CHEM 496 Topics in Chem & Biochem: Biochemistry, Physiology & Neurochemistry of Beer, Wine & Alcohol

Block IV – EtOH impact on brain, addiction and treatment

1

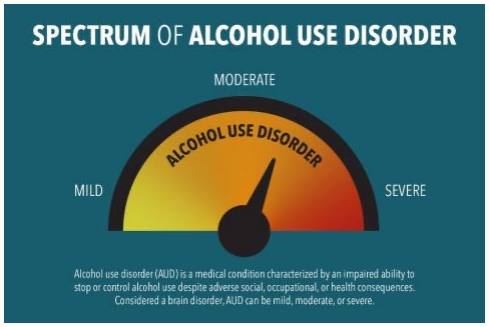
Basics of Neurobiology

Alcohol Use Disorder (treating alcohol addiction as a disease and not a choice).

- Alcohol use disorder is a medical condition involving frequent or heavy alcohol use. People with alcohol use disorder can't stop drinking, even when it causes problems, emotional distress or physical harm to themselves or others.

Is alcohol use disorder a disease?

- Alcohol use disorder is a medical condition. It's a disease of brain function and requires medical and psychological treatments to control it.
- Alcohol use disorder can be mild, moderate or severe. It can develop quickly or over a long period of time. It's also called alcohol dependence, alcohol addiction or alcohol abuse.



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What Are the Symptoms of AUD?

Diagnostic and Statistical Manual of Mental Disorders, DSM-5: Severity is based on the number of criteria a person meets based on their symptoms—mild (2–3 criteria), moderate (4–5 criteria), or severe (6 or more criteria).

In the past year, have you:

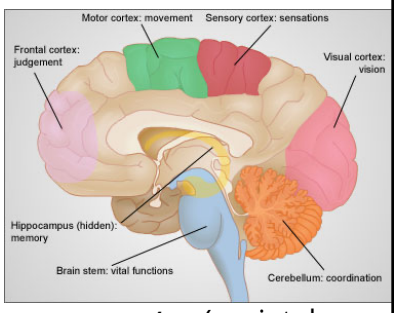
- Had times when you ended up drinking more, or longer, than you intended?
- More than once wanted to cut down or stop drinking, or tried to, but couldn't?
- Spent a lot of time drinking? Or being sick or getting over other aftereffects?
- Wanted a drink so badly you couldn't think of anything else?
- Found that drinking—or being sick from drinking—often interfered with taking care of your home or family? Or caused job troubles? Or school problems?
- Continued to drink even though it was causing trouble with your family or friends?
- Given up or cut back on activities that were important or interesting to you, or gave you pleasure, in order to drink?
- More than once gotten into situations while or after drinking that increased your chances of getting hurt (such as driving, swimming, using machinery, walking in a dangerous area, or having unprotected sex)?
- Continued to drink even though it was making you feel depressed or anxious or adding to another health problem? Or after having had a memory blackout?
- Had to drink much more than you once did to get the effect you want? Or found that your usual number of drinks had much less effect than before?
- Found that when the effects of alcohol were wearing off, you had withdrawal symptoms, such as trouble sleeping, shakiness, restlessness, nausea, sweating, a racing heart, or a seizure? Or sensed things that were not there?

3

Basics of Neurobiology

Alcohol affects the entire brain because it reaches all areas of the brain. Several of the intoxicating effects of alcohol and the corresponding brain regions affected by alcohol:

- Excitation (disinhibition): a normally functioning ***frontal cortex*** helps to suppress or inhibit behaviors that are socially inappropriate and impulsive. Alcohol releases this "brake" within the frontal cortex.
- Pleasure: the "pleasure or reward pathway" extends from the ***midbrain*** area to the ***limbic system*** (involved in emotion). Although not actually a symptom of intoxication, it is the pleasurable feeling that reinforces someone to keep drinking.
- Loss of judgment: the ***frontal cortex*** also controls judgment, thinking, decision-making, and risk-taking behavior.



- Slowed reaction time: the ***motor cortex*** (frontal lobe) and the ***sensory cortex*** (parietal lobe) or ***visual cortex*** (occipital lobe) work together to coordinate sensory information coming in to the brain with the reaction instructions going out of the brain to perform some type of movement.
- Unsteady gait: the ***cerebellum,*** located underneath in the back of the brain, controls balance and coordination.

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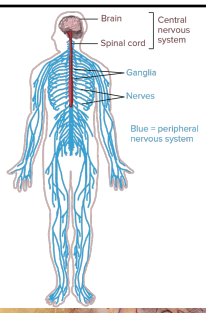
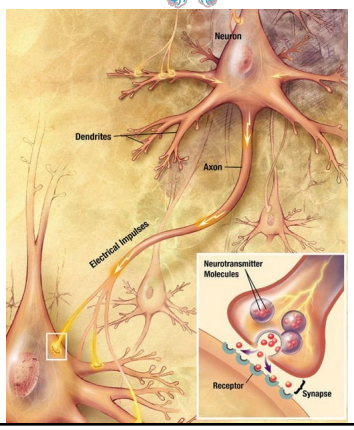
Basics of Neurobiology-Nerves, Neurons, Nerve Cells and Signal Transmission



The nervous system is a complex interconnected network of specialized cells that allow us to perceive and act on inputs from the world around us. How these nerves, with over 100 trillion connections generate electrical signals.

Weighing about 3 lbs., the brain is a complicated network of over 100 billion neurons interspersed with support/caretaker glial cells (astrocytes, schwann, microglial oligodendrocytes…) and myelin the insulating cells that support cognitive learning and memory processes.

- In the CNS, peripheral or brain system, neurons communicate by electrochemical signals. An electrical signal from one neuron is chemically transmitted to the next neuron in the pathway to the brain and back



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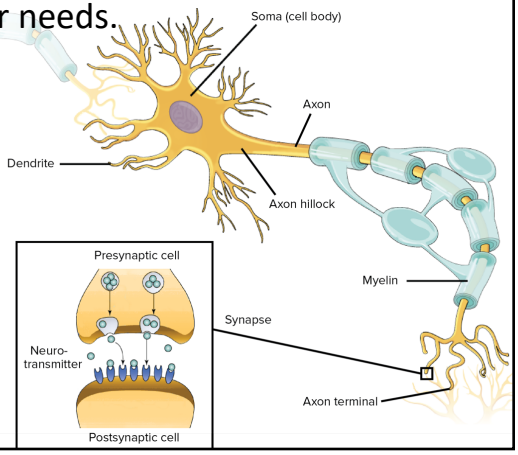
Basics of Neurobiology-Nerves, Neurons, Nerve Cells and Signal Transmission

Neurons – Cells that form the commutation wiring. The main components of a neuron are:

- **Soma** (cell body) contains the nucleus and most of the other organelles needed to synthesize proteins and other cellular needs.
- **Dendrites** are the acceptors of signals from other nerve fibers (neurons). These signals are placed into excitatory (generate electrical impulses down the axis) or inhibitory (blocking an electrical impulse from continuing).
- **Axons** leave the soma at the "axon hillock" and are where the electrical signal is propagated to the terminus of the nerve.
- **Axon terminal** splits into many branches ending in bulbous swellings (nerve terminals) where they connection with the next neurons are made.



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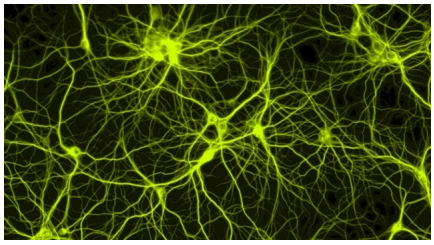
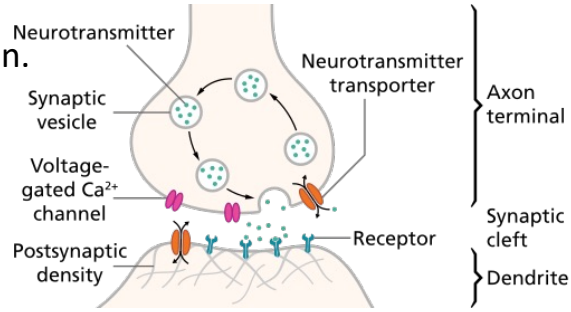
Basics of Neurobiology-Nerves, Neurons, Nerve Cells and Signal Transmission



Synapses – The connection between two neurons/nerve fibers. The signal is transferred by the release of chemical neurotransmitters.

- **Presynaptic neuron** – the sending neuron/axon terminus.
- **Postsynaptic neuron** – the receiving neuron.

Receptor activation in the postsynaptic neuron starts/blocks an action potential which travels down the axon to the next synapse.



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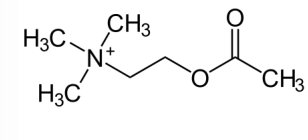
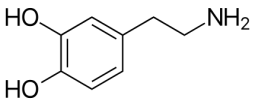
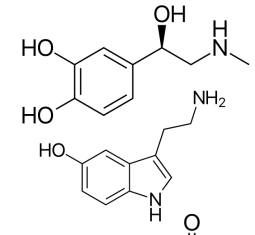
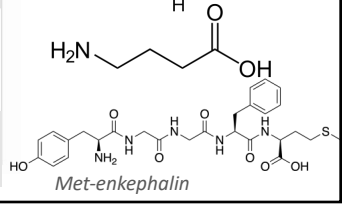
Neurotransmitter	Role
Acetylcholine *Choline esterified acetic acid*	Acetylcholine is a very widely distributed excitatory neurotransmitter that triggers voluntary muscle contraction and stimulates the excretion of certain hormones. It is involved in wakefulness, attentiveness, learning, memory, sleep, anger, aggression, sexuality, and thirst.
Dopamine *Monoamine amino acid derivative (Tyr)*	Dopamine correlates with movement, attention, and learning. Dopamine is involved in controlling movement and posture. It also modulates mood and plays a central role in positive reinforcement and dependency.
Norepinephrine *Monoamine amino acid derivative (Phe/Tyr)*	Norepinephrine is associated with alertness. neurotransmitter that is important for attentiveness, emotions, sleeping, dreaming, and learning. Norepinephrine is also associated with the "fight or flight" response.
Serotonin *Monoamine amino acid derivative (Trp)*	Serotonin plays a role in mood, sleep, appetite, and impulsive and aggressive behavior.
GABA (Gamma-Amino Butyric Acid)	GABA is the major inhibitory neurotransmitter in the CNS, contributing to motor control, anxiety regulation, vision, and many other cortical functions.
Opioid System - peptides	Opioids effect is to alter other neurotransmitter release. Resulting in pain relief, euphoria and other behaviors including alcohol consumption. This is key to craving alcohol in addiction/withdraw.









