

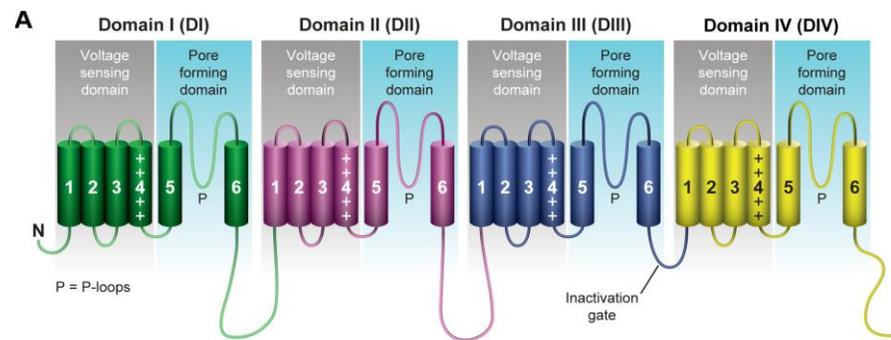
In 'excitable' cells the operation of specialized membrane ion channels allows the generation of action potentials

Na_v channels initiate and conduct action potentials in nerve and muscle, they set the threshold for action potential generation

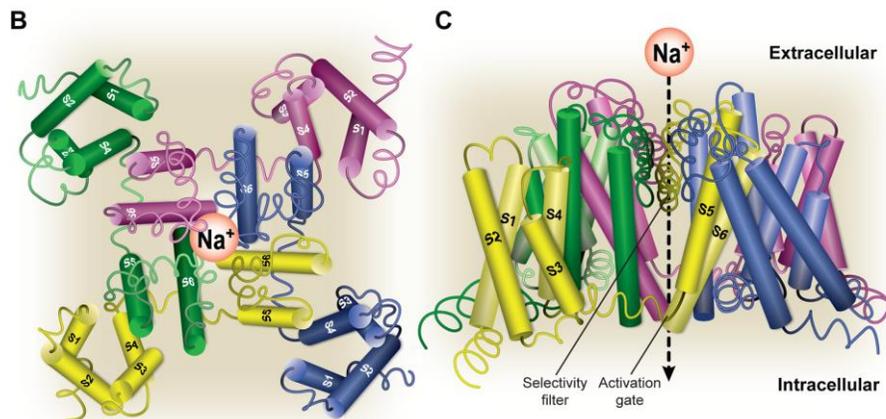
Voltage-gated Na⁺ channel-

1 pore forming α subunit associated to 1 or 2 β subunits

Domains, segments, loops..



The intracellular link between **DIII** and **DIV** underlies 'fast' inactivation



S4 is the **voltage sensor**

outer pore (AA link between the S5 and S6 segments in each domain = **selectivity filter**)

β 1- β 4 subunits modulate opening and inactivation kinetics, channel trafficking

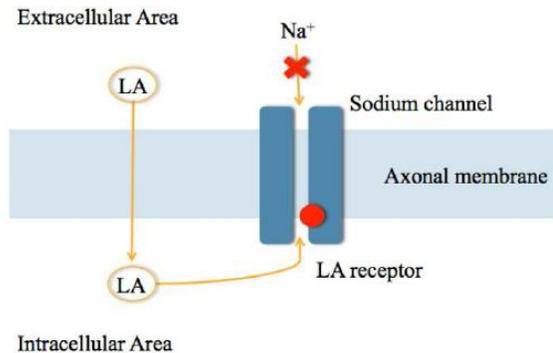
Na⁺ channels are molecular targets for a broad range of natural **neurotoxins** isolated from the venoms of sea anemones, cone snails, spiders, scorpions.

Local anaesthetic to anaesthetise the gums



Lidocaine: drug affinity is «voltage- and use-dependent»

Strong binding to the open state



Tetrodotoxin, TTX (puffer fish) binds to the **outer pore**

<p>A</p> <p>Tetrodotoxin</p>	<p>B</p> <p>Batrachotoxin</p>	<p>C</p> <p>Anthopleurin B</p>
<p>D</p> <p>μ-Conotoxin KIIIA</p>	<p>E</p> <p>ProTx-II</p>	<p>F</p> <p>OD1</p>

Different sodium channel isoforms (Nav1.1 – Nav1.9) exhibit unique biophysical properties, protein expression, and tissue distribution

	Tissue	Na _v Subtype	Effect of Na _v Dysfunction on Physiology
A	Central nervous system	1.1, 1.2, 1.3, 1.6	Epilepsy, migraine, autism, ataxia ³⁶
B	Retina	1.8, 1.9	Altered visual processing ⁶²
C	Olfactory sensory neurons	1.7	Anosmia ^{40,42}
D	Sensory neurons and vagal sensory neurons innervating airways	1.7, 1.8, 1.9	Cough ^{36,68}
E	Heart muscle	1.5, 1.8	Brugada syndrome, QT syndrome, atrial fibrillation ^{66,67}
F	Nerves, musculature involved in ventilation	TTX-s Na _v s	Respiratory cessation (TTX poisoning) ⁶⁹
G	Pancreatic β-cells	1.7	Diabetes ³⁶
H	Skeletal muscle	1.4	Hyperkalaemic periodic paralysis, paramyotonia congenita, hypokalaemic periodic paralysis ³⁶
I	Skin	1.7, 1.8	Pain disorders, paroxysmal itch ^{37,39}
J	DRG neurons	1.6, 1.7, 1.8, 1.9	Pain disorders, paroxysmal itch ^{37,39,51}
K	Metastatic cancer cells	1.1-1.9 and β-subunits	Ovarian, cervical, prostate, breast, colon, small cell lung cancer, melanoma, lymphoma ^{35,70,71}

Nav1.5 SCN5A TTX **Resistant** Heart muscle
 Nav1.8 SCN10A TTX **Resistant** PNS sensory
 Nav1.9 SCN11A TTX **Resistant** PNS (DRG)

