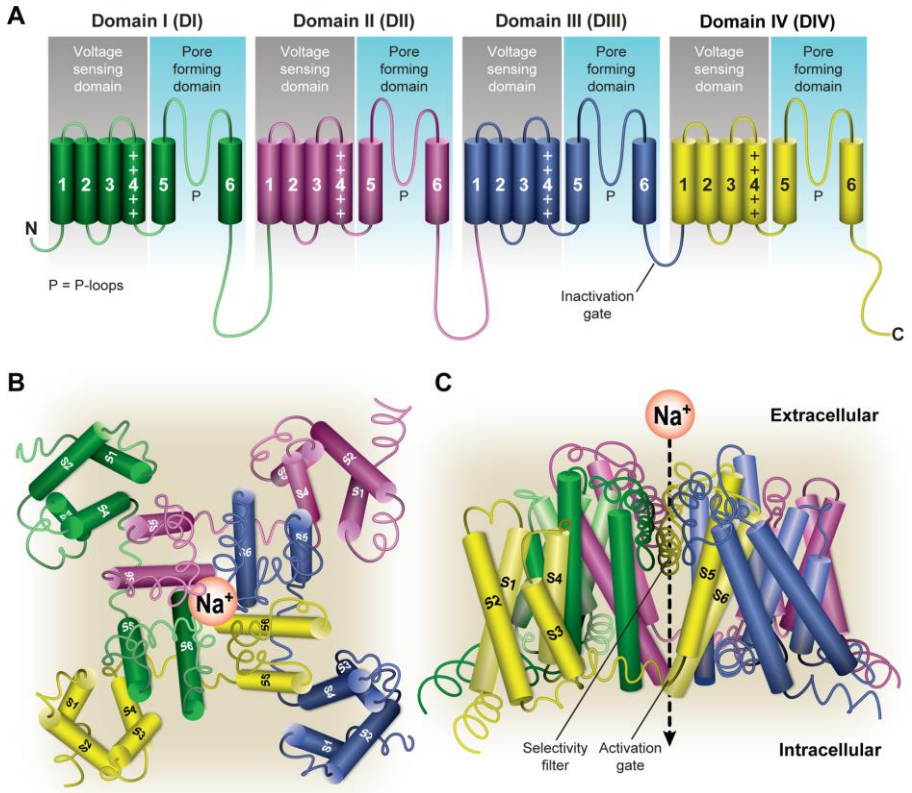
In ‘excitable’ cells the operation of specialized
membrane ion channels allows
the generation of action potentials

$Na_v$ 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 β subunities

Domains, segments, loops..




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

S4 is the **voltage sensor**

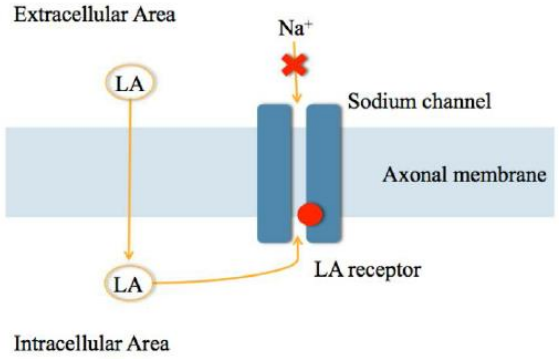
**outer pore** (AA link between the S5 and S6 segments in each domain = selectivity filter)

