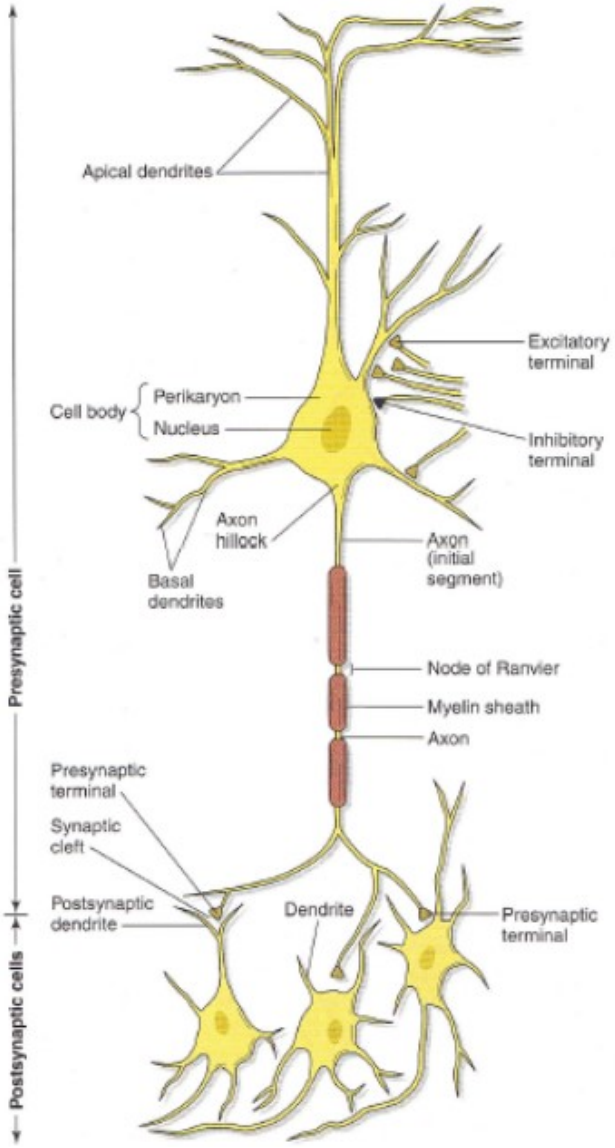
Synapses as Drug Targets

▪Revision
-All different types of neurons have unifying components: dendrites → cell body → axon
-Dendrites: receive input from excitatory and/or inhibitory axon terminals → fine regulation of input (excitatory/ inhibitory) into cell body
-Soma: contains machinery for transcription and translation (nucleus, ribosomes, sER, rER, mitochondria, golgi complex, lysosome) → high turnover of ATP and energy expenditure
-Axon hillock: location of integration and summation of various input from various dendrites → output of single electrical signal through the axon
-Axon: extension of cell body enclosed in myelin sheath and delivers AP to and activates presynaptic terminal → release of neurotransmitters from their vesicles → neurotransmission to neighbouring neurons



Q) Identify the key cellular features of a neuron, include the locations of VGIC, LGIC, GPCR, Transporters, myelin sheath, dendrites, axon, soma.

•Role of glial cells in brain function
1) Astrocytes
-25-50% of cell volume in most regions of the brain
-Shape varies – some star-like, some fibrous
-Glial Fibrillary Acid Protein (GFAP) is a marker for astrocytes

-Astrocytic filaments provide structure to the brain: determine where neurons sit, move and make connections
-Extend processes to the cell bodies of neurons
-Form part of the blood brain barrier
-Maintenance of neuronal homeostasis (environment around neurons)
-Regulate extracellular environment – K+, glutamate, GABA
-Secrete neurotrophic factors (directing growth and movement of neuron)
-Regulate neuronal migration, axon and dendrite growth
-Glycogen storage (glucose and ATP production)
-Differentiation

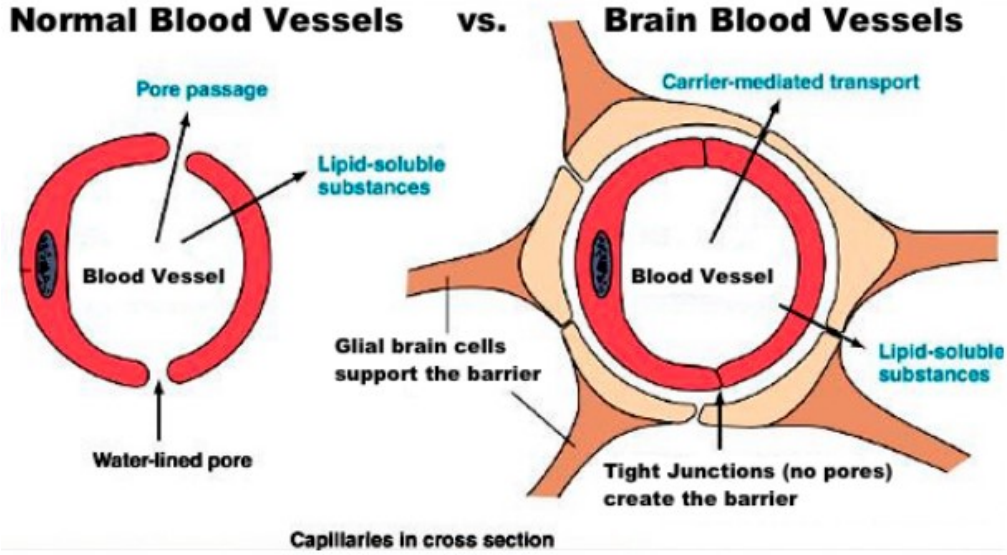
2) Oligodendrocytes and Schwann cells
-Produce and ensheath axons with myelin
-Myelin forms an insulating layer for axons to allow rapid electrical conduction

-Multiple sclerosis is a result of destruction of the myelin sheath

3) Microglia
-Immune cells of the brain and spinal cord
-Defence against infection
-Respond to insult or injury by changing their morphology and proliferating
-Excessive activation can be deleterious – may contribute to neurodegenerative disorders such as Alzheimer's Disease

Q) Describe the roles of the different types of glial cells.

•Role of the Blood Brain Barrier (BBB)



-Formed by tight junctions between endothelial cells of the capillaries in cerebral vascular beds (c.f. gap junctions in normal BV), which restrict the passage of soluble molecules

-Astrocytes give the BBB its structure and inhibit penetration

-Continuous lipid bilayer that prevents exchange between the brains extracellular fluid and the general circulation

-BBB required to isolate the extracellular fluid of the brain from the general circulation to allow very precise control of conditions and prevent the entry of potentially toxic substances (extra protective layer)

