**Ion Channels** are pore-forming membrane proteins whose function is

- *establishing a resting membrane potential*,
- *shaping action potentials and other electrical signals by gating the flow of ions across the cell membrane*,
- *controlling the flow of ions across membranes*,
- *regulating cell volume*.

Their activation translates into a rapid physiological effect.
The ion channel selectivity discriminates cations (Na, K, Ca) from anions (Cl), and allow selectivity among cations (Na, K, Ca).
ION CHANNELS: mechanisms of selectivity

Despite the small differences in their dimension, ions rarely go through the "wrong" channel. For example, sodium or calcium ions rarely pass through a potassium channel.

One hypothesis about selectivity considers that hydrated ions behave differentially and postulates that the pore lining could efficiently replace the water molecules that normally shield specific ions. Based on this, potassium ion are allowed in certain channels; conversely, sodium ions are too small to allow such shielding, and therefore could not pass through.
GATING THE FLOW OF IONS ACROSS THE CELL MEMBRANE MODIFIES THE MEMBRANE POTENTIAL AND THE CELL PHYSIOLOGICAL STATE

Membrane potential is the difference in electric potential between the interior and the exterior of a biological cell.

With respect to the exterior of the cell, typical values of membrane potential range from $-40 \text{ mV}$ to $-80 \text{ mV}$. 
4 or 5 subunits closely packed around a water-filled pore through the plane of the membrane or lipid bilayer

Each polipeptidic subunit has 4 hydrophobic regions (M1-M4) spanning the cell membrane

The M2 region of each subunit faces each others on the inner side of the channel and determines ion selectivity
ION CHANNELS: ROC and VOC

• Ion entry into cells (particularly neurons) occurs mainly either through receptor-operated channels (ROC) or voltage-operated channels (VOC).

• The function of ROC depends crucially on the action of agonists, antagonists or compounds modulating particular types of receptors (GABA A, NMDA, ACh N receptors).

• The function of VOC is closely connected with the activity of protein kinases and the processes of phosphorylation of membrane proteins (K+, Na+, Ca2+ channels).
Receptor-operated (ROC) CHANNELS

<table>
<thead>
<tr>
<th>LIGAND</th>
<th>RECEPTOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetilcholine</td>
<td>Nicotinic R</td>
</tr>
<tr>
<td>Glutamate and other excitatory aa</td>
<td>NMDA R</td>
</tr>
<tr>
<td></td>
<td>AMPA R</td>
</tr>
<tr>
<td></td>
<td>KAR</td>
</tr>
<tr>
<td>Serotonin</td>
<td>5-HT3 R</td>
</tr>
<tr>
<td>ATP and purines</td>
<td>P2X</td>
</tr>
<tr>
<td>Ciclic nucleotides (cAMP and cGMP)</td>
<td>CNG</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>LIGAND</th>
<th>RECEPTOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>GABA</td>
<td>GABA \textsubscript{A}</td>
</tr>
<tr>
<td>Glicine</td>
<td>Gly R</td>
</tr>
</tbody>
</table>
Acetylcholine (ACh) is the neurotransmitter of cholinergic system. These nerve cells are activated by or contain and release acetylcholine.

ACh is the **preganglionic** neurotransmitter for both the sympathetic and parasympathetic nervous system, and the **postganglionic** neurotransmitter in the parasympathetic nervous system.

The brain **cholinergic** system has been associated with a number of cognitive functions, including memory, selective attention, and emotional processing.
ACH RECEPTORS

Acetylcholine binds to both muscarinic and nicotinic receptors.

Nicotinic receptors are **Receptor-operated channel receptors** and get their name from nicotine, which selectively binds to the nicotinic receptor.

Muscarinic receptors are **G-protein coupled receptors** and get their name from a chemical that selectively attaches to that receptor, called muscarine.
ROC: Nicotinic receptor

- **5 transmembrane subunits:** α (2), β, γ, δ or ε
- Each subunit possesses 4 TSM
- They form a pentameric structure, with a γ subunit interposing the 2 α subunits

**Muscular** nicotinic receptor is a cation channel allowing cell entrance of NA, and to a lesser extent, of K and Ca

**Neuronal** nicotinic receptor preferentially permits Ca entry
• The 2 α subunits represent the **BINDING SITE** for the ligand (Ach). Both subunits must be occupied by Ach to allow receptor activation. The first binding facilitates the second (**cooperation**).