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

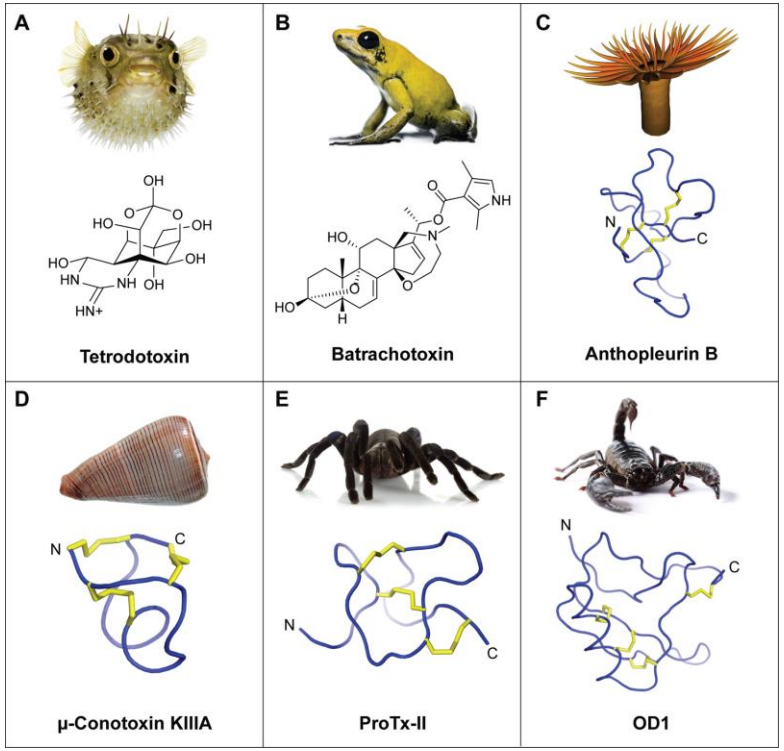
## Local anesthetics

«Drug affinity is voltage- and use-dependent

Strong binding to the open state




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



# Different sodium channel isoforms exhibit unique biophysical properties, protein expression, and tissue distribution

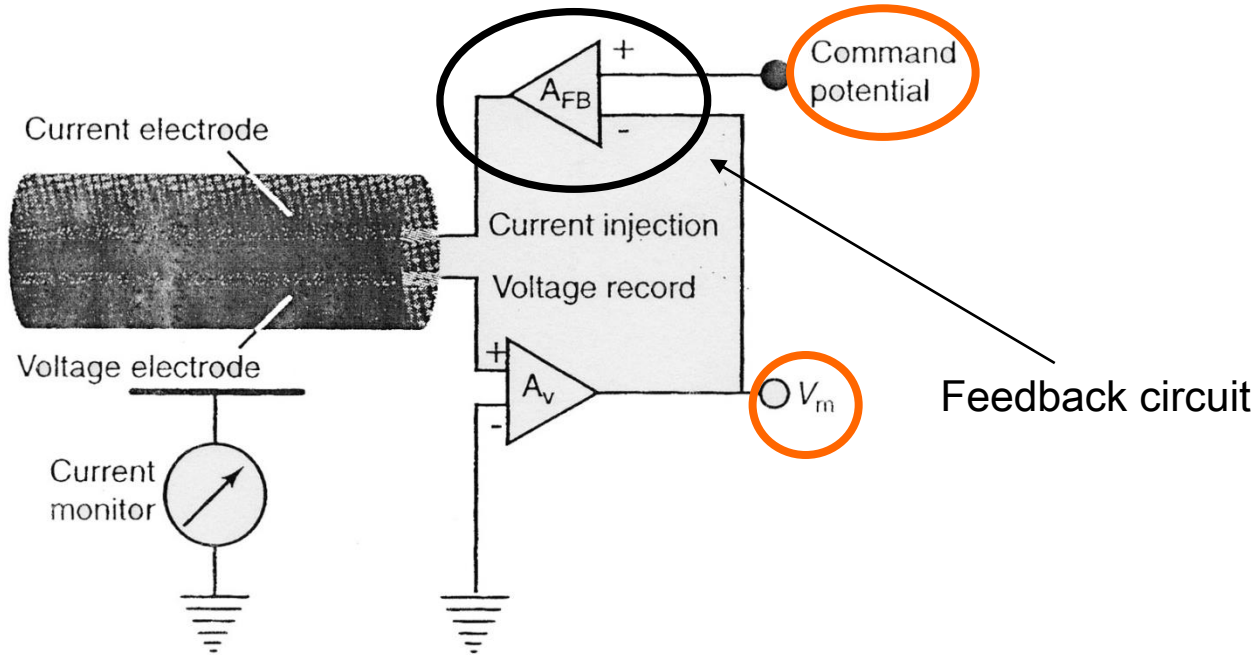
| | Tissue | $Na_v$ Subtype | Effect of $Na_v$ Dysfunction on Physiology |
|---|---|---|---|
| A | Central nervous system | 1.1, 1.2, 1.3, 1.6 | Epilepsy, migraine, autism, ataxia[36] |
| B | Retina | 1.8, 1.9 | Altered visual processing[62] |
| C | Olfactory sensory neurons | 1.7 | Anosmia[40,42] |
| 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] |

To date, nine Nav1 channel subtypes named Nav1.1 through Nav1.9 have been cloned
50% homology

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

*De Lera Ruiz & Kraus, J Med Chem, 2015*

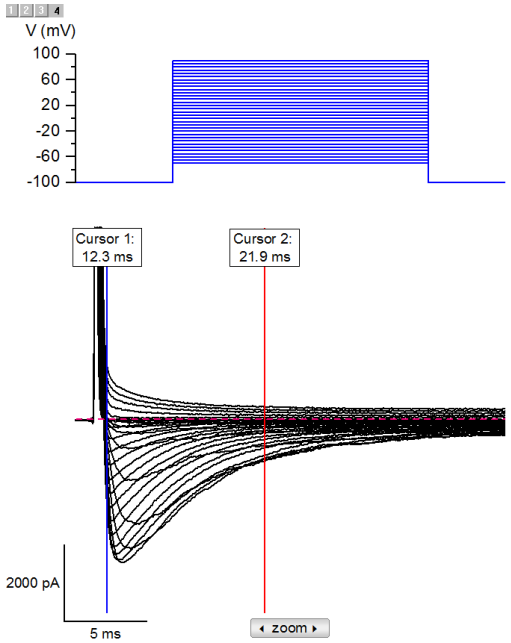
# Experimental arrangement
## **Two-electrode voltage-clamp** experiments on squid axons



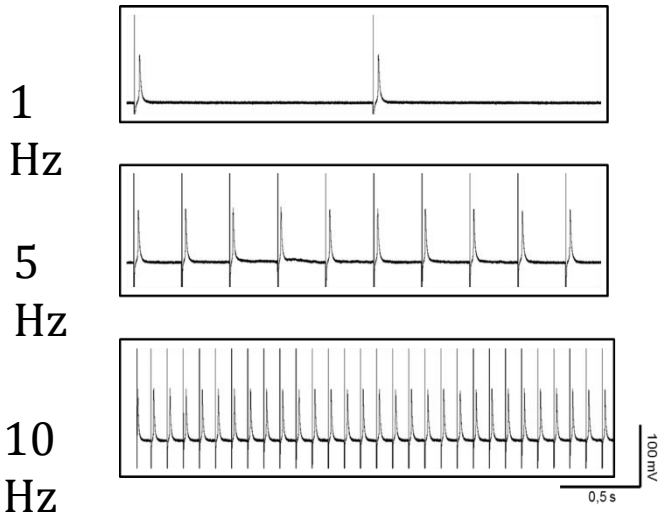

Two silver wires are inserted longitudinally. One of the wires provides a measure of the membrane potential, Vm inside the fiber with respect to that of the seawater (which is grounded); the other to inject current in the axon. These electrodes are connected to a feedback circuit to compare the Vm with the Vc, set by the person doing the experiment. If Vm is different by Vc, 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 Vm 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.