▪Drug and BBB

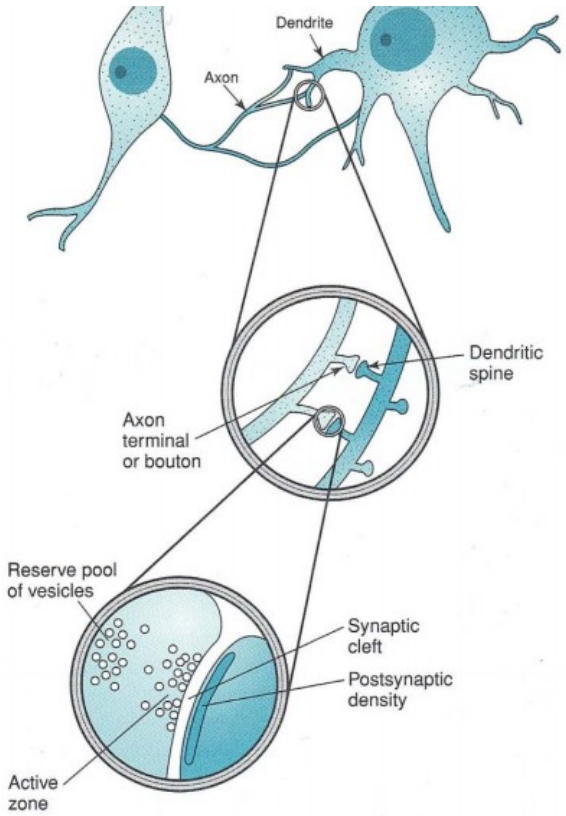
-All CNS drugs must cross the BBB

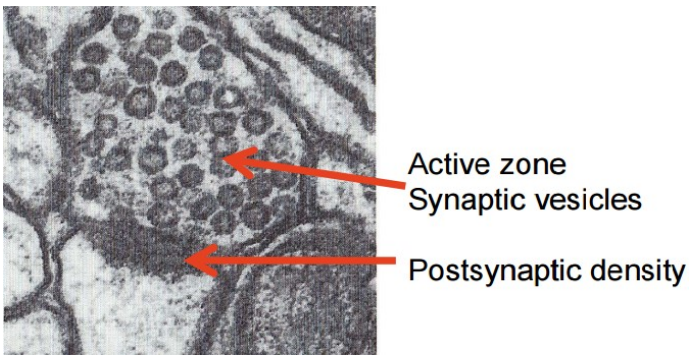
1) Prodrugs – lipophilic (lipophilic moiety attached) and then metabolised after crossing the BBB to become active
2) Lipophilic drugs – pass through the hydrophobic lipid membrane of BBB
3) Drug transporters – hijack normal transport system in the BBB (e.g. glucose transporter, organic cation transporter)

Q) What is the blood brain barrier, how does it regulate the brain environment, and how does it influence drug actions in the brain?

•Cellular and ionic basis of synaptic transmission

▪Synapse

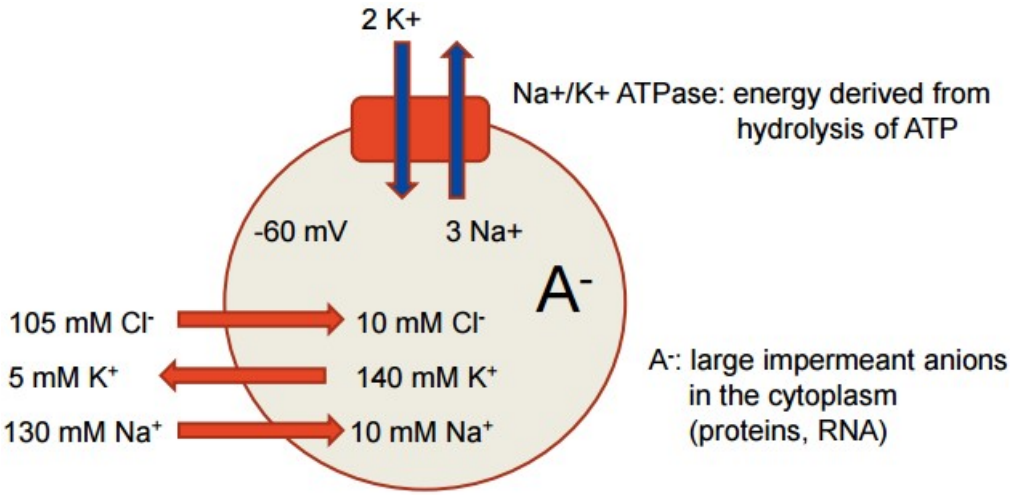




-Axon terminal contains vesicles that house neurotransmitters
-When electrical impulse arrives at the presynaptic terminal and activates it, vesicles fuse with membrane in active zone and dump content to synaptic cleft (fine gap between pre and post synaptic membrane)
-Postsynaptic density = tightly compacted region of postsynaptic membrane containing a lot of scaffolding proteins to hold receptors and signalling molecules → high fidelity signalling and efficient transduction

▪Synapse types
1) Axo-dendritic/ Axo-somatic synapses
2) Axo-axonic synapses: presynaptic bouton acts on another bouton to directly regulate transduction (bypassing events up in the cell body)
3) Autoreceptors: neurotransmitter gets released, diffuses and acts on the same bouton for self-regulation (e.g. NA being released and acting on NA receptors on the presynaptic terminal)
4) Complex synapses involving multiple neurotransmitters – multiple synapses converging on one presynaptic terminal causing one integrated and summated response

▪Electrical excitability of neurons
-At resting state, there is net negative charge (-60 mV) inside the cell:
    -ATPase – hydrolysis of ATP provides energy to pump out 3 Na^+ and pump in 2 K^+ (net +1 out)
    -Large excess of impermeant anions (proteins, RNA, DNA)
    -Combination of ion gradients across the membrane
-Changes membrane potential → Changes ion gradient → Opens ion channels → Movement of ions → further changes in membrane potential → Electrical excitation of neuron



Ion	Equilibrium potential
Na+	+50 mV
K+	-80 mV
Cl-	-60 mV

Nernst equation

$$E = \frac{RT}{ZF} \log \left\{ \frac{P_A[A^+]_o}{P_A[A^+]_i} \right\}$$

GHK equation

$$E = \frac{RT}{ZF} \log \left\{ \frac{P_K[K]_o + P_{Na}[Na]_o + P_{Cl}[Cl]_i}{P_K[K]_i + P_{Na}[Na]_i + P_{Cl}[Cl]_o} \right\}$$

= -60 mV (resting state)

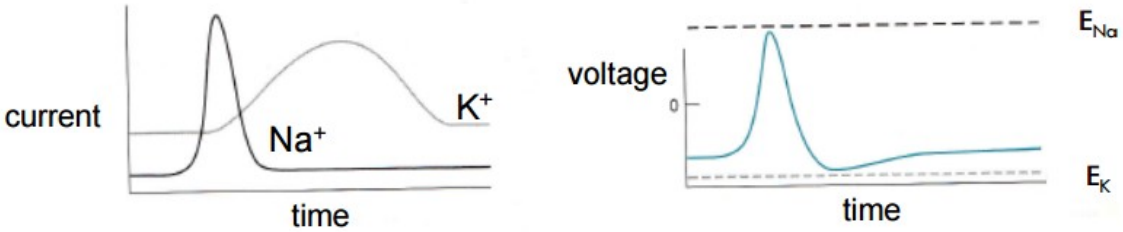
R: gas constant
T: temp in ºK
Z: charge
F: Faraday's constant
A: Ion (eg. Na^+)
P_A: Permeability of ion A

-At voltage above the equilibrium potential, the ion moves out of the cell
    -E.g. when K^+ channel opens at resting state, K^+ flows OUT until membrane potential reaches -80 mV
-At voltage lower than the equilibrium potential, the ion moves into the cell
    -E.g. when Na^+ channel opens at resting state, Na^+ flows IN until membrane potential reaches +50 mV

-Nernst equation: calculates the equilibrium potential for each ion individually
-GHK equation: combines contributions of all ions (K, Na and Cl)

