Potassium channels
&
Firing properties

Potassium channels

- **K^+ channels** are integral membrane proteins that facilitate the rapid and selective flow of K^+ ions across cell membranes. They are the most diverse group of the ion channel family. *~80 different genes encode the principal pore-forming subunits.*

- Voltage-gated (K_v) K^+ channels present in excitable cells, open and close in response to changes in the transmembrane potential and generally allow passive net efflux of K^+ ions from the cell (down their electrochemical gradient).

They are monstly blocked by TEA and Cs^+

Almost every type of excitable cell has its own unique set of K^+ channels.

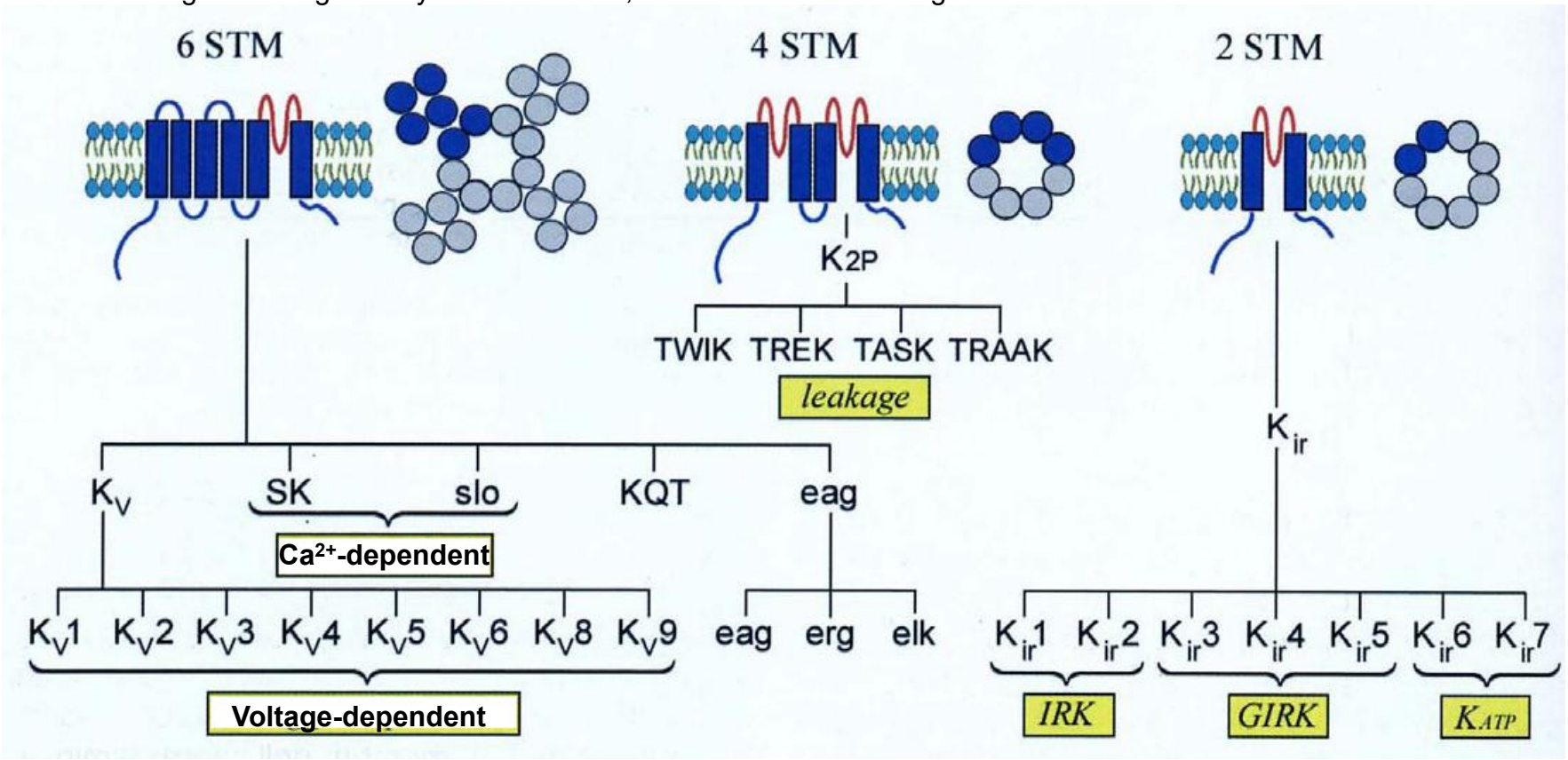
In terms of:

- voltage dependence
- rates of activation and inactivation
- pharmacology,

Furthermore, the same type of cell, but with a different role, can have its K^+ channels “tuned” by splice variants

Three categories of K^+ channels based on their molecular structure.

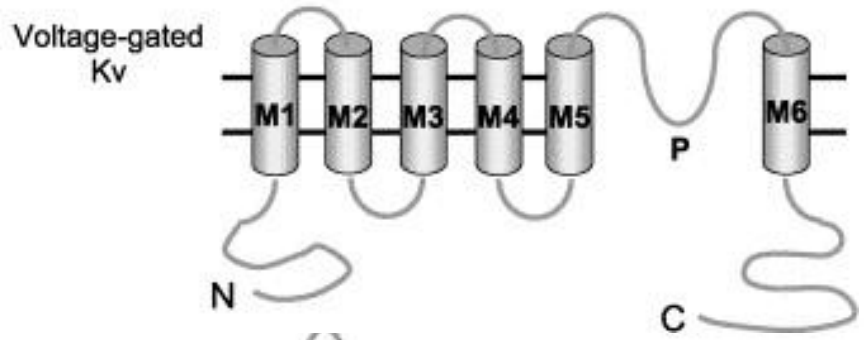
Circular arrangement of generally four α subunits, each of them with a single domain

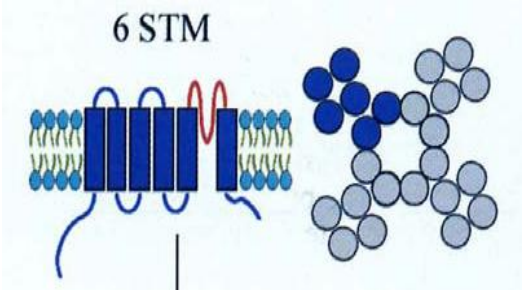


There is little homology among TM domains between the three kind of channels. However, there is short stretch of about 20 amino acids known as the **P (for pore forming)** region which is highly conserved: it is **responsible for the channel selectivity** and facilitate the trans-membrane passage of more than a million ions per second.

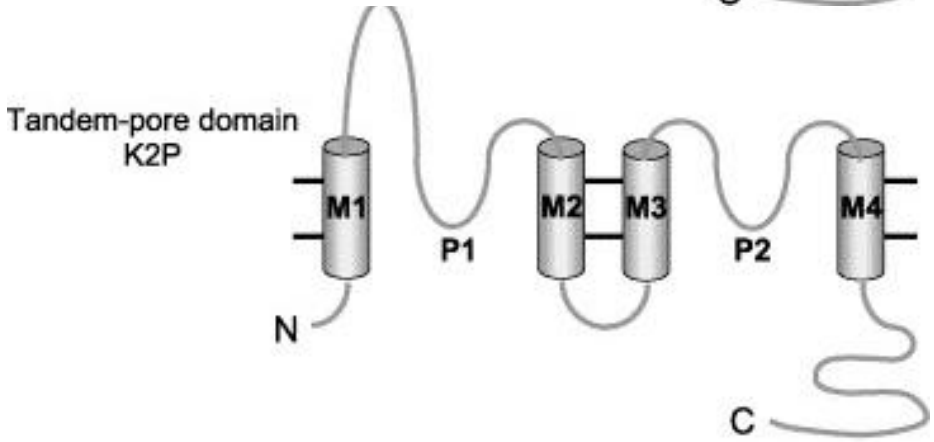
Taglietti, Casella, Principi di Fisiologia e Biofisica della cellula, la Goliardica pavese

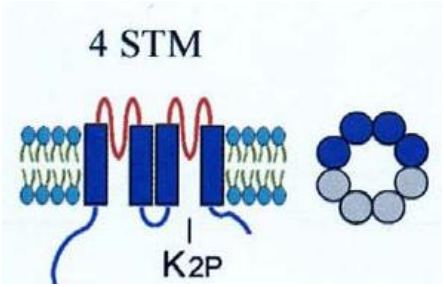
4 pore-forming domains form a functional channel





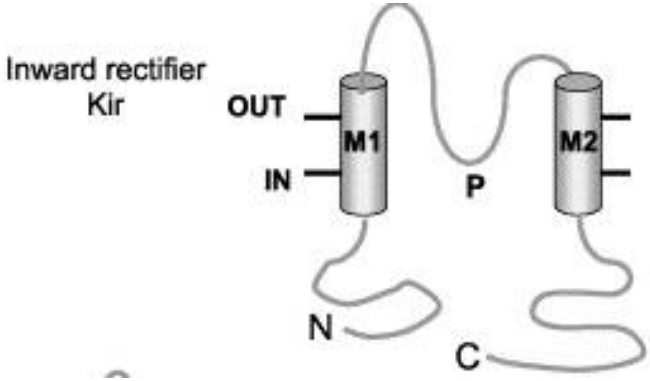
4 subunits, 1P/sub.

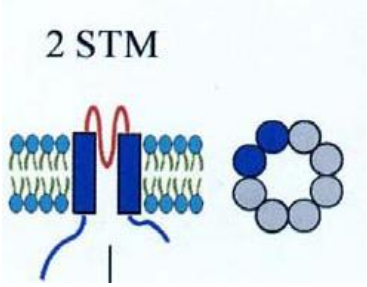




dimers

2 subunits, 2P/sub.





4 subunits, 1P/sub.

A.D. O'Connell et al. / Biochimica et Biophysica Acta 1566 (2002) 152–161

K^+ channels subunits with 6 TM, 1P

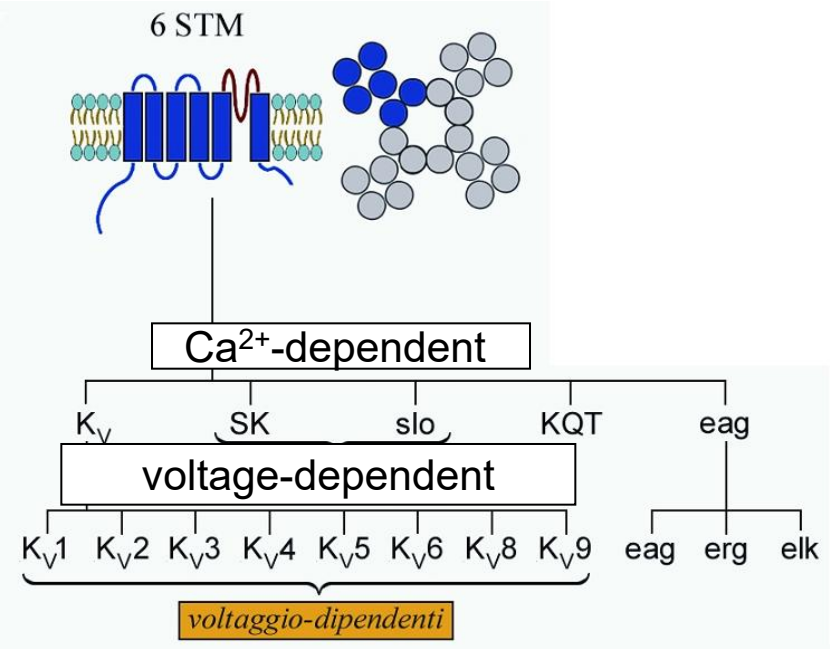
The largest family, the earliest discovered, and the best characterised

a) the family of Ca^{2+} - dependent K^+ channels (SK, BK, IK_{Ca}).

b) the family of K_V (K_V1-K_V12)
with various variants (Kv 1.1, Kv 1.2…Kv 5.1, Kv 5.2)

The Kv 1-4 subfamilies are the vertebrate homologues of the channels encoded by the *Drosophila Shaker, Shal, Shab and Shaw* genes.

Delayed rectifier and A-type channels



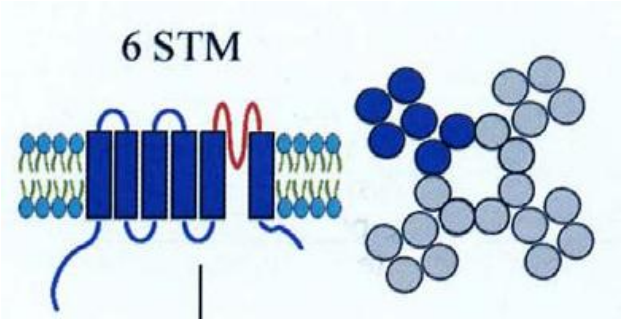
c) others, involved in various cardiac, muscular and nervous disease.