Met-enkephalin

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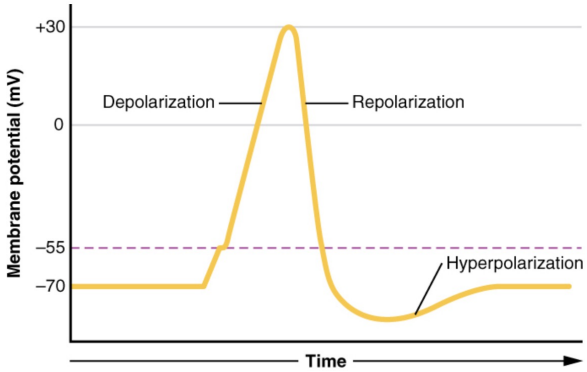
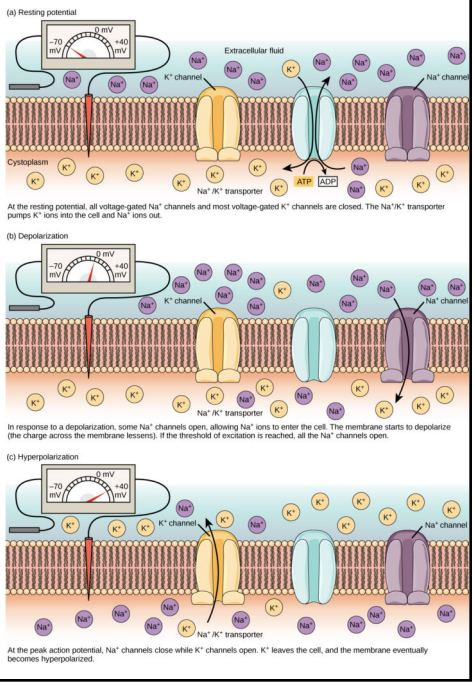
Action Potential – The electrical potential across a membrane. Sending the electrical signal down the axon resulting in a release of neurotransmitters.

Inside neuron: Na^+ and Cl^-

Outside neuron: K^+ & organic anions





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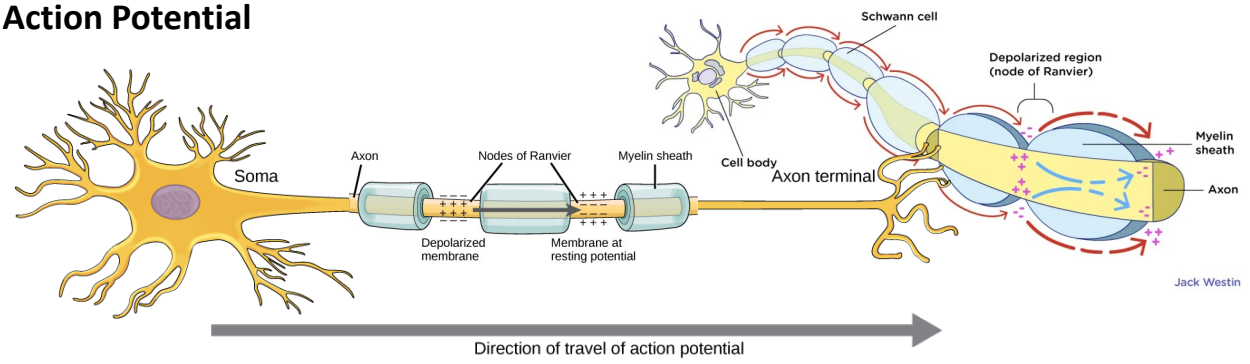
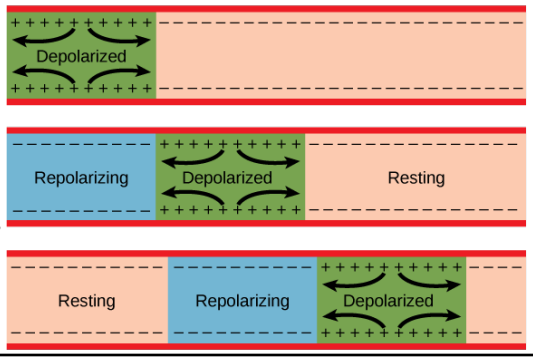
Action Potential

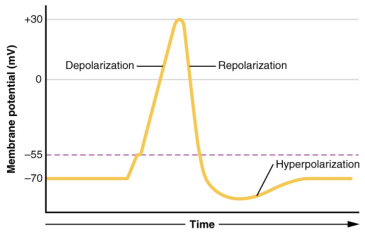


a. In response to a signal, the soma end of the axon becomes depolarized.

b. The depolarization spreads down the axon. Meanwhile, the first part of the membrane repolarizes. Because Na^+ channels are inactivated and additional K^+ channels have opened, the membrane cannot depolarize again.

c. The action potential continues to travel down the axon.





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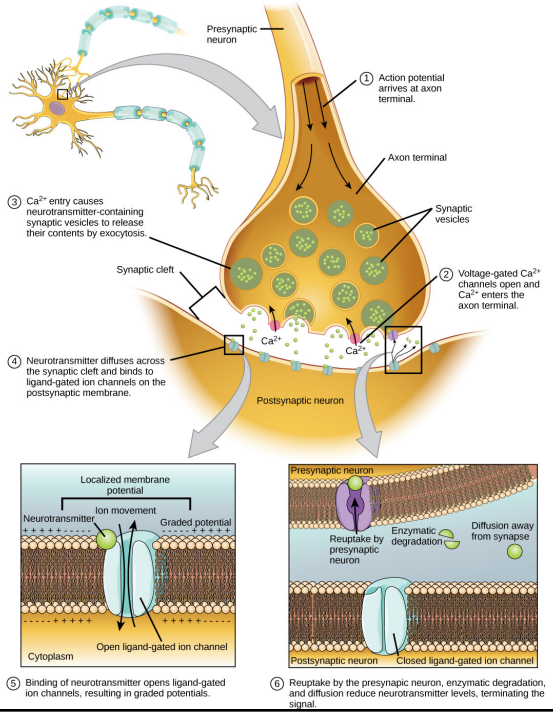
Basics of Neurobiology-

Transmission

When an action potential reaches the axon terminal it depolarizes the membrane and opens voltage-gated Na^+ channels. Na^+ ions enter the cell, further depolarizing the presynaptic membrane. This depolarization causes voltage-gated Ca^{2+} channels to open. Calcium ions entering the cell initiate a signalin cascade that causes small membrane-bound vesicles, called **synaptic vesicles**, containing neurotransmitter molecules to fuse with the presynaptic membrane.

Fusion of a vesicle with the presynaptic membrane causes neurotransmitter to be released into the **synaptic cleft**, the extracellular space between the presynaptic and postsynaptic membranes. The neurotransmitter diffuses across the synaptic cleft and binds to receptor proteins on the postsynaptic membrane.



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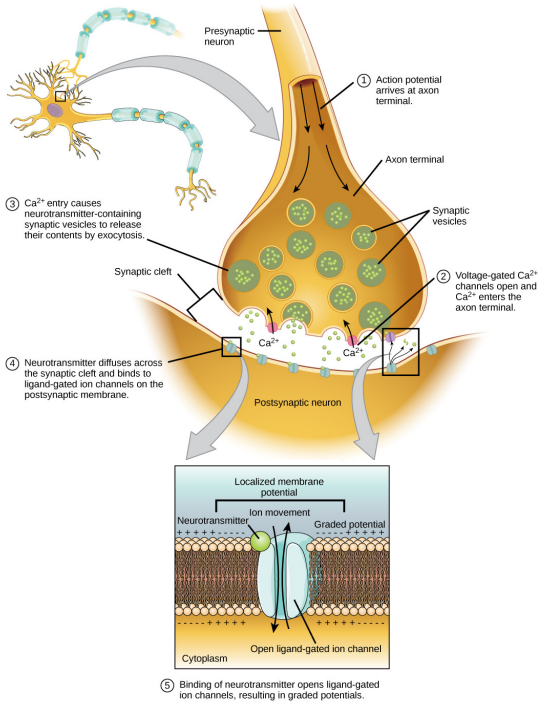
Basics of Neurobiology

The binding of a specific neurotransmitter causes particular ion channels on the postsynaptic membrane to open.

- Neurotransmitters can either have excitatory or inhibitory effects on the postsynaptic membrane.
- When Na^+ enters the postsynaptic cell and causes the postsynaptic membrane to depolarize. This depolarization is called an **excitatory postsynaptic potential (EPSP)** and makes the postsynaptic neuron more likely to fire an action potential.
- Release of neurotransmitter at inhibitory synapses causes **inhibitory postsynaptic potentials (IPSPs)**, a hyperpolarization of the presynaptic membrane.



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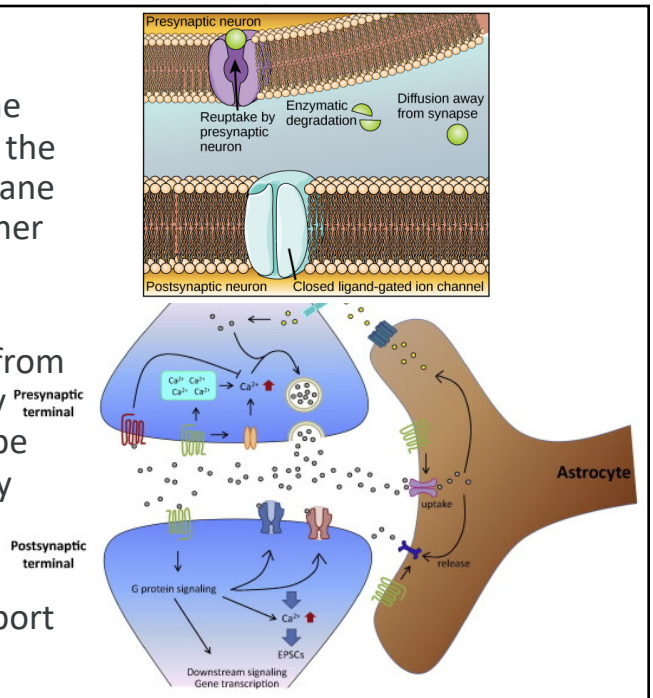
Basics of Neurobiology

Once neurotransmission has occurred, the neurotransmitter must be removed from the synaptic cleft so the postsynaptic membrane can "reset" and be ready to receive another signal. This can be accomplished in three ways:

- the neurotransmitter can diffuse away from the synaptic cleft, it can be degraded by enzymes in the synaptic cleft, or it can be recycled (sometimes called reuptake) by the presynaptic neuron.
- Astrocytes can release some neurotransmitters (glutamine) and support removal by uptake and recycling



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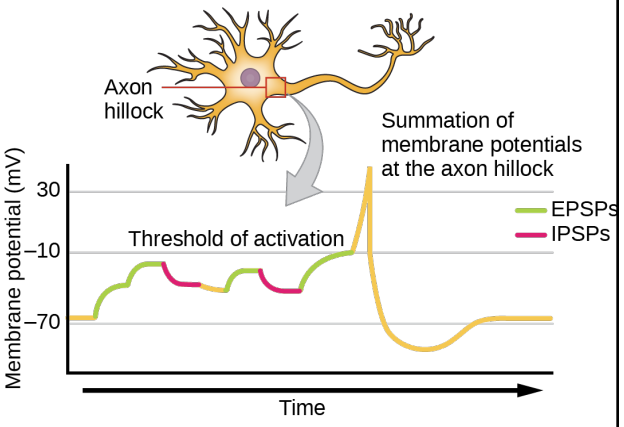
Basics of Neurobiology

Sometimes a single EPSP is strong enough to induce an action potential in the postsynaptic neuron, but often multiple presynaptic inputs must create EPSPs around the same time for the postsynaptic neuron to be sufficiently depolarized to fire an action potential. This process is called **summation** and occurs at the axon hillock..

