NEUR30002 Neurophysiology: Neurons and Circuits

Lecture 1 – Introduction

Neurons

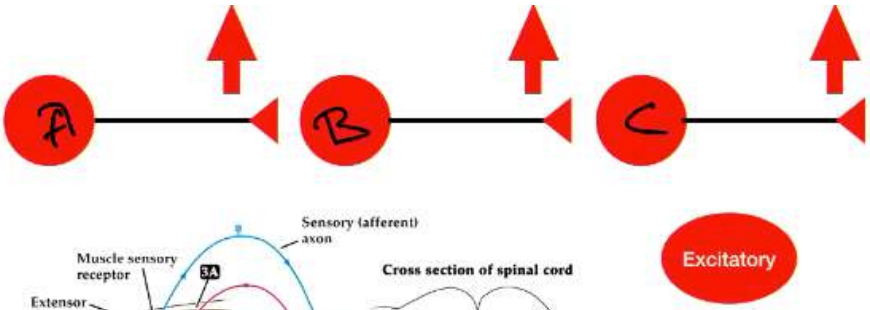
- Excitable cells with a resting membrane potential (Both electrical and chemical)
- Receives input through dendrites, which is then processed and passed down the axons of the neuron

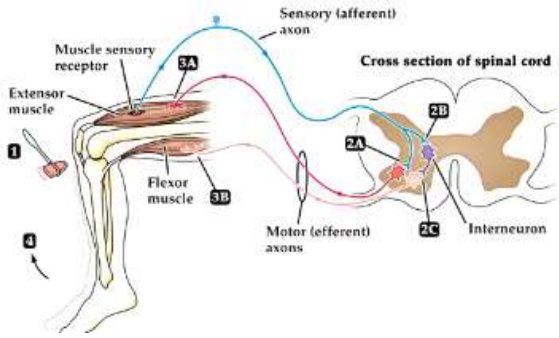
Neural Circuit

- Sensation is carried by an afferent into an integrating site, and the effect is carried out by output neurons
- All the information in a circuit is carried through via action potentials
- Neuronal activity can be measured
- Synapses are chemical communication sites between neurons from presynaptic release to postsynaptic receptor

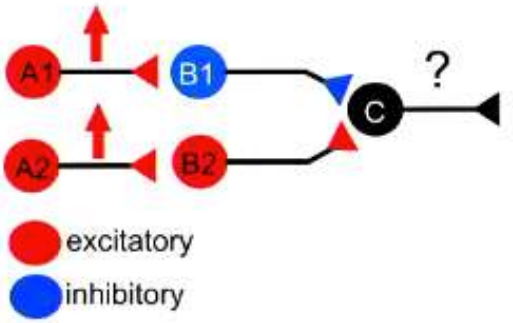
Simple Neural Circuit

- Whether or not a neuron is excitatory or inhibitory is determined by its effect on its downstream neuron, not about the function of the neuron itself

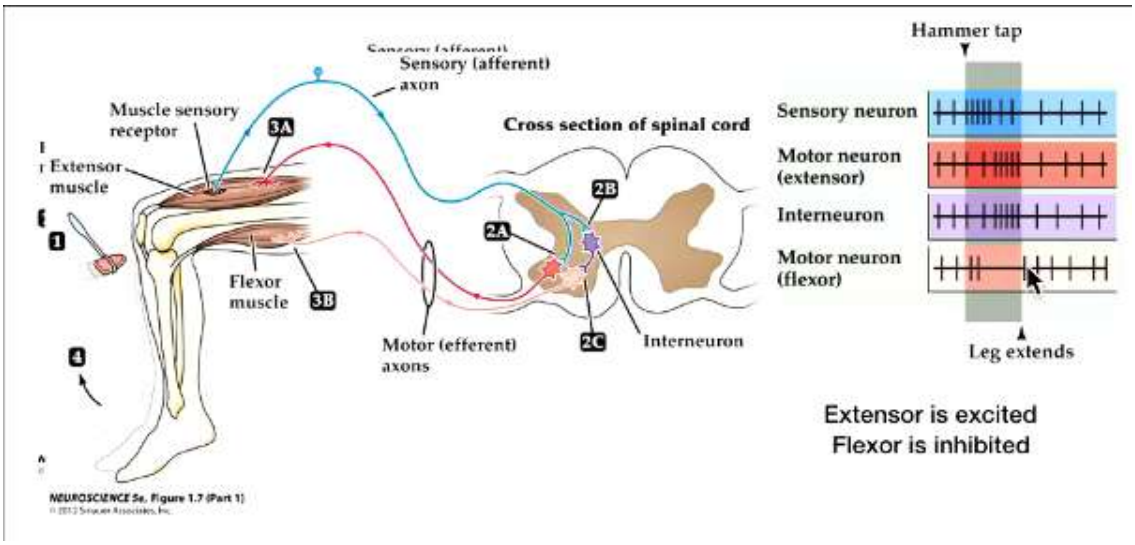




- In the diagram on the right, there is not enough information to determine the effect on neuron C. This is due to lack of information with spatial summation (proximity of inputs), weighting (contribution of each input), timing (temporal nature) and frequency



- If assuming all parameters mentioned above are equal, the effects of B1 and B2 would cancel each other out and there will be no change in the activity of C



- Using the information of neural activity, we can predict that the interneuron is inhibitory as the motor neuron downstream of it has reduced activity

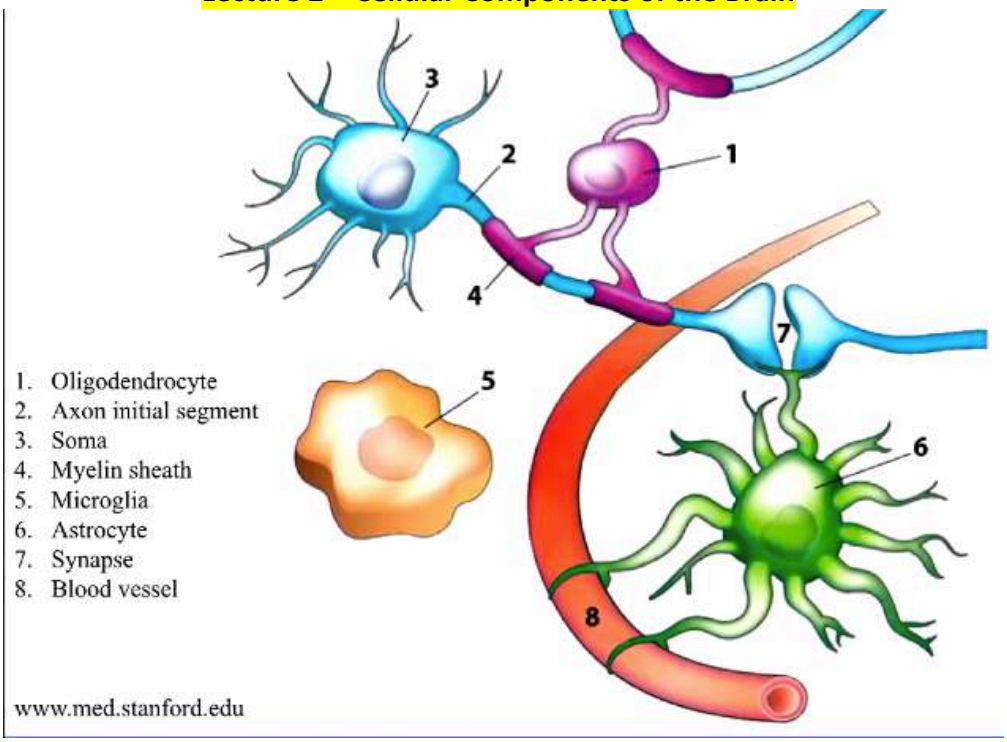
Glial Cells

- Oligodendrocytes → Make the myelin sheaths in the CNS
- Ependymal Cells → Source of neural stem cells in the CNS
- Microglia → Modified immune cells
- Astrocytes → Diverse range of function, from supporting the CNS to assisting in neurotransmission. Ratio of astrocytes to neurons increase with increase complexity of the nervous systems

Key Points

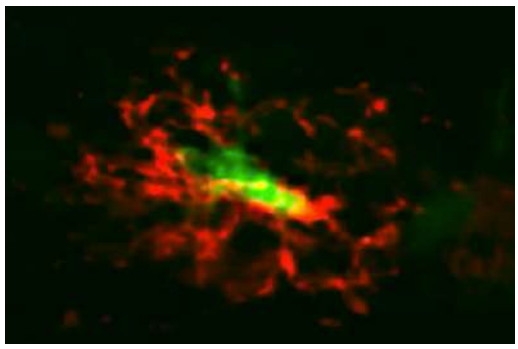
- Describe the key cells types within the CNS
- Identify the basic components of a neuron
- Understand the influence excitatory and inhibitory inputs on the output of a basic neural circuit

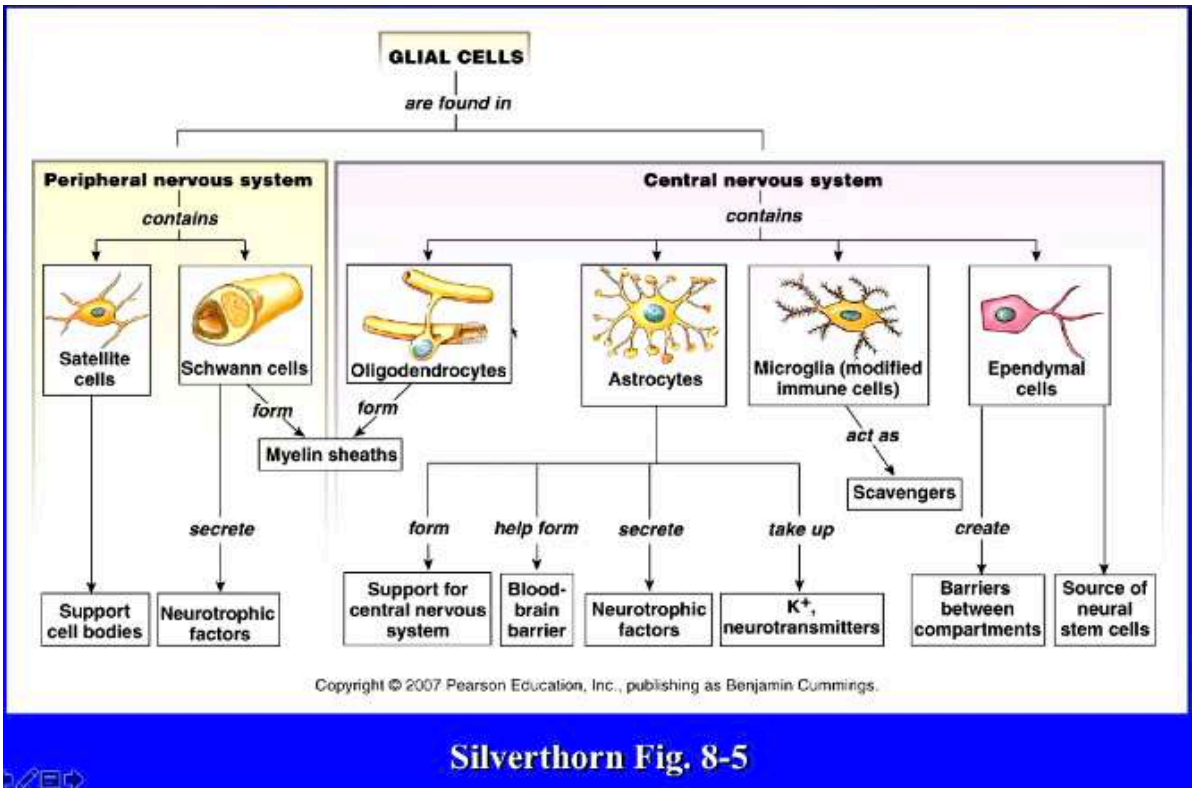
Lecture 2 – Cellular Components of the Brain



