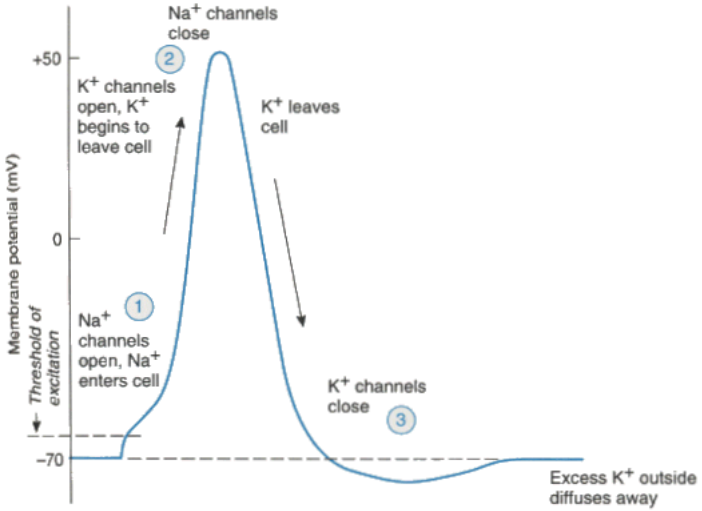
Electrophysiology:
The study of Electricity in the Body

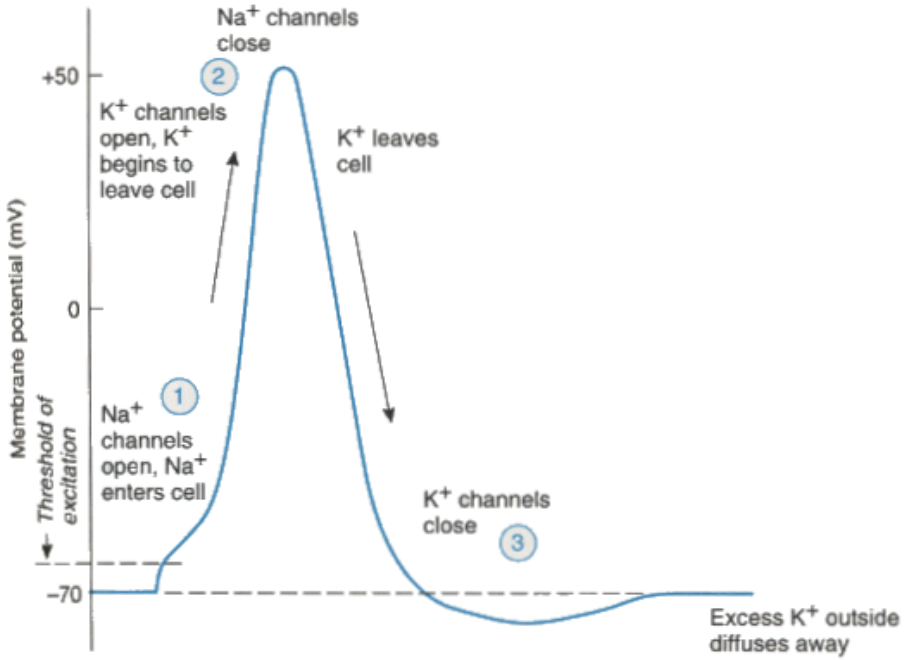
We measure currents and voltages in single cells



Equipment costs ~$100,000 to build, ~$70,000/year to operate
Emory / Jenkins lab only has 1 dedicated CSF set up.

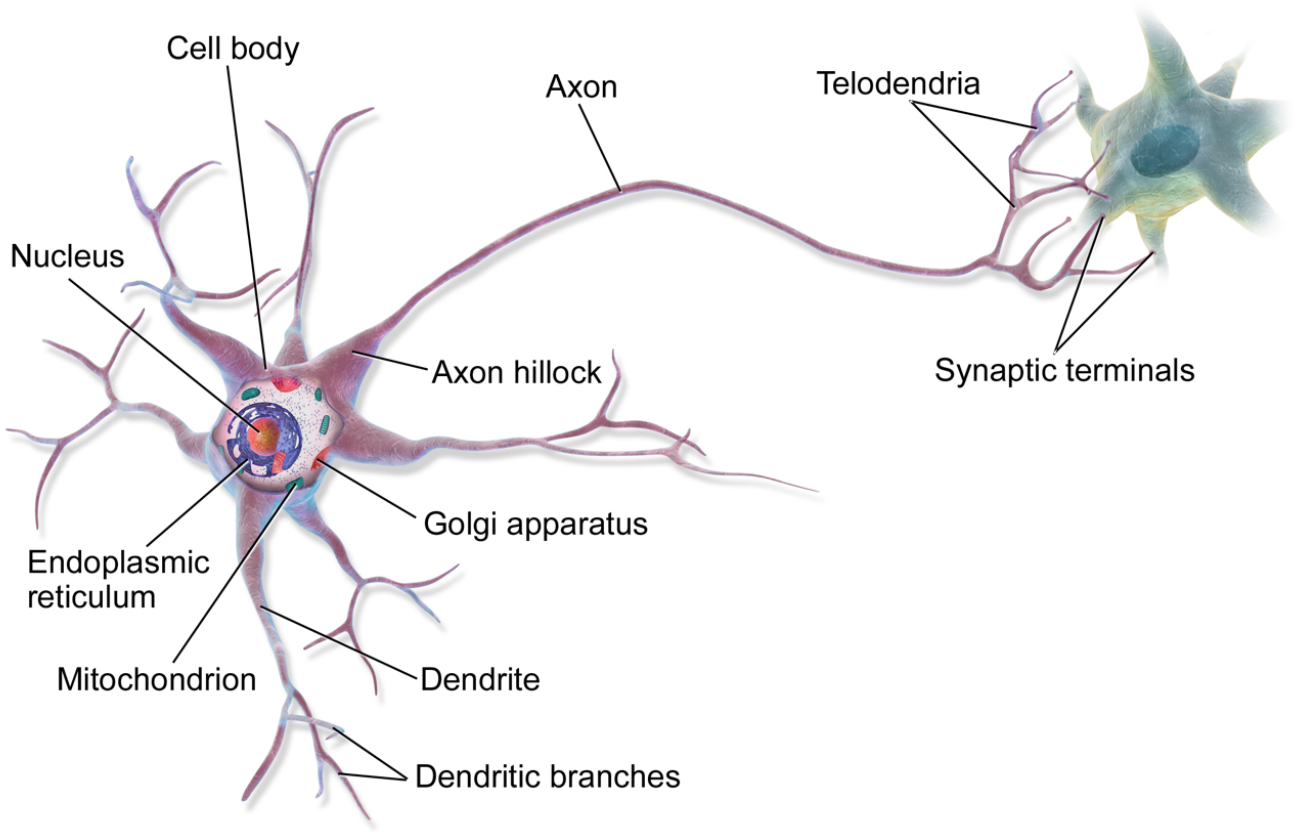
Electrophysiology:
The study of Electricity in the Body

Action Potentials: waves of voltage moving across a cell



Neuron

85 billion (85 x 109)
per human brain



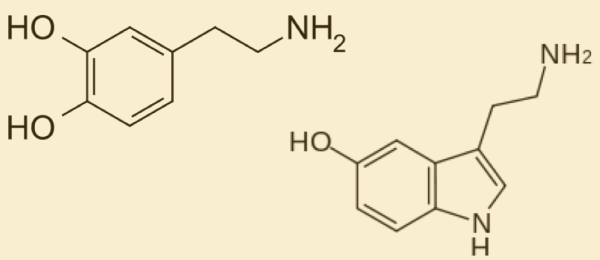
Our Brains

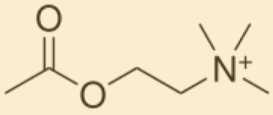
- 85 billion neurons (85 x 109)
- 1 million billion synapses (1 x 1015) (or 1 thousand trillion)
- ~90% synapses are excitatory
- only ~10% are inhibitory

Some Definitions

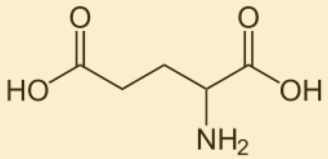
- *neuron*
- **neurotransmitter**
- **synapse**
- **receptor**
- agonist
- antagonist
- modulator
- *ion channel*

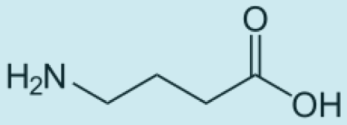
The Brain Uses Different Neurotransmitter Molecules for Excitation and Inhibition



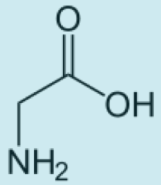


GO!





