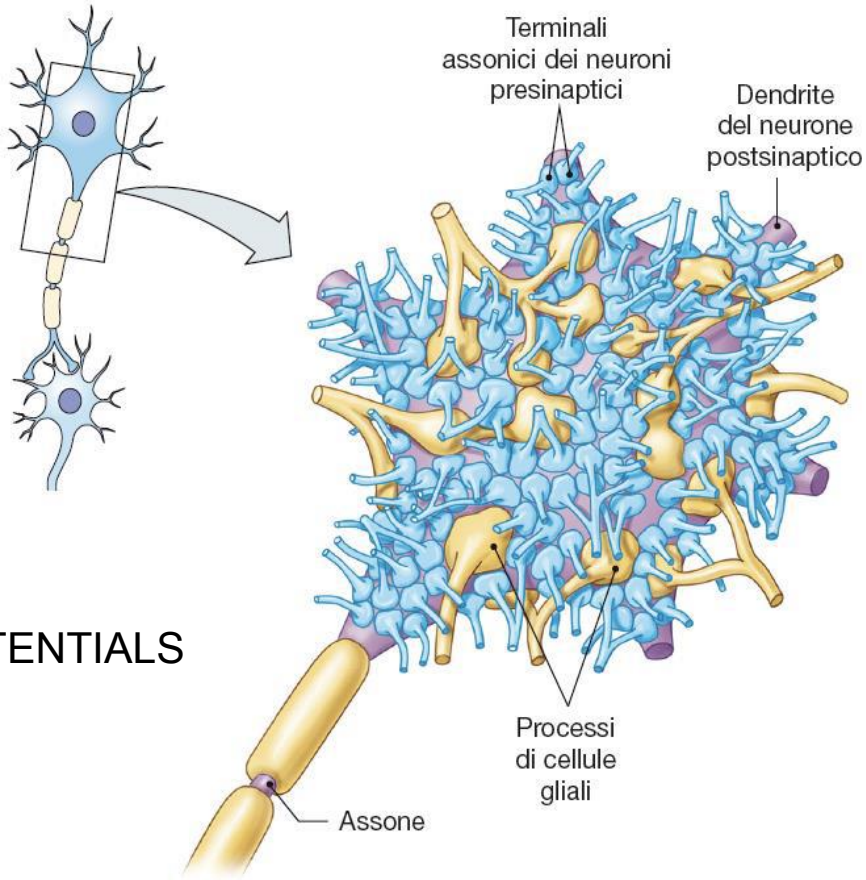
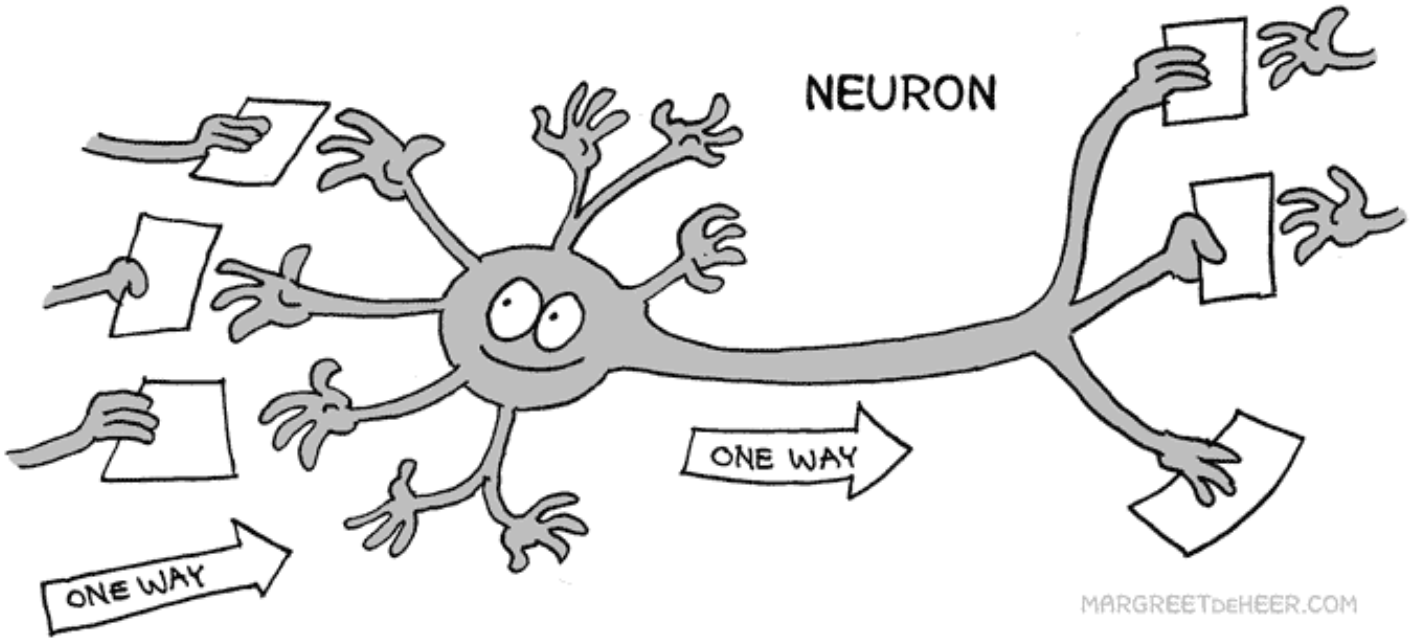
The operation of a neural network depends upon interactions among multiple non-linear processes…



CHEMICAL SYNAPSES
INTEGRATION OF SYNAPTIC POTENTIALS

Common properties of **Neural networks**:

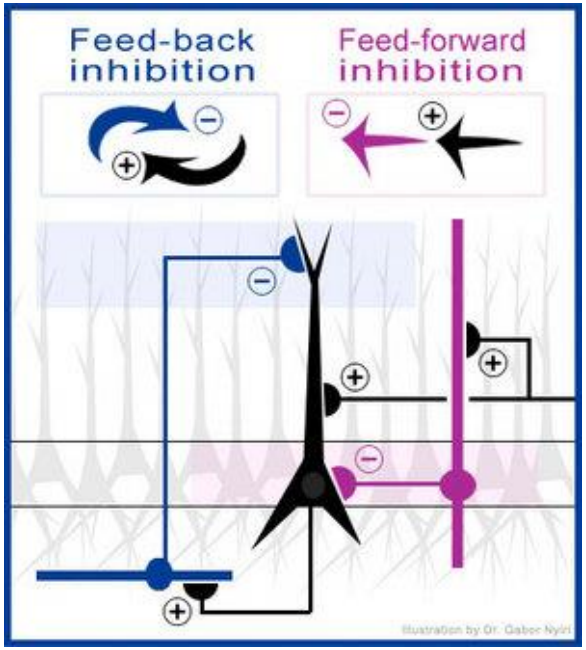
Each neuron participates in many synapses and can be connected with 1000 other neurons



Despite difference in the anatomy or function of neuronal circuits, two basic motifs emerge:

Recurrent inhibition

To regulate the firing in populations of excitatory principal neurons

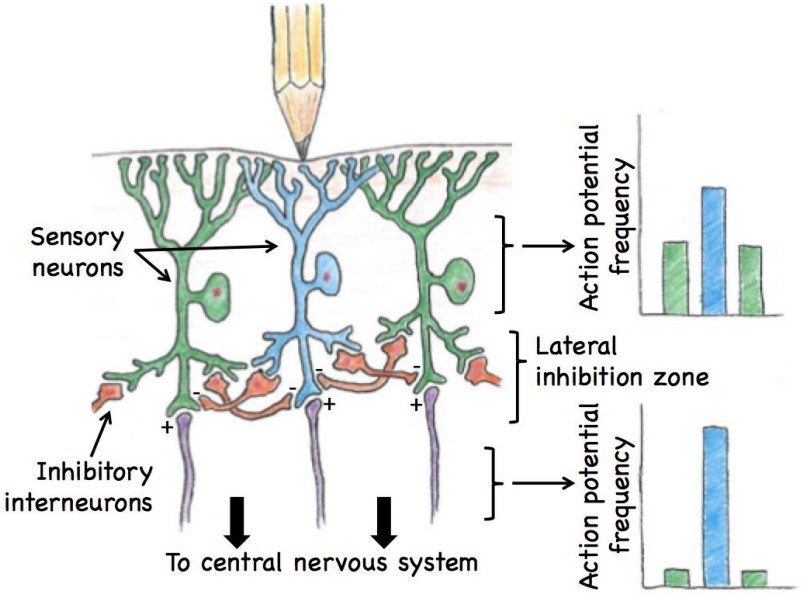


Upstream excitatory neurons

Feedback inhibition allows at a region to control its own output,
Feedforward inhibition regulates the integration of incoming information

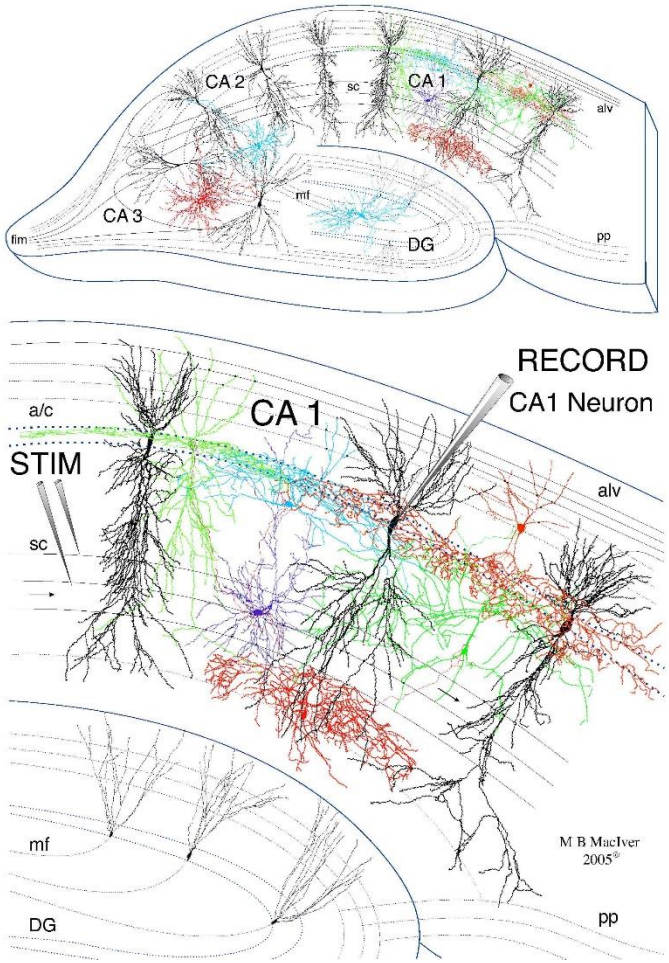
Lateral inhibition

Not linear relationship between stimulus intensity and AP frequency

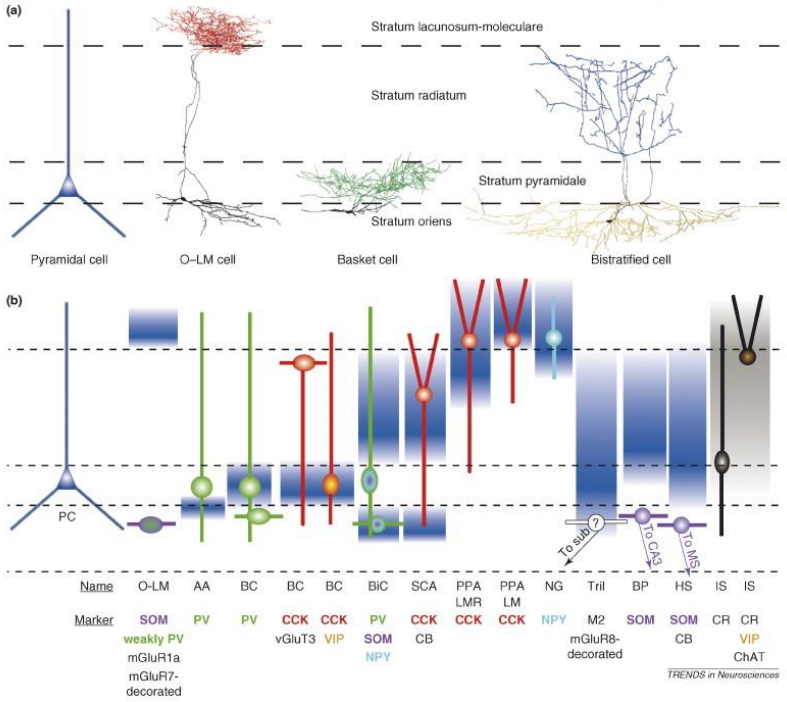


It is primarily designed to **block the lateral spread** of the excitatory signals and therefore increase the degree of contrast

PYRAMIDAL CELLS AND INTERNEURONS



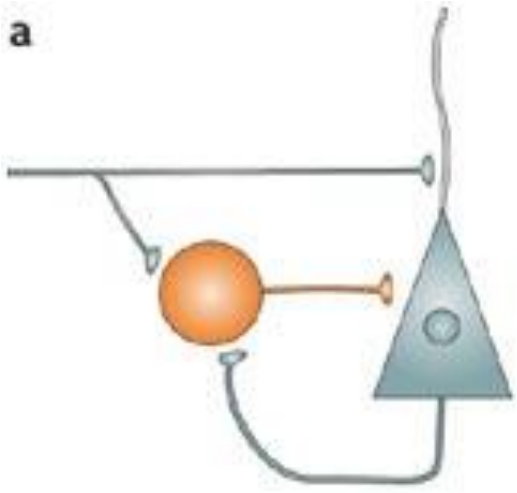
Inhibitory interneurons are a broad class of anatomically and neurochemically diverse cell types with different functional roles.