Glial Cells

- Considered the "Glue" of the brain to maintain structure and function of neurons
- **Astrocytes** form connections between synapses and the blood vessels
- **Oligodendrocytes** in CNS (Schwann Cells in PNS) myelinate axons to increase conduction velocity
- **Microglia** are a macrophage lineage cell which act as the immune cell of the brain, surveying for any invasion or damage which may require a response

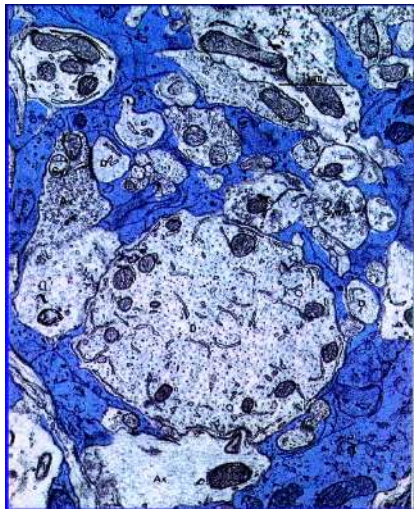




- In the PNS, **Satellite Cells** also exist to support the ganglia
- **Ependymal cells** line ventricles and the surfaces of the brain, creating barriers in the neuronal compartments of the CNS to ensure that soluble factors within each compartment are confined and do not spread out to neighboring areas
- **Ependymal cells** are also sources of neural stem cells

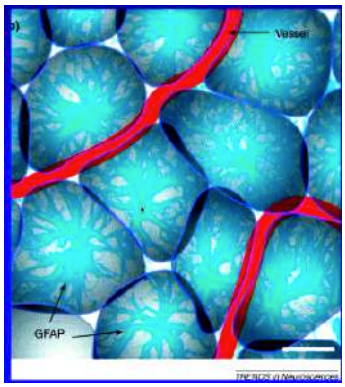
- **Astrocytes** maintains the environment for the nervous system to act
- Form the Blood-brain barrier: Most of brain is impenetrable to large factors within circulation (e.g. amino acids) for it to be able to function in a constant environment. The Brain requires this barrier to selectively uptake certain factors such as glucose when required. Prevention of amino acids entering the brain such as Glutamate preserves normal function, as it is the primary excitatory neurotransmitter
- Oxygen and Carbon Dioxide can move across it freely, but there are transporters that exist in the barrier which selectively transports factors into the brain. The barrier itself is formed from a relationship between astrocytes and blood vessels

- Astrocytes secrete neurotrophic factors which support neurons and ensure they survive, and also helps maintain the extracellular ion concentration

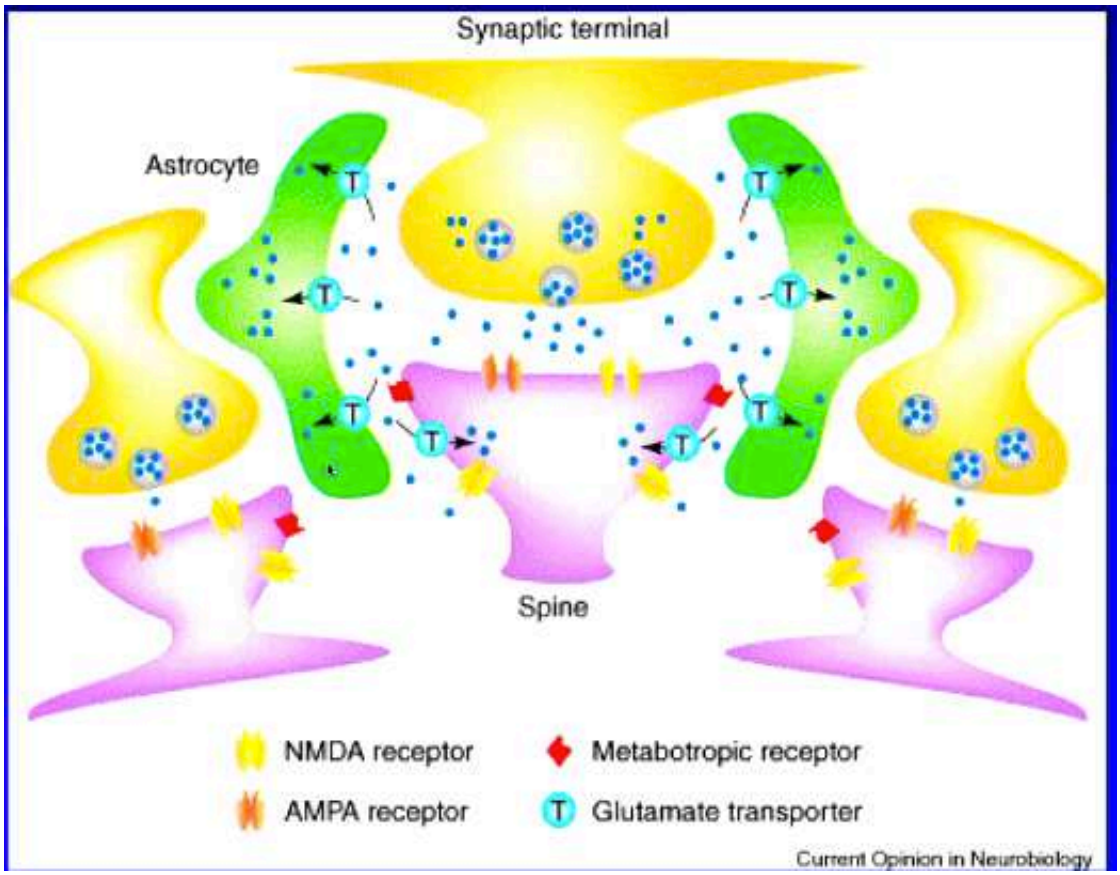


- Spaces between neurons are taken up by astrocytes and neuronal elements. Astrocytes are everywhere that the synapse is not, therefore the extracellular space of the brain is very small. The only direct point of connection between neurons is at the synapses

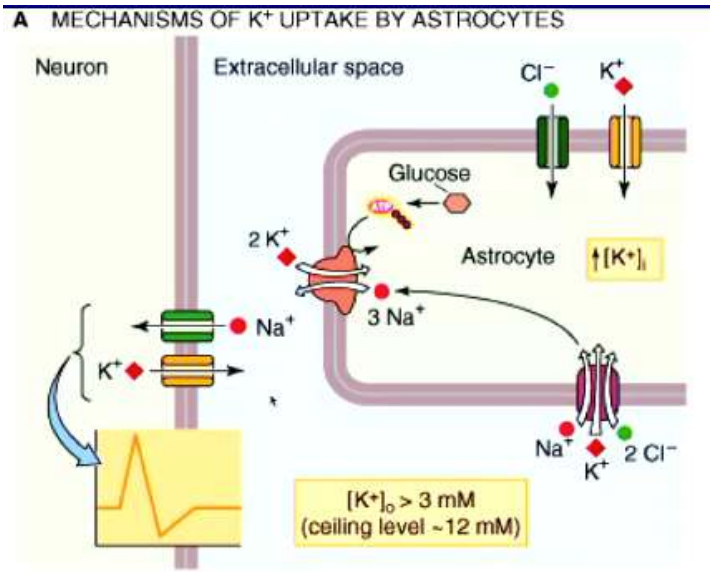
- Each individual astrocyte has its own territory that does not overlap with any meaningful degree with any other astrocytes. Siphon (?) processes of astrocytes make contact with blood vessels, which functions in forming the blood brain barrier and controlling blood flow



- Changes in astrocyte function lead to changes in uptake of glucose, therefore a change in blood flow of that area → fMRI uses this change in blood flow to determine active regions in the brain. Active neurons lead to change in blood flow, which is partly neuronal and partly due to an increased demand in glucose uptake

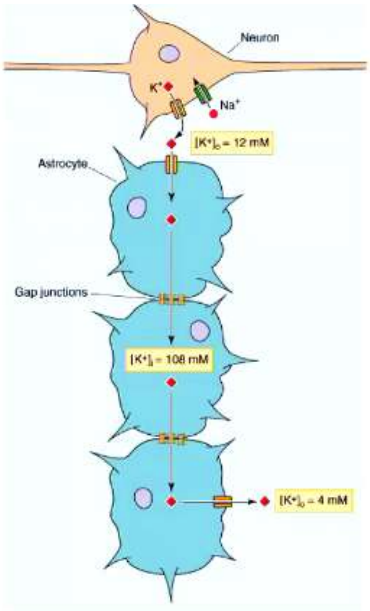


- Astrocytes have transporters that maintain the feudality of information transfer across a synapse → Prevents neurotransmitters from leaking out to other synapses



- Astrocytes, via active transport/co-transporters/channels, take up ions from the extra-cellular space into the intra-cellular space to maintain a constant, homeostatic concentration in the extra-cellular space, which maintains the neuronal excitability of the membrane

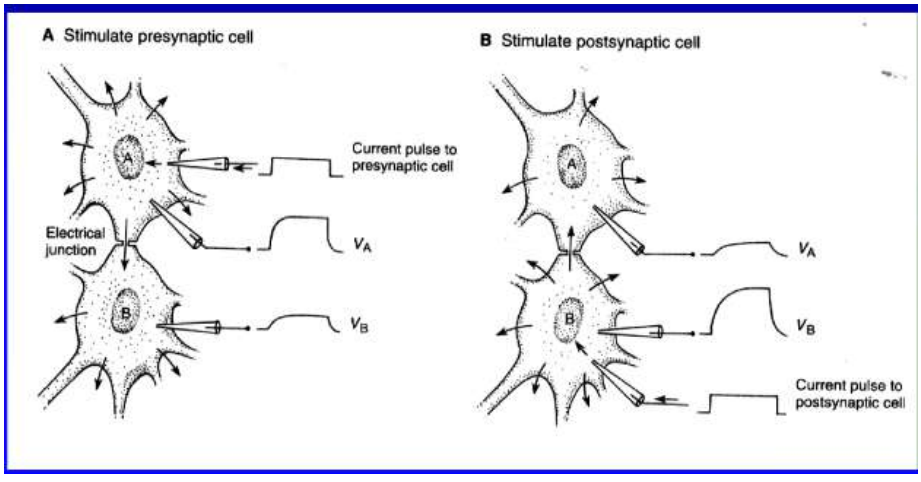
- Astrocytes are connected by gap junctions → When extra-cellular potassium is taken up, it travels to adjacent astrocytes with lower potassium concentrations via gap junctions



