Synaptic integration in the central nervous system

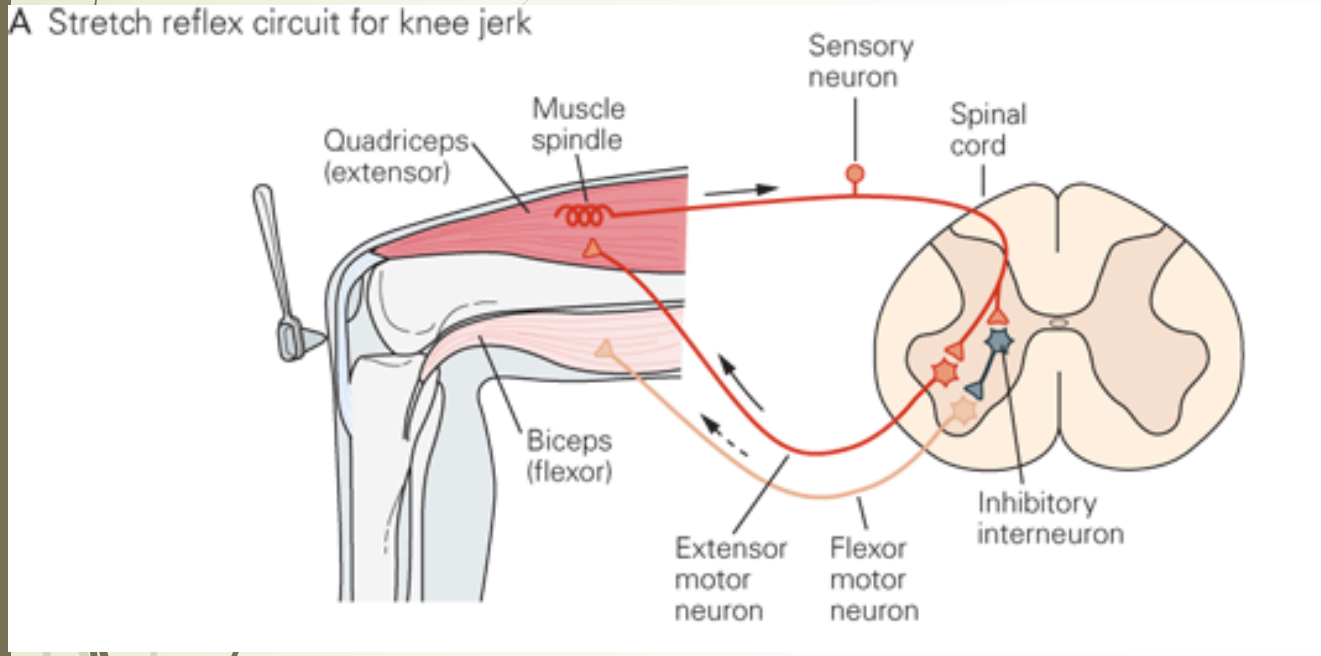
Based on Ch 10 Principle of Neural Science Kandel

See also Ch 9, 10, 13, 14, 15 Cellular and molecular neurophysiology Hammond

- Like synaptic transmission at the neuromuscular junction, most rapid signaling between neurons in the CNS involves ionotropic receptors in the postsynaptic membrane.
- Many principles that apply to the synaptic transmission between motor neuron and skeletal muscle fiber at the neuromuscular junction also apply in the fiber at the CNS

- Synaptic transmission between central neurons is more complex:
  - **Muscle fibers** are innervated by only one motor neuron while **ventral nerve cells** receive connections from hundreds or even thousands of neurons.
  - **Muscle fibers** receive only excitatory inputs whereas **central neurons** receive both excitatory and inhibitory inputs.
  - All synaptic action on **muscle fibers** are mediated by one neurotransmitter, Ach, which activates only one type of receptor (nAChR); a single **central neuron** can respond to different types of receptor (ionotropic or metabotropic).
  - **Neurons** must integrate diverse inputs into a single coordinated action.
  - **Nerve-muscle** synapse is very efficient: 1 action potential in the motor neuron produce 1 action potential in the muscle fiber. In contrast connections made by **presynaptic neuron into motor neuron** are only modestly efficient: often 50-100 excitatory neurons must fire together to produce a synaptic potential in a motor cell

Most of the initial studies were performed on the synaptic inputs onto spinal motor neurons that **control the stretch reflex**