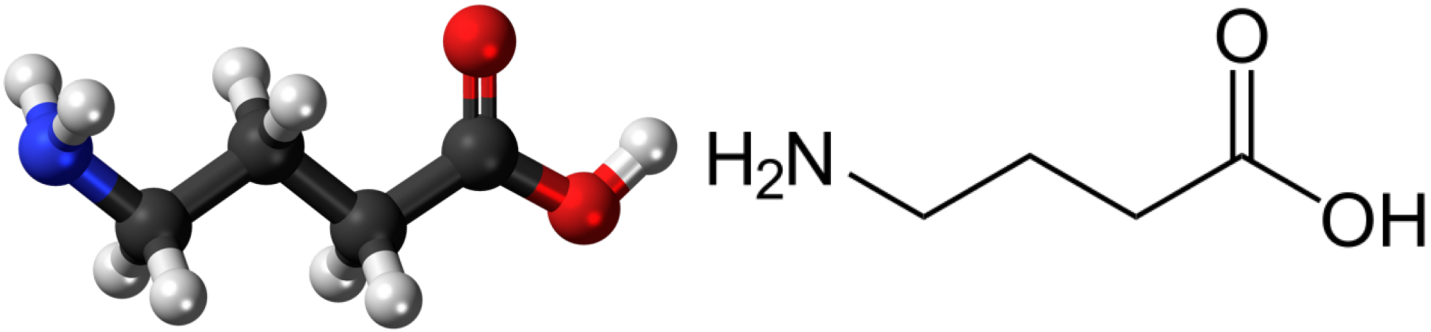
STOP!



Synapses Contain Receptors That Are Designed To Receive Excitatory Or Inhibitory Information Or Signals (GO! Or STOP!)

Receptors in ~10% synapses are specialized to recognize GABA and react to it by initiating a electrochemical response in the brain

Gamma Amino Butyric Acid (GABA)



- GABA is an inhibitory neurotransmitter.
- It carries information between neurons.
- It is generally associated with calming things down, hence “inhibitory”

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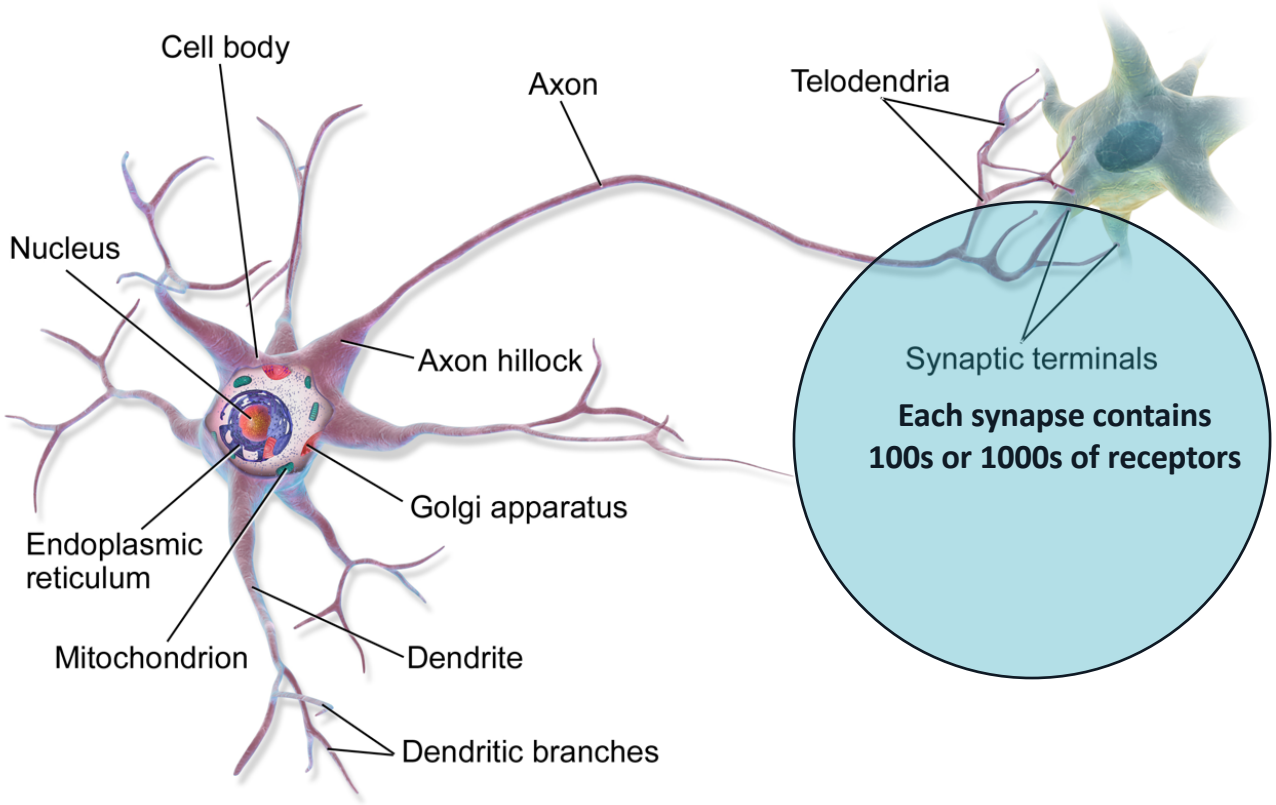
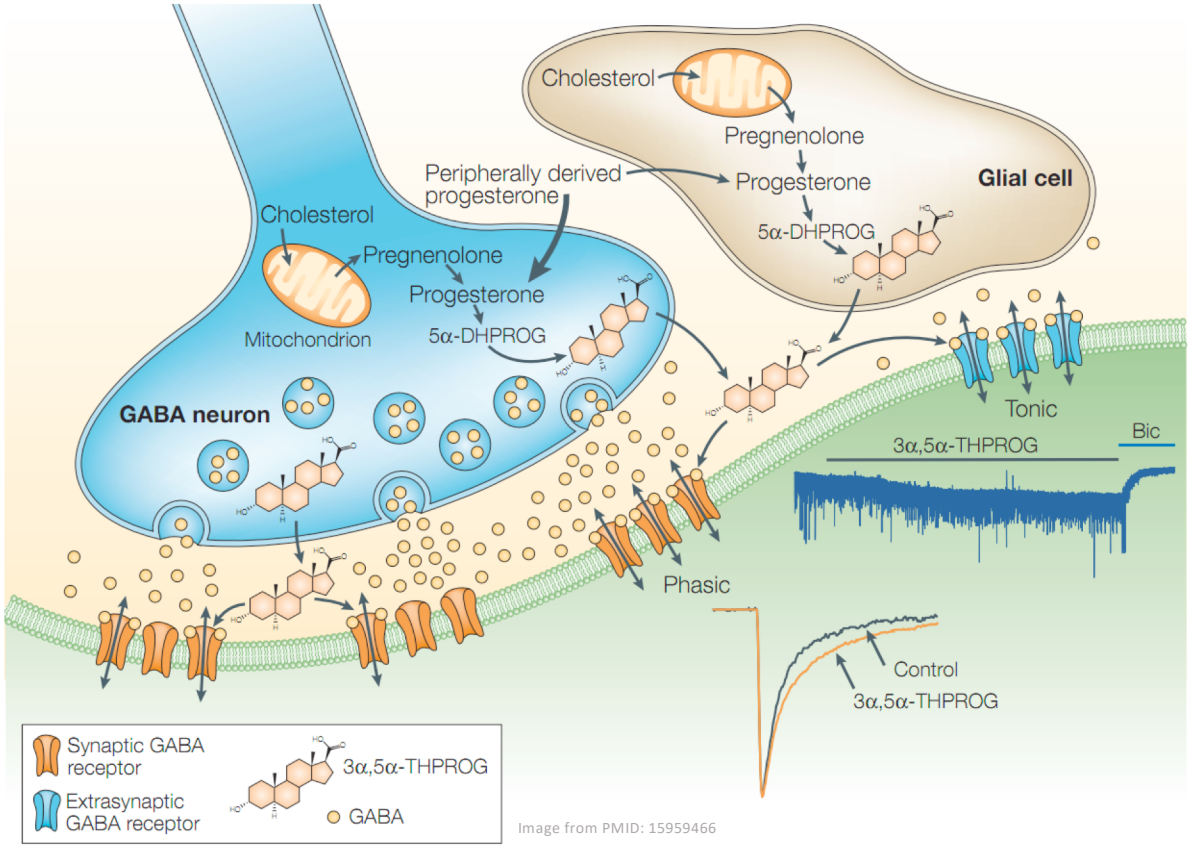
Synapses

