Human Brain intellectual challenges:

How do we remember something? What is a memory? What is a thought?
Why do we sleep? What is consciousness? Can we build a synthetic brain?
How does the brain build itself?

Health**:**
Circuitry problems: Schizophrenia, Autism, Depression, Addiction
Cell biology problems: Alzheimer's. Parkinson, Multiple Sclerosis

World: Honey bee navigation and memory – memory maps poisoned by insecticides – no pollination of agriculturally important plants– starvation for us

There are different levels at which brain can be studied. Group of cells formed in brain circuit, generating network human behaviour.
Analogous to physics: explain properties of large aggregates of atoms assume molecules or statistical mechanics (from one molecule extrapolate the property of gas)
Start from neurons and then brain (reductionism, bottom up =
From brain to neuron (holism, top down)

Evolution of nervous systems: The need for neurons: diffusion time & specificity
Why do we have a nervous system? Plant evolved strategy to survive in one place, no need to move, no need to develop nervous system.
Some bacteria/single cell eukaryotes however behave in complex ways, propulsion, complex behabiors etc.. without nervous sytem (they have ion channels however)
Challenge comes when more than one cell is present.

Hypothetical multicellular organism (spherical shell of cells) with sensory cells (S) that control motor cells (M) by releasing a chemical transmitter or hormone into the common fluid space.
Direct connections between sensory and motor cells by means of nerve axons:
Communication is quicker and more specific; the ultimate action on the motor cells is still chemical
Some cells specialise in sensing a molecule, then secrete messenger into inside, diffuse to action cells. This causes some change in properties (excitation etc…) and cilia move. Not a great way to survive if want to do fast actions (time taken for diffusion in solution is limiting factor). Instead nervous system evolved, through neuron communication (there can be speed but also specificity, it is wired in)

What are the timescales for diffusion in cells? (first paper of Einstein)
The time t for a molecule to travel a distance x is given on average by:
$t \approx \frac{x^2}{D}$ Dimensions of D are length^2/time
D is the diffusion coefficient (mm^2/s). A larger value indicates a higher rate of jiggling around of the molecule. Its value is determined by the velocity of the molecule and the mean time between collisions, and is inversely proportional to the viscosity of the medium (e.g. cytoplasm or water)- Take into account diffusion in 3D
For a monomeric protein of ≈ 30 kDa in cytoplasm, D ≈ 10 mm^2/sec
For very large protein, D ≈ 1 mm^2/sec

The diffusion time increases quadratically with distance.
This has major implications if you have to go further than small distant

Time (t) taken for diffusion is proportional to the square of the distance (x) travelled (Stokes-Einstein law)

$\tau = x^2/\mathbf{D}$

It goes up logarithmically. Scale up distance to 1 mm → 9 mins (slow response).

Time for protein diffusion along 1 cm of axon

$$\tau \approx \frac{x^2}{6D}$$

Assume D = 10 mm^2/sec, x = 1 cm
$\tau \approx 10^6$ sec $\approx$ 10 days!
Neuron assume that molecule has to go down axon from 1 cm → take 10 days.

Moving material along an axon needs to be an active process!
It does not rely only on diffusion. Neurons would not function
Demonstrated by injecting tracer (fluorescence)in neurons → find active transport. Time is dependent on cargo being transported by motor proteins on microtubules (kinesins).

Filamentous actin and microtubules are two major cytoskeletal elements
Actin and microtubules are very plastic, can assemble and disassemble fast (by phosphorylation etc…)

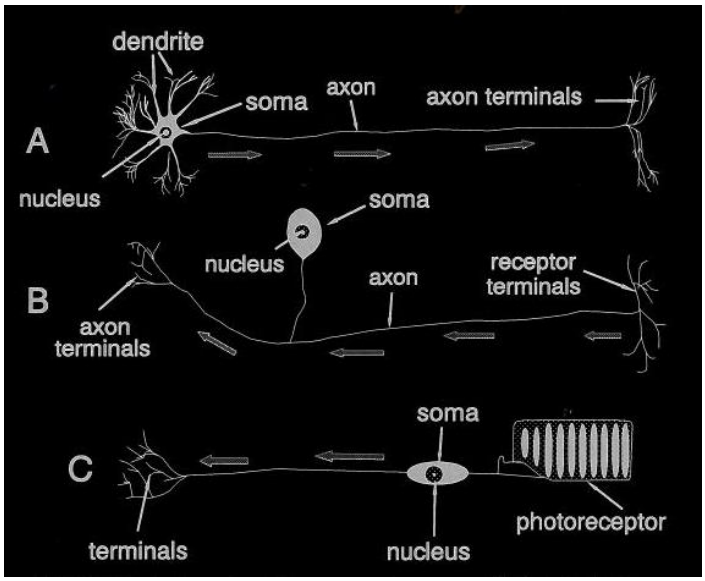
Cytoskeletal organization and motor proteins in axons and dendrites
Along the microtubule there is a series of specialised myosin motor proteins which attach to microtubule and different types of cargos (mitochondria, synaptic precursors etc…). They walk down axon to synapse. Damaged material from synapses is transported back up the axon, together with some growth factors → double traffic
Traffic also in dendrites. In small parts of neurons such as spines, there is also microtubules which are polymerizing and depolarising.

So how does any nervous system actually work?
most neurons signal to each other using chemical transmission at synapses.
A minority of specialized neurons use electrical connections via gap junctions (instead of synapses).
Chemical synapses relay unidirectional message (pre-post)
Electricals synapses (gap junctions) are bidirectional → tend to be involved in synchronizing group of neurons with same electrical activity.

Neurons are extremely polarized cells
Most polarised cell in body.

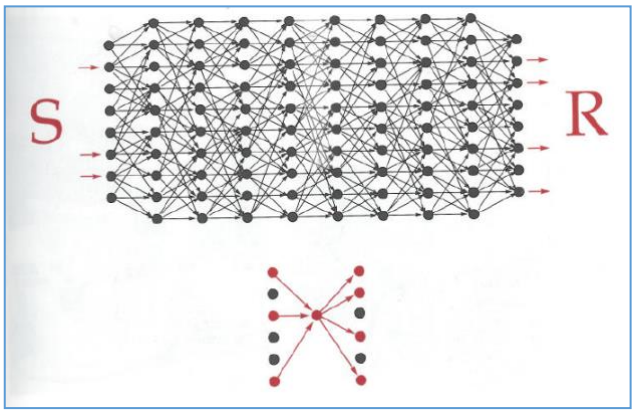
Differences between axons and dendrites

Axons	Dendrites
Take information away from the cell body	Bring information to the cell body
Smooth Surface	Rough Surface (dendritic spines)
Generally only 1 axon per cell	Usually many dendrites per cell
No ribosomes	Have ribosomes
Can have myelin (not always though)	No myelin insulation
Branch further from the cell body	Branch near the cell body



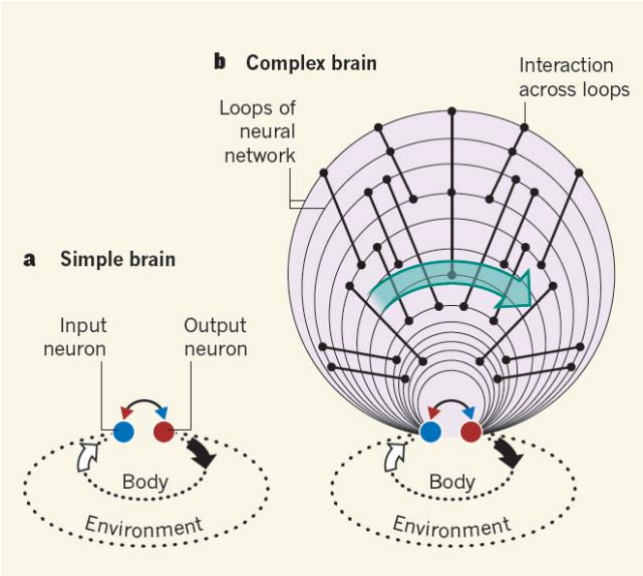
Morphological features of neurons
There is usually considerable structural diversity of neuronal types in any nervous system. This is particularly evident when comparing the shape and size of the dendritic arbor or tree. This structural specialization is thought to reflect functional specificity of these neuronal types. Dendrites constitute a kind of "neural microchip" for complex computations.
In all cases there is a single axon projecting from the soma. However, this axon can spit (axon collatorals) or form a complicated axonal plexus at its termination site.

84% of all genes are expressed, or turned on, somewhere in the human brain.
Most of the genes are expressed in the brain. Transcriptionally the brain is the most complex organ. Whole genome is recruited for the construction or maintenance of the brain (unlike liver or muscle).
Many previously uncharacterized genes are turned on in specific brain regions and localize with known functional groups of genes, suggesting they play roles in particular brain functions.
Synapse-associated genes --those related to cell-to-cell communication machinery in the brain - are deployed in complex combinations throughout the brain, revealing a great diversity of synapse types and remarkable regional variation that likely underlies functional distinctions between brain regions".
An anatomically comprehensive atlas of the adult human brain transcriptome.
Nature, 2012; 489 (7416)



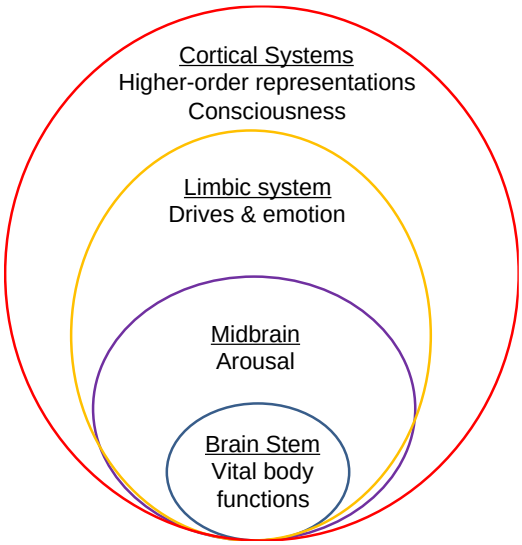
Representation of the nervous system as a series of neuronal levels, through which sensory patterns (S) are transformed into patterns of response (R). Each neuron responds to particular patterns amongst the neurons of the preceding layer (diagram below)

Take some kind of stimulus, then gives a response, information is processed through series of connection.
We are generating actions and thoughts but rain reacts to this. There is a series of loops which feedback on themselves. Brain is continuously sensing its own actions and feeding back on them.



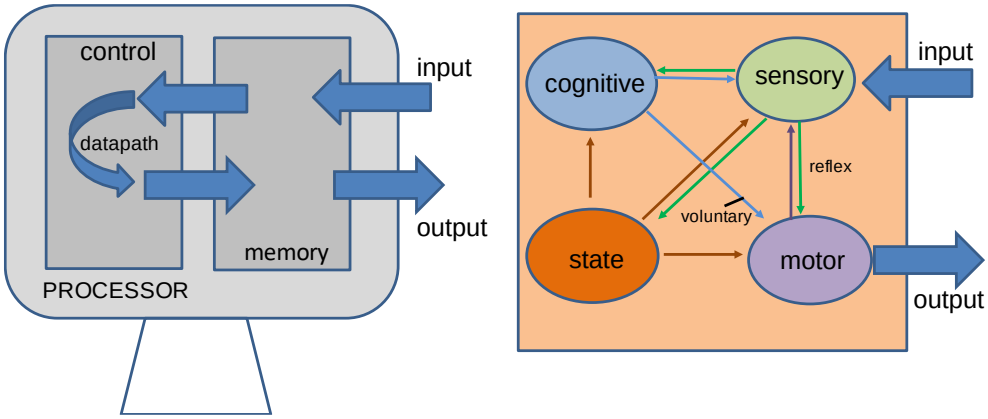
Evolutionary simple brains contain simple neural networks. Sensory input from the body and the environment activates input neurons, which interact with output neurons to generate appropriate reflex actions in a short time window.
In more complex brains, multiple interacting
loops of increasing length improve prediction of more elaborate events that occur at longer time
scales. (diagram and text from Buzsaki, G (2013) Nature 497)

Structural & Functional Hierarchy of Mammalian Brain Systems



Brain has different regions specialised different things.

Architectures of the computer and nervous system



Input: keyboard, mouse
Output: screen & printer
Memory: where data & programs are kept when programs are running
Datapath: arithmetic operations
Control: tells Datapath, Memory, Input and Output what to do according to instructions of programs
Control & Datapath together are the Processor

Motor systems controls output of the nervous system
(behaviour)
The motor system is controlled by three other systems:
Sensory system (input from external environment)
Cognitive system mediates voluntary behaviour
Behavioural state system: e.g. wake/sleep
Memory is stored as synaptic connection strengths of neural circuits

Computer is linear system; brain has 4 components. All of these regions are interacting in a very plastic way. Make parallel processing robot that has plastic ability to feedback on itself.

Electrical excitability

Moving a charge from water to lipid costs a lot of energy

Cannot put ions across a membrane without energy. Whole basis of neuronal signaling is working with differential ion concentrations. Channels and pumps have evolved.

Why membrane channels are needed

The membrane presents an energy barrier to ion crossing. (A) Schematic of a positively charged cation ("+" symbol) solvated by polar water molecules and of a membrane bilayer. (B) Simple graph showing that movement of the cation through the hydrophobic portion of the membrane bilayer is energetically unfavourable (*see also "Lecture 1 & 2"*).

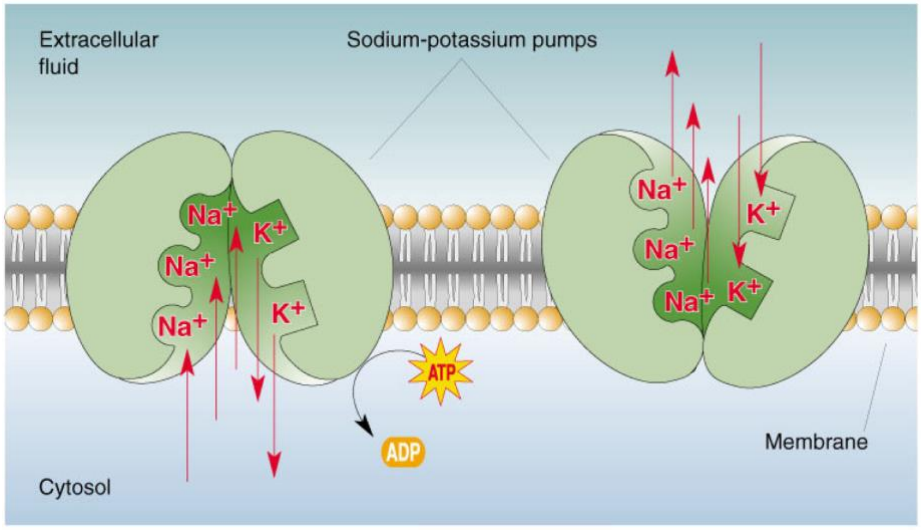
Gouaux E & Mackinnon R (2005) Principles of selective ion transport in channels & pumps. Science 310: 1461-1465 (read this review)

Active pumping

- The action of the pumps is crucial for the maintenance of ionic concentration differences.
- There are many different kinds of pumps. Most use ATP as an energy source to build up a gradient of ions (that's why mitochondria in axon termini)
- A large proportion of the energy intake of a human is devoted to the operation of ion pumps.

