Neurotransmission:

An Introduction to neurotransmitters, channels, and transporters

Blanton

SLIDE 1(title slide No.1): Good afternoon, you may recall that in the last block I lectured about NSAIDs, but just in case let me reintroduce myself- my name is Michael Blanton, I am a Professor in the Department of Pharmacology and Neuroscience.

Today- I will present a rather general introduction into neurotransmission with a focus on the diversity, structure, and function of channels and transporters. In the next hour- Dr. Josh Lawrence will provide a review of neurotransmission that focuses on membrane potential, action potentials, as well as synaptic plasticity.

The material that I will cover is presented in **Chapters 4 and 6 of the Purves Neuroscience** text (*Neuroscience 5th Edition, Dale Purves, et al. 2012*) and indeed most of the slides I am going to use come directly from the textbook.

With that said- you may recall from my NSAIDs lecture- I have written down my ***lecture script*** and this should be available on Sakai.
So to study my material- I would start by reading the two chapters in the *Neuroscience* textbook and then focus most of your time on my ppt and lecture script.

Channels and transporters are of course key players in neurophysiology and synaptic transmission and the majority of CNS drugs target these proteins. But let me try and drive home why I believe it is so important that you have a good understanding of these players, using just one example:

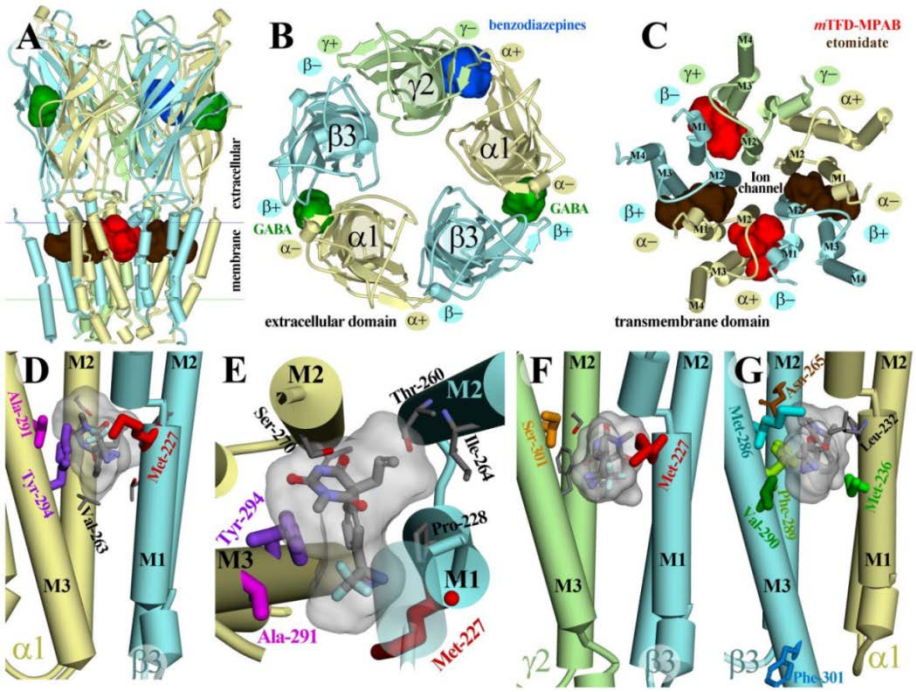
SLIDE 2: $GABA_AR$ A Chloride Conducting Ligand Gated Ion Channel: Gamma aminobutyric acid or GABA is the major inhibitory neurotransmitter in the CNS and the $GABA_A$ receptor is a major target for many important drugs-

Example 1: When I lecture to you on General Anesthetics in the next hour- the consensus view is that for the most part the effects of general anesthetics (Propofol, Isoflurane, Etomidate, etc) are mediated by their action on the $GABA_AR$, which is a chloride conducting ligand gated ion channel.
The movement of chloride ions into the neuron, hyperpolarizes the membrane, making it more difficult for excitatory currents to lead to an action potential;

general anesthetics potentiate the inhibitory chloride currents and therefore suppress neuronal firing- continuum- *sedation-anesthesia-coma/death.*

Recent crystal structures of mammalian $GABA_AR$s and other structural studies have revealed the binding sites for several general anesthetics (point to **Brown** etomidate) and this information may pave the way for the development of general anesthetic antagonists that reverse the effects of general anesthesia- which given the narrow therapeutic index of these drugs (concentration that is therapeutic vs toxic) - this is a very important development.