6 STM K^+ currents involved in the repolarizing phase of AP

Voltage-dependent

I_A Kv channels

I_{DR}



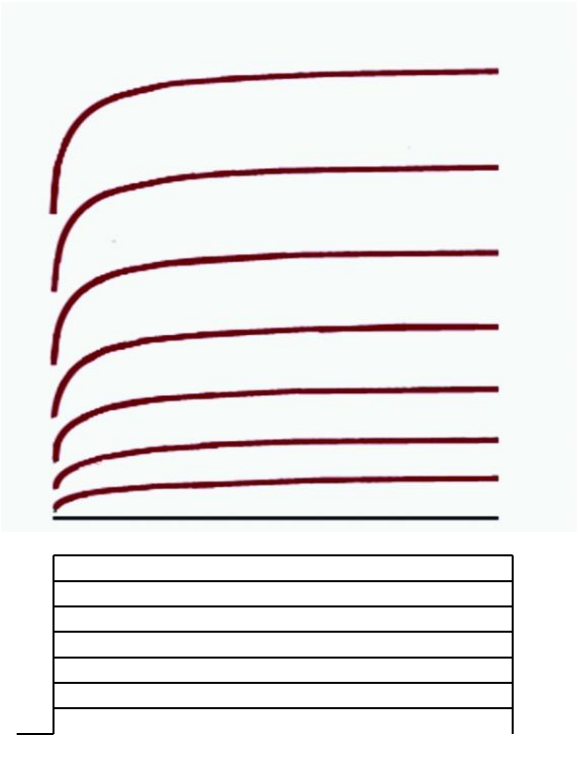
Ca^{2+}-dependent

I_{BK} (K_{Ca}1.1)

I_{SK} (K_{Ca} 2.1, 2.3)

Voltage-clamp

Delayed rectifier K^+ channels (Kv1,3,7,10)

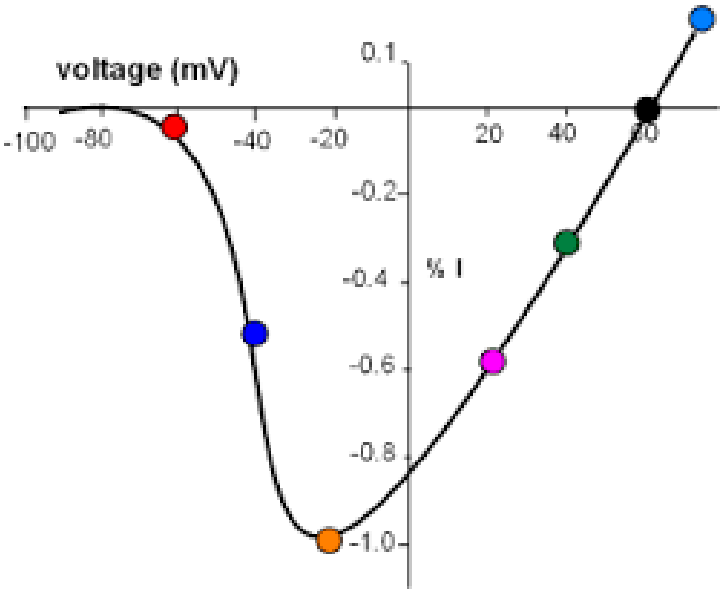


TEA, Ba^{2+} and Cs - sensitive

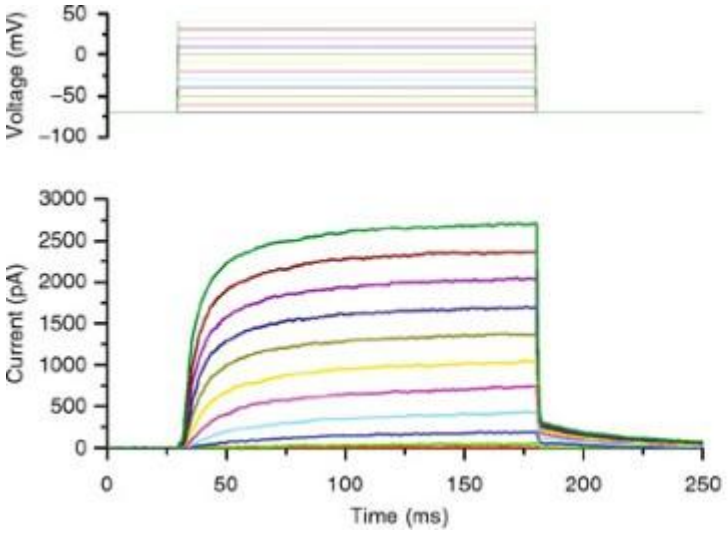
Functional diversity is generated by multiple mechanisms, including the expression and subcellular distributions of channels encoded by different Kv α subunits, interactions of this subunits with cytosolic and/or transmembrane accessory subunits and regulatory proteins and post-translational modifications of channel subunits.

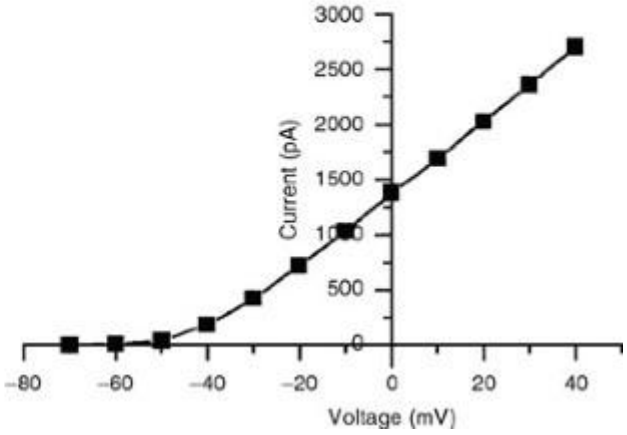
I-V curves

Na^+ channels



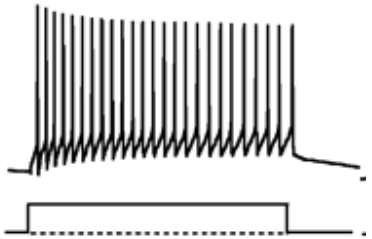
Delay rectifier K^+ channels



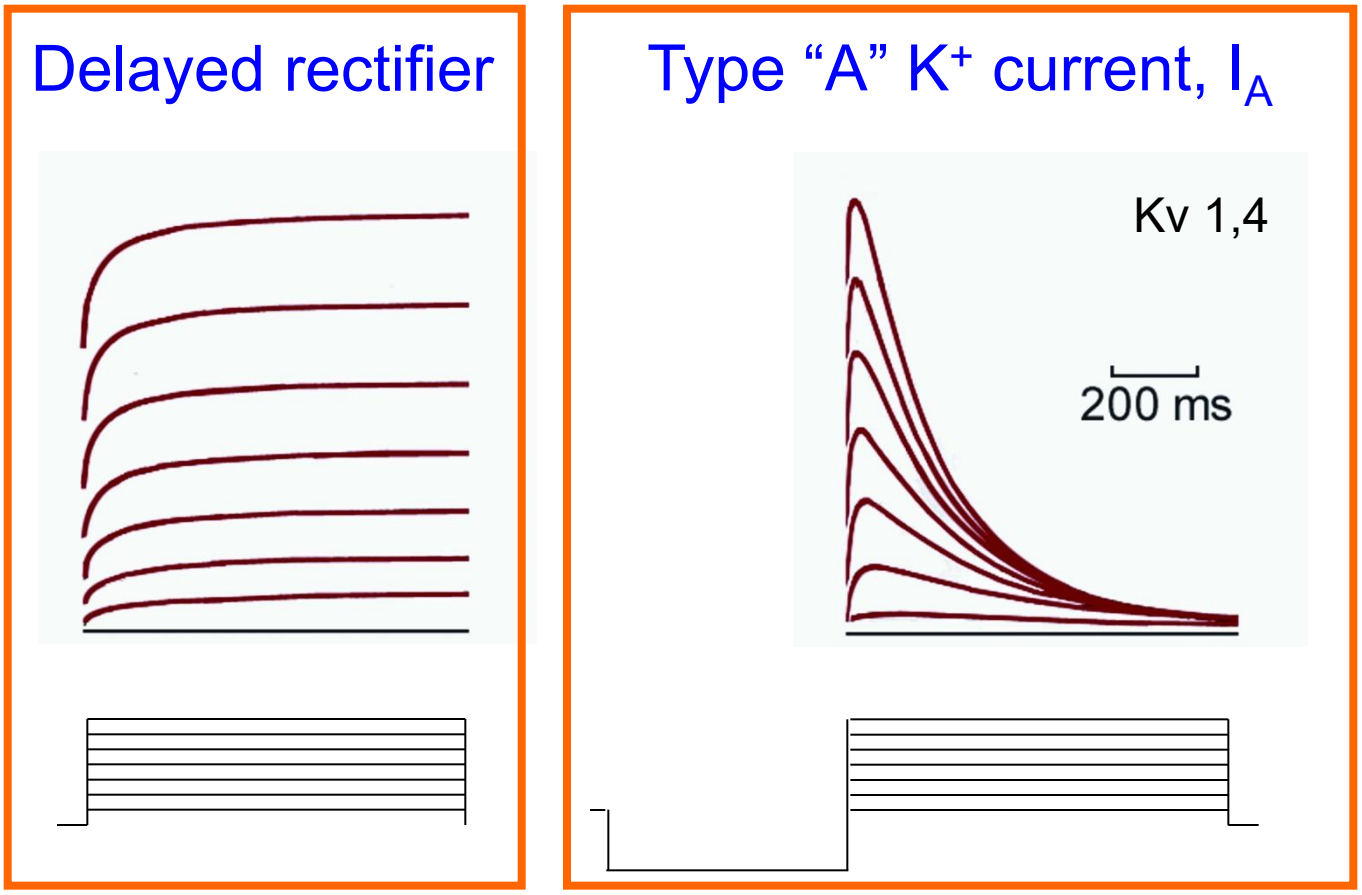


The fast-spiking phenotype has been related to the expression of the Kv3 family, delayed rectifier, voltage-gated K^+ channels

The **rapid and steep activation and deactivation kinetics** are well suited for generating **narrow AP and short refractory periods.**



Interneurons
Purkinje cells
Glutamatergic neurons in subthalamic nucleus



-K_{DR} (delayed rectifier) and K_A channels are both selective for K^+, but they have different properties.

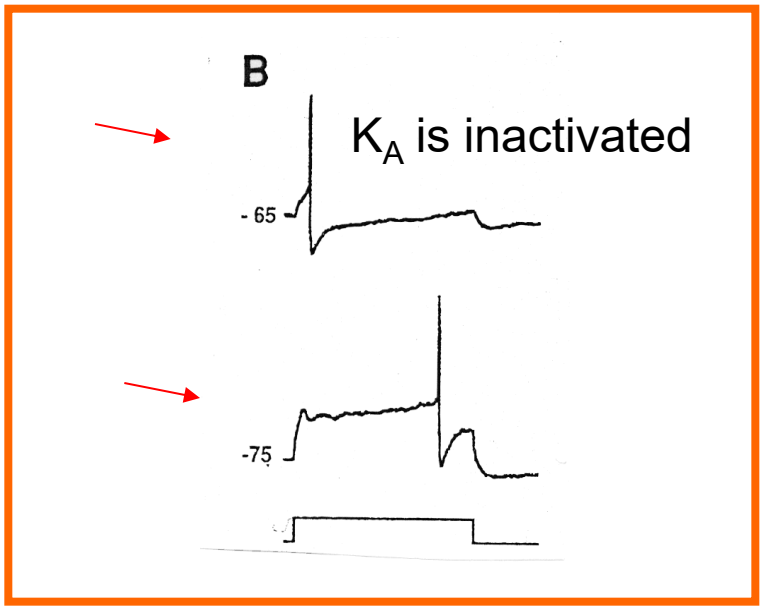
K_A rapidly activates at more negative potentials (early activation), inactivated at positive potential and inactivates fastly (100 ms)

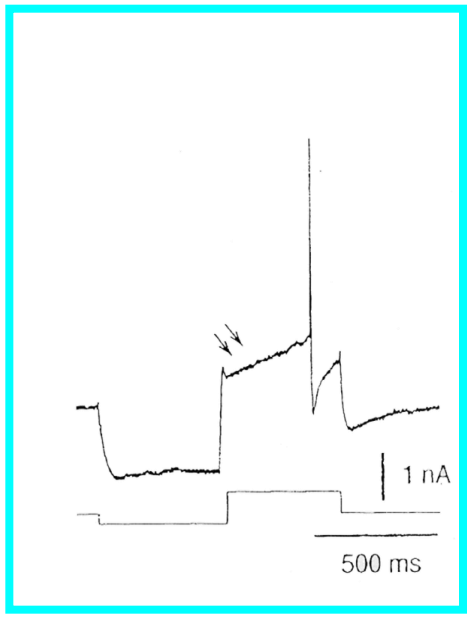
K_{DR} are blocked by TEA, Ba^{2+} and Cs^{2+}. K_A are blocked by 4-AP