To date, nine Nav1 channel subtypes have been cloned
 50% homology

β subunits modulate Na^+ channels inactivation kinetics

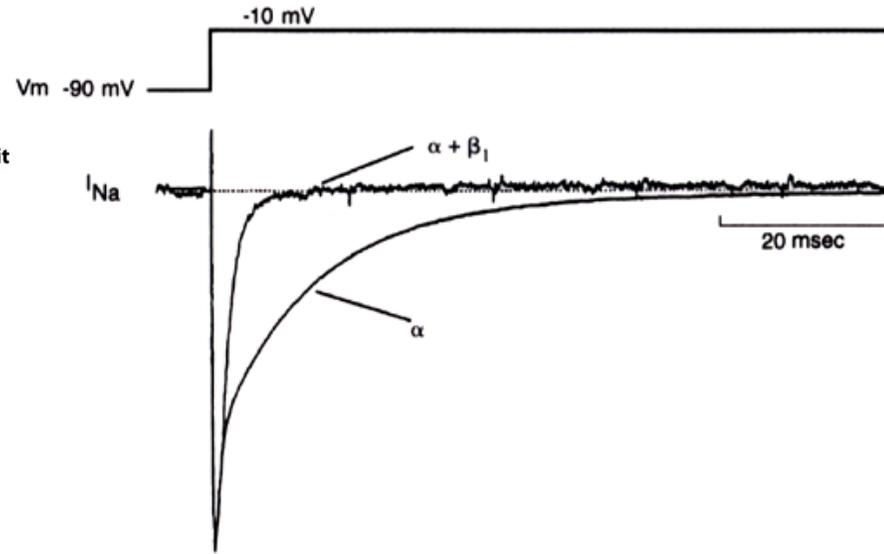
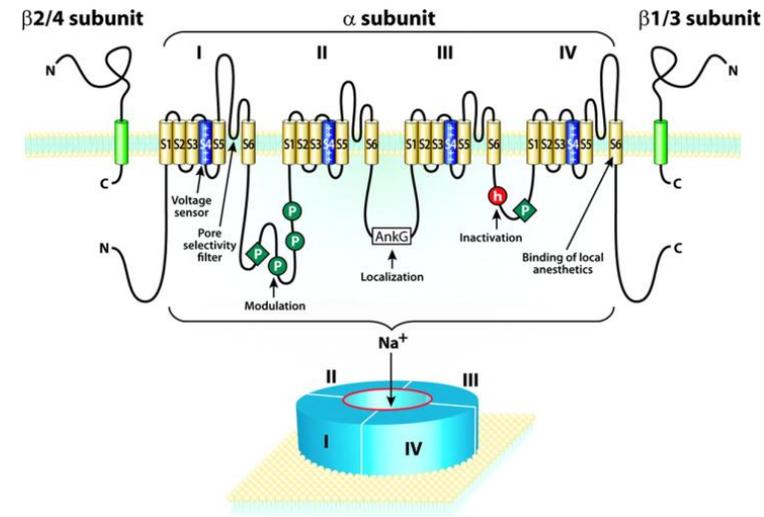
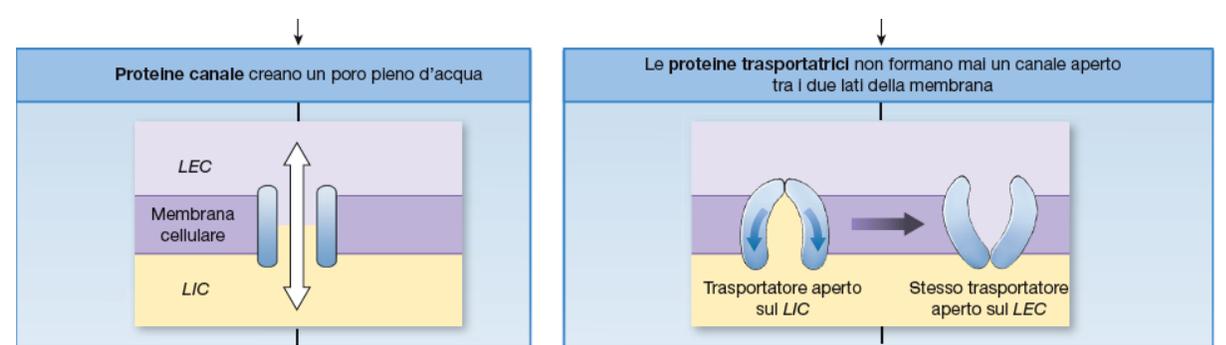


FIGURE 5.1 THE β -SUBUNIT REGULATES Na^+ CHANNEL INACTIVATION

Na^+ currents recorded from two different *Xenopus* oocytes expressing either the α -subunit of the rat Na^+ channel alone, or the α -subunit in combination with the β_1 -subunit. The traces have been normalized to the peak current level. From Ji *et al.* (1994).



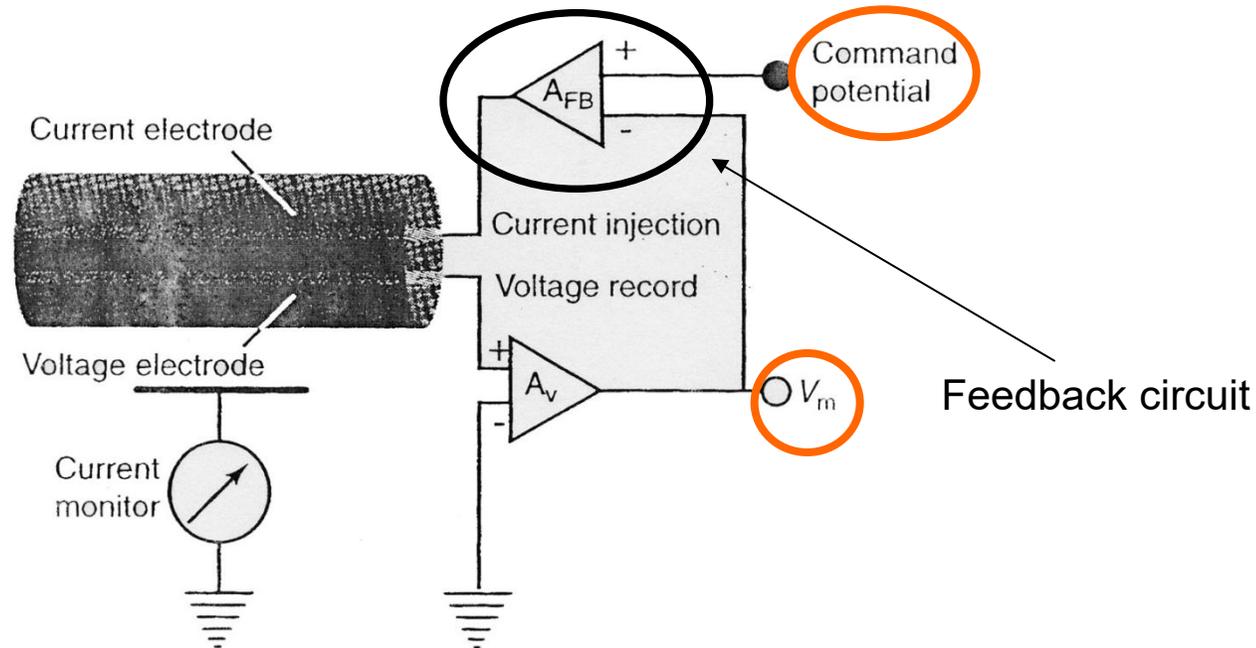
Ion channels & Transporters

In the CHANNEL, the electrostatic bond that forms between the selectivity filter and the ion is weaker than the bond that forms between the transported particle and the active site in TRANSPORTER proteins.