$GABA_AR$: A Chloride-Conducting Ligand Gated Ion Channel



Example 2: Benzodiazepines (point to Blue benzodiazepine) are another important class of drugs the act thru the $GABA_AR$- as you will hear from me later and from Dr. Manning in Psychiatry- these drugs are widely used for treating Anxiety disorders.

Example 3: The barbiturates (RED) like phenobarbital and pentobarbital, which are used as anticonvulsant or antiseizure drugs act thru the $GABA_AR$- as does Alcohol.

SLIDE 3: *-Overview:* Let me get back to the big picture-

The generation of electrical signal in neurons, that is synaptic transmission, requires the participation of numerous proteins. These proteins include, perhaps most importantly ion channels and ion transporters. The ion channels allow for the rapid movement of ions across the membrane in order to generate the electrical signal and the active ion transporters are important in both establishing and maintaining the ion gradients.

-Ion Channels:

-Ion channels, as the name implies are membrane proteins, which have pores that allow ions to cross the membrane.

Some ion channels open in response to the binding of neurotransmitters, such as acetylcholine or gamma aminobutyric acid. These are l**igand-gated ion channels**.

SLIDE 4: Neurotransmitters

As you may recall there are over 100 different neurotransmitters (Figure 6.1)that include small molecules like acetycholine and gamma aminobutyric acid but also peptides like the enkephalins and endorphins.

While neurotransmitter activation of ligand gated ion channels (Panel A) have a robust effect on synaptic transmission; these neurotransmitters often activate G-protein coupled receptors (Panel B) which may then indirectly modulate synaptic transmission.

SLIDE 5: *-Overview:*

Other ion channels open in response to a change in the membrane potential; these are **voltage-gated ion channels** *(when I lecture about Anticonvulsant drugs (early December) such as Phenytoin and Carbamazepine, used to treat Epilepsy –I will talk extensively about voltage-gated Na channels)*

Still other ion channels open in response to intracellular signals, to mechanical stimuli, or to pH or temperature.

-Transporters:

-**Active transporters or 'ion pumps'** are membrane proteins that expend energy to transport ions across the membrane to produce and maintain ion concentration gradients.

Perhaps the most important of these active transporters is the Na,K-ATPase, which utilizes energy derived from the hydrolysis of ATP to transport sodium ions to one side of the membrane and potassium ions to the other.

Other active transporters establish concentration gradients for ions such chloride, calcium, and protons.

Then there are transporters (**ion exchangers and co-transporters**) which use these gradients to move other ions or molecules.

For example- there are co-transporters that use the movement of sodium down its concentration gradient to move neurotransmitters like dopamine (DAT), serotonin (SERT), and norinephrine (NET) from the synaptic cleft back into the presynaptic terminal. *We will talk about these transporters when I lecture about drugs such as Prozac (fluoxetine) and Bupropion which are used to treat depression.*

While ion channels tend to get more of the attention, clearly all these players- channels and transporters are necessary for synaptic transmission to occur.

SLIDE 6 (Box A:patch clamp method)

-Ion Channels Underlying Action Potential

The first direct evidence for the presence of ion channels in nerve cell membranes came from measurements of the ionic currents flowing through individual ion channels.

A technique capable of measuring the currents flowing through single channels was devised in 1976 by Erwin Neher and Bert Sakman, for which they were subsequently, awarded the Nobel Prize. The technique of patch clamping, involves using a glass pipette with a very small opening and connecting it to a tiny area or patch of a neuronal cell membrane. After the application of a small amount of suction the seal between the pipette and the neuronal membrane

becomes so tight that no ions can flow between the pipette and the membrane, thus all the ions that flow when a single ion channel is open must flow into the pipette. The resulting current though extremely small can be recorded with an ultra-sensitive electronic amplifier.

In studying different ion channels, ligands such as acetylcholine can be added to the pipette to activate ligand-gated ion channels or the neuronal membrane in the patch can be depolarized or hyperpolarized to activate or inactivate voltage-gated ion channels.

SLIDE 7 (Box A:patch clamp method)

Different configurations of the patch clamp method (whole-cell; inside-out; excised patch) can be used to further study the functioning and pharmacology of these channels.