Additionally, one neuron often has inputs from many presynaptic neurons—some excitatory and some inhibitory—so IPSPs can cancel out EPSPs and vice versa.

It is the net change in postsynaptic membrane voltage that determines whether the postsynaptic cell has reached its threshold of excitation needed to fire an action potential.

Together, synaptic summation and the threshold for excitation act as a filter so that random "noise" in the system is not transmitted as important information.



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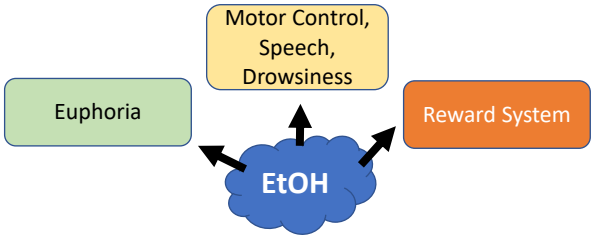
Addiction, vs addiction, vs adaptation vs withdrawal

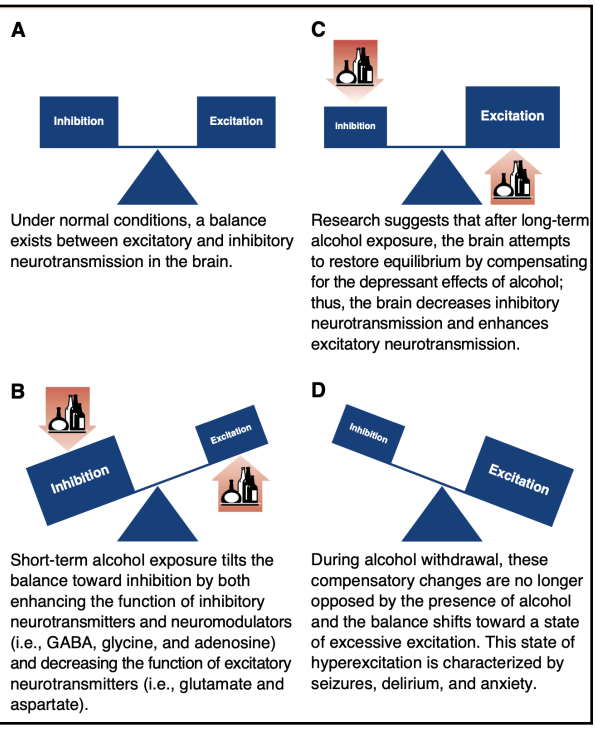
- Different systems are effected… differently (confusing isn't it?)
- Ethanol is considered a dirty drug as it affects many receptors producing a wide range of effects.



Cycle of Addiction

Intoxication → Withdrawal → Anticipation

Intoxication	Withdrawal	Anticipation
Spikes in DA release in mesolimbic reward center	Use shifts from gaining a euphoric feeling to avoiding dysphoria (unease). DA release is decreased.	DA and Glu disruption impairs decision making. Genomic shifts in neuronal protein expression supports need for more!



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Alcohol and Neurochemical signaling

- Alcohol consumption is promoted through the activation of neural circuits that produce stimulation and positive mood responses (reward) and that relieve stress and negative moods (relief). The rewarding effects of alcohol occur through the activation of mesolimbic circuits—the same system that regulates responses to natural reinforcers such as food, sex, and social interactions. Rewarding stimuli release the neurotransmitter dopamine into the nucleus accumbens, amygdala, and prefrontal cortex, resulting in increased salience of the stimulus, increased attention to the stimulus, and increased wanting or desire for it.

- At the same time, alcohol produces unpleasant effects—such as sedation, negative moods, and motor impairments—that typically deter alcohol consumption. The sedation results from enhanced inhibitory GABA neurotransmission via alcohol's direct action on GABA-A receptors. Alcohol reduces excitatory glutamatergic neurotransmission by attenuating excitatory glutamate responses at N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) glutamate receptors. Alcohol also releases dynorphins, which produce negative mood effects and cognitive dysfunction

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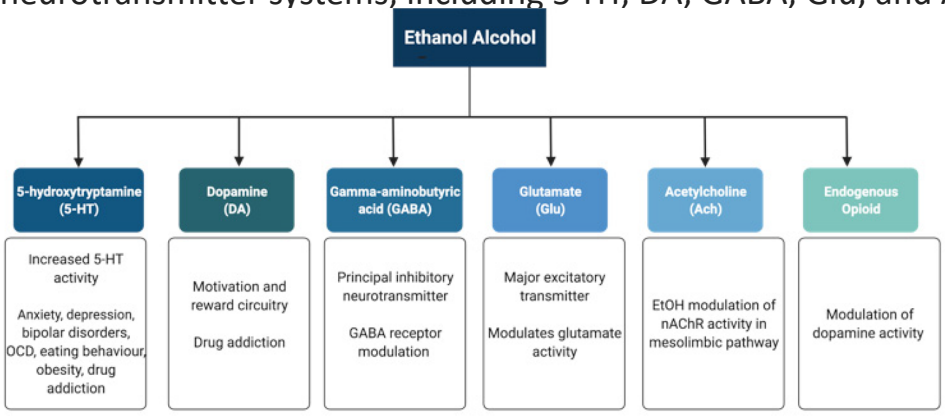
Basics of Neurobiology

Molecular Targets of Ethanol in Brain

- Alcohol molecules can directly interact with components of the cell membranes, such as receptors and transporters, as well as with several intracellular molecules and structures, thus impacting multiple cellular processes and functions. In particular, alcohol is able to alter synaptic function by impacting multiple neurotransmitter systems, including 5-HT, DA, GABA, Glu, and ACh



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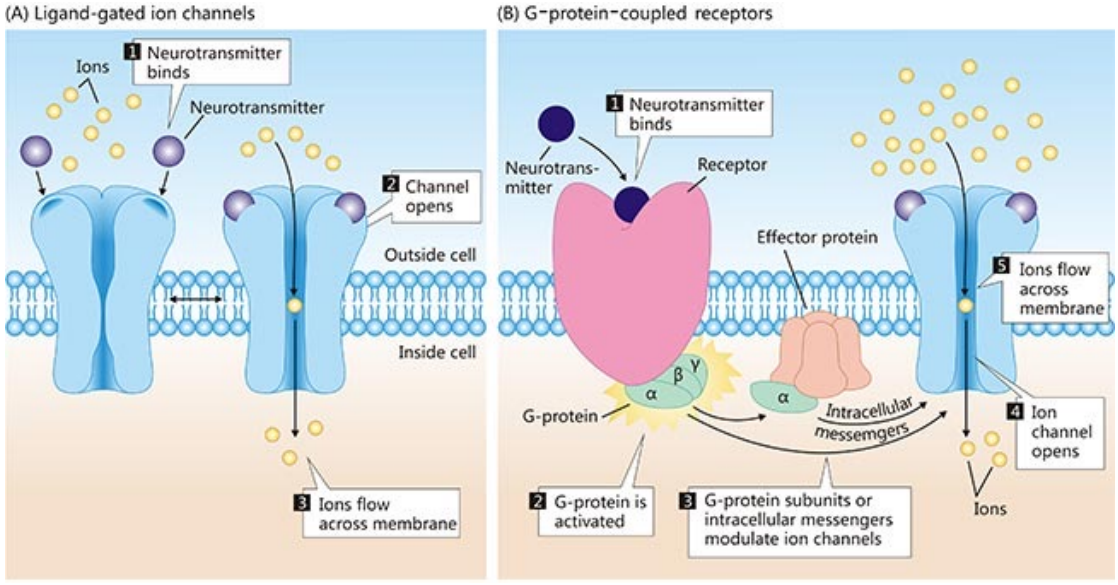
Basics of Neurobiology

Neurotransmitters – Receptors (GPCR and Voltage Gated Ion Channels)



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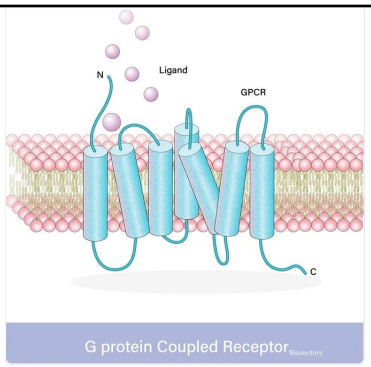
GPCR Signaling
(G Protein Coupled Receptors)



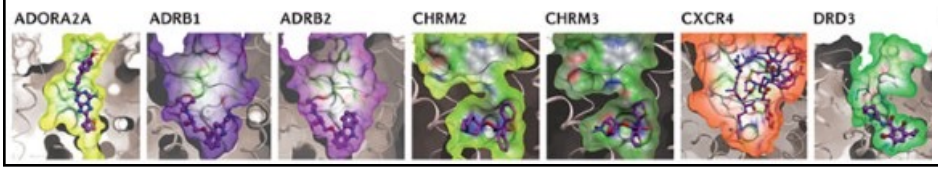
Neurotransmitters bind to proteins (receptors) on the surface of neurons.

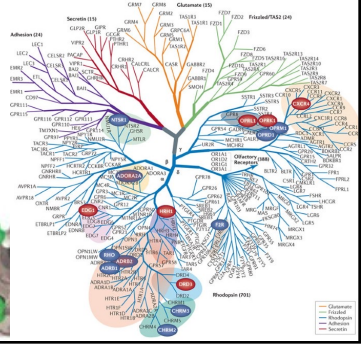
GPCR proteins are "seven pass" plasma transmembrane proteins that interact with their ligand at or in the cytosolic surface of the transmitter.

GPCRs bind specifically to one or a limited group of agonists (transmitters / hormones / signals).

There are about 1000 different receptors in humans.

The neurotransmitter "sends the signal" into the cell via the GPCR and the proteins bound to the receptor inside of the cell.





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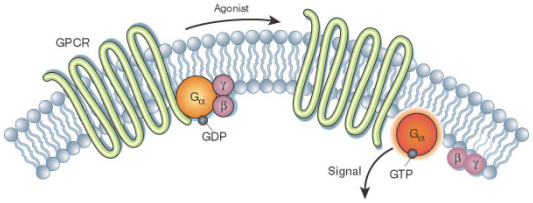
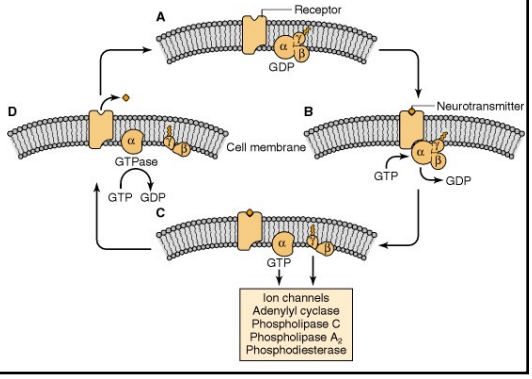
GPCR Signaling

GTP binding protein – Three subunits making a "heterotrimeric G protein (α - binds GTP/GDP), and $\beta\gamma$. Called a G protein as α subunit binds Guanine nucleotide (GTP or GDP).