-R = 8.314 $JK^{-1}mol^{-1}$
-T = 36.5 + 273.15 = 309.65 K
-Z = +1/+2/-1
-F = 96485 $Cmol^{-1}$
-$[X]_o$ = concentration of X in the extracellular fluid
-$[X]_i$ = concentration of X in the intracellular fluid
-E is in volts

▪Generation of action potential =Coordinated activity between Na^+ and K^+ ion channels

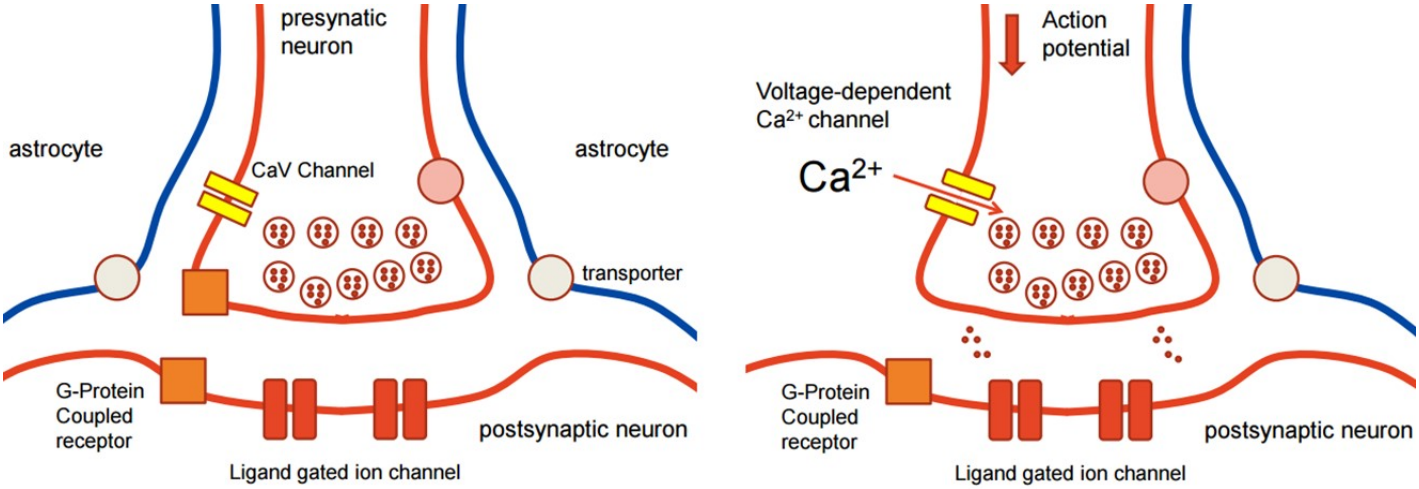


-Initial activation of voltage-dependent Na^+ channel when there is sufficient membrane depolarisation at axon hillock
-Na^+ channel opens (in response to depolarisation) and Na^+ flows into the cell = spike in activity
-Na^+ channel closes and is inactivated after being open for 1-2 msec
-Depolarization also causes opening of K_V channels
-Slower activation of K^+ channel (takes longer to be activated) which peaks when Na^+ channel is turned off
-K^+ ions flow out of the cell

-Na^+ influx causes the membrane potential to shift from hyperpolarised potential (-60 mV) to further depolarised potential +50 mV (reverse potential of Na^+)
-Na^+ channel closes and membrane potential starts to return slowly towards the resting state
    -Slow reversal correlates with the slow activation and opening of K^+ channel
-Membrane potential overshoots the resting state (below the starting membrane potential) and this level is close to the reverse potential of K^+
    -Membrane potential change is dominated by action of K^+ ions
-With time, as K^+ channels start to close, the membrane potential returns to the resting state (due to activation of Cl^- channels and leakage of K^+ going back out)

-Depolarisation begins at axon hillock
-Membrane further down the axon senses the change in membrane potential
-Activation of next set of ion channels
    -Nodes of Ranvier = concentrated Na^+ and K^+ channels propagating the depolarisation and hyperpolarisation
-Action potential reaches the presynaptic terminal

▪Neurotransmission



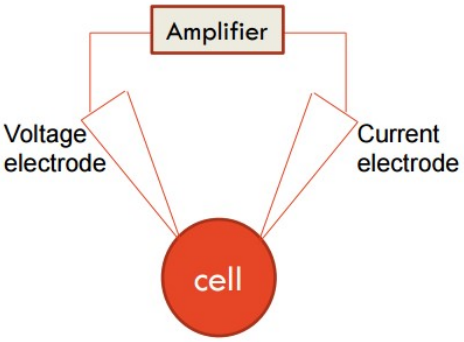
-Action potential (wave of depolarisation) arrives at the presynaptic terminal
-Voltage-dependent Ca^{2+} channel senses the change in membrane potential in its close vicinity
-Opening of Ca^{2+} channel and Ca^{2+} ions flow into cell and influence the properties of presynaptic terminal
-Ca^{2+} trigger fusing of synaptic vesicle to the presynaptic membrane (via a series of events)

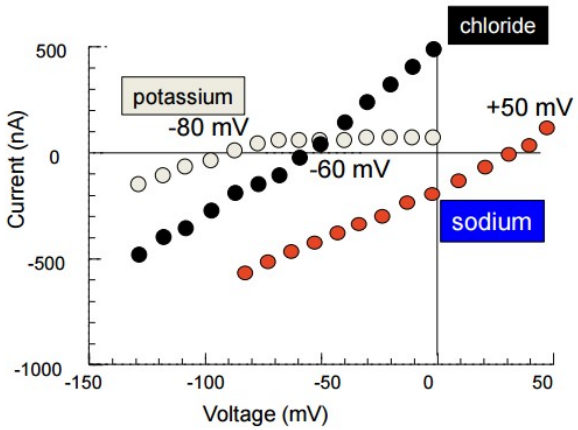
-NTs are released into the synaptic cleft
-NTs can bind to GPCR or LGIC on postsynaptic membrane to cause neurotransmission

-To terminate neurotransmission, NTs need to be removed from the synapse
-NTs can be metabolised and inactivated (e.g. ACh and cholinesterase)
-NTs can diffuse out of synapse until the concentration is insufficient to cause receptor activation
-NTs can be removed by transporters and moved into astrocytes or back into presynaptic terminal (repackaged and reused)

→All proteins and processes in the synapse are potential drug targets

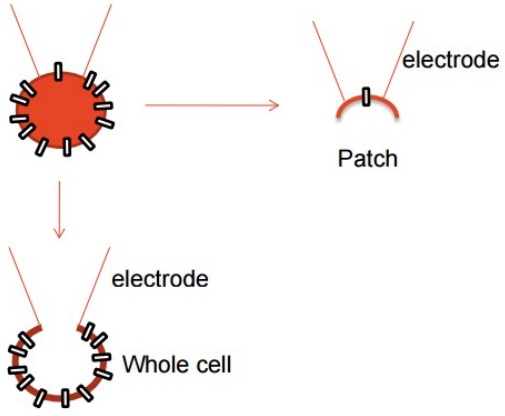
▪Measurement of ion channels
1) Two electrode voltage clamp