SLIDE 8 (Figure 4.1, Na^+ channel currents)

Using patch clamp measurements, individual ion channel types have been characterized.

For example (Figure 4.1), in this patch clamp of a squid giant axon, when a depolarizing voltage is applied (panel A), we can see individual openings of sodium channels (panel B).

SLIDE 9 (Figure 4.1, Na^+ channel currents)

In panel C you can see that while the membrane remains depolarized, the channel opens and then inactivates.

The opening and closing of the sodium channel are voltage-dependent, as you can see in panel D, if you apply different membrane potentials and then sum up the number of open channels what you find is a nice Boltzmann relationship (panel E) between membrane potential and the probability of channel opening.

SLIDE 10 (Figure 4.2, K^+ channel currents)

Now the previous patch clamp recordings with the squid giant axon were done in the presence of the ion cesium, which blocks all the potassium channels that are present. Now if instead we add the neurotoxin tetrodotoxin we can knock out all the sodium channels and look at the K channel currents.

Figure 4.2 now if we apply a depolarizing voltage (panel A) we see the openings of individual K channels (panel B). Notice the currents are now flowing outward, indicating that the K is flowing from the intracellular space outwards, down the potassium concentration gradient.

SLIDE 11 (Figure 4.2, K^+ channel currents)

These potassium channels in contrast to the sodium channels are not inactivated, as you can see in panel C the channel remains open as long as the membrane remains depolarized, but the channel closes when the membrane potential is returned to normal. Also, again like the sodium channels, channel opening is voltage-dependent (panel E).

SLIDE 12 (Figure 4.3, Functional states of channels)

Patch clamping has allowed direct observation of the currents that flow through individual ion channels that contribute to action potentials. This has allowed a detailed characterization of the functional properties of these and other ion channels.

As shown in this Figure 4.3 we see that the membrane depolarization is sensed by a voltage-sensor in the Na channel which then leads to opening of the ion channel pore, sodium ions flow inward, and then an inactivating domain then closes the channel. The receptor then transitions to activatable closed state and can reactivated. This cycle underlies- neuronal firing… cognition… high frequency firing is instead what we see during a seizure.*These functional states will become very important when I talk about anticonvulsant drugs to treat seizures/epilepsy. Explain Carbamazepine MOA- stabilizes inactive state and suppresses high frequency firing.*

SLIDE 13 (Figure 4.4, Types of voltage-gated ion channels)

-The Diversity of Ion Channels

Genetic analysis in combination with patch clamp studies as well other techniques that allow for the expression genes that code for ion channels have all contributed to the identification of an ever-growing number of ion channels. To date over 100 different ion channel genes have been discovered.
This diversity not only applies to a diversity of channels which conduct different ions, that channels that conduct sodium or potassium or chloride or calcium; but even within sodium channels there is diversity such that there are different sodium channels that have different functional properties, ones that inactivate more quickly than others, and so forth.

Let's explore this in more detail:
-Voltage-gated Ion Channels (Figure 4.4, Types of VG channels)

(Panel A). Na Channels: Voltage-gated ion channels that are selectively permeable to each of the major physiological ions, that is sodium, potassium, calcium, and chloride, have been discovered. To date over 10 genes coding for voltage-gated sodium channels have been identified. These sodium channels differ slightly in their structure, their functional properties, and in their distribution in specific tissues or regions of the brain. There are sodium channels that inactivate very rapidly, such as those in the squid giant axon, and there are ones that do not inactivate and which give rise to prolonged action potentials. These particular sodium channels are particularly sensitive to local anesthetics such as benzocaine and lidocaine.

(Panel B) Ca Channels: There are also voltage-gated calcium channels. In many neurons calcium channels can control the shape of action potentials generated by sodium channels and therefore modulate the electrical signaling.

For example- T-type VG Calcium channels in the thalamus help establish a 3 hertz spike and wave rhythm seen in absence or petit mal seizures- the ant seizure drug ethosuximide stabilizes the inactive state of these particular calcium channels and inhibits absence seizures.

Calcium channels regulate an enormous range of biochemical processes within cells, including the release of neurotransmitters from the presynaptic membrane.

-16 different calcium channel genes have been discovered.
(Panel C) K Channels: The largest and most diverse class of voltage-gated ion channels is the K channels. Nearly 100 K channel genes have been discovered.