- In the resting state (no transmitter) the α subunit has GDP in the center of the protein and as a result binds to the $\beta\gamma$ subunits and the receptor.
- Upon agonist binding to the receptors, the α subunit releases the GDP in exchange for a GTP. This decreases the binding of α subunit for the receptor and the $\beta\gamma$ subunits
- "active" α subunit and $\beta\gamma$ subunits then interact with other proteins (effector proteins) leading to intracellular changes – examples include turning on protein kinases, opening channels, increasing calcium release into the cytosol and increasing cAMP levels…





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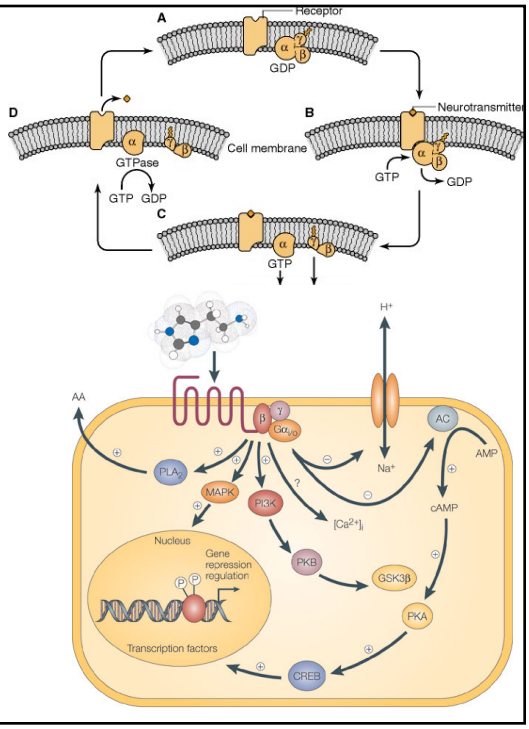
GPCR Signaling

GTP binding protein – Three subunits making a "heterotrimeric G protein (α - binds GTP/GDP), and βγ. Called a G protein as α subunit binds Guanine nucleotide (GTP or GDP).

- Eventually the α subunit hydrolyzes the GTP to GDP and PO_4^{-3}. This results in GDP rebinding of the α subunit to the βγ subunits and in combination re-bind to the unoccupied GPCR.

All results in a complicated and coordinated response inside of the cell.

Not all receptors are expressed on all cells! Different areas of the brain will have one group of GPCR vs another area – leaving specific responses to the same message (transmitter)



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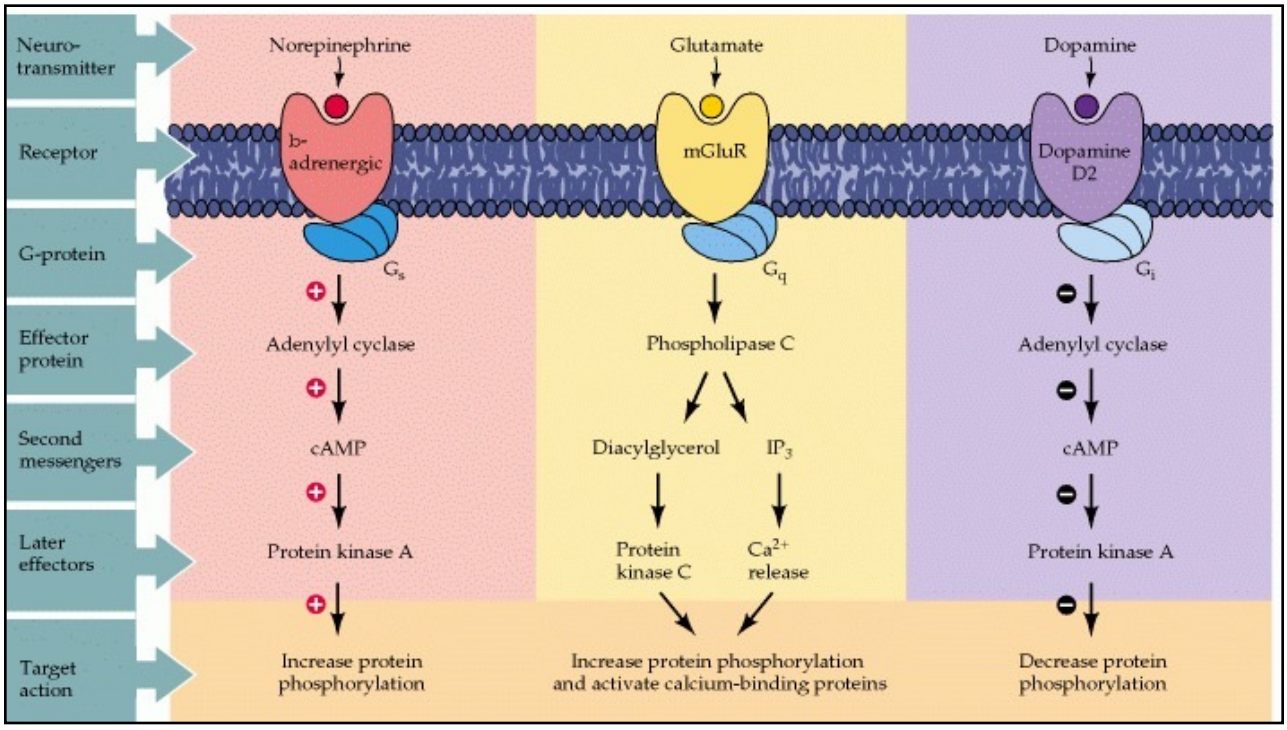

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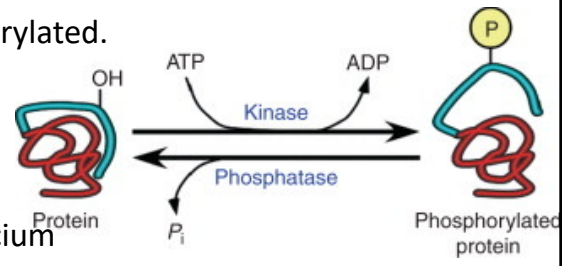
What are protein kinases?

Protein kinases are enzymes that transfer a phosphate from ATP to an R-OH (Ser/Thr/Tyr) of other proteins.

There are ~1000 kinases in the human genome – each activated in unique ways.

When a protein is phosphorylated, the structure and function of the targeted protein will change – some proteins are activated some are silenced

- In neurons – ion channels are often the target opening/closing the channel
- Many of the receptors are themselves phosphorylated.



Common Kinases

PKA – protein kinase A. Activated by cAMP

PKC - protein kinase C. Activated by DAG and calcium

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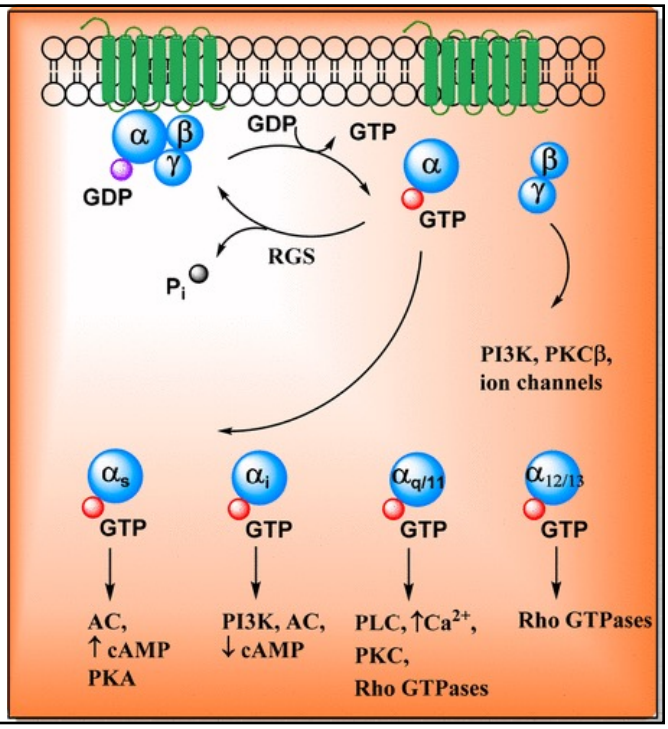
GPCR Signaling

Each receptor signals to specific G proteins which are responsible for a particular response.

Each G protein has a set of downstream effectors/signaling pathway

- Gαs – Increases cAMP/PKA, Calcium and PKE
- Gαi– decreases cAMP/PKA (antagonizes Gs) and activates PI3Kinases
- Gαq/11 – Activates lipases, PKC and small G protiens
- Gα12/13 – Involves cytoskeletal changes and small G proteins
- βγ subunits – stay together and act on PKCb and ion channels



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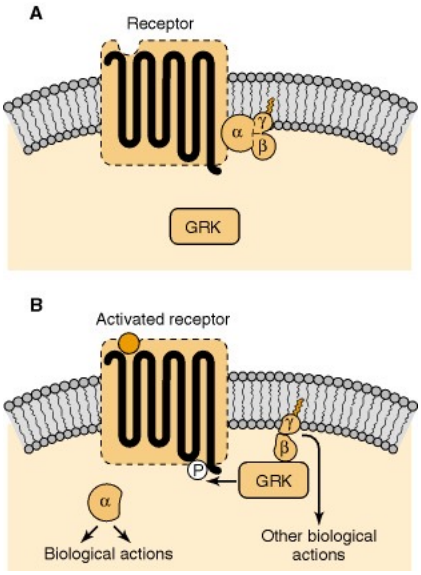
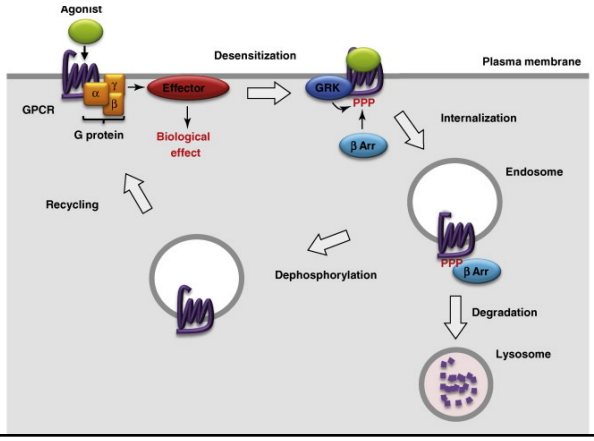
GPCR Signaling



GPCRs can be **desensitized** following activation by agonists by becoming phosphorylated by members of the family of G protein-coupled receptor kinases (GRKs). Phosphorylated receptors are then bound by arrestins, which prevent further stimulation of G proteins and downstream signaling pathways.



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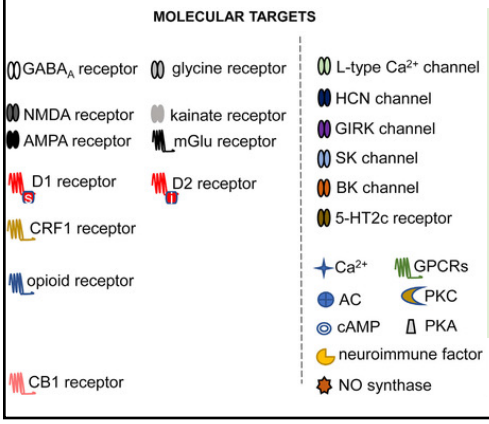
Molecular Targets of Ethanol in Brain

While we know the impact of EtOH on neuronal signaling, the pharmacological mechanism of EtOH on proteins in the brain are much less understood.