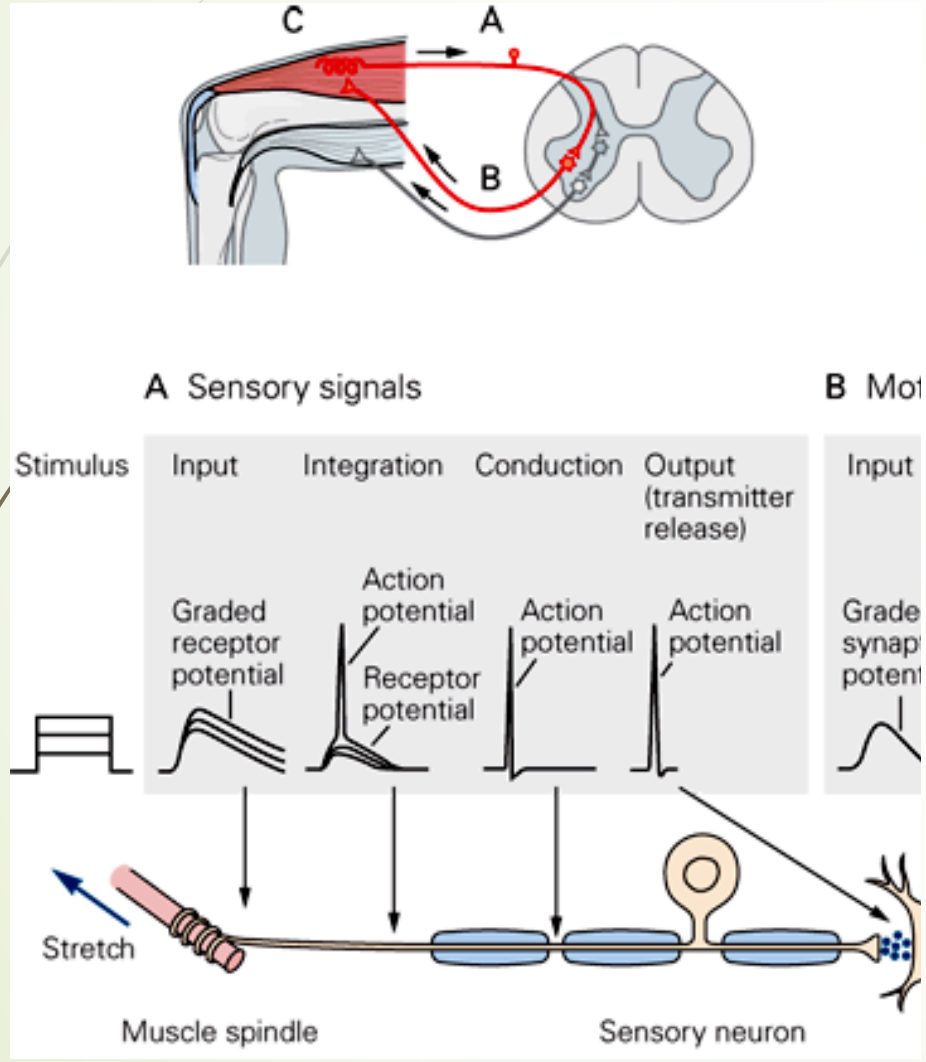


The stretch receptor at the extensor muscle makes an excitatory connection with an extensor motor neuron that innervates the same muscle group.

It also make an excitatory synapse with an interneuron, which in turn makes an inhibitory connection with a flexor motor neuron that innervates the antagonist biceps femoris muscle group.

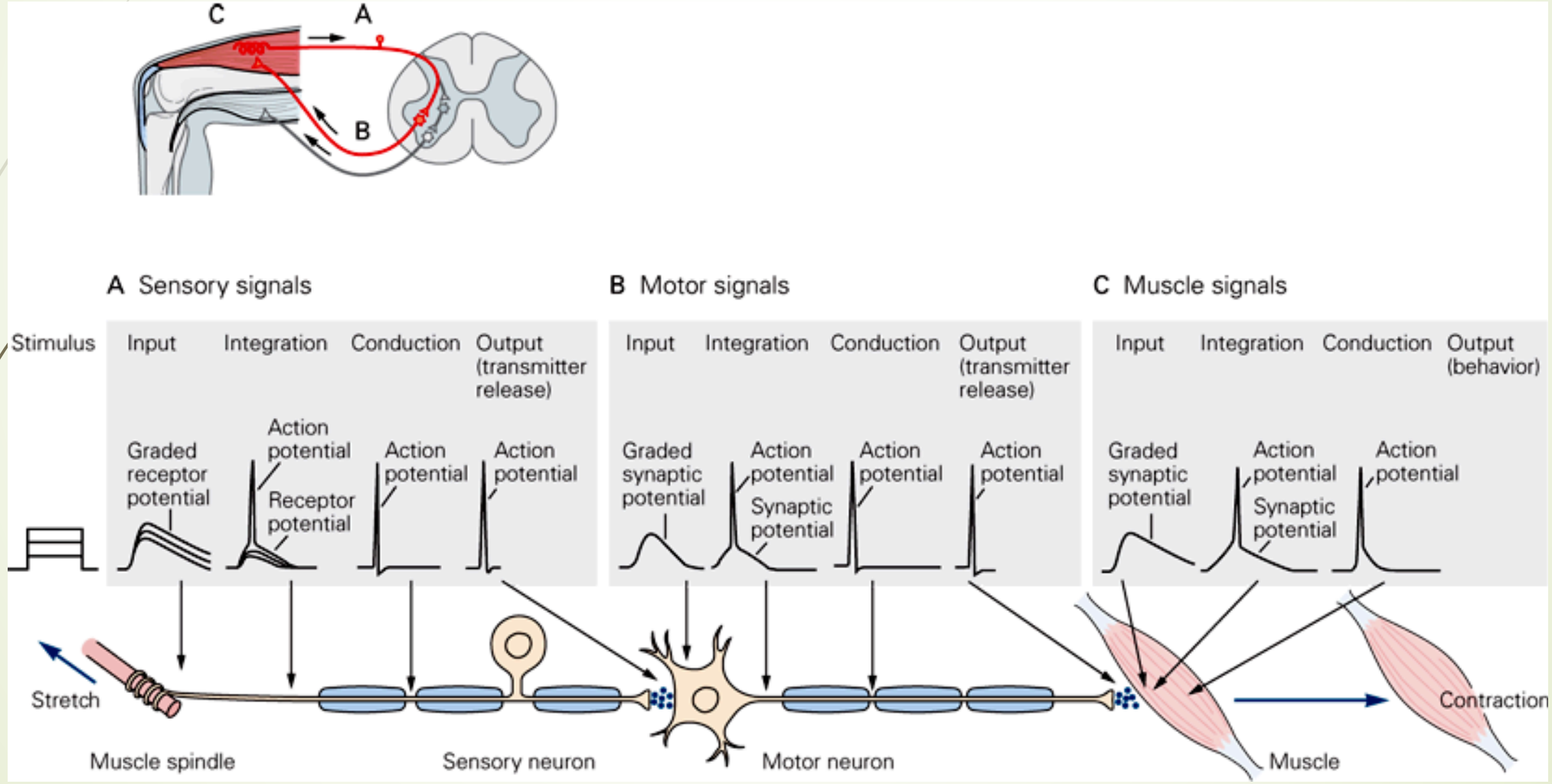
Conversely an afferent fiber from the biceps excite an interneuron that makes an inhibitory synapse on the extensor motor neuron.