SLIDE 14 (Figure 4.5, Diverse properties of K channels)

These K channels fall into distinct groups:

(A) Some like the Kv2.1 channel, show very little inactivation
(B) Some like the Kv4.1 channel, inactivate within 10's of milliseconds
(C) The HERG channel inactivates so rapidly those current flows only when the inactivation is removed at the end of a repolarization.

SLIDE 15 (Figure 4.5, Diverse properties of K channels)

(D) Inward rectifier type K channels allow potassium to flow at hyperpolarized membrane potentials rather than at depolarizing potentials…the potassium currents flowing through these channels are an important component of the resting membrane potential

SLIDE 16 (Figure 4.4)

(Panel D) Cl Channels: Finally several types of voltage-gated chloride channels have been identified. These channels are present in every type of neuron and play an important role in establishing the resting membrane potential. Chloride channels also play a role in regulating cell volume etc.

SLIDE 17 (Figure 4.4; *Ligand-gated Ion Channels)*

(Figure 4.4, Types of channels)

Ligand-gated ion channels play an essential role in synaptic transmission; such channels include (E) the nicotinic acetylcholine receptor, the GABA receptor, the glutamate receptor family and so forth. Unlike the voltage-gated channels the ligand-gated channels typically allow for the passage of more than one ion, for example both sodium and potassium and in some cases calcium ions can pass through the acetylcholine receptor.

(Panel F) The assembly of tetrameric or pentameric channels with different subunit combinations provides for a tremendous diversity in function, which is also relevant to drug interactions and side-effects.

Since I work on nicotinic acetycholine receptors- let me use this receptor to provide an example. $\alpha 4\beta 2$ nicotinic receptors in the brain are activated by nicotine and lead to increased release of dopamine-pleasure. These receptors have a very high affinity for nicotine while $\alpha 1\beta 1\gamma 1\delta 1$ receptors at the neuromuscular junction do not…which is why smoking/ nicotine does not result in muscle spasms, but other nicotinic receptors are activated leading to a range of side-effects….

(Panel E) Other ligand-gated ion channels are opened by chemical signals from within the cytoplasm, such as calcium (F) as well as cyclic nucleotides (G). These types of channels play an important role in sensory transduction.

-Stretch- and Heat-Activated Channels

Other channels (TRP channels) are activated by physical signals, for example heat-activated channels contribute to the sensation of pain and temperature and help mediate inflammation. Other signals include light, odors, sound etc

SLIDE 18 (Figure 4.7, Structure of a simple bacterial K channel)

Understanding the structure of ion channels is important for many different reasons, for understanding the basis for how they function that is how do ions traverse the membrane, how does voltage or ligand cause the channel to open and so forth. But structure is also important for understanding how the different variants result in differences in function as well has how genetic disorders affect the function and finally how drugs such as anesthetics, anticonvulsants, etc how they work.

A critical research discovery occurred in 1998 when the x-ray crystal structure of a primitive bacterial K channel was determined by Rod McKinnon and colleagues at the Rockefeller Institute. Dr McKinnon was awarded the Nobel Prize for this work in 2003. Because this K channel is present in bacteria, large amounts of protein could be produced, amounts necessary to make protein crystals used for x-ray crystallography.

The basic structure of the channel consists of 4 identical subunits arranged together as a tetramer. Each subunit contains 2 segments, which traverse the lipid bilayer in a α-helical secondary structure. The association of these membrane-spanning segments forms a tunnel through the lipid bilayer called a pore through which the K ion traverses. At the outside mouth of the channel were the K ions enter, there is a restriction in the channel pore that is formed by what is called the selectivity filter domain. Here the diameter of the pore is just narrow enough to allow a single K ion to pass through. The pore is too small to allow a larger Cs ion to pass through but is too big to interact with a sodium ion and allow it to pass through. Hence this region determines which ions can pass through the channel.

This bacterial K channel is very primitive, for example unlike other K channels it does not open in response to changes in the membrane potential and there is no inactivation process. Nonetheless it is strongly believed that the structure of this K channel represents the basic structural unit of the pore domain of not only all K channels but also for voltage-gated ion channels.

SLIDE 19 (Figure 4.6, Topology of VG channels)

(Panel C) By adding other structural domains, it is believed that voltage-activation and inactivation are added (C, E) and auxiliary subunits further alter the function.