- Gap junctions: Contains a transmembrane spanning protein made up of 4 membrane spanning units connected by intra and extra cellular loops. These membrane spanning units form a Connexin, and then 6 connexins come together and form a connexon (Channel). The pre and post synaptic connexon then join up to form a pore known as the Gap Junction

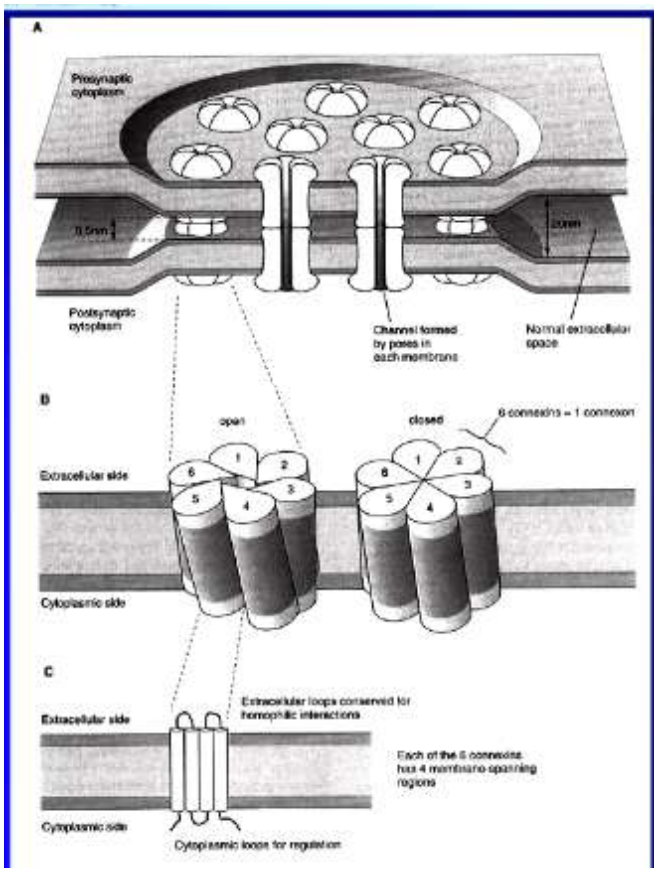
- Gap Junctions are regulatable through increasing and decreasing cell conductance, which ultimately functions to exclude movement on the basis of size. Also non-directional

- Ions that carry a current can pass through these gap junctions. Therefore, when one astrocyte depolarizes, adjacent depolarization in both directions occur in the other astrocytes as well



ECF ion homeostasis

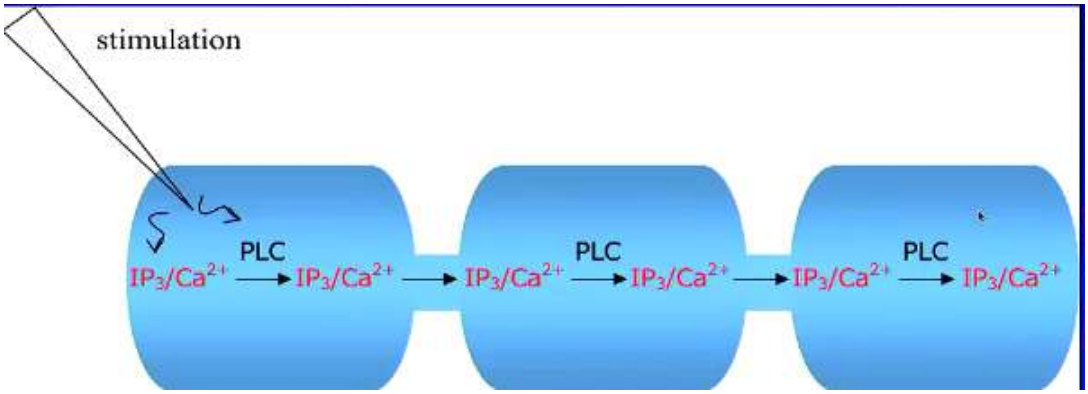
- Synaptic activity results in extravasation of K+, under physiological conditions several mM change. For glia, under physiological conditions, membrane potential is largely determined by K+, as small changes in ECF K+ produces changes in voltage
- Depolarization occurs wherever K+ concentration changes, which is different from localized synaptic inputs. It does not reflect excitation or inhibition, just altered activity
- Current will flow along a potential gradient. The inward current due to increased ECF K+ induces local depolarization that will spread throughout the glial cell and, through gap junctions to neighboring glia. At other regions, K+ is released to the ECF
- This is called **Spatial Buffering**



- In addition, astrocytes possess Na+/K+ ATPase, an anion pump and a Na+/K+ symporter to assist in spatial buffering
- Neuronal activity also induces considerable changes in ECF pH (increase)
- Astrocytes also possess Na+/H+ exchanger, Na+/HCO3- cotransporter (bidirectional, electrogenic pump which is unique to glial cells in the nervous system), Cl-/HCO3- exchanger and an active H+ ion extrusion mechanism

Responsive Astrocytes

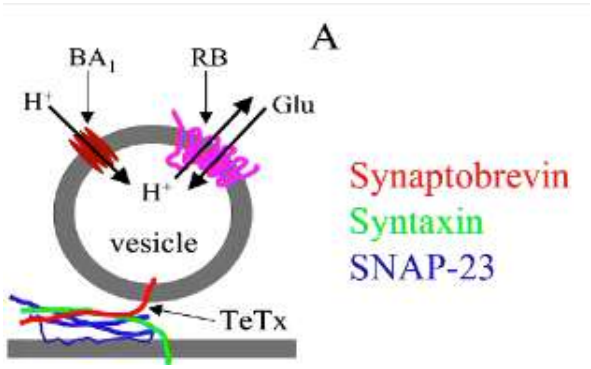
- Astrocytes respond to specific inputs via neurotransmitter receptors
- This response includes activation of intracellular calcium via IP3
- Activation of neighboring astrocytes occur not just via Gap Junction Communication, but also through release of extra-cellular substances



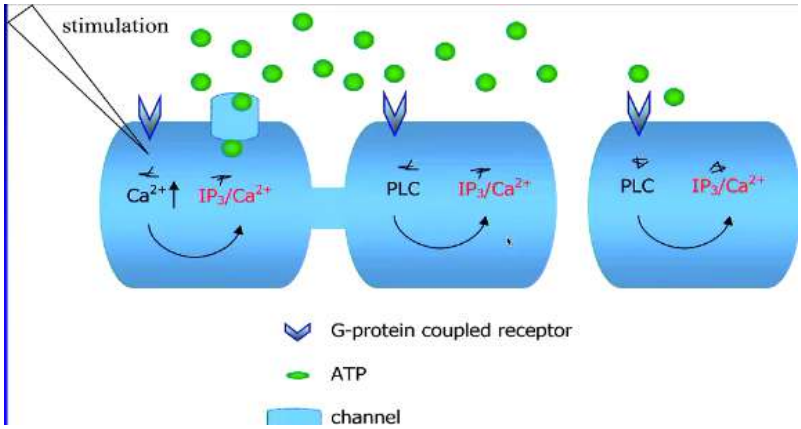
Reactive Astrocytes

- In response to stimulation, glia release neuroactive substances that have been dubbed "gliotransmitters"

- Gliotransmitter release is through exocytosis of synaptic-like microvesicles → Glutamate appear to have VGlut. Cluster at release sites associated with exocytotic proteins such as SNAP, SNARE and VAMP. Dense core secretory granules are also present for nucleotides (ATP) and peptides. The process of exocytosis is slower than in neurons, as it is triggered by IP3 induced changes in intracellular calcium



- Various channels may also play a role in release → Hemichannel, Anion Channel, P2X7 Receptor



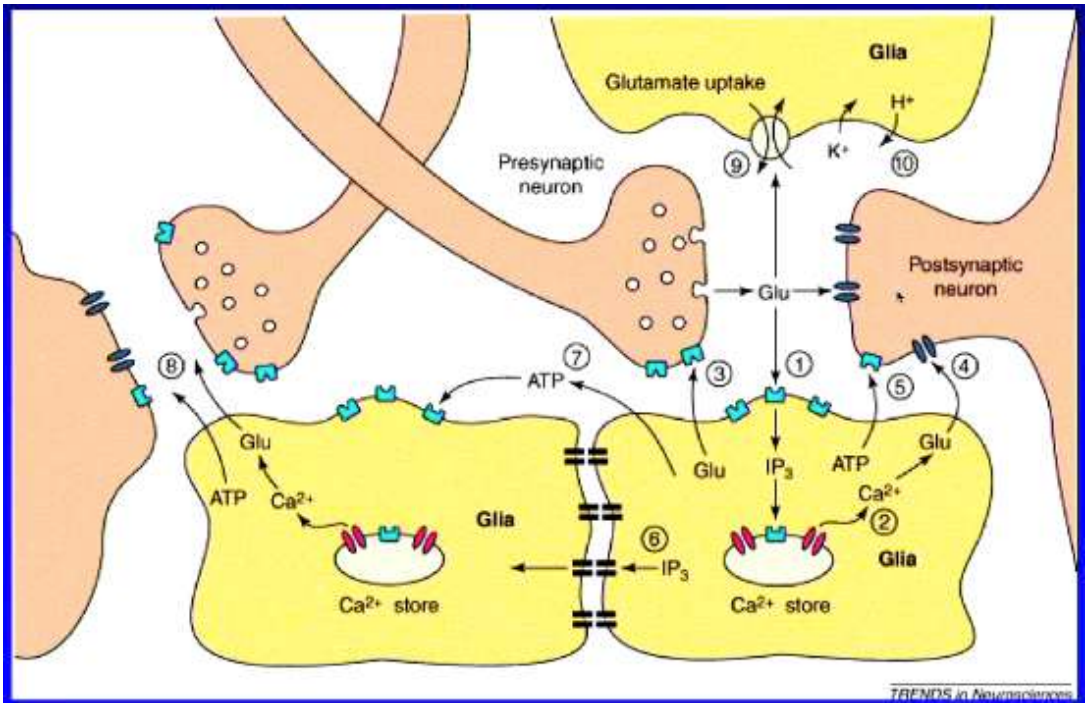
Astrocyte Influence

- Astrocytes have the ability to take up the glutamate from the extra-cellular space to ensure it does not cause a response from other neurons → Maintains feudality of information transfer between two neurons

- The astrocytes also ensure extra-cellular ion concentration remains constant e.g. exchanging potassium with hydrogen ions