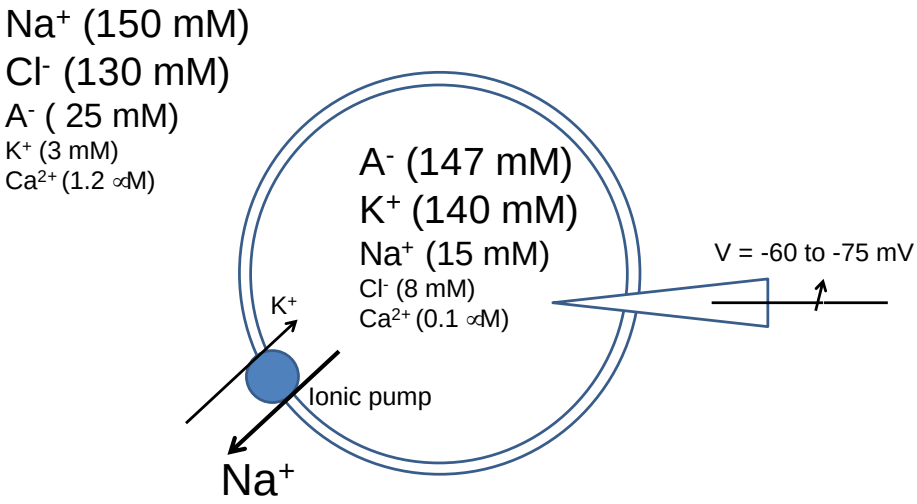
The sodium pump (sodium-potassium ATPase)

It pumps out 3 Na, counter changed by pumping in of 2 K. Build up ion gradient in neuron.



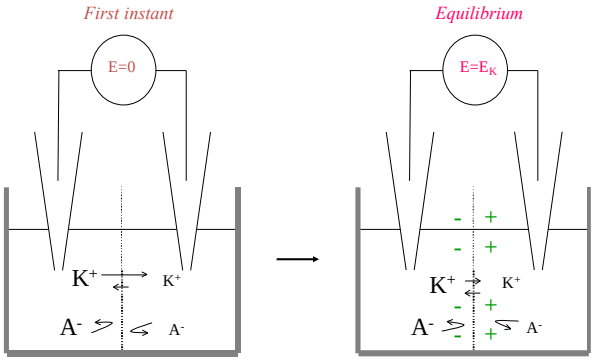
Distribution of ions across neuronal membranes

Amount of K ions inside neurons is much higher than outside. Na inside is lower than concentration outside, maintained by Na ATPase. Neurons use differential ion gradient for signaling. Ion gradient → voltage gradient → energy.



Equilibrium Potentials

- All systems move towards equilibrium – where the tendency for further change vanishes
- Consider a bath separated by a membrane permeable only to K_+ ions. A high concentration of a salt (KA) is introduced into one side (the left hand side) and a low concentration on the other side.
- We also have a voltmeter to measure membrane potential



- In the first instance the voltmeter reads 0 mV as both sides are neutral.
- However, K ions start to diffuse down their concentration gradient (just statistic as there are more ions) from one side to the other – left to right.
- This gives an excess of positive charge on the right hand side of the membrane and an electrical potential difference builds up across the membrane as it becomes charged

- The chemical forces causing a net diffusion of K from left to right are now countered by a growing electrical force which opposes the flow of K_+
- Eventually an equilibrium potential is reached where the electrical force equals the chemical (or diffusional) force and no exchange occurs (net flow is zero as negative

charge is holding K+ back, equilibrium between concentration gradient and electrical force pulling back charged ions)

- This is the potassium equilibrium potential (E_K) but how can we calculate it?

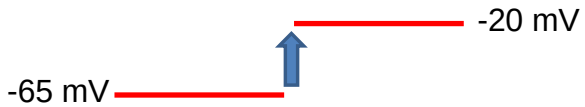
The Nernst equation (Nernst, 1888)

Electrical difference is energy difference. Energy can be discharged across the membrane in various way.

$$V_m = \frac{RT}{zF} \ln \frac{C_{out}}{C_{in}}$$

Many things can open channels (voltage difference, ligand binding, mechanical force, temperature difference). Energy is discharged when many stimuli open channels.
Change in membrane potential stores information.
Membrane potential (voltage) across a membrane is equivalent to work per unit charge (see later slide).
A change in membrane potential is used by neurons to signal information. The change in potential can be graded or sharp. The sharp changes are called Action Potentials, but graded changes are also important (e.g. post-synaptic potentials).
A voltage change causes specific channel proteins to change shape and conduct ions across the cell membrane, or some types of channel to shut (more detail later).
A voltage change will also cause Ca^{2+} ions to enter the presynaptic terminal and induce conformational changes promoting fusion of vesicles carrying neurotransmitter

Depolarization: a reduction in difference of electrical potential across the plasma membrane of a nerve or muscle cell. Potential becomes more positive inside the cell. **Depolarization is usually an excitatory signal.**



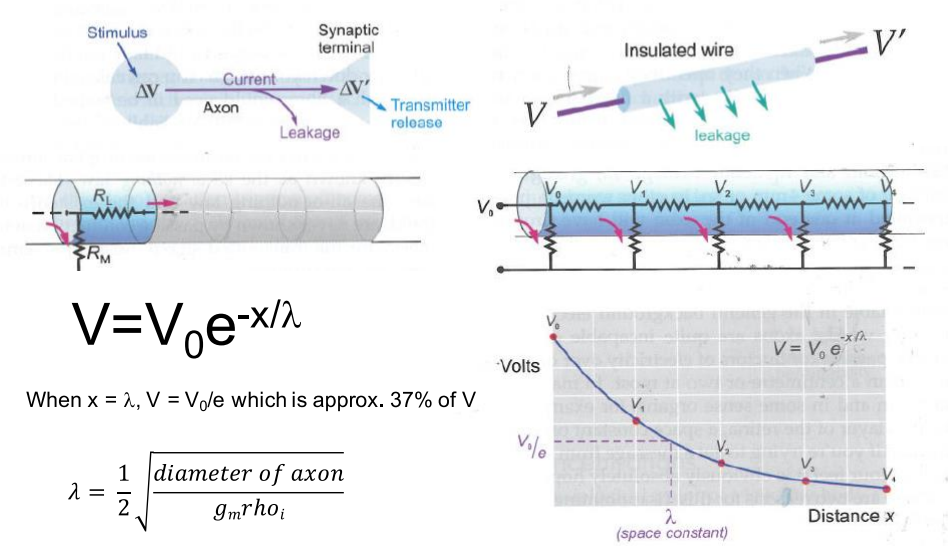
Hyperpolarization: an increase in difference of electrical potential across the membrane. Potential becomes more negative inside the cell. **This is usually an inhibitory signal.**

Main conductance is potassium channel. Neurons is always negatively charged inside.

Lecture 2 - Action potentials - Neurotransmitter release

Difference in ion concentration across membrane is energy source of electric field which then neurons use for signaling.
How the signals are transmitted down the axons?

Axons are needed:
The space constant,
Simple copper wire insulated with voltage difference, voltage gradient is energy gradient (ions move down the wire). A lot of the current goes down the wire but some leaks out – shown by resistance (this is why insulation, to stop leak). With length, leak dissipate signal.



Shown mathematically that if voltage is applied, decays of signal exponentially with length.
g_m is the conductance per unit area of membrane (Scm^{-2}); conductance is the reciprocal of resistance
r_i (rho$_i$) is the resistivity of the internal cytoplasm, typically 100 Ohmcm

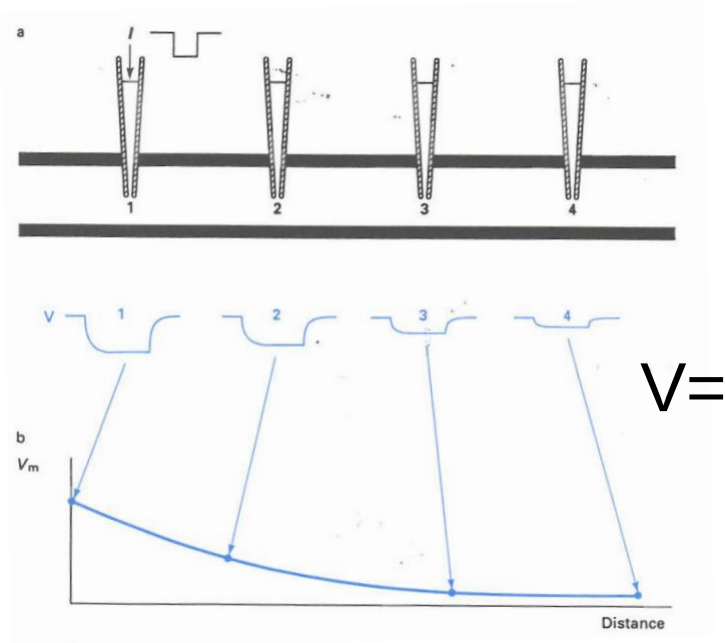
The longer the axon, change in voltage is not enough to trigger vesicle release.

Implications
λ is the characteristic length at which the signal, V, decays to 37 % (in other words, *1/e*th) of its original value, V0. Often referred to as the "Space constant".

λ is typically 0.1 to 2 mm in nerve fibres. If g_m = 1.25 x10^{-4} S per cm^2 and the resistivity of intracellular space is 100 Ohmcm,
Check for yourself that for an axon 20 mm in diameter, $\lambda \approx 2\ mm$
c. elegans does not often use action potentials in neurons (there are no voltage-gated sodium channel genes in its genome), small animals do not need to solve this probel as they are short enough.
Larger animals evolved a solution to overcoming:
Myelin and **action potentials**

Hypothetical action: Stick electrode to measure voltage. inject some current to make current more negative (1), generate electric field and measure what happens down the action. Voltage change picked up by electrodes, get exponentially smaller.

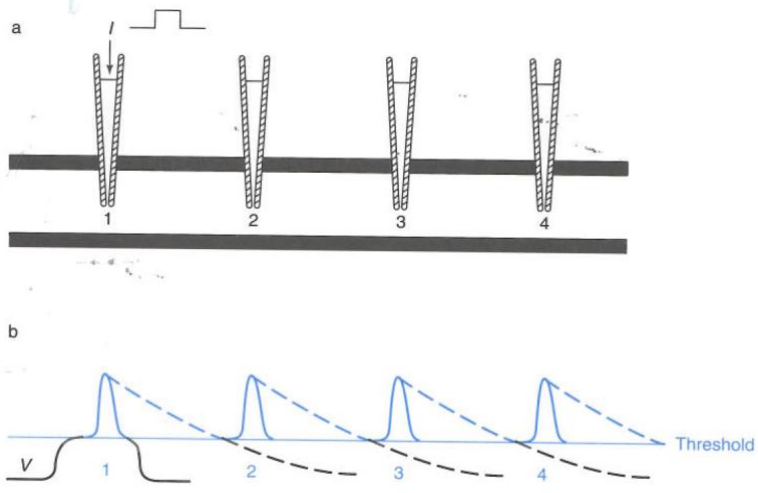
Passive spread. A, When current (I) is injected at one point (1) in the axon, a voltage change (V) can be measured at that point. Electrodes in other parts of the axon some distance away (2, 3, 4) measure smaller voltage changes. b, plot in decrease of voltage (Vm) over distance. Levitan & Kaczmarek, 2001



$$V=V_0e^{-x/l}$$

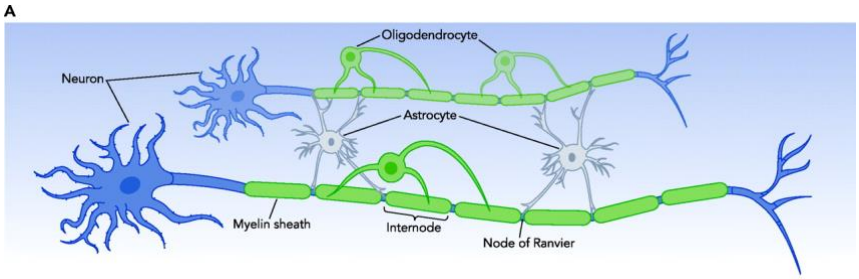
In real axon, instead of passive voltage change there is action potential (very rapid depolarization (more negative to positive membrane potential) of the membrane to a peak and then repolarization. Signals is the same, same change is picked up. All-or-none change (digital phenomenon)

Propagation of the action potential. Although the large voltage change (V) due to an action potential at one point decreases with distance (dashed lines), the depolarization that spreads to the adjacent region of the axon is still above threshold. Thus a full-size action potential is generated at each point in the axon. I, current.



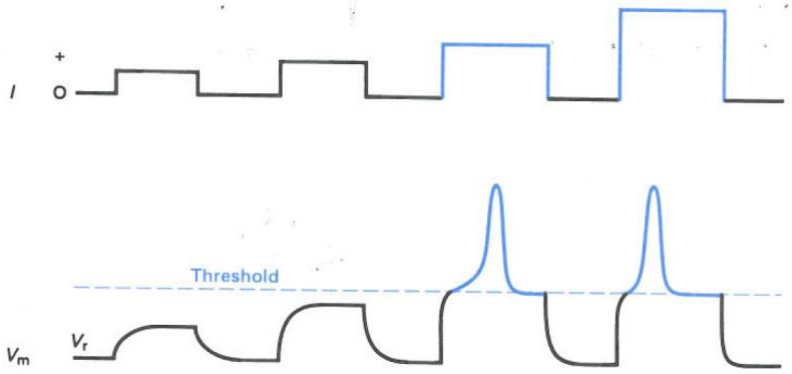
Myelination

Myelin is formed by glial cells and creates a high-resistance, low-capacitance, sheath. This, effectively, greatly increases the space constant (l) and the action potential jumps from node to node, thus increasing the velocity of the action potential by 20 times or more.
In the central nervous system, the glial cells are *oligodendrocytes*, in the peripheral nervous system they are *Schwann cells.*

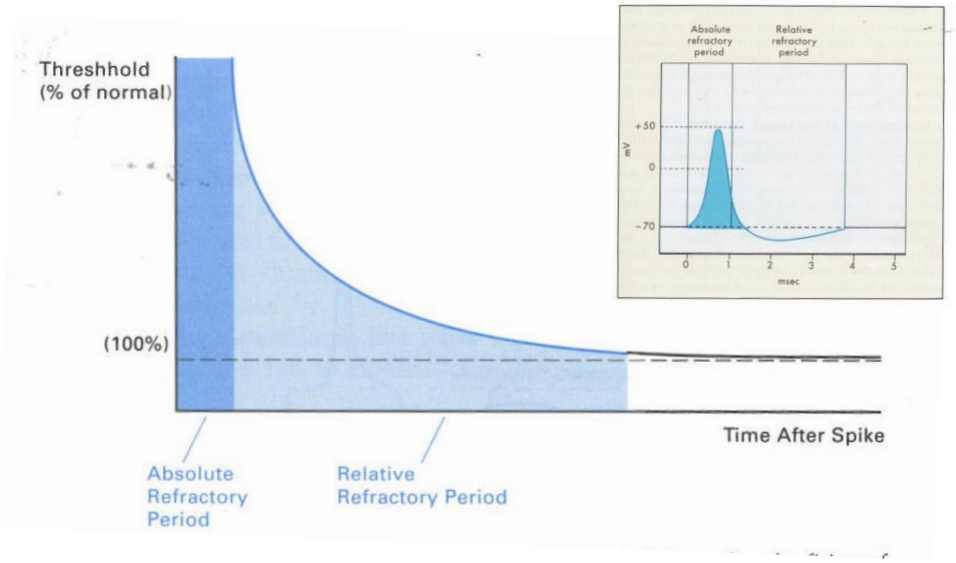


In long axons myelin sheath increase space constant but is not enough yet to convey signal (too much leakage), that is why you also need action potential to relay signal.