K_A current mediates the delayed excitation

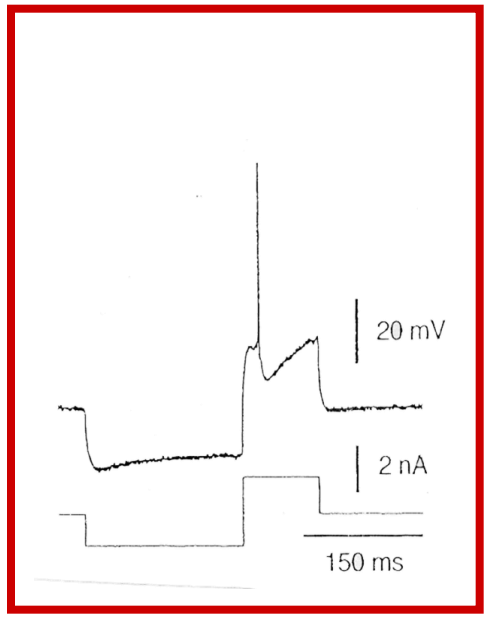
Vagal motoneuron

Hypoglossal motoneuron



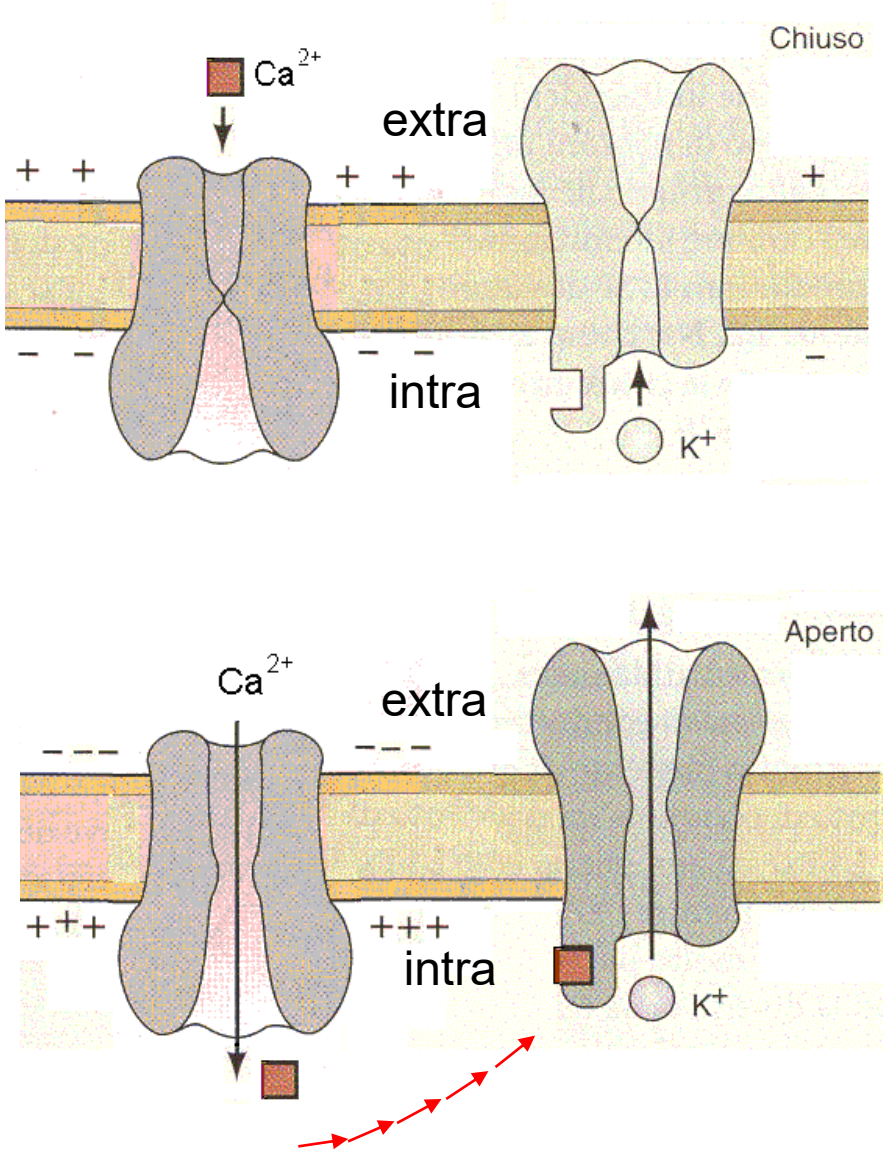


no K_A

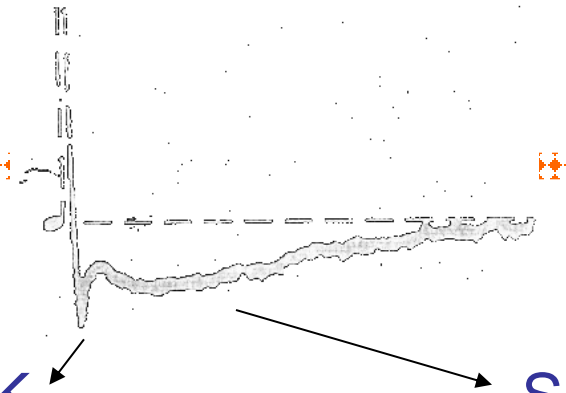


b)

Ca^{2+} -dependent K^+ channels



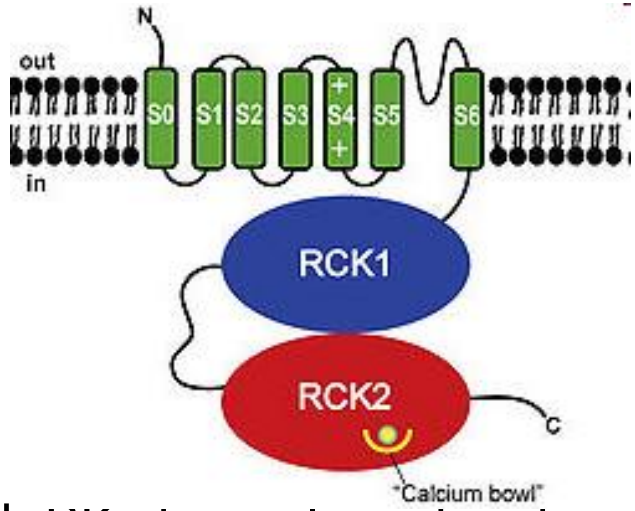
K_{Ca} channels



Main properties

	BK	SK
Single channel conductance	100-200 pS	8-20 pS
Voltage-dependence	yes	not
Ca^{2+} sensitivity	1-10 µM	50-900 nM
Pharmacological blockers	TEA charibdotoxin iberiotoxin	apamin (nM)
AHP activation	1-10 ms	10-1000 ms
AHP deactivation	tens of ms	sec

BK potassium channels can be activated by depolarization or/and by $[Ca^{2+}]_i$ increase



BK channels are essential for the regulation of several key physiological processes including neuronal excitability and transmitter release.

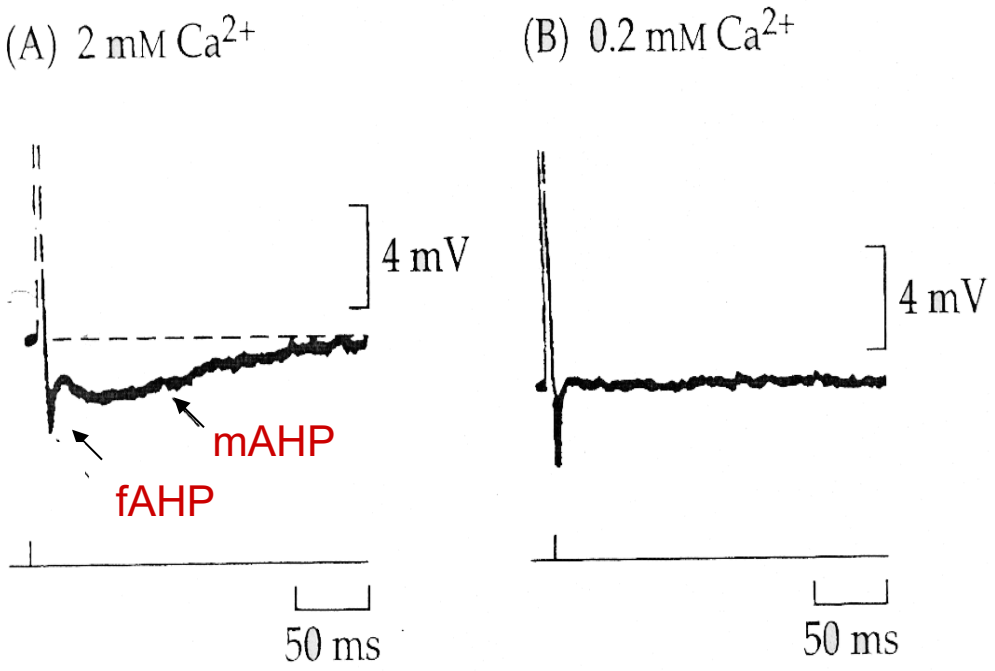
Each BK channel α subunit consists of:

A unique transmembrane domain (S0) that precedes the 6 transmembrane domains (S1-S6) has binding site for β subunits (1-4).

A cytoplasmic C-terminal consisting of a pair of RCK domains that contain the primary binding sites for Ca^{2+}.

The voltage-sensing mechanism of the BK channel is independent of Ca^{2+} binding but an allostering coupling of the activation gate with Ca^{2+} binding site and voltage sensor is present. They cooperate in opening the channel.

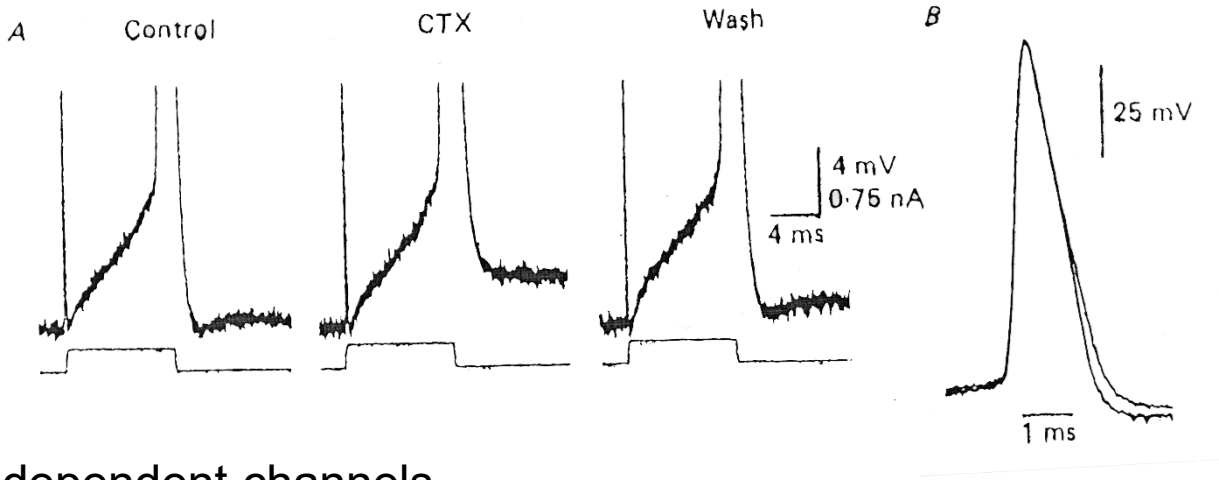
Ca^{2+} -dependent K^+ currents mediate fast and medium AHPs



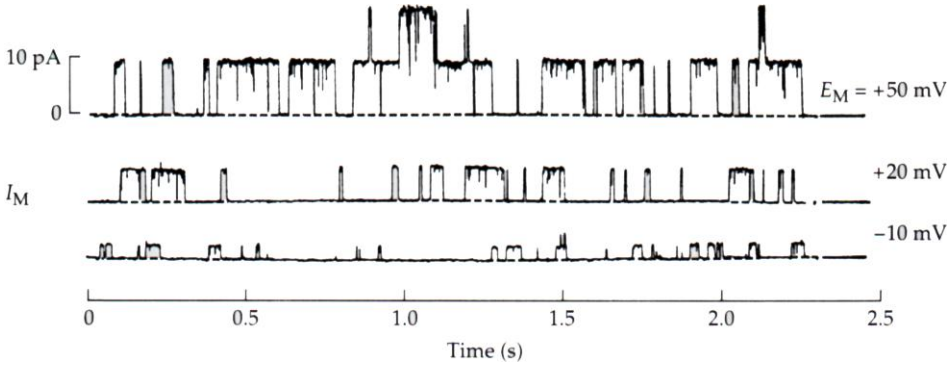
fAHP mediated by BK channels

mAHP mediated by SK channels

BK channels mediate the fAHP



Big voltage-dependent channels



Hille, Sinauer Ass.

After hyperpolarizing potential

For APs in mammalian neurons AHPs are common, but by no means universal.

AHP typically has three components: [1] the fast AHP; [2] the medium AHP; and [3] the slow AHP.

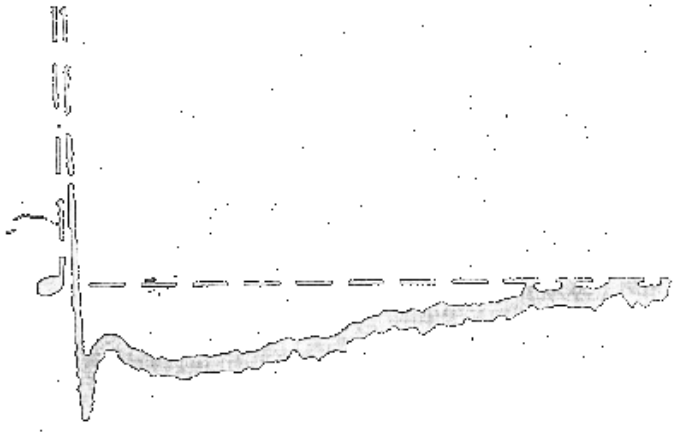
Ionic currents contributing to AHP include:

I_{BK} *caribdotoxin and iberiotoxin-sensitive*

I_{SK} *apamin-sensitive*

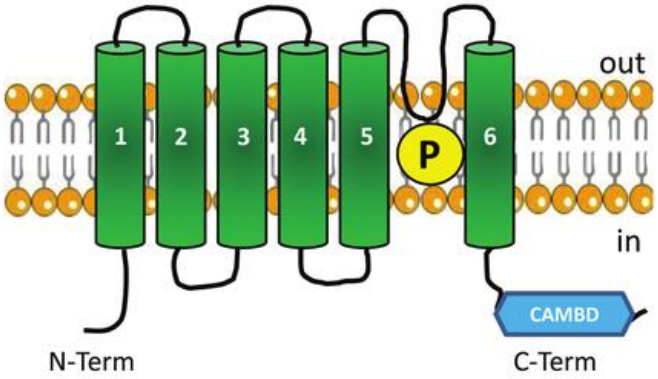
I_M Kv7.2, 7.3
Blocked by activation of mAChRs

I_{Kslow} *sensitive to neurotransmitters*



The SK channel family contains 4 members - SK1, SK2, SK3, and SK4.

