IH & GABA

Andy Jenkins Ph.D.
Associate Professor, Anesthesiology & Pharmacology
Emory University School of Medicine
Personal/Professional Financial Relationships with Industry

<table>
<thead>
<tr>
<th>External Industry Relationships *</th>
<th>Company Name</th>
<th>Role</th>
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<tr>
<td>Equity, stock, or options in biomedical industry companies or publishers</td>
<td>Balance Pharma / pending</td>
<td>IP license agreement</td>
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<td>Board of Directors or officer</td>
<td>Somnolytics</td>
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<td>Royalties from Emory or from external entity</td>
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<td>Industry funds to Emory for my research</td>
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<td>Other</td>
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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**
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
• GABA is an inhibitory neurotransmitter
• GABA binds to GABA<sub>A</sub> receptors
• Receptors are proteins that:
  • Bind chemical messages
  • After binding, the receptor changes shape
  • It initiates changes in the neuron
• GABA<sub>A</sub> receptors are ion channels
  • They let negative charge into neurons
  • This inhibits neuronal activity
GABA\textsubscript{A} receptors are ion channels

GABA\textsubscript{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
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
Your Brain Contains 85 Billion Neurons
Neuron

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

$\text{Na}^+ \text{ ions}$  & $\text{Cl}^{-} \text{ ions}$

$\text{Na}^{+} + \text{Cl}^{-}$
Every Neuron Has a Cell Wall. This is a Barrier to Water and Salts.
Ion Channels Let the Salts Cross the Cell Wall

Chloride (-)
Neurons Have Many Different Ion Channels

Chloride (-)
Sodium (+)
Potassium (+)
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?
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 10^9) per human brain
Our Brains

- 85 billion neurons (85 x 10^9)
- 1 million billion synapses (1 x 10^{15}) (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 an 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”
Synapses

on average more than 100,000 per neuron
1 thousand trillion per human brain
Synaptic Transmission Via GABA<sub>A</sub> Receptors

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

Image from PMID: 15959466
GABA\textsubscript{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
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?

![Diagram showing receptor activation by agonist](image)
How do Receptors Get Activated and Modulated?

**Pharmacology 2/4**

- How do antagonists block a GABA<sub>A</sub> receptor?

![Diagram showing activation and modulation of receptors with antagonists and agonists](image)

- 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<sub>A</sub> 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<sub>A</sub>Rs go wrong?

**MINIREVIEW—50<sup>th</sup> ANNIVERSARY SPECIAL ISSUE**

Ionotropic GABA and Glutamate Receptor Mutations and Human Neurologic Diseases

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 Anesthesiology, Yong Loo Lin School of Medicine, National University of Singapore Graduate School for Integrative Sciences and Engineering, and Neurobiology/Aging Programme, National University of Singapore, Singapore (C.-M.L.)

Received January 19, 2015; accepted April 23, 2015

Human GABA<sub>A</sub> 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.

<table>
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<th>Gene, Subunit</th>
<th>Total</th>
<th>RVIS&lt;sup&gt;a&lt;/sup&gt;</th>
<th>AD</th>
<th>ASD</th>
<th>DD/MR</th>
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AD, Alzheimer’s disease; ADD, addiction; ASD, autism spectrum disorder; DD, developmental delay; Epi, epilepsy; MR, mental retardation; SZ, schizophrenia.
Pathological Sleep: Idiopathic Hypersomnbia

- 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
Electrophysiology Experiments Show that IH CSF Enhances GABA$_A$ Receptor: resulting in too much inhibition!
IH CSF experiments are difficult and take time

- scientists have to be well trained
- scientists have to be very careful
IH CSF is a PAM, but Not a Conventional Benzodiazepine Agonist

α1β2γ2(F77I)  αβ

Pooled affected CSF/α1β2γ2s receptors

Pooled affected CSF/α1(H102R)β2γ2s receptors

SUBJECTS KNOWN TO BE TAKING CLONAZEPAM

MIDAZOLAM Ki = 3.08 nM

% TOTAL BINDING

MIDAZOLAM (log [M])
“...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
Many Clinically Approved Drugs Like Clarithromycin Have “Side Effects” that Alter Sleep and Arousal

<table>
<thead>
<tr>
<th>Antibacterials</th>
<th>Side Effects</th>
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<tbody>
<tr>
<td>Penicillins</td>
<td>Encephalopathy, irritability, sedation, anxiety, seizures, hallucinations</td>
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<tr>
<td>Cephalosporins</td>
<td>Sleep disturbances, hallucinations</td>
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<tr>
<td>Cycloserine</td>
<td>Dose-dependent side-effects: depression, irritability (common); psychosis</td>
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<tr>
<td>Quinolones</td>
<td>Sleep and mood disorders, psychosis</td>
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<tr>
<td>Nitrofurans</td>
<td>Euphoria, psychosis, sleep disturbance</td>
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<td>Tetracyclines</td>
<td>Decreased concentration, mood and sleep disorders</td>
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<tr>
<td>Chloramphenicol</td>
<td>Depression</td>
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<td>Clofazimine</td>
<td>Major depression and suicide</td>
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<td>Rifampicin</td>
<td>Sedation</td>
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<tr>
<td>Ethionamide</td>
<td>Sedation, irritability, agitation, depression, psychosis</td>
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The Macrolide Antibiotic Clarithromycin Inhibits GABA$_A$R Receptors

300 µM clarithromycin
5 µM GABA
Clarithromycin makes GABA work less well
Clarithromycin Reverses Modulation by IH CSF

100 µM clarithromycin
50% PH CSF
5 µM GABA
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 $p = 0.01$
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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**“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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</table>
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 protein 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