All or none: Small depolarizing currents produce a passive membrane response, but the voltage response to larger currents is very different, and gives an “all or none” action potential. I, current; V_m, membrane potential



The threshold is not fixed. For a short period of time after the firing of the action potential, the threshold is much greater than normal



Leak potassium channels are open, more K inside than outside, negative charged inside. Transiently open some sodium channels (much higher outside than inside), but normally it cannot get in as there are no NA channels open. So leak K channels are open all the time in neurons. Membrane largely only permeable to K. Each time K goes out, more negative inside. K more inside so it goes out but then negative charge pulls is back inside again. So at rest, equilibrium, no net flow.
Na in resting state, cells energy to pump out. Na are concentrating outside. If transiently open Na channels→ Na moves down its concentration gradient into neurons, positive charge inside → more positive inside → more na the more membrane potential becomes more positive, eventually if process goes on, Na influx stops → equilibrium (if Na channels were open all the time would reach the Na reversals potentials, determined by Nernst equation.)
Put a bit Na ions axon artificially, membrane becomes more positive and then it decays as na is pumps out. K eak channels are open, everything returns to K resting membrane potential. At certain point t (threshold), Not enough Na added , action potential created (if above threshold). If membrane becomes more positive to threshold negative value --< irreversible all-or-none action potential is triggered.
(if you put even more na, signal is the same, not increased – digital signaling)

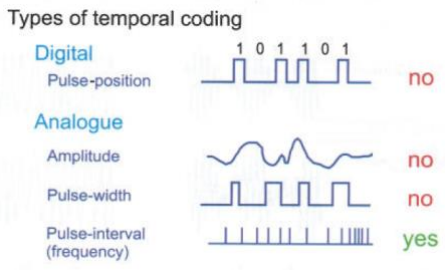
Milliseconds, absolute refractory period → not possible to trigger another action potential .if you wait you can trigger another one but it needs more current. Here shows how much Na iyou need to inject to have another action potential. Channels that generate action potential becomes transiently inactivated (desensitized). Practical consequence is that how much info can a neuron transmit.

Rate of action potential firing is information
Frequency of action potential is info. It is a rate code in the nervous system. Some neurons can have high rate codes (hearing for example detect high frequency). Other fire at low frequency. Frequency modulation. Our brain use FM frequency modulation code. But the actual signal is all-or-none.
After receiving a signal, next neuron than has to decide whether to fire as well or wait for another signal (integration).

Axonal information transfer

There is a **threshold** for the generation of action potentials.
The **all-or-none law** guarantees that once an action potential is generated, it
Is always full size (no information lost).
The refractory period, together with the threshold, allow the coding of information
as a **frequency code.**

This type of signaling is called **frequency modulation**
The size of the spikes (action potentials) is always the same, but what varies is how often they happen

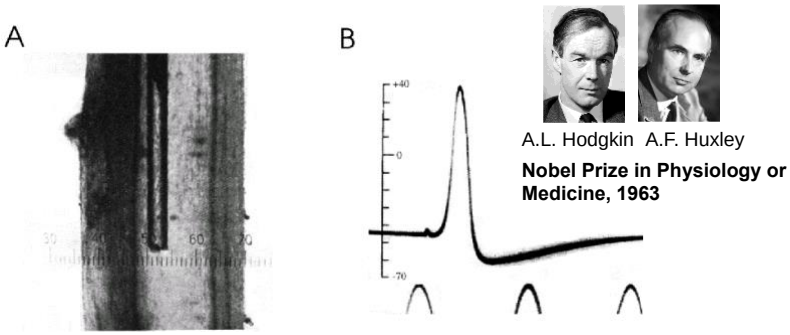


The squid giant axon

Axon is too thin to study in most organisms. Giant axon helped elucidate mechanism of action potential. Squid has incredibly fast escape response. An electrode can be inserted into middle of giant axon and see what is the actual electrical current.

The mechanism of the action potential

The squid giant axon can be up to 1 mm in diameter – a thousand times thicker than in humans. The first recording of an action potential using a microelectrode inserted into an axon was done by Alan Lloyd Hodgkin and Andrew Fielding Huxley in Plymouth UK in 1939.

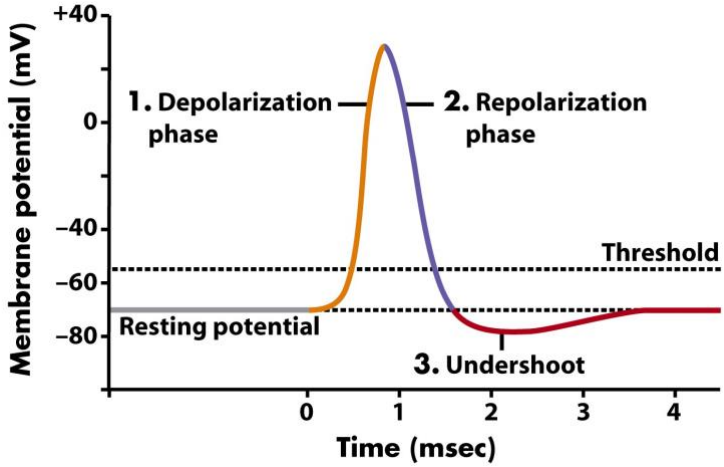


A.L. Hodgkin A.F. Huxley

Nobel Prize in Physiology or Medicine, 1963

Voltage clamping is electronic technique used by them to measure.

Phases of the action potential



When action potential starts there is very rapid depolarization then peak then repolarizes then undershoot than threshold again.

What generates this?

2 types of channels are needed (voltage gated Na channel, when things become more positive, amino acid on channel change shape and open. Na goes down its concentration and flows inside until electrochemical equilibrium potential is reached, this happens very quickly.)

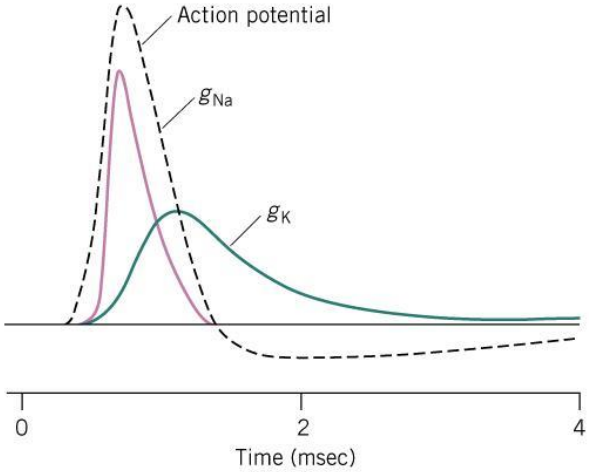
The if Na channels were to stay open, membrane would be depolarised, would stay positive. But voltage gated channels have in built lock, after certain amount of time they change shape and they shut stochastically. Some Na current decays.

Then voltage gated K channels. Sense electrical field. When positive, K channels open and it takes longer than Na channels. So whole Na conductance increases, eventually K channels open. Leak K channels rebalce the membrane potential back to potassium (K is winning now). They stay open longer than needed and causes an overshoot towards the potassium.

Overshoot is when there are extra K channels open. Then K channels shut and returns to zero

- After reaching threshold gNa increases quickly, but inactivation then reduces g_{Na} to zero.
- g_K increases more slowly, and only decreases once the voltage has hyperpolarized.
- The *absolute* refractory period is when the sodium channels are inactivated
- The *relative* refractory period is when g_K dominates following the action potential

At beginning of action potential. More positive, Na comes in. Things become more positive and more na channels open. All happening in half a millisecond. Na channels than stochastically switch off. Meanwhile Voltage gated k channels open. Allow K to go down gradient, restore potential to negative value.

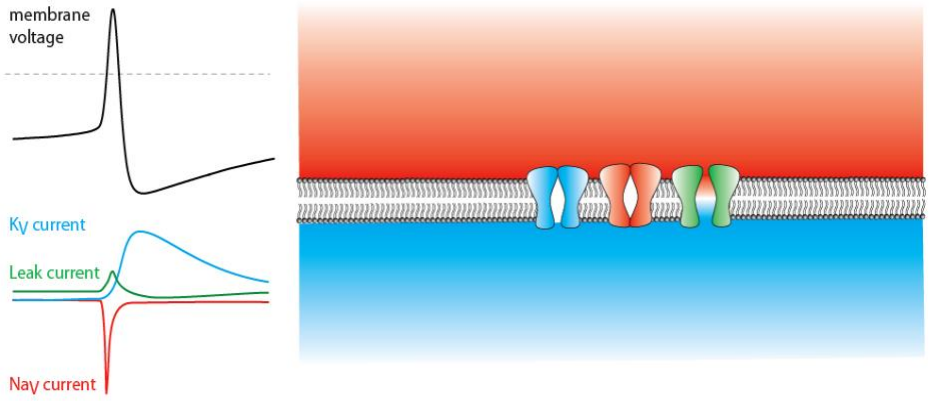


Kinetics of Na and K currents under voltage clamp

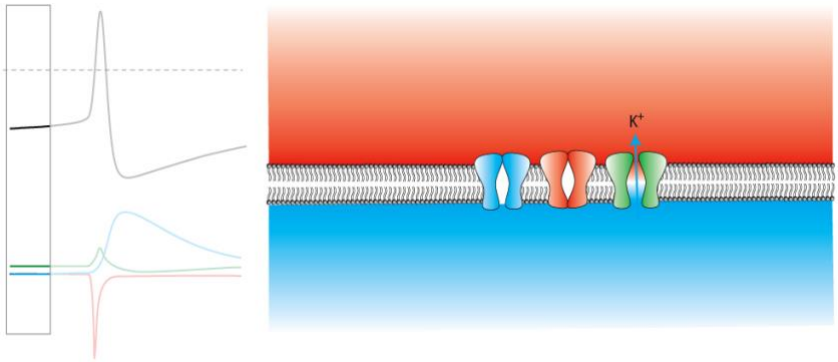
- K potassium currents activate following depolarisation but show little inactivation
- Na currents are governed by two kinetic processes activation and inactivation following depolarisation
- Both Na and K channels deactivate (*i.e.* close) when the membrane potential is hyperpolarised

Activation – opening following depolarisation
Inactivation – closing independent of voltage
Deactivation – closing following hyperpolarisation

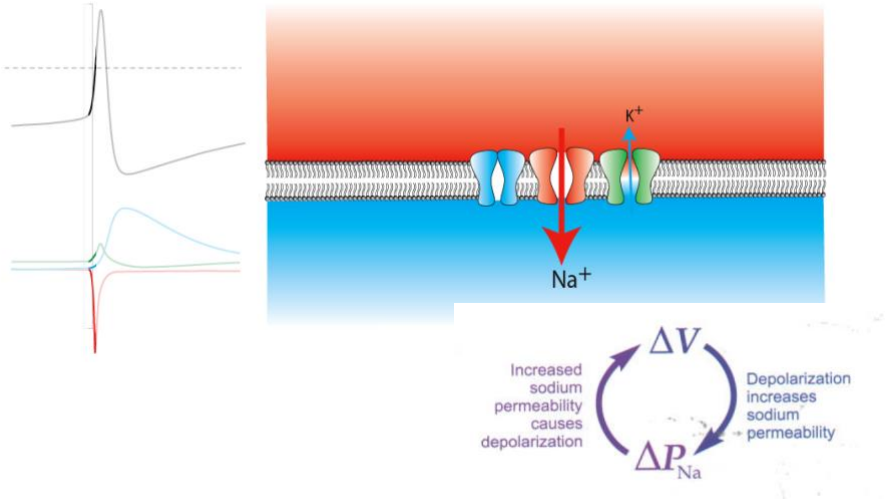
Voltage-gated ion channels experience activation, deactivation and inactivation during the action potential.



At rest there is a small resting potassium permeability through voltage-independent leak channels.

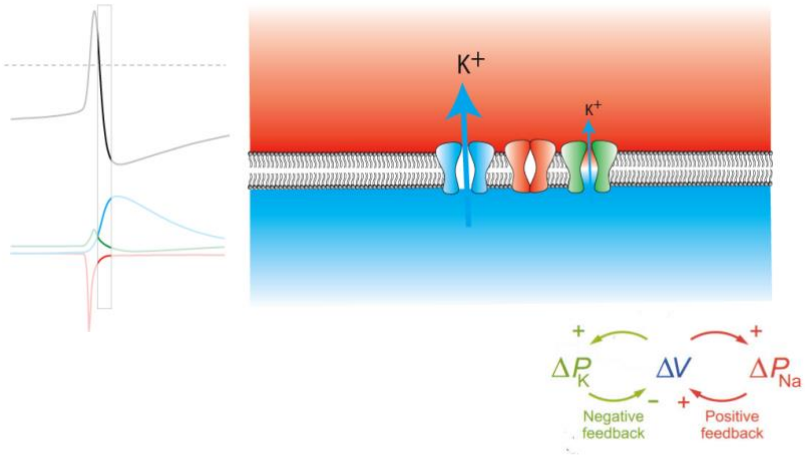


During the up-shoot of the AP voltage gated sodium channels open increasing sodium permeability of the membrane.

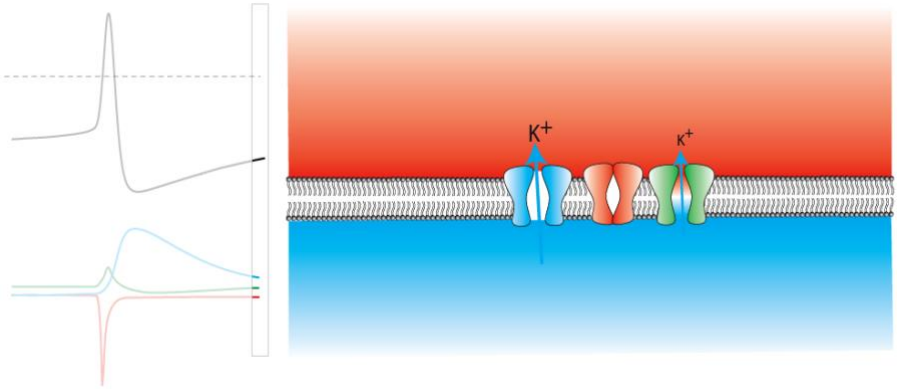


Threshold for Na^+ channels to open
is about -20 mV

Repolarisation of AP involves sodium channel closure (inactivation and deactivation), and the activation of voltage-gated potassium channels

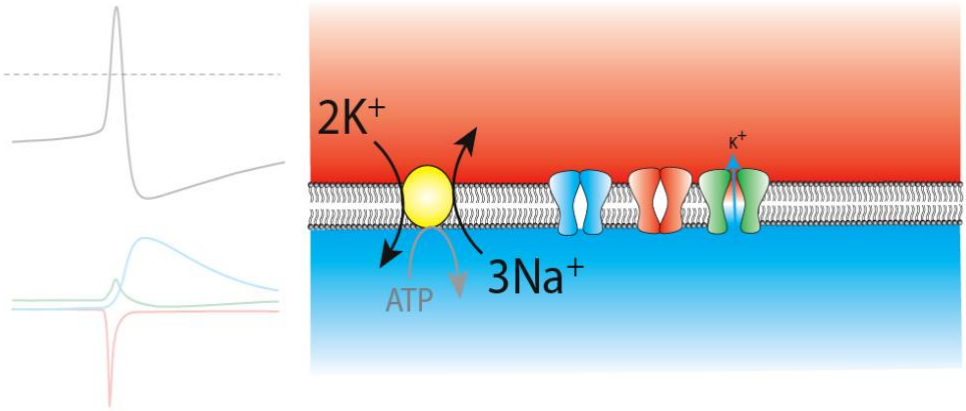


A refractory period persists until the voltage gated sodium channels have recovered from inactivation.