• **GATING:** Binding of the 2 Ach molecules induces a conformational change that opens the channel.
ROC: Nicotinic receptor

- The M2 sequence of each TSM is a segment enriched in Ser or Thr (negatively charged) amino acids, forming three rings.

- These three consecutive rings represent the SELECTIVITY FILTER facilitating the entry of cations and excluding anions.

- The cytoplasmic region of the receptor includes a REGULATORY P SITE that can be modulated by phosphorylation.

- At this level additional regulatory sites have the role to anchor the receptor to specific regions of the cell membrane.
L-Glutamate is the major excitatory neurotransmitter in the mammalian CNS. L-glutamate is responsible for basal excitatory synaptic transmission and many forms of synaptic plasticity such as long-term potentiation (LTP) and long-term depression (LTD), mechanisms that are thought to underlie learning and memory.

Glutamate is contributing to normal neural transmission, development, differentiation, and plasticity.
L-glutamate acts via two classes of receptors, ligand gated ion channels (ionotropic receptors, iGluR) and G-protein coupled (metabotropic, mGluR) receptors.

**iGluR** are named after their respective more specific agonist:
- **NMDA R**, *N*-methyl-\(D\)-aspartate
- **AMPA R**, \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
- **KA R**, kainate

**mGluR** are G-protein coupled receptors and trigger a second messenger cascade. They are found both at the pre- and post-synaptic neurons.
AMPA receptors mediate **fast synaptic transmission** in the CNS and are composed of subunits GluA1-4. Like all the iGlu R subunits, GluA subunits have an extracellular N-terminus and an intracellular C-terminus. The ligand binding domain is made up from N-terminal region S1 and S2.

**GluA2 subunit**
- The C-terminus of the GluA2 subunit contains binding sites for a large number of interacting proteins. The effects of these protein-protein interactions is crucial in localisation and trafficking of these receptors so that they can fulfill their roles in plasticity.

**AMPA R are permeable mostly to Na and, to a lesser extent, to Ca ions.**
- The calcium permeability of the GluA2 subunit is determined by the so called Q/R post-transcriptional editing site - GluA2(Q - glutamine) is calcium permeable whilst GluA2(R - arginine) is not.
• AMPA and kainate R are grouped together in the 'non-NMDA receptor' family. Kainate R are similar to AMPA R, and are formed by multimeric assemblies of GluK1-3 and GluK4,5 subunits. GluK1-3 subunits can combine to form functional heteromeric and homomeric assemblies.

• Until relatively recently, KAR functional and physiological role in the mammalian CNS was unclear. They seem present at both sides of the synapse.
• Pre- and postsynaptic KAR can regulate transmission at many synapses in a specific manner, and seem to be involved in short- and long-term plastic phenomena.

KA R are permeable to Na and Ca . However, their kinetics is much lower than AMPA R
Unusually for the iGluR, **L-GLUTAMATE IS NOT THE ONLY AGONIST FOR THE NMDA R**. Another amino-acid, **GLYCINE, IS A CO-AGONIST** and both transmitters must bind to their respective binding site in order for the receptor to function.

The binding sites for glutamate and glycine are found on different subunits:
- **GLYCINE** binds to the GluN1 subunit while
- **GLUTAMATE** binds to the GluN2 subunit.

The GluN2B subunit also possesses a **BINDING SITE** for **polyamines**, regulatory molecules that modulate the functioning of the NMDA receptor.

**NMDA R are highly permeable to Ca and, to a lesser extent, to Na.**
The **NMDA R** channel is normally blocked by **Mg$^{2+}$** in a voltage- and use-dependent manner.

Although necessary, binding of both **L-GLUTAMATE** and **GLYCINE** is still not sufficient to completely activate **NMDA receptor** and favor **Ca$^{2+}$** entry.

Depolarization of the postsynaptic neuron via AMPA R activation releases the Mg$^{2+}$ block on the NMDA R.

**Ca$^{2+}$** entry is therefore subsequent to complete post-synaptic depolarization (required to remove Mg$^{2+}$). This effect is obtained by early activation of AMPA R.
Glutamate RECEPTORS summary

A

Resting Synapse

Channel effectively blocked by magnesium ions attracted into channel by negative potential across membrane.