on average more than
100,000 per neuron
1 thousand trillion per
human brain





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Synaptic Transmission Via $GABA_A$ Receptors

Receptors are specialized to recognize GABA and react to it by initiating a electrochemical response in the brain

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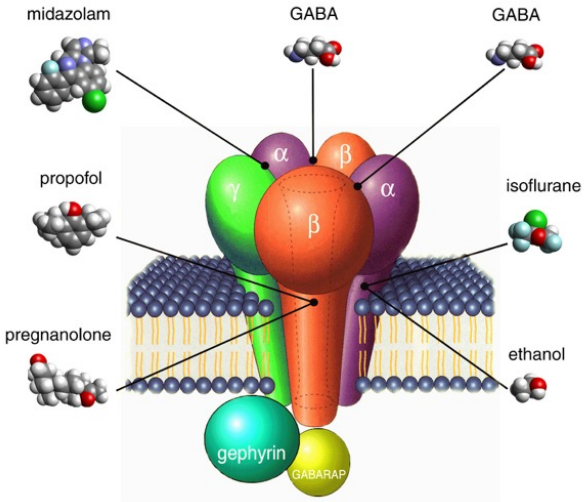
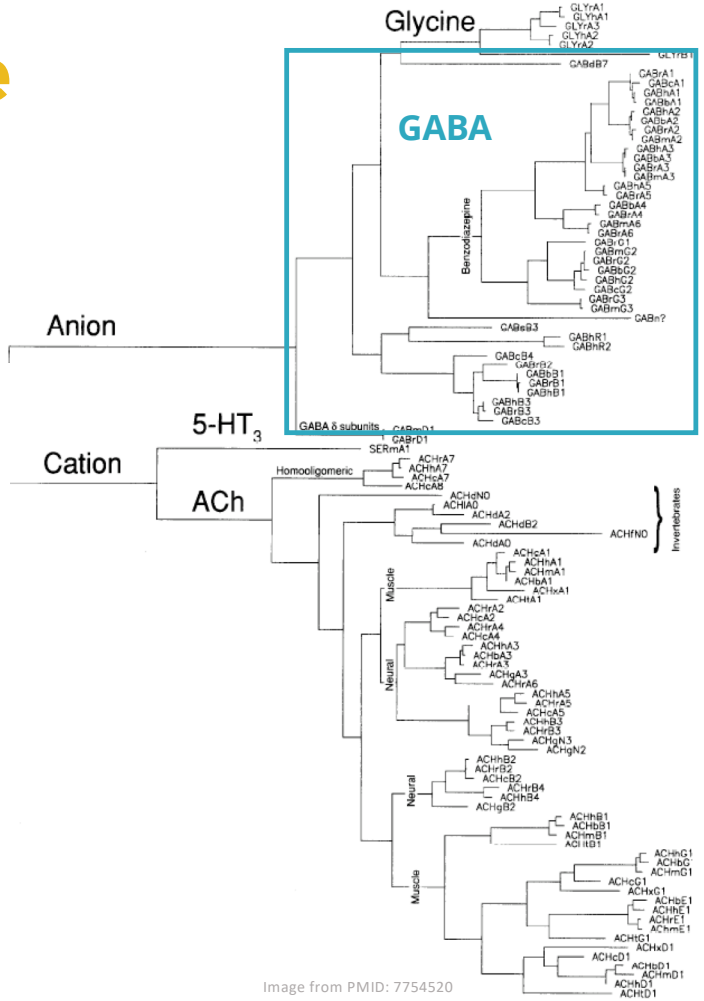
$GABA_A$Rs are Members of the Superfamily of Pentameric Ligand Gated Ion Channels

- most GABAARs are heteropentamers formed from 3 different subunits
- receptor function is modulated by many endogenous and exogenous molecules





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Some Definitions

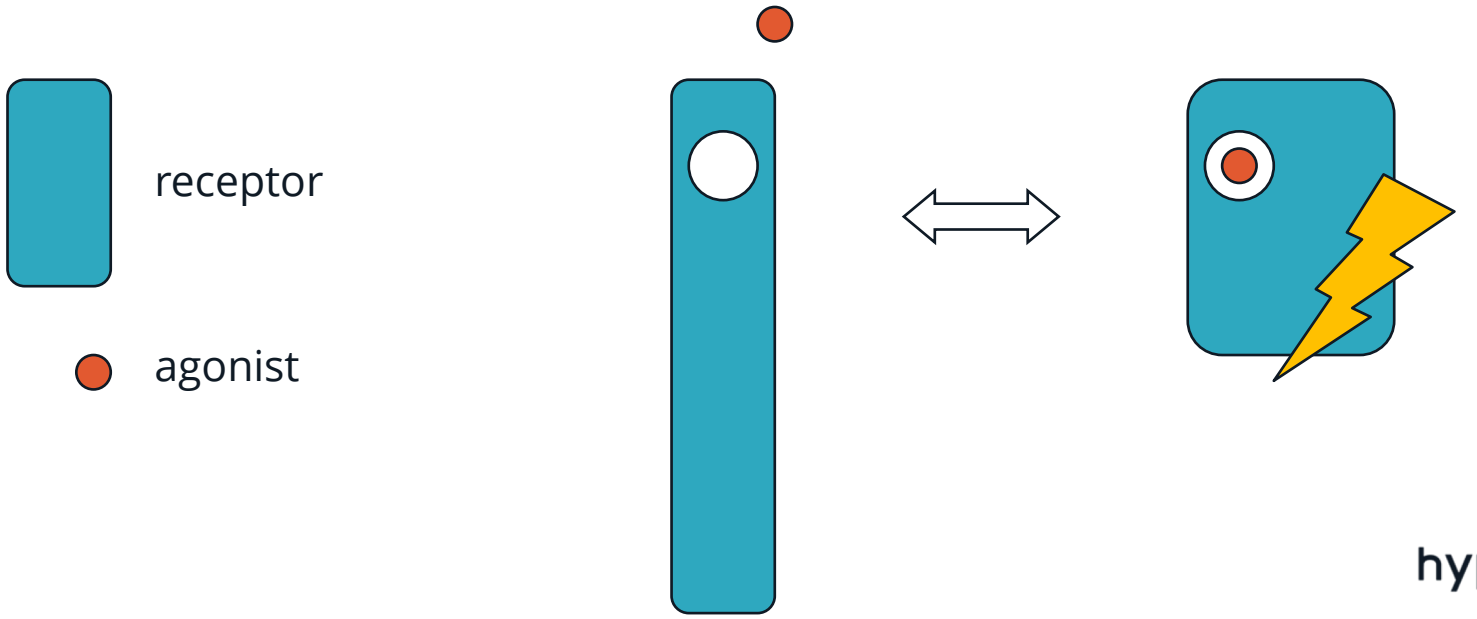
- neuron
- neurotransmitter
- synapse
- receptor

- **agonist**
- **antagonist**
- **modulator**
- ion channel

How do Receptors Get Activated and Modulated?