Calmodulin (CaM) binds to SK channels at the CaM binding domain. Ca^{2+} binding to CaM induces channel gating.

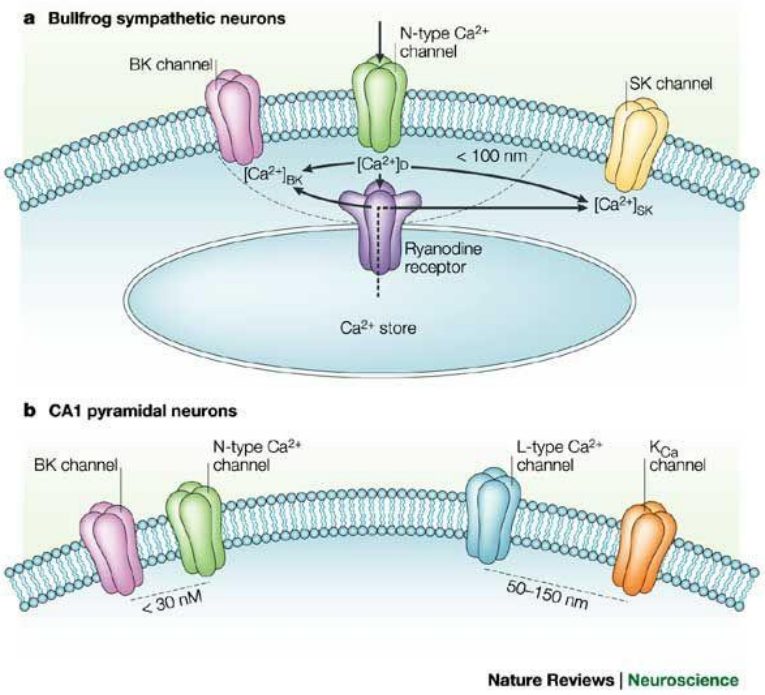


SK channels are blocked by apamin, with different sensitivity.

Apamin acts on the SK channel outer pore through an allosteric mechanism.

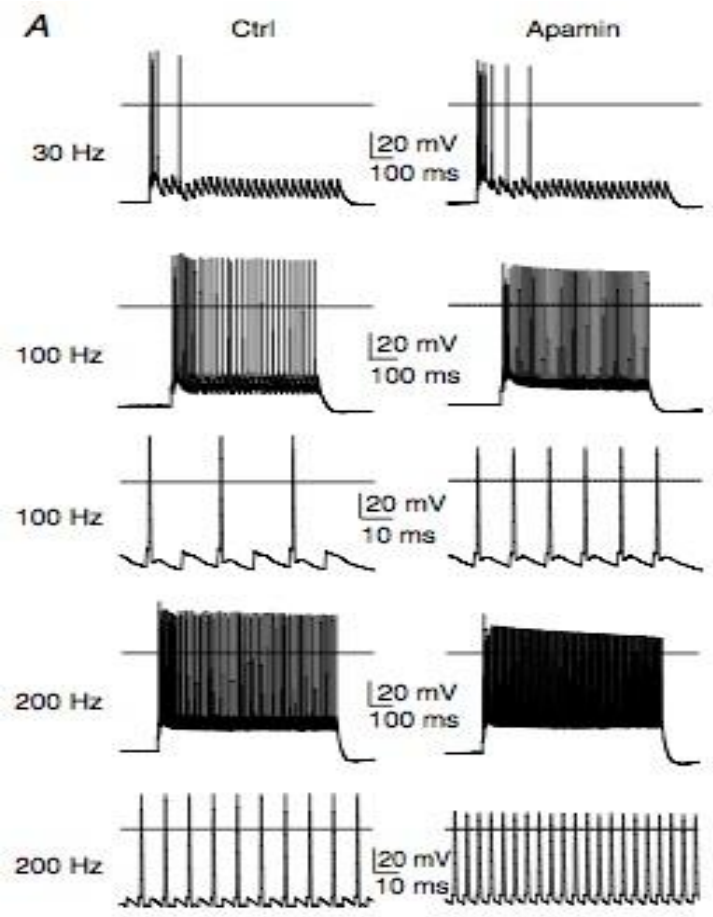
SK1 is apamin insensitive

Colocalization of BK or SK channels & Ca^{2+} channels translate Ca^{2+} entry rapidly into membrane hyperpolarization.

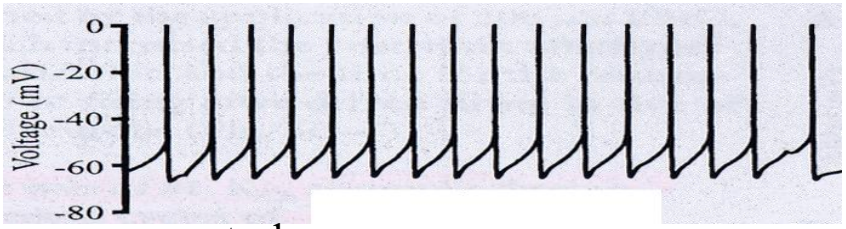


Neuronal firing is regulated by SK channels

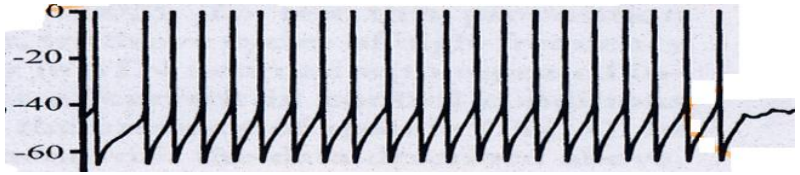
The AHP gives a time limit to the neuron response



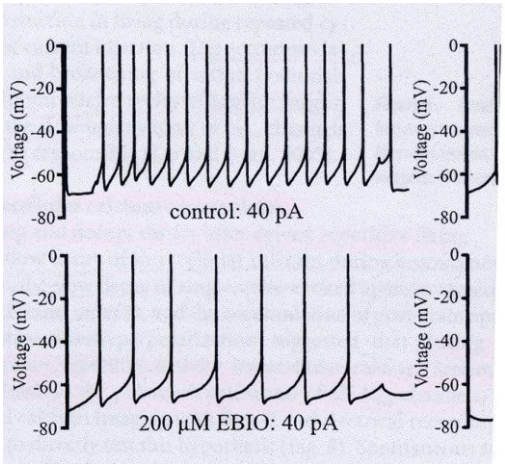
SK channels influence the *firing* of subthalamic neurons.



control



apamin 100 nM

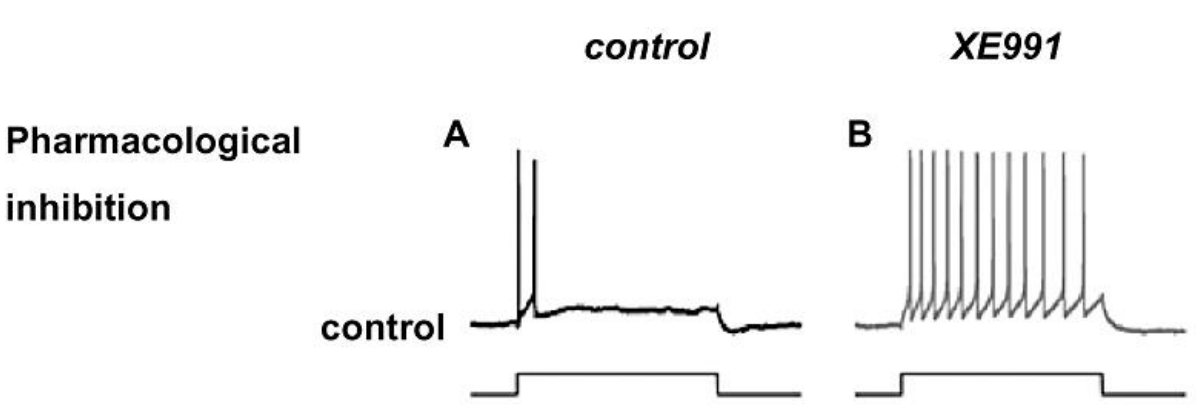


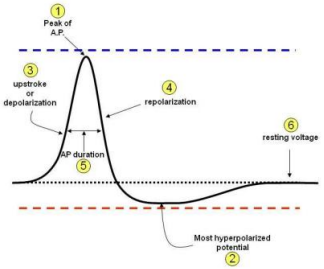
The benzimidazolinone 1-EBIO increases the apparent sensitivity of SK channels to Ca^{2+}

Hallworth et al. J. Neurosci., 23: 7525-7542, 2003

Inhibition of I_M currents induces a tonic firing

Activation of Kv7/M-channels during the initial stages of an action potential discharge serves to suppress later action potentials and abbreviate the duration and frequency of the spike train induced by sustained depolarization. Thus, inhibition of channel activity (by mAChR activation or blocking drug such as XE991), strongly enhances repetitive firing

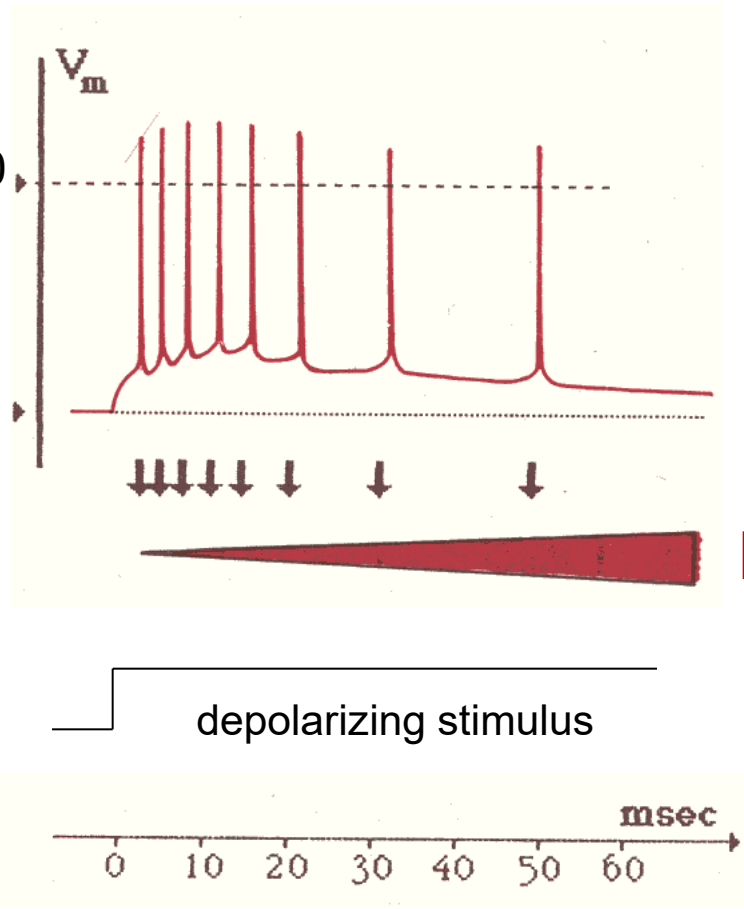


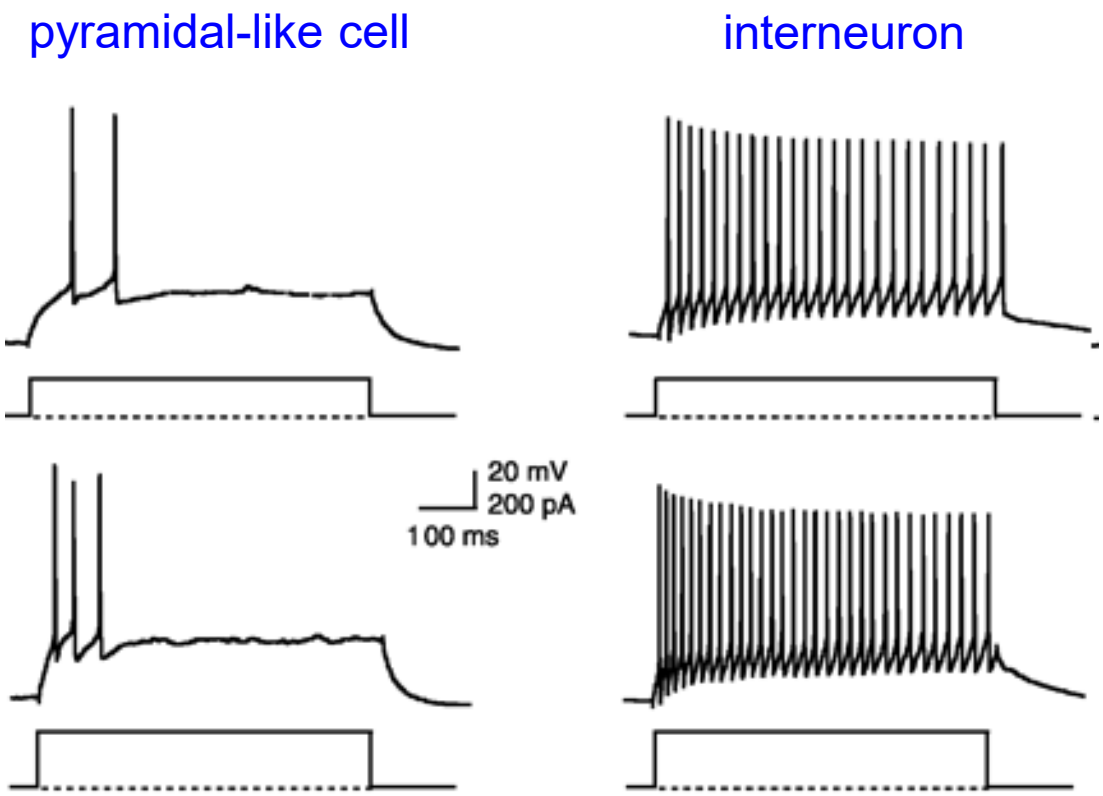


David A Brown and Gayle M Passmore, **Neural *KCNQ* (Kv7) channels,** Br J Pharmacol. 156(8): 1185–1195, 2009.