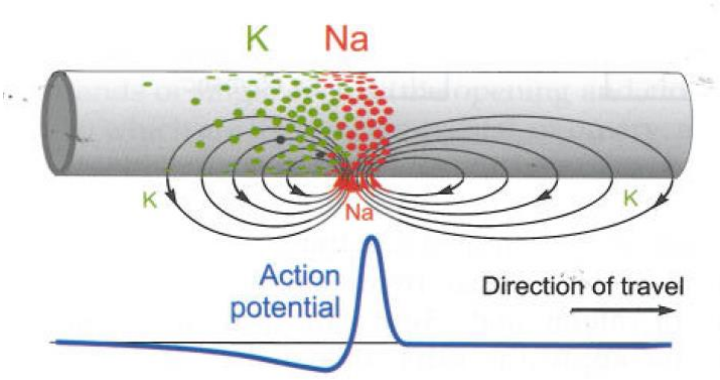
Action potentials propagate unidirectionally in the axon. What ensures this? Refractory period:
the delayed activation of the K channel and stochastic inactivation of the Na channels create a refractory period after an action potential just occurred, during which time another action potential cannot be reinitiated. Where the depolarization wave front has just passed, is experiencing a refractory period which prevents action potential from backpropagating from back from B to A.

Ionic gradients are re-established by Na/K ATPase activity.

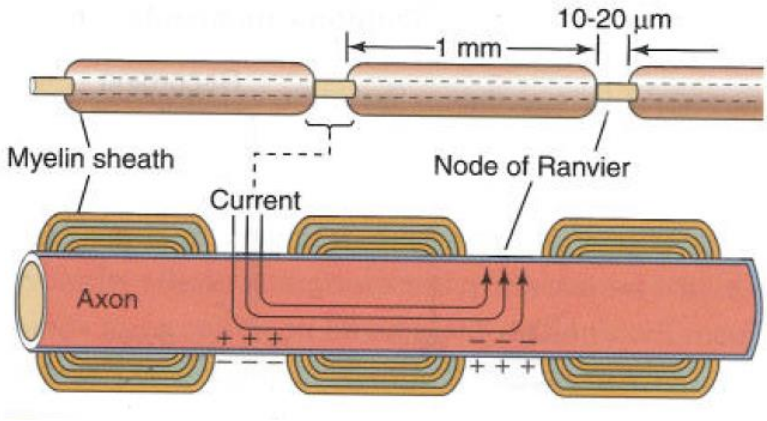


Very few ions are needed to change the membrane potential
This is because of the capacitance of the cell:
spherical cell - radius 25 mm
surface area - 8 x 10^{-5} cm^2
total capacitance - 8 x 10^{-5} mF (membrane capacitance is about 1 mF/cm^2)
Suppose we want to depolarise the membrane from -65 mV to 0 mV by sodium ions moving into the cell.
Then the charge Q needed is given by $Q = C_m \times DV = 5.2 \times 10^{-12}$ Coulombs.
Since Faraday's constant is 96,400 Coulombs/mol, then this is equivalent to 5.4 x 10^{-17} moles of sodium.
But, the cell volume is about 65 x 10^{-9} litres, which, with an internal Na^+ concentration of 14 mM, is about 9 x 10^{-10} moles of Na^+.
So, the sodium ion concentration changes by 10^{-5} %

The speed of the action potential depends on how far the *passive* depolarisation spreads. This is determined by the Space Constant l (see earlier)



Saltatory Conduction



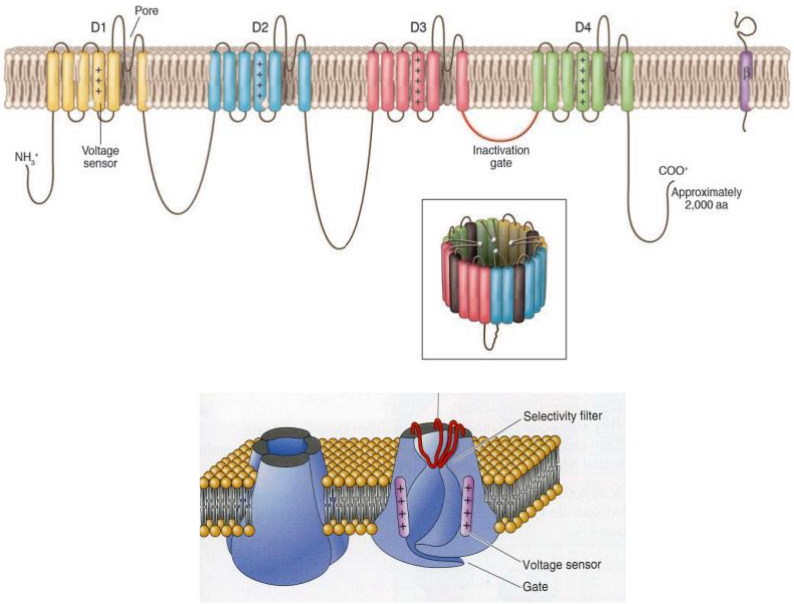
Process is spread out even more as potential jumps from node to node. Voltage gated channels are only found in the gaps of myelin (nodes of ranvier). Electrical field jumps down the action.

Neurons that sense pain and temperature are slow as they do not have myelinated axons.

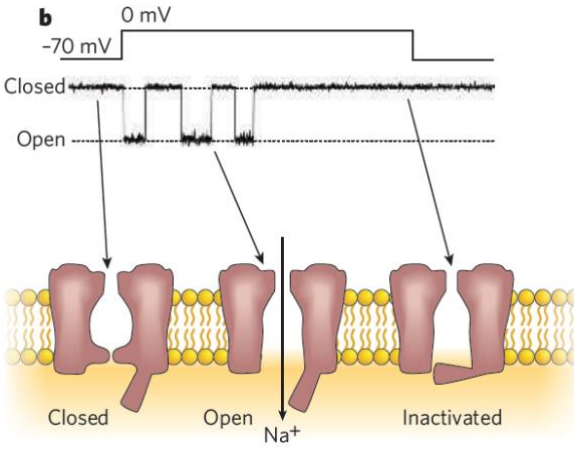
The importance of myelin: multiple sclerosis
Episodic autoimmune destruction of myelin surrounding the nerves of the central nervous system leads to a progressive burden of neurological deficits from
monocular blindness to total paralysis.
Poor nerve conduction
Antibodies attack myelin. Transfer of information is fucked.

Ion channel structure

Voltage-gated sodium channel.



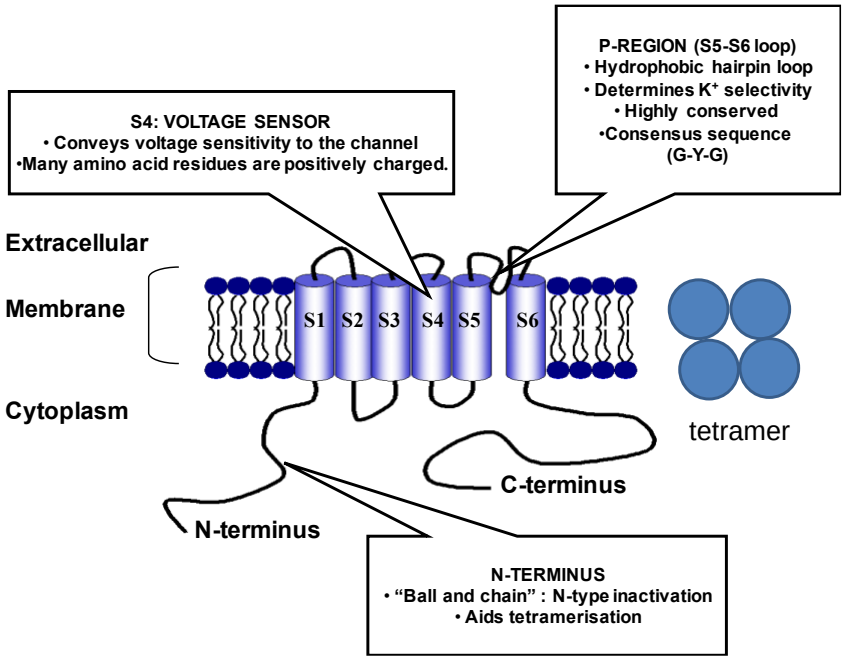
Na channel is composed of 4 repeating modules. Each module consists of 6 helical TM segments, with the fourth TM containing positively charged amino acid that play a key role in voltage sensing. The pore loop and the adjacent fifth and sixth TMs together constitute the ion conduction pore.



How Na^+ ion channels open and close, with associated single-channel recordings. Opening and closing of the channel are random events, but the frequency with which they occur is influenced by transmembrane voltage. The transition rate between open and closed states is <10 μs. The flux rate through the pore when it is open is of the order of 107 ions per second; Following opening, voltage-gated Na^+ channels enter an inactivated (non-conducting) state in which they are refractory to subsequent depolarization.

Voltage-gated potassium channels (Kv1 series)
Similar to Na but not 4 domains in 1 peptide, 4 domains get together separately to make channel. Part of protein is charged and senses changes in electrical field.

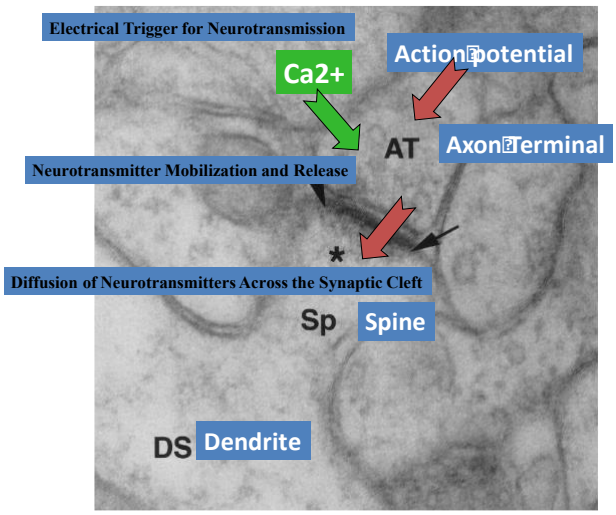
Voltage gated K channel protein resembles one of the four repeating modules of Na channel. 4 subunits constitute functional channel.



What happens once action potential invades synapse ?
Crossing the gap: The chemical synapse
Neurotransmission & neurotransmitters

By revealing the mode through which impulses communicate their signal across the miniscule gaps, or synapses, that separate nerve cells from each other and from their target destinations, **Sir Henry Dale** and **Otto Loewi** received the 1936 Nobel Prize in Physiology or Medicine.

Passing information between neurons



Synapses allow directional communication. Majority of synapses are chemical. Triggers influx of Ca through voltage gated Calcium ions. (higher inside cell than outside). Ca comes

in when membrane is depolarised and works as second messenger to trigger release of transmitter. Transmitter crosses synaptic cleft and binds to receptor in the spine in the dendritic shaft.

Chemical neuronal transmission (slower than electrical) and confers directionality of message. Transmitter goes across synapse.
AT SP DS Image
Axon terminal releasing glutamate onto a spine in dendritic tree of receiver neuron. The action potential comes to end of axon, invade it, depolarizing, NA comes in. Now a voltage gated calcium channels open (same gamily as Na and K channels). When action potential invades terminal, Ca enters from outside of the cell (higher concentration outside), moves down concentration gradient and acts as a second messenger.
Transmitters can be GABA, acetylcholine etc… A range of molecules that cross synaptic cleft and bind different ranges of receptors.

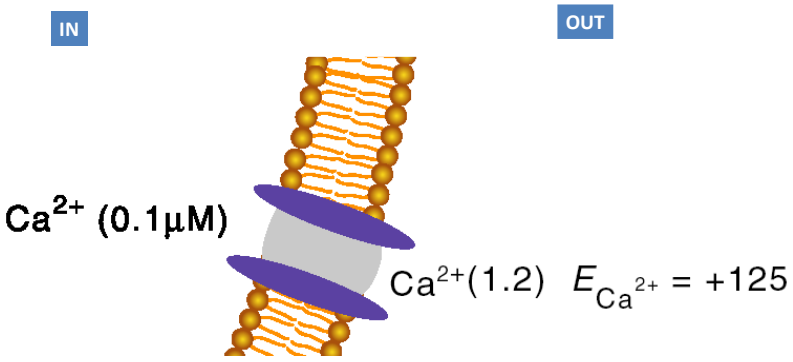
Vesicles in synapses are calcium sensitive, only fuse with membranes at right concentration of Ca ions. But vesicles are the same as normal trafficking vesicles in other cells. (ER, GOLGI, Clathrin mediated etc…).
There is a cloud of vesicles released to receptors.
What actually happens when vesicles fuse in the membrane? (kiss and run or fusion?)

Gap junctions -electrical transmission
- fast
- both directions

Chemical transmission
- slower - unidirectional
- integrative
- amplifies and regenerates the signal

Calcium entry is excitatory, and depolarizes the membrane

Calcium is also a second messenger which binds to target proteins



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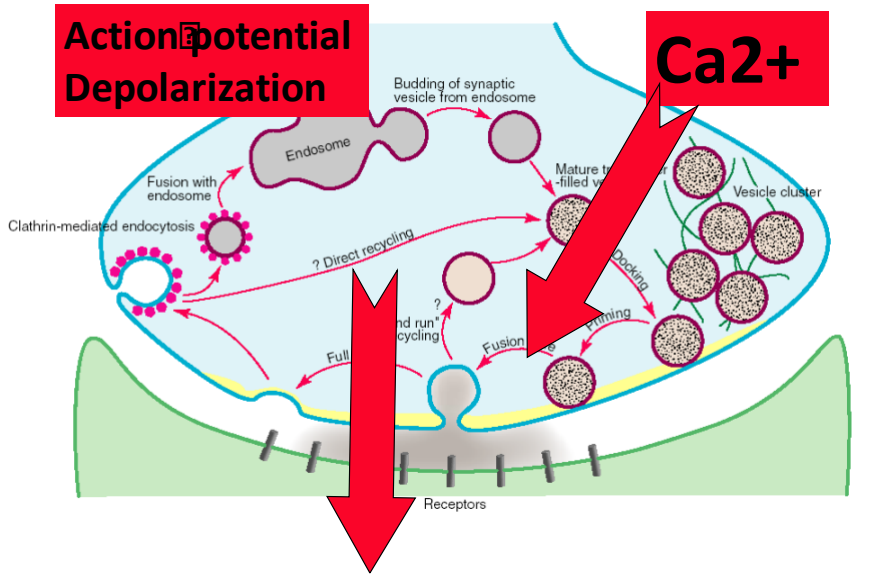
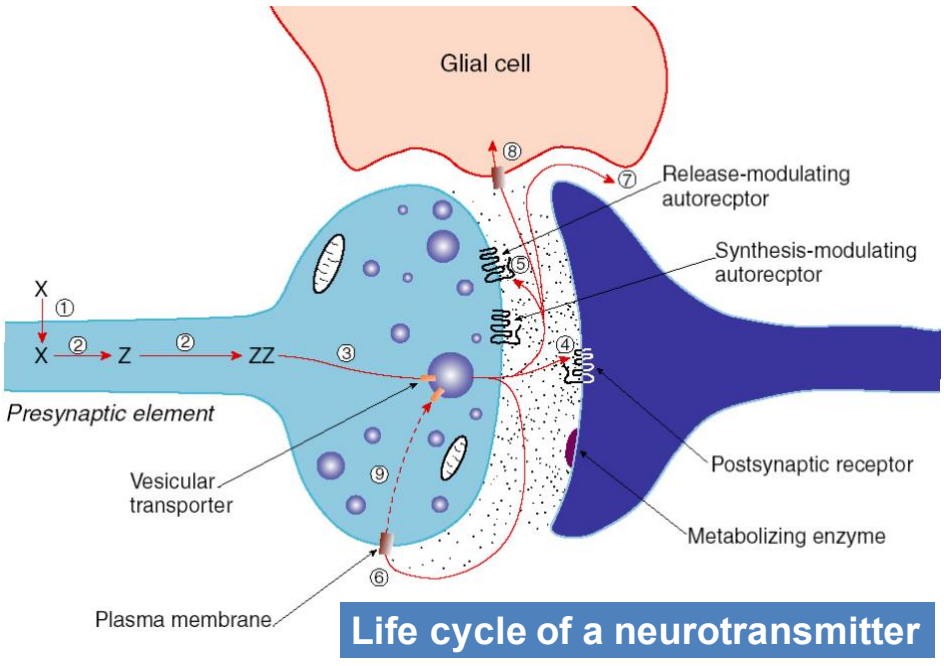