B

Weakly Active Synapse

extracellular

L-glutamate

intracellular

Na+

C

Strongly Active Synapse

NMDA Receptor (NMDAR)

Non-NMDA Receptor AMPAR/KAR

mGlu Receptor (mGluK)

NMDA Receptor

Non-NMDA Receptor

mGlu Receptor

Glu

Zn2+

PCP

Polyamine

Glu

Gly

Glu

Gly

A Current

PLC

Signaling
Gamma Amino Butyric Acid (GABA)

**GABA** is the chief inhibitory neurotransmitter in the mammalian central nervous system. It plays the principal role in reducing neuronal excitability throughout the nervous system. In humans, GABA is also directly responsible for the regulation of muscle tone.
GABA RECEPTORS

GABA binds to both GABA$_A$ and GABA$_B$ receptors.

GABA$_A$ receptors are ligand-gated channel receptors

GABA$_B$ receptors are members of the 7-TM G protein-coupled receptors
The γ-aminobutyric acid, type A (GABA_A) receptor is a chloride-conducting receptor composed of α, β, and γ subunits assembled in a pentameric structure forming a central pore.

The majority of GABA_A Rs are believed to be expressed as heteromeric complexes of 2α, 2β, and 1 γ subunit

The 2β subunits represent the BINDING SITE for the ligand (GABA).

Both subunits must be occupied by GABA to allow receptor activation. The first binding facilitates the second (cooperation)
Each subunit has a large extracellular agonist binding domain and four transmembrane domains (M1–M4), with the second transmembrane (M2) domain lining the pore.

The α subunits represent the **ALLOSTERIC SITE**, a modulatory region where binding of ligands different from GABA may facilitate/obstacolate the GABA/RECEPTOR interaction.

Another ligand site is present on the deep part of the channel, on the β subunit. Binding on this site allows a different modulation of channel opening that may exceed the GABA-mediated effects.
Voltage-operated (VOC) CHANNELS

The function of VOC is closely dependent on transmembrane electrochemical gradient. A gradient is represented by the different concentration of ions on either side of the membrane. The open conformation of the ion channel allows for the translocation of ions across the cell membrane, while the closed conformation does not. The activity of these channels is connected with the activity of enzymes and the processes of phosphorylation of membrane proteins.

Post-synaptic membrane with ROC’s

Muscle membrane with VOC’s
Electrophysiological studies indicate the existence of three main types of VOC (K\(^+\), Na\(^+\), Ca\(^{2+}\) channels). In number of neurons various subtypes of Ca\(^{2+}\) channels (P, T, N and L-type) occur together.
VOC CHANNELS

In each protein subunit, the membrane-spanning segments, designated S1-S6, all take the form of alpha helices with specialized functions. The **S1-S4 TMS** serve as the **voltage-sensing region**. The **S5-S6 TMS** and pore loop have a key role in ion conduction, and represent the **gate** and **pore** of the channel.

![Diagram of VOC channels]

**S1-4**
Voltage-sensing region

**S5-6**
Pore region
The inactive state, which is stable and non-conducting, is caused by the physical blockage of the pore. The blockage is caused by a “ball” of amino acids attached to the main protein by a string of residues on the cytoplasmic side. The ball enters the open channel and binds to the hydrophobic inner vestibule at the center of the channel.

The blockage causes inactivation of the channel by stopping the flow of ions.
**Na⁺ CHANNELS**

**VOC Na⁺ channels** are formed by a single subunit and are expressed in all excitable tissues.

**Na⁺ channels** are responsible for the rapid membrane depolarization during the action potential. Activation and inactivation are voltage-dependent, very fast processes (1-10 ms). During the inactivation state, the channel is in a refractory period.
**VOC Na⁺ CHANNELS**

**Na⁺ channels** are important drug targets. Drugs bind the Na channel inside the pore. Their binding maintains the channel in the inactivated state. Since they reach their binding site when the channel is open, their **inhibitory effect is use- and voltage-dependent.**

Drugs acting as Na⁺ channels blockers include Class I anti-arrhythmics, anticonvulsants, local anesthetics.
The epithelial Na\(^+\) channels (ENaC) are non-voltage gated, highly Na-selective channels. ENaC activity is rate limiting for Na\(^+\) reabsorption in the distal nephron. The long term control of blood pressure involves Na\(^+\) homeostasis through the precise regulation of ENaC in the aldosterone-sensitive distal nephron.

Inhibition of these channels results in reduced K excretion. This effect is obtained by some potassium-sparing diuretics such as amiloride.
K⁺ CHANNELS

K⁺ channels are found in virtually all living organisms. They form K⁺-selective pores that span cell membranes and conduct rapidly and selectively K⁺ ions down their electrochemical gradient.