The duration of the bond between the ion and the channel is very short. The operating cycle of an ion channel is extremely fast 10^4 - 10^5 ioni / sec vs 10^2 particles / sec for the carriers.

Experimental arrangement

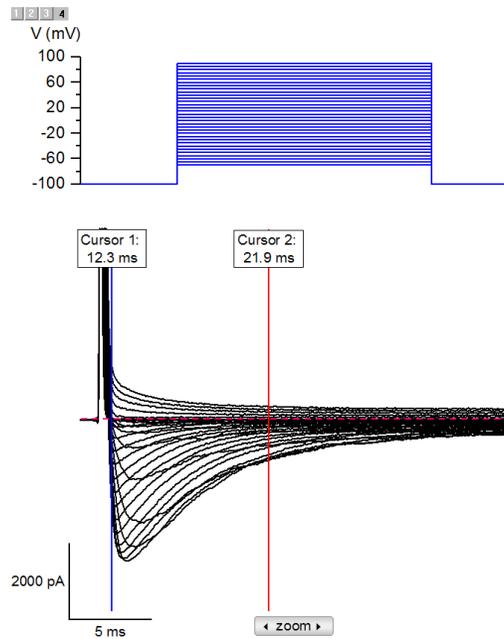
Two-electrode voltage-clamp experiments on squid axons



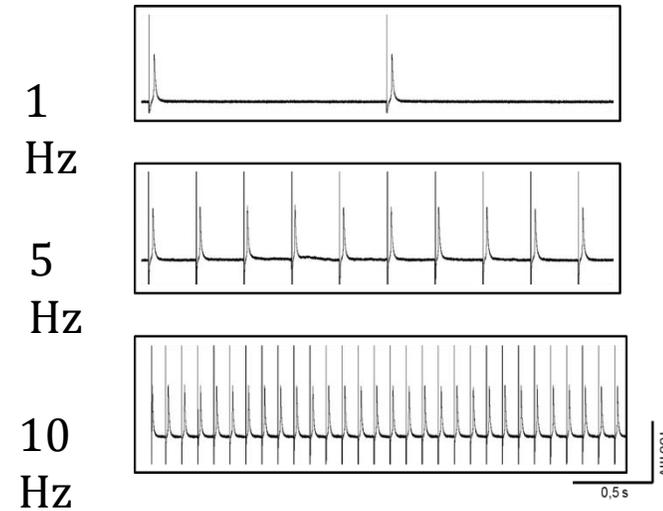
Two silver wires are inserted longitudinally. One of the wires provides a measure of the membrane potential, V_m inside the fiber with respect to that of the seawater (which is grounded); the other to inject current in the axon. **These electrodes are connected to a feedback circuit to compare the V_m with the V_c , set by the person doing the experiment. If V_m is different by V_c , output current is injected in the axon to remove the voltage difference between the two inputs.** If the circuitry is properly designed, the change in V_m is achieved within a few microseconds. The delivered current is equal to the current flowing through the channels and it is that measured by the experimenter.

Functional properties of ion channels are identified with electrophysiological studies using the *voltage-clamp* and *current-clamp* modes.

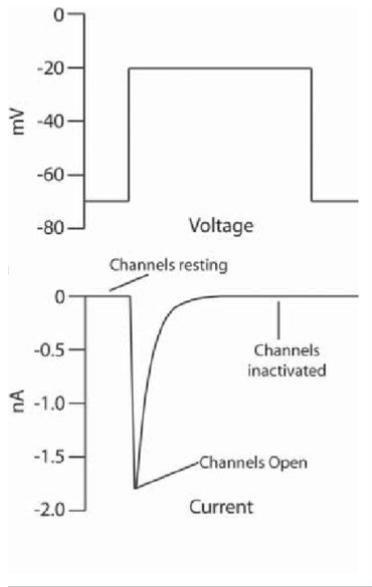
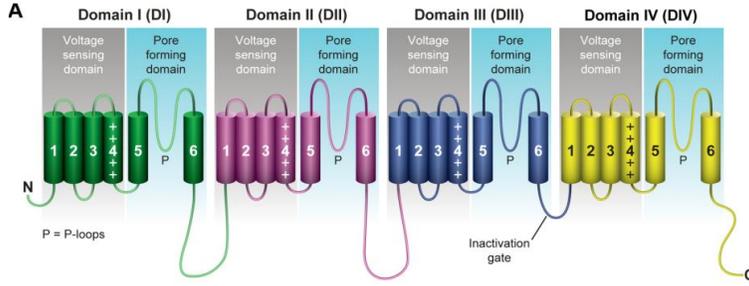
Voltage-clamp



Current clamp

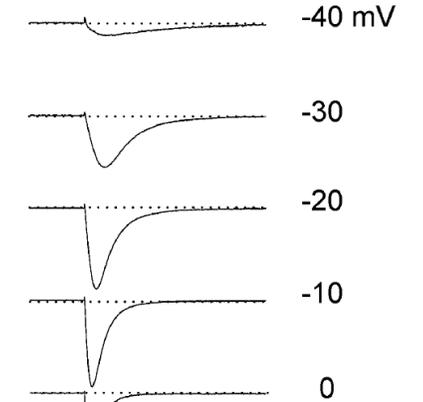
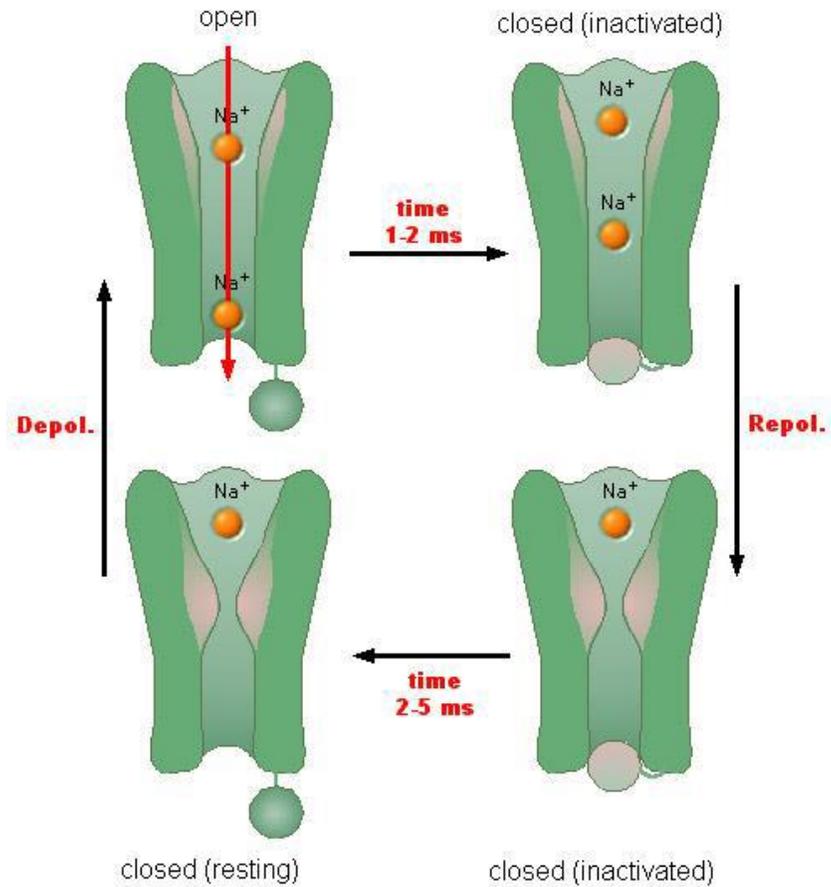