Pharmacology 1/4

- How does GABA activate a $GABA_A$ receptor?



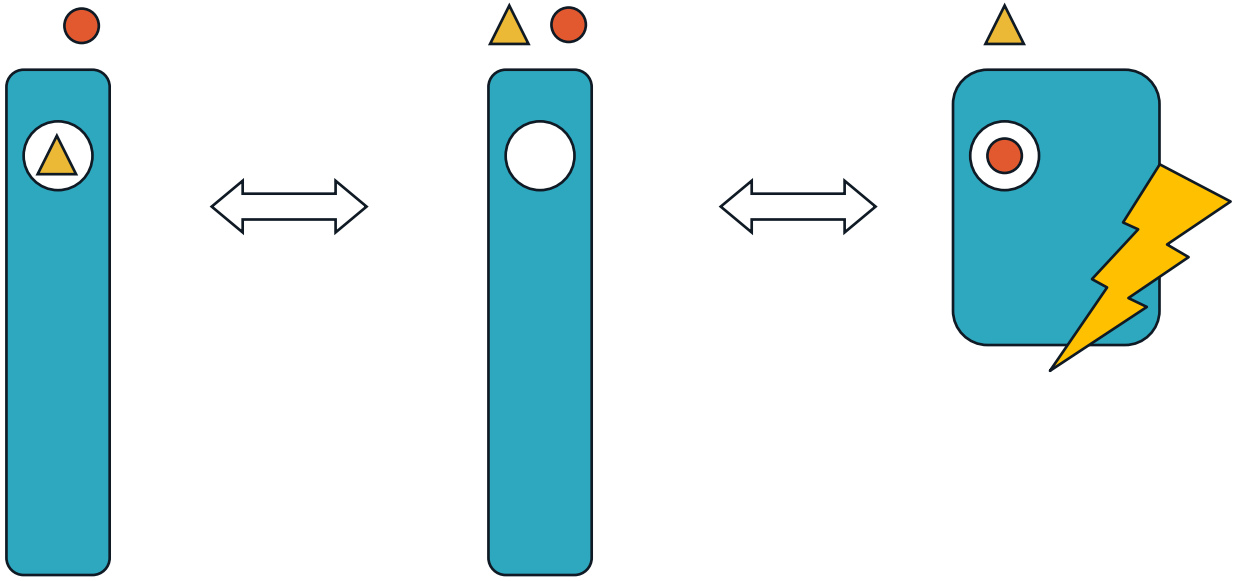
How do Receptors Get Activated and Modulated?

Pharmacology 2/4

- How do antagonists block a $GABA_A$ receptor?

competitive antagonist

agonist



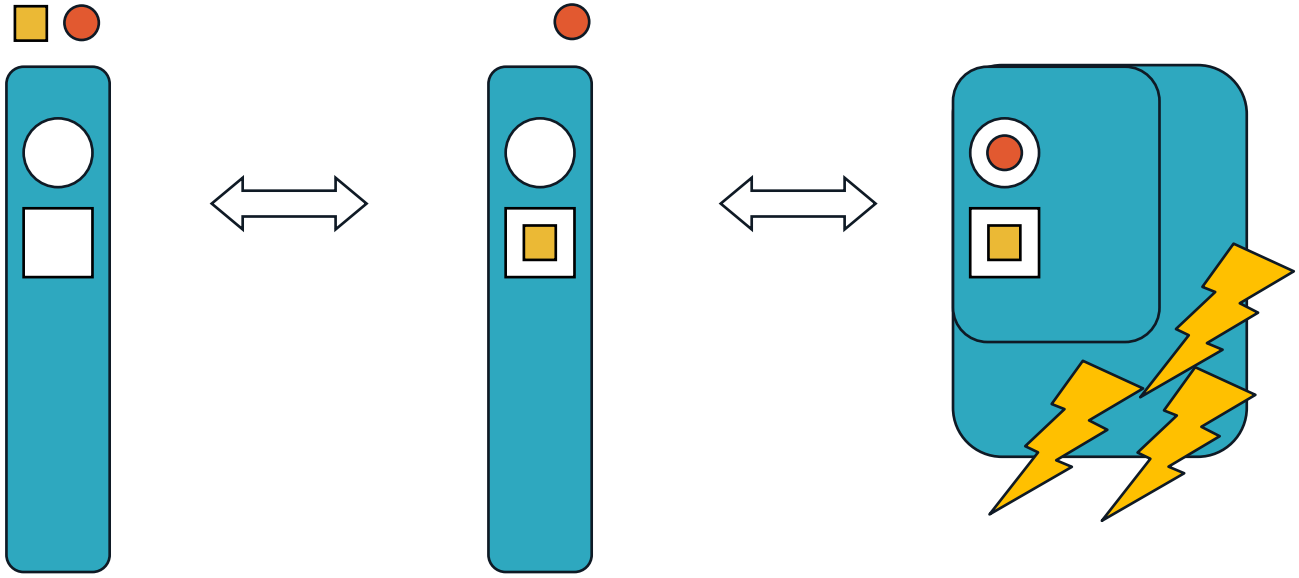
How do Receptors Get Activated and Modulated?

Pharmacology 3/4

- How do modulators enhance a $GABA_A$ receptor

modulator

agonist



How do Receptors Get Activated and Modulated?

Pharmacology 4/4

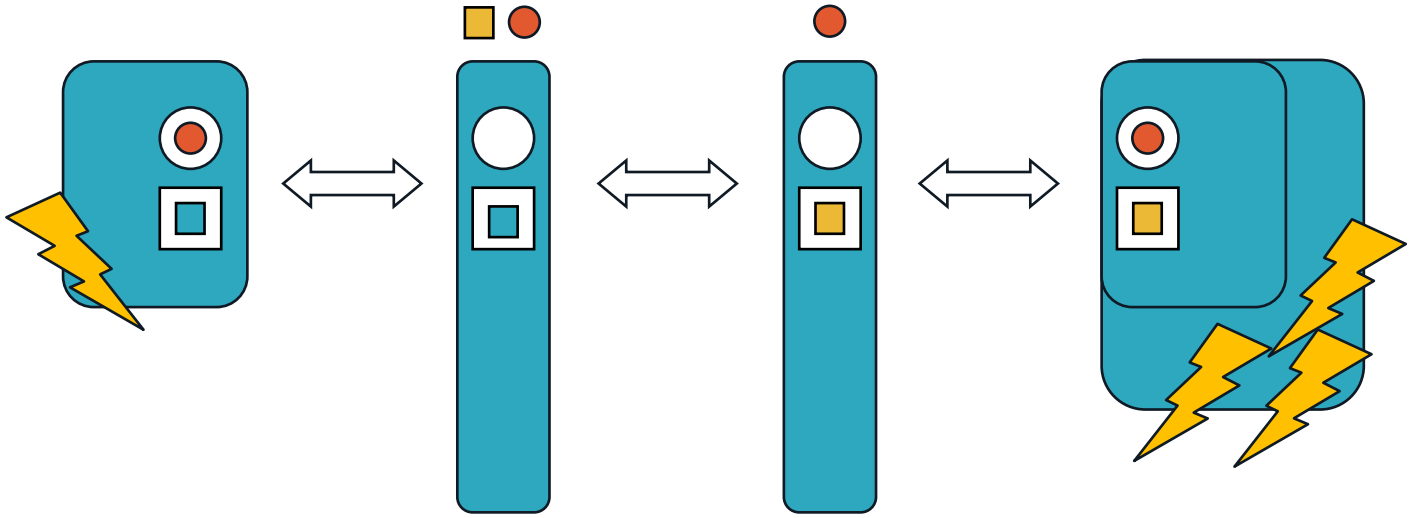
- How do we block an enhanced $GABA_A$ receptor?