Various GABAergic cells contain different Ca^{2+}-binding proteins (e.g., calbindin, calretinin, and parvalbumin) or peptides (e.g., cholecystokinin, somatostatin, and neuropeptide Y; Klausberger and Somogyi, 2008)

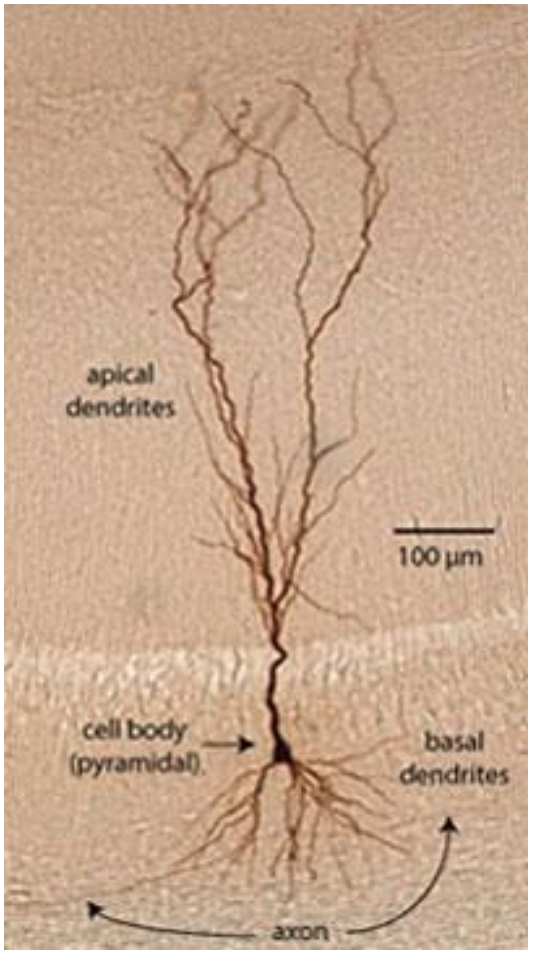
A simplistic classification of interneurons as either feedback or feedforward has limited value.

Many cells (see hippocampal basket cells), are excited by both the axon collaterals of pyramidal cells and by the excitatory axons of more remote structures.



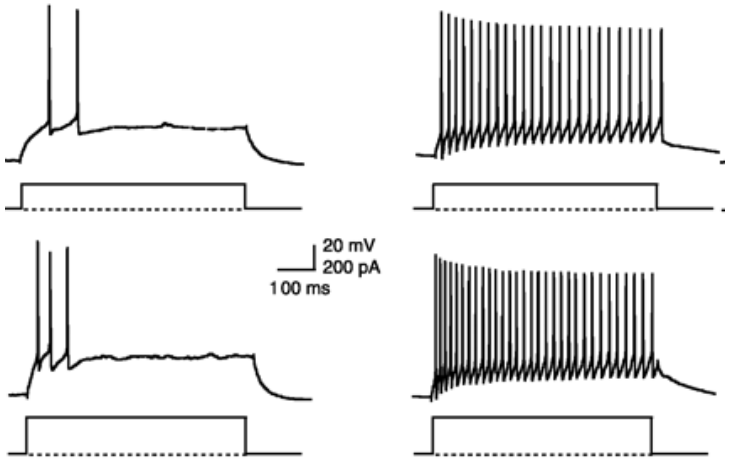
Nature Reviews/Neuroscience

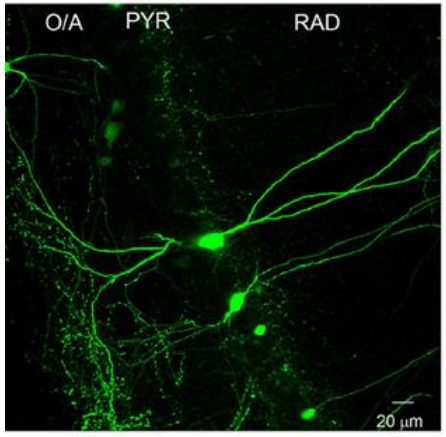
It is possible to identify interneurons by cell morphology or firing properties



pyramidal-like cell

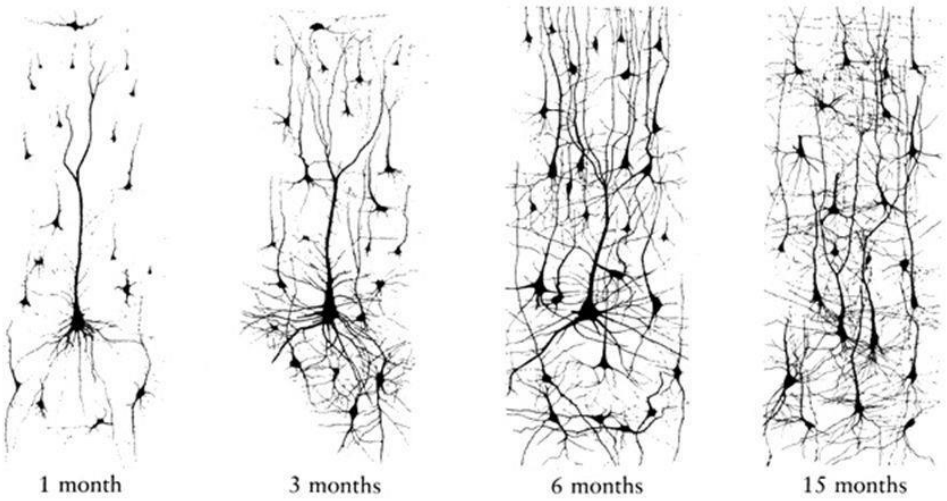
interneuron



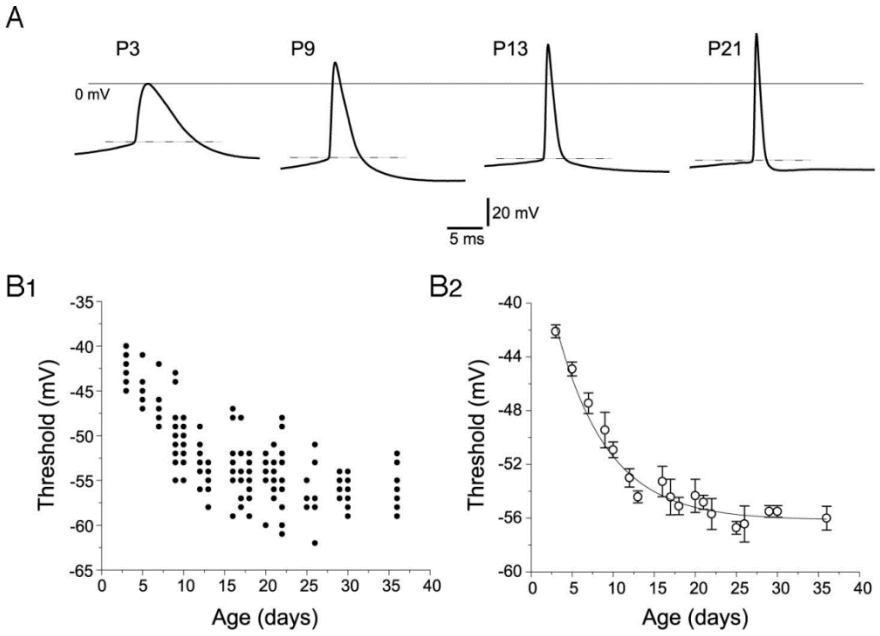


Neural networks change during development.

Neural networks in postnatal life



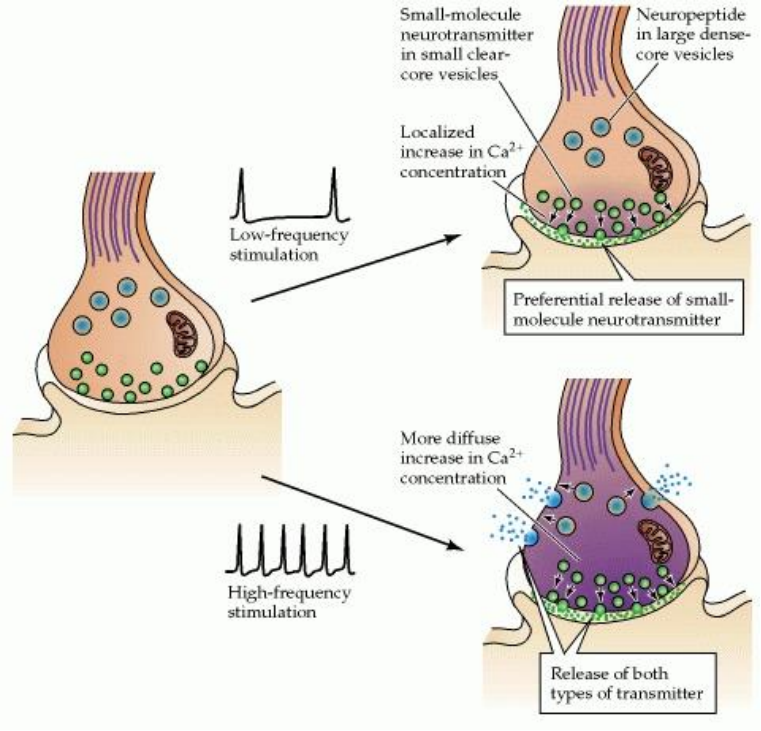
Action potential-development



Zhang, J Neurophysiol 91: 1171–1182, 2004.

There is a convincing evidence, that many types of neurons release neuropeptides.

It was established that a cell could co-release a fast-acting “classical” neurotransmitter and a modulatory factor, that could be a peptide, nucleotide (e.g., ATP), Zn^{2+}, neurotrophic factor, nitric oxide, or endogenous cannabinoids – among other modulators.

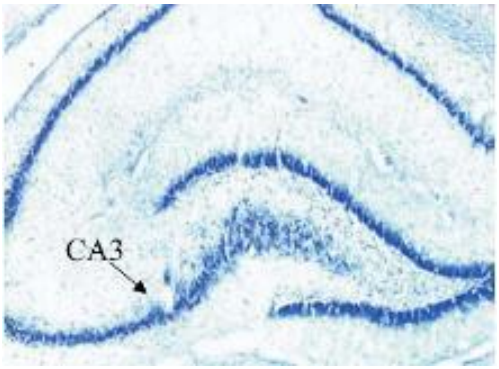


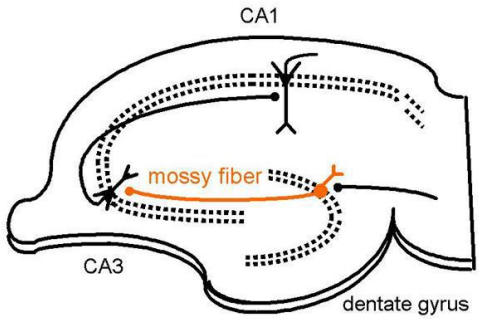
Neuroscience, Sinauer Associates; 2001.