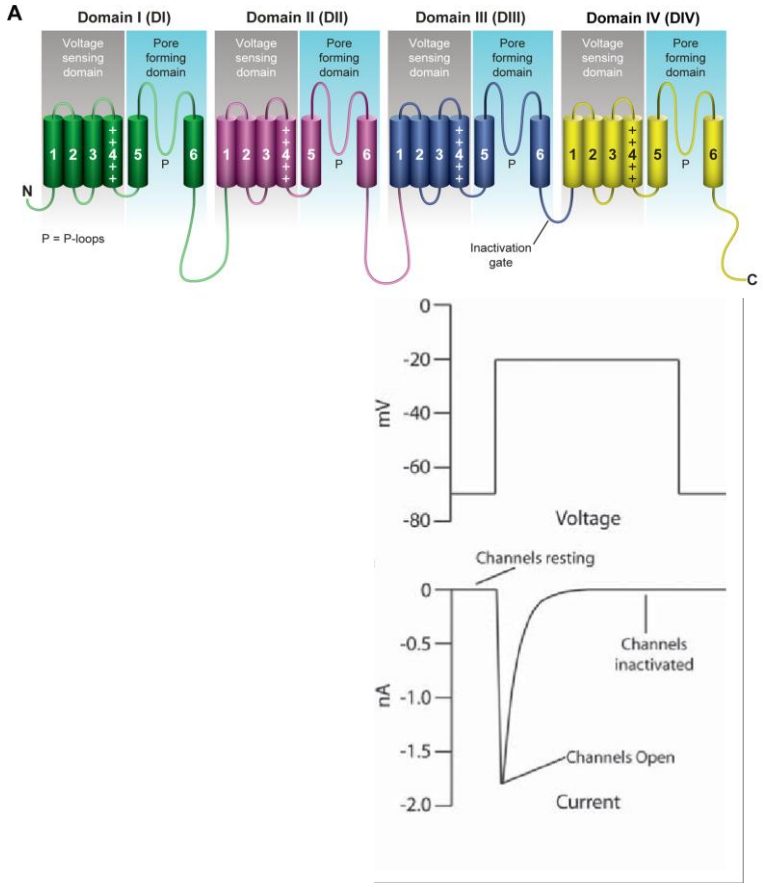
Voltage-clamp




Current clamp

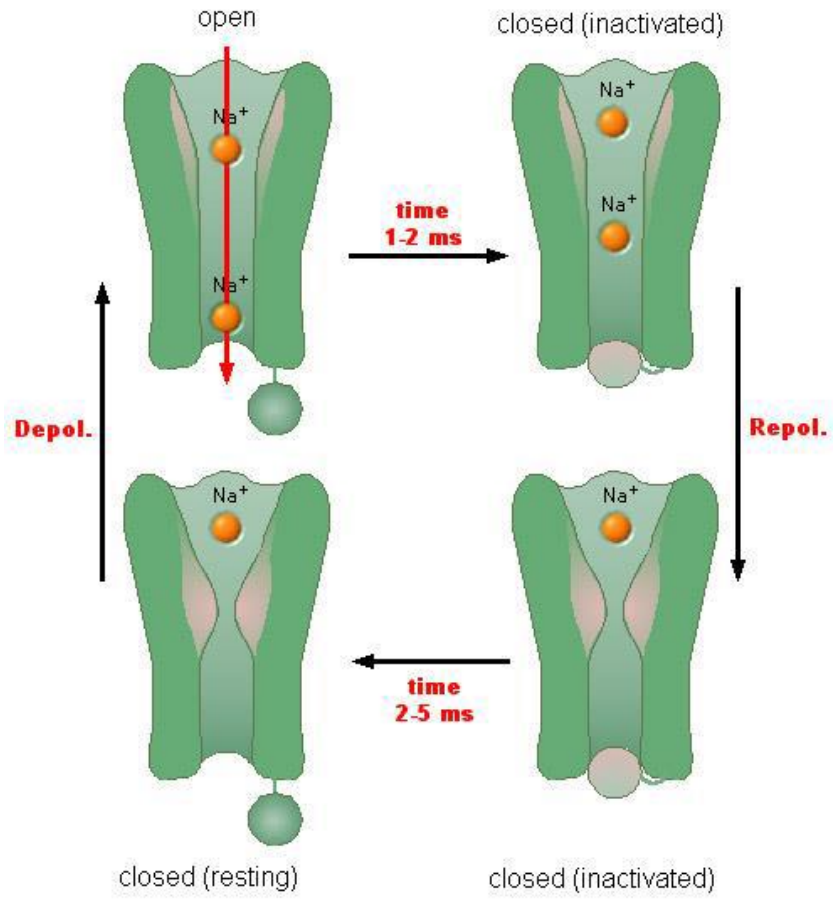


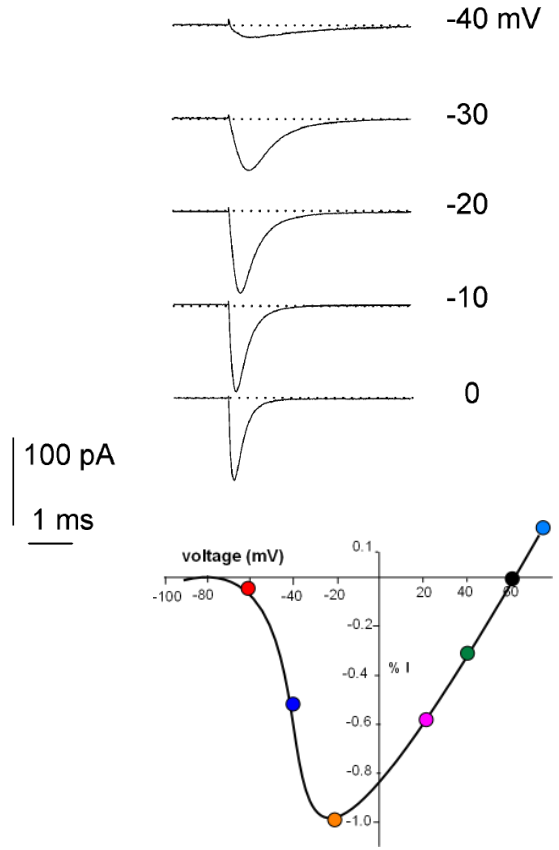
# The $P_o$ of $Na^+$ channel is strongly time-dependent