Life cycle of a neurotransmitter

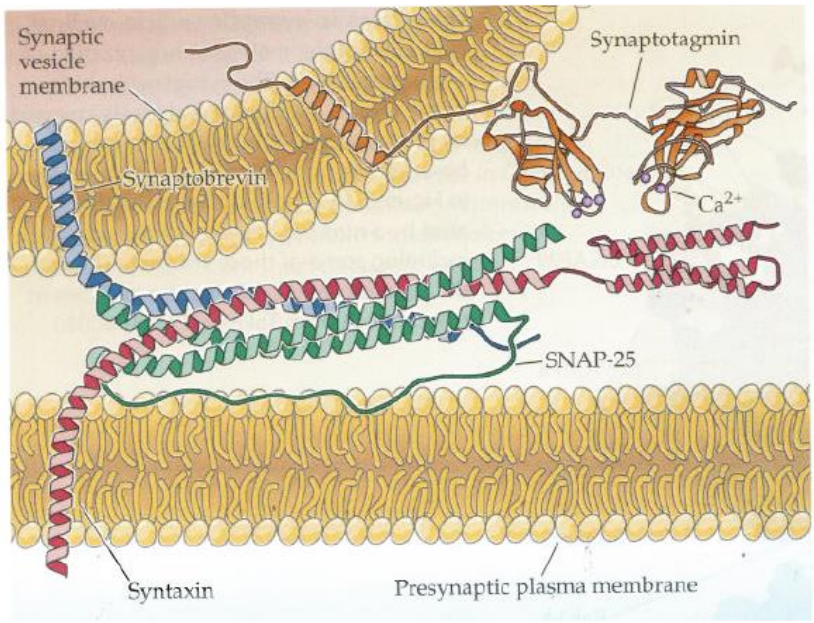
The process of chemical neurotransmission can be divided into five steps
1. Synthesis of the neurotransmitter in the presynaptic neuron
2. Storage of the neurotransmitter and/or its precursor in the presynaptic nerve terminal
3. Release of the neurotransmitter into the synaptic cleft
4. Binding and recognition of the neurotransmitter by target receptors
5. Termination of the action of the released transmitter

Neurons evolved special transporters to package molecules driven by ATP into vesicles. Depending of the type of transmitters, it is taken away from synapse (diffuse away or degraded actively, as acetylcholine-esterase). Astrocytes (glial cells) wrap around synapses, often involved in taking up transmitters.

Fusing naked vesicles with other lipid membranes would require substantial energy.
SNARE proteins, the engine of membrane fusion

Mechanism is SNARE pin. There is an anchored synaptic vesicle close to membrane by triple helix complex of proteins:

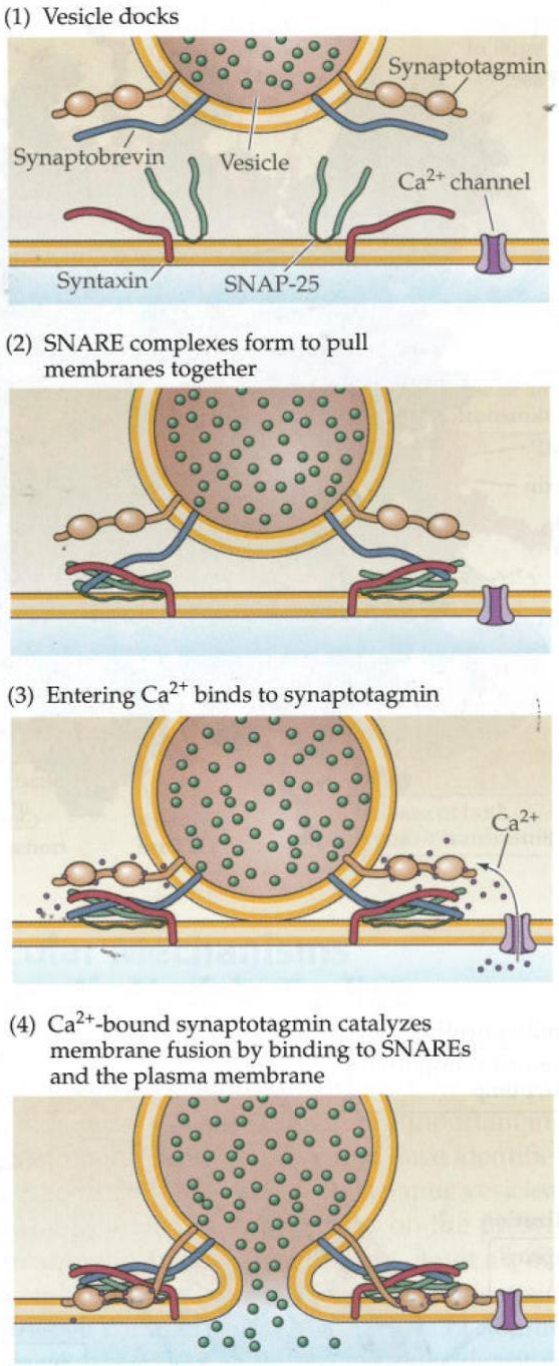
1. Synaptobrevin
2. SNAP-25
3. Syntaxin



How does Calcium release vesicles?
Ca comes to terminal, binds Synaptotagmin and somehow causes a conformational change in protein (which is tethered to membrane) → helical bundle change shape and pull the vesicle into the membrane. This is energy dependent (already used energy before to assemble the SNARE complex). It is very ATP expensive (that why brain has highest metabolic rate among organs, synaptic vesicles are very costly).

Ca^{2+}-dependent vesicular release

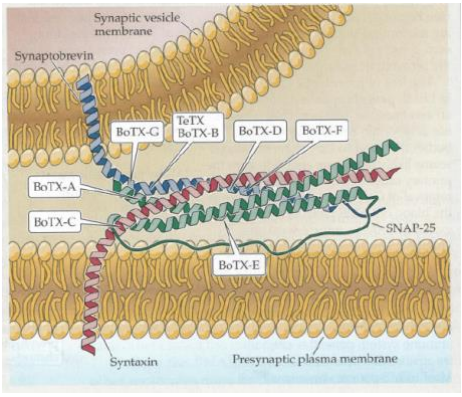
Action potential from the axon → Depolarization of the presynaptic terminal → Opening of voltage-gated Ca^{2+} channels → Ca^{2+} entry into the presynaptic terminal → Fusion of synaptic vesicle with presynaptic plasma membrane → Neurotransmitter release



Energy is used in the docking process

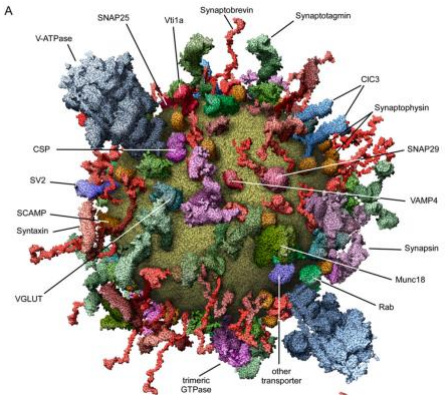
Each SNARE pin releases about 35 k_BT of energy (equivalent to about 20 kcal/mol) as it zippers up. The activation energy for lipid bilayer fusion is about 50 to 100 k_BT , and so three or more individual SNARE pins suitably arranged provide enough energy to drive fusion.

Tetanus toxin (or Botox) → promote cleavage of SNARE pin complex (no more synaptic transmission takes place). Botox would work if injected in the skin and abolishes acetylcholine release, muscle relax and no more windows.
Cleavage of SNARE proteins by clostridial toxins. Indicated are the sites of proteolysis by tetanus (TeTX) and various types of botulinum toxin (BoTX). Purves et al., 2012
(Don't mix up TeTX with TTX (Nav channel blocker, from Puffer fish)



Synaptic vesicles are rapidly released after Ca^{2+} entry. Neurotransmitter release is transiently released so that presynaptic terminal can respond to future action potentials → need to remove free Ca rapidly after entry. Indeed, Ca-binding proteins and pumps quickly sequester free calcium after entry.
These mechanisms ensure that neurotransmitter release is only triggered transiently and locally at the site of ca entry.

Our current view of the structure of synaptic vesicles



Many proteins on the coat of vesicles. Many of which are not understood.
The proton gradient (high in vesicle and low in the cytosol) is created by **V-ATPase**→ largest molecule on the synaptic vesicle membrane → pumps proton into the synaptic vesicle against an electrochemical gradient using ATP.

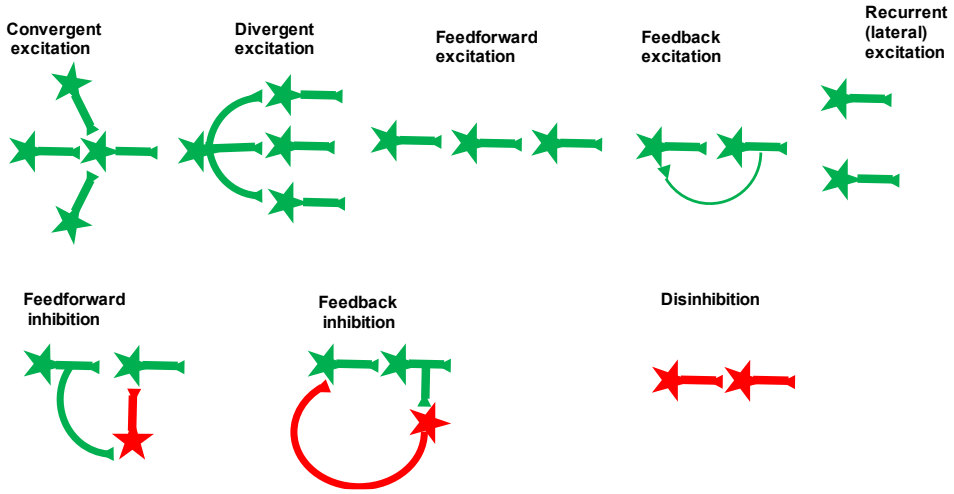
"Despite many years of research, it is still controversial as to how calcium influx brings about the extraordinary and highly cooperative acceleration of exocytosis."
Jahn & Fasshauer (2012) Nature 490: 201-207

Lectures 3-4

The synapse, receptors and circuits, memory and LTP.

GABA, Glutamate (AMPA & NMDA) receptors, neuromodulators (e.g. dopamine).

Examples of commonly used neuronal circuit motifs



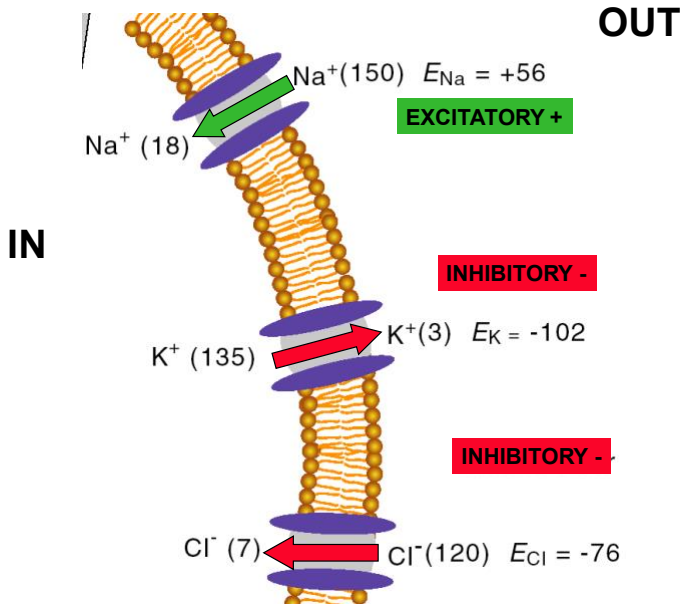
Green: excitatory neurons; Red: inhibitory neurons

Many circuits exist. Excitatory talking to inhibitory ey… Any kind of combination has probably evolved in brains.

Information flow need also feedback inhibition. Inhibitory neurons allow this. Brains are built by millions of these models, micro circuits. Require different transmitters. We require a "yes" molecule, a "no" molecules. Neuromodulators (such as dopamine) give context to circuit.

Simple transmitters:

g-aminobutyric acid (GABA)
glutamic acid (glutamate)
acetylcholine (Ach)

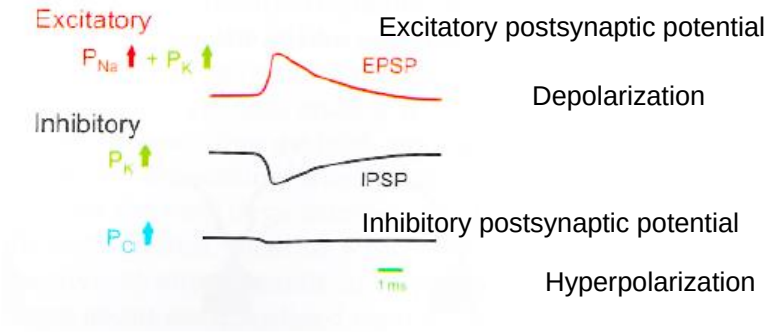


Distribution of ion concentrations across the membrane.

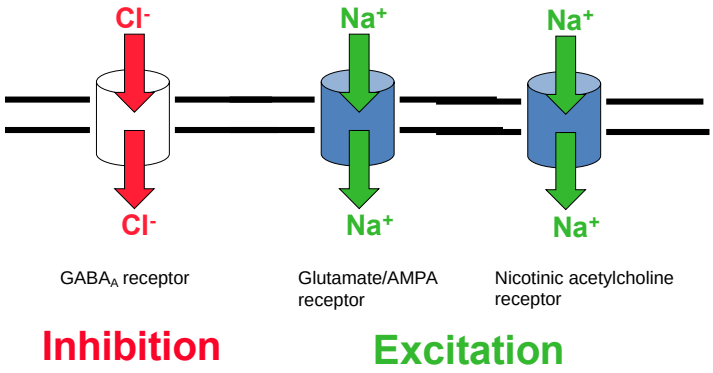
Na channel open →excitatory response
K channel opens is no response
Cl comes out → inhibitory response.

Ion concentrations in millimoles; E is equilibrium potential (mV) calculated from the Nernst equation

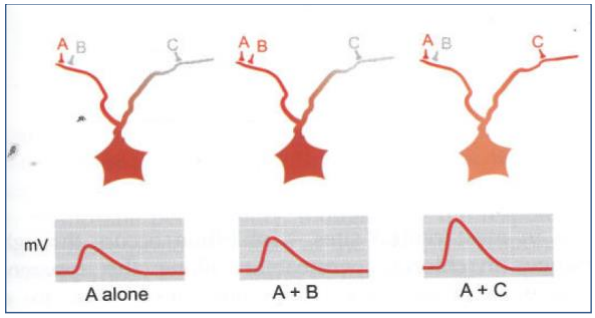
Postsynaptic potentials



Not an action potential (not all-or-none). it is a graded response. A smooth amount of depolarization. P is a shorthand for conductance. Hyperpolarization is inhibitory. Depolarization is excitatory.