- Questions remain: is the affect of ethanol on membrane fluidity, ethanol non-specific interaction on water solvent protein surfaces or specific ethanol binding sites. Likely answer is yes: some or all of the above!



EtOH binding sites (direct effect of alcohol) must have the following criteria

- The target protein should be affected by ethanol at both low and high concentration – should be a saturating effect (signaling or allosteric)
- Molecular binding site and ethanol interaction should be characterized biochemically. i.e. Mutation of possible amino acids involved in EtOH binding that make the binding site should alter the ethanol interaction and the biological effect of ethanol.
- Structural biological evidence should indicate that ethanol inhabits the binding site (protein shifts in tertiary structure w/ethanol vs w/o)
- Genetic alteration of the target (knock out) should result in a readily discernable ethanol-related phenotype

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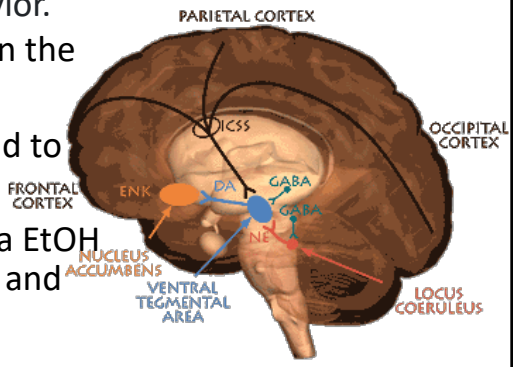
Molecular Targets of Ethanol – Dopamine Signaling

Dopamine (DA) signaling influences how organisms orient toward incentive changes in the environment and is key in addiction behaviors.

- The positive reinforcing action of alcohol comes from the **activation of the dopaminergic reward pathway in the limbic system**. Dopamine is a neuromodulating compound that is released in the **ventral tegmental area** (VTA) and projects to the **nucleus accumbens** (NA) where it is acutely involved in motivation and reinforcement behavior.
- Acute alcohol injection increases in DA release in the mesolimbic center
- Several mutations of the DA receptor(s) may lead to increased vulnerability to AUD.
- The ethanol-induced DA release is influenced via EtOH impact on GABAergic neurons, opioid receptors and acetylcholine receptors all driving DA release.



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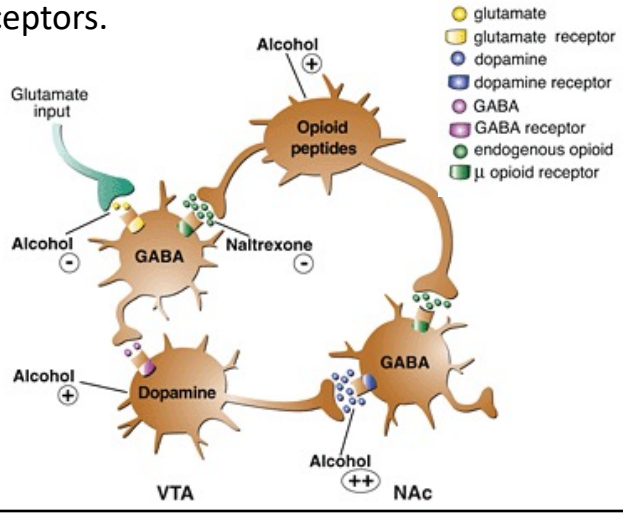
Molecular Targets of Ethanol – Dopamine Signaling

Immediate (acute/short term) ethanol and dopamine signaling on REWARD:

- Alcohol, promotes GABA subtype A receptor inhibits GABAergic transmission in the VTA – This relieves (disinhibits) the blocking of DA neurons. Resulting in the activation of DA release to the NA receptors.
- Opioid signaling (endorphin) is enhanced in acute alcohol intake and blocks the GABA inhibition of DA neurons in the VTA and activate DA release in the NA.
- Ethanol inhibits glutamate neuronal input to DA neurons via GABA

While mechanism isn't clear – this is one possible effect of EtOH on reward



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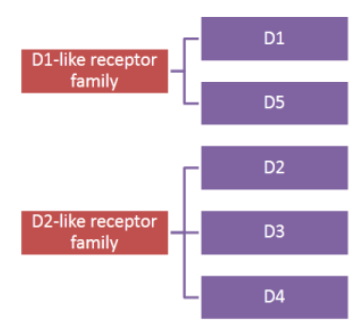
Molecular Targets of Ethanol – Dopamine Signaling

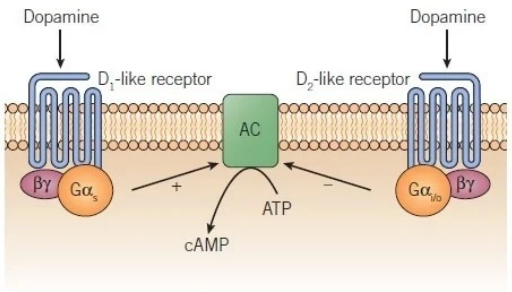
The hallmark of an addictive disorder is its chronic relapsing course characterized by a strong resistance to change an apparent self-destructive behavior. Although alcohol affects many neurochemical systems, its effects are primarily mediated by the brain's reward system with dopamine and endogenous opioids playing major roles

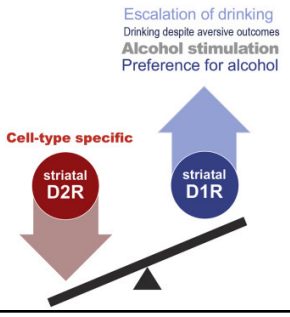
There are two classes of DA receptor D1 and D2 (many members of each class) and each class has an opposite signaling mechanism.

AUD increases D1 and decreases D2 receptor density.

D1 receptors seem to be the major effector in encoding alcohol reward processes, while D2 receptors counteract these processes both at the post- and presynaptic level. Available dopamine levels are further controlled by presynaptic reuptake of dopamine via the dopamine transporter (DAT).







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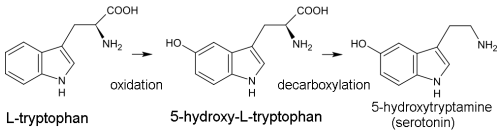
Molecular Targets of Ethanol – Serotonin Signaling

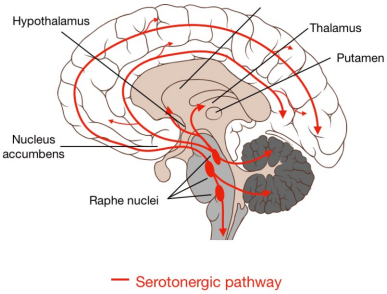
The serotonergic (5-hydroxytryptamine or 5-HT) system is involved in nearly every aspect of mammalian physiology including neurogenesis, motor control, sleep, mood, and cognition; it also plays a key role in regulating alcohol consumption, dependence, and withdrawal.

Similar to GABA and glutamate receptors, 5-HT receptors come as either ligand-gated ion channels (5-HT3) or metabotropic GPCRs

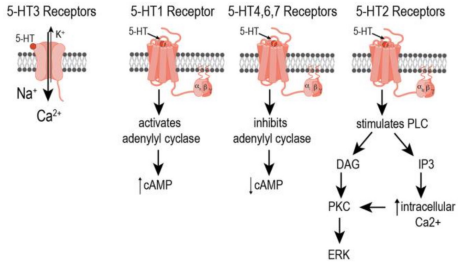
- There are many subtypes of 5-HT3 receptors in the body.



Serotonin in the brain is synthesized in the dorsal raphe nuclei and is subsequently dispersed throughout the rest of the brain, most notably to the mesolimbic and frontal cortices.



Pharmacological compounds that target the serotonin system by inhibiting neuronal reuptake of serotonin, thereby prolonging its actions, or by blocking specific serotonin receptor subtypes have been shown to suppress alcohol-reinforced behavior in rats



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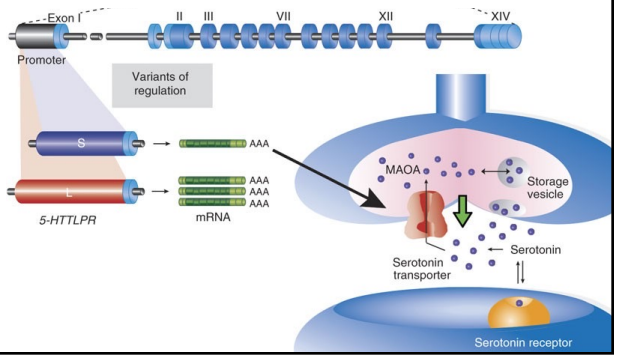
Molecular Targets of Ethanol – Serotonin Signaling

Ethanol elevates 5-HT levels in several brain regions associated with the reward circuitry.

Implications for serotonergic dysfunction in both AUDs and affective disorders have been found at multiple levels of serotonergic functioning, from synthesis to terminal projections. For example, serotonin's rate limiting enzyme, TRH, demonstrates increased immunoreactivity in the dorsal raphe of alcohol dependent victims of depression and suicide compared to psychiatrically normal controls

Alcohol triggers serotonin release in the nucleus accumbens - a hallmark characteristic of drugs of abuse.

- The amount of alcohol consumed correlated negatively with basal levels of serotonin and dopamine, indicating that an inherent dearth of these neurotransmitters, due to an increase in 5-HT release rather than uptake, can prompt greater alcohol.
- Mutations in the receptor For example, in humans, individuals with the less active short allele of the 5HT transporter (resulting in increased 5HT in synapse) consumed less alcohol wild-type.



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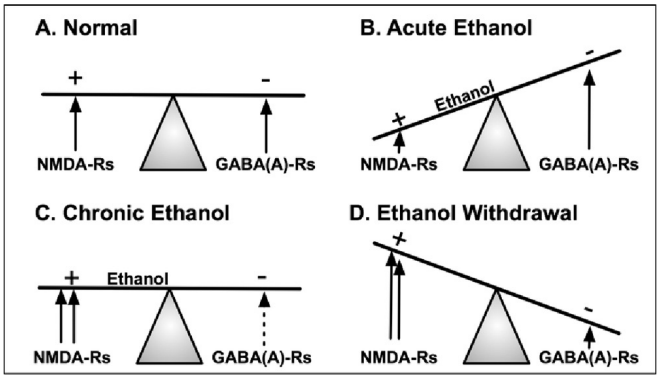
GABA and Glutamate Signaling in AUD

Balancing GABA and Glutamate Signaling

GABA is the major inhibitory neurotransmitter in the CNS and is allosterically modified by EtOH where in general, alcohol increases the effects of GABA signaling.

Glutamate is the major excitatory neurotransmitter in the human brain. Acute alcohol exposure inhibits glutamate neurotransmission, while chronic exposure and acute withdrawal activates the excitatory signals.