*Voltage-clamp*

closed-open-inactivated states

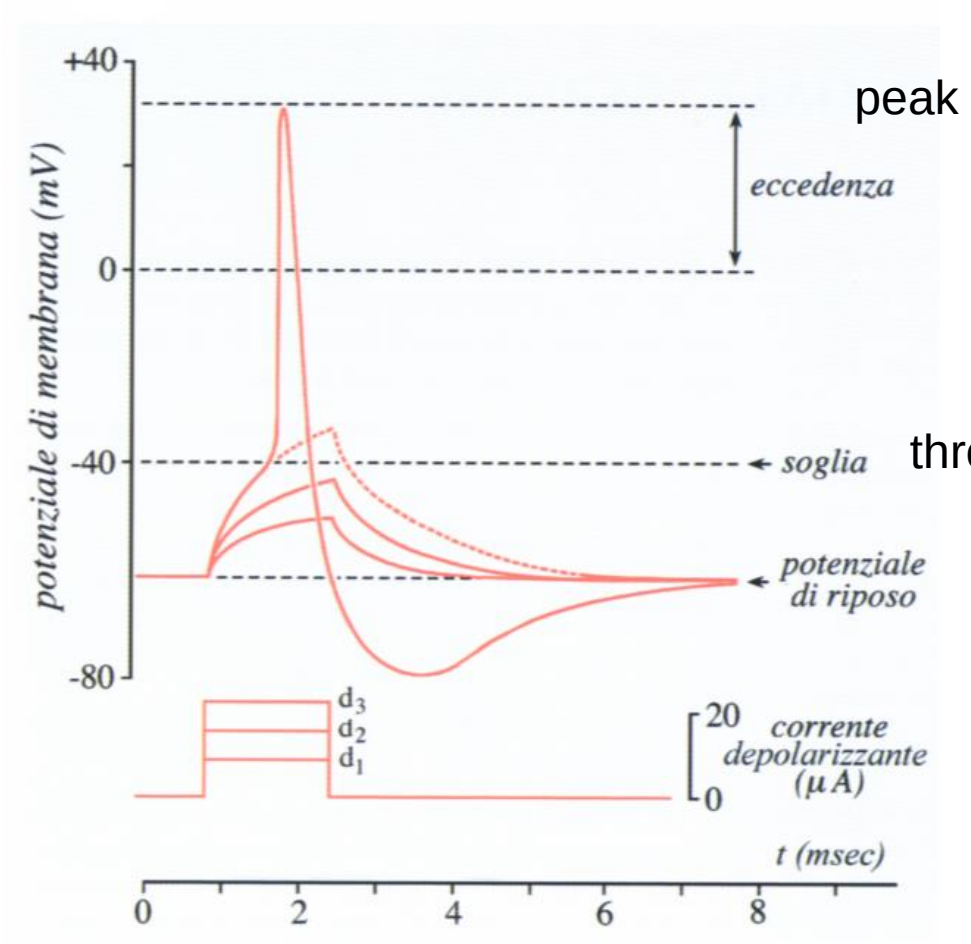



activation and inactivation gates alternate between keeping the channel “conducting and non condacting”