Modulator Antagonist = FLUMAZENIL

modulator

agonist

modulator competitive antagonist



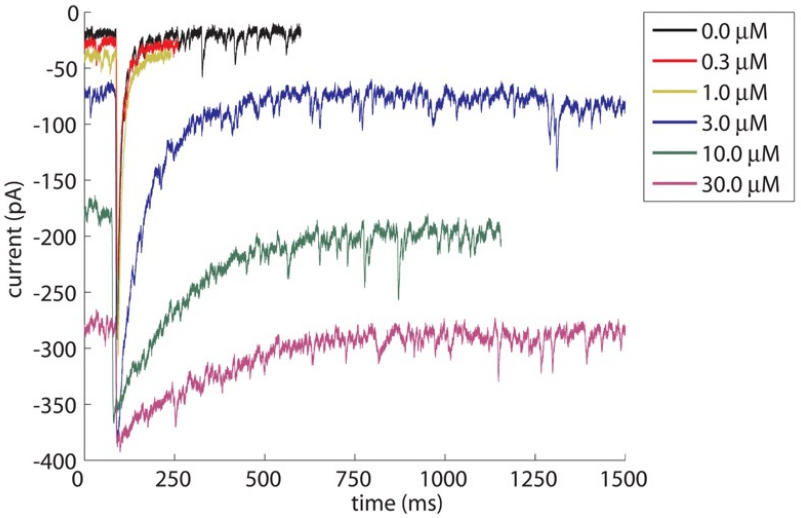
Some Definitions

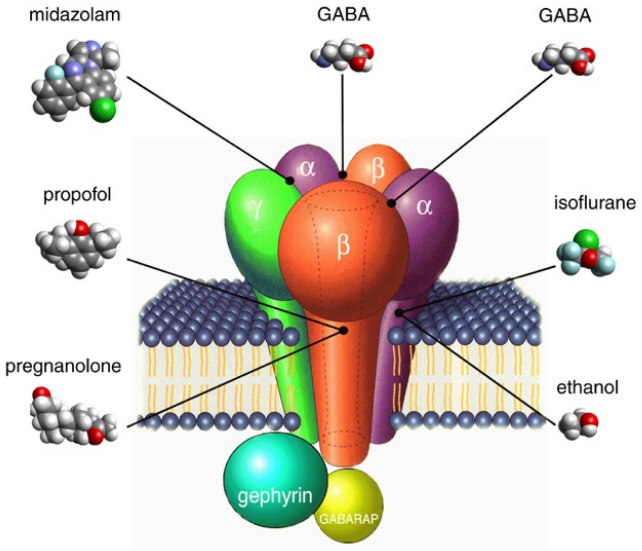
- neuron
- neurotransmitter
- synapse
- receptor
- agonist
- antagonist
- modulator
- ion channel

What About GABA & Drugs?

Drugs that enhance GABA activation are:

- Sedatives
- Sleep aids
- General Anesthetics





What Happens When $GABA_A$Rs go wrong?

MINIREVIEW—50th ANNIVERSARY SPECIAL ISSUE

Ionotropic GABA and Glutamate Receptor Mutations and Human Neurologic Diseases[S]

Hongjie Yuan, Chian-Ming Low, Olivia A. Moody, Andrew Jenkins, and Stephen F. Traynelis

Departments of Pharmacology (H.Y., A.J., S.F.T.) and Anesthesiology (O.A.M., A.J.), Emory University School of Medicine, Rollins Research Center, Atlanta, Georgia; and Departments of Pharmacology and Anaesthesia, Yong Loo Lin School of Medicine, National University of Singapore Graduate School for Integrative Sciences and Engineering, and Neurobiology/Ageing Programme, National University of Singapore, Singapore (C.-M.L.)

Received January 19, 2015; accepted April 22, 2015

Human $GABA_A$ receptor mutations in neurologic disorders

All missense mutations with a frequency of <1% as well as stop codons and splice junction mutations are included. Total indicates the number of published de novo or inherited mutations in each subunit. Many mutations have more than one phenotype.

Gene, Subunit	Total	RVIS[a]	AD	ASD	DD/MR	Epi	SZ	ADD
		%						
GABRA1, α1	13	24	0	0	0	12	1	0
GABRA2, α2	11	34	0	1	1	0	0	9
GABRA6, α6	3	68	0	0	0	0	2	1
GABRB2, β2	7	15	0	2	0	0	5	0
GABRB3, β3	7	22	0	1	0	5	0	1
GABRG1, γ1	4	12	0	0	0	0	0	4
GABRG2, γ2	9	25	0	0	0	8	1	0
GABRG3, γ3	2	46	1	1	0	0	0	0
GABRR2, ρ2	6	59	0	1	0	0	0	5
GABRD, δ	2	59	0	0	0	2	0	0
Total	64		1	6	1	27	9	20

AD, Alzheimer's disease; ADD, addiction; ASD, autism spectrum disorder; DD, developmental delay; Epi, epilepsy; MR, mental retardation; SZ, schizophrenia.

Pathological Sleep: Idiopathic Hypersomnia

- Excessive daytime & prolonged nocturnal sleepiness affects up to 5% of the population
- Sleep is not restorative
- Psychostimulants, most are ineffective, bringing variable but insufficient relief
- No genetic or chemical assay for IHS
- Diagnosis arises seemingly by default from a set of clinical signs and symptoms
- This nosology relies heavily upon arbitrary, subjective and non-quantifiable measures, it provides no insight into the pathogenesis of I.H.S. or its treatment

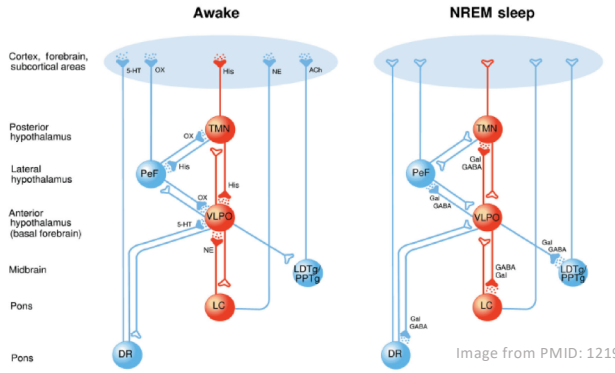
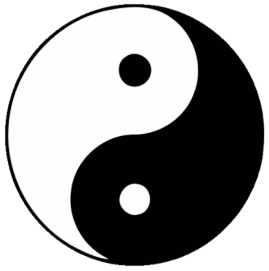


Image from PMID: 12195434

Fig. 1. Simplified NREM sleep-promoting pathway. An inhibition of noradrenergic neurons in the LC, which accompanies endogenous NREM sleep[22,33], releases a tonic noradrenergic inhibition of the VLPO[35]. The activated VLPO[16] is believed to release GABA into the TMN[17,23–25], which inhibits its release of arousal-promoting histamine into the cortex, and thus induces loss of consciousness[18,45]. A number of pathways are involved in NREM sleep; the sleep-active VLPO[16] projects to all the ascending monoaminergic, cholinergic and orexinergic arousal nuclei (TMN, LC, DR, PPTg, LDTg, PeF)[17,25], which project to the cortex where they release arousal-promoting neurotransmitters to promote wakefulness[16]. We focused on the TMN as representative of the arousal centers inhibited by the VLPO during sleep. The LC widely innervates the brain, but only projections associated with NREM sleep are shown here. A simplified version of this circuitry—the portion of the pathway highlighted in red—was the focus of our research. ACh, acetylcholine; GABA, γ-aminobutyric acid; DR, dorsal raphe nuclei; His, histamine; 5-HT, serotonin; LC, locus coeruleus; LDTg, laterodorsal tegmental nuclei; NE, norepinephrine; NREM, non-rapid eye movement; OX, orexin (hypocretin); PeF, perifornical area; PPTg, pedunculopontine tegmental nuclei; TMN, tuberomammillary nucleus; VLPO, ventrolateral preoptic nucleus.



