

Transcript for Dr. Amanda Freeman's Video: Learning About GABA, Part 2

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Hello, I am Dr. Amanda Freeman. I am the Director of Undergraduate Studies and a lecturer in the Center for the Study of Human Health at Emory University. In this video, I will describe how GABA inhibits neurons from firing and how the GABA_A receptor is related to hypersomnia.

If you are unfamiliar with GABA, I recommend watching the GABA 101 <u>Part 1</u> video before viewing this one to provide the context for this information.

How does GABA inhibit neurons?

To answer this question, we first need to understand the general properties of neurons and how they function.

The most common way for neurons to communicate with other neurons is through the release of a neurotransmitter which conveys a message from the presynaptic neuron to the postsynaptic neuron. In order to release neurotransmitter, the neuron must be activated. When a neuron is not active, we say that it is in a resting state.

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Neurons are surrounded by, and filled with, ions, which are charged particles.

At rest, there is a regulated balance of positively charged ions inside and outside of the neuron. This balance of ions is carefully maintained by proteins in the membrane of the neuron and, as a result, there are more positively charged ions outside the neuron than inside.

When a neuron is active, there is a shift in this ion balance.

In the initial stage of activation, a few positive ions cross the membrane and enter the neuron.

More positive ions follow and the end result is a lot more positive charged ions are inside the neuron compared to outside. Another way to think about this is to pretend that you are throwing a party and you invite a few friends over. Once your friends arrive, they are having such a great time that they call more friends to come over. Now your house is packed with people, similar to the neuron which is packed with positive charges.



However, all good things must come to an end, and eventually your friends leave and go home. The extra positively charged ions also cross the neuronal membrane and exit the neuron which allow it to return to the resting state. We refer to the momentary shift of ions, rushing in then out of the neuron, as an action potential.

What I've just described is a simplified explanation of an action potential because we have only been talking about "positive ions". In reality, an action potential involves the movement of multiple types of positive ions, for example sodium and potassium, and also negative ions, like chloride.

In order for a neuron to be activated, a sufficient number of positively charged ions must enter the neuron to reach the threshold for an action potential. Without an action potential, no neurotransmitter will be released. The neuron can be prevented from reaching the threshold for an action potential by keeping the number of positively charged ions inside and outside the cell in balance. A few ions may cross the membrane, but not a sufficient number to alter the balance of charges inside and outside the membrane. If we think back to our party analogy, this would be like having just one or two friends come over, but not having a house packed with people.

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In neurons, the inability to reach threshold for an action potential could occur because too few positively charged ions enter the neuron or it could occur because negatively charged ions enter the neuron and cancel out the positively charged ions that enter.

For example, if one positively charged ion and one negatively charged ion both enter the neuron, they will cancel each other out and there will be no net increase of positive charges within the neuron. It would be the same as not having any positively charged ions enter the neuron. This is how GABA works to inhibit neurons from firing action potentials. It makes it harder for the neuron to reach the threshold of internal positive charges required to trigger the action potential.

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When a presynaptic neuron releases GABA, it will bind with a GABA receptor on the postsynaptic neuron and inhibit the postsynaptic neuron from firing an action potential. There are two primary types of GABA receptors. Both are inhibitory, but operate by different mechanisms.



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GABA_A receptors are ligand-gated ionotropic receptors. When GABA binds, the receptor creates a pore in the membrane (which is like opening a door) and allows negatively charged Chloride ions to enter the neuron.

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GABA_A receptors are important targets for tranquilizers, anesthetics, and anticonvulsants. [GABA_C receptors are so similar to structure and function of GABA_A receptors that they are considered a subclass of GABA_A receptors and the name was changed several years ago to GABA_A- ρ receptors.]

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 $GABA_B$ receptors are metabotropic receptors. When GABA binds to a $GABA_B$ receptor, this indirectly opens a pore that allows a positively charged potassium ion to exit the neuron. A positively charged ion leaving the neuron or a negatively charged ion entering the neuron have the same effect, both make it difficult to increase the number of positively charged ions inside the neuron to the threshold necessary for an action potential.

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GABA_B receptors are important targets for muscle relaxants and antiepileptics.

While the end result is the same, the different mechanisms of action allow for more nuanced communication between neurons which can have functional consequences. When we communicate, "No" means something different than a sharp "No!" which is also different from a "Nooooo". Individual neurons are capable of similar subtleties in communication. Metabotropic receptors, like GABA_B, take a longer to activate but can have a wider range of responses compared to the rapid open/shut activities of ionotropic receptors like GABA_A.

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Not only do these GABA receptor subtypes work differently, they are expressed in different areas of the brain. In these images of a rat brain, the location of each receptor subtype is indicated by black staining. The staining is darker wherever more receptors are present.