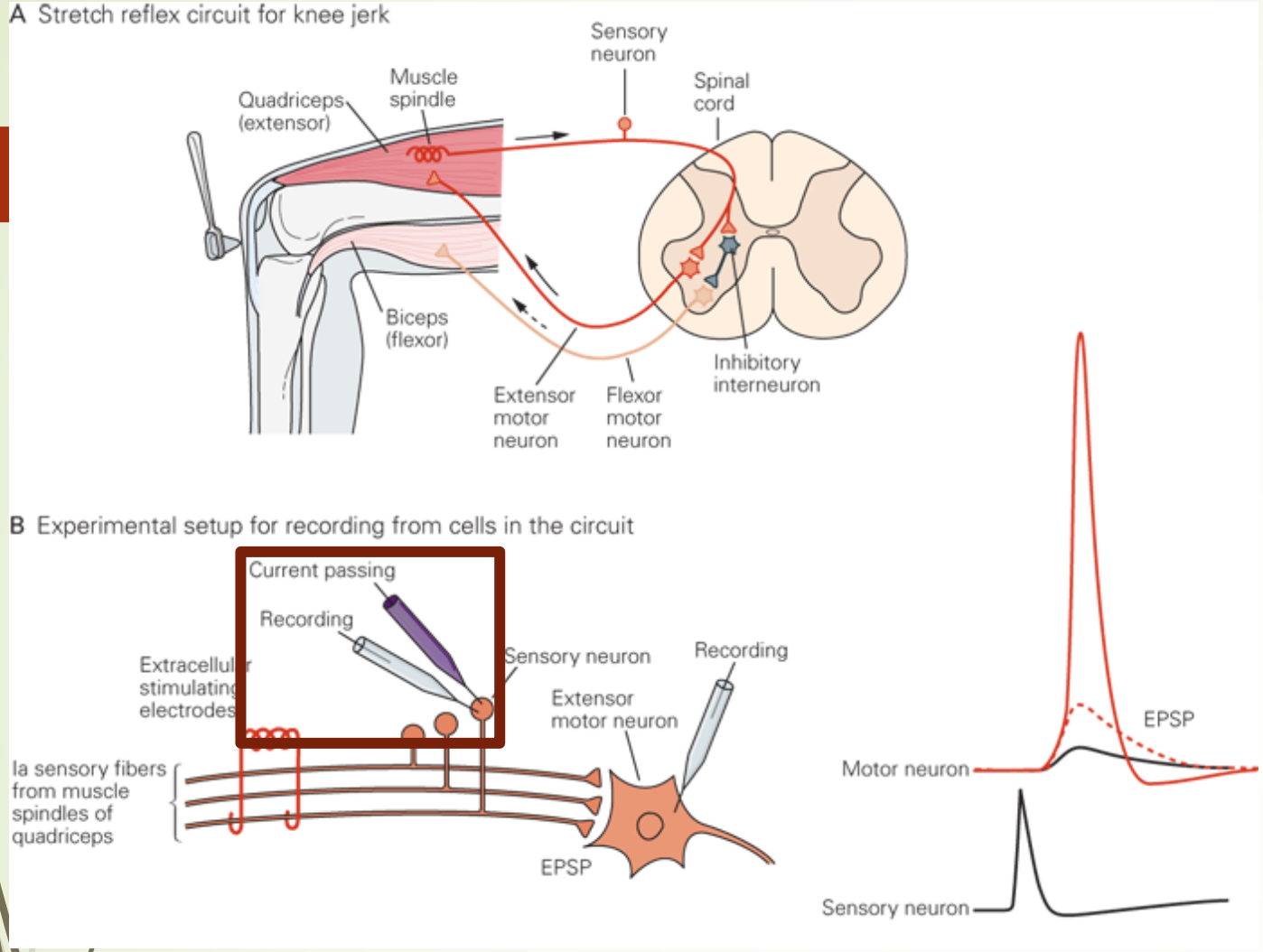
- When a muscle is stretched, the amplitude and duration of the stimulus are reflected in the amplitude and duration of the receptor potential generated in the sensory neuron



- If the receptor potential exceeds the threshold for an action potential in that cell, the graded signal is transformed at the trigger zone into an action potential.
- The more the receptor potential exceed the threshold, the greater the depolarization and consequently the greater the frequency of action potentials in the axon
- The duration of the input signal also determines the duration of action potential

- The information encoded by the frequency and duration of firings is conveyed along the axon to its terminals, where the firing of action potentials determines the amount of transmitter released. These staged of signaling have their counterparts in the motor neuron (B) and in the muscle (C)

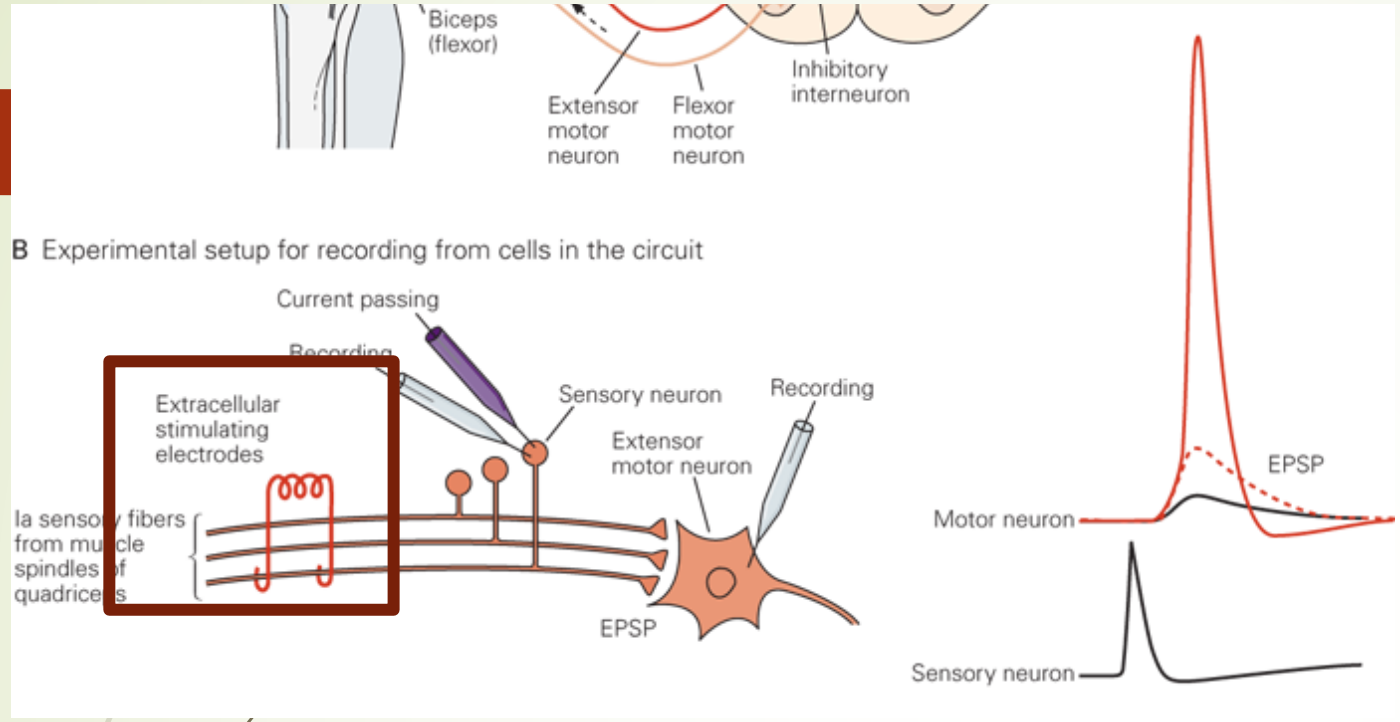




Experimentally we can stimulate with one electrode the cell body of a stretch-receptor neuron that innervates the extensor muscle:

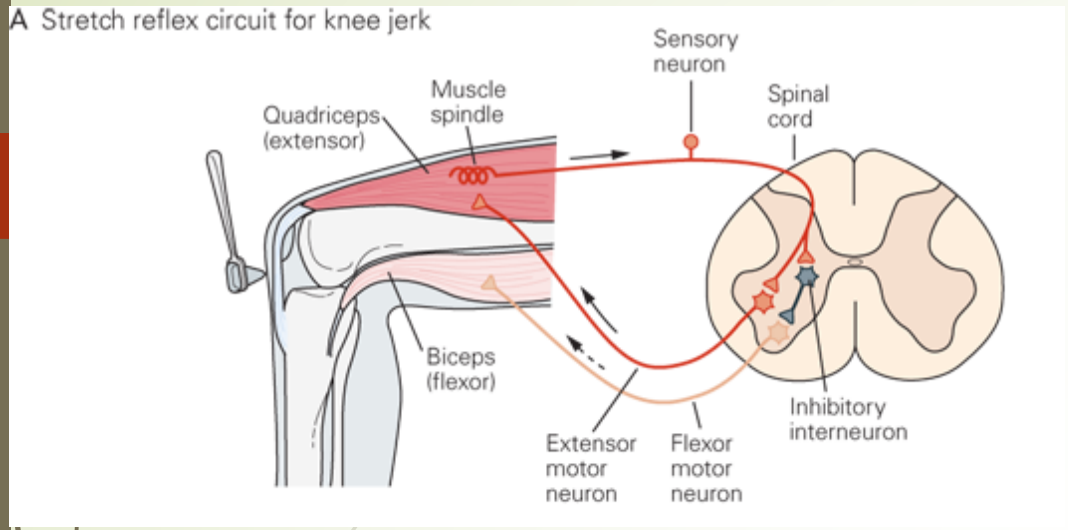
- this will produce a small EPSP in the motor neuron that innervates precisely the same muscle (quadriceps) monitored by the sensory neuron.

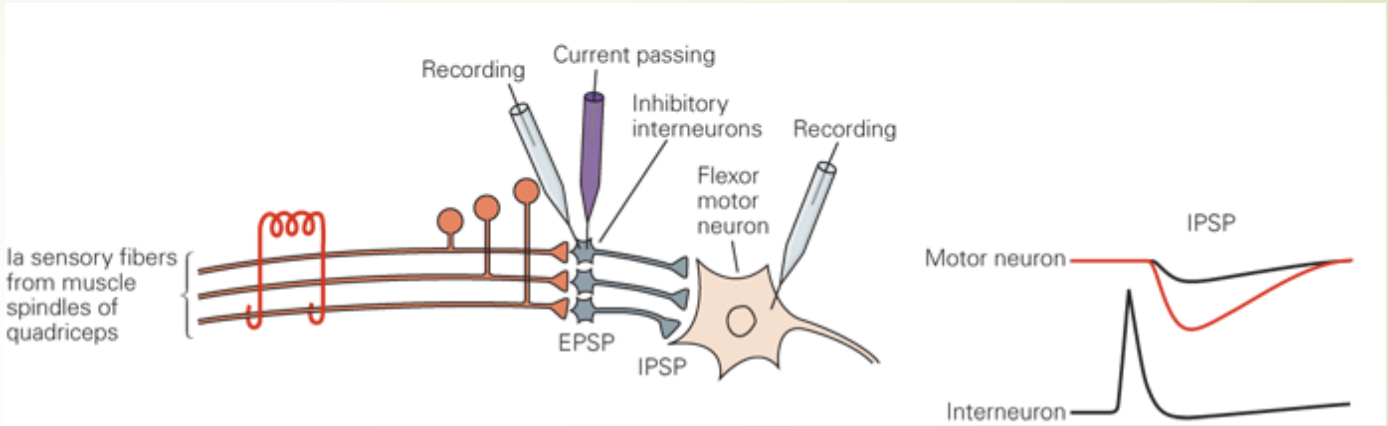
The EPSP produced by the sensory neuron, depolarize the extensor motor neuron by less than 1mV (0.2-0.4mV) far below the threshold for generating an action potential (typically, a depolarization of at lest 10mV is required). The generation of an action potential requires a near synchronous firing of a number of sensory neurons.



This can be achieved in an experiment in which a population of sensory neurons is stimulated by passing current through an extracellular electrode.

As the strength of the extracellular stimulus is increased, more sensory afferent fibers are excited and the depolarization produced by the EPSP becomes larger enough to bring the membrane potential of the axon initial segment (the integrative component of the motor neuron)to the threshold for an action potential