Neuropeptides-high MW, «slow acting» may diffuse some distance to act on G protein-coupled receptors, even modulating gene expression

The co-release of two classical neurotransmitters has been less studied

MFs can switch, in a developmentally and activity-dependent regulated way, the type of neurotransmitter released *(Gutierrez, 2005).*



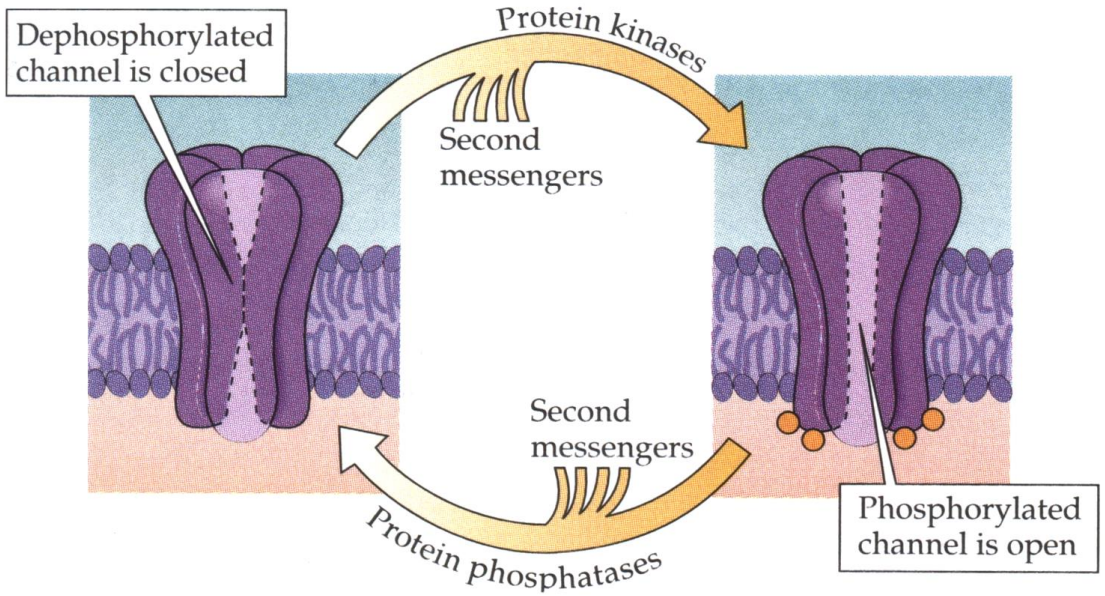


Early in Postnatal Life the main neurotransmitter released from Mossy Fibre (MF) terminals is GABA while at later developmental stages they use exclusively glutamate.

MFs express GAD65 and GAD67 as well as the mRNA for the vesicular GABA transporter, VGAT *(Safiulina et al., 2010).*

In some conditions, such as kindling or activity-dependent processes, MFs can transiently resume a GABAergic phenotype in adulthood.

Channels can be directly modulated by phosphorylation which can cange the circuit properties



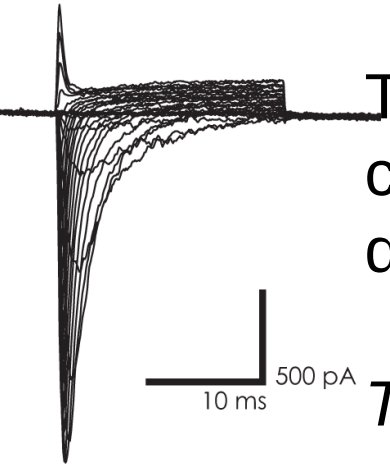
Channel subunits are good substrates for phosphorylation by PKA and PKC

Phosphatases dephosphorylate residues regulating channel activity.

The use of exogenous kinases demonstrates that phosphorylation can produce modulation, but does not address the question of whether the cell actually uses this mechanism under normal physiological conditions.

The introduction of highly specific inhibitors does ask whether endogenous kinase activity is necessary for a given physiological response

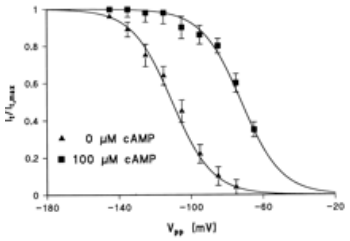
What aspect of channel function is altered by phosphorylation?



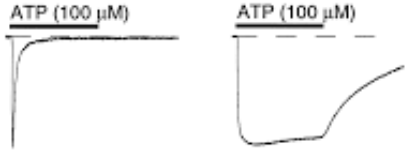
The biophysical mechanisms modulated by channel phosphorylation exhibit a striking diversity, phosphorylation may affect:

Total peak current. I = Npi

It may enhance the affinity of the ligand binding, perhaps by inducing a conformational change in the binding site.



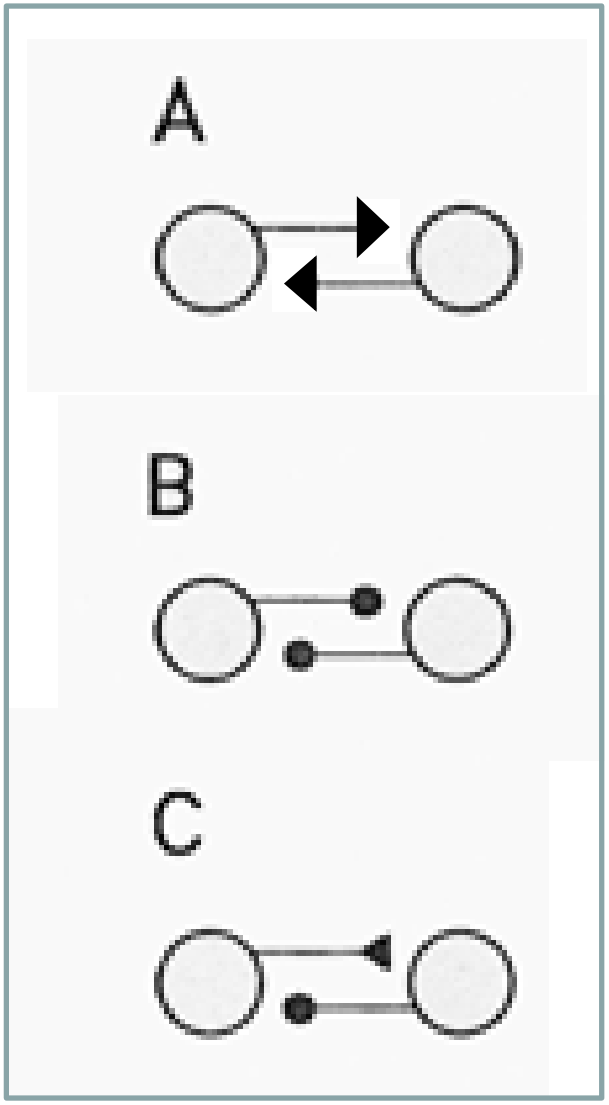
It can change desensitization rates



It can shift the voltage dependence and affect kinetics of channel activation and inactivation

NETWORK CONNECTIVITY PATTERN

Most commonly encountered features



Reciprocal (or recurrent) excitation

promotes firing synchrony

Reciprocal inhibition

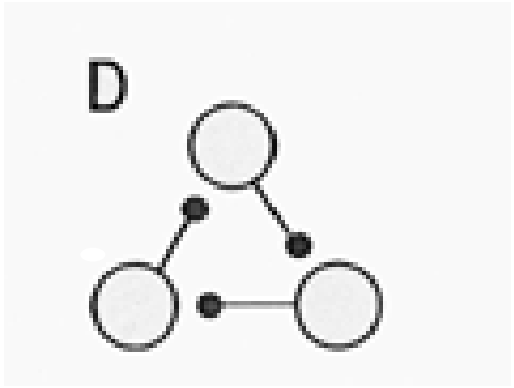
Neurons fire in alternation (fast synaptic inhibition)

Recurrent inhibition

Regulate excitability

▶ excitatory

● inhibitory

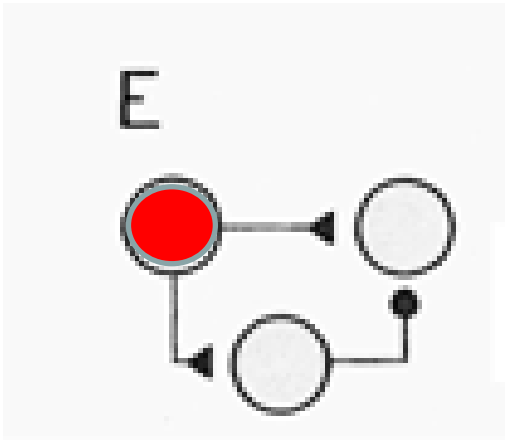


Recurrent Cyclic inhibition

promotes oscillatory burst pattern

► excitatory

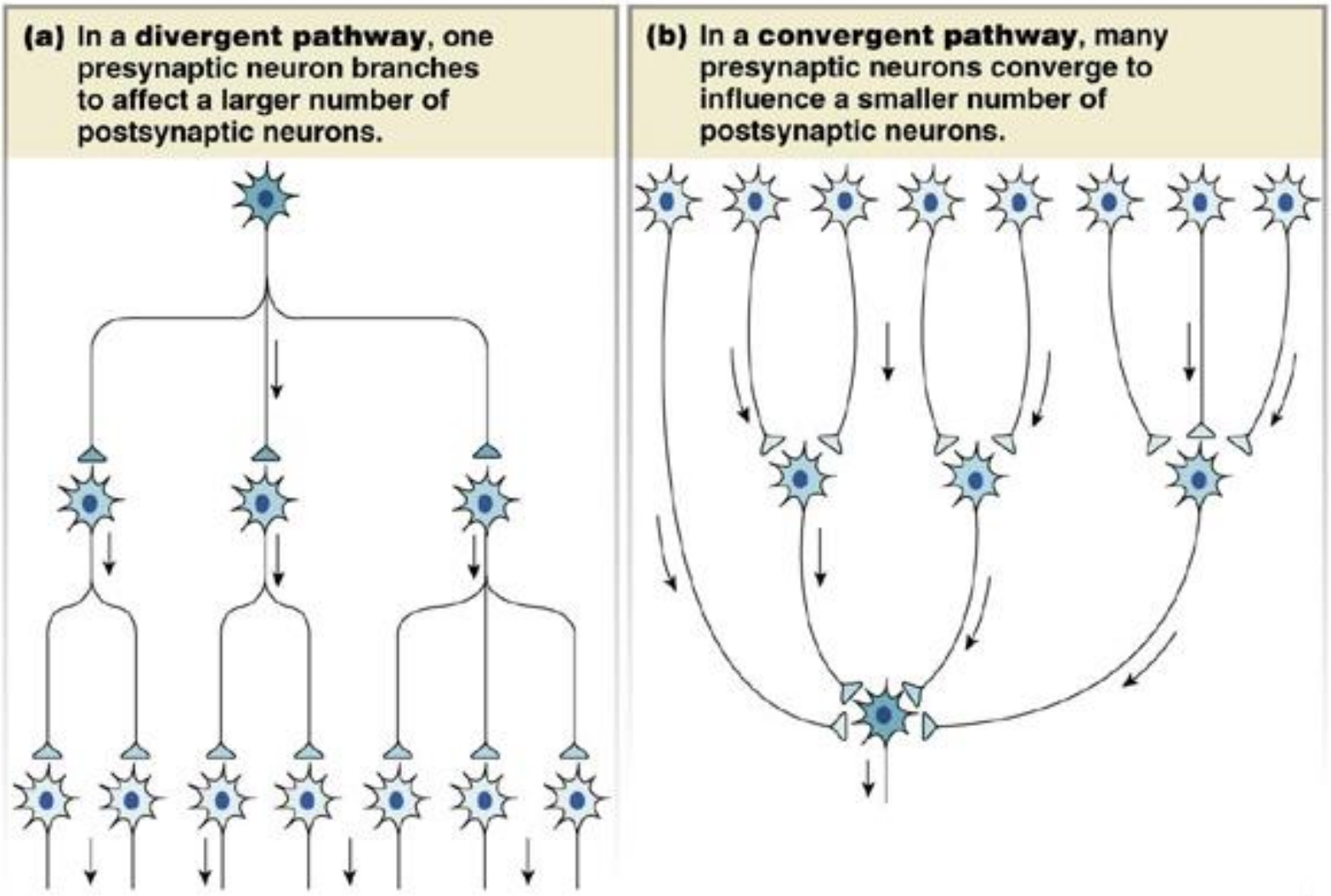
● inhibitory



Parallel Excitation and Inhibition

The cell may mediate
more than one action on its target

Communication is not always one-to-one

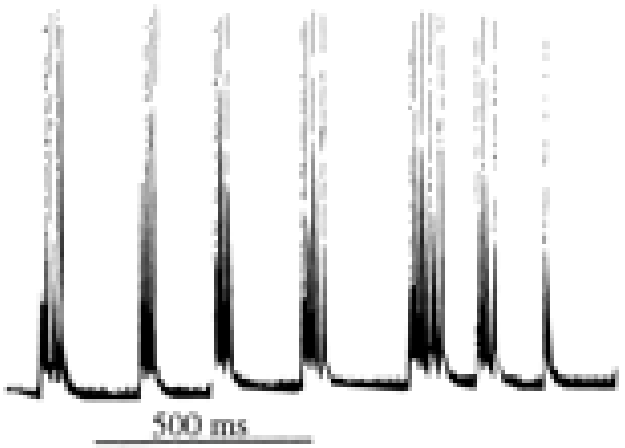


Neurons assembled into neuronal networks generate different rhythms (e.g., theta, gamma etc) which sign specific brain states (learning, sleep).