GABA$_A$ receptor (No)
Glutamate/AMPA receptor (Yes)
Nicotinic acetylcholine receptor (motor neuron excite muscles)
Gaba and Nicotinic are in the same gene family.



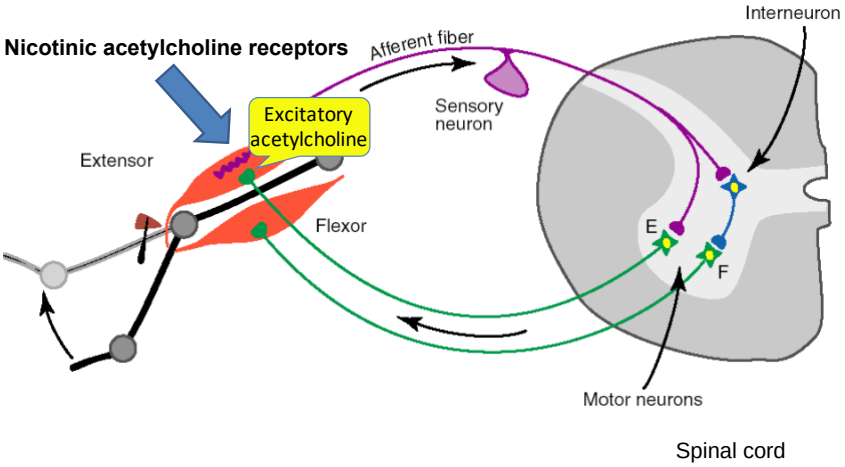
Integration of inputs from the dendrites determines if an action potential initiates at the axon hillock.

Net amount of depolarization spreads into the cell body and if net effect is enough, it starts a classic action potential. A neuron is sampling the total amount of excitation or inhibition and decides whether there is going to be action potential or not.

Record action potential in neuron, releases GABA onto motor neurons flexor (inhibitory).

Nicotinic acetylcholine receptors
Acetylcholin release from muscles, Bind pentameric channel. Each on of each has 4TM domains (C-loop). Dicystein loop provides structural stability. TM2 lines up the channels. 2 acetylcholine molecules bind. Different types of subunits, work together to make pore. It allows na to come in and not chloride, there is charge aminoacids that govern relative flor of Na and Cl.

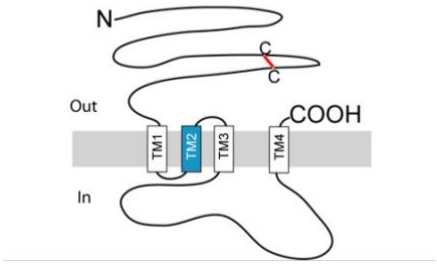
Knee jerk reflex in humans.



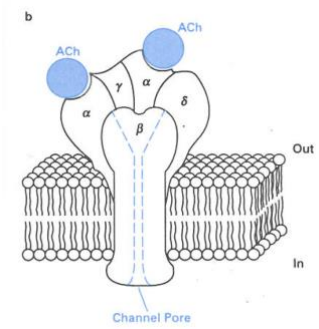
There is a sensory fiber in muscle which use stretch receptors (special kind of ion channel), receptors open, Na comes in, depolarizes neuron, action potential goes down into top part of spinal cord. There is an excitation (acetylcholine relesead), muscle contract and move. To stop other muscle from working, --A at the same time motornueron that gives excitory to flexor muscle, is inhibited. Splitting of message.

Nicotinic acetylcholine receptor: a ligand-gated ion channel that gates mainly Na^+ ions
When acetylcholine opens the channel, Na^+ ions flow down their electrochemical gradient into the muscle cell to depolarize it
Each binding induces the rotation of the alpha subunits, which causes an alternative conformation of the M2 helices and opens the gate to allow the passage of cations.

The nicotinic acetylcholine receptors - C-loop receptor superfamily

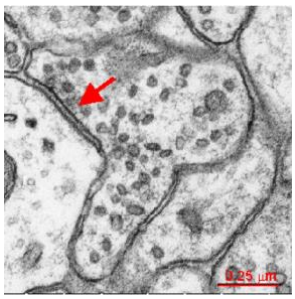


A subunit from the nicotinic acetylcholine Receptor. TM, transmembrane domain; C, cysteine



Five subunits come together to form the channel: two alpha subunits, one beta, one gamma and a delta; the two acetylcholine binding sites are at the interface of the a & g and a and d subunits.

Electronmicrograph of a GABAergic synapse in the central nervous system
All glutamate synpapses have large postsynaptic densities, GABA do not have this. Called a symmetric synapse (under the microscope, you cannot see any shadow of the post synaptic density)

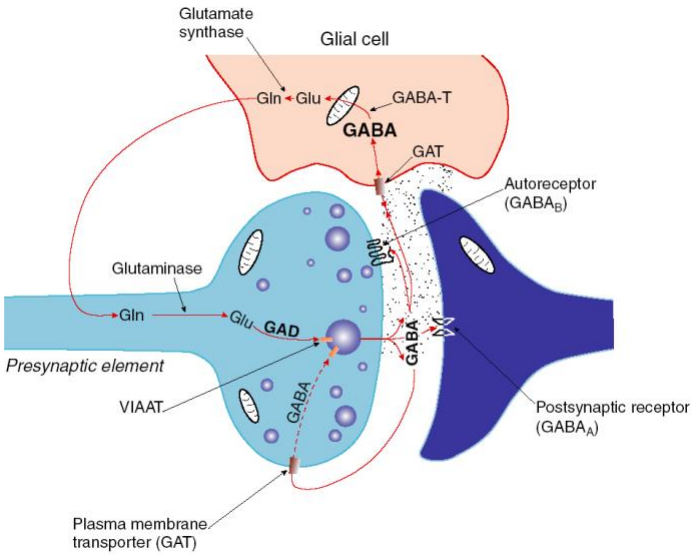


Grey type II/symmetric synapse: GABA --- Arrow is presynaptic side

$GABA_A$ receptor: a ligand-gated ion channel that gates mainly Cl^- ions
Selectively gate Cl ions when GABA binds and cause shape change. Cl is inhibitory signal and hyperpolarises the neuron. Making downstream neuron less likely to fire action potential. When GABA opens the channel, Cl^- ions flow down their electrochemical gradient into the motor neuron to hyperpolarize it

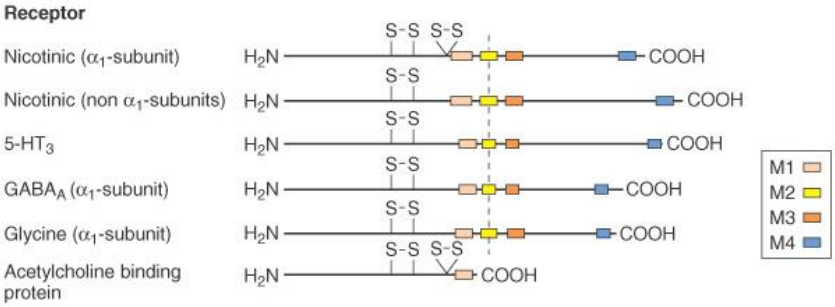
A synapse using g-aminobutyric acid (GABA)
In order to make glutamate into GABA neuron → transcribe glutamic acid decarboxylase gene (glutamate into GABA) + make specific transporter (vesicular inhibitory aminoacid transporter). Package GABA (or glycine) into vesicles, GABA released. No GABA specific

enzyme degrades GABA but GABA is taken up by GABA transporters in glial cells. In mitochondrial glial is turned into glutamine, which is then returned to neurons and reconverted into GABA.



Some Cys-loop receptor subunits

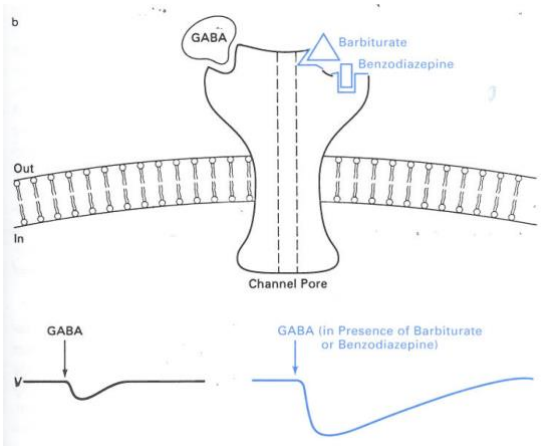
There is a large family of these receptors. Family is remarkable flexible. Basic plan has evolved to bind whole range of different transmitter. Acetylcholine, serotonin…

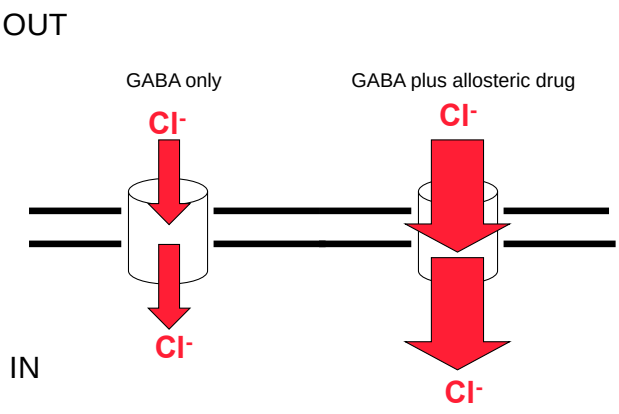


The gene family is found in all animals:
e.g. the glutamate-gated chloride channel in *C. Elegans*; the histamine-gated chloride channel in invertebrate eyes

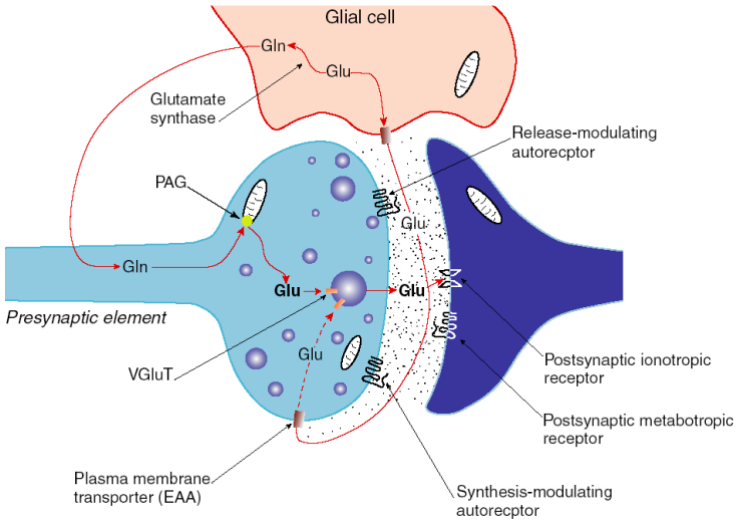
The GABA receptor is a major drug target for anaesthetics. Sleeping pills are allosteric modulator, bind channels onto different side than AGBA and force GABA to open channel more efficiently. Net effect is more Cl flow into neuron:

Benzodiazepines, barbiturates, some steroids and propofol all allosterically enhance GABA's ability to open the GABA$_A$ receptor.

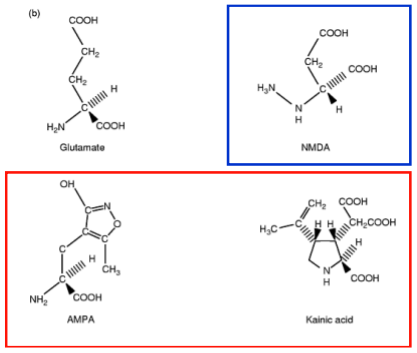




An excitatory (glutamatergic) synapse
Glutamate comes from Krebs cycle. Releases onto range of post synaptic ion channels and is recycled to glutamine back into glutamate in mitochondria.



Artificial drugs bind. 2 molecule mimic the shape of glutamate: used to dissect different types of glutamate ion channel:
N-Methyl-D-aspartate: synthetic ligand which partially mimic glutamate but open only NMDA types of receptor → have the unusual property of not only being gated by glutamate but also influenced by membrane potential (Mg blocking channel outside, released by depolarization), they also require glycine as co-agonist.
AMPA and Kainite (found in seaweeds): activate AMPA receptors,



N-methyl-D-aspartate

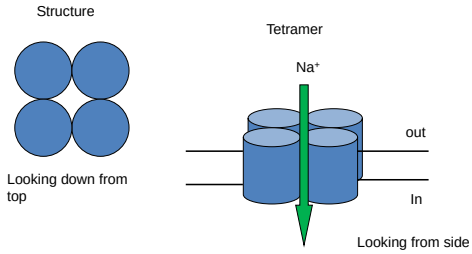
Kainate – monster from the sea (Japanese)

Agonists that work at different subtypes of glutamate-gated ion channels

Glutamate AMPA receptor: a ligand-gated ion channel that gates mainly Na^+ ions
It is a Glutamate-gated Na ion channel. It is not in the same gene family as GABA and Ach gene family. It is in the pore loop family (AMPA, NMDA,). P-loop (4 types) contribute to the channel. It is inside out configuration inverted topology but same gene family than voltage gated Na and K ion channel. These however are not voltage gated but ligand gated.
When glutamate opens the channel, Na^+ ions flow down their electrochemical gradient into the motor neuron to depolarize it.

AMPA receptors

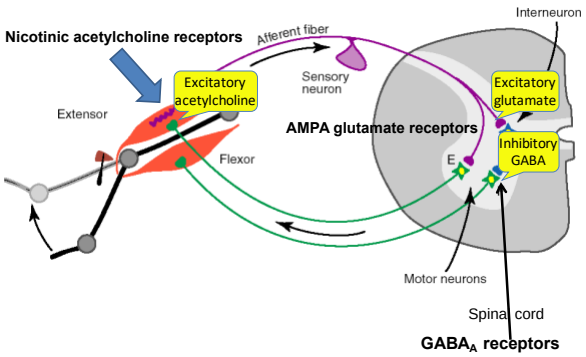
4 types of subunits. Different genes are combinatorially expressed in the brain

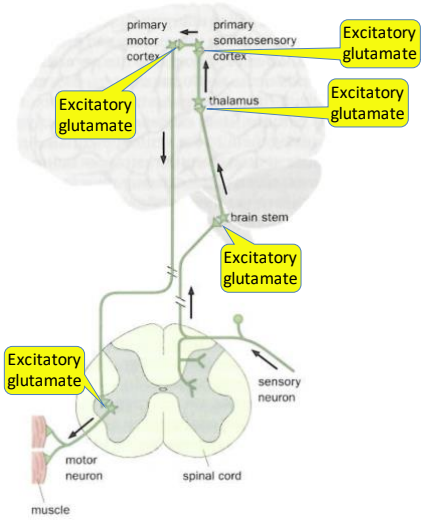


4 subunit genes:
GluR1 or GluRA or GluA1
GluR2 or GluRB or GluA2
GluR3 or GluRC or GluA3
GluR4 or GluRD or GluA4

These receptors are also called iGluR-type receptors. The "i" stands for "ionotropic".

Voluntary control of muscles





In addition to participating in the spinal cord reflex circuit, sensory neurons also send an ascending branch to connect with relay neurons in the brainstem and thalamus to deliver information to neurons in the primary somatosensory cortex. Through inter-cortical connections, information can be delivered to neurons in the primary motor cortex, which send descending output via their long axons directly to motor neurons for the voluntary control of muscle.