The P_o of Na^+ channel is strongly time-dependent

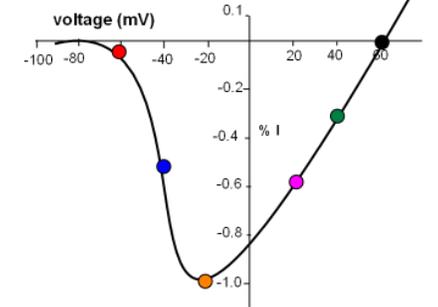


Voltage-clamp

closed-open-inactivated states

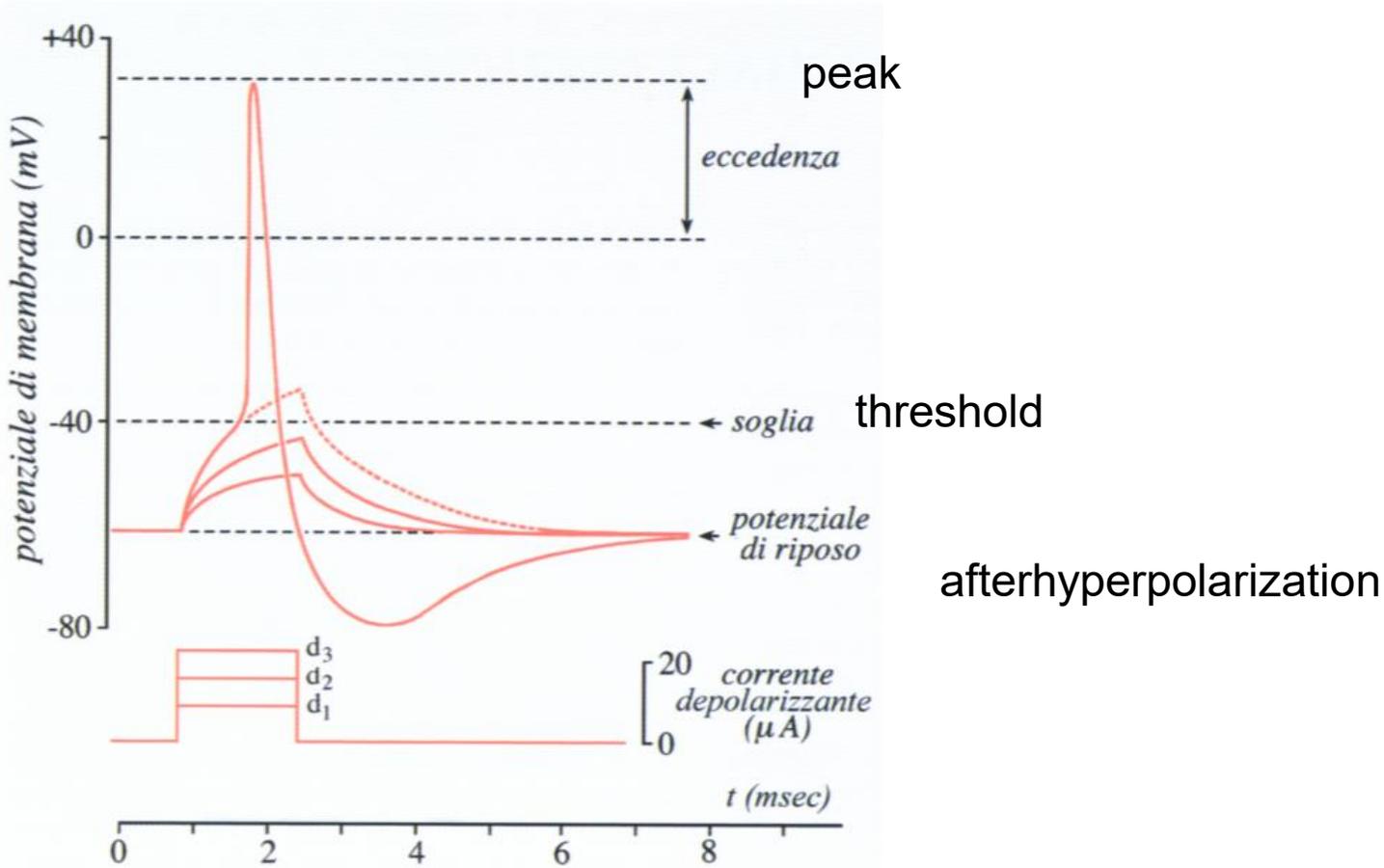


100 pA
1 ms



activation and inactivation gates alternate keeping the channel “conducting and non conducting”