Also by making subtle changes in the structure of the selectivity filter, the ion selectivity can be changed from K to sodium or to calcium. (A, B).

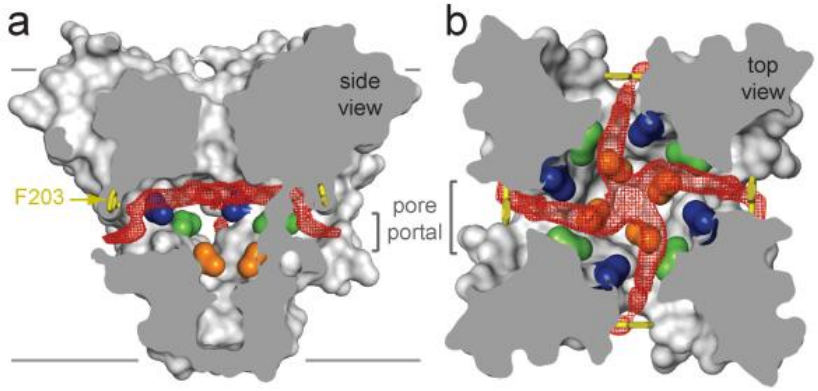
SLIDE 20 (Figure 4.8, Voltage-sensor)

A special property of voltage-gated ion channels is a sensor that detects changes in the membrane potential and which then opens the channel. This sensor is a α-helical transmembrane segment, which contains positive charges along the length of it.

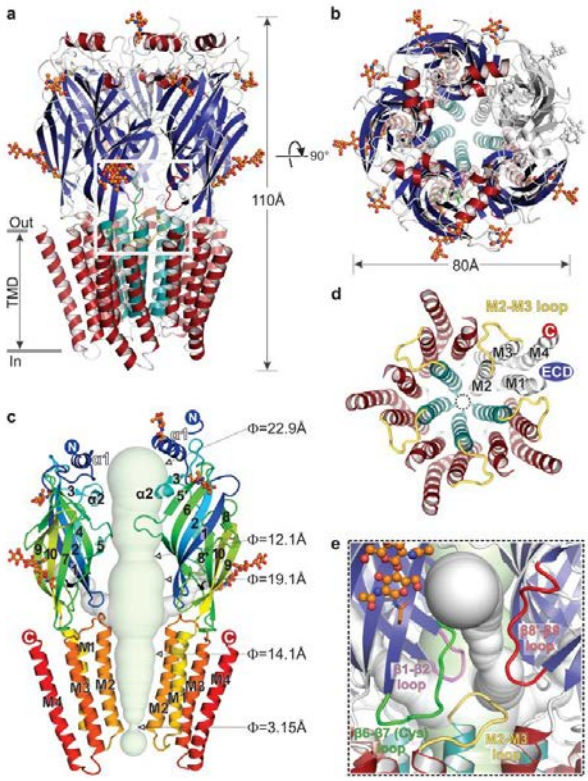
(panel C) Depolarization pushes the sensor outward, while hyperpolarization pulls the sensor inward. The movement of the sensor exert force on the helical linkers connecting the sensors to the channel pore, pulling it open or pushing it closed.

SLIDE 21 (NavAb crystal structure; Payandeh et al 2011, *Nature* 475)

Recent crystal structures of bacterial sodium channels, have confirmed the same structural motifs found in K channels and revealed among other features- the binding sites for local anesthetics and anticonvulsants such as phenytoin and carbamazepine (Figure 4- showing pathway for phenytoin and pore binding site).



SLIDE 22 (3-D Structure $GABA_AR$: Ligand-gated ion Channel Structure)