# The action potential

Threshold -55/-50 mV




peak

threshold

afterhyperpolarization

Action potential in neurons

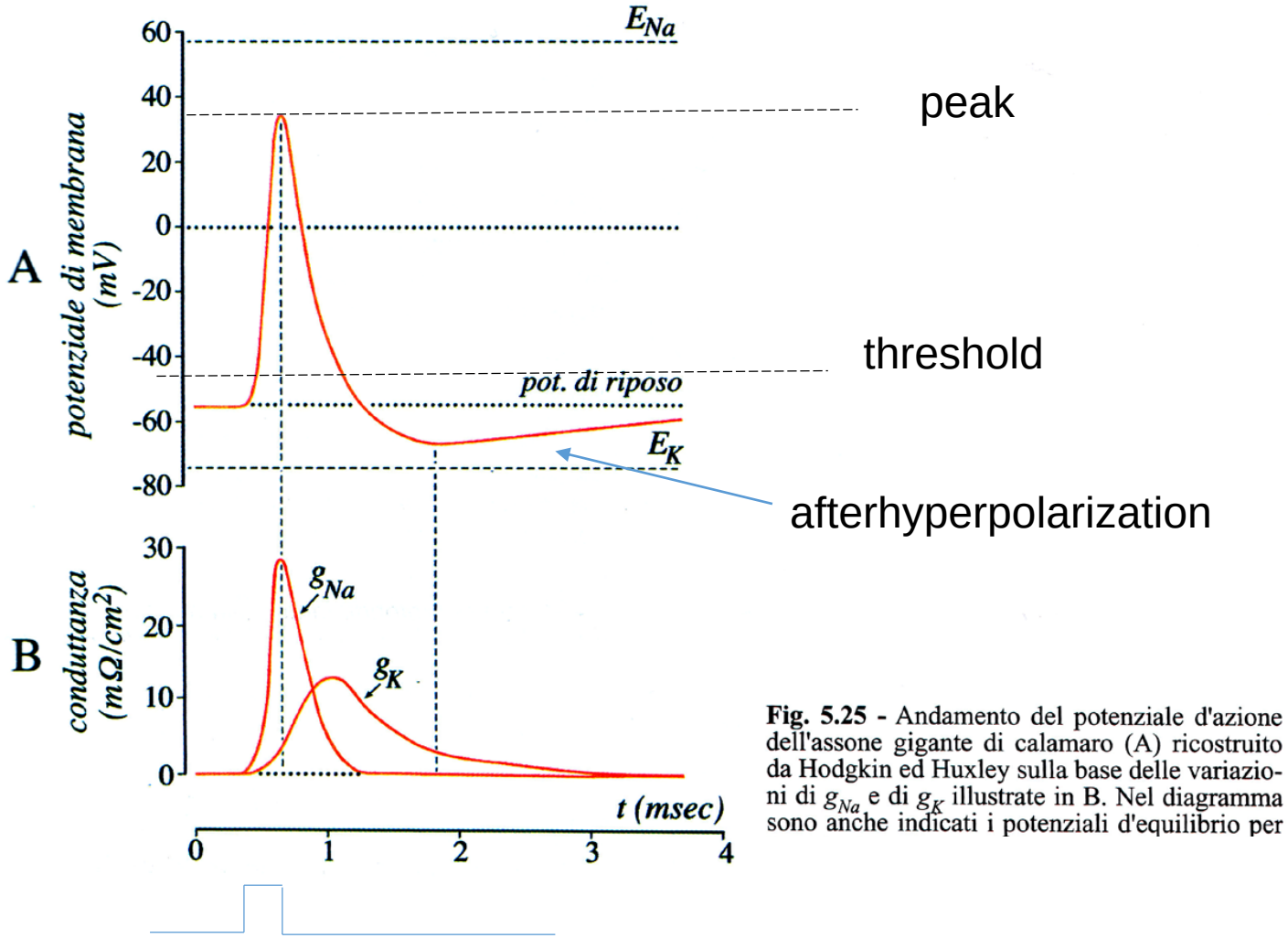
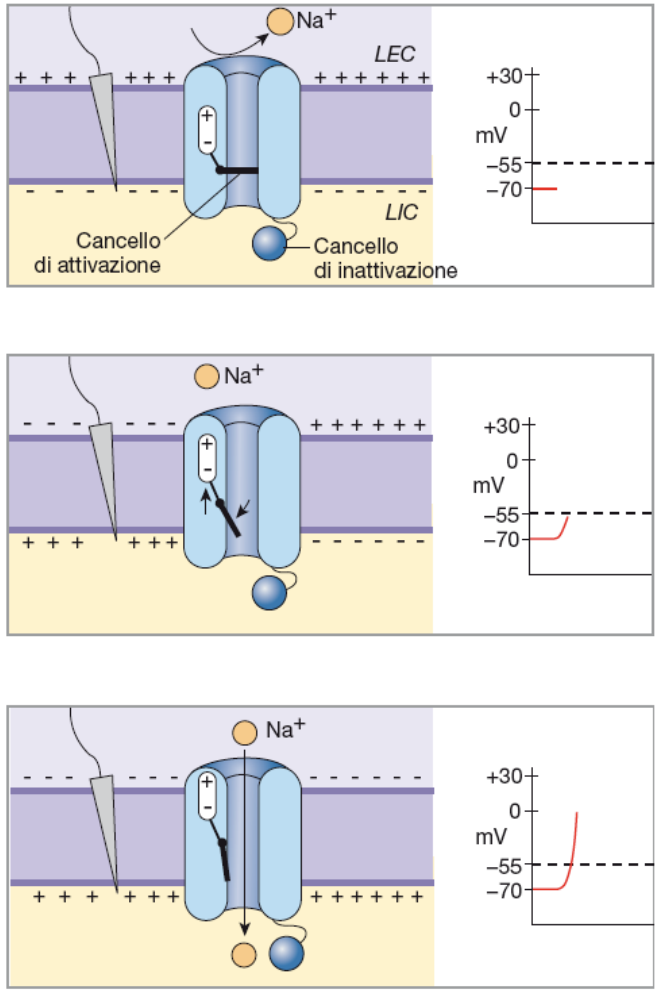


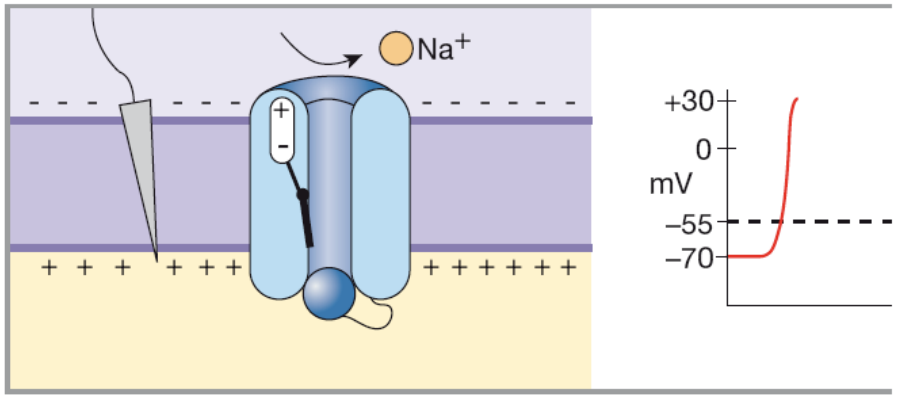

**Fig. 5.25 -** Andamento del potenziale d'azione dell'assone gigante di calamaro (A) ricostruito da Hodgkin ed Huxley sulla base delle variazioni di $g_{Na}$ e di $g_K$ illustrate in B. Nel diagramma sono anche indicati i potenziali d'equilibrio per

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.

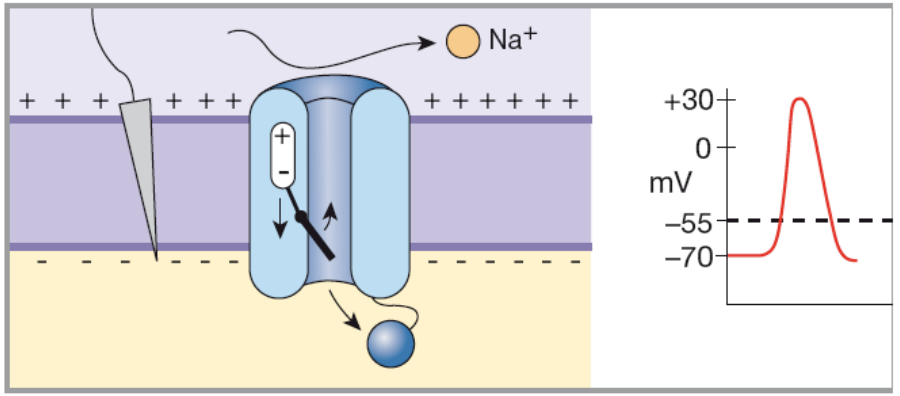
## $Na^+$ channel activation during action potential