The action potential



Action potential in neurons

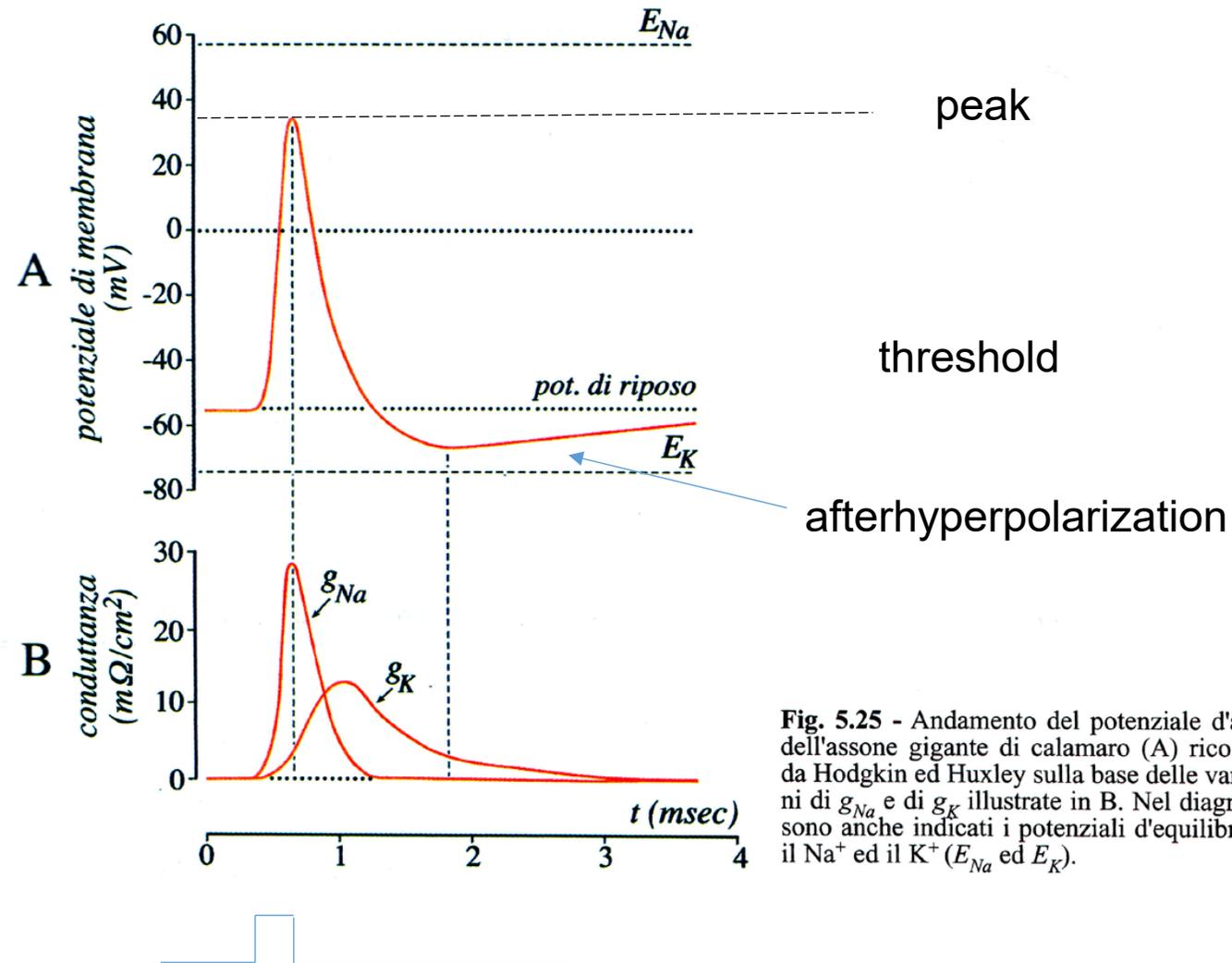
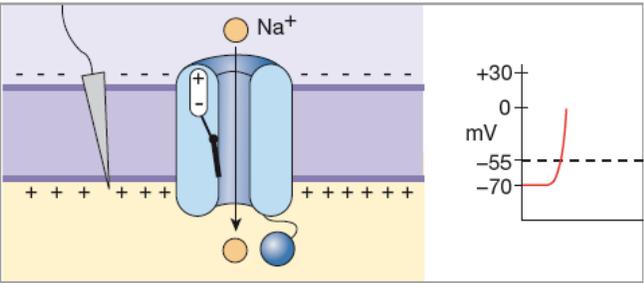
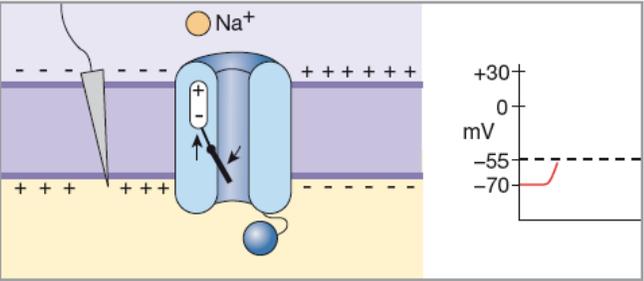
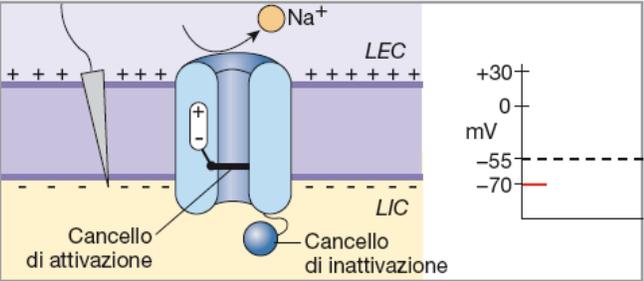


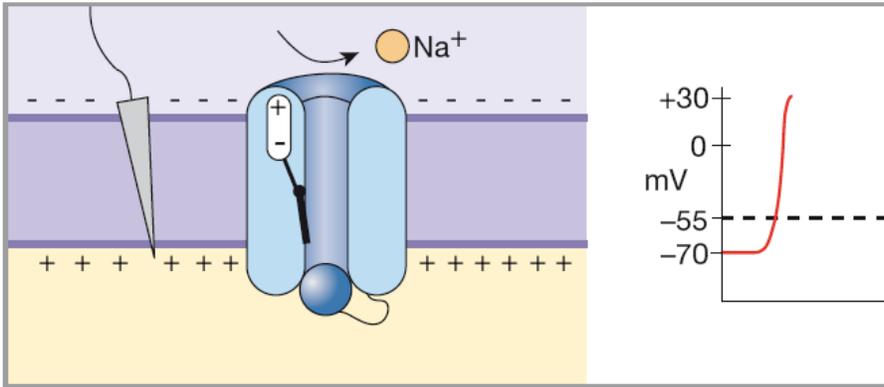
Fig. 5.25 - Andamento del potenziale d'azione dell'assone gigante di calamaro (A) ricostruito da Hodgkin ed Huxley sulla base delle variazioni di g_{Na} e di g_K illustrate in B. Nel diagramma sono anche indicati i potenziali d'equilibrio per il Na^+ ed il K^+ (E_{Na} ed E_K).

The overall ions that move across the membrane during the action potential tend to remain close to either surface. Their number is very small relative to the total concentrations in the cytosol and interstitial fluid.

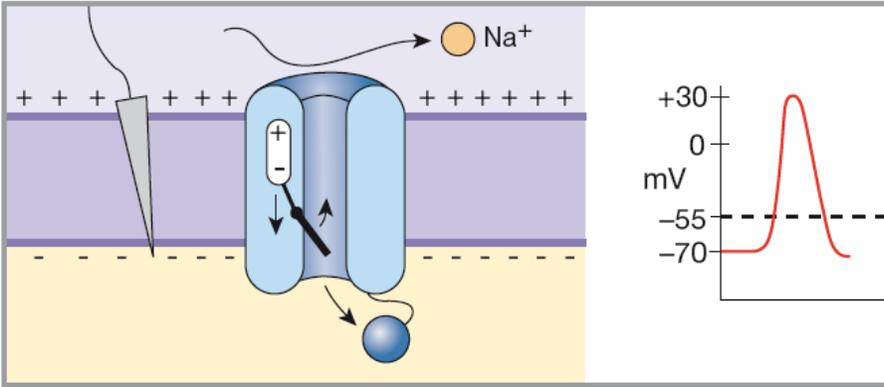
Na⁺ channel activation during action potential



Na⁺ channel inactivation



K⁺ conductance increase



Channel-related terms

'**activation**' is the process of opening the activation gate.