A. **In a normal state** without alcohol, neuronal excitability and inhibition are in balance with NMDA and GABA.
B. **With acute alcohol intake**, there's an overall inhibitory state from alcohol's agonism of GABA receptors. The NMDAs are overpowered, and inhibition wins (sleepy).
C. **With chronic alcohol use**, NMDA receptors are up-regulated to compensate for alcohol's inhibitory effects on GABA. This increased number and function of NMDA receptors is why people who chronically use alcohol are able to function
D. **When alcohol is removed** in a chronic user, the up-regulated NMDA receptors are left unopposed and as excitable as they want to be.

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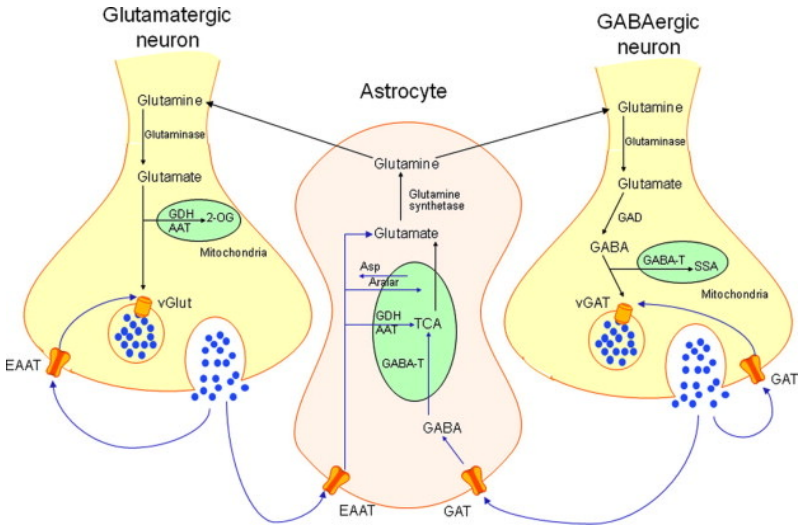
GABA and Glutamate Signaling in AUD

Balancing GABA and Glutamate Signaling



The synthesis, release, reuptake, and metabolism of the excitatory and inhibitory neurotransmitters glutamate and GABA, respectively, are tightly controlled

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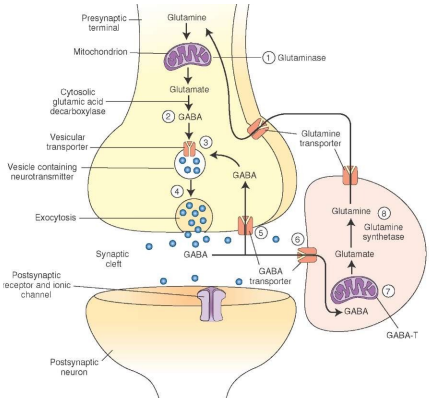
Molecular Targets of Ethanol – GABA

Synthesis and Receptors

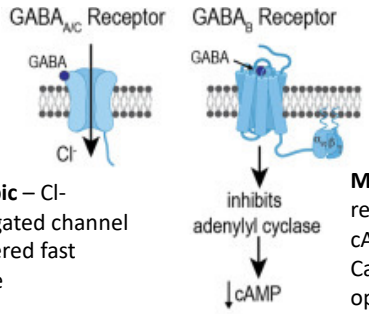


GABA is formed from glutamate via the addition of glutamate decarboxylase and vitamin B6. GABA can then be used to form succinate, which is involved in the citric acid cycle. Once GABA is formed, is it released into the post-synaptic terminals of neurons.

γ-aminobutyric acid (GABA): prime inhibitory neurotransmitter and regulates fear and anxiety by blocking the excitatory signals that create these feelings.

- Acute alcohol consumption increases the levels/signaling of GABA. Less fear and inhibition.
- This is why alcohol is called the "social lubricant of alcohol" or…. "Ten feet tall and bullet proof (Travis Tritt)"… or, hey guys, hold my beer ! (1983 - Freshmen J Provost)

Depending on how organized there are two (three) classes of GABA receptors



Ionotropic – Cl- voltage gated channel - considered fast response

Metabotropic – GPCR receptor. Decrease cAMP/PKA, decrease Ca transport and open K+ channels.

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Molecular Targets of Ethanol – GABA

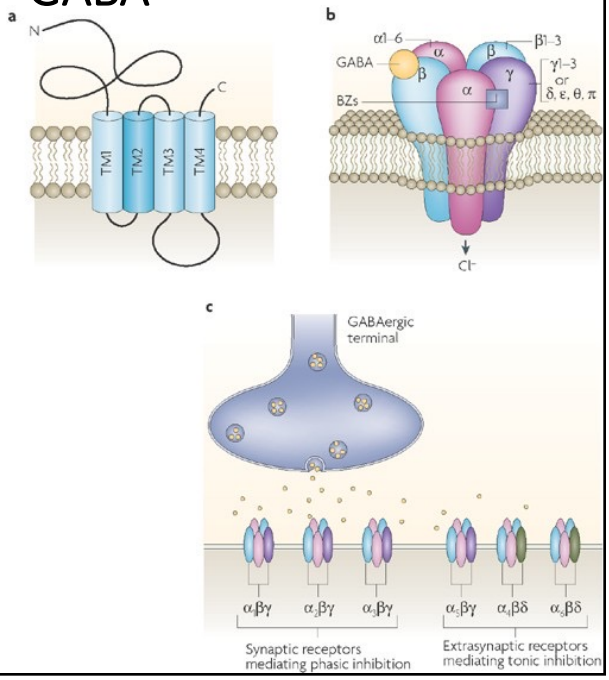
GABA A Receptors, Signaling and Ethanol

GABA-A receptors **control the majority of inhibitory signaling in the central nervous system**. They exist as hetero-pentameric, ligand-gated ion channels and conduct chloride ions following activation by GABA, which results in neuronal hyperpolarization and inhibition of neuronal signaling.

In the VTA, GABA A blocks reward system (DA) by inhibiting the DA release from Ach and Glu excitatory neurons. – Alcohol increases GABA activity (inhibition) and decreases Glu inhibitory signals. ??? WTH???

The receptor is a four (hepta) transmembrane spanning channel with an extended amino-terminus that include "cys loops).

- There are five subunits (Gene products) that increase diversity and signaling of GABA A receptor (α, β, γ & δ)
- Receptors exist at both post and presynaptic locations.
- Binding of GABA to subunits open the chloride pore and allowing an inward migration of Cl- leading to hyperpolarization of the neuron.



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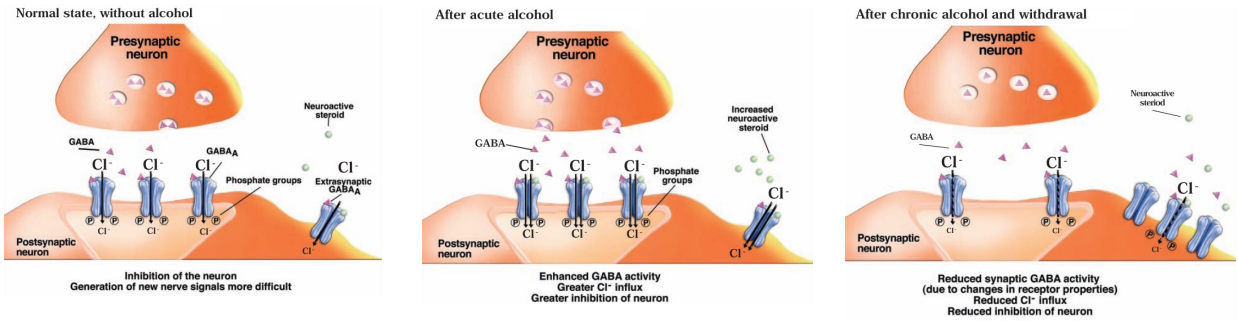
Molecular Targets of Ethanol – GABA

GABA A Receptors, Signaling and Ethanol



- **Normal state** – regulated GABA activation leads to inward chloride influx reducing the ion potential and blocking axon signal
- **Acute alcohol** – through several mechanisms, possibly direct binding of EtOH to the channel, GABA ion channels increases the flux of chloride into neurons making it even more difficult for neurons to start a signal.
- **AUD and Withdrawal** – A decreased activity and lower expression (density) of GABA A receptors leads to a loss of inhibition (think loss of breaks on a fast idling car) and increased neuronal signaling in several areas of the brain. This is a major source of *increases in anxiety and hyperexcitability in both AUD and during withdrawal*. It is important to know that in VTA/NA (DA reward system) of AUD patients INCREASE and contributes to decrease in DA release associated with withdrawal.

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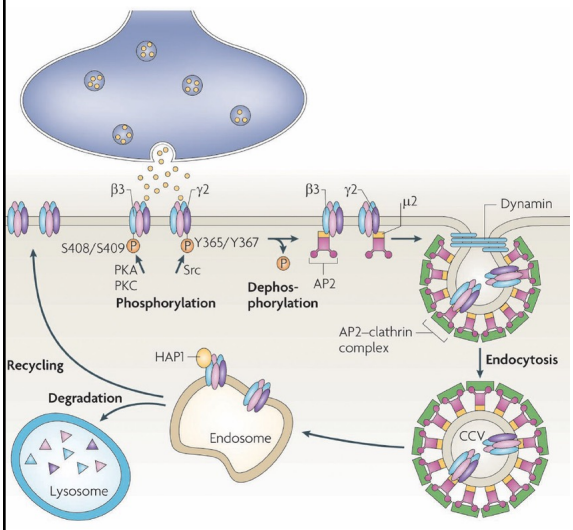
Molecular Targets of Ethanol – GABA

GABA A Receptors, Signaling and Ethanol



A person with alcoholism engages in risky or dangerous drinking despite experiencing serious negative physical and social consequences. Such persistence in pursuing damaging behaviors suggests that the short-term "appetitive" results of drinking (such as intoxication and losing one's inhibitions) have greater control over the alcoholic's behavior than do the negative consequences. From a neurobiological perspective, this pattern implies weak "top-down"—or knowledge-driven—executive control over impulsive and compulsive urges to consume alcohol and a strong "bottom-up"—or stimulus-driven—appetitive drive to consume alcohol, both impulsively and compulsively.

Neuroplasticity, **the remarkable ability of the brain to modify and reorganize itself**, is affected by or in response to excessive alcohol, whether through individual consumption or exposure in the womb. It is now well accepted that the birth and integration of new neurons continue beyond development and into adulthood

The mechanism studies have shown that the behavioral changes are primarily due to the plastic changes of $GABA_A$Rs that occur after chronic EtOH exposure, which include significantly reduced postsynaptic α1 and increased α4-containing $GABA_A$Rs. The subunit composition of $GABA_A$R subtypes is expected to determine their physiological properties and pharmacological profiles. Thus, these $GABA_A$R subunit composition changes are a mechanism underlying the behavioral changes after chronic EtOH exposure. ***The end result is loss of GABA activity with long term EtOH exposer.***

Short and long-term GABA A receptor involves the phosphorylation by PKA and PKC (PKC zeta expression, location and activity is increased in chronic AUD)

GABA A receptors are also targeted for endosomal trafficking and degradation by ubiquitination.