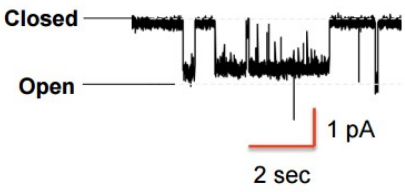


-Stick a voltage electrode and a current electrode into a cell
-Set the voltage electrode at the desired membrane potential
-Current electrode measures how much current is required to keep the cell at the set membrane potential
-If you're at the resting membrane potential, no current will be required
-If you make the cell more hyperpolarised, you need to inject current to keep it at that membrane potential
-Cl^- channel: at negative membrane potentials, Cl^- flow out (inward current of negative charges)
-Na^+ channel: at negative membrane potentials, Na^+ flow in (inward current of positive charges)

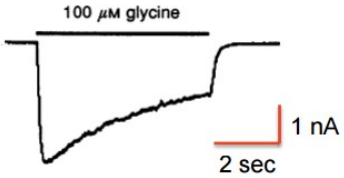
2) Patch clamp



Patch clamp glycine-gated chloride channel current



Whole cell glycine-gated chloride channel current



-A cell expressing glycine-gated chloride ion channels on the membrane + glass electrodes attached

a) Whole cell current
-Apply suction within the electrode and suck out a portion of membrane
-The solution that is in the electrode is now in contact with the whole cell
-The whole cell is essentially stuck on the end of the electrode
-Applying glycine to extracellular solution will activate the chloride ion channel and allow influx of current
-In continued presence of glycine (~4s), it will slowly decay to a steady state level
-Removing glycine will return it to the baseline
-Whole cell current is a summation of 1000 patch clamp currents

b) Patch clamp current
-Pull a patch/ fragment of the membrane while it is still attached to the electrode
-A single ion channel is essentially sitting on a patch of membrane
-The ion channel is exposed to the solution within the electrode
-The bath solution (where the rest of the patch is/ the inside of the cell is being exposed) can be perfused with various intracellular solutions
-Add glycine inside the electrode with the single ion channel
-In the continual exposure to glycine, the channel opens (to allow influx) and spontaneously closes in a fluctuating manner
-The amount of fluctuation depends on the amount of glycine within the electrode
    -High [glycine] → the channel will be open with glycine bound most of the time
    -Low [glycine] → glycine will be hopping on and off
→ Elucidate how a single ion channel behaves in a cell membrane

Q) Calculate the reverse potentials for Na+, K+ and Cl- using the Nernst equation with standard resting intracellular and extracellular ion concentrations.
Q) Describe the role of the Na/K-ATPase in setting the resting potential of a neuron.
Q) Describe the roles of Ca^{2+} channels in triggering synaptic vesicle release.

•Major NT and NT receptors

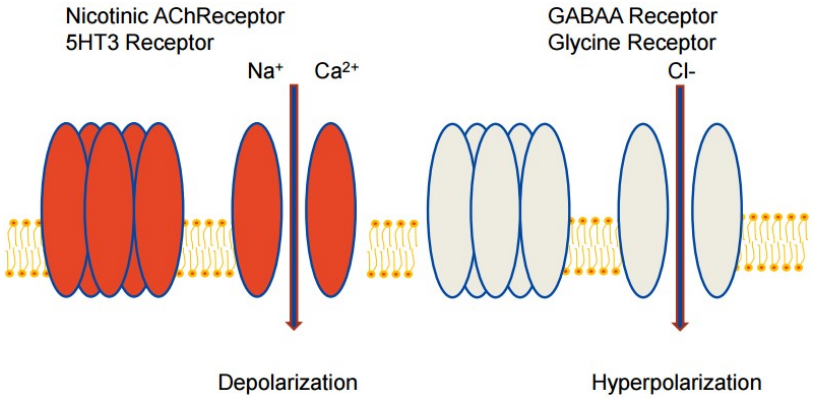
Neurotransmitter	Ligand-gated ion channels	G-Protein coupled receptors
Glutamate	AMPA-R, NMDA-R	mGluR
Acetylcholine	nACh-R	mACh-R
GABA	$GABA_A$-R	$GABA_B$-R
Glycine	GlyR	
Dopamine Serotonin Noradrenaline	5HT-3 R	D-R 5HT-R A-R
Peptides (enkephalins) Nucleosides (ATP)	P2XR	OR (μ, κ, δ) P2YR

-Glycine only activates LGIC
-Glycine is a co-transmitter at the NMDA receptor (glutamate + glycine → activation)

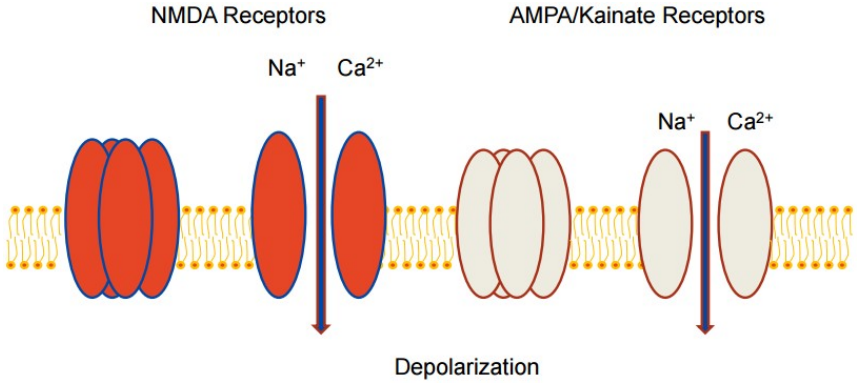
-1 LGIC for catecholamine = serotonin type 3 receptor (similar to nACh-R and $GABA_A$-R)

•Major NT receptors
1) Ligand-gated ion channels (LGIC)
-Fast synaptic transmission
-Mediate fast impulses (e.g. getting injured)

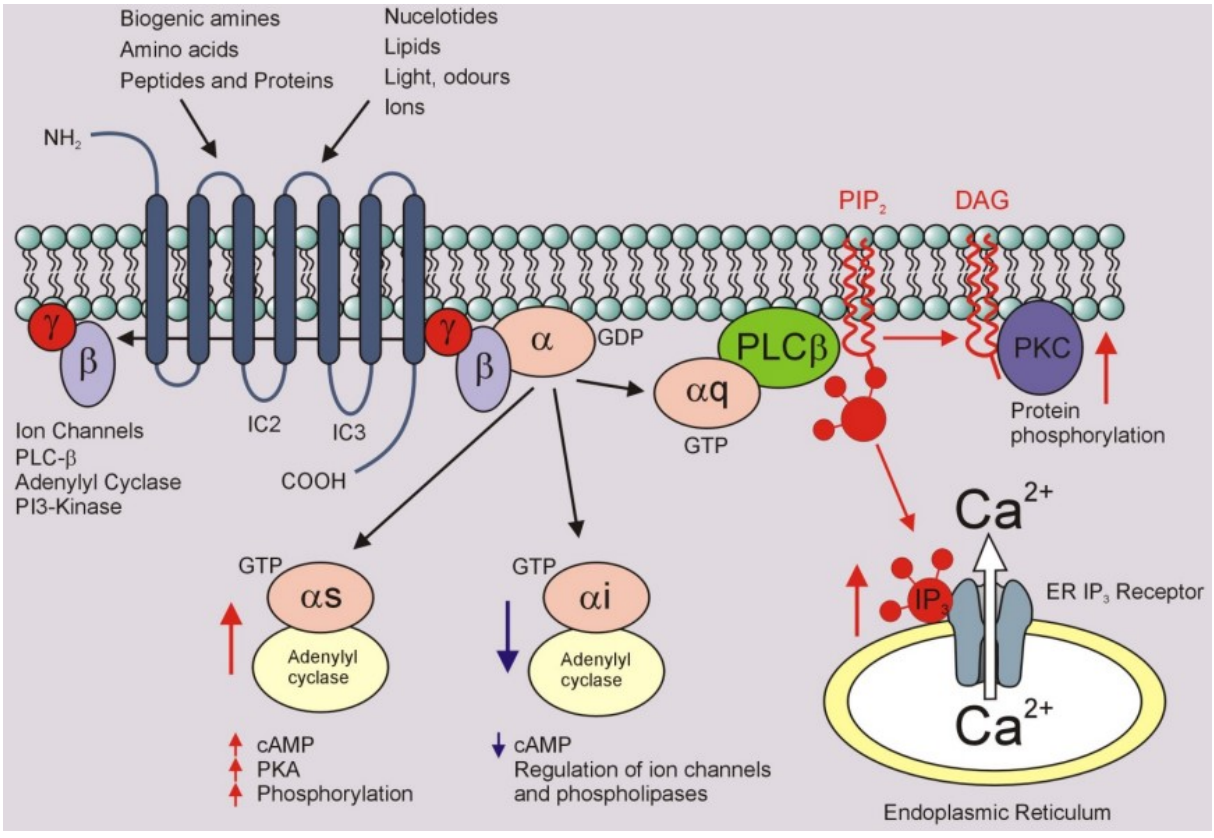
a) Cys loop receptors – Pentameric
-nAChR and 5-HT_3R generate depolarising/excitatory signal by allowing Na^+ and Ca^{2+} ions in
-$GABA_A$ and Glycine receptors generate hyperpolarising/inhibitory signal by allowing Cl^- ions in
-Similar structures but strategically placed amino acid residues at the edges of the pores of the channels allows sensitive selection of ions