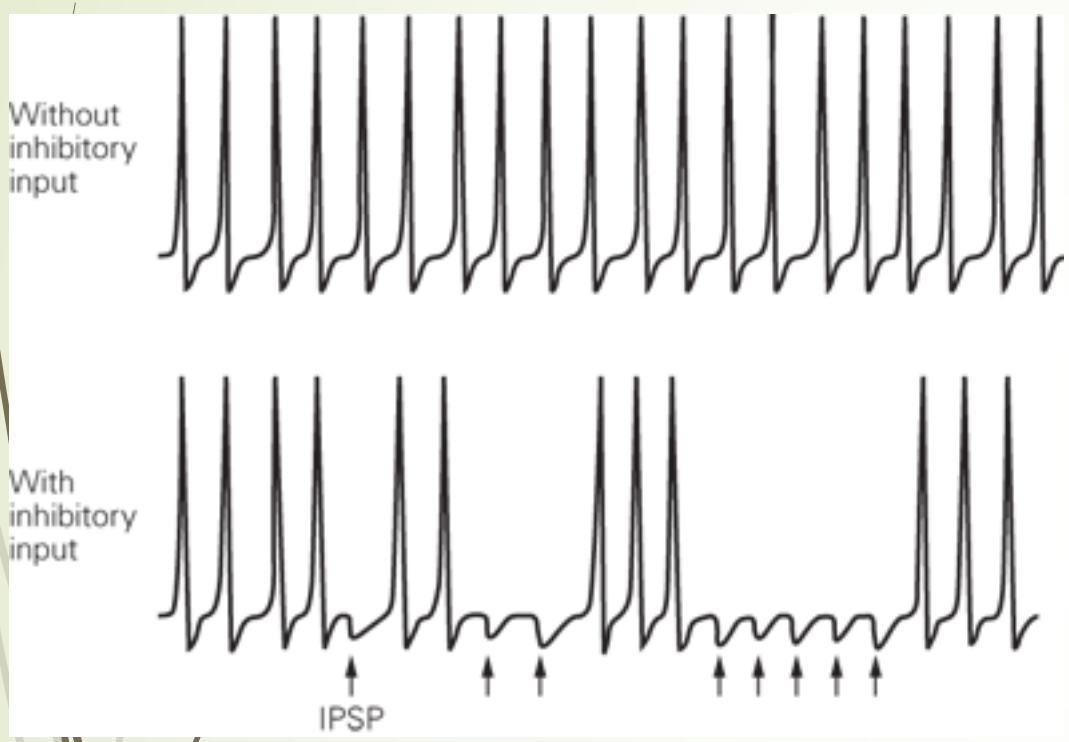




In contrast to the EPSP produced in the extensor motor neuron, stimulation of the stretch-receptor neuron produces **a small IPSP in the motor neuron that innervates the flexor muscle**, which is antagonistic of the extensor muscle.

This hyperpolarizing action is mediated by an inhibitory interneuron, which receive excitatory input from the sensory neurons of the extensor muscle and in turn makes synapses with the motor neurons that innervate the flexor muscle.

IPSP if strong enough can counteract the sum of excitatory input and prevent the membrane potential to reach the threshold.



In addition to counteracting synaptic excitation, synaptic inhibition ca exert a powerful control over action potential firing neurons that are spontaneously active because of the presence of intrinsic pacemakers channels.

This function is called **sculpturing role** of inhibition, shapes the pattern of firing in such cells

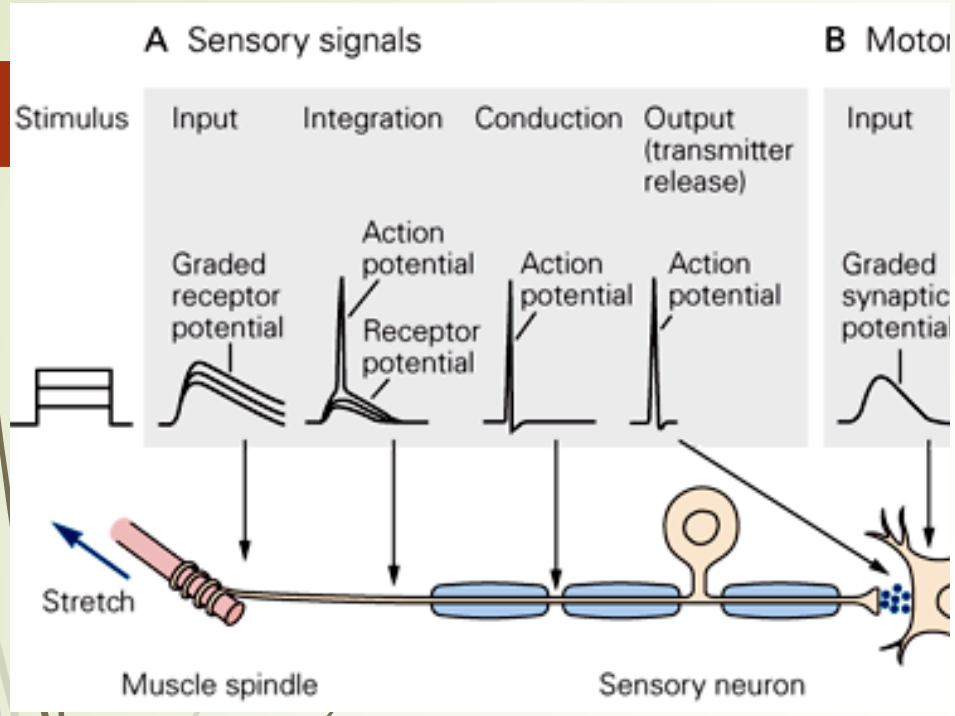
The effect of a synaptic potential – whether excitatory or inhibitory – is determined not by the type of transmitter released from the presynaptic neuron but by the type of channels in the postsynaptic cell activated by the neurotransmitter.

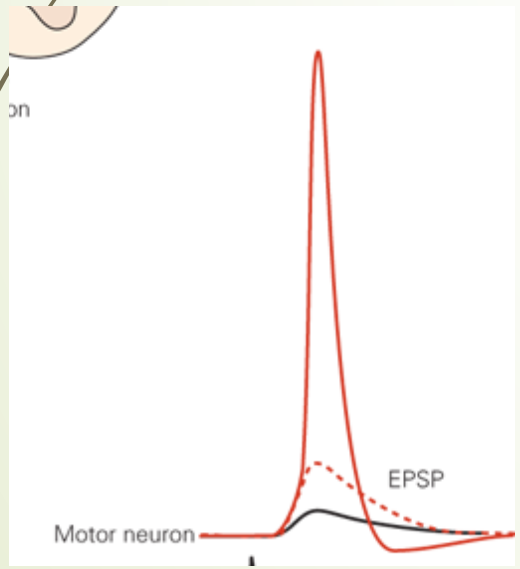
Although some transmitter can produce both excitatory and inhibitory postsynaptic potentials, by acting on distinct classes of ionotropic receptors at different synapses, most transmitters produce a single predominant a type of synaptic response

A transmitter is usually inhibitory or excitatory

Ex: in vertebrate brain neurons that release **Glutamate** typically act on receptors that produce **excitation**;