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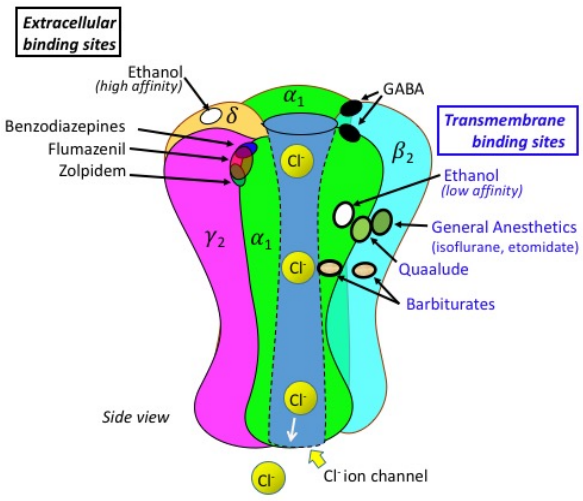
Molecular Targets of Ethanol – GABA

GABA A Receptors, Signaling and Ethanol



$GABA_A$Rs are heteropentamers formed from a selection of 19 subunits: six α (alpha1-6), three β (beta1-3), three γ (gamma1-3), three ρ (rho1-3), and one each of the δ (delta), ε (epsilon), π (pi), and θ (theta) which result in the production of a considerable number of receptor isoforms. Each isoform exhibits distinct pharmacological and physiological properties. However, the majority of $GABA_A$Rs are composed of two α subunits, two β subunits, and one γ subunit arranged as γ2β2α1β2α1 counterclockwise around the center.

There are two apparent binding sites for Ethanol, a high and low affinity site.

- High concentrations of EtOH (greater than 60 mM EtOH) is a low affinity, high Km binding site on the delta subunit at the transmembrane domain. THIS EtOH concentration is well above the mM concentrations of EtOH that are found after 6 shots (23 mM) or even higher than the ~45mM that brings a coma and possibly death!
- There is a second high affinity, low Km site on the surface of the receptor/channel that has a 1-3 Km

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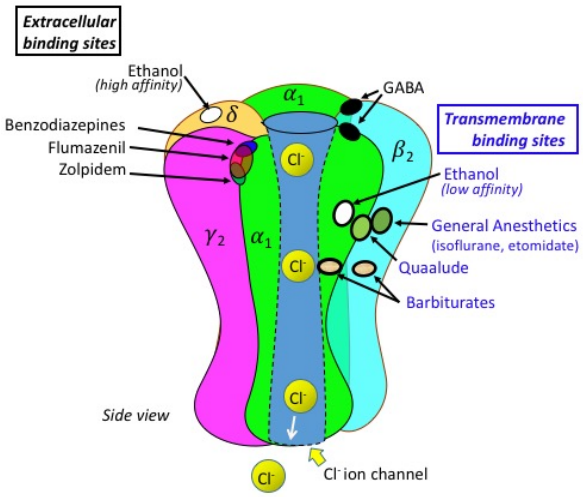
Molecular Targets of Ethanol – GABA

GABA A Receptors, Signaling and Ethanol



Social drinking can produce a low-to-moderate concentration of EtOH (≤30 mmol/L) in the brain.

Animal studies have shown that a low dose of EtOH (0.01 g/kg, <10 mmol/L) applied to VTA could significantly increase GABA neuron firing rate and afferent-evoked synaptic responses.

Acute EtOH (~13 mmol/L) consumption induced an elevation of dopamine in the rat mesolimbic reward pathway.

Social levels of EtOH consumption (<10% EtOH) could induce an increase of $GABA_AR$ α4/δ subunits in the hippocampus. These α4/δ-containing $GABA_ARs$ have been shown to exist on extrasynaptic membrane sites, which are sensitive to as low as 3 mmol/L EtOH (approximately 0.3% EtOH).

Therefore, EtOH entering the brain by each episode of social drinking will target and stimulate $GABA_ARs$, primarily extrasynaptic $GABA_ARs$; these $GABA_ARs$ respond to the alcohol, then $GABA_AR$ dynamic changes occur.

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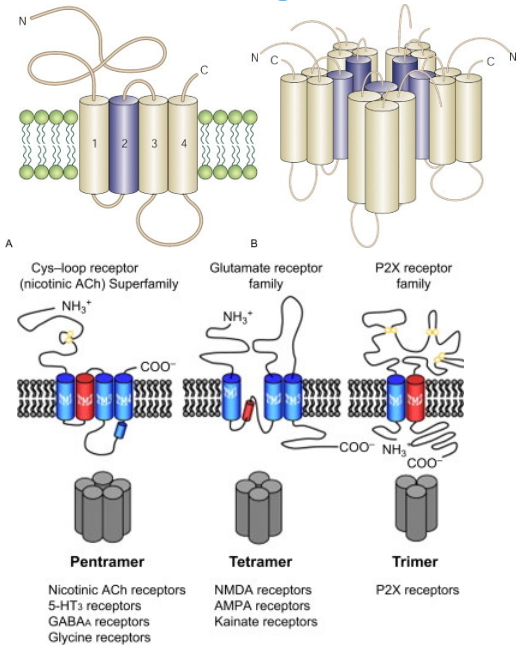
Molecular Targets of Ethanol – GABA



Studies of neurological actions of alcohols and anaesthetics have focused on ligand-gated ion channels such as GABA(A), glycine and nicotinic acetylcholine receptor.

Receptors in this family are assembled from five identical or similar subunits arranged around a C5 symmetry axis perpendicular to the membrane; each subunit contains a large ligand-binding extracellular domain, a transmembrane domain comprised of four helices (M1–M4) from each subunit and a variable intracellular loop.

These receptors carry neurotransmitter-binding sites within the extracellular domain, while the ion channel is located in the transmembrane domain. The coupling of agonist binding and channel opening involves critical regions between the extracellular and transmembrane domains, particularly extracellular loops 2 and 7 and the upper portion of the pore-lining M2 helices. Mutagenesis and labelling studies have identified a few specific residues critical to alcohol and anaesthetic effects, primarily in the transmembrane domain[3].

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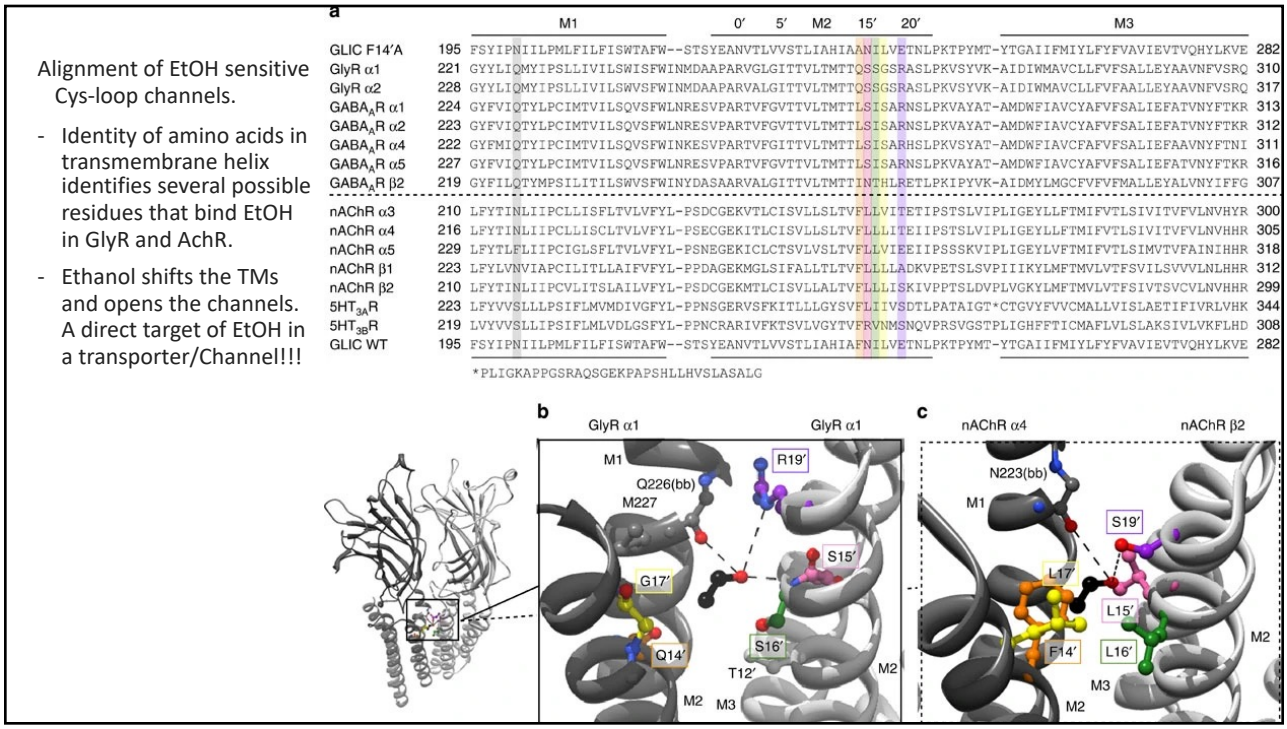
Alignment of EtOH sensitive Cys-loop channels.

- Identity of amino acids in transmembrane helix identifies several possible residues that bind EtOH in GlyR and AchR.
- Ethanol shifts the TMs and opens the channels. A direct target of EtOH in a transporter/Channel!!!



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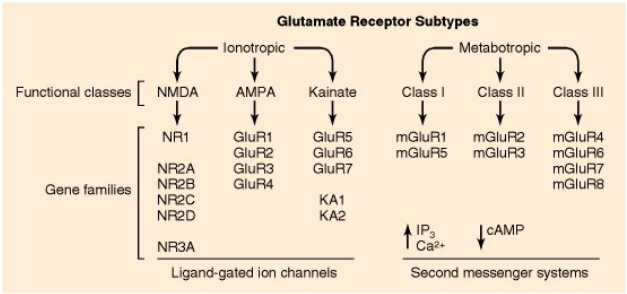
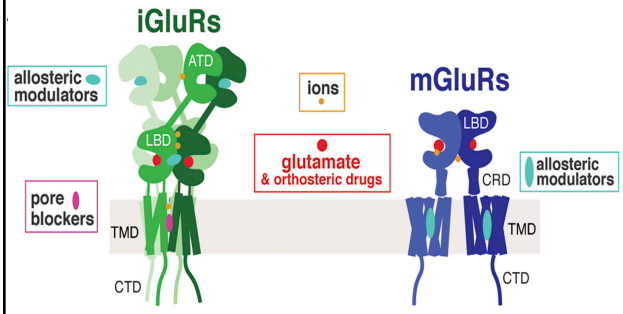
Molecular Targets of Ethanol – Glutamate

Glutamate Receptors, Signaling and Ethanol

Molecular families of **glutamate receptors**. Each of the two main **glutamate receptor** divisions comprises three functionally defined groups (classes) of **receptor**. These are made up of numerous individual subunits, each encoded by a different gene.





Ionotropic GluRs (iGluRs) are ligand-gated ion channels that produce excitatory glutamate-evoked currents

Metabotropic (GluRs) are G protein-coupled receptors (GPCRs) that control cellular processes via various cascades.