These channels act to set or reset the resting potential in many cells.

Voltage-gated K⁺ channels

Calcium-activated K⁺ channels

Inward rectifier K⁺ channels

“Leak” or background K⁺ channels
**K⁺ CHANNELS: structure**

2TM/P channels (which consist of two transmembrane (TM) helices with a P loop between them), exemplified by **inwardly rectifying K⁺ channels**. Channel is formed by (2TM/P \(\times 4\)) tetramer

6TM/P channels, which are the predominant class among **ligand-gated and voltage-gated K⁺ channels**. Channel is formed by (6TM/P \(\times 4\)) tetramer

4TM/2P channels, which consist of two repeats of 2TM/P channels. 4TM/2P channels are far more common than was originally thought. Channel is formed by (2TM/P \(\times 2\)) dimer
**K⁺ CHANNELS**

**Voltage-gated K⁺ channels**

*Kv* channels are one of the key components in generation and propagation of electrical impulses in nervous system and in the heart. Upon changes in transmembrane potential, these channels open and allow passive flow of K⁺ ions from the cell to restore the membrane potential. They are the target of Class III anti-arrhythmic drugs.

**Calcium-activated K⁺ channels**

*KCa* channels can be grouped into three distinct subfamilies,

- large-conductance (BK),
- intermediate conductance (IK),
- small conductance (SK) channels.

These channels are activated largely in response to calcium influx during action potentials. Activation of these channels is to hyperpolarize the membrane.
**K⁺ CHANNELS**

**Inward rectifier K⁺ channels**

\[
\text{K}_\text{IR}
\]

include several subfamilies, among which the most important are

- **IRK** (strong inward rectifier K⁺ channels)
- **GIRK** (G-protein-activated inward rectifier K⁺ channels)
- **K_{ATP}** (ATP-sensitive K⁺ channels)

These channels are responsible for **repolarizing a cell following an action potential**

<table>
<thead>
<tr>
<th><strong>CARDIAC MYOCYTES</strong></th>
<th>( \text{K}_{ir} ) close upon depolarization, slowing membrane repolarization and helping maintain a more prolonged cardiac action potential.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ENDOTHELIAL CELLS</strong></td>
<td>( \text{K}_{ir} ) are involved in regulation of nitric oxide synthase.</td>
</tr>
<tr>
<td><strong>KIDNEYS</strong></td>
<td>( \text{K}_{ir} ) export surplus K⁺ into collecting tubules for removal in the urine, or reuptake K⁺ back into the body.</td>
</tr>
<tr>
<td><strong>NEURONS AND HEART</strong></td>
<td>G-protein activated IRKs ((\text{K}_{ir}, 3)) are important regulators, modulated by neurotransmitters.</td>
</tr>
<tr>
<td><strong>PANCREAS BETA CELLS</strong></td>
<td>( \text{K}_{ATP} ) channels control insulin release.</td>
</tr>
</tbody>
</table>
“Leak” or background K⁺ channels

This type of potassium channel is formed by two homodimers that create a channel that leaks potassium out of the cell. They are all voltage-independent and can be opened by heat, membrane stretching, intracellular acidosis, and certain anesthetics. These channels are responsible for a high K⁺ conductance under basal state conditions and therefore contribute to resting potential.

- **TRAAK channels** are mechanically activated when there is a convex curvature in the membrane that alters the channel’s activity (membrane stretching).
- **TASK channels** are sensitive to changes in extracellular pH and inhibited by extracellular acidification.
- **TALK channels** are primarily expressed in the pancreas, and activated at alkaline pH.
Calcium is an ubiquitous intracellular messenger, controlling cellular processes ranging from gene transcription, muscle contraction and cell proliferation.

The Ca2+ signaling apparatus involves various channels, pumps, and transporters.
Calcium regulatory mechanisms

CALCIUM ENTRY ("ON") MECHANISMS
VDCCs - voltage-dependent calcium channel; ROC - Ligand-gated channels, SOCE - store-operated calcium entry; TRP - transient receptor potential channels; ASIC - acid-sensing ion channels; IEIC - inward excitotoxic injury current calcium-permeable channels; NCX - sodium-calcium exchanger (operating in entry mode).

CALCIUM INTRACELLULAR SEQUESTERING AND RECYCLING MECHANISMS
SERCA - Sarcoplasmic-Endoplasmic Reticulum Ca\(^{2+}\)-ATPase; RyR - ryanodine receptors.