Although the relationship between neuronal output (firing pattern) and function (during a task) is not fully understood, there is evidence that **a given neuron can show very different firing patterns according to brain state**.

Neuronal rhythm in the circuits can be central to brain function

Despite the decades of intensive investigations, the neuronal mechanisms responsible for rhythmic bursting in many CNS regions are still debated in the literature.



Studying bursting activity in neuronal networks…

in vivo or *in vitro* recordings?

In vivo studies sometimes fail to describe the "real" physiology because neurons can be sensitive to commonly used solvent narcotic agents.

In particular, **barbiturates** potentiate inhibitory synaptic processes and depress:

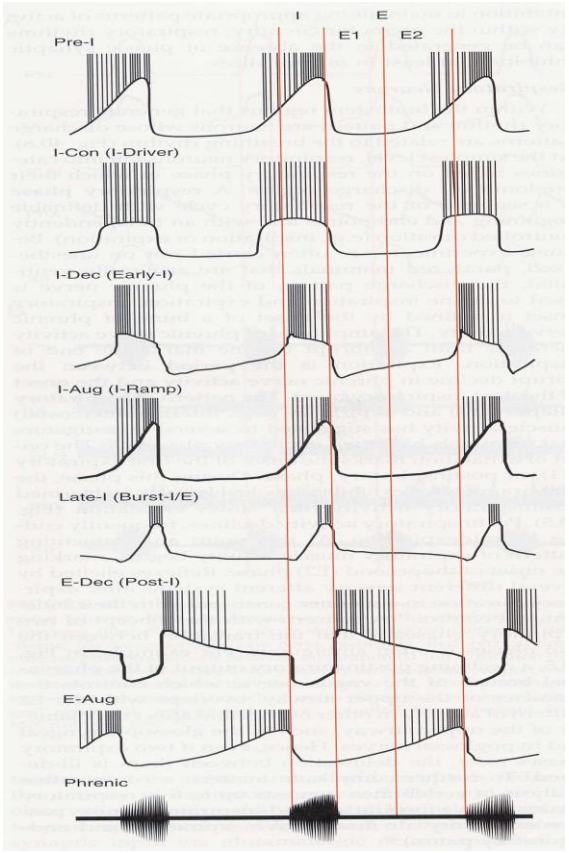
- ✓ **Fast inactivating Na_f channels**
- ✓ **Rectifying K^+ channels including K_{ATP}**
- ✓ **Pre- and post-synaptic Ca^{2+} channels**
- ✓ **Ca^{2+}-activated K_{Ca} channels**

All conductances that can be involved in cellular bursting.

Reciprocal Inspiratory and expiratory patterns controlling respiratory rhythm

BRAINSTEM slice

Increase of external K+ to induce the rhythm



Fundamental Neuroscience

Looking at your *in vitro* scientific model…

You must be sure to maintain all the structures necessary for the rhythm generation

Respiratory neuroanatomy of
the rat brainstem

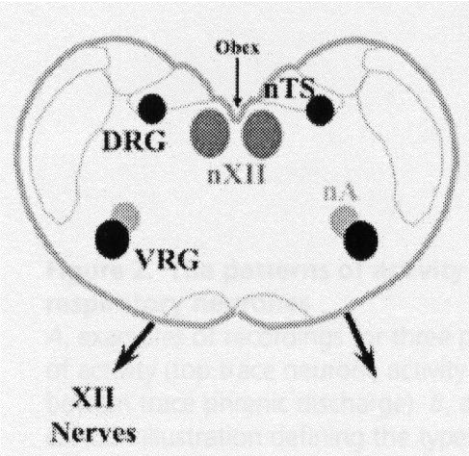
INSPIRATORY NEURAL NETWORK

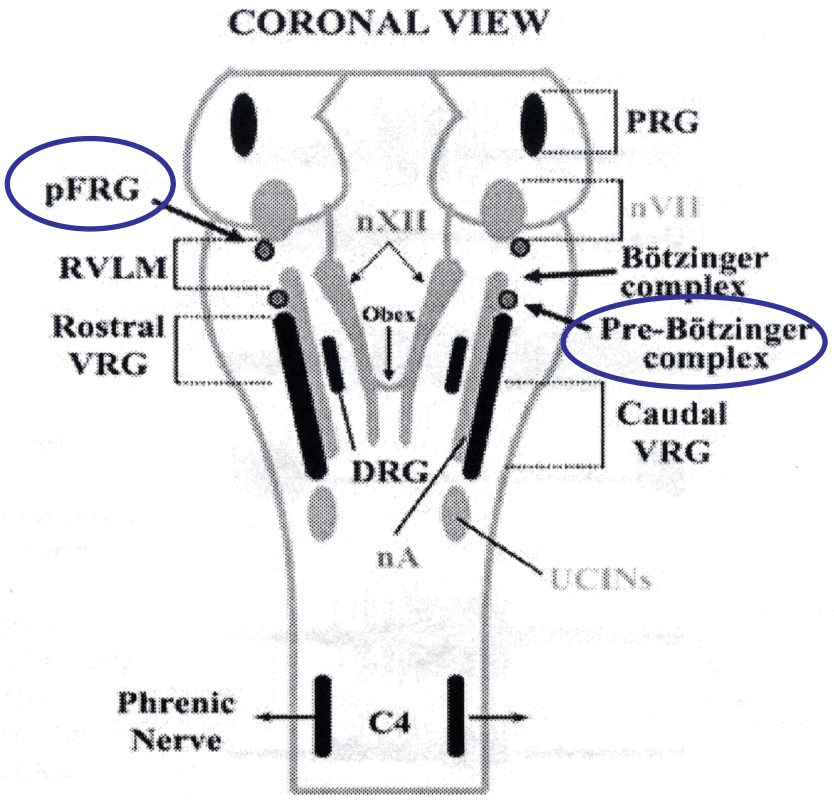
PBC in ventro-lateral midolla

PRE-INSPIRATORY NEURAL NETWORK

Rostrally in ventro-lateral midolla
Parafacial respiratory group (pFRG)