Slow AHP in pyramidal cells limits the firing frequency of repetitive action potentials (**spike-frequency adaptation**) and is mediated by Ca^{2+}-dependent K^+ channels

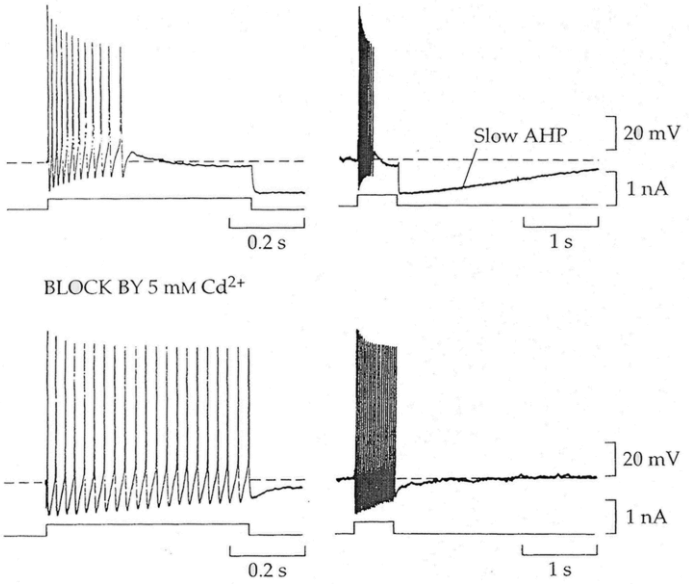
sI_{AHP} is characterized by a slower time course (in the range of seconds), by its **lack of sensitivity to apamin or any other classical K^+channel blocker,** and by its modulation by several neurotransmitters





sAHP in hippocampal CA1 and CA3 pyramidal cells, neurons of the locus coeruleus, myenteric neurons

In some neurons a long hyperpolarization follows the "burst activity" mediated by slow Ca^{2+}-dependent K^+ conductances



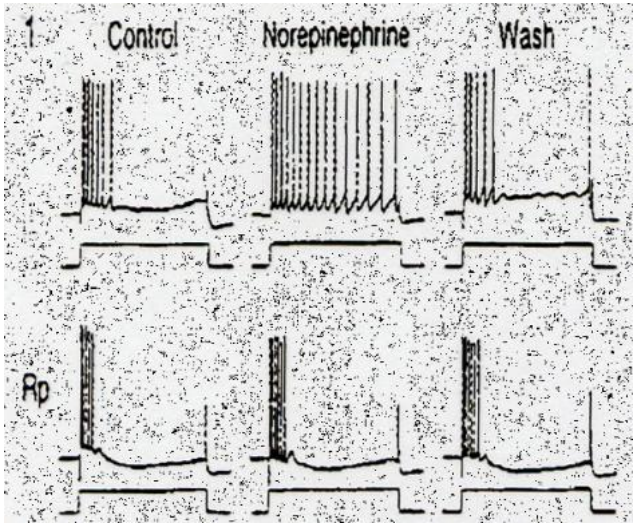
Small single channel conductance, high affinity for Ca^{2+}

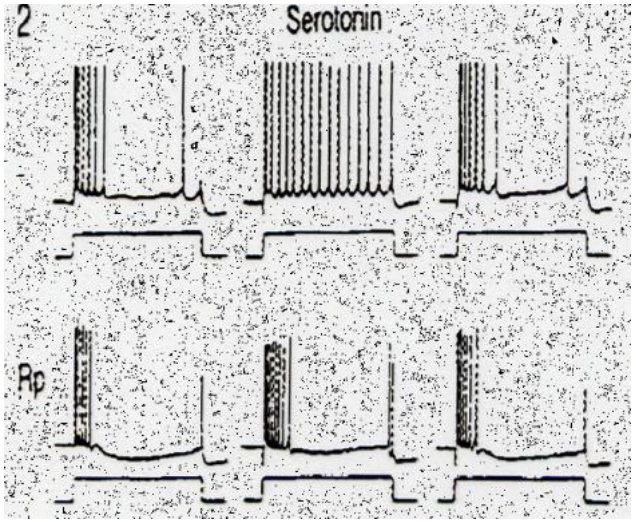
The molecular identity of the sAHP channels remains unknown.

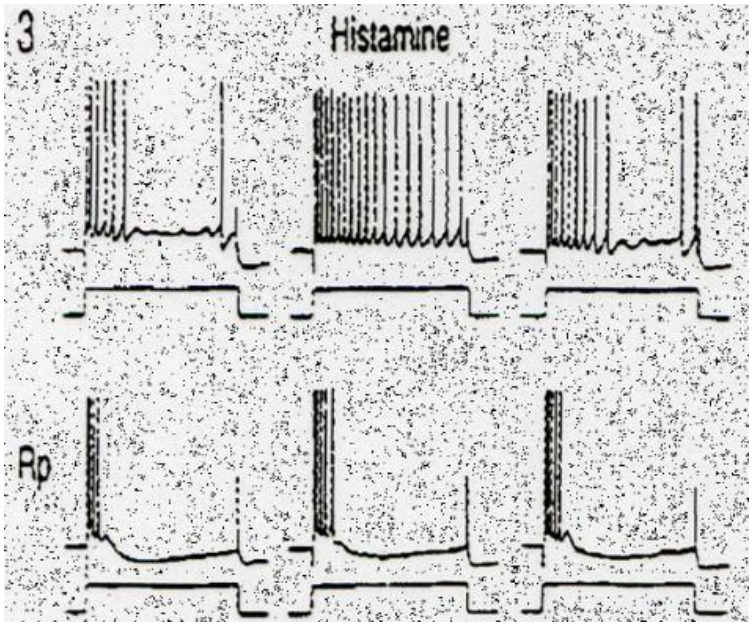
sAHP currents prevent the origin of new action potentials limiting the hyperexcitation of neurons in response to long lasting or repetitive stimulus.

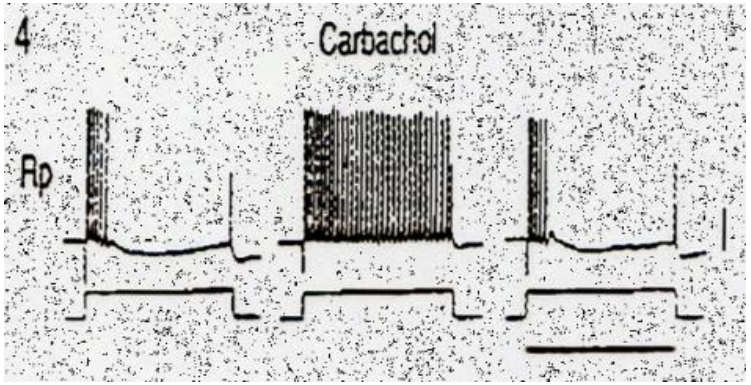
The high sensitivity of sAHP to neurotransmitters and hormones may allow a change in *firing frequency:*
from *burst* of action potentials to tonic *firing*.

Neurotransmitters activate metabotropic R & modulate s AHP





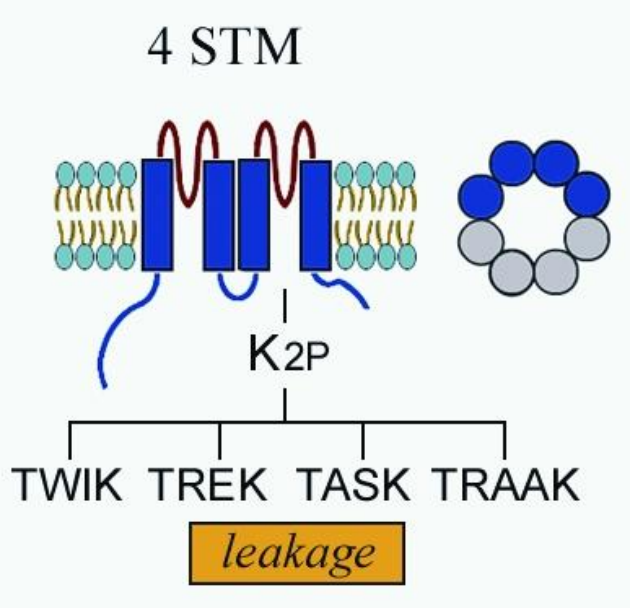




The PKA inhibitor Rp-cAMP blocks the effects of many neurotransmitters

Second group K2P: 2 subunits form a functional channel, each with *4 STM and two P regions*.

Functional channels forms as dimers with a single pore



Increased leak currents stabilize cells at hyperpolarized voltages whereas leak suppression permits depolarization and excitation.

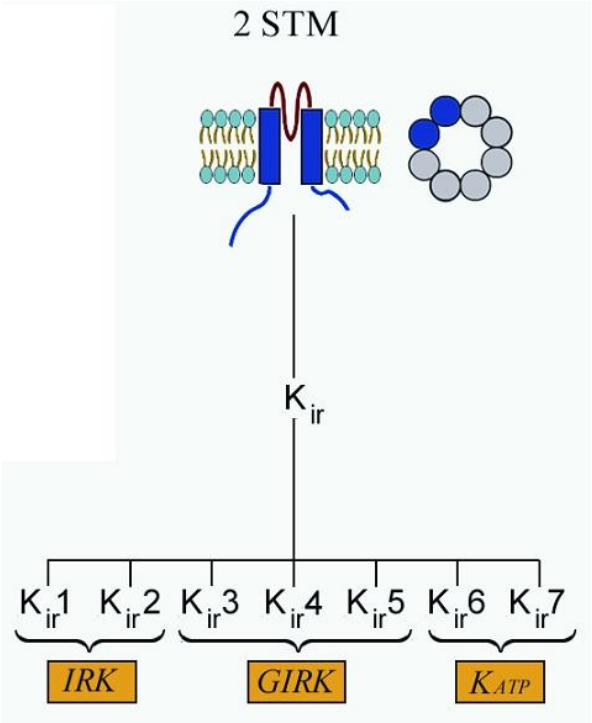
Although they are always open, they operate under tight control of agents:
- molecular oxygen
- cyclic nucleotides
-noradrenaline,serotonin, GABA

Since the K2P field is relatively new, and several of the channels were cloned in parallel, there has inevitably been some duplication, e.g. TASK-1 has also been called cTBAK.

The pharmacological sensitivities of mammalian K2P channels are varied, but generally they are relatively insensitive to classical K^+ channel blockers such as TEA, Ba^{2+} and Cs^{2+}.
They are often modulated by anaesthetics.
TASK-1 channels are reported to be selectively inhibited by submicromolar concentrations of the endocannabinoid anandamide.

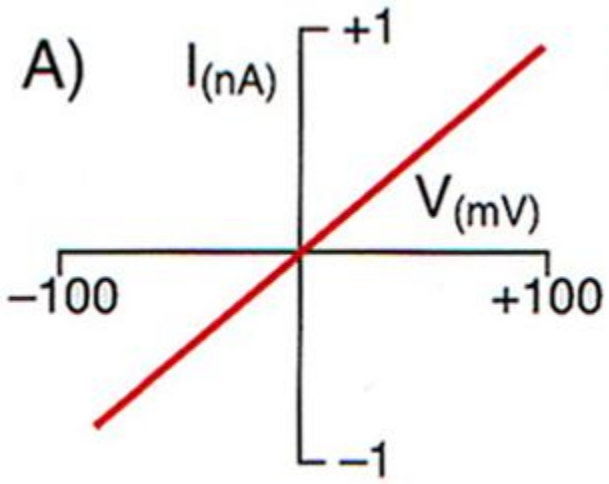
A number of novel regulatory properties have been uncovered. For example, TREK-1 is stretch-activated and heat-sensitive

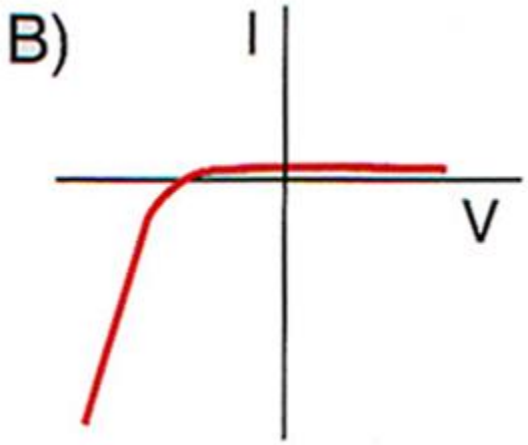
Third group: tetrameric structure, 4 α-subunits, each with *2 TMS*.