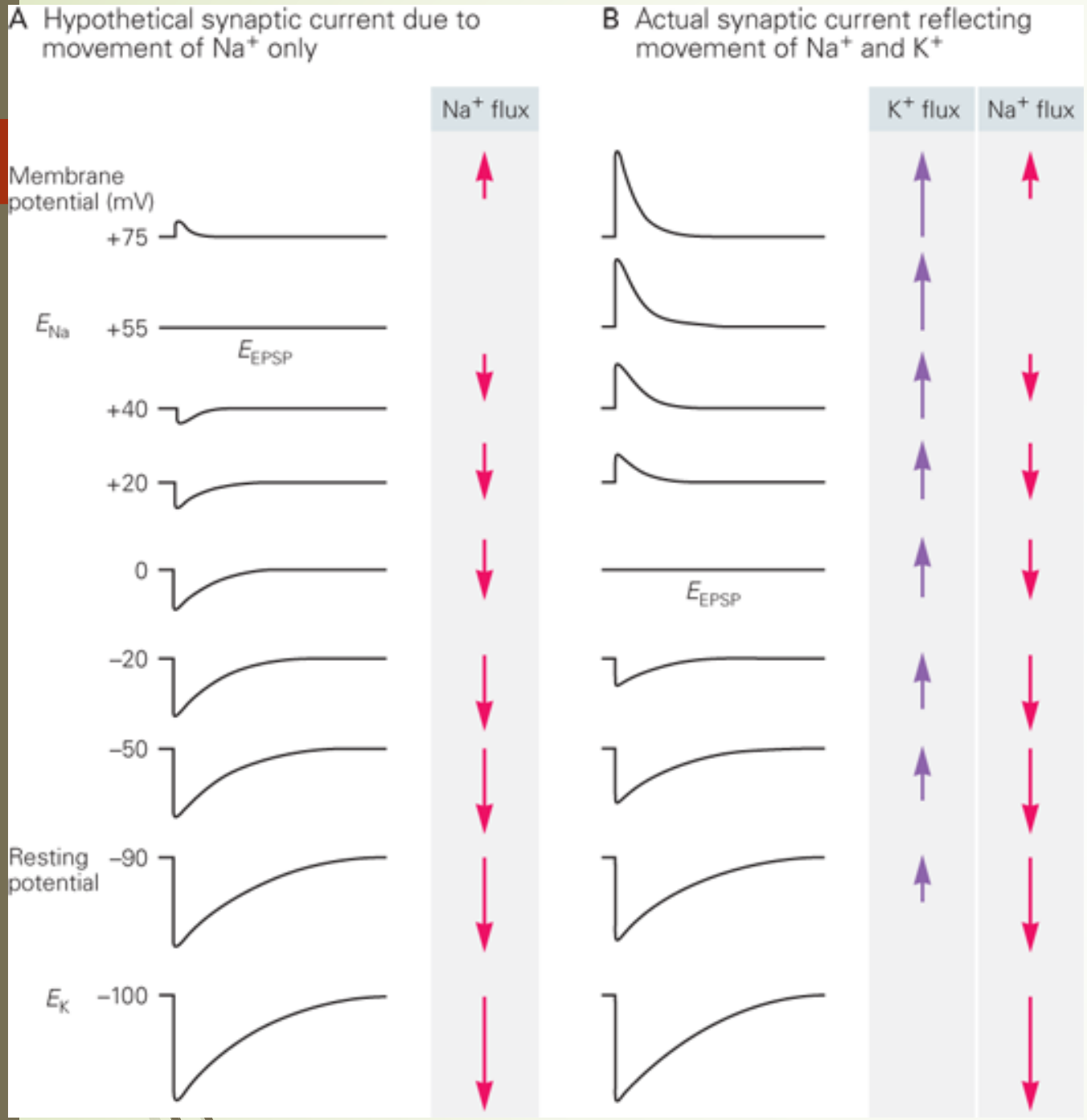
Neurons that release **GABA or glycine** act on receptors that produce **inhibition**





The excitatory trasnmitter relaesed from the presynaptic terminals of the **stretch-receptor neurons is L-glutamate**.

The EPSP in the spinal motor neuron results from the opening of the glutamate-gated channels permeable to Na^+ and K^+.



The ionic mechanism is similar to that produced by ACh

Like nAchR, the glutamate-gated channels conduct both Na^+ and K^+ with nearly equal permeability. As a result, the reversal potential from current flow through these channels is 0mV

Glutamate receptor can be divided

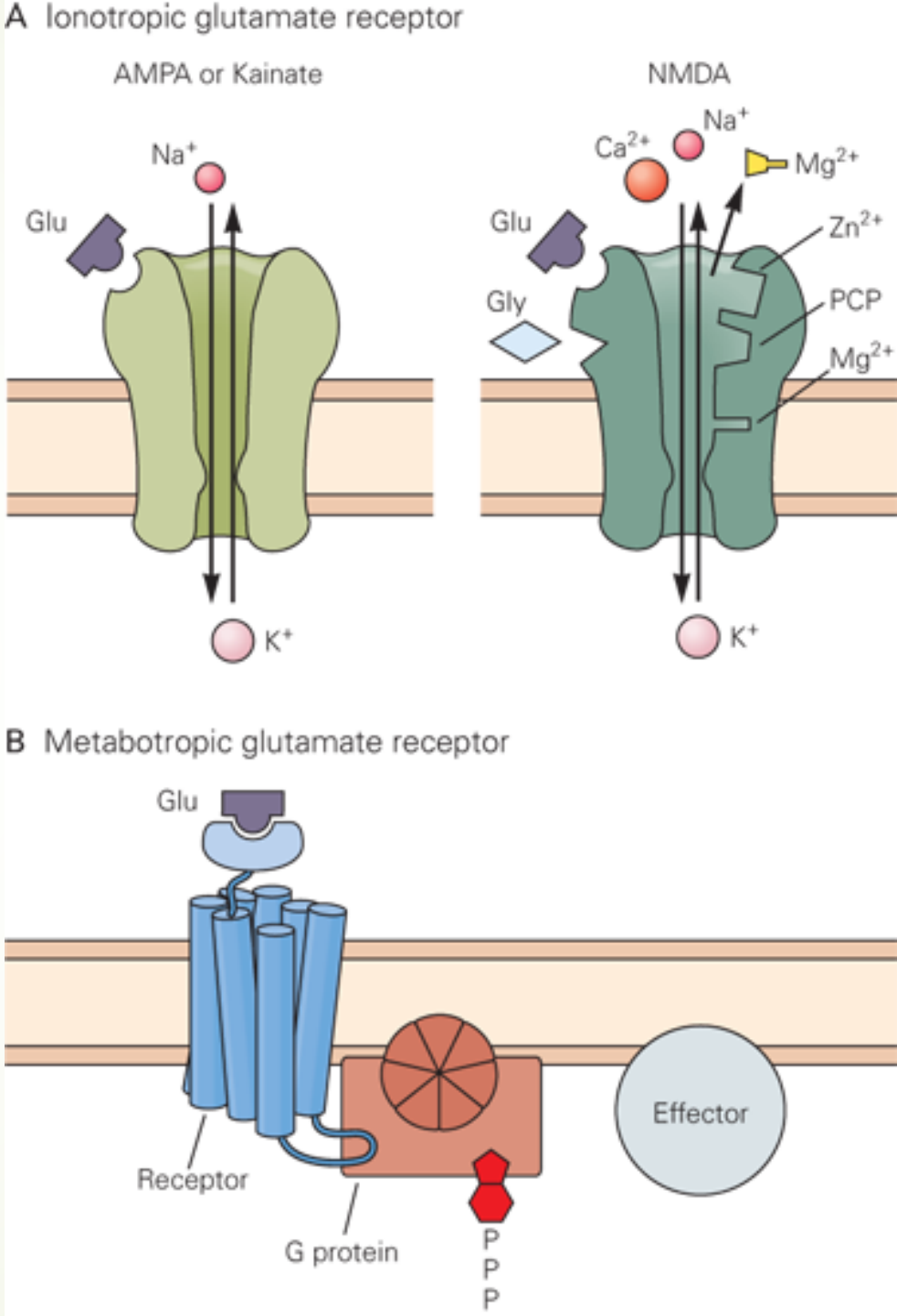
- Ionotropic receptors always excitatory