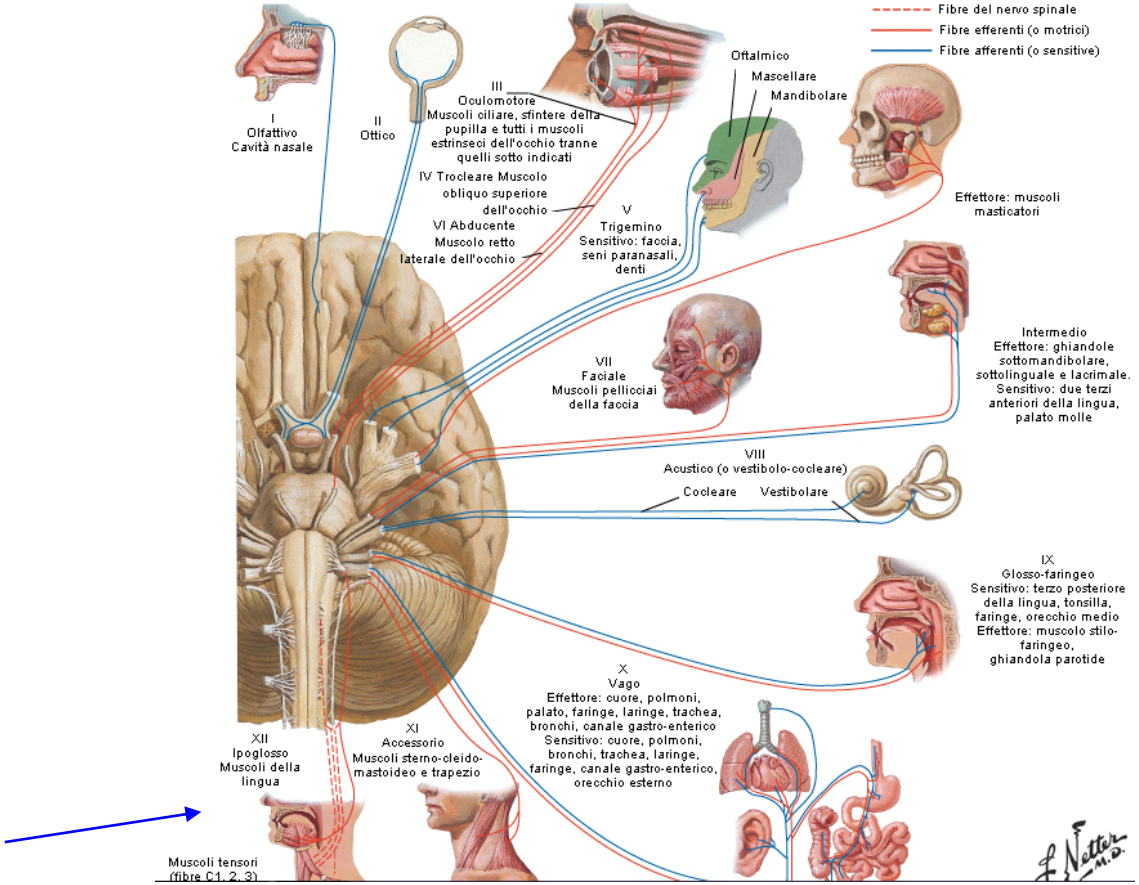
TRANSVERSE VIEW



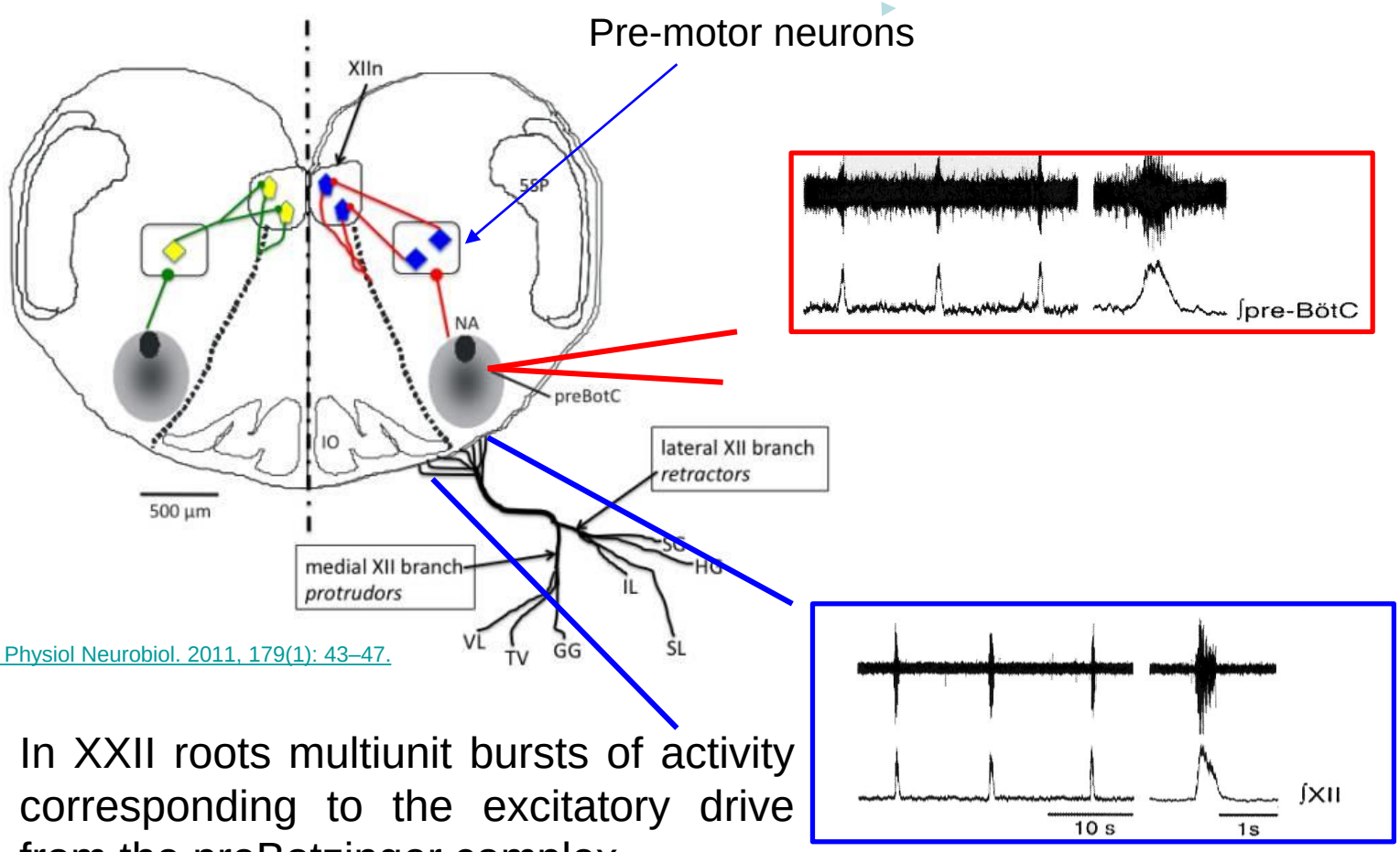


Duffin, Exp. Physiol. 89.5: 517-529, 2004

Cranial nerves



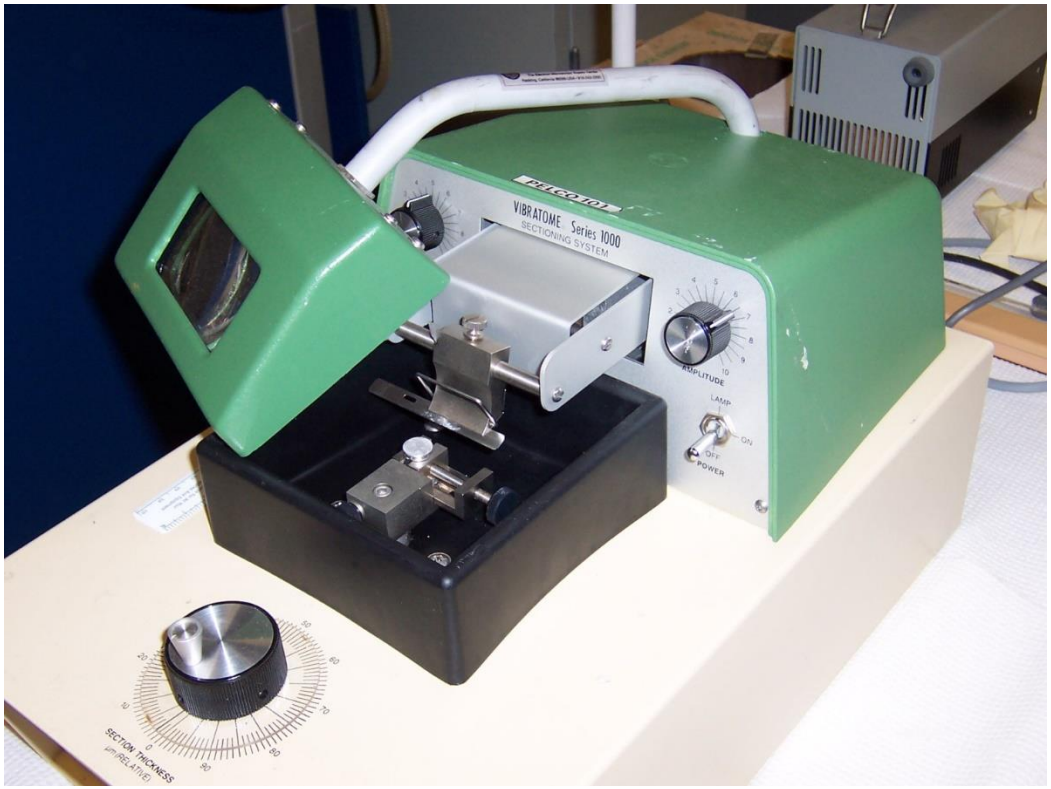
RESPIRATION-RELATED MOTOR DRIVE TO HYPOGLOSSAL MOTONEURONS

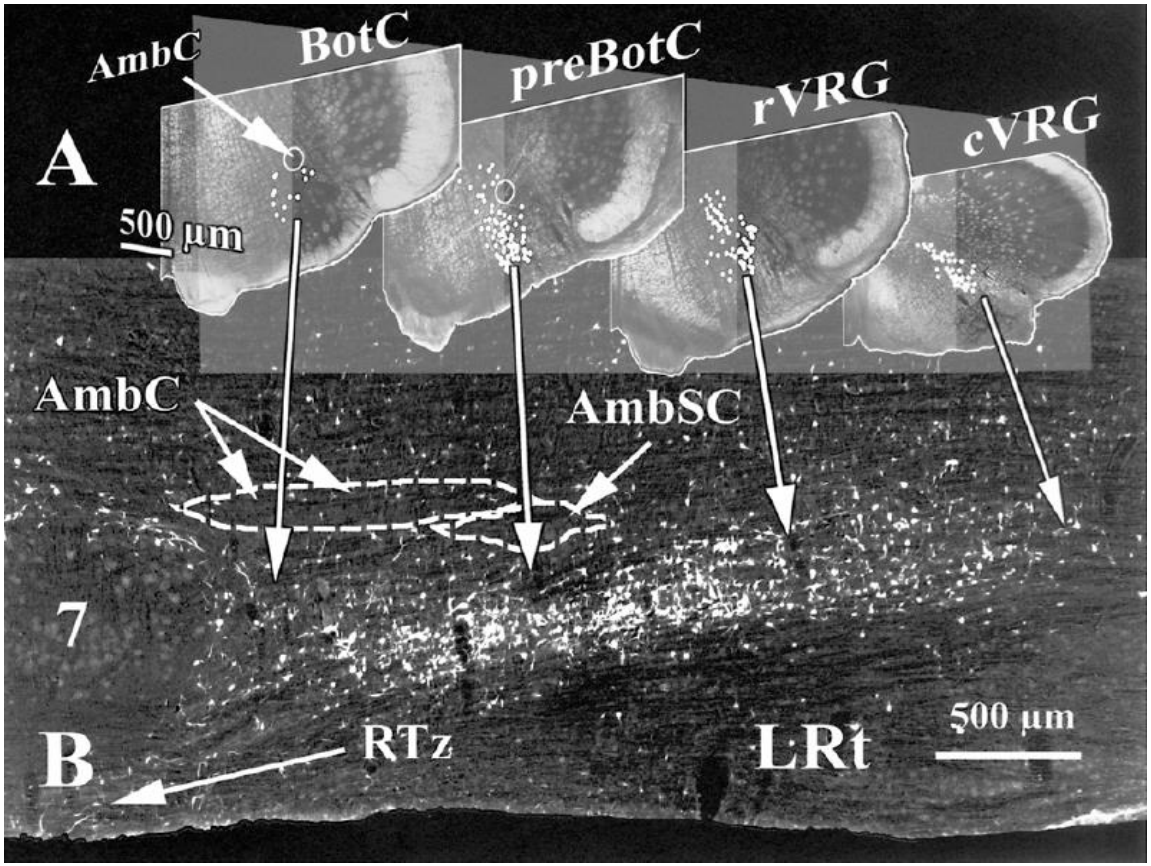


Fregosi, Respir Physiol Neurobiol. 2011, 179(1): 43–47.

In XXII roots multiunit bursts of activity corresponding to the excitatory drive from the preBotzinger complex

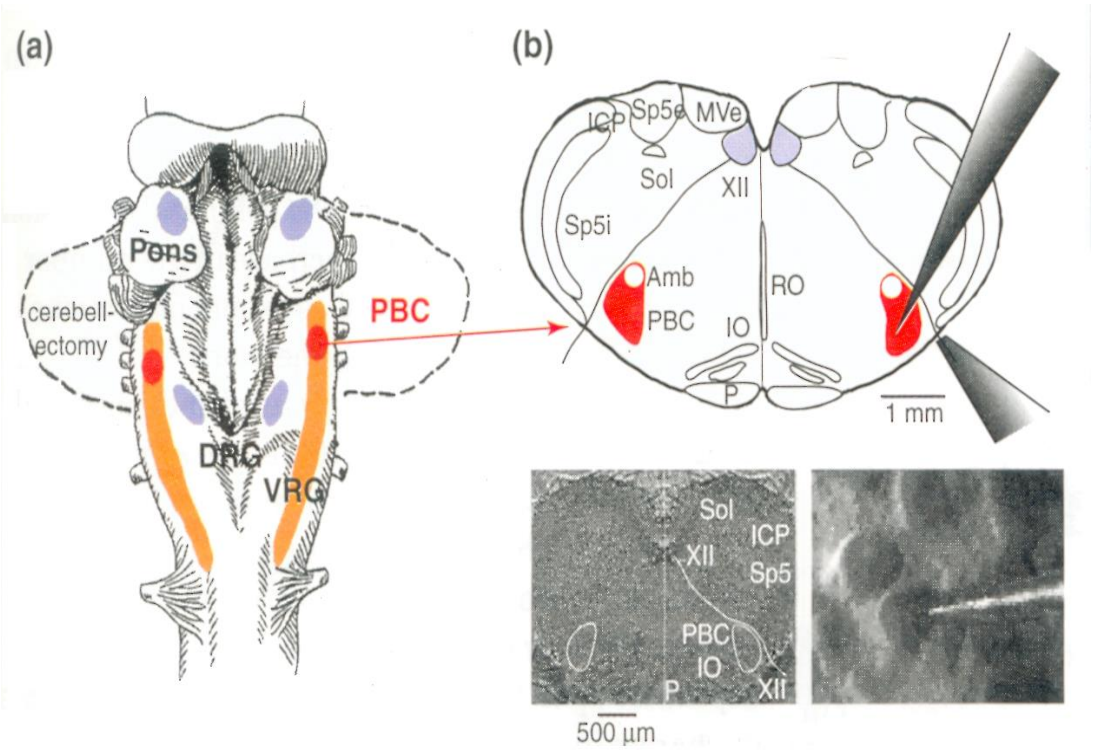
Brain slice preparation to study the control of respiratory rhythm must be prepared from the neonatal brainstem, because in adult brains the thickness needed to capture the entire network results in anoxia within the tissue core




AmbC
BotC
preBotC
rVRG
cVRG
A
500 µm
AmbC
AmbSC
7
B
RTz
LRt
500 µm

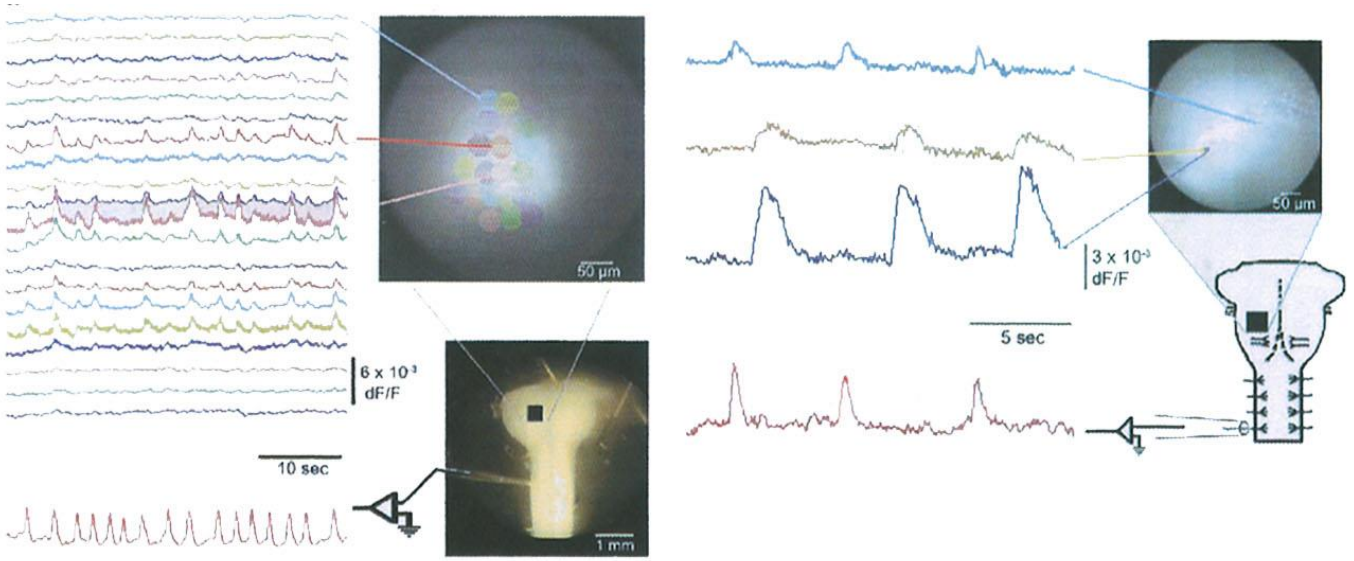
Brain slices

This preparation disrupts the extensive dendritic arbors of neurons that extend over 0.5 mm
even in the neonatal rats.
The restriction to a small number of neurons will limit synaptic interactions,
which results in **low frequency firing**.