- Astrocytes can not only maintain, but also RESPOND: Receptors for glutamate are found on the Astrocytes that initiates a change in signaling (e.g. metabotropic g-protein coupled receptor) → Calcium release via IP3. 1) Can feedback into the post-synaptic neuron via calcium mediated glutamate or ATP transfer, which could enhance the response in the post-synaptic neuron. 2) Can feedback into the pre-synaptic neuron; 3) Can effect neighboring astrocytes via gap junctions or release of extra-cellular substances, and thus ultimately influencing neighboring synapses

- Astrocytes receive inputs and response, release neuroactive substances, and are positioned as a bridge between many elements in the CNS such as neurons, blood vessels and neighboring domains



Summary

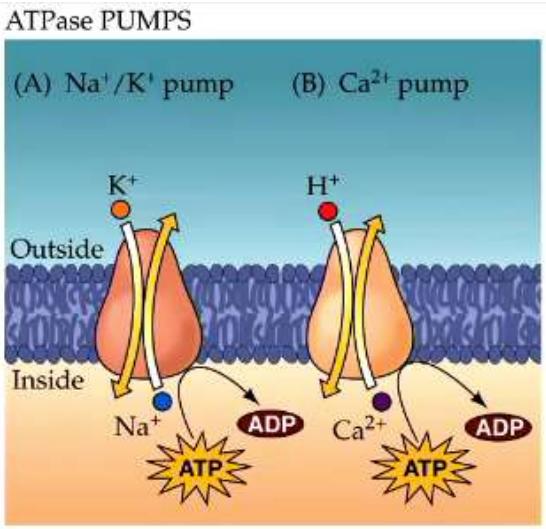
- Glia (glue) have a complex structure
  - Cover the surface of neurons with the exception of the synapse
  - Contact blood vessels and are an interface between neurons and blood
  - Fill the space of the nervous system, making small intercellular spaces of ~20 nm
- Vastly outnumber neurons (10:1)
  - Increased number per neuron with increasing nervous system complexity
- Glia are present in different types
  - Astrocytes (many functions)
    - fibrous (among bundles of myelinated axons)
    - protoplasmic (mostly in gray matter)
  - Oligodendrocytes and Schwann cells (myelination)
  - radial glia (developmental)
  - ependymal cells (line the ventricles)
  - microglia (injury response)

Lecture 3 – Modulation of Membrane Potential I

- Resting membrane potential is established via a concentration gradient difference between intracellular and extracellular ions → Potassium has a high intracellular concentration, Sodium has a high extracellular concentration, Chloride has a high extracellular concentration, and lastly, Calcium has low extracellular concentrations and extremely trace amounts within the axon. Important because changes in Calcium level are what provides information within the cell, so a low starting point allows the cell to detect the changes to transfer the information

- These concentration gradients combined with a resting permeability to potassium contribute to setting up the resting membrane potential and is critically important for a neuron to generate an action potential → At equilibrium, due to the concentration gradients, even though the membrane is selectively permeable, there is no movement of potassium

- Ion Transporters (Energy Dependent) → Pumps that actively move ions against concentration gradients, and thus set up ion concentration gradients. Uses ATP to move ions from areas of low to high concentration e.g. Na+/K+ pump and Ca2+ pump



- Na+/K+ Pump → Electrogenic pump that binds 3 sodium and 2 potassium. Creates a net positive and negative on each side, therefore creating an electrogenic potential

- Ca2+ Pump → Transports Calcium into the cell whilst pumping out Hydrogen ions

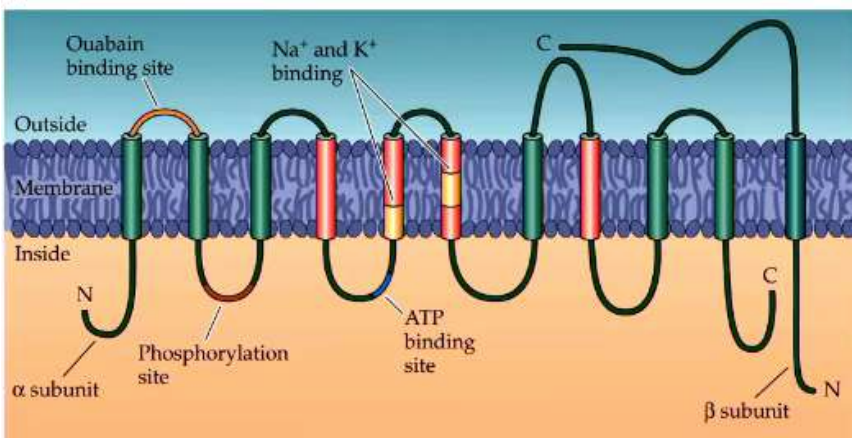
TABLE 2.1 Extracellular and Intracellular Ion Concentrations

Ion	Concentration (mM) Intracellular	Extracellular
Squid neuron		
Potassium (K^+)	400	20
Sodium (Na^+)	50	440
Chloride (Cl^-)	40–150	560
Calcium (Ca^{2+})	0.0001	10
Mammalian neuron		
Potassium (K^+)	140	5
Sodium (Na^+)	5–15	145
Chloride (Cl^-)	4–30	110
Calcium (Ca^{2+})	0.0001	1–2

Molecular Organisation of the Na+/K+ Pump

- Two subunits: Alpha and Beta. The Alpha subunit is crucial for the function of the Ion Pump, whilst the Beta subunit is only involved in transport and modulatory effects

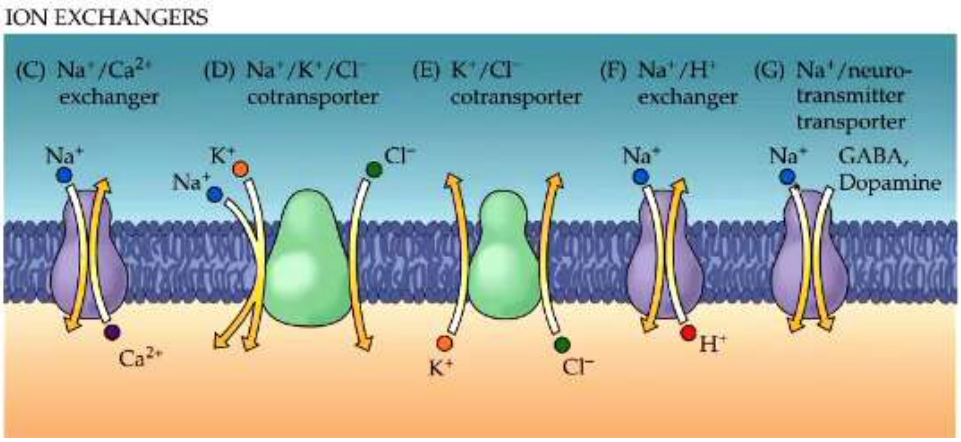
- It is a transmembrane spanning domain protein with multiple transmembrane spanning domains → When Sodium and Potassium bind to the binding sites, it undergoes conformational changes in order to transport the ions across the membrane. An ATP binding site is located in the intracellular, which acts as an energy source, whilst an Ouabain Binding Site exists in the extracellular, which functions to modulate the activity of the Na+/K+ ATPase. Phosphorylation site in the intracellular also functions to modulate activity



Ion Exchangers

- Energy Independent
- The key difference between ion exchangers and ion transporters/pumps is that they do not directly use energy/ATP themselves. Instead, it utilizes the potential energy that has been set up by the concentration gradients to exchange ions e.g. Na+/Ca2+ exchanger follows the concentration gradient to transport Sodium into the axon, and then utilizes this energy to move Calcium out of the axon

- Energy generated by moving Sodium into the intracellular can also be used to transport neurotransmitters such as GAPA and Dopamine into the cell

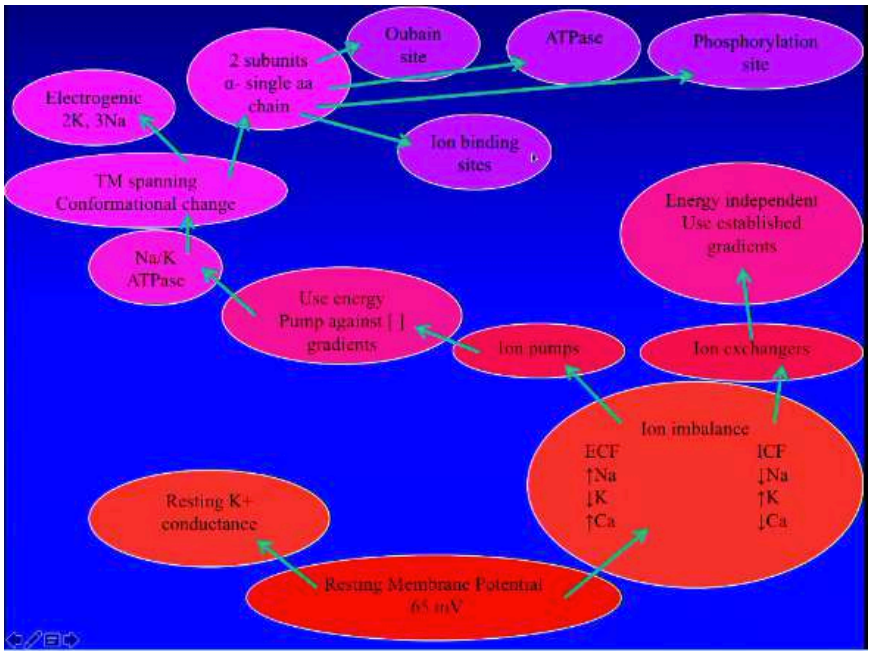


<u>Summary</u>

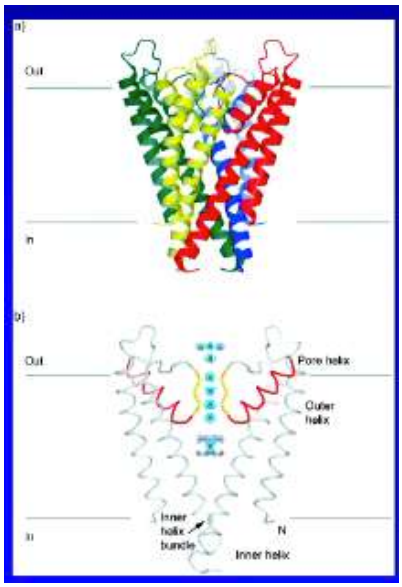
- The cell membrane forms a barrier to the movement of ions
- Pumps use energy to establish concentration gradients across the neuronal membrane. Transporters use energy stored in ion gradients to transport ions
- At rest, the neuronal membrane is selectively permeable to potassium
- Potassium is close to equilibrium with little net movement due to a balance between the concentration gradient and the electrical gradient forces

- This results in a membrane potential of approximately 65 mV with the interior of the neuron negative (-65 mV), which is close to the K+ equilibrium potential

- Resting membrane potential is SET UP by the ion concentration gradients and is induced by the resting permeability to potassium