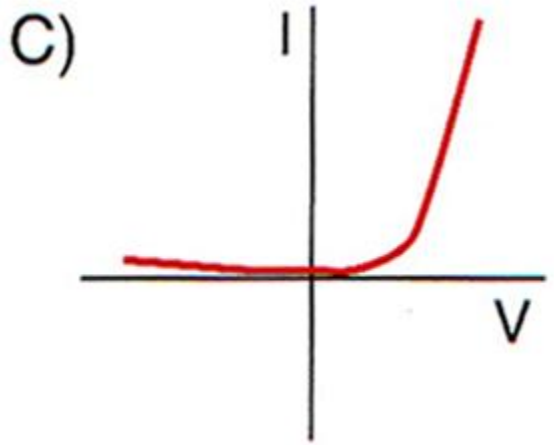


IRK are "*inwardly rectifier*" (K_{IR}) (o "anomalous rectifier"), because they are responsible, if present, for the "inward rectification".
GIRK are opened by G proteins activated by neurotransmitters acting at mRs.

I-V relationship







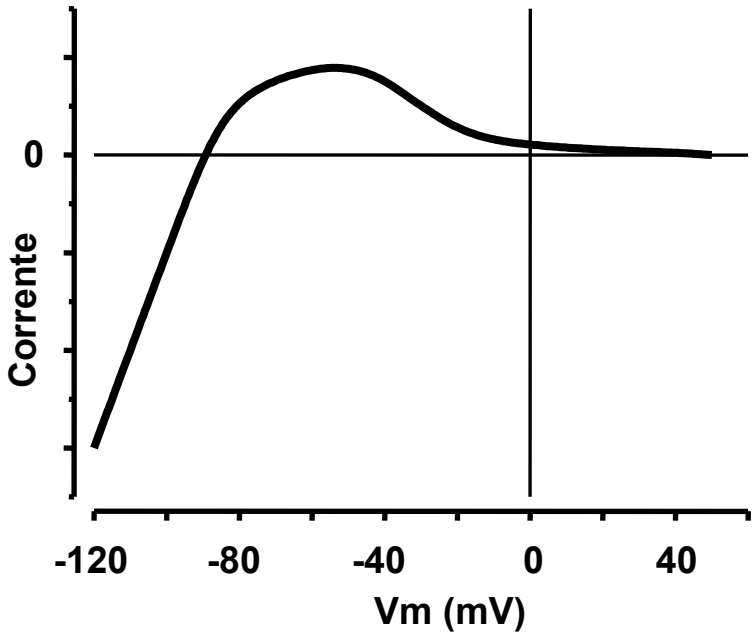
CONSTANT CONDUCTANCE

INWARD RECTIFIER

OUTWARD RECTIFIER

Farmacologia generale e molecolare, UTET

K_{IR} channels do not have *voltage sensor* (S4).



The voltage-dependent block does not derive from a “gating” process but by the *voltage-dependent block induced from the intracellular side by* Mg^{2+} and positive charged organic molecules as the *polyamines*.

K_{IR} stabilizes the membrane potential close to the K^+ equilibrium potential

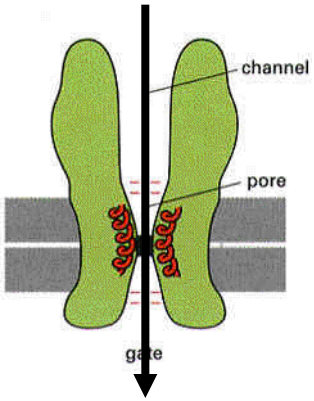
A) If the membrane is hyperpolarized, an inward K^+ current goes against the hyperpolarization. B) If the membrane is *depolarized*, a outward K^+ current goes against the membrane depolarization.

E_K = -90 mV

K^+

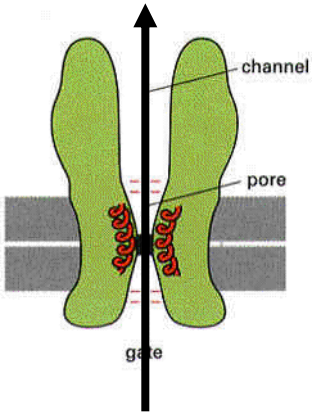
A

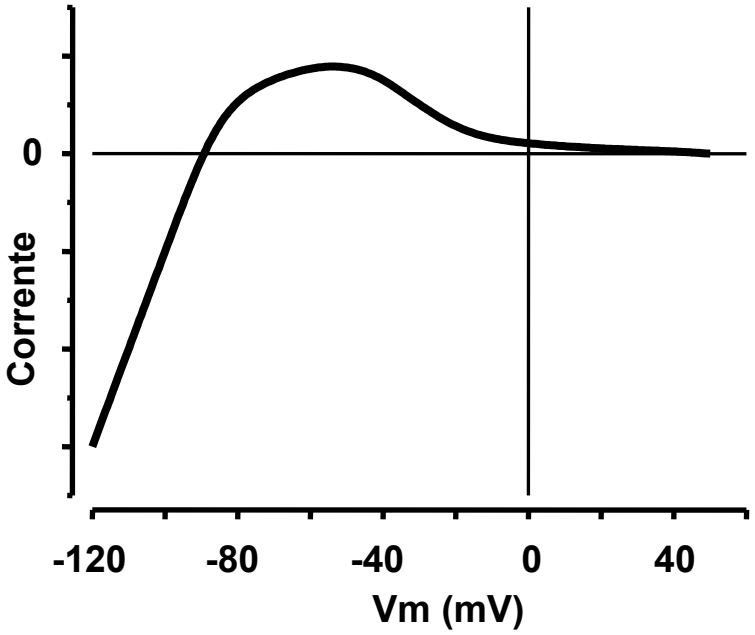
Vm = -100 mV



B

Vm = -50 mV





K_{ATP} link the K^+ permeability to the cell metabolism, they are *closed* when ATP_i increases.

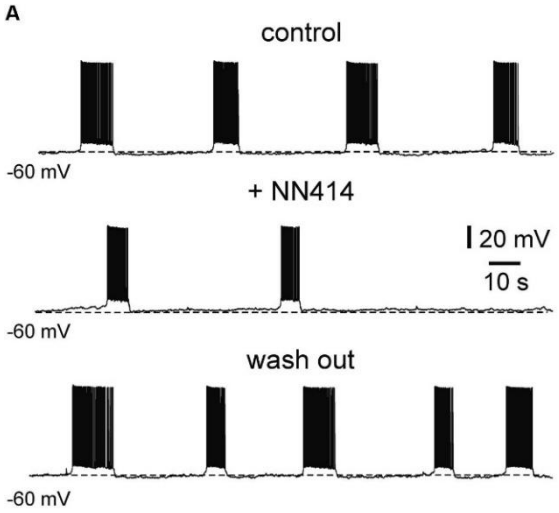
Physiologically

K_{ATP} channels are gated by ATP.

In young cells high firing induces a decrease of ATP, opening of K_{ATP} and hyperpolarization.

They show inward rectification due to intracellular Mg^{2+}.

NN414 open K_{ATP} channels



Lemak et al., Front. Cell. Neurosci., 2014

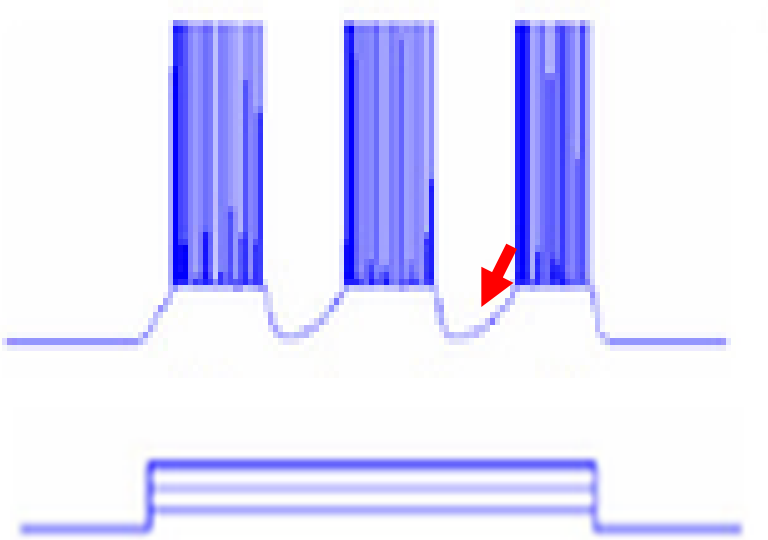
There are other inward rectifier channels

Functional properties of I_h channels put them in a class of their own.

Almost as permeable to Na^+ and K^+.

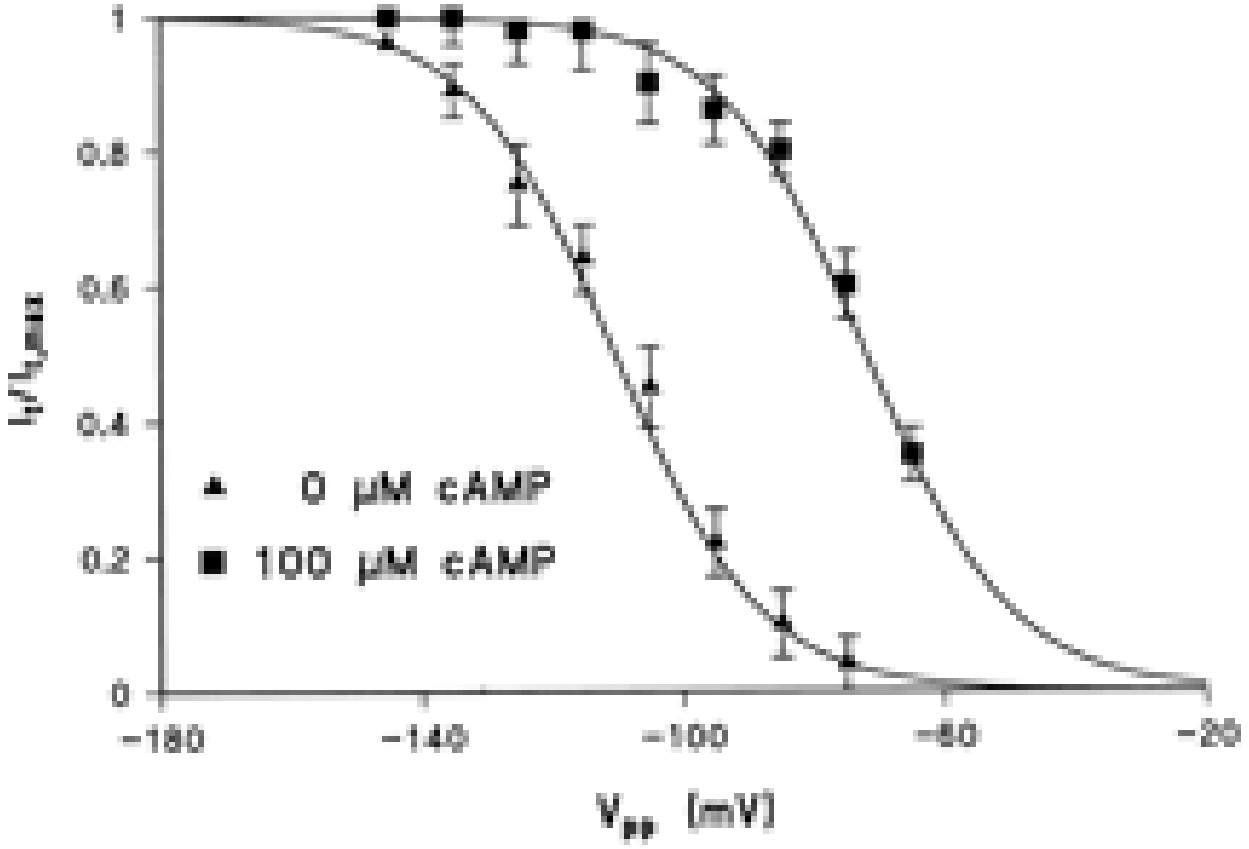
E_{rev} = -20 mV

They are activated at membrane potentials negative to the resting.



In central neurons I_h participates in rhythmic and burst firing and can initiate spontaneous activity (rebound) following strong inhibition.