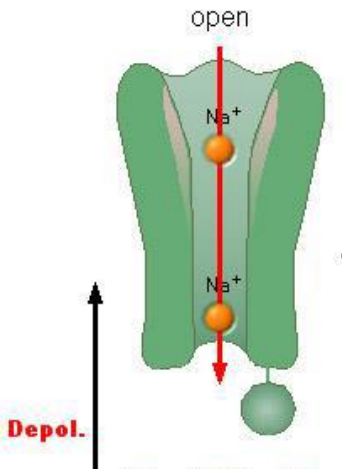

## $Na^+$ channel inactivation




## $K^+$ conductance increase



# Channel-related terms




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

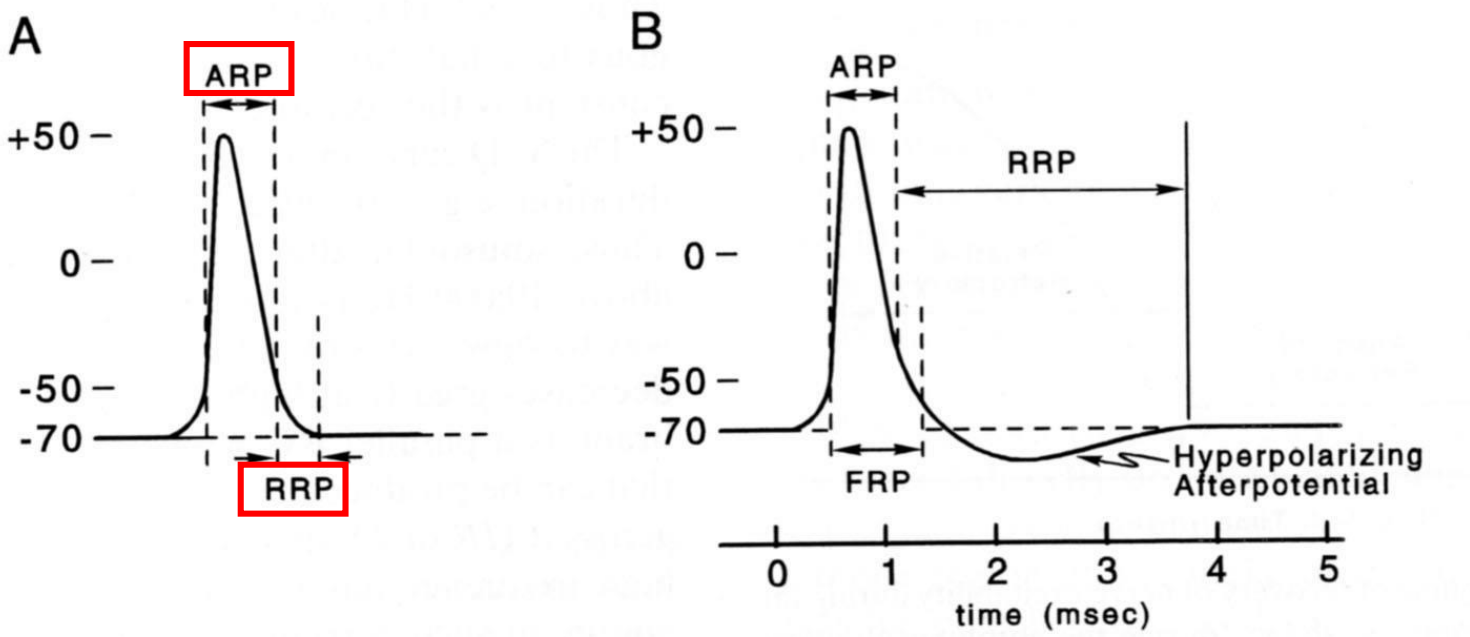
'deactivation' is the opposite process of the activation (gate closing)

'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.

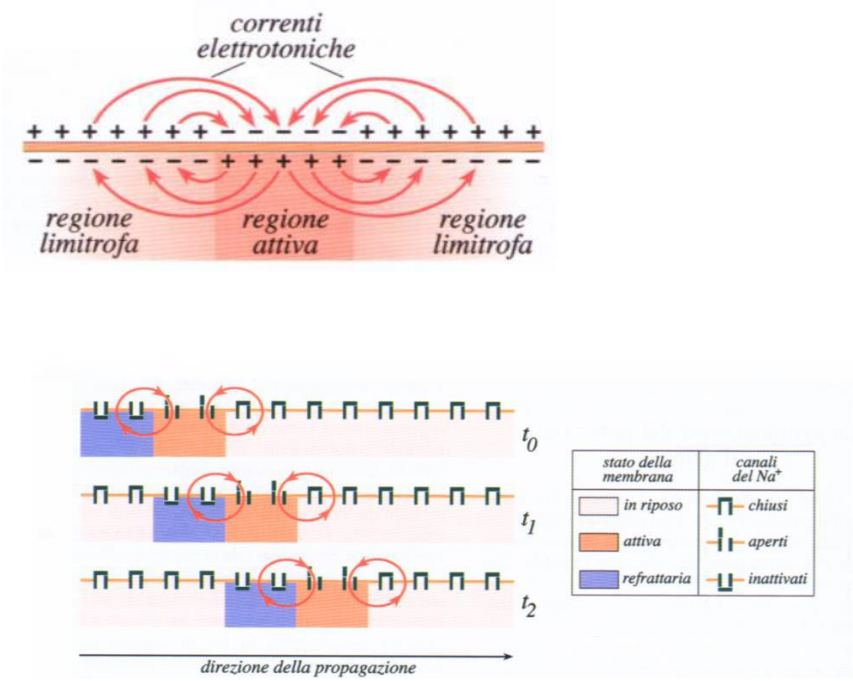
# Absolute and relative refractory periods