Cannot describe the wiring of the brain in the same detail as in the knee-jerk reflex. But conceptually is the same mechanism, just (way) more interconnected.

Lecture 4 - Memory

Motor memory (play the piano, cerebellum)
declarative memories (cognitive, facts, relationships etc…)

Hebb's postulate: When an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased.

Persistent activity to A and B leads to the strengthening of the connection (can also be the decreasing of connection).

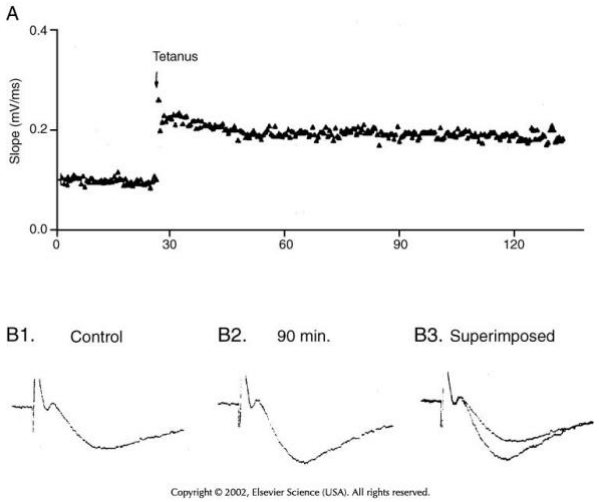
What are the molecular mechanisms?
Synaptic plasticity: changes of the strengths of synaptic connections in response to experience and neuronal activity.
Hebbian synapse: synapse whose strength can be enhanced by co-activating pre- and post-synaptic partners.
These Hebbian synapses exhibit **NMDA receptor-dependent LTP**

LTP :
Bliss: how memory is formed in vitro in rat hippocampus. Took an axon pathway (performant path – glutamate transmitter) → artificially simulate with particular frequency. the axon bundle and record electrical postsynaptic responses. They studied what happened to glutamate response. They found LTP phenomenon.
One electrical pulse to axon so that glutamate is released in a brief period of time → record and found that it and EPSP (excitatory post-synaptic potential with specific amplitude, it is not an action potential but graded potential, causes depolarization of membrane). Can measure the height or rate of this potential (at which potential increases with time, the slope).

Associativity: potentiation of synapses that experience a weak stimulus by a coincident strong stimulus
Cooperativity: presynaptic cell fires and releases neurotransmitters to a postsynaptic neuron that is depolarised. It explains why high-frequency stimulation can induce LTP.

The opening of the NMDA receptor channel requires simultaneous glutamate release from the presynaptic terminal and depolarization of the postsynaptic neuron to remove the blockade by Mg^{2+}. This property accounts for the cooperativity and associativity of lTP. Indeed, evidence supports a key role for the NMDA receptor in the establishment of LTP at the CA3 → CA1 synapse.

LTP at a hippocampal synapse
Instead of giving 1 pulse but 10 pulses → tetanus (a burst of stimulation); then waited then a few minute later another pulse again. Result: following the tetanus, can see that response is larger (higher peak and increases more rapidly –steeper slope). It stays permanently elevated → long term potentiation.
It is a permanent change ins synaptic strength, could be the basis of memory (enhancement is stable and persist up to 48 hours).



A, test stimuli delivered every 10 seconds while the strength of the synaptic connection (rate of rise of the EPSP) is monitored; to induce LTP, two 1 s, 100 Hz tetani were delivered with a 20 s interval. Subsequent test stimuli produce enhanced EPSPs. The enhancement is stable and persists for at least two hours (Fundamental Neuroscience, 2nd ed, Squire et al., 2003)

We now know that this response is given by NMDA receptors

NMDA receptors (natural agonist glutamate)
NMDA is a ligand that partially mimics conformation of glutamate
Mg binds to channel and block ion current.

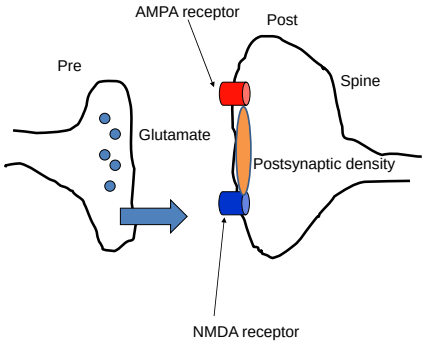
It is a ligand gated calcium channel as well as sodium and unlike AMPA receptor stays open for long (sustained influx of Ca when NMDA opens). Mg can be displaced if membrane become excited.

Voltage-dependent magnesium block

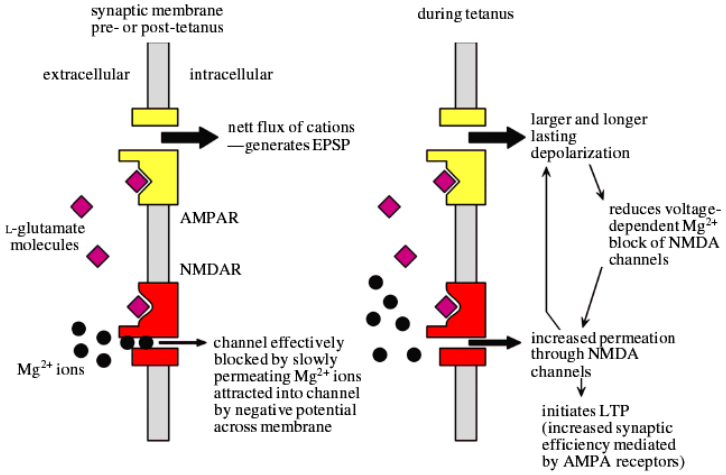
- → $Ca2+$ permeable
- → Slow activation (hundreds of milliseconds)
- → Stay open for a long time (up to several seconds)
- → Blocked by the antagonist AP5
- → NMDA receptor subunits are in the same pore loop family as AMPA receptors

The distinct properties of AMPA and NMDA receptors can be dissected with selective antagonists.
AP5 or APV ((2R)-amino-5-phosphonovaleric acid) is a selective NMDA receptor antagonist that competitively inhibits the ligand (glutamate) binding site of NMDA receptors.
CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) is a competitive AMPA receptor antagonist.

Schematic of glutamate synapsis in brain.



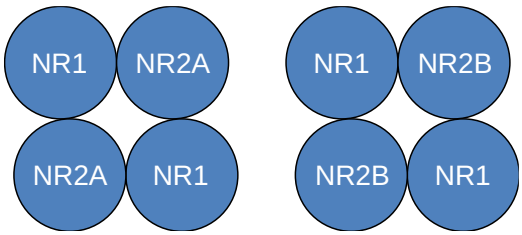
2 types of glutamate ion channel in parallel: AMPA and NMDA recpetor. Linked together by postsynaptic density. First is AMPA receptor, second is NMDA. Linked together by post-synaptic density. On a spine in the cortex or hyppocampus



If just a few molecule (equivalent of a pulse, one stimulation of the axon) → glutamate released on postsynaptic neuron → binds to AMPA receptor and allows in an influx of Na down electrochemical gradient which causes depolarization of the neuron (EPSP). Mg ion blocks NMDA so nothing happens.
But if we give a tetanus (blast stimulation to the axon). → now flood of glutamate. More Na come to AMPA receptor → more depolarization (graded, not action potential).
Mg ions then leave the channels as NMDAR has a voltage sensor (when it senses amount of depolarization changes conformation and opens) → Mg comes out of receptor, giving a free pathway for Ca and Na ions. Then Na and Ca can come in through NMDAR. It is the Ca that is then triggering the long term changes in the structure of the synapses.

Structure of NMDA receptors
Same broad family of AMPA and voltage gated Na, K, Ca channel (P loop). Closest family member of AMPA.
All NMDAR contain NR1 subunit. In different types of neurons there is differential transcription of genes (2,3 etc…).
They have different times in which they stay open (different amount of Ca let in -> different response).

Same gene family as AMPAreceptor subunits
Five genes differentially expressed
NR1 in every receptor



NR1 = GluN1
NR2A = GluN2A
NR2B = GluN2B
NR2C = GluN2C
NR2D = GluN2D

How does the brain encode memories?
The best candidate mechanism is LTP or an LTP-like process.
How do AMPA and NMDA receptors contribute to memory formation in the Hippocampus?
Persistence and apparently permanent change in synaptic strength (seems to follow Bliss hypothesis).

The NMDA receptor can function as a molecular integrator
It can work as a coincidence detector (if cell has already been depolarised by other stimulus, only need weaker stimulus to excite cell. It can detect two pathways working together and function as integrator). Can explain Pavlov's Dogs (dog salivating when hear bell, converging stimuli.
NMDAR→ pair stimuli (integrate neuronal circuits)
The induction of LTP requires NMDA receptors & hippocampal-dependent learning requires NMDA receptors

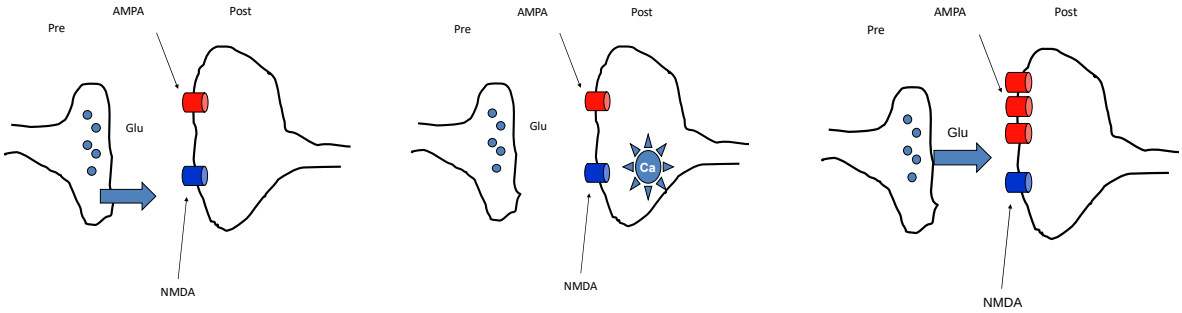
Morris water maze: an assay for hippocampal-dependent learning
Early in training, rats search for the submerged platform for extended periods. After training the rat swims directly to the platform

AP5 interferes with the ability to remember the location of a hidden platform in the Morris water maze
AP5 is nmda antagonist. If you inject it –> it interferes with memory.

How does LTP work at the molecular level?
AMPA and NMDAR are working together. Calcium enters the postsynaptic terminal through NMDAR. Neuroscientists cannot agree what causes the long term potentiation. One explanation: LTP is due to increased number of AMPA receptor.

Image

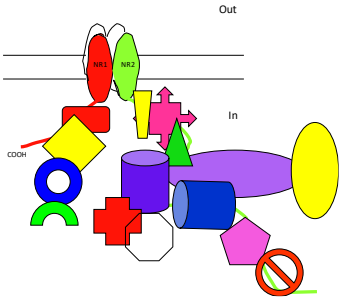
LTP induced – AMPA receptors up-regulated



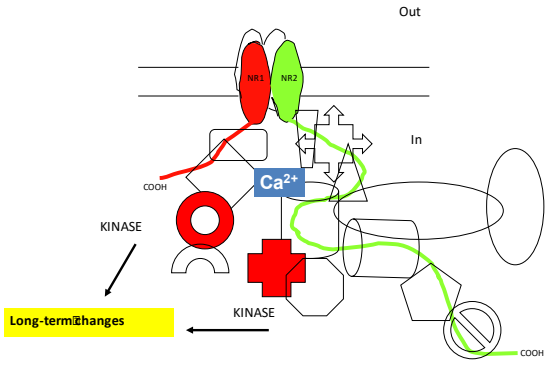
Bigger response is given by the increased number of AMPA receptors postsynaptically. Memories may be encoded on dendridic spines, the site where LTP occurs

Post-Synaptic Density

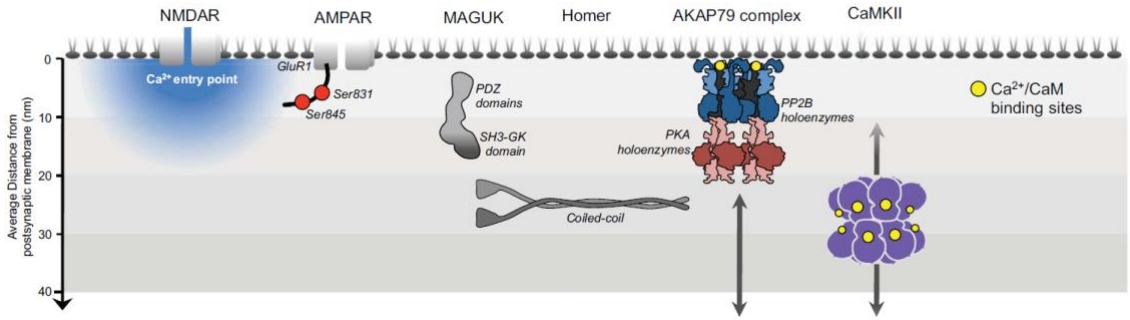
Structure of the NMDAR (4 subunits). C-terminus inside the cell of NR2 subunit is extraordinary long with no precise structure. There are motifs on the C-termini which bind particular proteins (one is PSD-95, protein which selectively binds C-terminus). NR1 and NR2 so has huge number of proteins bound to C-termini → so there is a large complex permanently anchored to C-terminuns of NMDAR and to some extent to AMPA. Big complex explains the shading in the EM → that is the post-synaptic-density



NMDAR stays open relatively long time. AMPA is short time. NMDAR stays a hundred of milliseconds → during that time Ca is entering through NMDAR. Ca functions as second messenger and produce long term changes (scientist do not know precisely which changes). Believes that most of the complexes are kinases which phosphorylate other kinase and outcomes is that more AMPA are expressed on membrane surface.



Laminar organisation of PSD signalling molecules involved in AMPA receptor phospho-regulation.



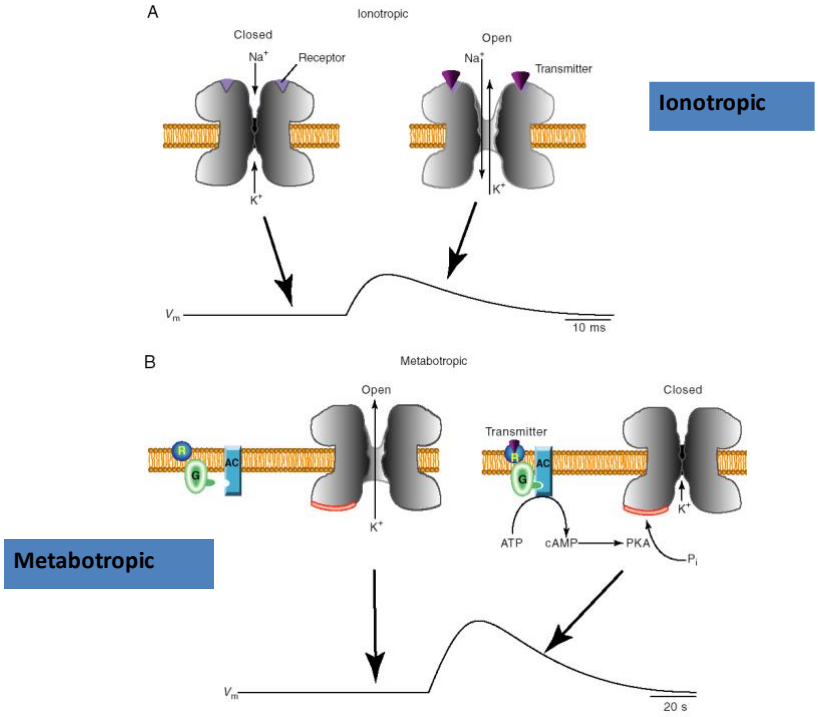
Array of complex proteins is not entirely random. As you move through PSD by NMDAR, there are enzymes at particular level within density. Some proteins are bound to C-terminus and are thought to be structural domains, other are coiled-coil. Other are the famous PKA complexes (AKAP complex, linked structurally to NMDA receptor) and Calmodulin dependent protein kinase.
Analogy to signaling scaffold (link cell signaling).