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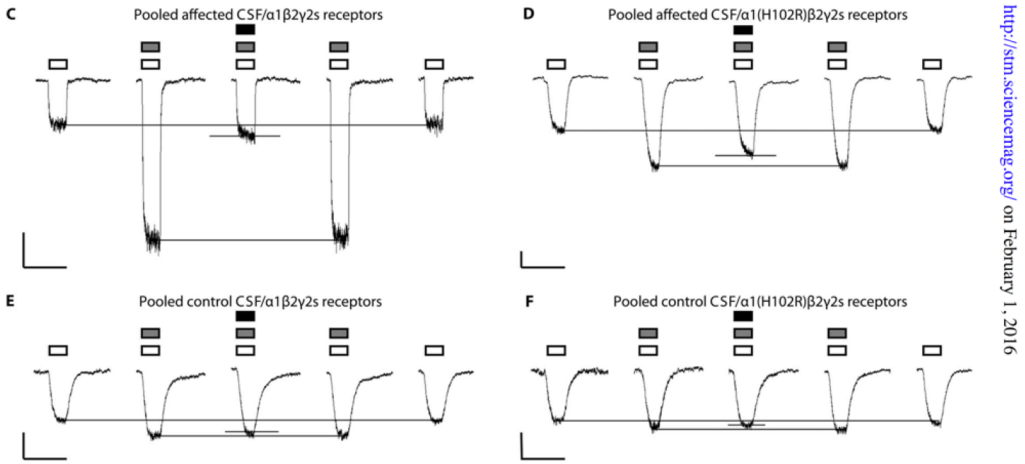
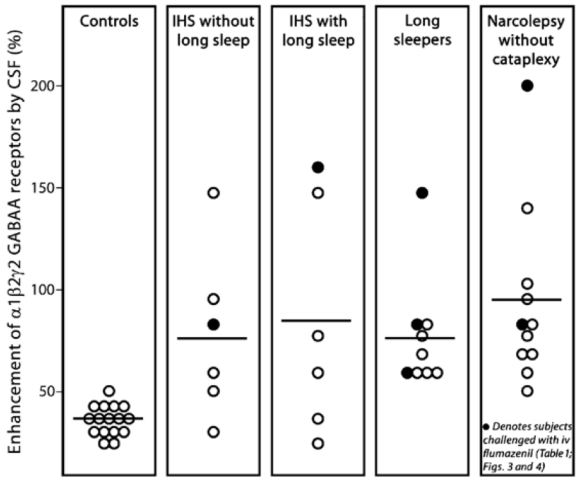
Electrophysiology Experiments Show that IH CSF Enhances $GABA_A$ Receptor: *resulting in too much inhibition!*





SLEEP

Modulation of Vigilance in the Primary Hypersomnias by Endogenous Enhancement of $GABA_A$ Receptors

David B. Rye,[1]* Donald L. Bliwise,[1] Kathy Parker,[2] Lynn Marie Trotti,[1] Prabhjyot Saini,[1] Jacqueline Fairley,[1] Amanda Freeman,[1] Paul S. Garcia,[3,4] Michael J. Owens,[5] James C. Ritchie,[6] Andrew Jenkins[3,7]

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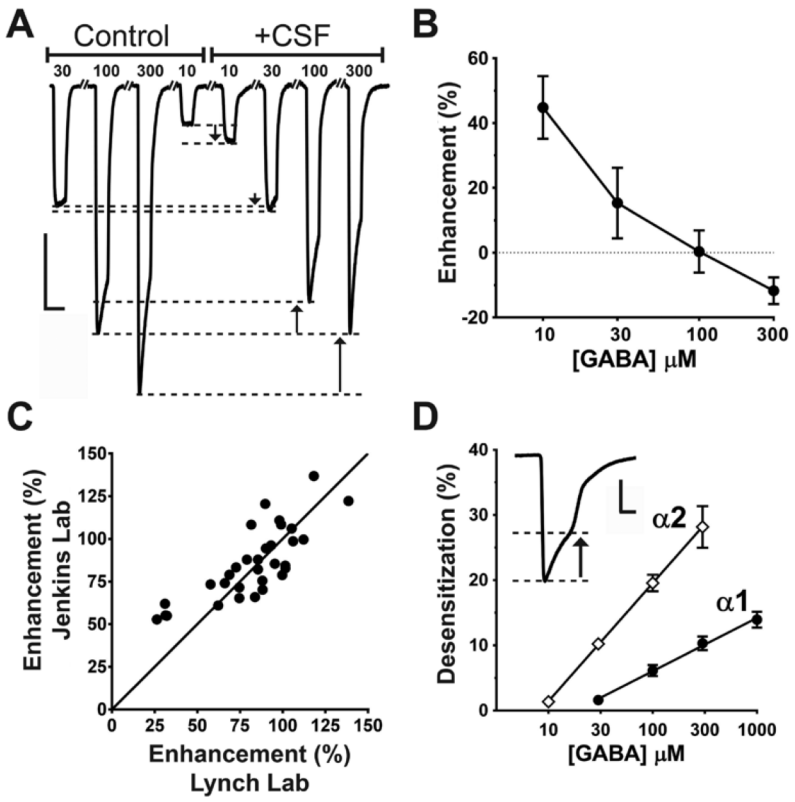
IH CSF experiments are difficult and take time

- scientists have to be well trained
- scientists have to be very careful



Rigor, Reproducibility, and In Vitro Cerebrospinal Fluid Assays: The Devil in the Details

Olivia A. Moody, BA,[1] Sahil Talwar, PhD,[2] Meagan A. Jenkins, PhD,[3] Amanda A. Freeman, PhD,[4] Lynn Marie Trotti, MD, MSc,[5] Paul S. García, MD, PhD,[1,6,7] Donald Bliwise, PhD,[5] Joseph W. Lynch,[2,8] Brad Cherson, RPh,[9] Eric M. Hernandez, MD, PhD,[10] Neil Feldman, MD,[11] Prabhjyot Saini, MSc,[5] David B. Rye, MD, PhD,[1,5] and Andrew Jenkins, PhD[1,7,12]

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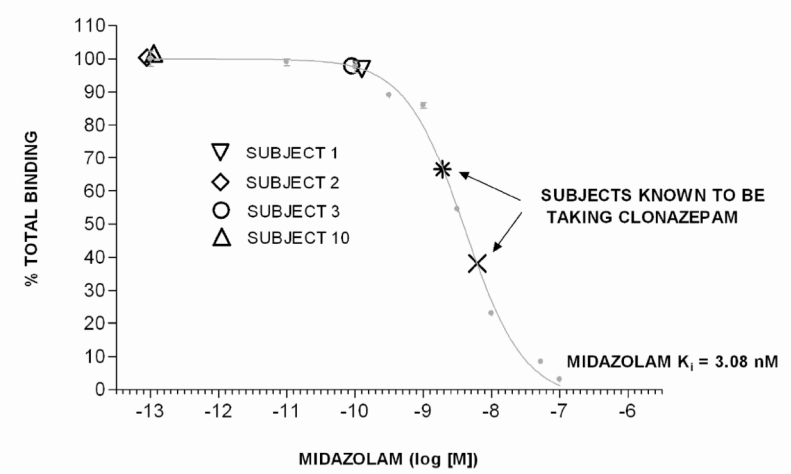
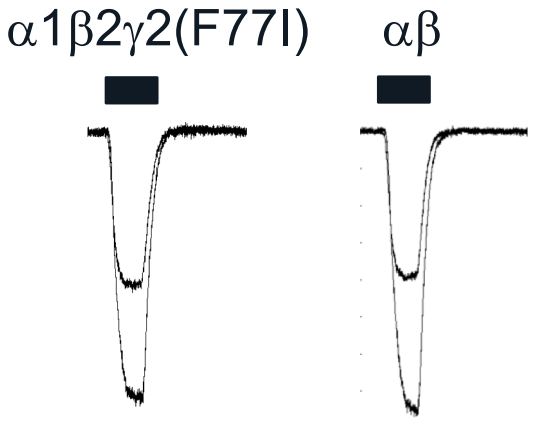
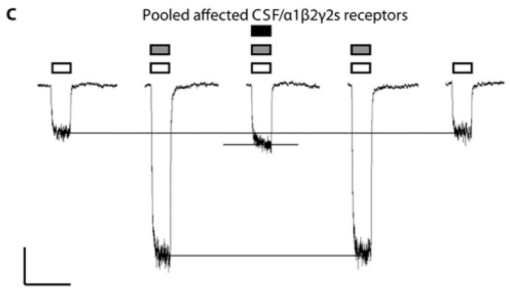
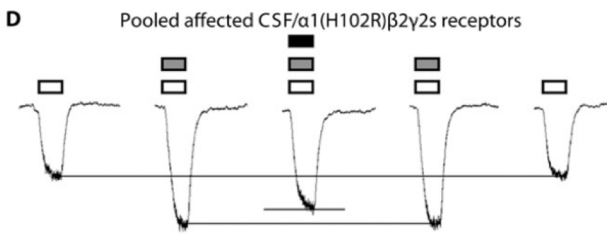
IH CSF is a PAM, but Not a Conventional Benzodiazepine Agonist









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“...dans les champs de l'observation, le hasard ne favorise que les esprits préparés...”