Richter and Spyer TRENDS in Neuroscience vol 24, 2001

Ca^{2+}-imaging to monitor the rhythm

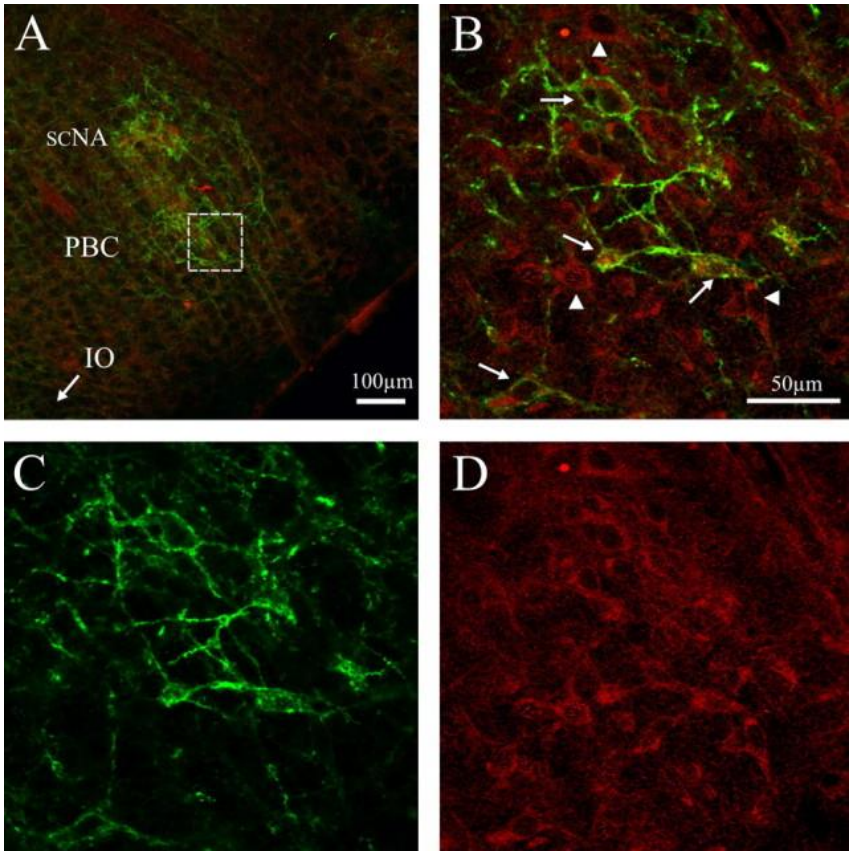


C4

Fig. 1. (a) Fluorescence changes in rostral medulla (traces on the left) and integrated C4 "phrenic" ventral root burst activity (bottom trace) in brainstem–spinal cord preparation (E17 mouse, 5% CO_2, pH 7.4), stained with calcium-sensitive dye. Scheme and photograph of the preparation (right) indicate, in colors corresponding to optical traces, the areas for averaging the fluorescence (right, top). Blue, red and pink lines, for example, join medullary areas surveyed for averaging with corresponding optical traces. Note that spots showing respiratory-related activity are distributed along a rostrocaudal column. A photograph of such a preparation is shown (right, bottom) with attached suction electrode and the approximate region of view indicated with a black square. (b) Inspiratory-(top trace) and expiratory-related (second and third traces) fluorescence changes recorded optically from rostral medulla (E20 brainstem–spinal cord). Integrated C4 ventral root activity is shown below. Fluorescence signals were averaged over areas corresponding to cell bodies of fluorescent cells. Colored lines join areas in medulla used with their respective optical recordings. A schematic representation of the preparation showing the regions of optical and electrical recording is drawn to the approximate scale of the photograph in a.

Eugenin et al., 2006

Markers can label the neural population of interests. Immunolabelling for specific receptors can help to localize the area of interest



NK1 (for SP in green) and 2PY1 (for ATP in red) receptors in N. ambiguous and PBC

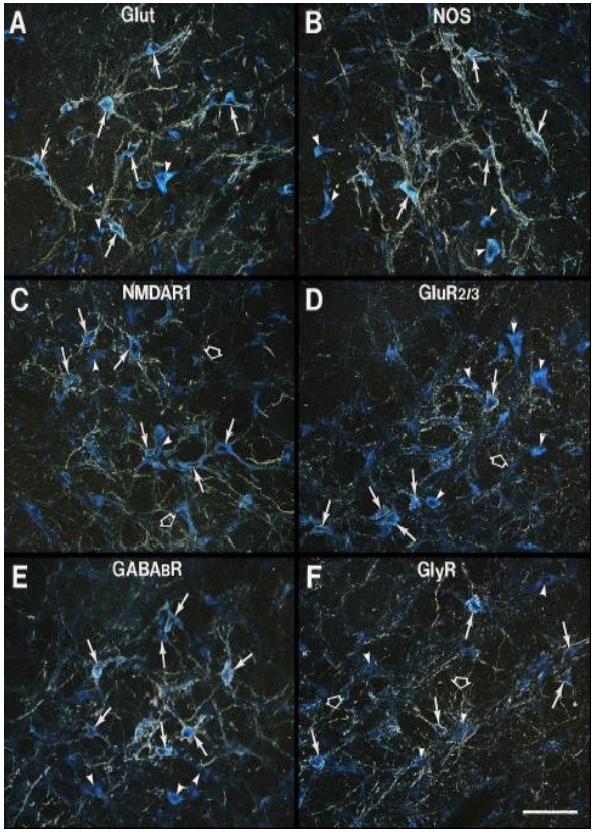
Lorier et al. J Neurosci. 2007 Jan 31; 27(5): 993–1005.

Neuromodulation of the rhythm

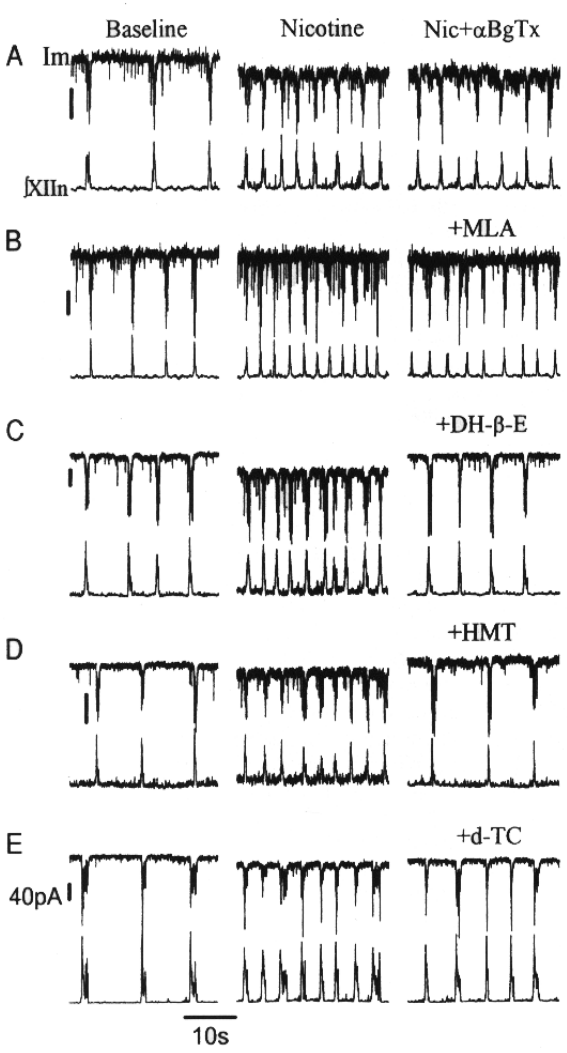
Firing activity can be modulated in duration and frequency

by various substances:

- 5-HT
- Histamine
- Acetylcholine
- Glutamate
- Substance P
- TRH (Thyroid Releasing Hormone)
- Noradrenaline
- GABA
- Glycine
- Enkephalin



Scala=50mm



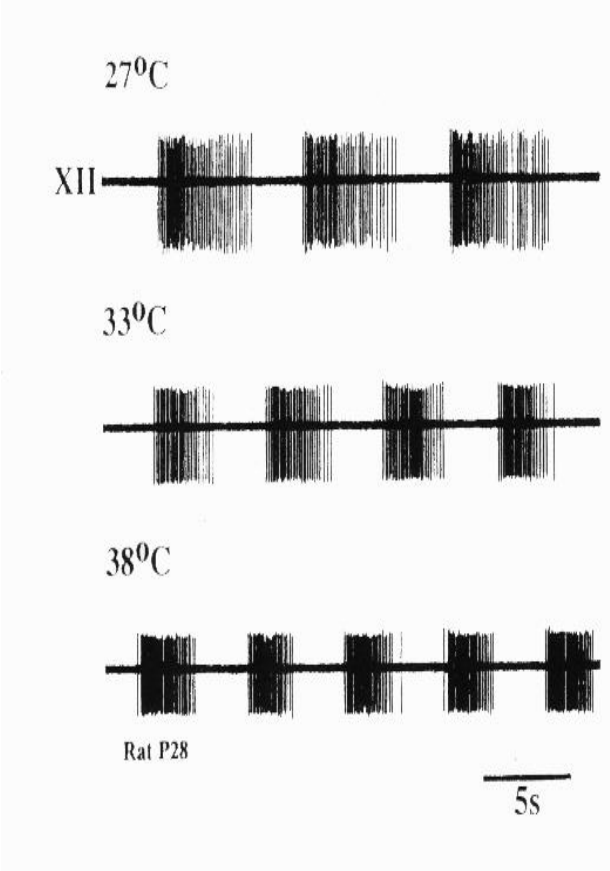
Nicotinic and muscarinic AChRs can modulate the rhythm

Various nicotinic antagonists suggest the presence of $\alpha 4\ \beta 2$ subunits.