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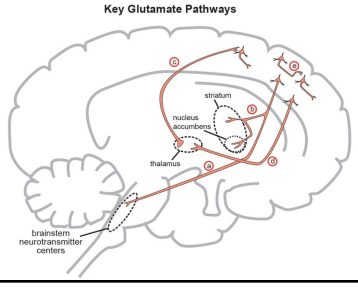
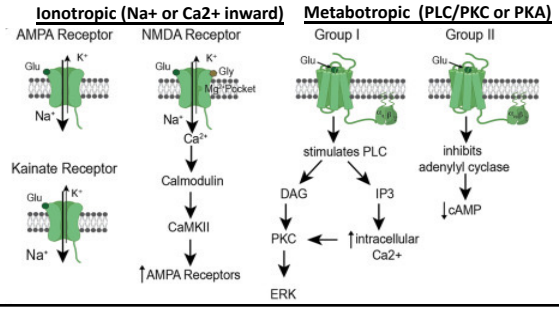
Molecular Targets of Ethanol – Glutamate

Glutamate Receptors, Signaling and Ethanol

Glutamate pathways are linked to many other neurotransmitter pathways, and **glutamate receptors are found throughout the brain and spinal cord in neurons and glia**. As an amino acid and neurotransmitter, glutamate has a large array of normal physiological functions.

- Ionotropic receptor channels are formed by assemblies of heterotetrameric or homotetrameric protein subunits. Glutamate receptors are best known for mediating glutamate's role in learning and memory through plasticity, or modification. NMDA receptors highly expressed on neurons, but they are also expressed on astrocytes. The human brain's expansive capacity for plasticity, learning, memory, and recovery from injury is attributed to improvement in synaptic anatomy and physiology of NMDA signaling, most notably in the hippocampus and other regions of the mammalian CNS.
- Metabotropic glutamate receptors are slower acting; they exert their effects indirectly, typically through gene expression and protein synthesis. Those effects are often to enhance the excitability of glutamate cells, to regulate the degree of neurotransmission, and to contribute to synaptic plasticity. Once glutamate binds with a metabotropic receptor, the binding activates a post-synaptic membrane-bound G-protein, which, in turn, triggers a second messenger system that opens a membrane channel for signal transmission. There are three broad groups of glutamate metabotropic receptors, distinguished by their pharmacological and signal transduction properties. Altogether, a total of eight metabotropic glutamate receptor subtypes have been cloned thus far.





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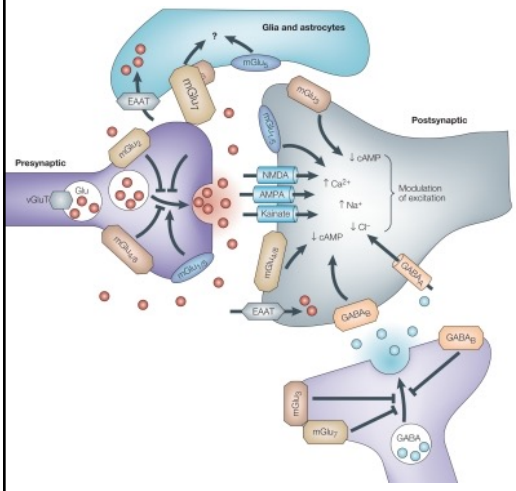
Molecular Targets of Ethanol – Glutamate

Glutamate Receptors, Signaling and Ethanol

Glial Cells & Glutamate Transporters



- Tight regulation of extracellular glutamate concentrations at both the synapse and in extra-synpatic locations is critical for normal synaptic transmission and to prevent excitotoxicity.
- Glutamate transporters regulate glutamate concentrations and are situated on both pre- and post-synaptic neurons as well as on surrounding astrocytes, a type of glial cell. Five excitatory amino acid transporters (EAATs), previously known as glutamate transporters, have been cloned: EAAT-1 to EAAT-5, with EAAT-2 expressed predominantly on cells in brain regions rich in glutamate. Glutamate transporters on glial cells are primarily responsible for maintaining extracellular glutamate concentrations.
- Glial cells, most often astrocytes but also microglia and oligodendrocytes, perform a key role in modulating extracellular glutamate levels. Under normal conditions, glutamate is recycled continuously between neurons and glia in what is known as the glutamate–glutamine cycle.
- Excess glutamate in the synapse is taken up by glial cells via EAAT transporters, where it is converted to glutamine. Glutamine is then transported back into neurons, where it is reconverted to glutamate.
- However, glial cells, under certain conditions, may also release glutamate by at least six mechanisms, one of which is reversal of uptake by glutamate transporters. This kind of reverse transport may be involved in brain damage and stroke.
- During withdrawal from chronic alcohol intake EAAT2 expression is decreased leading to hyper-activation of several nervous pathways, making it more difficult to handle the lack of alcohol. There are studies that suggests genetic variation of the gene confers vulnerability to risk-taking behavior in AUD patients.

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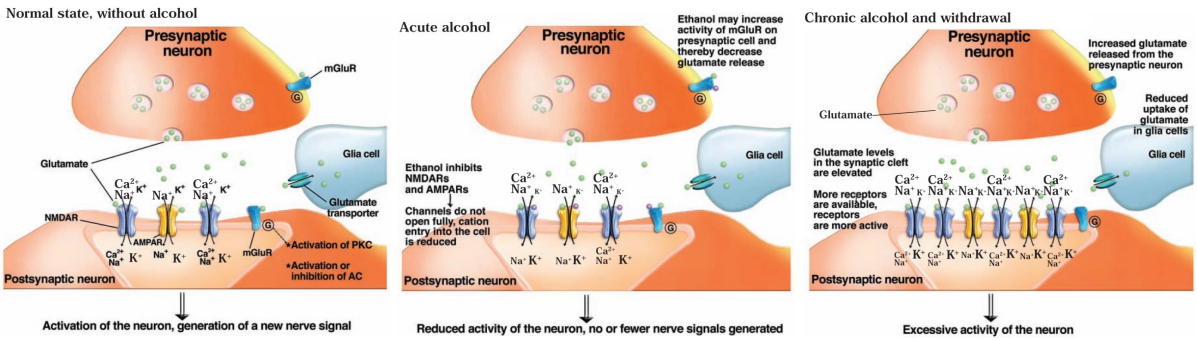
Molecular Targets of Ethanol – Glutamate

Glutamate Receptors, Signaling and Ethanol

In general – EtOH reduces the level of glutamate and glutamate signaling (as opposed to the activation of GABA).



- **Normal State** – NMDA and AMPA receptors are activated by release of pre-synaptic Glu. Metabotropic GluRs lead to activation of PKA and PKC for short term regulation. Glial cells remove Glu from synapse.
- **Acute Alcohol** – Ethanol binds and inhibits receptor reducing excitatory effects on post-synaptic neurons. Pre-synaptic GluR (both types) are inhibited by EtOH further reducing Glu secretion and increasing the inhibitory effect of EtOH on Glu signals.
- **AUD and Withdrawal** – Increased expression (plasticity) of ionotropic receptors and reduced glial uptake via EEATs. Presynaptic impact as well.

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Molecular Targets of Ethanol – Glutamate

Glutamate Receptors, Signaling and Ethanol

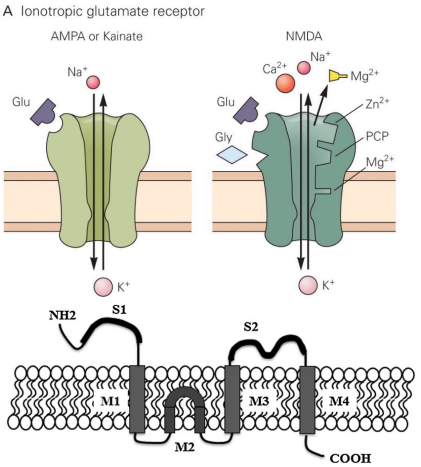
Ionotropic Receptors and AUD

NMDA receptors – mostly presynaptic nerve terminals and glial cells and are involved in plasticity.

- Blocking NMDA block the inhibitory effect of EtOH on of alcohol on NMDA.
- Alcohol withdrawal increases NMDA activity and expression in the NA which was associated with further alcohol intake. Blocking NMDA decrease EtOH effects on sedative and locomotor properties.

AMPA and Kainic acid Receptors – Glu binds any two subunits but the receptor isn't open until both binding sites are occupied. AMPA receptors modulate most of the excitatory neurotransmissions in the brain, making it a target for drug discovery of neurological disorders and AUD.

- Moderate alcohol intake upregulates AMPA expression in brain associated with mood, and increased alcohol craving. And like NMDA impairs motor coordination.
- Inhibitors of AMPA reduced alcohol consumption in rats.



Schematic diagram of ionotropic glutamate receptors (iGluRs) subunits. iGluRs contain a large extracellular amino-terminal (N) domain and an intracellular carboxy-terminal (C) domain. These receptors constitute four transmembrane domains (M1-M4), wherein the M2 domain forms a re-enterant loop. Two distinct extracellular loops containing S1 and S2 form the ligand-binding region in the receptor.

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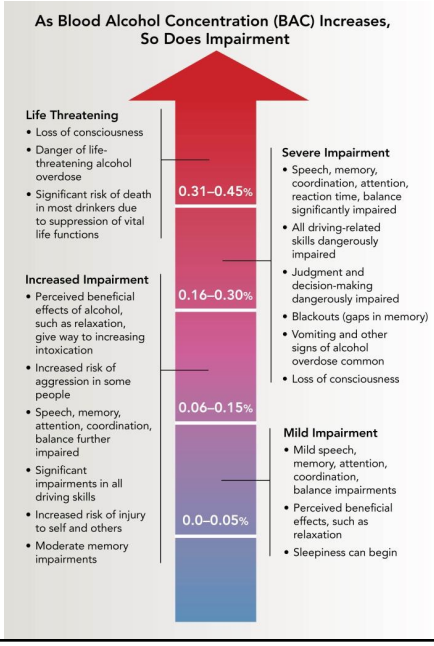
Molecular Targets of Ethanol - Toxicity

- Alcohol intoxication results in CNS depression by enhancing the effect of GABA and the inhibition of NMDA receptors, resulting in an incautious and dull state of mind.
- Intoxication by ethanol also gives rise to slurred speech, stupor, and gait abnormalities and may even result in a coma. Clinicians should correct thiamine deficiency, which can accompany chronic alcohol misuse, by supplementing B1.
- Some drugs focused on promoting alcohol cessation include 'naltrexone' (via antagonism of mu-opioid receptors), disulfiram (via creating negative conditioning through pathways mentioned above), topiramate, and gabapentin.
- Alcohol withdrawal is another common morbidity that arises as a sequela to alcohol use disorder (AUD). It results from the abrupt cessation of alcohol consumption after binge drinking or long-term dependence. The signs and symptoms usually arise within 6 to 24 hours of stopping alcohol consumption. It may range from milder symptoms like anxiety, headache, palpitations to severe symptoms like withdrawal seizures and delirium tremens. The treatment should focus on providing supportive therapy for all the complaints. Any associated comorbidities should have a 'banana bag' therapy of essential vitamins. The severe symptoms require the use of benzodiazepines



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