- Index PH patient, while receiving FLZ, contracted bronchitis
- prescribed antibiotic: macrolide clarithromycin
- Result: insomnia for 4 days

“...in science, fortune favors the prepared mind...”
Louis Pasteur

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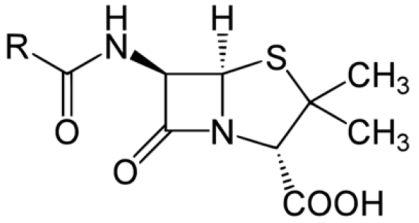
Many Clinically Approved Drugs Like Clarithromycin Have "Side Effects" that Alter Sleep and Arousal

Antibacterials	
Penicillins	Encephalopathy, irritability**, sedation,** anxiety, seizures, hallucinations
Cephalosporins	**Sleep disturbances**, hallucinations
Cycloserine	Dose-dependent side-effects: depression, irritability (common); psychosis
Quinolones	**Sleep** and mood disorders, psychosis
Nitrofurans	Euphoria, psychosis**, sleep disturbance**
Tetracyclines	Decreased concentration, mood and **sleep disorders**
Chloramphenicol	Depression
Trimethoprim	Depression, psychosis and sulphonamides

Antimycobacterials	
Isoniazid	Cognitive impairment, mood disorder, psychosis
Clofazimine	Major depression and suicide
Rifampicin	**Sedation**
Ethionamide	**Sedation,** irritability, agitation, depression, psychosis



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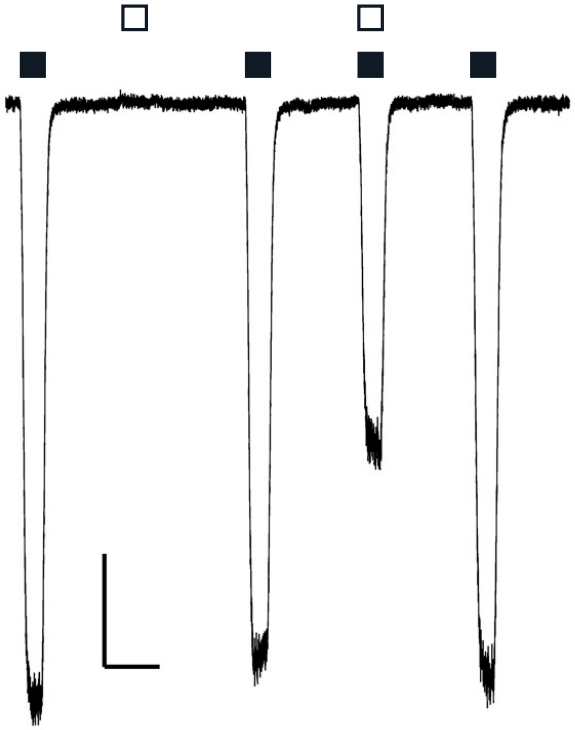
The Macrolide Antibiotic Clarithromycin Inhibits $GABA_AR$ Receptors



300 μM clarithromycin
5 μM GABA

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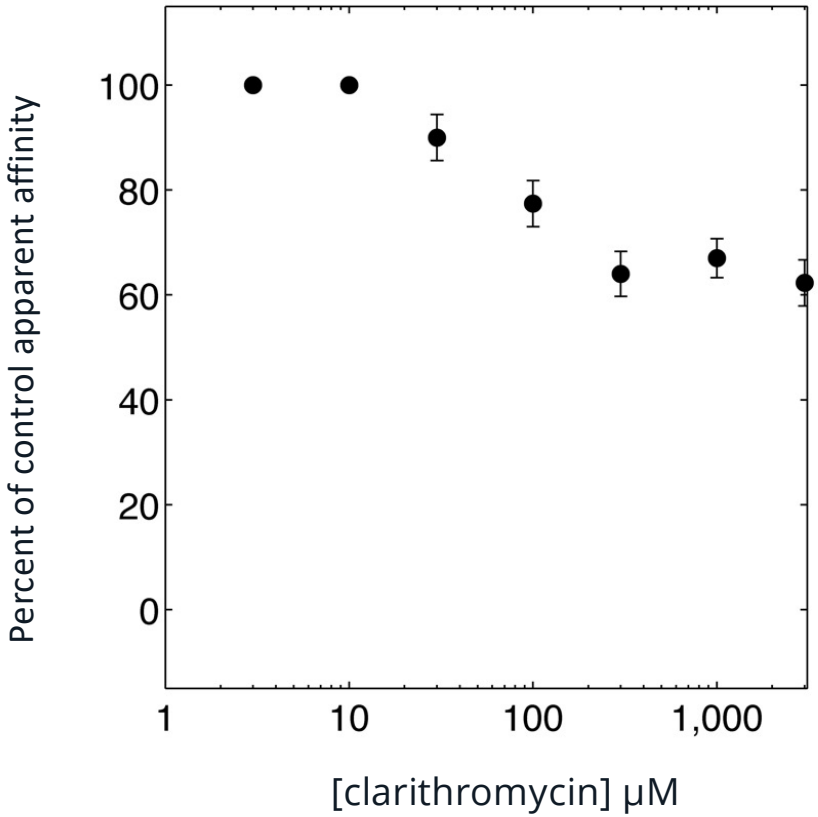
Clarithromycin makes GABA work less well



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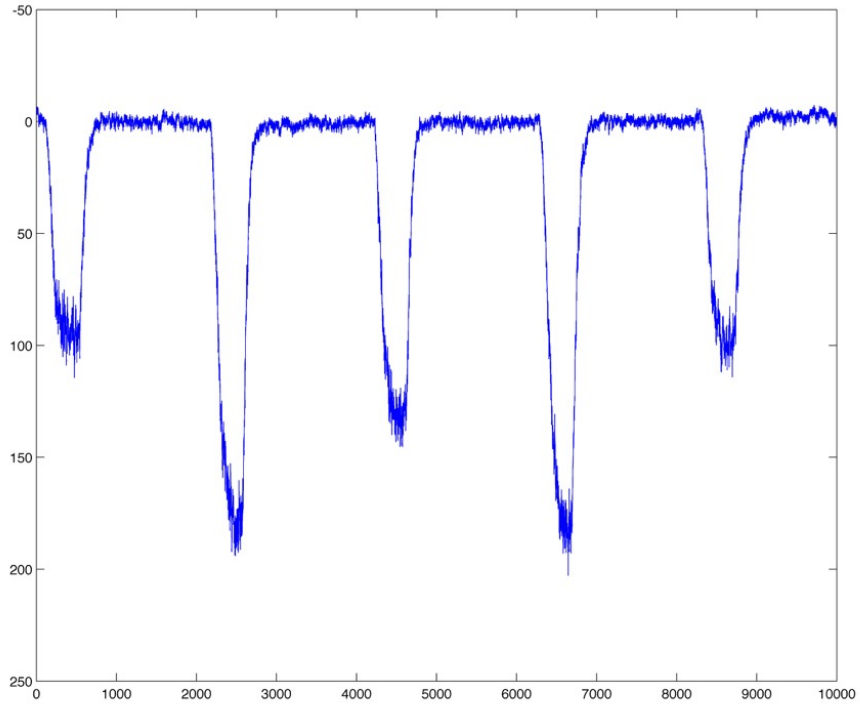
Clarithromycin Reverses Modulation by IH CSF



100 μM clarithromycin
50% PH CSF
5 μM GABA

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Clarithromycin Phase II Clinical Trial Double Blind, Randomized

1. Patients say they feel better with Clarithromycin
2. PVT results did not change

Objective:

- Psychomotor Vigilance Task (PVT) Reaction Time $p = 0.47$
- PVT Median Reaction Time $p = 0.6$
- PVT Number of Lapses $p = 0.6$

Subjective:

- Epworth Sleepiness Scale $p = 0.002$
- Functional Outcomes of Sleep Questionnaire $p = 0.002$
- SF-36 Vitality Subscale $p = 0.01$

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The Future?