Both the mechanism and the location play a role in why the receptor subtypes are targeted for different purposes.

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The structure of GABA receptors is more complicated than the previous illustrations indicate. Let's look at the structure of the GABA_A receptor in more detail. The receptor is made up of five subunits which surround a central ion channel. There are multiple subunit types, but the major isoform of the GABAA receptor found in adults is composed of two alpha subunits, two beta subunits, and 1 gamma subunit. When the receptor is activated by the binding of GABA, the ion channel opens allowing chloride ions to pass in to the neuron.

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GABA is known to bind between the alpha subunit and the beta subunit. That means that there are two potential binding sites for GABA on the most common $GABA_A$ receptor.

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Ethanol and volatile anesthetics also bind between the alpha subunit and the beta subunit, but in a different location from GABA. (Indicated by the blue stars on this illustration.)

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On the other hand, there is only one binding site for Benzodiazepines, such as Valium or Xanax, between the gamma and alpha subunit. (Indicated by the purple star on this illustration.)

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To make things even more complicated, there are multiple versions of each subunit type. For example, there are 6 different forms of the alpha subunit. The majority of $GABA_A$ receptors include $\alpha 1$, $\alpha 2$, $\alpha 3$, or $\alpha 5$ subunits and each subtype is associated with a different function.



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These images of staining in rat brains demonstrate that the different subunits are expressed in different brain regions.

The diverse functional properties of the GABA_A receptor are influenced by the variety of subunits, the arrangement of the subunits, and the location in the brain where the receptor is expressed. Going back to the analogy of human communication, this potential variety in receptor structure, function, and pathway is critical to provide neurons with a larger vocabulary than a simple "yes" or "no".

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So you may be asking, how is all of this information about the $GABA_A$ receptor relevant to sleep?

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Let's briefly review the neurotransmitter systems regulating wake and sleep that were presented in the 1st GABA video. There are 5 different neurotransmitter systems that all act to promote wakefulness via diffuse projections throughout the brain: Histamine, Serotonin, Norepinephrine, Acetylcholine, and Hypocretin or Orexin.

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GABA, however, is the only neurotransmitter system that promotes sleep and does this by inhibiting the activity of all of the wake-promoting neurotransmitters.

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Research by Dr. David Rye and colleagues at Emory University, identified an endogenous molecule in the cerebral spinal fluid of patients with hypersomnia that increases the inhibitory activity of GABA_A receptors. What exactly does this mean and what are the consequences of this?



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As we discussed earlier, when GABA binds to a GABA_A receptor, the receptor opens a pore in the membrane that allows negatively charged chloride ions to enter. The influx of these negative charges inhibits the neuron from firing an action potential because the neuron is prevented from reaching the required threshold of positive charges inside the neuron.

When benzodiazepines bind to GABA_A receptor, they increase the efficiency of the receptor. Now, when GABA binds to the receptor, more chloride ions flow into the neuron compared to when GABA binds alone. This results in increased inhibition of the neuron which is responsible for the sedative, anti-anxiety, and muscle relaxant properties of the Benzodiazepines.

Although the exact mechanism has not been determined, the endogenous molecule in the cerebral spinal fluid of patients with hypersomnia also acts to increase the influx of chloride ions through the GABA_A receptor leading to increased inhibition of the neuron. Similar to a benzodiazepine, this molecule is unable to activate the receptor itself. GABA is required to bind to the receptor, but the endogenous molecule makes the receptor more efficient.

Flumazenil is a benzodiazepine antagonist which prevents the binding of benzodiazepine to the GABA_A receptor. It is used to reverse benzodiazepine-induced sedation. When GABA_A receptors are exposed to the endogenous hypersomnia molecule and Flumazenil simultaneously, the function of the receptor returns to normal. Fewer negatively-charged chloride ions enter the neuron and the neuron returns to a normal level of inhibition.

Although the endogenous molecule underlying hypersomnia and benzodiazepines have similar effects on the GABA_A receptor, this does not mean that the endogenous substance is a benzodiazepine. Dr. Rye and colleagues have experimentally tested the effects of the endogenous molecule on GABA_A receptors which lack a benzodiazepine binding site and found that the effects remain. The endogenous molecule is still able to increase the efficiency of the receptor, allowing a larger influx of chloride ions than when GABA alone is bound to the receptor. This means that while both the endogenous molecule and benzodiazepines are able to increase GABA_A receptor efficiency, they are not the same molecule and the mechanisms are different.

Although these are very important details to know, they are only the first pieces of the puzzle. Much research is still needed to fully understand the endogenous molecule in the cerebral spinal fluid of patients with hypersomnia and its effects on the GABA_A receptor.