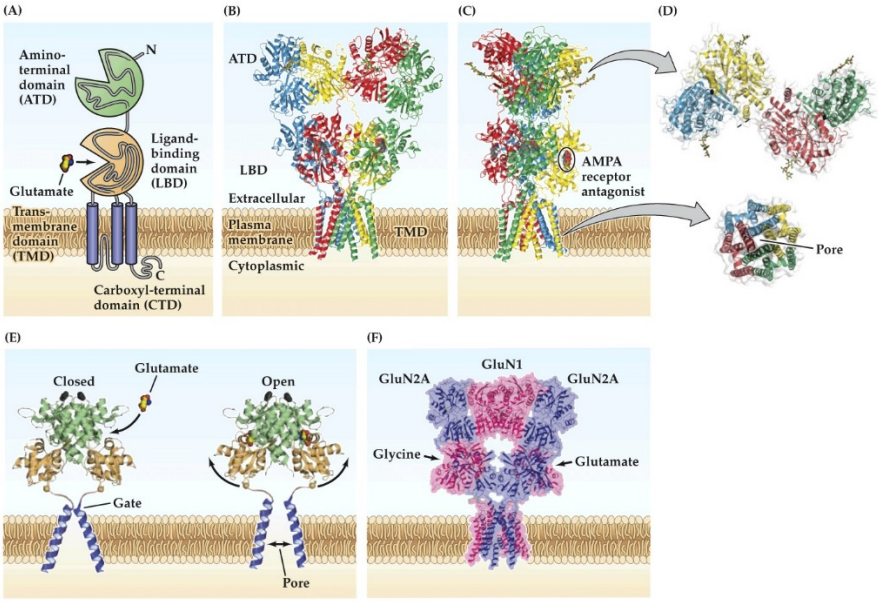
Very recent crystal structures of a human $GABA_AR$ (2014), mouse 5-HT_3R (2014), and zebrafish GlyR (2015) have further demonstrated that these ligand gated ion channels all share very homologous three-dimensional structures and as I

described at the beginning of my lecture- have provided detailed molecular insight into the binding sites for a number of important drugs- general anesthetics (both intravenous and inhaled, as well as for benzodiazepines, barbiturates, alcohol etc.

I wanted to point out that the AMPA, NMDA, and Kainate family of LGICs have a very different structure than the $GABA_AR$/ Nicotinic/5-HT3/Glycine receptor LGIC family, as do the purinergic or P2Y family of receptors.

SLIDE 23 (3-D Structure AMPAR: Ligand-gated ion Channel Structure)

Here you can see the molecular details of the AMPAR…..



NEUROSCIENCE 5e, Figure 6.7

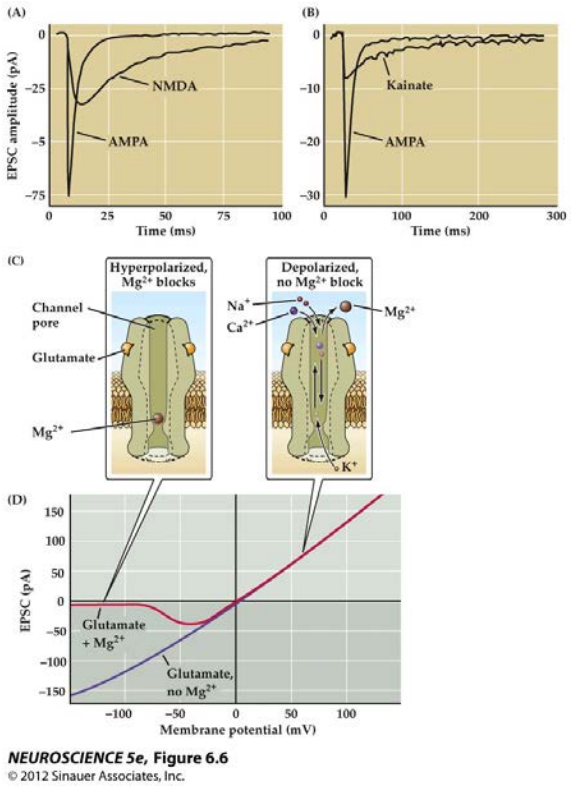
SLIDE 24 (Figure 6.6. NMDA currents)

NMDA receptors have physiological properties that set them apart:

1) The NMDA pore allows passage of calcium in addition to Na and K and therefore NNMDA EPSPs also include calcium activation of second messenger systems.
2) Mg blocks the pore of this channel at hyperpolarizing potentials and this block is released during depolarization. The coincident presence of both glutamate and postsynaptic depolarization to open NMDA receptors is

believed to underlie forms of synaptic information storage such as long-term synaptic plasticity.

3) NMDA receptor activation requires the presence of the co-agonist glycine which provides another layer of regulation.