CALCIUM EXIT ("OFF") MECHANISMS
PMCA - Calcium ATPase pump; NCX - sodium-calcium exchanger (operating in exit mode).
CALCIUM “ON” MECHANISMS: VOCCs

Voltage-Operated Calcium Channels are slightly permeable to Na (also called Ca\(^{2+}\)-Na\(^{+}\) channels), but their permeability to Ca\(^{2+}\) is about 1000-fold greater. At resting membrane potential, VOCCs are normally closed. They are activated (i.e., opened) at depolarized membrane potentials and this is the source of the "voltage-dependent" definition.

Activation of particular VOCCs allows Ca\(^{2+}\) to rush into the cell, which, depending on the cell type, results in activation of calcium-sensitive potassium channels, muscular contraction, excitation of neurons, up-regulation of gene expression, or release of hormones or neurotransmitters.
**Voltage-operated Ca\(^{++}\) CHANNELS (VOCCs)**

<table>
<thead>
<tr>
<th>TYPE</th>
<th>VOLTAGE</th>
<th>MOST OFTEN FOUND IN</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>L-TYPE CALCIUM CHANNEL</strong></td>
<td>HVA (high voltage activated)</td>
<td>Skeletal muscle, smooth muscle, bone (osteoblasts), ventricular myocytes (responsible for prolonged action potential in cardiac cell; also termed DHP receptors), dendrites and dendritic spines of cortical neurones. <strong>L-Type channel blockers are used as antihypertensive and antiarrhythmic drugs</strong></td>
</tr>
<tr>
<td><strong>P-TYPE CALCIUM CHANNEL</strong></td>
<td>HVA (high voltage activated)</td>
<td>Purkinje neurons in the cerebellum / Cerebellar granule cells</td>
</tr>
<tr>
<td><strong>N-TYPE CALCIUM CHANNEL</strong></td>
<td>HVA (high-voltage-activated)</td>
<td>Throughout the brain and peripheral nervous system.</td>
</tr>
<tr>
<td><strong>R-TYPE CALCIUM CHANNEL</strong></td>
<td>intermediate-voltage-activated</td>
<td>Cerebellar granule cells, other neurons</td>
</tr>
<tr>
<td><strong>T-TYPE CALCIUM CHANNEL</strong></td>
<td>low-voltage-activated</td>
<td>neurons, cells that have pacemaker activity, bone (osteocytes). <strong>T-Type channel blockers are used as antiepileptic and neuropathic painkiller drugs</strong></td>
</tr>
</tbody>
</table>
**CALCULUM INTRACELLULAR RECYCLING MECHANISMS**

**Store-operated Ca$^{2+}$ Channels (SOCC)**
SOCC can be activated by any procedure that empties the stores.

**Receptor-operated Ca$^{2+}$ Channels (ROC)**
- nACh R
- NMDA R, kainate R
- 5HT$_3$
- P2X

**Transient-receptor-potential Channels (TRP)**
activated by cAMP, cGMP, Arachidonic acid, sphingosine, ADPribose

**InsP3-Receptor-Ca$^{2+}$-Channels**
allow Ca$^{2+}$ release form intracellular stores

**Ryanodine receptors (RYR)**
activated by cADPR
- Ryr1 (muscle cells)
- Ryr2 (heart)
- Ryr3 (neurons)
**CALCIUM “OFF” MECHANISMS**

**Na+/Ca²⁺ exchanger (NCX1-3)**
is an antiporter membrane protein that removes Ca²⁺ using the energy from the electrochemical gradient of Na⁺. The NCX removes 1 single Ca²⁺ ion in exchange for the import of 3 Na⁺ ions. **Low affinity but high speed** (Ca/Na 1:3)

**SERCA**  
(Sarcoplasmic-Endoplasmic Reticulum Ca²⁺-ATPase)
It is a Ca²⁺ ATPase that transfers Ca²⁺ from the cytosol of the cell to the lumen of the SR at the expense of ATP hydrolysis during muscle relaxation. The pump transports 2 Ca²⁺ ion/1 molecule of ATP hydrolysed and it is CaM-independent (Ca/ATP 2:1)

**PMCA (Plasma Membrane Ca²⁺ ATPase)**
the plasma membrane pump is powered by the hydrolysis of ATP, with a stoichiometry of 1 Ca²⁺ ion/1 molecule of ATP hydrolysed. **High affinity but low speed.** Its activity is modulated by the calmodulin (CaM) protein (Ca/ATP 1:1)