b) Ionotropic receptors – Tetrameric
-Glutamate receptors (classified by pharmacological agonists)
-All allow Na^+ and Ca^{2+} ions to flow in and cause depolarisation/excitation
-NMDA receptors require glutamate and glycine (or D-serine)
-AMPA/Kainate receptors only require glutamate



2) G-protein coupled receptor (GPCR) - 7 transmembrane domain receptors
-Slow synaptic transmission
-Modulate the fast transmission (accelerate/decelerate) by changing metabolism within the cell

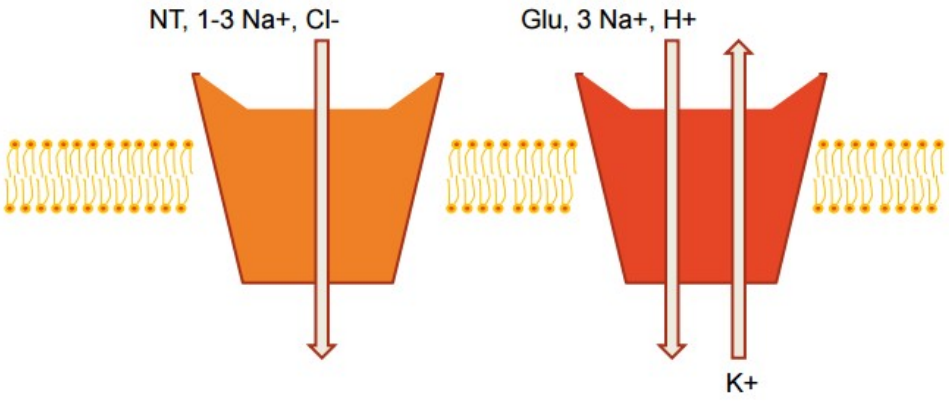


-The receptor activates the G-protein which then couples to GTP
-At GTP-bound state of G-protein, the α-subunit separates from the β and γ subunits
-The activated α-subunit then modulates the activity of enzymes
    -αq activates PLC which regulates lipid metabolism (cleaves PIP_2) to generate IP_3 → IP_3 activates intracellular Ca^{2+} channels in ER → elevate intracellular Ca^{2+} level → change responsiveness of cell or affect gene expression
    -αq activates PLC which regulates lipid metabolism (cleaves PIP_2) to generate DAG →DAG activates PKC → PKC alters protein phosphorylation → altered cellular activity
    -αi inhibits adenylyl cyclase → downregulates cAMP → regulates ion channels and phospholipases
    -αs stimulates adenylyl cyclase → upregulates cAMP and hence PKA → PKA alters protein phosphorylation
-The activated β and γ subunits regulate activity of other enzymes and ion channels

•Major NT transporters
-2 Families of Neurotransmitter Transporters
-Transporting NTs against their concentration gradients (into the cell where there is higher [NT]) is an active transport process to drag NTs across the membrane → NTs are coupled to co-transported ions

1) Family 1: GABA, Glycine, Dopamine, Noradrenaline, Serotonin
-NTs are coupled to 1-3 Na^+ ions and a Cl^- ion
-Na^+, Cl^- have natural tendency to go into the cell (following concentration gradient) and drag the NTs

2) Family 2: Glutamate, Aspartate
-NTs are coupled to 3 Na^+ ions, a H^+ ion and a counter-transported K^+ ion
-More ions are involved in glutamate transport → more powerful transporter



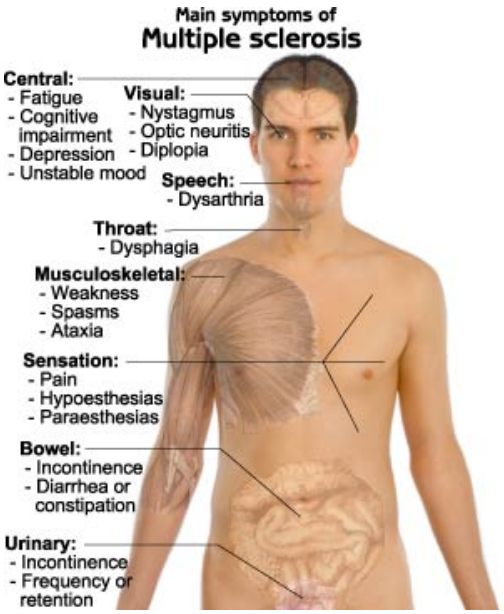
Q) Relate the location of VGIC, LGIC, GPCR and Transporters to their roles in synaptic transmission.