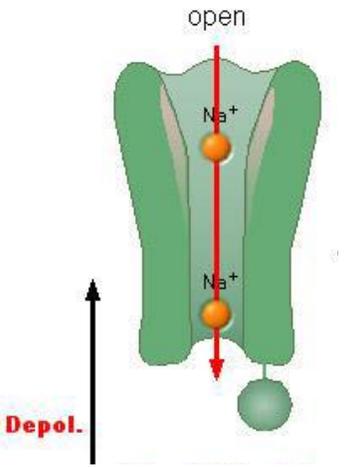
'**deactivation**' is the opposite process of the activation (gate closing)

'**Inactivation**' is the closing of the inactivation gate.

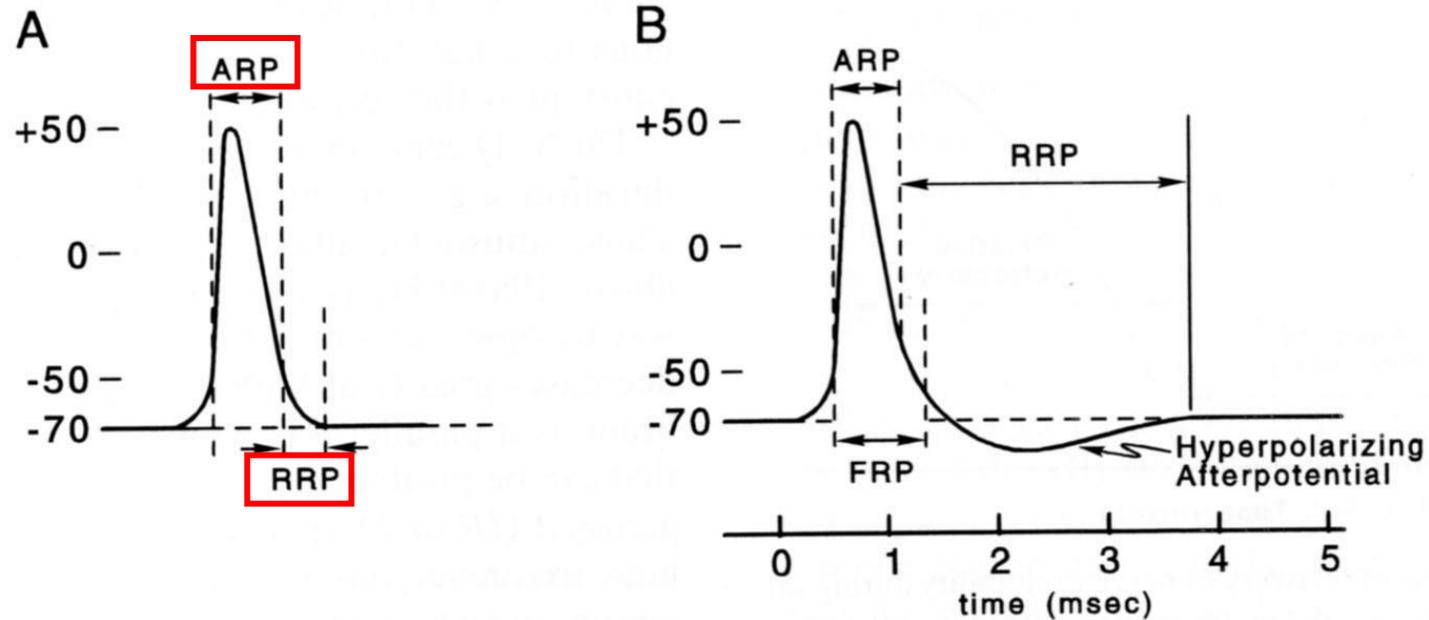
As with activation, inactivation can occur in response to the voltage inside the membrane becoming more positive, but inactivation is delayed in time compared to activation.

Both inactivation and deactivation are processes that lead to the channel becoming non-conducting.

'**Recovery from inactivation**' is the opposite of inactivation.



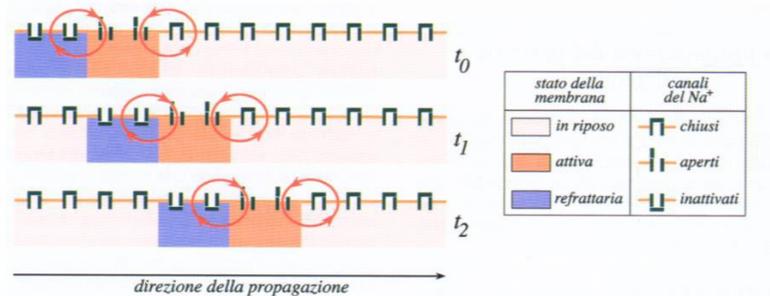
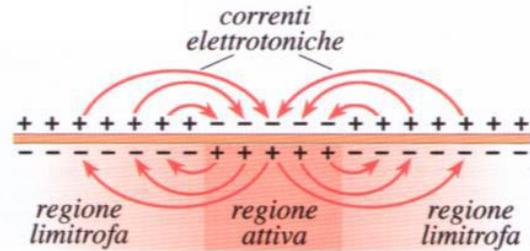
Absolute and relative refractory periods



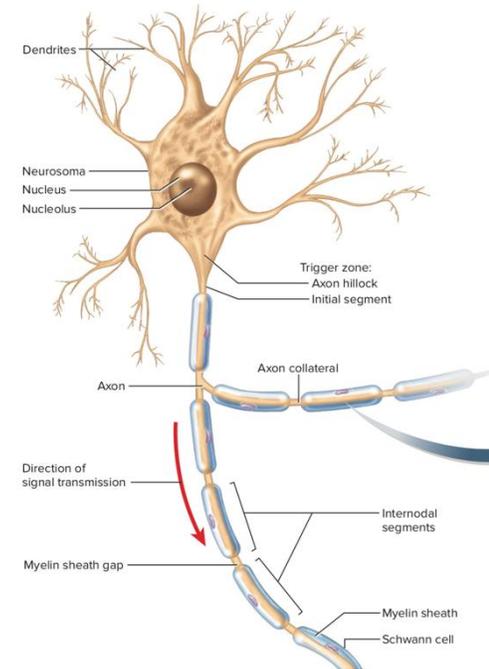
Cell Physiology, Academic Press

Once an AP is initiated, a finite and characteristic time must elapse before a second AP can be generated. This time interval is the **refractory period**.