***NEUROSCIENCE 5e*, Figure 6.6**

The dissociative general anesthetic- Ketamine acts via the NMDA receptor

SLIDE 25 (Figure 4.9, Ion Transporters)

Active Transporters Create and Maintain Ion Gradients

For ions such as sodium or potassium to flow through channels to create an electrical signal, ion transporters are necessary to establish concentration gradients for these ions as well as to reestablish those gradients after these channels open.
Active transporters form complexes with specific ions, and the process of binding the ion on one side of the membrane and then unbinding on the other side

requires several milliseconds, which is relatively slow compared to ion channels where thousands of ions cross the membrane each millisecond.

Several types of transporters have been identified, but for all energy must be consumed to transport ions against their electrochemical gradient.

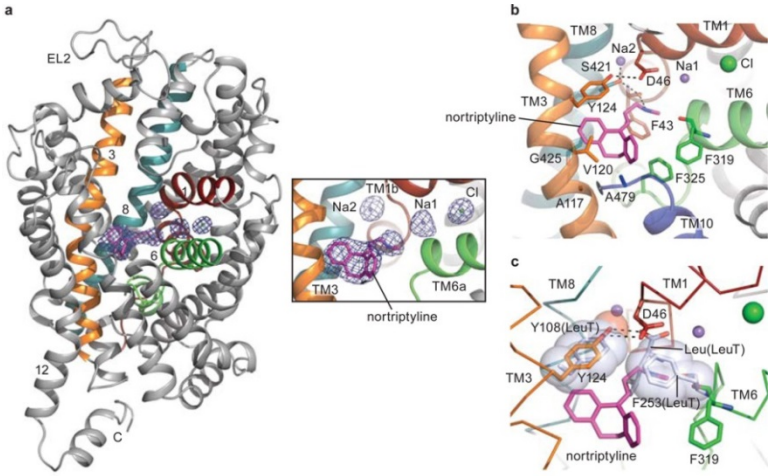
1) (panel A/B) Some transporters use energy directly in the form of ATP hydrolysis to transport ions: these transporters are called ion-motive ATPases or pumps. (Panel A) One of the most prominent examples of this type of active transporter is the Na,K-ATPase or the sodium pump. The sodium pump is responsible for maintaining the electrochemical gradients for sodium and potassium. Another example is the Ca-ATPase or Ca pump.

2) (panel C/D)The second type of transporter uses energy indirectly. These transporters allow one ion to flow down its electrochemical gradient in exchange for transporting one or more ions against their electrochemical gradient. These transporters are called ion exchangers. For example the Na/Ca exchanger allows sodium to flow down its electrochemical gradient and uses the free energy of this process to transport calcium against its electrochemical gradient.
There are many types of ion exchangers, such as the sodium proton exchanger and so forth.

3) (panel E/F) The co-transporters are another very important class of transporters- included in this family are those that remove the neurotransmitters dopamine (DAT); serotonin (SERT); and norepinephrine (NET) from the synapse in exchange for sodium moving down its concentration gradient.

Recent crystal structures of these co-transporters have provided insights into the binding of antidepressant drugs to their therapeutic targets:

SLIDE 25B (DAT/TCA structure)
Shown here is the structure of a dopamine transporter complexed with a tricyclic antidepressant (TCA) Penmatsa et al. (2013) *Nature* 503.



SLIDE 26 (Figure 4.10, Ion Movements)
-Functional Properties of the Na^+/K^+ Pump

The functional properties of the Calcium and Na,K pumps are the best understood of ion transporters.

The activity of the Na,K pump is estimated to account for 20-40% of the brains energy consumption, which underscores the importance of its activity for brain function.

First three sodium ions bind to sites on the pump located on the intracellular side of the membrane. The pump then undergoes a series of conformational changes that includes the hydrolysis of ATP and phosphorylation of a specific aspartic acid residue. The conformational changes in the pump result in the sodium ions being released on the extra cellular side of the membrane.

Next two potassium ions bind to sites now exposed on the extra cellular surface of the pump. This time the phosphate group is released, and the conformational changes result in the potassium ion being released to the intracellular medium and the pump is now back to the starting position of the cycle.