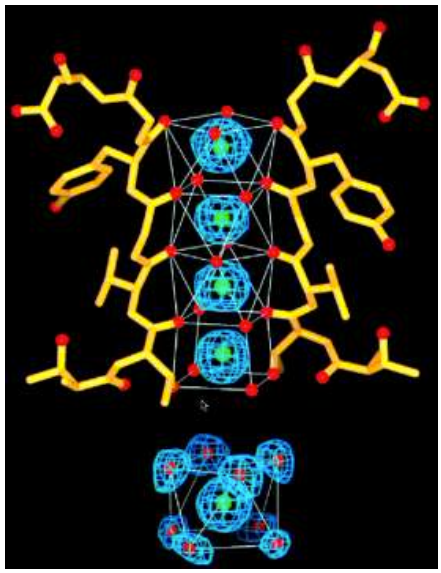


Ion Selectivity



- Size difference between partially hydrated Na+ ion compared to a partially hydrated K+ ion is only slightly smaller → The Infrastructure of the Potassium Ion Channels make it possible to differentiate between them

- The Ion Selectivity Filter of the channel dehydrates the hydrated Potassium Ions. As it enters the pore as only positively charged potassium ions, the Oxygens of the R- amino acids R-groups that line the pore are spaced so they perfectly mimic the positioning of the water molecules that surrounded the potassium ion when it was in its hydrated/soluble state → This allows the potassium ions to be in their most relaxed conformation. Any small change in charge or size of the potassium ion would mean that the oxygens will be in the wrong position, and the ion will be energetically unfavourable, hence why any other ion such as Sodium or Calcium cannot pass through this same channel (ION SELECTIVITY BUILD UP)

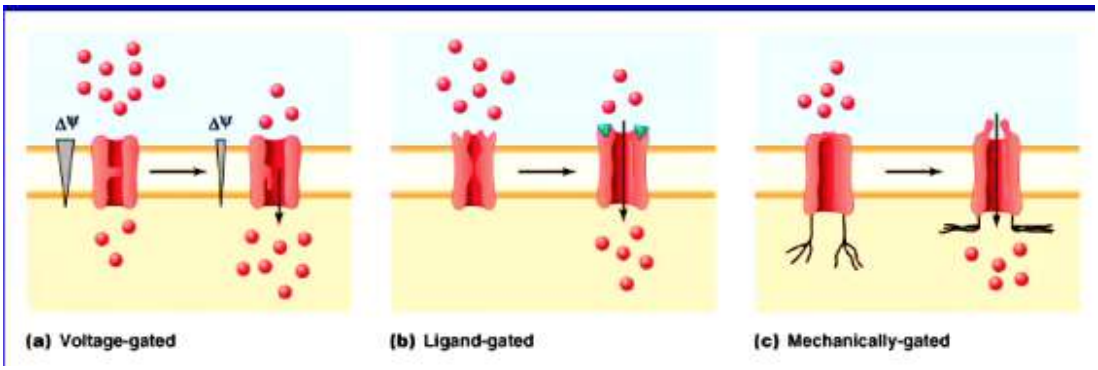


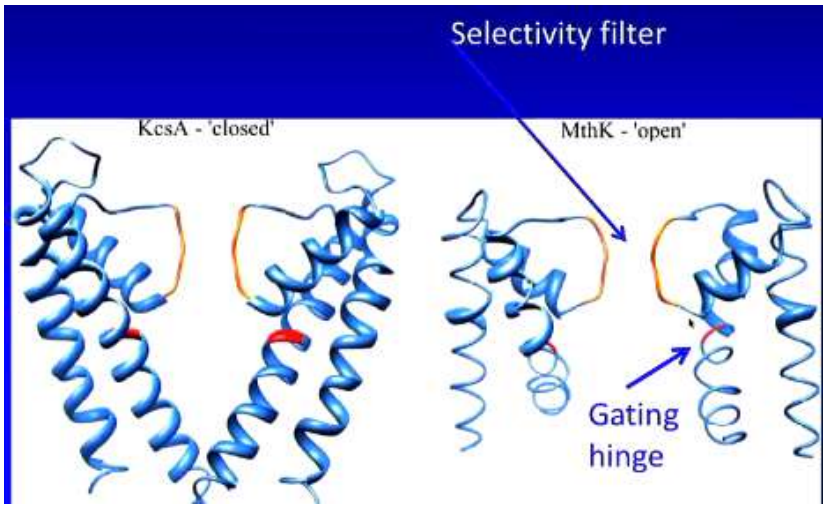
- When the potassium channel opens, the ions line up like 4 billiard balls and knock the furthest ion out into the extracellular space one at a time, whilst replacing the vacant space with another ion from the intracellular space → This maintains the energetically favourable and stable position of the potassium ions within the pore

Gating

- The opening and closing of ion channels
- The Potassium Channels relative to the resting membrane potential, are open during the resting membrane potential (-65 mV), whilst Sodium Channels at the resting membrane potential are not open

- Voltage Gated → Changes in potential across the membrane leading to the opening or closing of voltage gated ion channels
- Ligand Gated → Binding of a chemical messenger (e.g. neurotransmitter) leads to the opening of ligand gated ion channels
- Mechanically-gated → e.g. Stretch receptors in the skin. In response to defamation of the skin, the mechanically-gated ion channel will open and send information

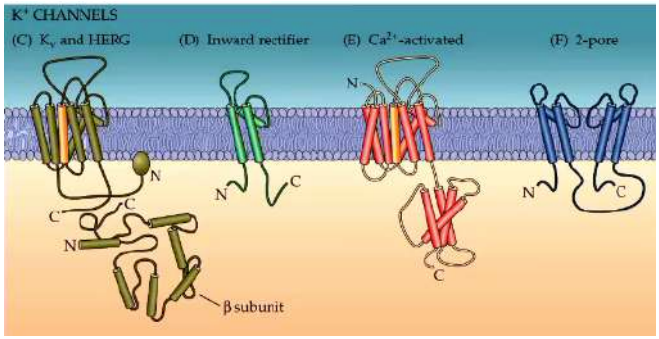


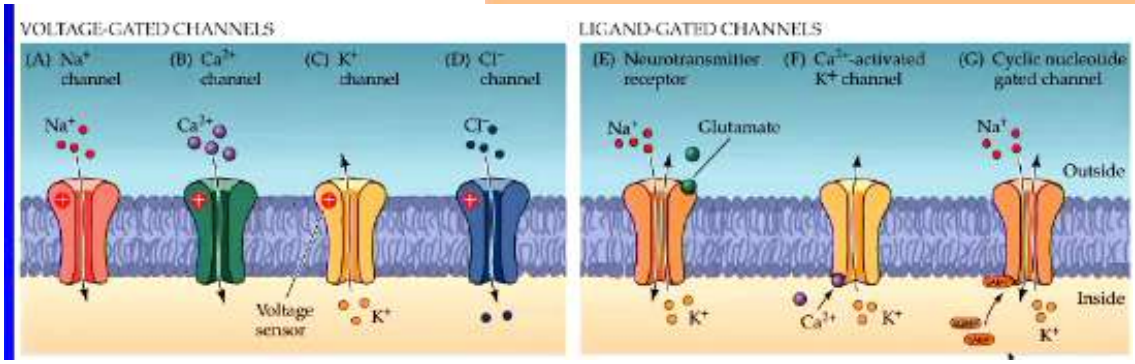


Vast Array of Ion Channels with Different Characteristics

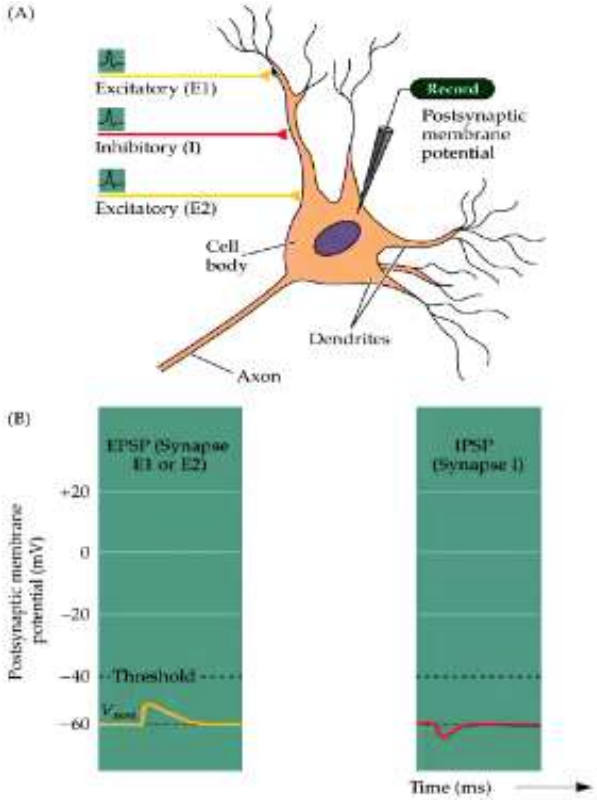
- Large diversity of ions, structures and gating mechanisms
- Voltage Gated Channels → Na+, Ca2+, K+, Cl-
- Ligand Gated Channels → Neurotransmitter receptor, Ca2+ activated K+ channel, Cyclic Nucleotide gated channel

- K+ channels set up the resting membrane potential, whilst a completely different voltage gated K+ channel functions to repolarize the membrane back to resting membrane potential after an action potential has occurred





Lecture 4 – Modulation of Membrane Potential II

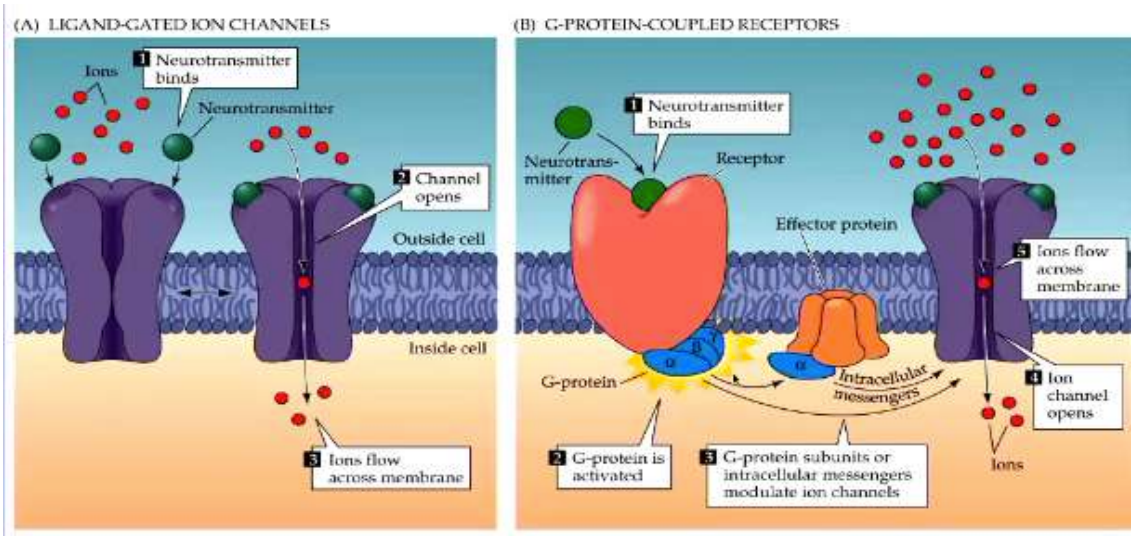


Lecture Outline

- Changes in neuronal activity are enacted by multiple mechanisms. One important mechanism is synaptic transmission
- There are different classes of transmitters that act via different processes
- Fast excitatory transmission