The elevation of cAMP makes I_h channels easier to open (at more positive potentials) promoting depolarization



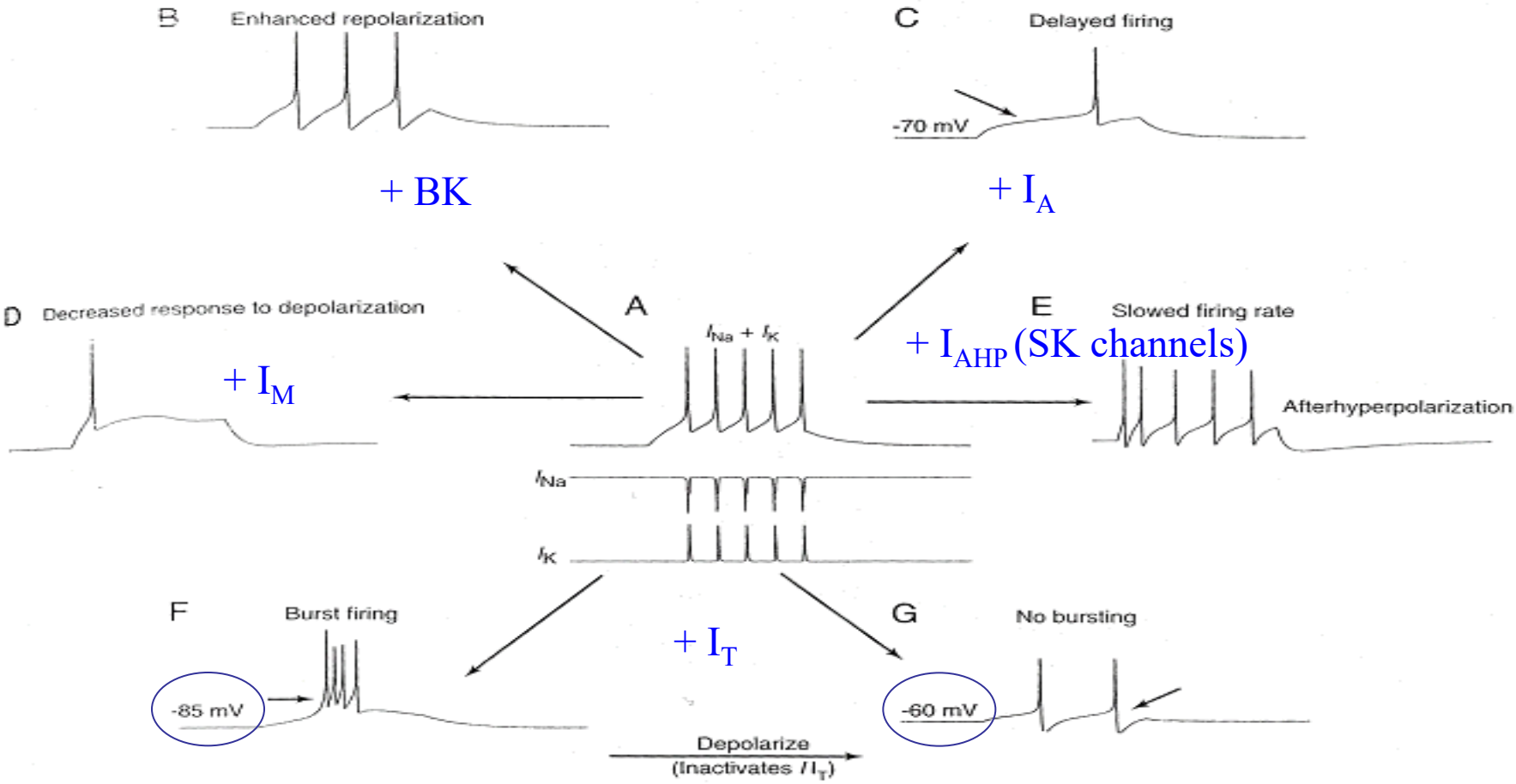
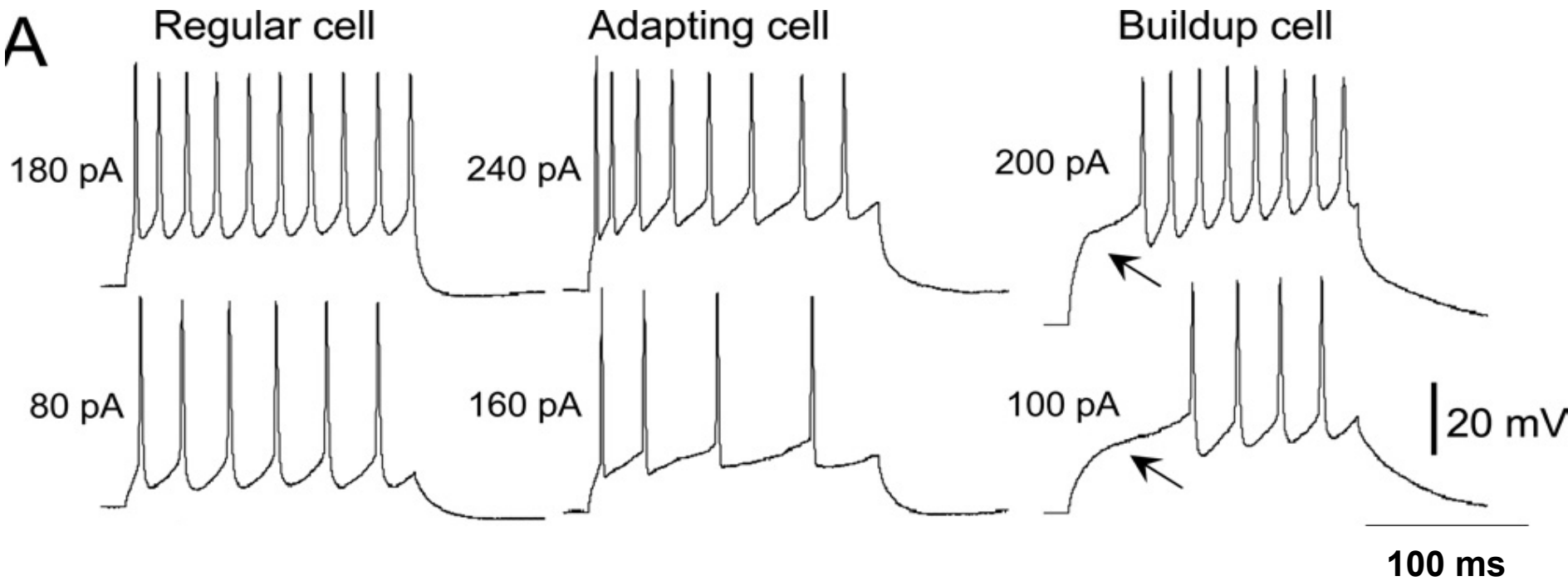
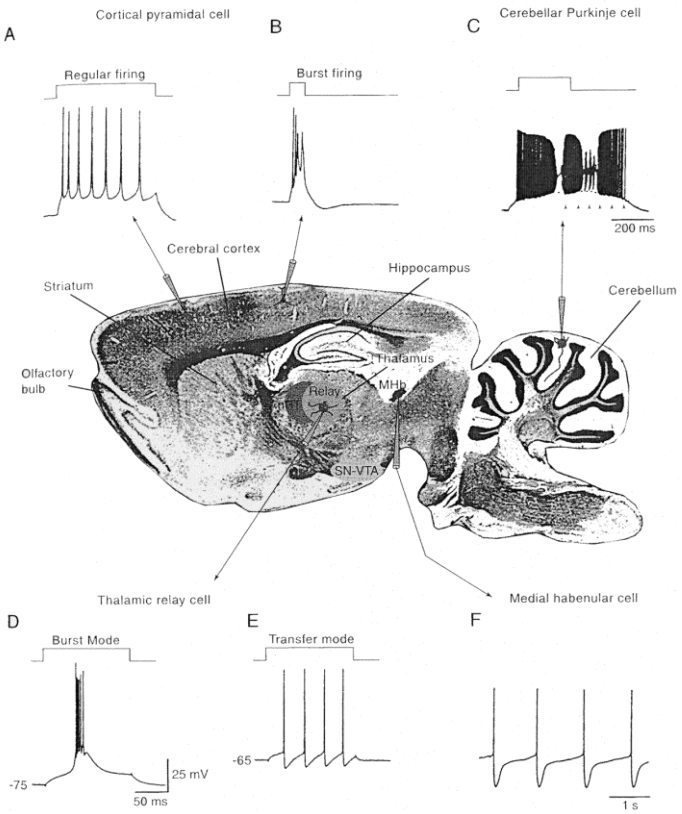


FIGURE 6.12 Simulation of the effects of the addition of various ionic currents to the pattern of activity generated by neurons in the mammalian CNS. (A) The repetitive impulse response of the classical Hodgkin–Huxley model . With only I_{Na} and I_K, the neuron generates a train of five action potentials in response to depolarization. Addition of I_C (B) enhances action potential repolarization. Addition of I_A (C) delays the onset of action potential generation. Addition of I_M (D) decreases the ability of the cell to generate a train of action potentials. Addition of I_{AHP} (E) slows the firing rate and generates a slow afterhyperpolarization. Finally, addition of the transient Ca^{2+} current I_T results in two states of action potential firing: (F) burst firing at −85 mV and (G) tonic firing at −60 mV. From Huguenard and McCormick.[51]

Fundamental Neuroscience, Academic Press


A
Regular cell
Adapting cell
Buildup cell
180 pA
240 pA
200 pA
80 pA
160 pA
100 pA
20 mV
100 ms

Neurons in the brain exhibit widely varying electrophysiological properties.

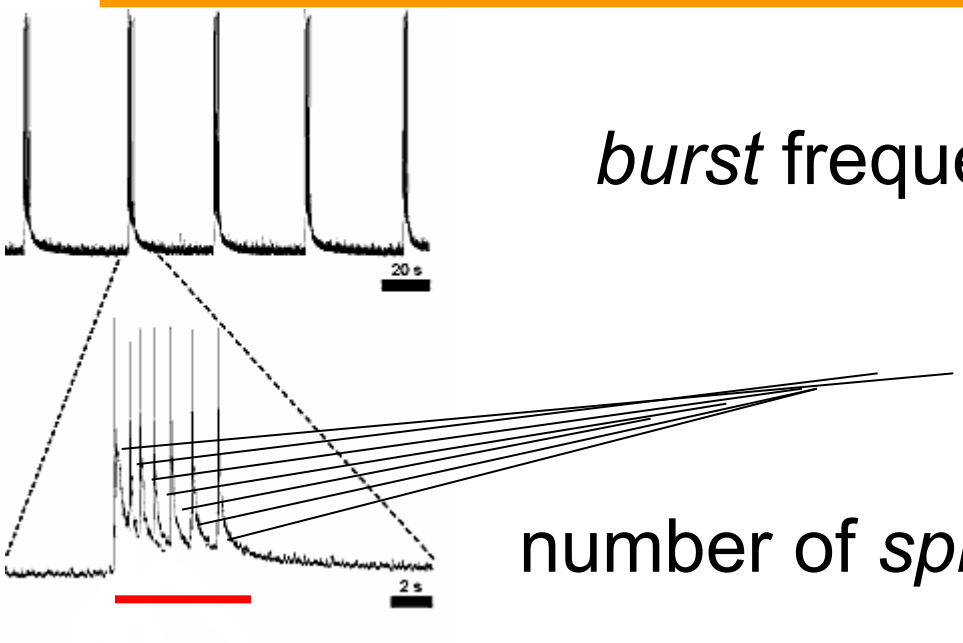


Fundamental Neuroscience, Academic Press

SINGLE SPIKES CONTAIN LITTLE INFORMATION IN THEMSELVES

The presence of specific intrinsic conductances may give origin to a more complex *firing pattern:*