Multiple Sclerosis and Neuro-inflammatory Diseases

•Multiple Sclerosis
-Inflammatory disease (autoimmune reaction) in which the **myelin sheath surrounding axons** is damaged
-Damage prevents neurons from functioning in the normal way

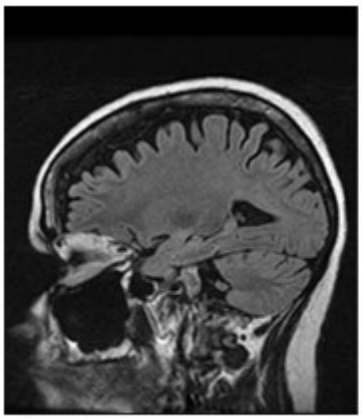
-Multiple sclerosis refers to multiple scars (plaques or lesions) in the CNS
-Lesions most commonly affect the white matter (myelin-coated axons) of the optic nerve, brain stem, basal ganglia and spinal cord
-Recurring episodes of MS can cause many scars to appear as a result of the breakdown of the myelin
-Episodes can occur at varying time intervals affecting different areas of the CNS – frequency of episodes increases as disease progresses

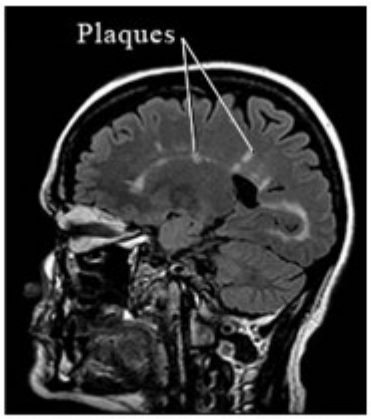
-Common onset between 20 and 50 years
-There is no one symptom that indicates the presence of MS (various organs involved)
-No single test can establish an accurate diagnosis



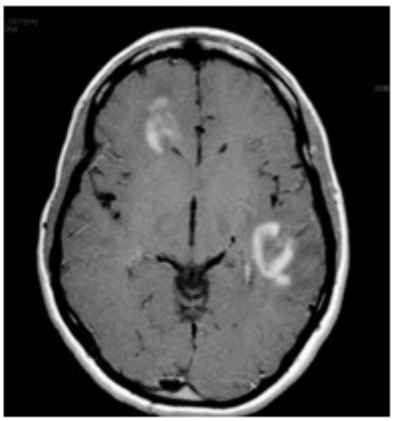
Q) What is the pathological basis for MS?
Q) What are the symptoms of MS?

▪Features
-Diagnosis by MRI of plaques/lesions
-Plaques/lesions (white spots) in the brain


Healthy brain



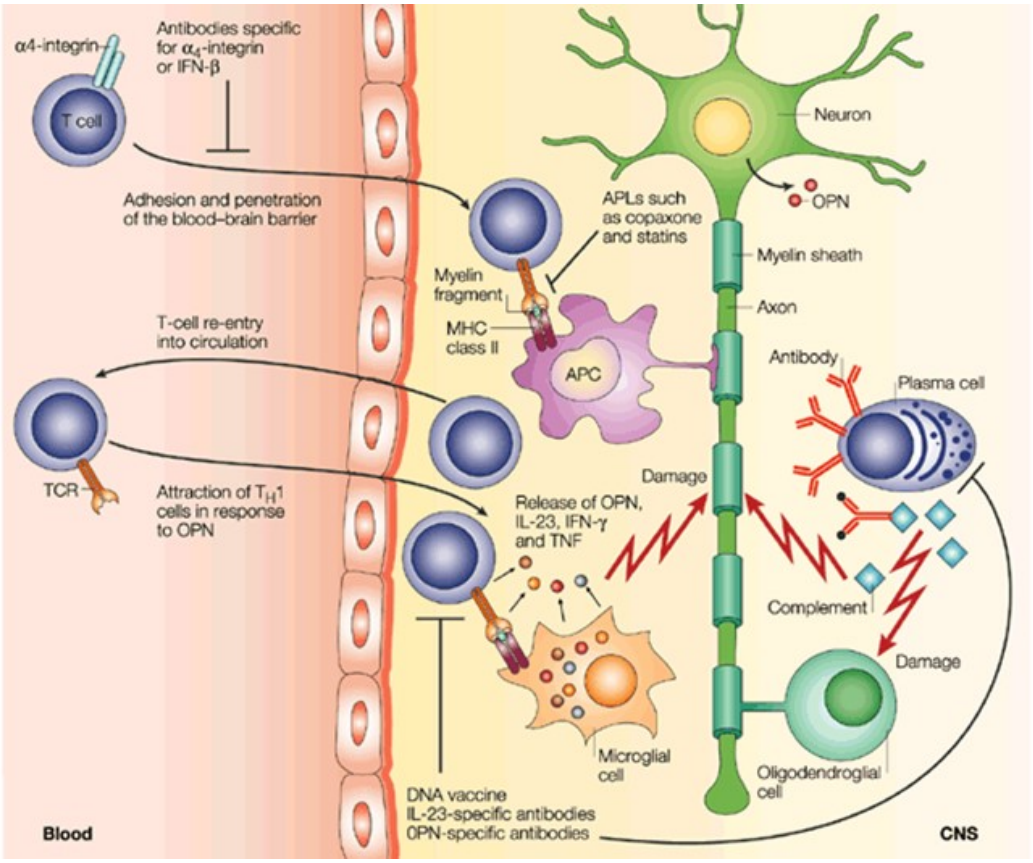
Brain with damage (lesions or plaques) caused by MS



-Areas of plaque surrounding the spinal cord = build-up of inflammatory reactions/milieu

▪Cause of plaques
-No clear trigger – maybe associated with post viral infection?
-Temporary breakdown of BBB
-BBB becomes susceptible to leakage and immune cells leak across the BBB and infiltrate into the CNS
-Immune cells recognise antigens they have never seen (normally protected area)
-Mount neuro-immune reactions against "foreign" proteins
-Most commonly, proteins in the myelin sheath such as myelin basic protein
-Immune system develops an aberrant response to its own myelin sheath and breaks down the myelin
-In the process, it triggers additional immune responses e.g. activation of complement pathway, recruitment of inflammatory mediators such as TNF, interleukin
-Activation of microglial cells
-Auto-immune reaction

-BBB reforms
-The immune response dampens down and recovery begins
-There are resident T cells that remain within the CNS and get reactivated from time to time → Episodes at various periods in time



•Drug treatment for MS
-There are no curative drugs for MS
-Current therapies are aimed at reducing the severity and frequency of symptoms (autoimmune reactions)

1) Beta interferons - Avonex
-Beta interferons are naturally occurring cytokines secreted by immune cells
-Inhibit viral replication via a variety of immunomodulating and antiviral activities
-Perform regulatory functions in the immune system, and are anti-inflammatory
-May restrict lymphocytes crossing the BBB and limit immune response

-Reduced inflammatory lesions by 50-80% (shown on brain MRI scans) and improve quality of life
-Most effective in early stages of MS
-Side effects: liver function abnormalities, leukopenia, thyroid disease, depression, flu-like symptoms are common

2) Sphingosine-1-phosphate receptor modulator – Fingolimod
-Sphingosine-1-phosphate is a lipid derived from membrane which triggers inflammatory reactions
-Prodrug is metabolized by sphingosine kinase to the active metabolite fingolimod phosphate which modulates sphingosine-1-phosphate receptor
-Inhibit inflammatory response
-Blocks the migration of lymphocytes from lymph nodes, thereby reducing the number of lymphocytes in peripheral blood

-Comparable or better than beta-interferons
-Most common adverse events: headache, elevated liver enzymes, influenza viral infections, diarrhea, back pain

3) Myelin basic protein mimic – Glatiramer acetate
-Copolymer polypeptide mixture consisting of l-glutamic acid, l-lysine, l-alanine, and l-tyrosine
-Mimics and competes with myelin basic protein (key protein to which autoimmunity develops)
-Bind up and inactivate all the antibodies directed against myelin basic protein
-Reduces inflammation

-80% of patients showed improvement over 15 years treatment with minimal long-term safety problems
-Recommended in patients who cannot tolerate beta interferons
-Glatiramer acetate–specific suppressor T cells are induced and activated in the periphery

4) K^+ channel blockers – Dalfampridine
-Contain 4-aminopyridine
-May improve the nerve conduction in fibers whose insulating myelin coating has been damaged by MS
-Modulates efficiency and fidelity of action potentials
-Improve walking in patients with MS