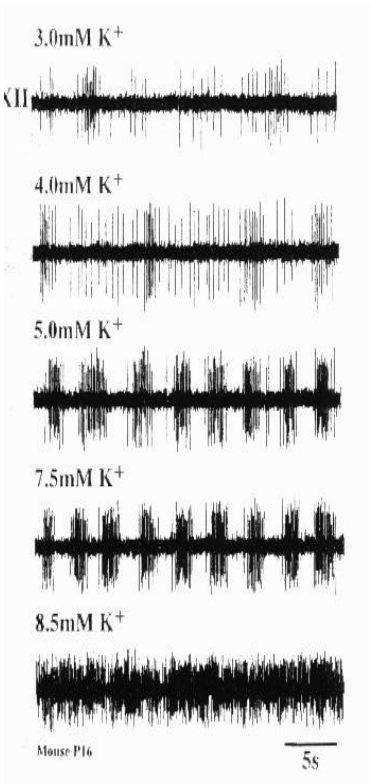
Shao and Feldman, J. Neurophysiol. 88: 1851-8, 2002; 83, 2000

In brain slices the rhythm frequency can change:

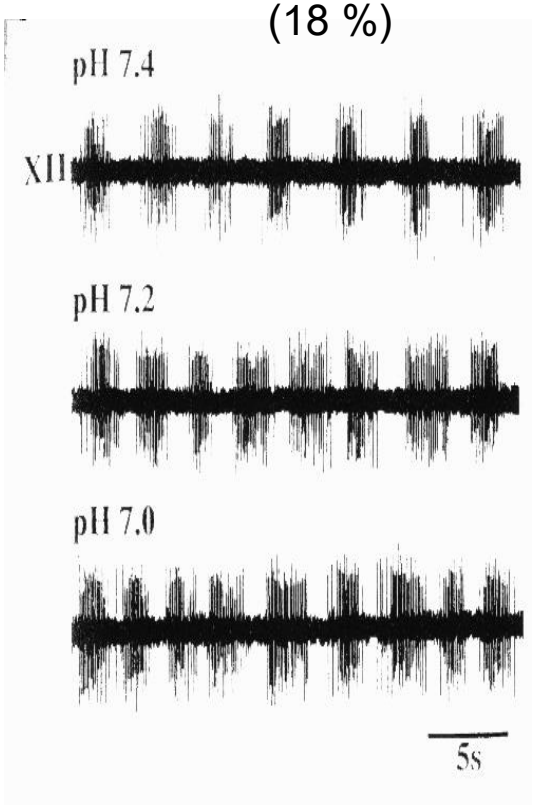
from 29 °C to 38 °C (54%)



from 4 to 7.5 mM $[K^+]_o$ (52 %)



from pH 7.4 to pH 7 (18 %)



JFR Paton et al., Pflűgers Arch, 428:250-260, 1994

To distinguish two neurons connected each other:

Anatomical methods consist in various labelling techniques but in this way it is not easy to identify a functional network.

Electrophysiological techniques give more immediate results and physiological information.

Cross correlation histograms allow a simultaneous recording of two cells activity thought to be synaptically connected and are able to identify syncronous events.

It is possible to detect if correlations are affected by stimulus drive, learning or experience or changes in behavioral context

Correlations can also provide important information about the functional architecture of neuronal networks.

The recent advent of recording techniques such as multielectrode arrays and two-photon imaging has made obtaining such recordings easier.

Theory for the generation of rhythm

Synaptic networks

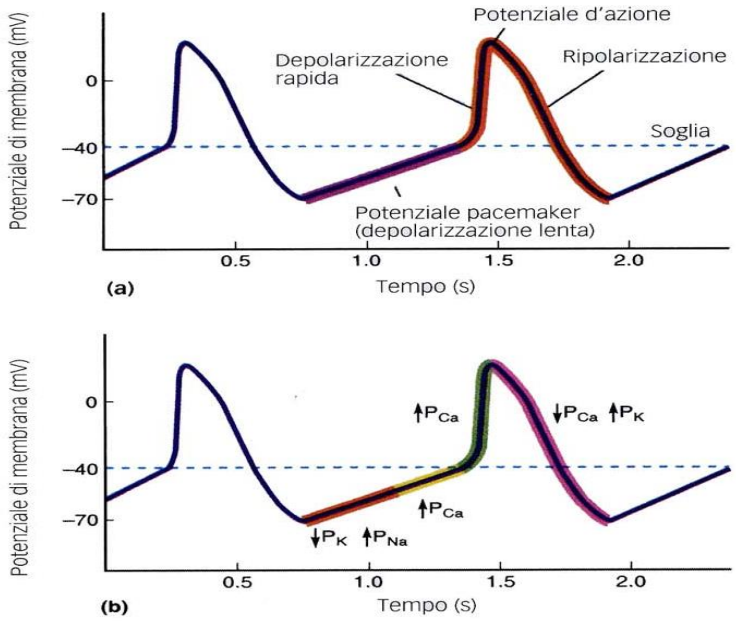
Rhythm is generated by excitatory and inhibitory interactions among neurons

Pacemakers intrinsic membrane properties cause rhythmic oscillations of membrane potential. This can be suggested by the persistence of the rhythm in the absence of synaptic inhibition.

Both models require a tonic input for generating the oscillations.

Hybrid models require pacemaker neurons and synaptic interactions.

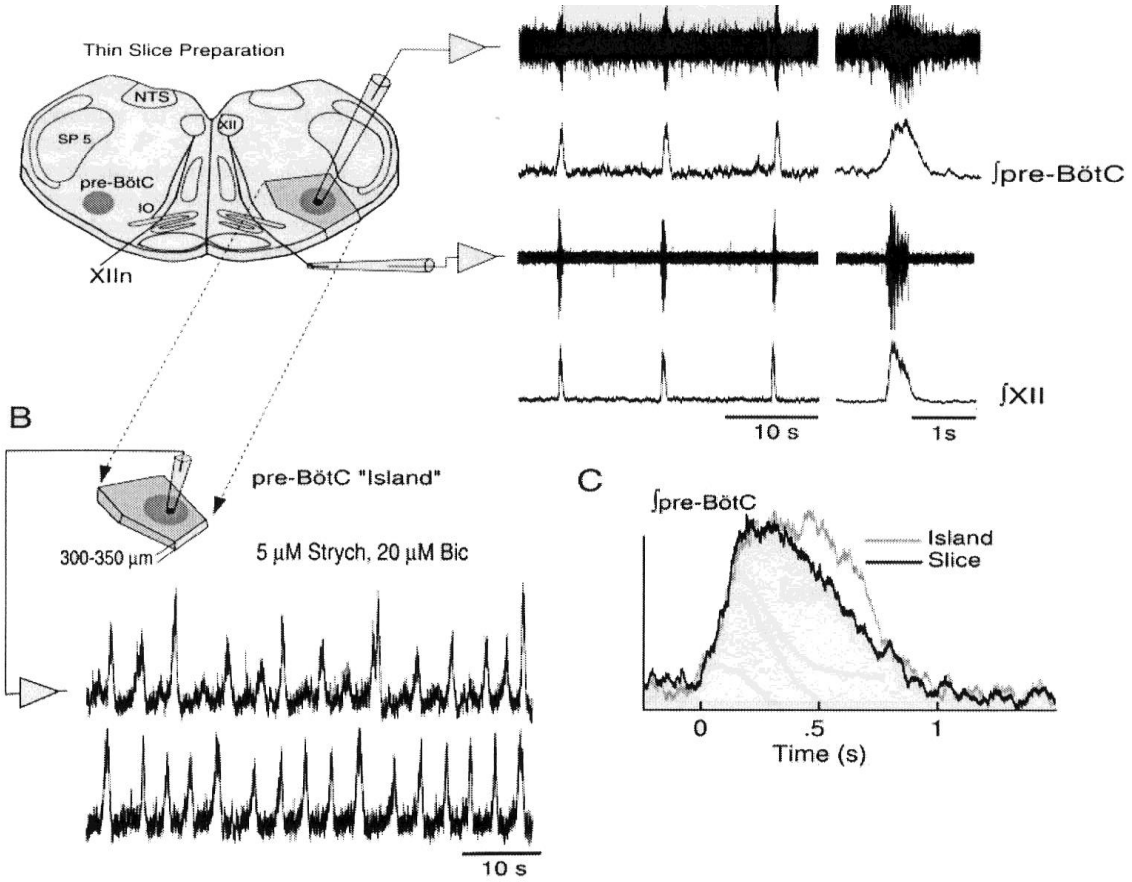
What is a pacemaker cell?



A cell with inherent bioelectric properties that enable it to rythmically burst

Bursting pacemaker neurons

Do these cells possess particular intrinsic properties that enable them to discharge with a rhythm?

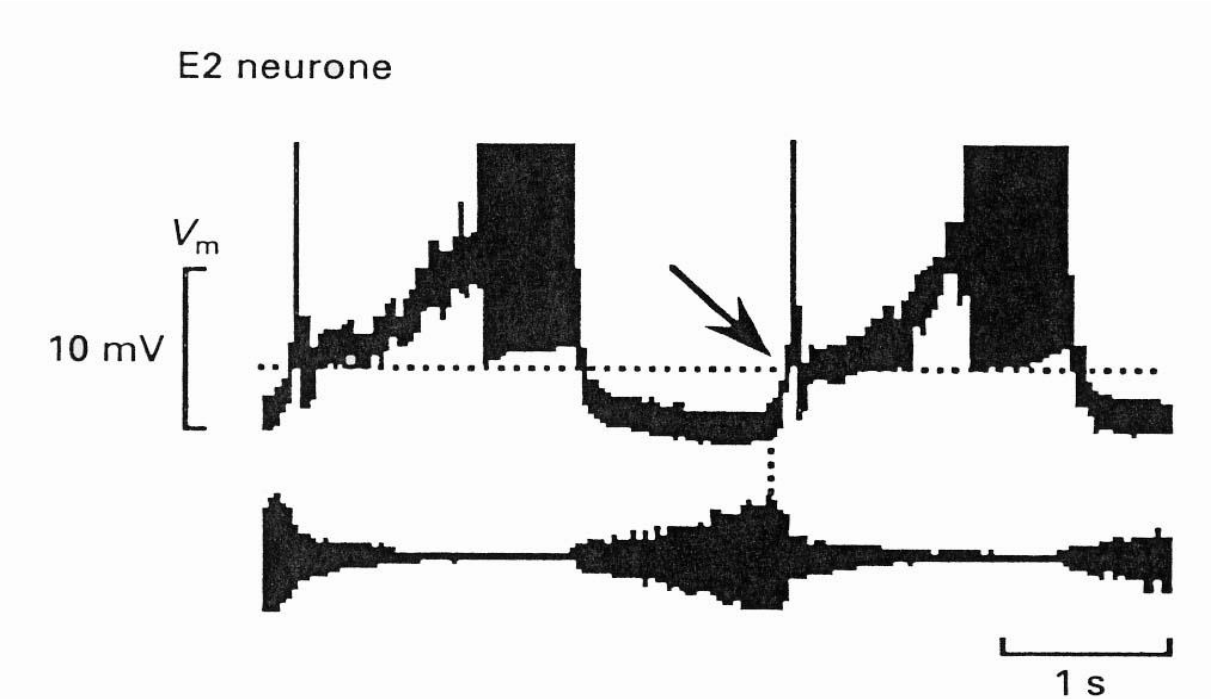


Microislands of pacemaker neurons in PBC

Johnson et al., J. Neurophysiol., 85: 1772-1776, 2001.

T-type Ca^{2+} currents are activated immediately after the synaptically-induced hyperpolarization is removed

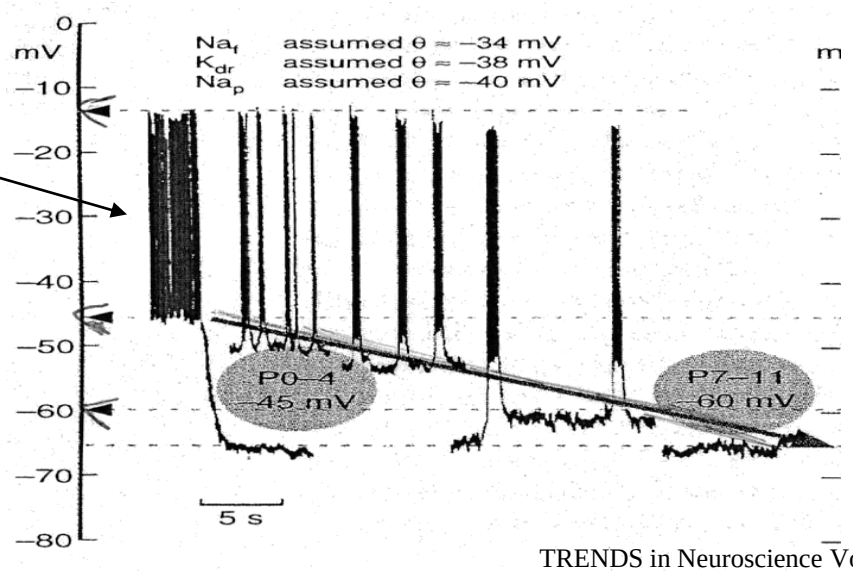
T-type currents might contribute to give the trigger for membrane depolarization during the rhythm change of phase

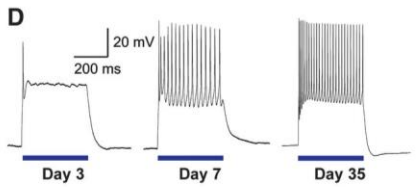


Richter et al., J. Physiol. 470: 23-3, 1993

Development of biophysical properties of neurons

High Rm





TRENDS in Neuroscience Vol. 24 N.8, 2001

Membrane potential in newborn animals is low. This increases the neuron excitability and potentiates the ability to develop voltage oscillations. Such oscillations may depend by a limited set of voltage-gated ionic conductances.