Ionotropic Receptors I – Excitation

- Small excitatory or inhibitory inputs that lead to subthreshold changes to the resting membrane potential are known as Passive Potential Changes → If the sum of all Passive Potential Changes reach threshold, then an action potential will occur. If they do not, an action potential will not occur
- Ligand-gated Ion Channels → Fast excitation and inhibition
- G-protein coupled receptors → Metabotropic, involves signaling within the cell



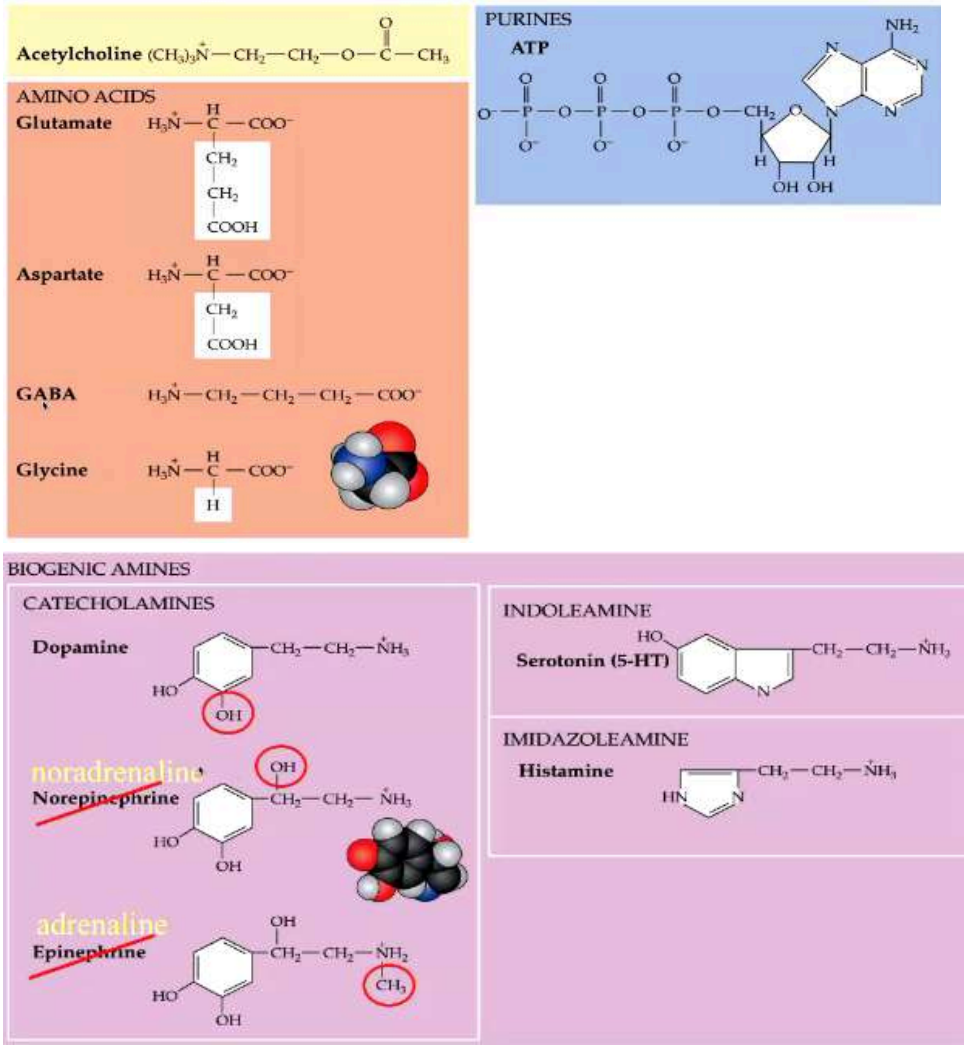
Small Molecule Neurotransmitters

- Amino Acids (Excitatory) → Glutamate and Aspartate
- Amino Acids (Inhibitory) → GABA and Glycine
- Acetylcholine
- Purines → ATP

- Biogenic Amines (Catecholamines) → Dopamine, Noradrenaline and Adrenaline
- Biogenic Amines (Idoleamine) → Serotonin (5-HT)
- Imdazoleamine → Histamine

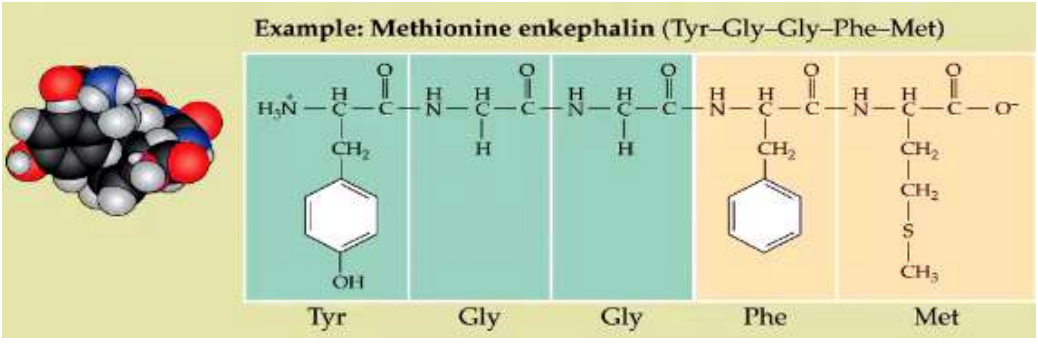
- Glycine is generally considered to be an inhibitory Neurotransmitter in the spinal cord, but is also active in the CNS

- The Biogenic Amines act via the Metabotropic receptors → Modulatory Class
- The Catecholamines all have a similar structure: Dopamine is derived from Tyrosine, with the addition of a Hydroxyl Group. Another Hydroxyl group added gives you Noradrenaline. A further methyl group added gives Adrenaline → Produced via enzymatic cascade starting with Tyrosine
- The Catecholamines can act in both the CNS and PNS



Peptide Neurotransmitters

- More than 100 peptides, usually 3-30 amino acids long
- Modulatory function with a diverse range of uses



Glutamate Receptors

- AMPA, NMDA and Kainate → All receptors bind to glutamate and aspartate, activating this class of receptors which lead to changes in the movement of ions across the cell membrane

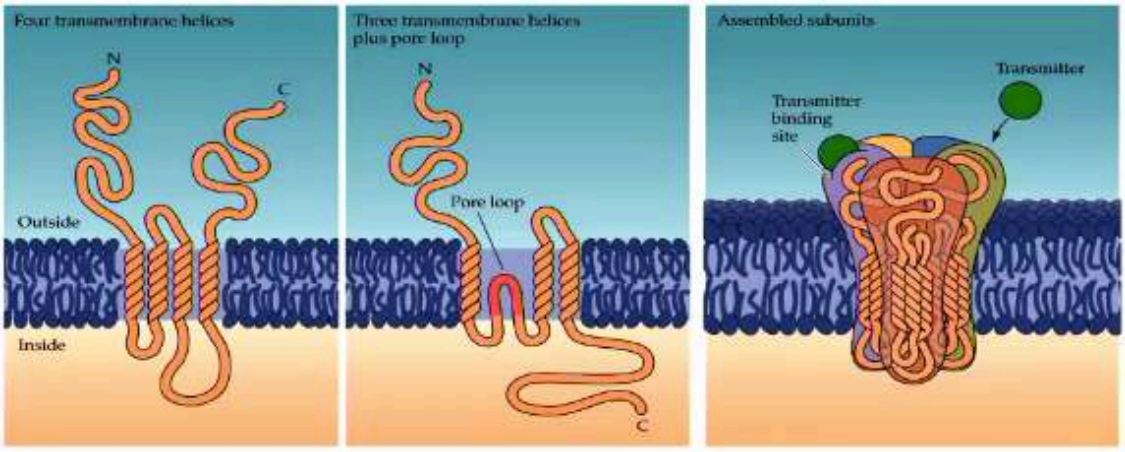
- AMPA receptors are made up of 4-5 subunits → Each subunit has an Amino terminus, a Carboxy intracellular terminus, 3 transmembrane spanning domains and a re-entrant Pore Loop. Subunits include Glu R1, R2, R3 and R4, which have different genes. They are all variants of each other with slightly different properties but ultimately all bind glutamate, and form a pore with the AMPA pharmacology

- NMDA Subunits → NR1, NR2A, NR2B, NR2X, and NR2D
- Kainate → Glu R5, Glu R6, Glu R7, KA1 and KA2

- All subunits assemble and combine to form the final receptor, with the pore in the middle → Contains a gating mechanism so when Glutamate binds it opens, and when nothing is bound, it is closed

- Subunits can assemble in multiple different combinations → This leads to variation in the receptors in terms of pharmacology, ion conduction rate and trafficking (Lots of complexity within Glutamate Receptors)

- When a glutamate receptor is activated, it makes a hole which is a non-selective cation channel → Allowing sodium, potassium and specifically for NMDA, calcium to pass through

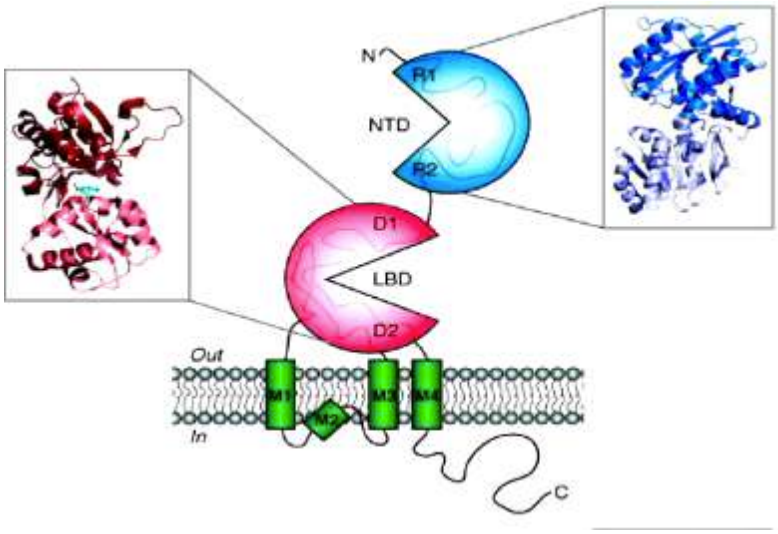


(C)