Unidirectional action potential propagation



Taglietti, Casella, *Principi di fisiologia e biofisica della cellula*, La Goliardica



The action potential is self-propagating (depolarization of a small region of the membrane triggers the opening of adjacent sodium channels such that a wave of depolarization travels along the length of the cell).

The propagation of an action potential is **unidirectional**, because the absolute refractory period prevents the initiation of an AP in a region of membrane that has just produced an AP.

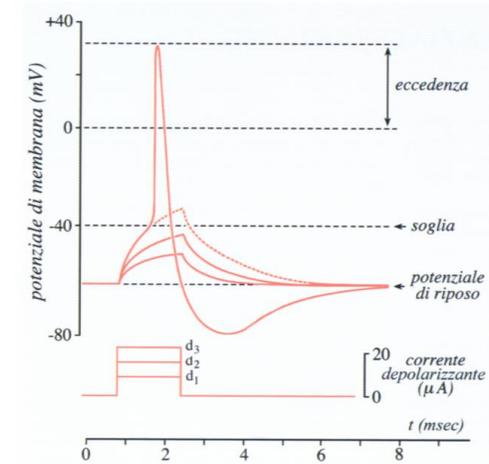
Speed of action potential propagation along neuronal axons is increased greatly by myelination.

Large-diameter myelinated axons in the human body can conduct action potentials at speeds of over 100 m/second

The key features of the action potential are that it is:

- (i) an all-or-none event, rather than a graded response;
- (ii) it is self-propagating, such that the wave of depolarization travels rapidly along the plasma membrane;
- (iii) it is transient, such that membrane excitability is quickly restored.

These features of the action potential allow rapid transfer of information along nerve axons in the Nervous System.



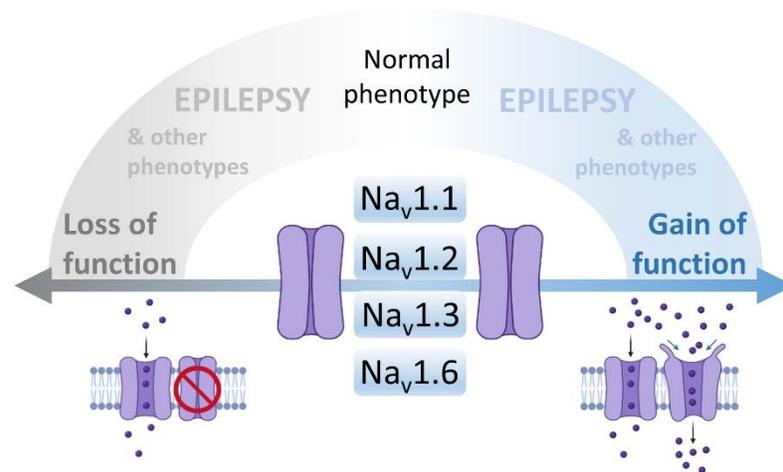
Mutation in AA sequence affects Na⁺ channel properties
changing cell excitability by influencing:

AP kinetics,
threshold,
peak,
rise time,
duration

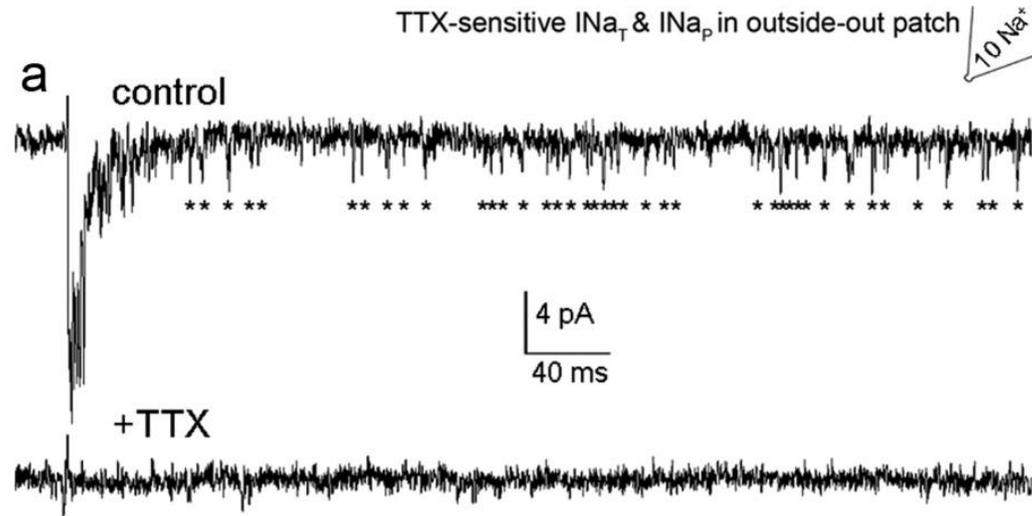
capacity to fire fastly.

The role of Na^+ channels in neurological, psychiatric and cardiovascular disorders is known, mutations in human genes encoding Nav channel subtypes have been linked to **channelopathies such as epilepsy, cardiac arrhythmias, and chronic pain syndromes.**

Non selective Na^+ channel blockers have been developed as therapeutic agents: anticonvulsant, antiarrhythmic, local anesthetics.



Rapid activation of a low-voltage activating “persistent” Na⁺ current



Hage & Salkoff, *J. Neurosci* 2012, 32: 2714-2721

Depolarization of many neurons results not only in the activation of a transient Na⁺ current (I_{Na}) but also in the rapid activation of a low-voltage activating “persistent” Na⁺ current (I_{NaP}) that plays a role in determining neuronal excitability and synaptic integration.

RILUZOLE highly reduces current I_{NaP}

Fast-inactivating, transient current through these channels plays a central role in initiation and propagation of action potentials ([Hodgkin and Huxley, 1952](#); [Stuart and Sakmann, 1994](#)). In addition, a more slowly inactivating, TTX-sensitive, “persistent” Na⁺ current (I_{NaP}) I_{NaP} operates in the subthreshold voltage range, where other large, voltage-gated conductances are not active ([Crill, 1996](#)).

Under certain circumstances, incomplete fast inactivation can generate persistent sodium currents.