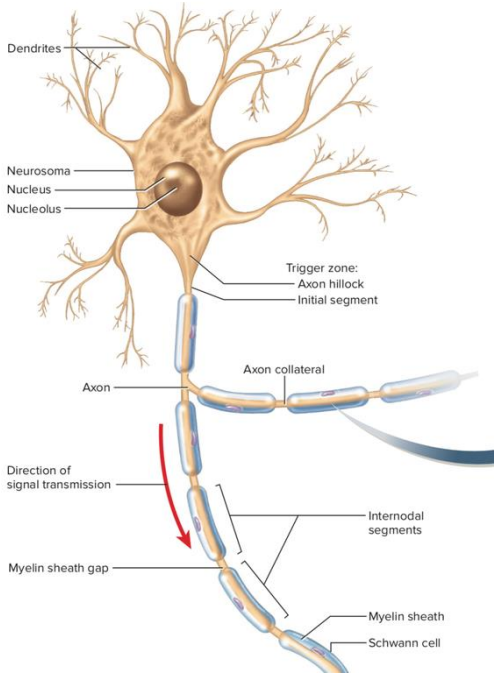

Once an AP is initiated, a finite and characteristic time must elapse before a second AP can be generated. This time interval is the refractory period.

*Cell Physiology, Academic Press*

# Unidirectional action potential propagation

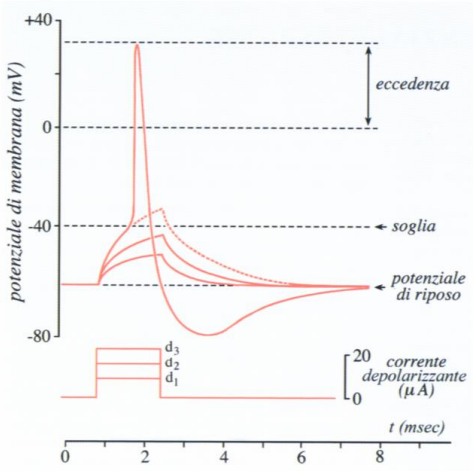



*Taglietti, Casella, Principi di fisiologia e biofisica della cellula, La Goliardica*




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 propogation 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.

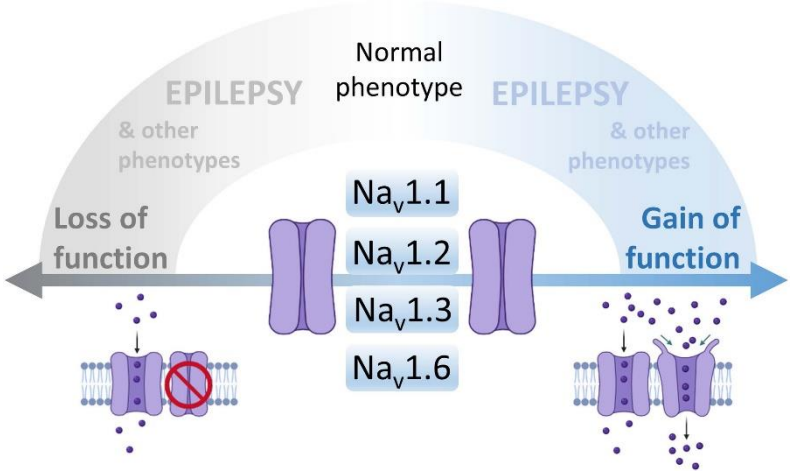
Mutation in AA sequence affects $Na^+$ channel properties
changing cell excitability by influencing:

AP kinetics,
threshold,
peak,
rise time,
duration

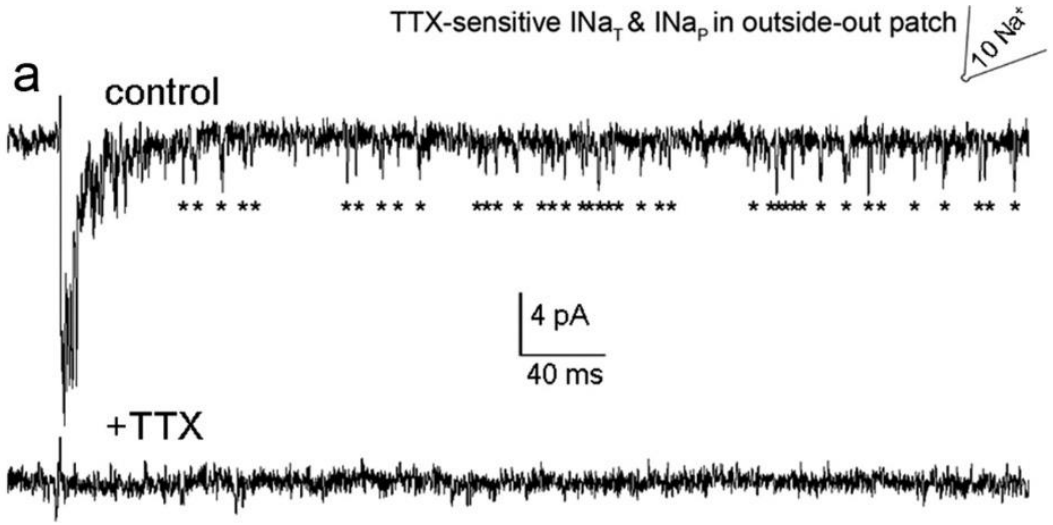
capacity to fire fastly.

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 vertebrate 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 $INa_P$

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, there is 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).