LTP epilogue

"The property of LTP that most commends it as a cellular mechanism for encoding information is its longevity. The studies we have discussed so far reveal little about the mechanism of enduring LTP in the intact animal. Ultimately, the search for expression mechanisms will need to be conducted in the context of the neural networks subserving memory and cognition, processes that can potentially operate over a lifetime. Here progress will depend on techniques to study the plasticity and structure of single synapses in the freely moving animal. If and when agreement on a long-term expression mechanism based on structural changes is reached, pre and postsynaptic mechanisms will converge, and a controversy that has already continued for nearly four decades may finally be put to rest".

Neuromodulators - Metabotropic signaling

Metabotropic response: ligand binds GPCR, which then open or close K channel affecting excitability of the cell. Used to modify over the long term the fast response of GABA. Metabotrobic signaling in CNS is slow and integrative response (contrast this to vision signaling in rod cells, fast as fuck).



Widely projecting neuromodulator transmitters

There is a range of small molecuels and also peptides that signal through GPCR and cause slow, integrative changes. **Not information but tone and context** (emotions).

Acetlycholine (ACh) (in CNS, Ach is working on metabotropic GPCR, not as in muscle tissues which is ionotropic)
Dopamine (DA)
Noradrenaline (NA)
Histamine (His)
Serotonin (5-HT)
Orexin (peptide)

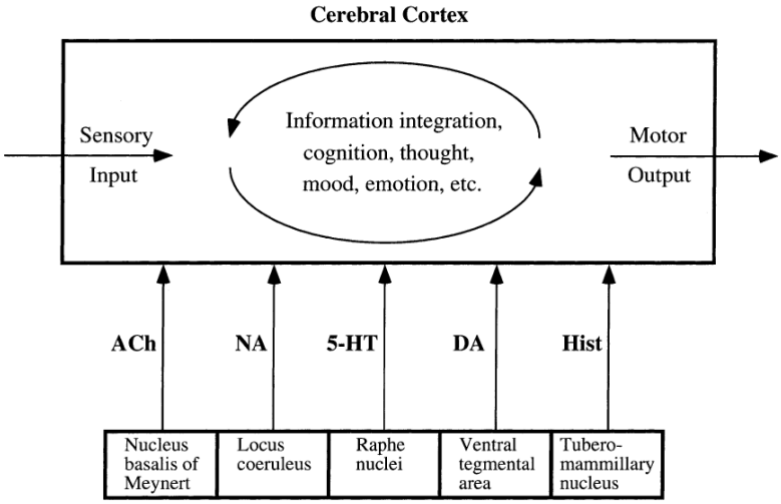
These neurochemicals are made in very specific neurons, not found everywhere. They have amazing axons, which release in general way the transmitters to the whole brain.

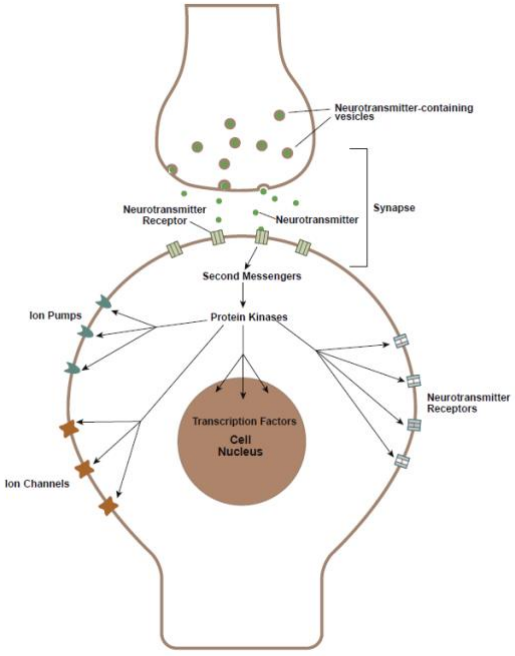
Neuromodulation: transmitters are released, work on GPCR, cause changes in second messengers (inositol 3 p or cAMP), kinases are activated, substrates for kinases tend to be ion pump. Net effect: phosphate groups are added or subtracted to ion channels (AMP, NMDR etc…). Phosphates on ion channel can cause increase or decrease in activity depending on the particular channel. Activity of the channel tend leads to excitation or inhibition of the neuron, depending on the channel.

Dopamine does not work at specific synapse but released widely on the brain. Works on metabotropic receptors, releasing cAMP. Released from varicosities in dopamine axons (volume transmission: dopamine diffuses out of the axon broadly to the brain).
Dopamine is released in substantia nigra and locus ceruleus (send axons to cortex).
Dopamine is decarboxylated tyrosine + hydroxyl group.
Axons go to cortex and other wide areas of the brain.

Local neuromodulator transmitters (often co-released with GABA or glutamate)
Many peptides (e.g. somatostatin, NPY, CCK, opioid peptides, neurotensin, sub. P)
Adenosine
Gases (nitric oxide, carbon monoide and hydrogen sulphide)
"Cannabinoid lipids" and prostaglandins
Steroids
Volume transmission: diffuse, not point to point as occurs at "fast" synapses.

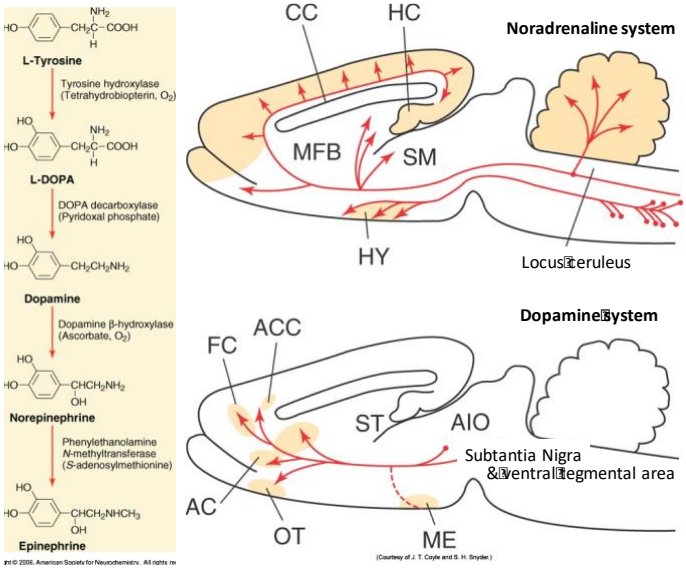
Neuromodulators





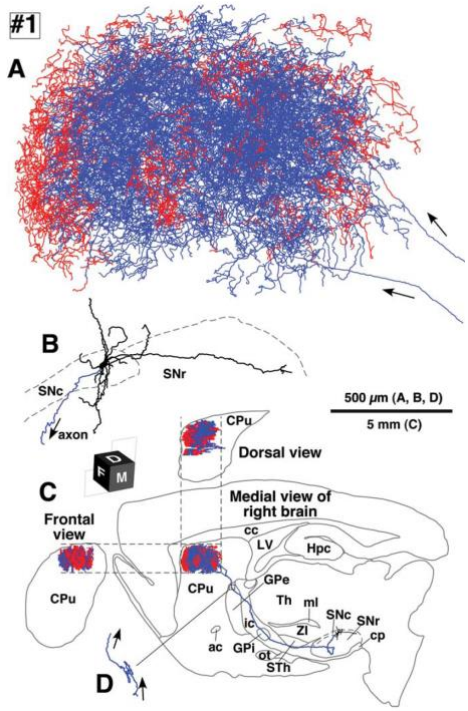
Neuromodulators work via second messengers
Seconds to minutes' timescale
Ligand-gated ion channels such as GABA-A and AMPA receptors work on millisecond timescale

Dopamine - Volume transmission



Single nigrostriatal dopaminergic neurons form widely spread and highly dense axonal arborizations in the neostriatum

A section of the brain and we are looking side way. One single dopamine virus infected by virus which has GFP (transported into axon) Axon assembles all the way to certain part of the brain.
Makes cloud (blue and red is axon form one neuron). Release dopamine as cloud to thousands of neurons simultaneously (volume transmission).

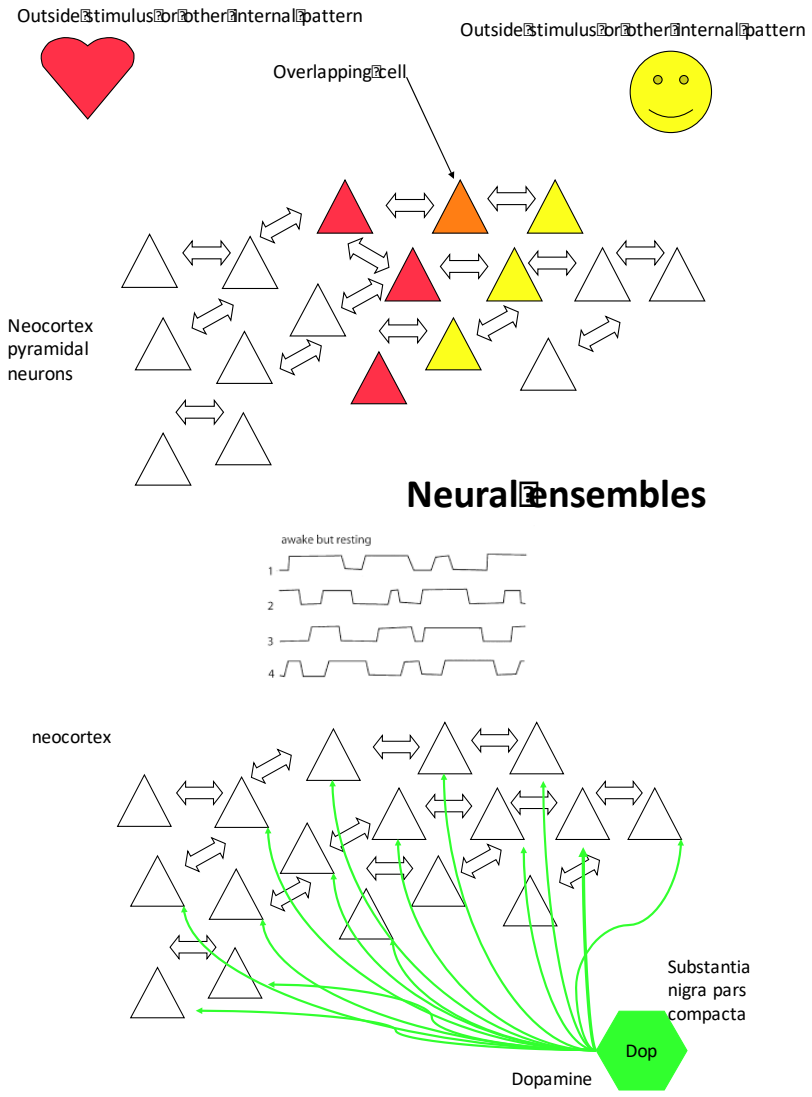


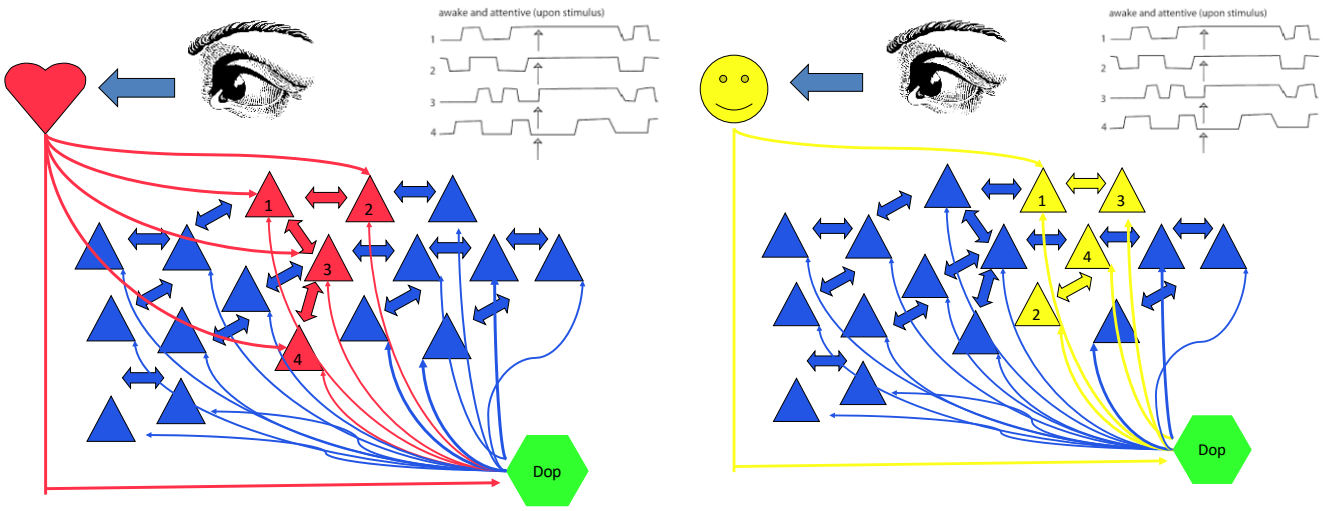
Matsuda W et al., (2009)
J. Neurosci. 29:444-453
Imaging with a viral vector expressing membrane-targeted GFP

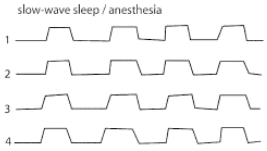
Dopamine transmission is "Volume transmission" – diffuse release over a wide area

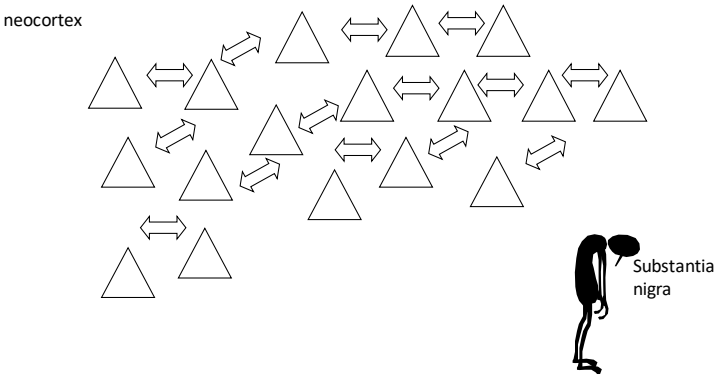
Dopamine cells project from the substantia nigra in the midbrain to the Striatum and neocortex; and from the ventral tegmental area (VTA) in the midbrain to the nucleus accumbens.
In the striatum, the dopamine released facilitates initiation of voluntary movement.
We don't understand how.
Degeneration of the dopamine-containing cells in the substantia nigra is all that is needed to produce the progressive motor disorder of Parkinson's disease.
Dopamine signalling in the VTA contributes to pleasure and addiction behaviours.

Neural Ensembles




awake and attentive (upon stimulus)
1
2
3
4
Dop
awake and attentive (upon stimulus)
1
2
3
4
Dop


slow-wave sleep / anesthesia
1
2
3
4


neocortex
Substantia
nigra