AMPA blocked by CNQX (6-cyano-7-nitroquinoxaline-2,3-dione)

Kinate

NMDA blocked by **APV** (2-amino-5-phosphonovaleric acid)

- Metabotropic receptors, can be excitatory or inhibitory

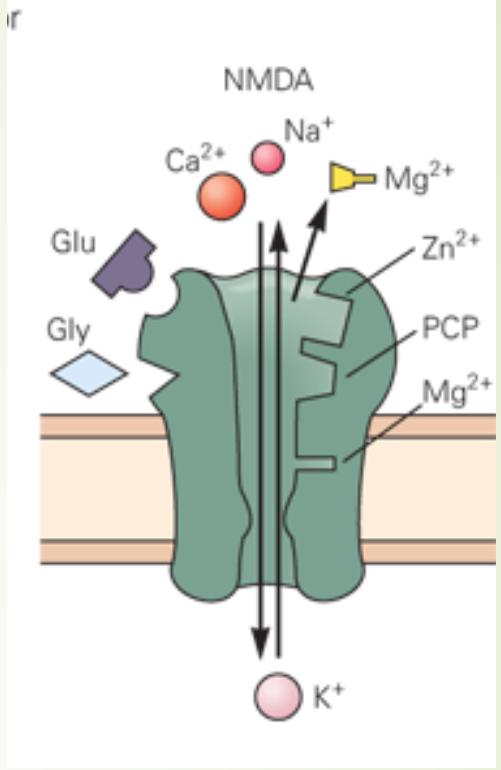


NMDA receptors

1. Permeate Ca^{2+} beside Na^{+} and K^{+}

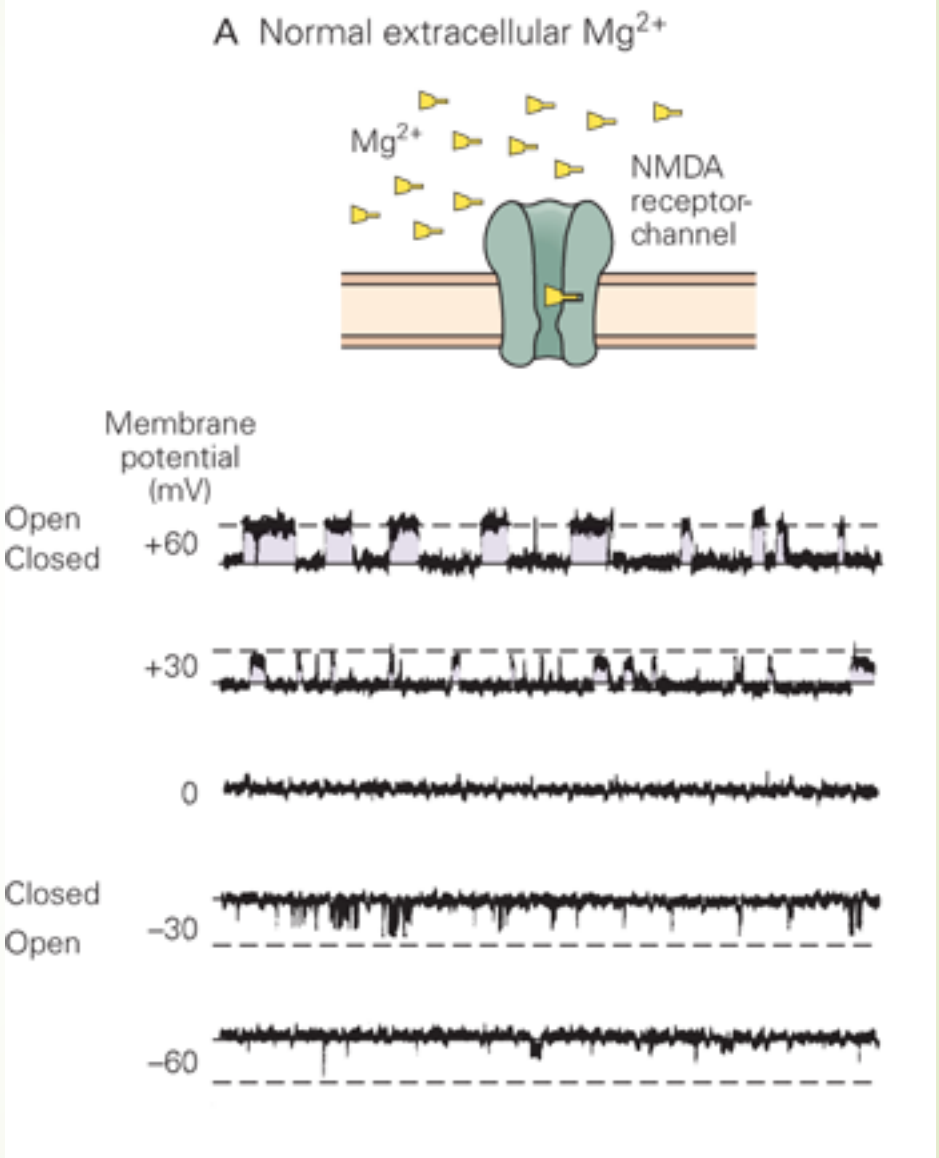
2. The presence of glycine is necessary as cofactor for the opening of the channel