-Recommended in later stage MS patients who have lost muscle control over their lower limbs and have considerable walking problems e.g. wheelchair-bound
-Overdose can cause seizures (hyperexcitable neurons as hyperpolarisation is reduced)
-Common adverse events include: urinary tract infections, insomnia, dizziness, headache, nausea, weakness, back pain, balance disorders, swelling in the nose or throat, constipation, diarrhea, indigestion, throat pain, and burning, tingling, or itching of the skin

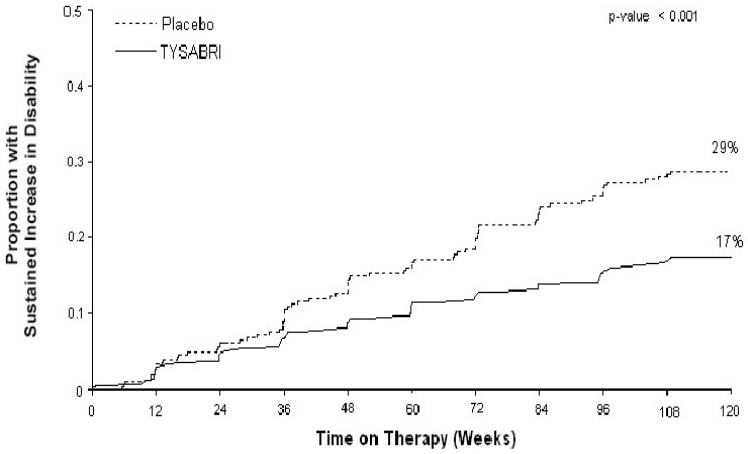
▪Not formally approved as MS drugs
5) Chemotherapeutic agents – Mitoxantrone
-Suppresses proliferation and activity of T cells, B cells, and macrophages that attack the myelin sheath
-Impairs antigen presentation via T cell as well as the secretion of interferon gamma, TNF-alpha, and interleukin-2

-Intercalates into DNA and RNA – disrupts DNA replication and prevents cell division and proliferation
-A potent inhibitor of topoisomerase II, an enzyme responsible for repairing damaged DNA

-Knockout all rapidly proliferating cells in a non-specific manner
-Common adverse events: nausea and vomiting, alopecia (hair loss), urinary tract infections, upper respiratory tract infections

6) Human immunoglobulins - Natalizumab
-Natalizumab is a humanized monoclonal IgG4 antibody
-Natalizumab binds to the alpha 4-subunit of alpha 4β1 and alpha 4β7 integrins expressed on the surface of leukocytes (except neutrophils), and it inhibits the alpha 4–mediated adhesion of leukocytes and makes BBB crossing more difficult
-Prevents lymphocyte migration across the BBB

-Natalizumab delays the progression of physical disability and reduces the frequency of relapse
-60% reduction in relapse rate
-Risk of progressive multifocal leukoencephalopathy (PML), an opportunistic viral infection of the brain that usually leads to death or severe disability



7) Immunosuppressants
-Azathioprine and Methotrexate
-Disrupt purine biosynthesis
-Cyclophosphamide
-Cytotoxic alkylating agent of DNA – may be beneficial when given with beta interferon
-Mycophenolate Mofetil and Cladribine
-Inhibition of purine synthesis in T and B cells
-Used alone or in combination with a beta interferon
-DNA synthesis, replication and proliferation are inhibited to dampen the immune response

Q) Outline the mechanisms of action of the drugs used for the treatment of MS.

▪Drugs in development
-Humanized monoclonal antibodies directed against only the immune cells that are attacking the myelin sheath:
-Alemtuzumab targets CD52, a broadly expressed cell–surface molecule on immune cells
-Daclizumab binds to the CD25 alpha subunit of the high-affinity IL-2 receptor on the surface of activated lymphocytes
-Rituximab and Ocrelizumab bind to the CD20 antigen, a hydrophobic transmembrane protein located on pre-B and mature B lymphocytes

▪Immunoablation and autologous haemopoietic stem-cell transplantation for aggressive multiple sclerosis
-Combination therapy
-Immunoablation with busulfan, cyclophosphamide, and rabbit anti-thymocyte immunoglobulin to get rid of immune cells contributing to autoimmunity
-Autologous haemopoietic stem-cell transplantation (aHSCT) devoid of components directed against myelin sheath
-Redevelop a new immune system

-No new episodes of MS and no new lesions
-Improve control of relapsing disease
-One of 24 patients died of transplantation-related complications. Most patients had substantial recovery of neurological function despite their disease's aggressive nature

•Inflammatory processes in other neurological disorders
1) Alzheimer's Disease
-In AD, the BBB is compromised
-Elevated Igs and T cells have been reported in AD brains
-Igs are associated with vessels in the AD brain tissues
-The Ig labeling correlates with neurodegenerative and apoptotic features
-A degree of chronic inflammation and autoimmunity may be present

-Loss of neurons in AD brain may be dependent on the presence, affinity/avidity of neuron-specific autoantibodies
-The more compromised the BBB is (possibly due to amyloid plaque deposition), the more neuron-specific Igs and T cells could pass into the brain parenchyma and gain access to their autoantigens → cell death

-Anti-TNF treatment: in a study of 3000 RA patients given anti-TNF, 55% lower incidence of AD compared to patients given other rheumatoid arthritis therapies or placebo

-Rheumatoid arthritis = chronic inflammatory condition of the joints

-Dampening down the immune reaction in RA benefits AD and maybe MS?

2) Chronic Fatigue syndrome

-Symptoms of CFS include: malaise after exertion, unrefreshing sleep, widespread muscle and joint pain, sore throat, headaches, cognitive difficulties, chronic and severe mental and physical exhaustion

-Rituximab (monoclonal antibody directed against CD20 on B cells) has been used to treat CFS

-Used in a pilot study: in a small cohort of patients, Rituximab reduces B cells and improves symptoms of CFS

Q) Identify common mechanisms of disease between MS, Alzheimers Disease and Chronic Fatigue syndrome.

Epilepsy

•Epilepsy
-Defined by International League Against Epilepsy (ILAE)
-"A disorder of the brain characterised by an enduring predisposition to generate epileptic seizures"
-"Occurrence of at least one unprovoked seizure" (c.f. older definition which required at least two unprovoked seizures)

-10% of people will have at least one seizure during their lifetime due to head trauma, high fever, alcohol withdrawal, dehydration
-Of those, 1/3 will go on to develop epilepsy and have recurrent seizures

-One of the most common neurological disorders with the same burden of disease as lung cancer in men or breast cancer in women

-A healthy brain can keep a fine balance between excitation and inhibition
-Too much excitation/ Too little inhibition → convulsions, anxiety, high blood pressure, insomnia
-Too much inhibition/ Too little excitation → sleep, sedation, depression, coma, low blood pressure

•Different types of seizures
1) Focal/ Partial = Activation of neurons in a relatively small, discrete region
-Clinical manifestation reflects region of brain in which they occur: sensory or motor
-Familiarity (déjà vu) or strangeness (jamais vu), automatisms (spontaneous motor behaviours), hallucinations (auditory and visual), temporal lobe epilepsy
-*Complex partial seizures* – impairment of consciousness