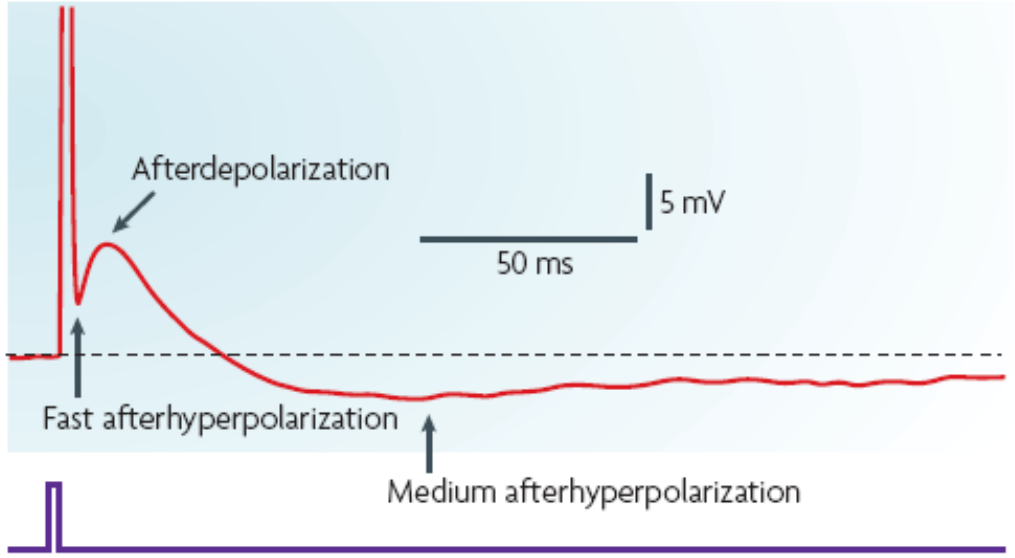
## Under certain circumstances, incomplete fast inactivation can generate persistent sodium currents.

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 INaP

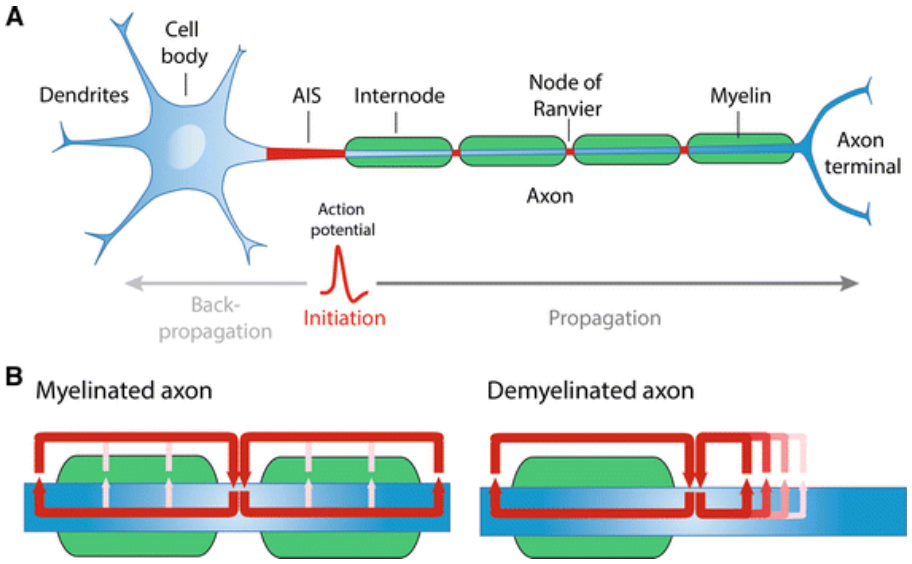
- Integration of synaptic potentials
- Acceleration of firing rates
- Promotion of subthreshold oscillations

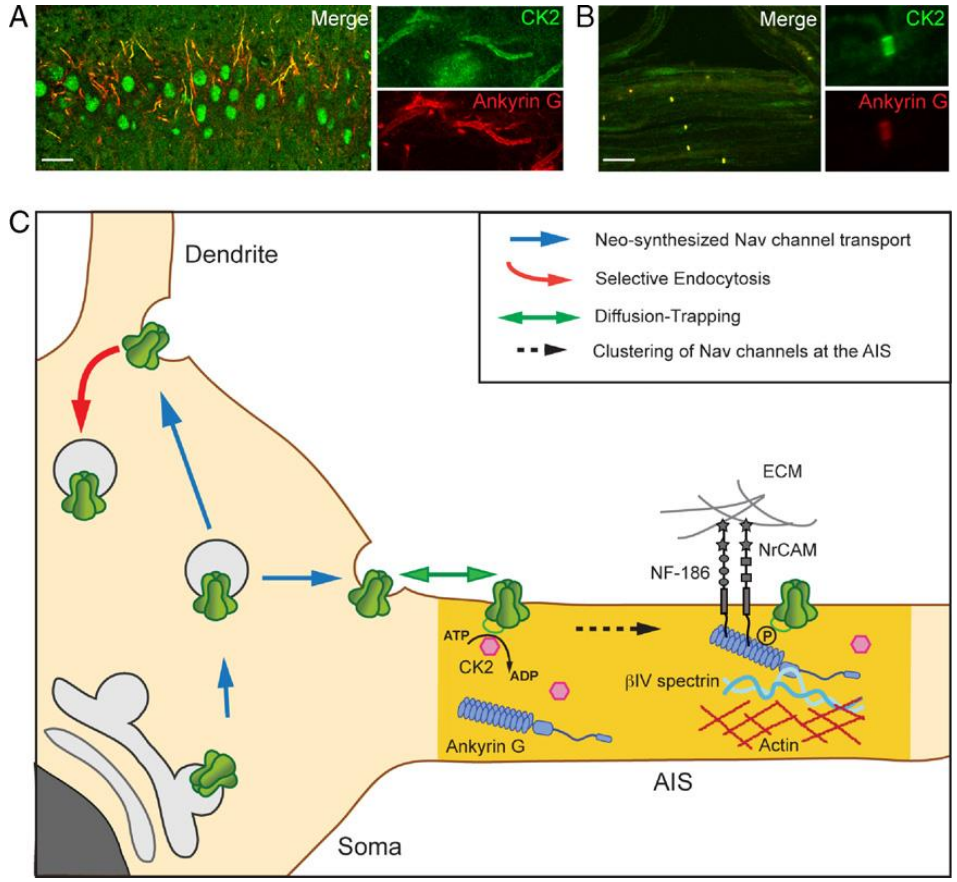



Persistent $Na^+$ conductances are the target of some antiepileptic drugs that reduce transient $I_{Na}$ as well as the $I_{NaP}$ and its ability to promote the high-frequency spike firing that occurs during seizures.

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