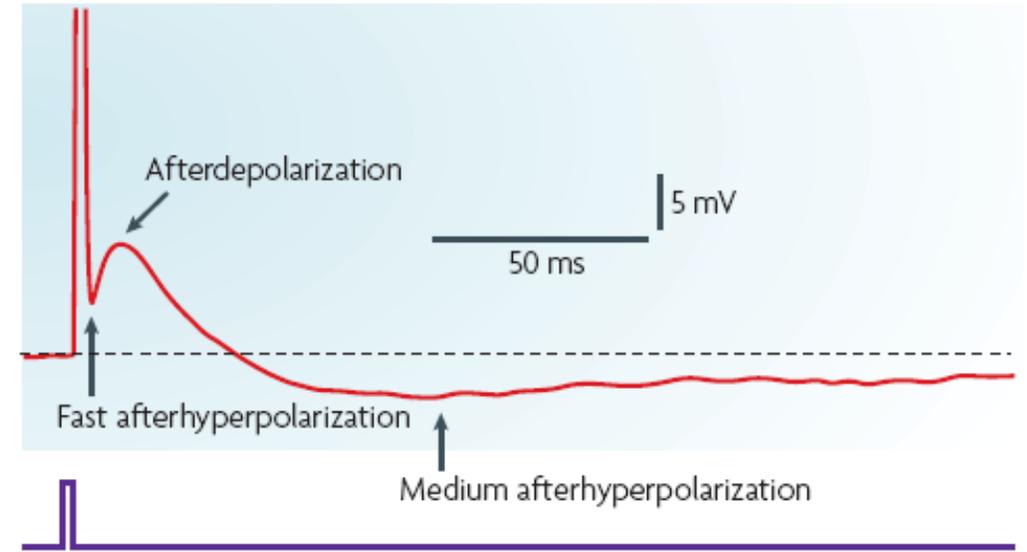
Subtype **Nav1.9** is known to give rise to low-threshold persistent I_{Na} **in sensory neurons**.

Also, **Nav1.4** and **Nav1.6** channels have been reported to generate persistent currents in **muscle fibers** and **Purkinje neurons**, respectively.

Mutations in voltage-gated Na^+ channels can cause defects in inactivation gating, **increasing persistent I_{Na}** leading to ataxia and epilepsy.

Functional consequences of I_{NaP}

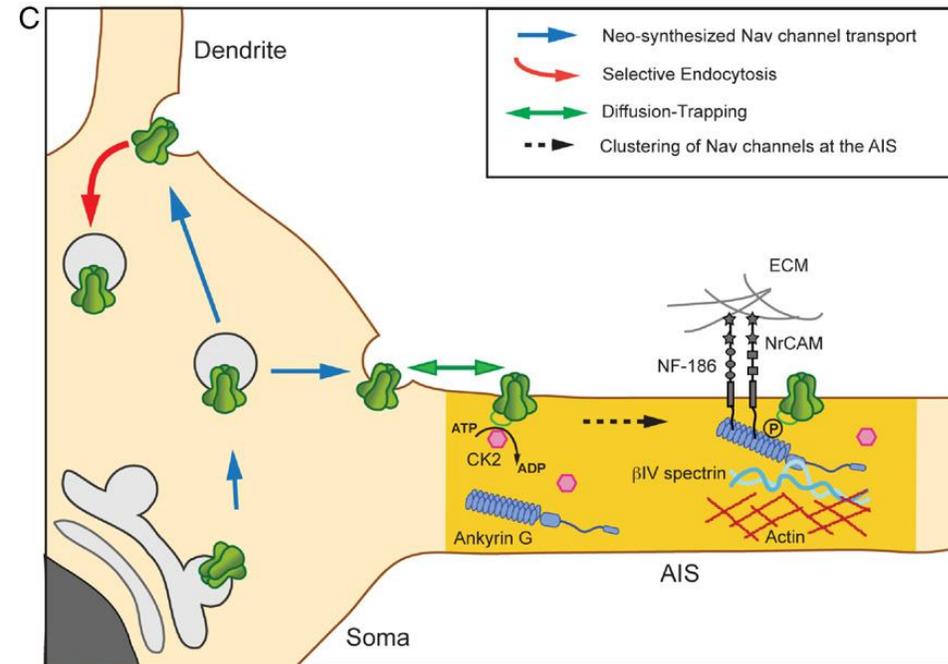
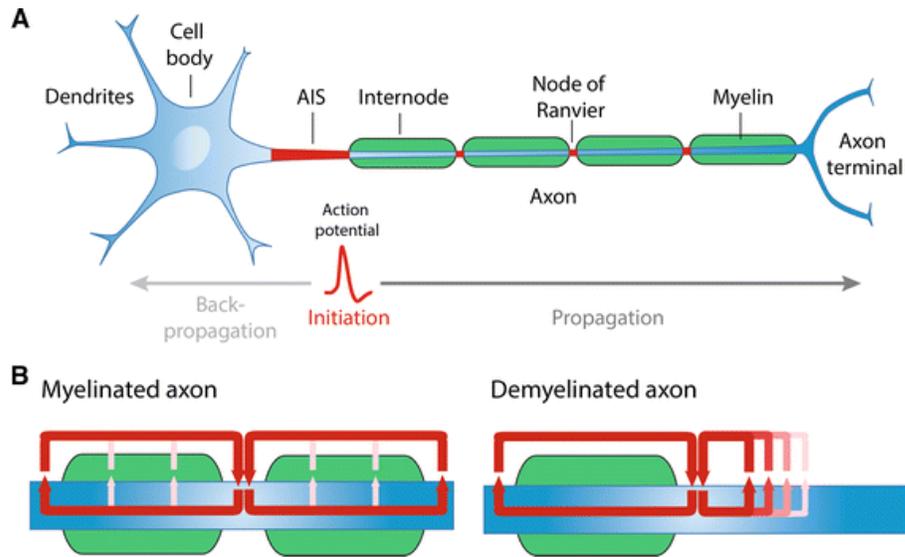
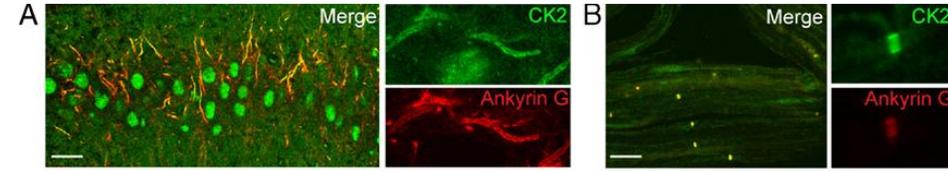
- Integration of synaptic potentials
- Acceleration of firing rates
- Promotion of subthreshold oscillations



Persistent Na⁺ conductances are the target of some antiepileptic drugs that reduce transient I_{Na} as well as the I_{NaP} and its ability to promote the high-frequency spike firing that occurs during seizures.

Dense clusters of voltage-gated sodium channels (Nav) have been found at the axonal initial segment and nodes of Ranvier underlying action potential generation and fast propagation

The core Na_v complex can interact with numerous other proteins that are important for trafficking, localization, and modulation of functional properties



Leterrier et al., 2010