Newborn animals use INa_P. INa_f are inactive. The activation of **INa_P** requires only a small depolarization from the resting potential.

I_h, I_{CaT} e I_{KA} channels are often inactive in neonatals.

As individual neurons mature….

their membrane potential becomes more negative

The R_m declines

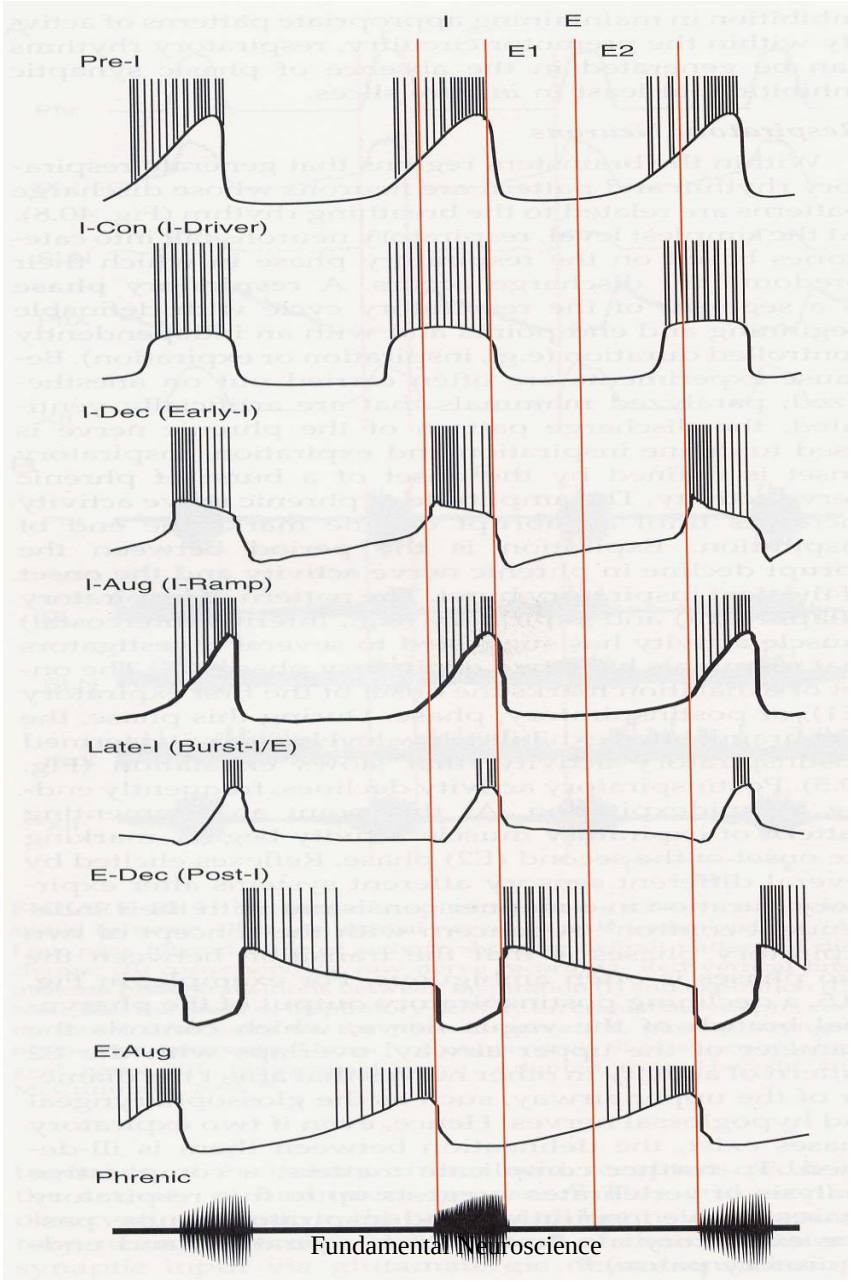
Some pacemaker neurons can become inactive

Synaptic processes as inhibitory synaptic activity become more effective

and hyperpolarize the membrane potential

The repertoire of ion conductances that become available is enriched.

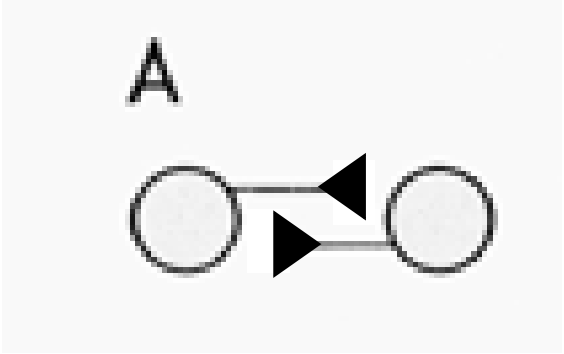
Let's try to explain the reciprocal Inspiratory and expiratory patterns



Fundamental Neuroscience

Different inspiratory neurons have different discharge patterns

This is because of the different roles of various neurons in controlling the respiratory activity

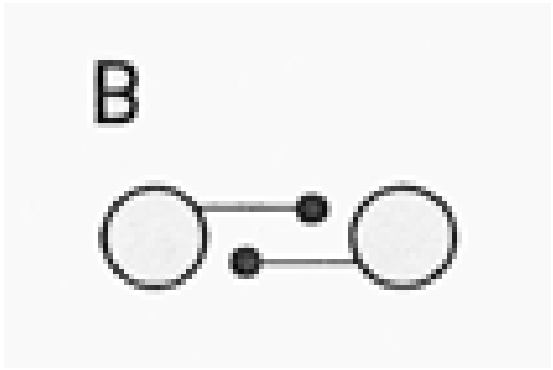


Reciprocal (or recurrent) excitation

promotes firing synchrony

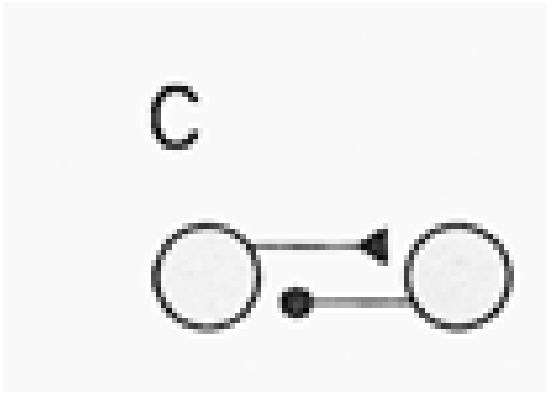
excitatory

inhibitory



Reciprocal inhibition

Neurons fire in alternation (fast synaptic inhibition) or synchronize (slow synaptic inhibition)



Recurrent inhibition

Regulate excitability

If neuronal firing activity **is** reciprocal in 2 types of neurons the rhythm can be generated by RECIPROCAL INHIBITION

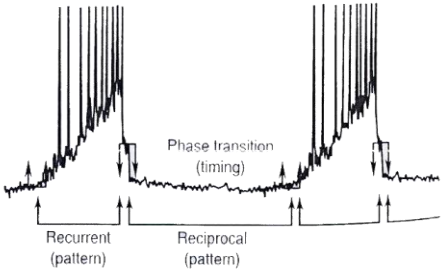
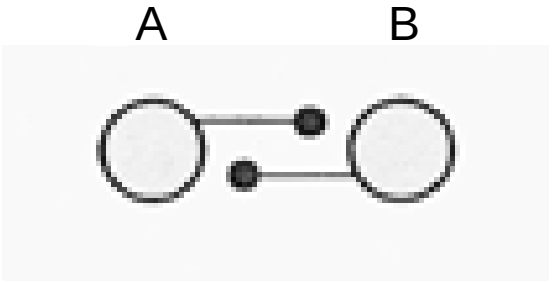


FIGURE 40.6 Membrane potential and discharge pattern of an augmenting inspiratory (or expiratory) neuron. The timing of three distinct types of inhibition is shown.



How the alternation of the respiratory phases is explained?

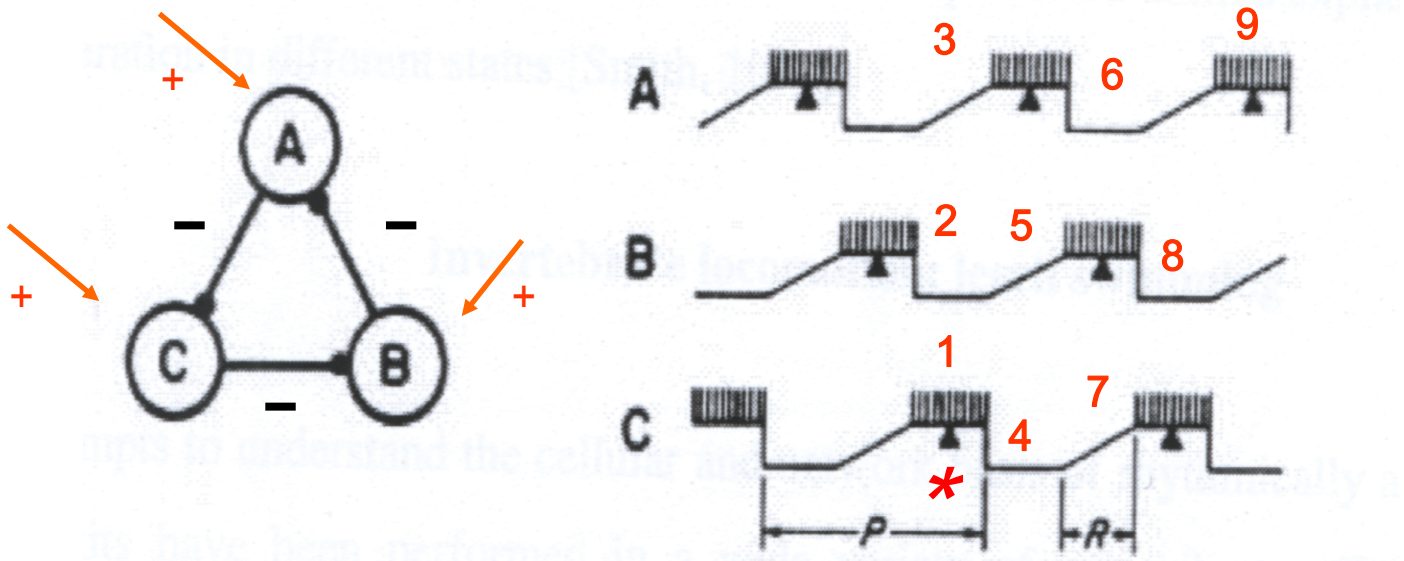
- One neuronal type can autonomously limit itself through a **synaptic fatigue mechanism.**

- Adaptation or rebound currents may decrease the inhibitory effect of one cell on the other one.

There is incomplete understanding about the neuronal activity of various circuits.

Computational modeling also on the modulation of bursting activity by various agonist and antagonists of neurotransmitters is a promising approach.
Not only predicts network behavior but allows theoretical and experimental testing of therapeutic strategies to recover neuronal–affected patients

CYCLIC RECURRENT INHIBITION



Inhibitory ring with three tonically excited neurons.

Each cell has an inhibitory contact with another cell and receives one of them. If C is depolarized, its postsynaptic cell, B must be hyperpolarized while its presynaptic cell A is just recovering from the past inhibition.

As the cell A recovers from inhibition and reaches the threshold to generate a pulse, cell C is inhibited. This disinhibits the cell B and allows it to enter in a “recovery” phase. When the cell B is recovered, it inhibits A, allowing to cell C to recover. When C is recovered, B enters an inactive phase and A starts a recovery phase.