Receptor	AMPA	NMDA	Kainate	GABA	Glycine	nACh	Serotonin	Purines
Subunits (combination of 4 or 5 required for each receptor type)	Glu R1	NR1	Glu R5	α_{1-7}	α1	α_{2-9}	5-HT_3	P_{2X1}
	Glu R2	NR2A	Glu R6	β_{1-4}	α2	β_{1-4}		P_{2X2}
	Glu R3	NR2B	Glu R7	γ_{1-4}	α3	γ		P_{2X3}
	Glu R4	NR2C	KA1	δ	α4	δ		P_{2X4}
		NR2D	KA2	ε	β			P_{2X5}
				ρ_{1-3}				P_{2X6}
								P_{2X7}

- Amino Terminus is important for the function of the receptor because it contains the Ligand Binding Domain → When Glutamate binds, it activates the receptor

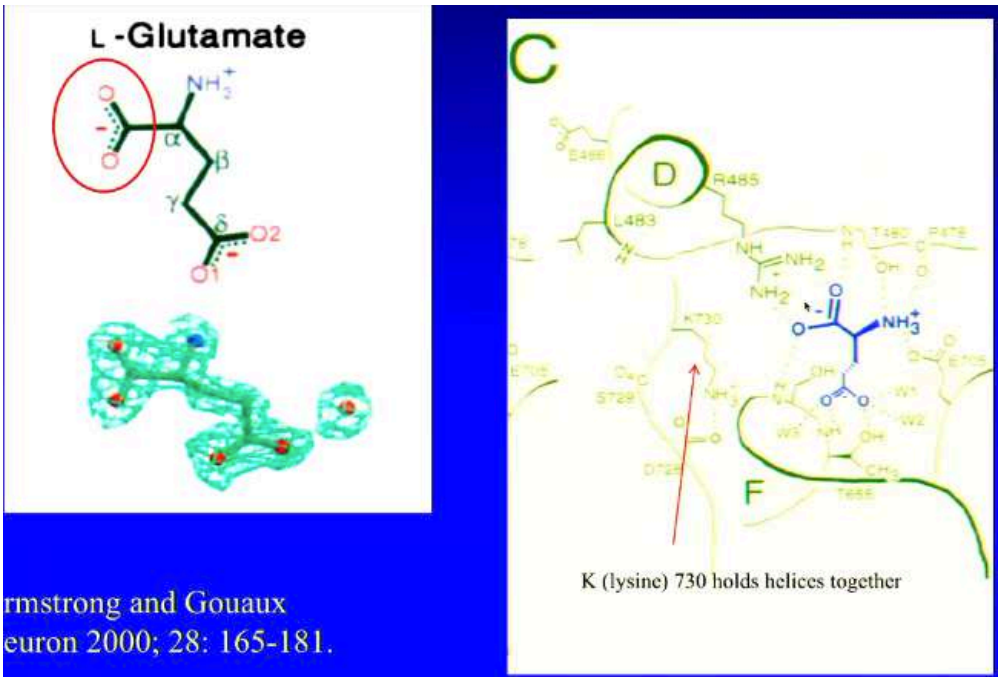
- Also at the Amino Terminus, is the Amino Terminal Domain which is important for Desensitization → Prevents over-excitation via excess glutamate. Down-regulates receptors in response to constant stimulation in order to protect the cell



Recognition of Ligand

- Glutamate is derived from Glutamine (Derived from Glucose), which can then be acted upon by an enzyme called Glutamic Acid Decarboxylase and a co-factor Pyridoxal Phosphate to produce GABA → Glutamate and GABA only differ by a single carboxyl group so a way to differentiate between the two is required

- Key role of Arginine 485 (R485) in recognition of Glutamate → The positioning of the NH2 groups of R485 are critical in allowing the Oxygens of Glutamate to interact with them, thus allowing the glutamate to sit comfortably in the pocket of the receptor

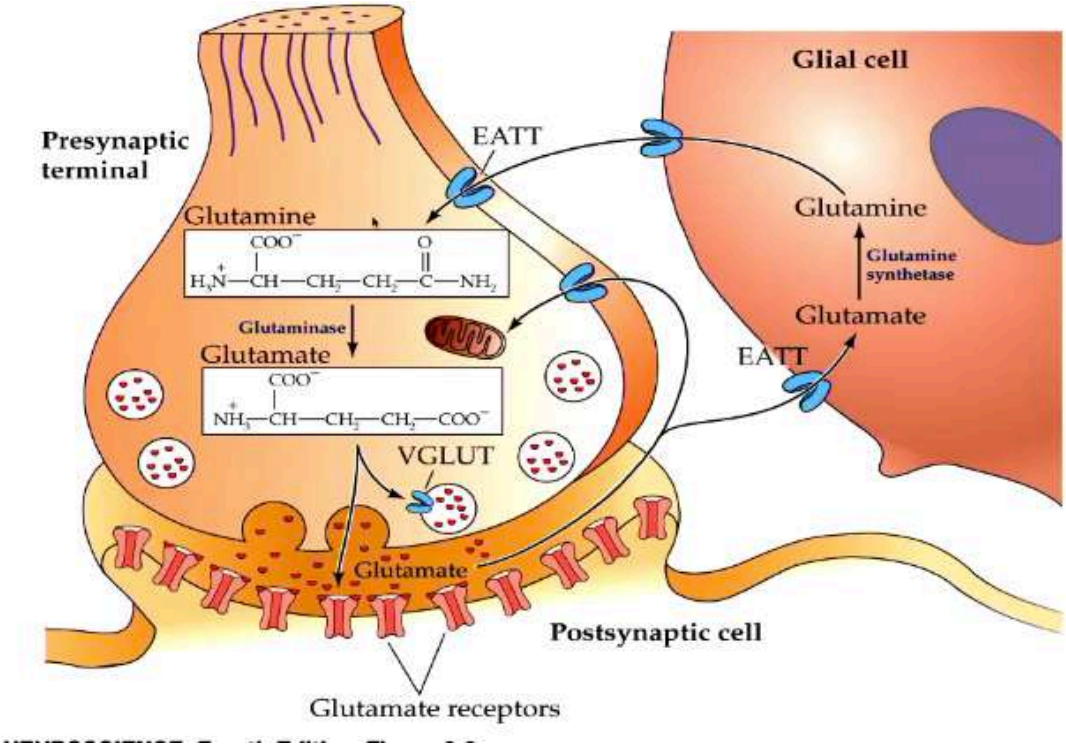


Transmitter Inactivation and Recycling

- Glutamine is acted upon by Glutaminase, which removes the Oxygen to produce Glutamate → It is then packaged into vesicles via VGLUT (Transporter), which in response to an action potential arriving at the terminal, will fuse with the membrane and release the Glutamate into the extracellular space in the synaptic cleft → Glutamate moves across the synapse to bind to the ionotropic receptors on the post-synaptic neuron, and thus facilitating depolarization of this neuron

- Glutamate in the synaptic cleft is then taken up into the Glial Cell predominantly and to a lesser extent the pre-synaptic terminal via EATT (Excitatory Amino Acid Transporters) → Glutamine Synthetase in the Glial Cell adds an oxygen to Glutamate to reform Glutamine, which is inactive. It is released back into the extracellular space and transported via EATT back into the pre-synaptic terminal

- Highly efficient Glutamine/Glutamate cycle → High firing rates for long periods
- Contrasting this with Peptide Transmitters → Made in the soma of the cell, transported down into the axon terminal and released. Once it is released, it is broken down in the synapse and new peptide transmitters must be made and transported. Therefore, peptide transmitters, unlike our principle transmitter glutamate, can be depleted very easily



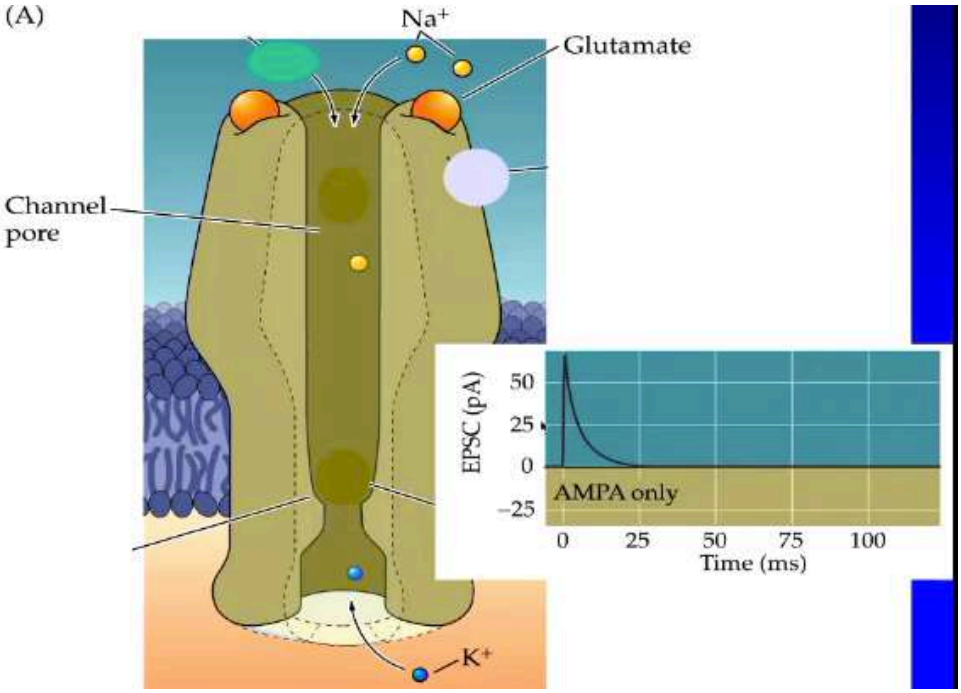
Ion Movement

- Upon binding of glutamate to the receptor, there is potential for the ion channel to allow Sodium, Potassium or even Calcium to move through → This is excitation because at rest, Sodium is impermeable to the membrane. Due to the concentration and electrogenic gradient, Sodium has a large drive and will flood into the cell whilst potassium will initially stay within the cell as it is at equilibrium. The membrane potential goes up and depolarizes as a result

- The most important response to binding of glutamate and opening of the Ligand-gated ion channels is the influx of sodium and depolarization of the cell → Fast excitation when the Sodium moves in. Once the Sodium flows in, it will give rise to a drive for the Potassium to move out

AMPA Receptor (Described above)