2) Generalised = Involvement of the whole brain (both hemispheres) and widespread neuronal activation
-*Tonic*: extension of the extremities, rigid stretching
-*Atonic*: sudden loss of muscle tone (collapse)
-*Clonic* (*Myoclonic*): repetitive muscle twitching
-*Tonic-clonic* (*grand mal*): distinct tonic phase followed by a clonic phase (full body spasms with intermittent relaxation)
-*Generalised absence seizures* (*petit mal*): brief lapse of consciousness; mild outward presentation but excessive activity is going on in the brain

•Mechanisms of action of anticonvulsants
->22 anti-seizure drugs (ASDs) on the market
-25-40% of newly diagnosed epilepsy patients are drug resistant ("failure of a patients seizures to respond to at least two ASDs" with distinct mechanisms) → New ASDs needed
-Drug combination and dose need to be individualised
-Huge variability in disease management: Compliance (ensured by monitoring plasma levels)
-Toxicity issues – long term use

Mechanism	Drug	Effect
Decrease excitation		
1) Enhance Na^+ channel inactivation – reduce firing frequency of neurons	Phenytoin Carbamazepine Lamotrigine (Lamactil)	-Enhance voltage-gated Na^+ channel inactivation (harder to open) -Use-dependent: binds to already open Na^+ channels; specific for rapidly firing neurons -Prevent initiation and propagation of action potentials -Reduce sustained high-frequency firing of action potentials
2) Inhibit excitatory amino acid release	Ethosuximide (Zarontin)	-Presynaptic T-type Ca^{2+} channel blocker -Prevent Ca^{2+} entering presynaptic neuron and synaptic vesicle exocytosis -Reduce presynaptic release of glutamate -Used in the treatment of generalised absence seizures
	Levetiracetam (Keppra)	-Binds to neuronal synaptic vesicle glycoprotein 2A protein (SV2A) which coordinates synaptic vesicle fusion with membrane and exocytosis -Inhibits presynaptic release of glutamate -Inhibits presynaptic Ca_V channels? -Reduce neuronal excitability
3) Block excitatory amino acid action	Topiramate (Topomax)	-AMPA/Kainate receptor antagonist
	Felbamate (Felbatol)	-NMDA receptor (NR2B) antagonist
Increase inhibition		
4) Enhance GABA action	Barbiturates Phenobarbitone	-May act alone or enhance actions of GABA as allosteric modulator -Act on all $GABA_A$ receptors -Increase affinity for GABA -Increase Cl- conductance -Prolongs the open time of the channel -Unwanted sedative side effects: tiredness, forgetfulness, confusion, dizziness, anemia, folic acid deficiency, decrease libido, erection problems
	Benzodiazepine	-Enhances the actions of GABA as allosteric modulator -Increases the frequency of channel opening -Selectively act on $GABA_A$ receptors containing α1, α2, α3, α5 and γ subunits -Binds at the interface between α and γ subunits -Patients can develop rapid tolerance
5) Inhibit GABA breakdown	Vigabatrin	-Synthetic structural analogue of GABA -Specific inhibitor of GABA transaminase -Inhibits metabolism of GABA and increase [GABA] in the brain
6) Inhibit GABA uptake	Tiagabine	-Non-transportable GAT1 inhibitor -Derivative of nipecotic acid (transportable inhibitor) -Inhibit GABA uptake into cells -Increase synaptic/ extracellular GABA levels

▪Multiple mechanisms

1) Sodium valproate (Epilum)

-1: Use-dependent Na^+ channel blocker (weaker than phenytoin and carbamazepine)

-2: Ca^{2+} channel blocker

-5: Increases levels of GABA in CSF (unknown mechanism)

2) Topiramate (Topomax)

-1: Inhibit voltage-dependent Na^+ channels

-3: Antagonist at AMPA/Kainate receptors

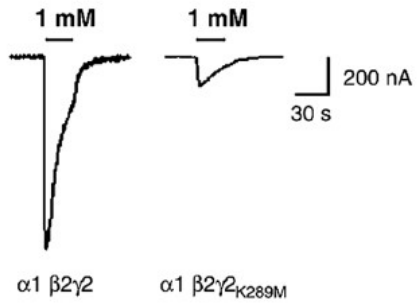
-4: Augment GABA at some $GABA_A$ receptors

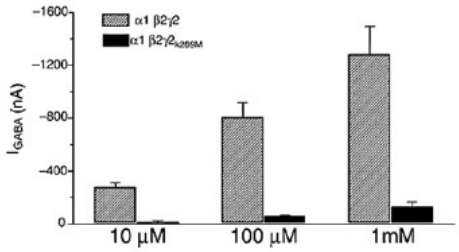
3) Felbamate (Felbatol)
-1: Inhibit voltage-dependent Na^+ channels
-3: Antagonist at NMDA receptors (NR2B)
-4: Positively modulates $GABA_A$ receptors

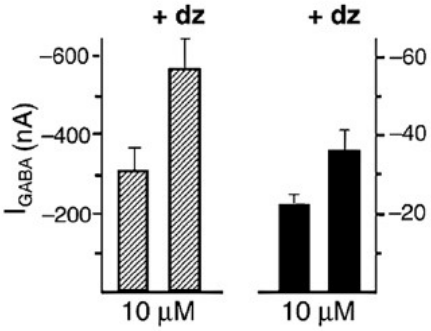
▪Unknown mechanism
-Gabapentin, pregabalin
-Synthesised as GABA analogues
-Increase total GABA concentrations in CSF
-Bind to the α2δ subunit of voltage-gated (L-type) Ca^{2+} channels (maybe associated with a decrease in NT release)
-No proof that they bind to GABA receptors or affect GABA breakdown or reuptake

•Genetics of epilepsy
-Brain is predisposed to seizure activity because neurons are so extensively interconnected to keep a fine balance
-More than 25 different genes cause epilepsy
-Virtually all known mutations are in ion channel subunits
-Others include Na^+/K^+-ATPase pump, EAAT1, GLUT1

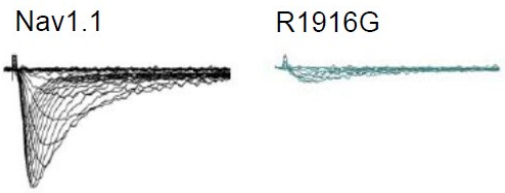
1) $GABA_A$ receptor mutation
-French family with GEFS+
-Lysine 289 to methionine mutation (K289M) in γ2 subunit of $GABA_A$ receptor
    -Between 2 transmembrane domains (M2 and M3)
-Decrease in $GABA_A$ receptor amplitude when GABA is administered → Reduced Cl^- flow and inward current
-Administration of diazepam restored the amplitude despite the overall current being smaller
-$GABA_A$ receptors still exist in the membrane, folded properly and have γ2 subunit but Cl^- ion flow is restricted
-Reduction of inhibitory neurotransmission → Epilepsy







2) Sodium channel mutation
-Family with GEFS+
-$Na_V1.1$ channel - arginine 1916 to glycine mutation (R1916G)
    -Intracellular tail (interact with other proteins and regulate cell membrane)
-R1916G renders $Na_V1.1$ non-functional – protein folding
-No functional $Na_V1.1$ is trafficked and inserted into the membrane
-$Na_V1.1$ is found in GABA interneurons → Reduction of inhibitory neurotransmission → Epilepsy



Q) Describe the mechanism of action of the following anticonvulsant drugs; Lamotrigine, Diazepam and Phenobarbitone.
Q) Discuss the consequences of a mutation that causes epilepsy.