- “New” Drugs
- New diagnostic techniques
- New science

Many Clinically Approved Drugs Like Clarithromycin Have "Side Effects" that Alter Sleep and Arousal

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Penicillins	Encephalopathy, irritability, **sedation**, anxiety, seizures, hallucinations
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Ethionamide	**Sedation,** irritability, agitation, depression, psychosis

"New" Drugs

1. Many neuroscience drug pipelines are being closed
2. Developing new drugs is slow and expensive
3. We must seek faster and cheaper alternatives

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New Techniques 1:

The patcherbot

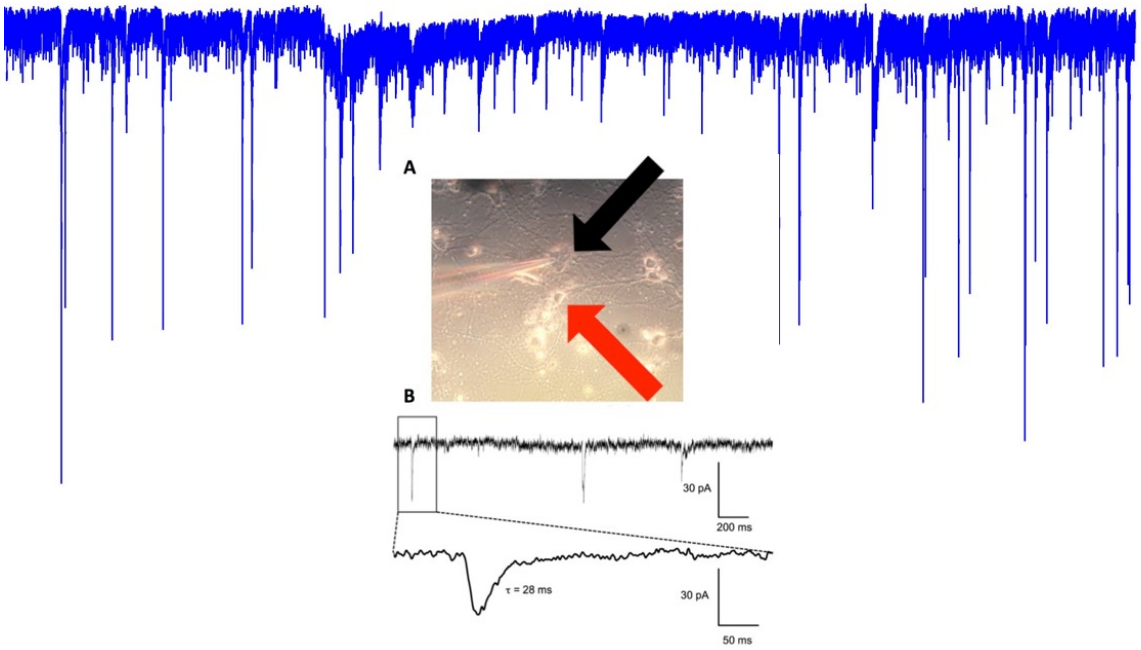
patcherBot

in-vitro operation

I Kolb, WA Stoy, E Rousseau, OA Moody, A Jenkins, CR Forest

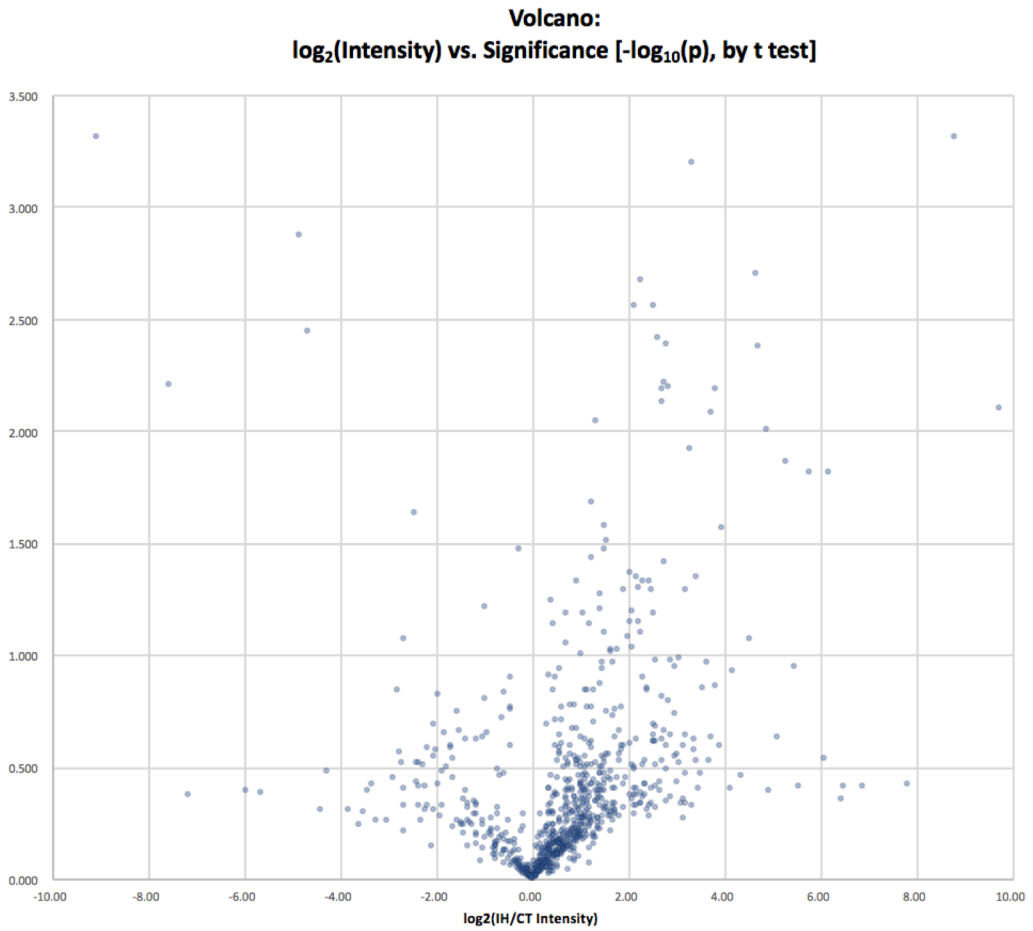
New Techniques 2:

Rebuilding your brain in my lab
Your cells, Your CSF…



Newest Science:

CSF Mass Spectroscopy
Each dot represents a protien in IH CSF that might be the cause of GABA-related sleepiness



Talk Overview

- How do our brain cells communicate?
- How do drugs and disease change this?
- What is GABA?
- Why is it important in IH?
- ***How can we relieve hypersomnia symptoms with drugs working on GABA?***
- ***What does the future hold for IH-GABA research?***

Thank you!

- You
- +600 sleep patients whose CSF has passed through my lab
- HF, Diane & Andrew Powell, Cat Rye
- Emory Sleep Center, esp. Drs. Rye, Trotti & Bliwise
- Research Funding: NIH (NINDS), Marigold Foundation
- Patent Licensing: Balance Pharmaceuticals & Emory OTT