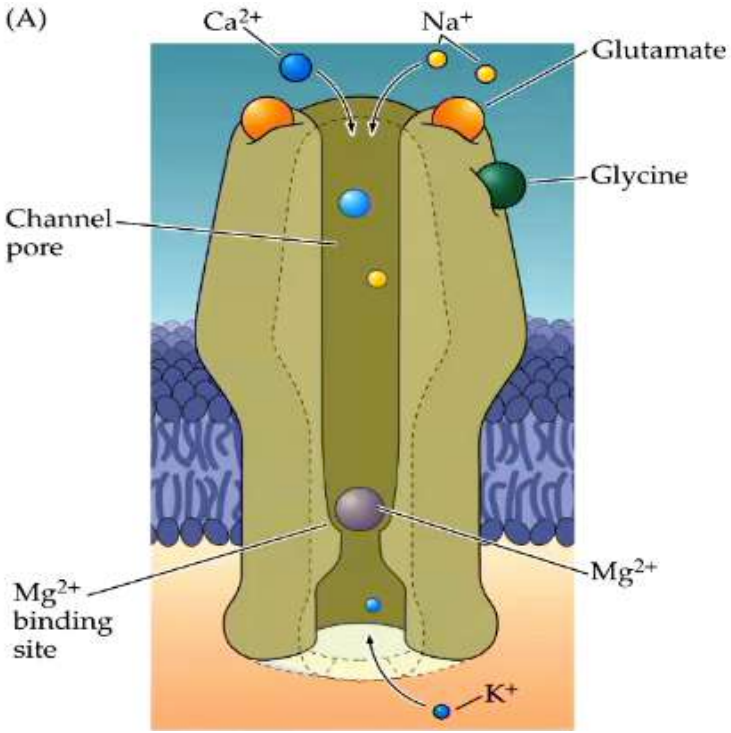
- EPSC = Excitatory Post-Synaptic Current



NMDA Receptor

- Primarily allows Calcium to flow through, which is important because as it enters the cell, it not only changes the membrane potential, but can also initiate secondary messenger pathways

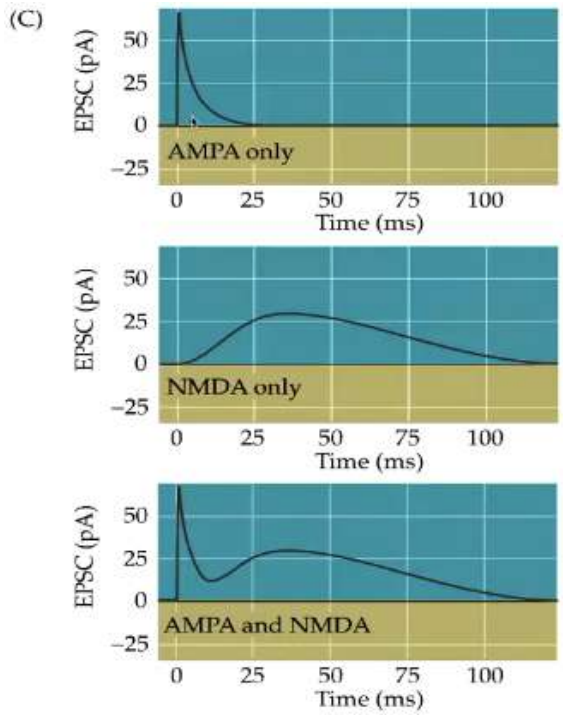
- Contains a Mg2+ binding site, where the Mg2+ ion blocks the Channel Pore. Binding of Glutamate alone will not move this ion, so Calcium and Sodium would not be able to move through → The positioning of the Mg2+ shows that it is not only Ligand-gated, but also voltage sensitive. Depolarization of the cell would make the inside of the cell more positive, and eventually repel the Magnesium, and thus opening up the ion channel



- Due to this voltage sensitivity, it usually works in conjunction with AMPA receptors as it would initiate depolarization that removes the Mg2+ ion

- The NMDA Receptor is also allosterically modulated by Glycine → It is a key inhibitory amino acid transmitter, but due to an unknown reason, it is essential for this excitatory pathway to function

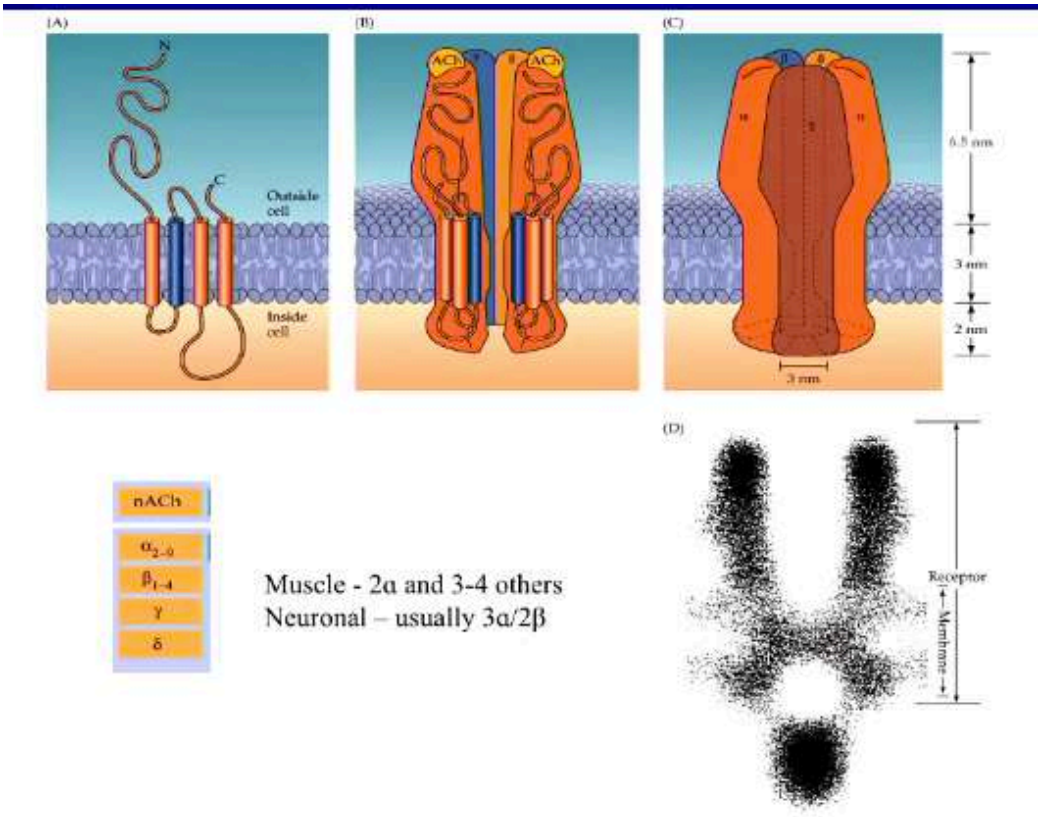
- AMPA receptor alone gives a fast, sharp EPSC, whilst NMDA alone gives a smaller but more prolonged EPSC → When they work together, transmission is achieved



<u>Nicotinic ACh Receptor (Acetylcholine)</u>

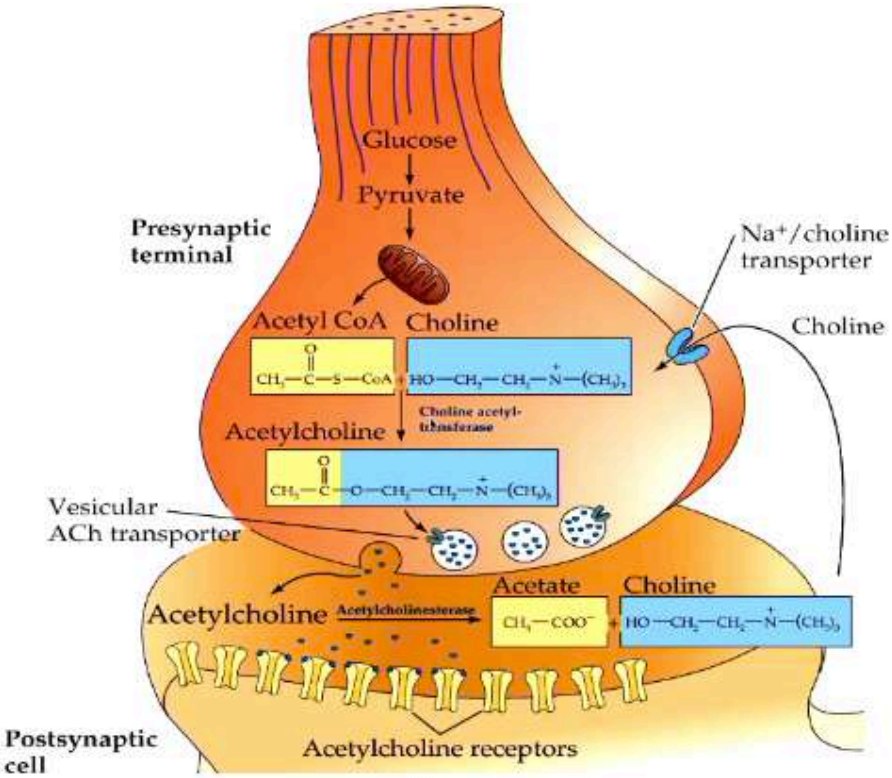
- ACh has an ionotropic receptor called the Nicotinic Acetylcholine receptor (nACh)
- Many different subunits of nACh → alpha 2-9, beta 1-4, gamma and delta. It is made up of a pentamers of 4 transmembrane spanning domains
- In the muscle, nACh contains 2 alpha subunits and 3-4 others, whilst in the neuron, the most common form contains 3 alpha subunits and 2 beta subunits

- Plays a crucial role in movement, and thus nACh is a target for toxins → alpha bungarotoxin (From the snake, Branded Krait) binds to nACh irreversibly and inhibits motor function. Cone shells from the Great Barrier Reef also contain a vast array of toxins, which also targets nACh



Transmitter Inactivation

- Acetylcholine is derived from Acetyl CoA binding with Choline, a reaction catalyzed by the enzyme Choline Acetyl-Transferase → Packaged into vesicles by Vesicular Ach transporter and released into the synaptic cleft in response to action potentials
- After it binds to Acetylcholine receptors in the post-synaptic cell, it is not recycled in the same way glutamate is, rather it is degraded and broken down back into Acetate and Choline via Acetylcholinesterase → The Acetate is removed and recycled elsewhere, whilst the Choline is taken back into the pre-synaptic cell via a Na+/Choline Transporter to be reused. Acetyl CoA is continuously made and supplied via the Pyruvate Cycle
- Neuromuscular Junction is packed with Acetylcholinesterase → Fast and efficient breakdown of Acetylcholine



Summary

- Synaptic inputs result in graded changes in membrane potential.
- There are two main classes of receptor – ionotropic and metabotropic
- Fast neuronal regulation is produced by ionotropic receptors.
- Glutamate is the principal fast excitatory neurotransmitter within the mammalian central nervous system.
- Glutamate ionotropic receptors are non-selective cation channels.
- Glutamate ion channels have 3 distinct families, AMPA, kainate and NMDA with each having different pharmacology and actions.
- The channels are made up of subunits which have three membrane-spanning domains and a re-entrant loop. Most models predict 4 subunits making up an ion channel.
- Nicotinic acetylcholine receptors are another important family of ligand-gated ion channels. These are also non-selective cation channels.
- They are made of pentamers of four transmembrane spanning domains.