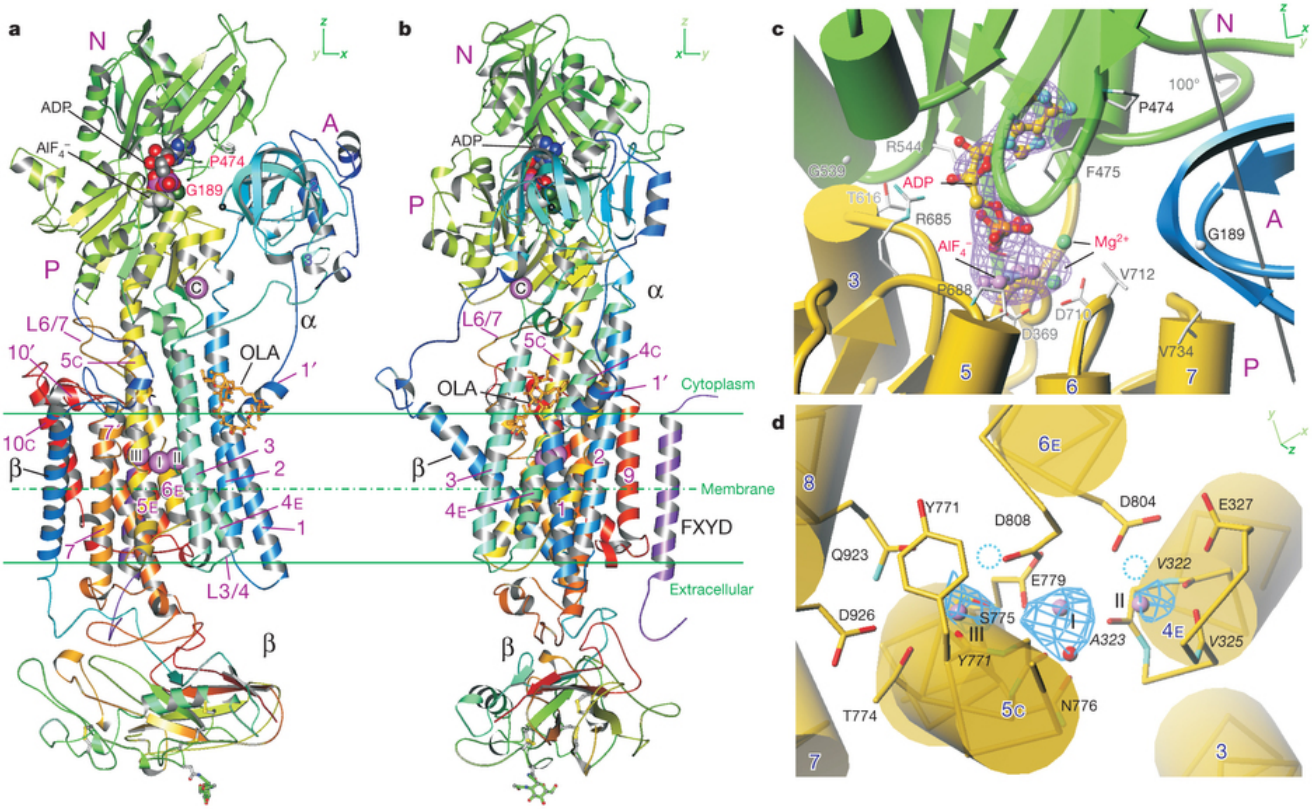
Because the pump translocates three sodium ions and only two potassium ions, it is electrogenic. That is, it is creating an electrical gradient, it is hyperpolarizing the cell. But this current is actually very small accounting for only about 1% of the

current flowing through the membrane at a given time and affecting the resting potential by only about a mill volt.

Like channels there are inhibitors of ion transporters, for example the plant glycoside, ouabain specifically inhibits the Na,K pump.

SLIDE 27 (Crystal Structure of Na/K Pump)
-The Molecular Structure of the Na^+/K^+ Pump

Again, the availability of crystal structures in recent years has provided tremendous insight into the molecular mechanisms of the pump- from the binding of sodium and potassium to modulation of the pump by ouabain and other drugs:



Kanai et al. (2013) *Nature* 502

SLIDE 28 (Study Guide)

Study Guide:
-no structures!

1. **Voltage-Gated Ion Channels-** what are the properties of these channels that contribute to synaptic transmission (ion-selectivity; voltage-sensing, etc),what are the functional states.
2. **Ligand-Gated Ion Channels-** what are the properties of these channels that contribute to synaptic transmission (neurotransmitter selectivity; ion-selectivity; subunit composition, etc.) that contribute to synaptic transmission.
3. **Transporters**- what are the different types of transporters and how do they each contribute to synaptic transmission.