BURST
rapid cluster of action potentials



burst frequency

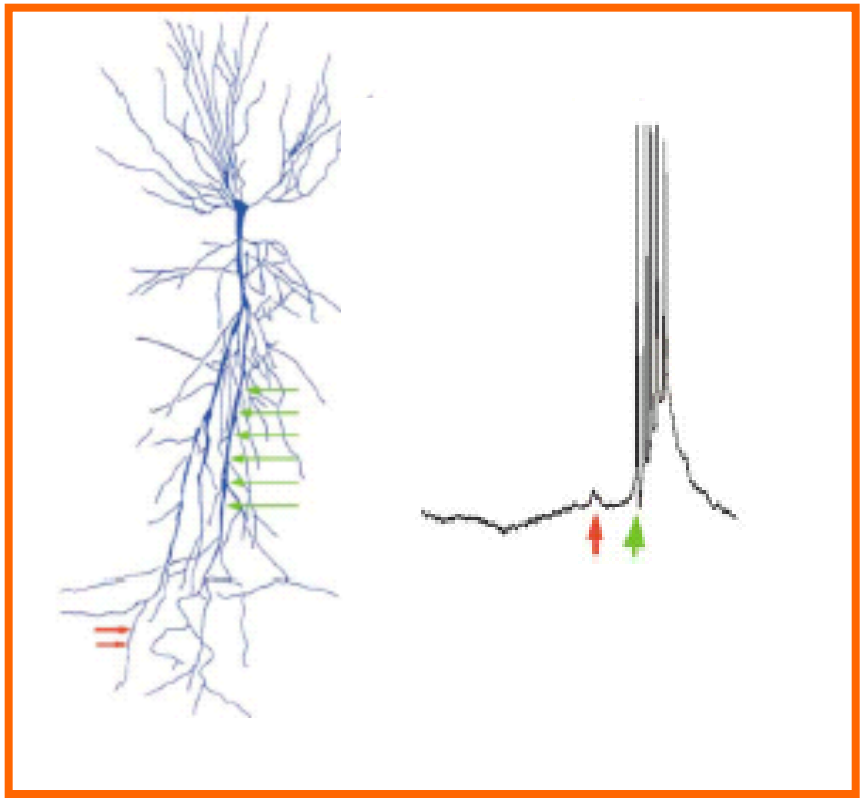
number of *spikes* per *burst*

burst duration

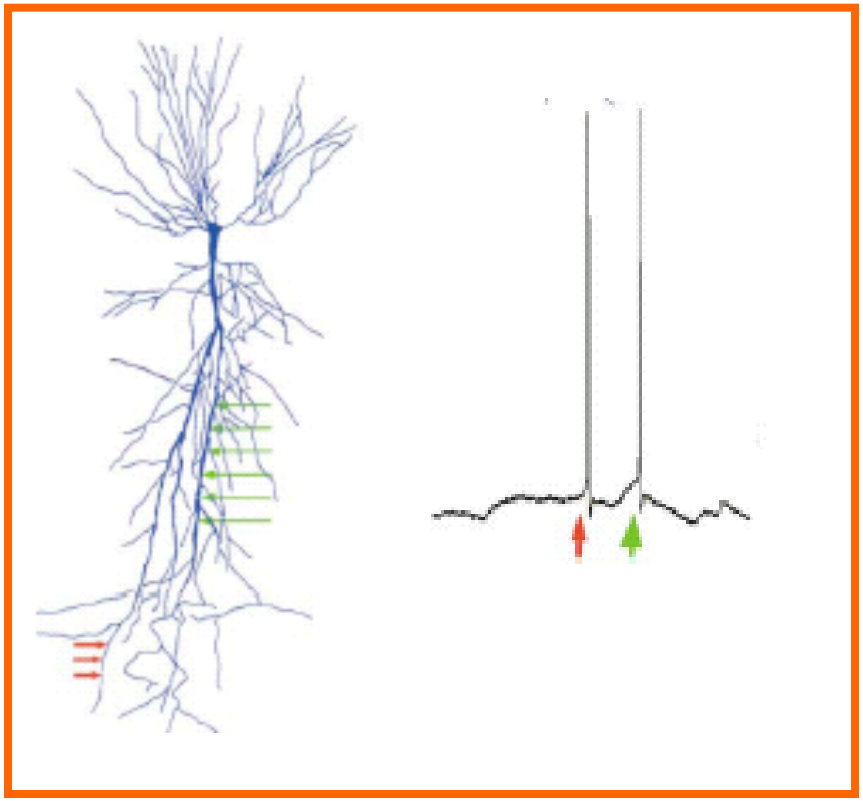
may code specific information

In many brain areas there is a tight relationship between the cell immaturity and “burst pattern”:
Hippocampus (Sperber et al., 1998)
Neocortex (Potier & Psarropoulou, 2001)

Integration of afferent inputs is complex

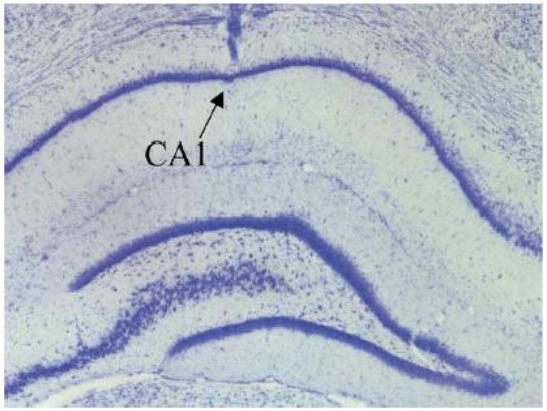


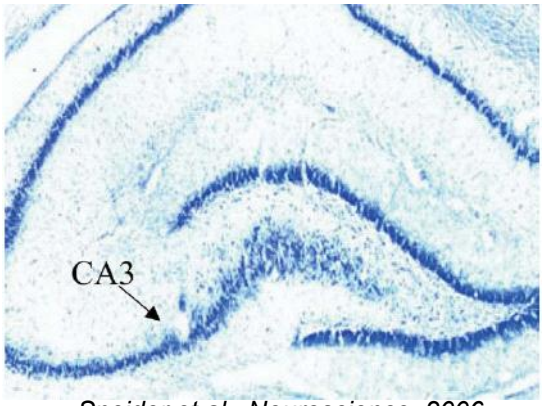
A weak input (red) is followed by a strong input (green).
If the weak input is subthreshold, the strong input can trigger a burst and can lead to strengthening of the weak input.



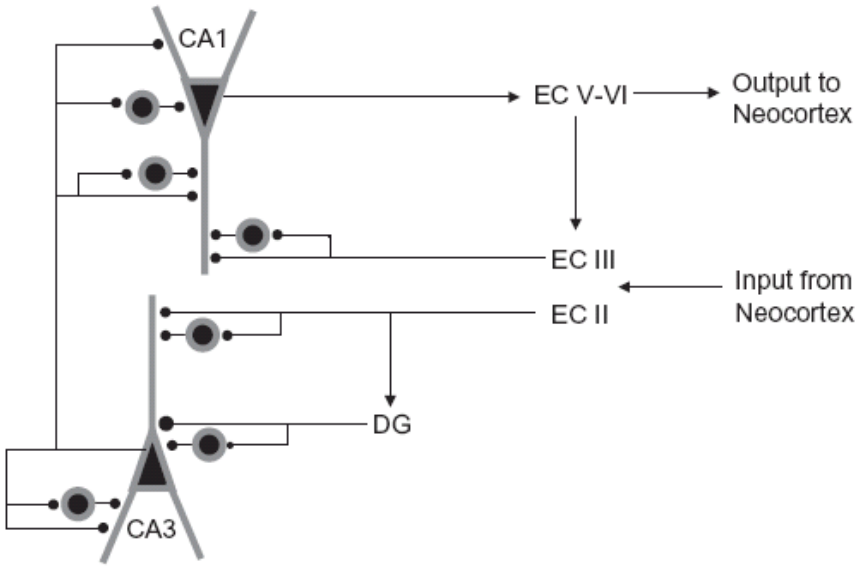
Evoked single spike can inhibit burst response to the strong input. Therefore, the firing of already potentiated afferents can reduce the efficacy of the strong input inhibiting further potentiation.

Bursts could be associated to behavioural states



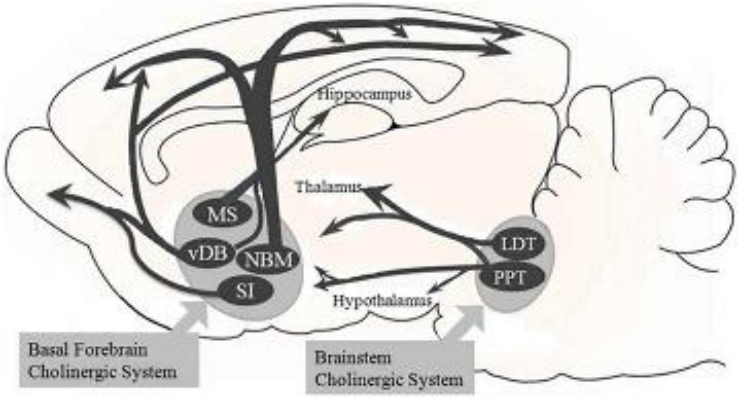


Sneider et al., Neuroscience, 2006



In rats CA1 e CA3 *cells burst* activity *are* associated to hippocampal theta rhythms (6-12 Hz) during the *locomotion and memory processes* .

Sensory-motor integration neural circuitry



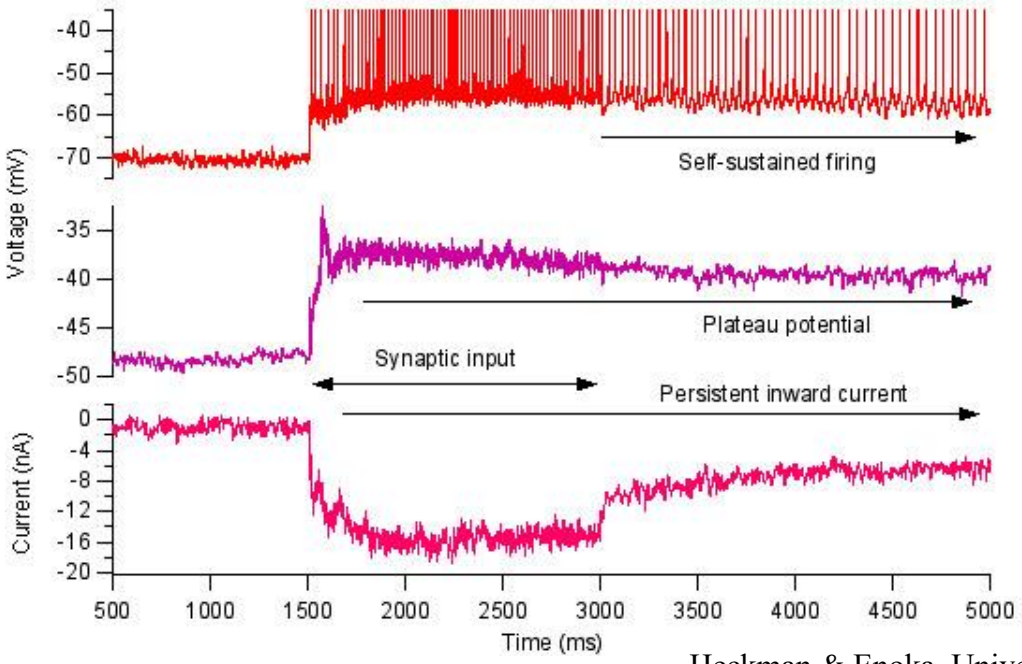
Hippocampal theta wave seems to originate by sensory input coming from brainstem, hypothalamus and medial septum.

It is a rhythm considered to be a timing mechanism to temporally organize movement sequences, memory encoding, trajectory for spatial navigation

…*after 50 years and hundred of experiments there is no widely accepted term that would unequivocally describe behavioral correlate of hippocampal theta oscillations* (Buzsaki, 2020)

Also the physiological input is undefined

Plateau potentials



Heckman & Enoka, University of Chicago and Colorado

In response to a short-lasting depolarization, prolonged depolarizations and AP discharges can be induced.

"Plateau" potentials can be originated mediated by neuromodulators like 5-HT, noradrenaline, ACh and Glu acting on metabotropic receptors and modulating the activity of L-type Ca^{2+} current or I_{CAN}, voltage-insensitive cationic current activated by Ca^{2+}.

The ability to produce “plateau potentials” could be useful

-in muscle for maintaining posture
inducing the necessity of a constant synaptic drive during the muscle contraction, protracting during the time.

-provides a mechanism for translating a transient input into sustained firing or a prolonged burst

Plateau Potentials in Vertebrates

Spinal cord

Motor neurons

Dorsal horn neurons

Hippocampus

CA1 pyramidal cells

Subiculum

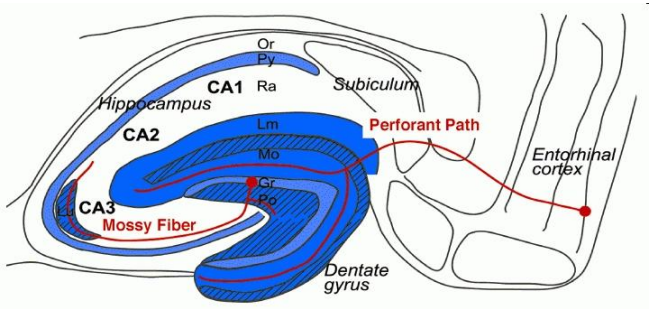
muscarinically-activated

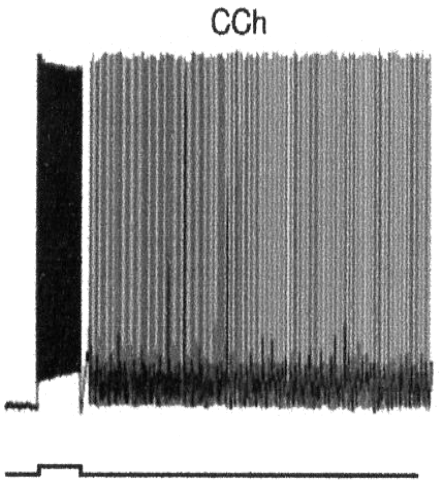
Subthalamus

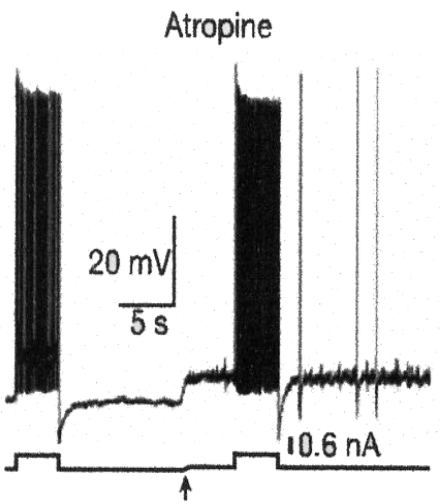
Thalamic relay neurons

Perigeniculate nucleus

Plateau potentials: good amplification mechanism







Enthorinal cortex is innervated by cholinergic fibers activating mAChRs and having a big influence on the intrinsic neuronal *firing pattern*.

Because EC is crucially involved in the acquisition, consolidation and recovery of memory traces, the "*plateau potentials*" are suggested to be an elementary mechanism utilized in the "*working memory*" the brain's ability to hold information for a short period of time.

It seems to depend on neuronal activity that lasts beyond the end of the stimulus that elicited it.

Egorov et al. Nature 420: 173-178, 2002

Although each neuronal type is different, there are undoubtedly general principles involving interactions among particular conductances waiting to be discovered.

Experimental advances (KO models, RNA interference, new pharmacological tools) will facilitate increasing details and realism of computer models.