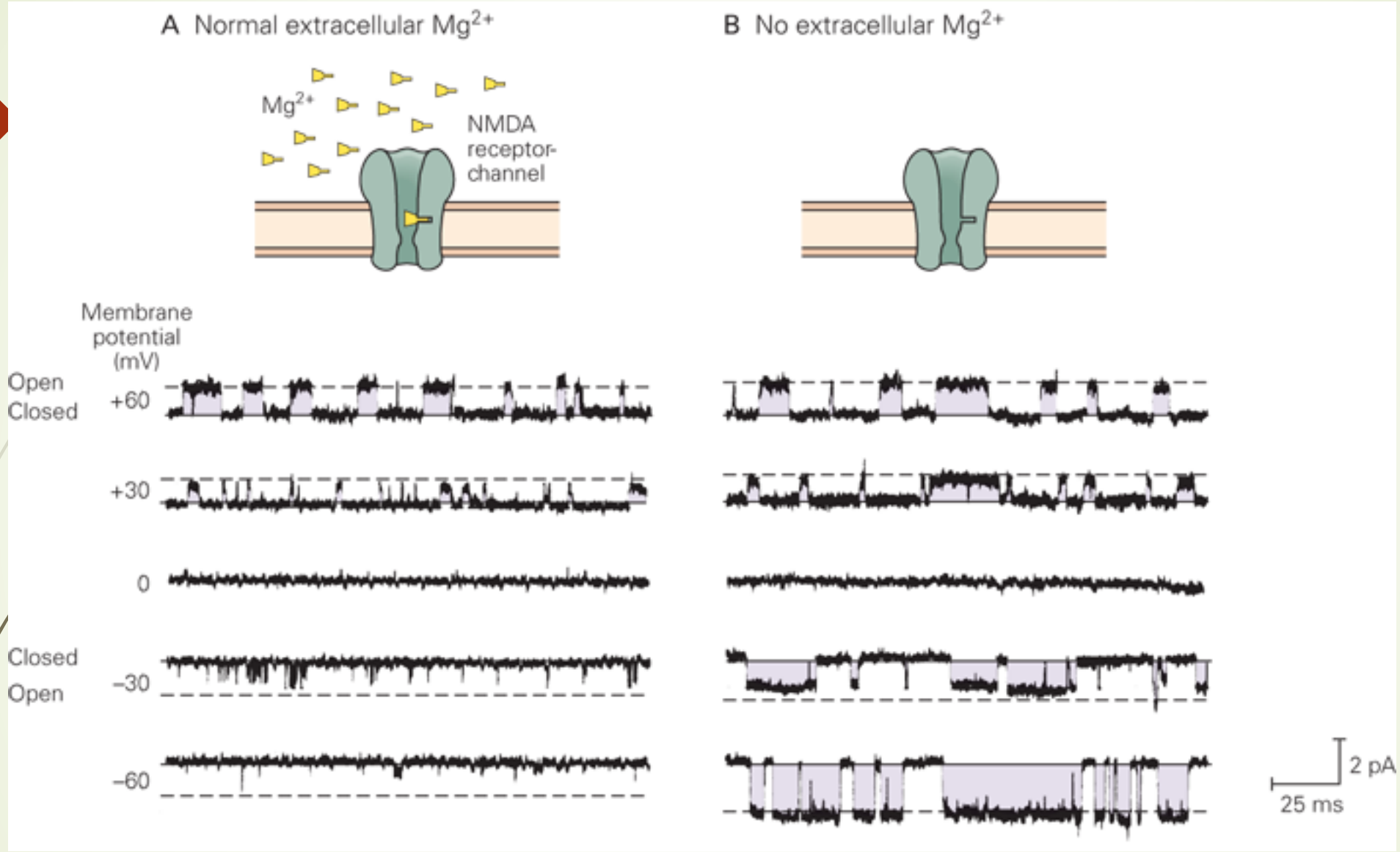
3. The opening depends on voltage as well as transmitter. The voltage-dependence is due to a mechanism that is different from that of the Voltage-gated channels involved in action potential: depolarization remove an extrinsic plug from the channel



At the resting potential (-65mV) extracellular Mg^{2+} binds tightly to a site in the pore of the channels, blocking ion current.

When the membrane is depolarized (fro example by opening of AMPA receptor), Mg^{2+} is expelled from the channel, allowing Na^{+} and Ca^{2+} to enter





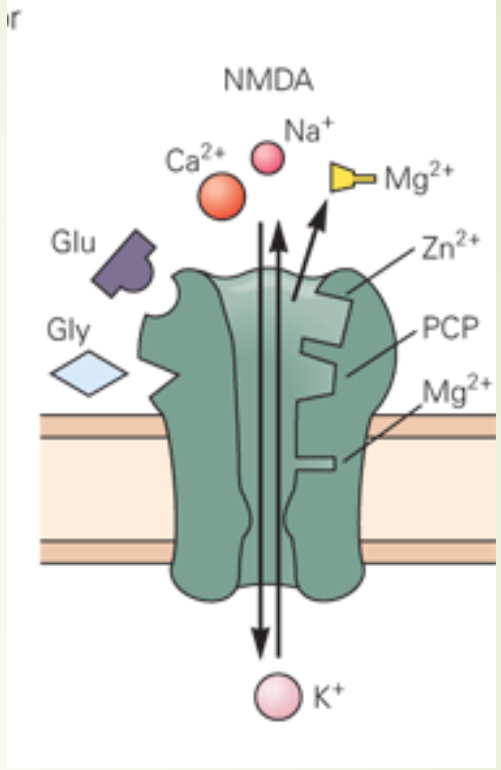
When Mg^{2+} is removed from the extracellular solution, the gating of the channels become independent from the voltage.

NMDA receptors

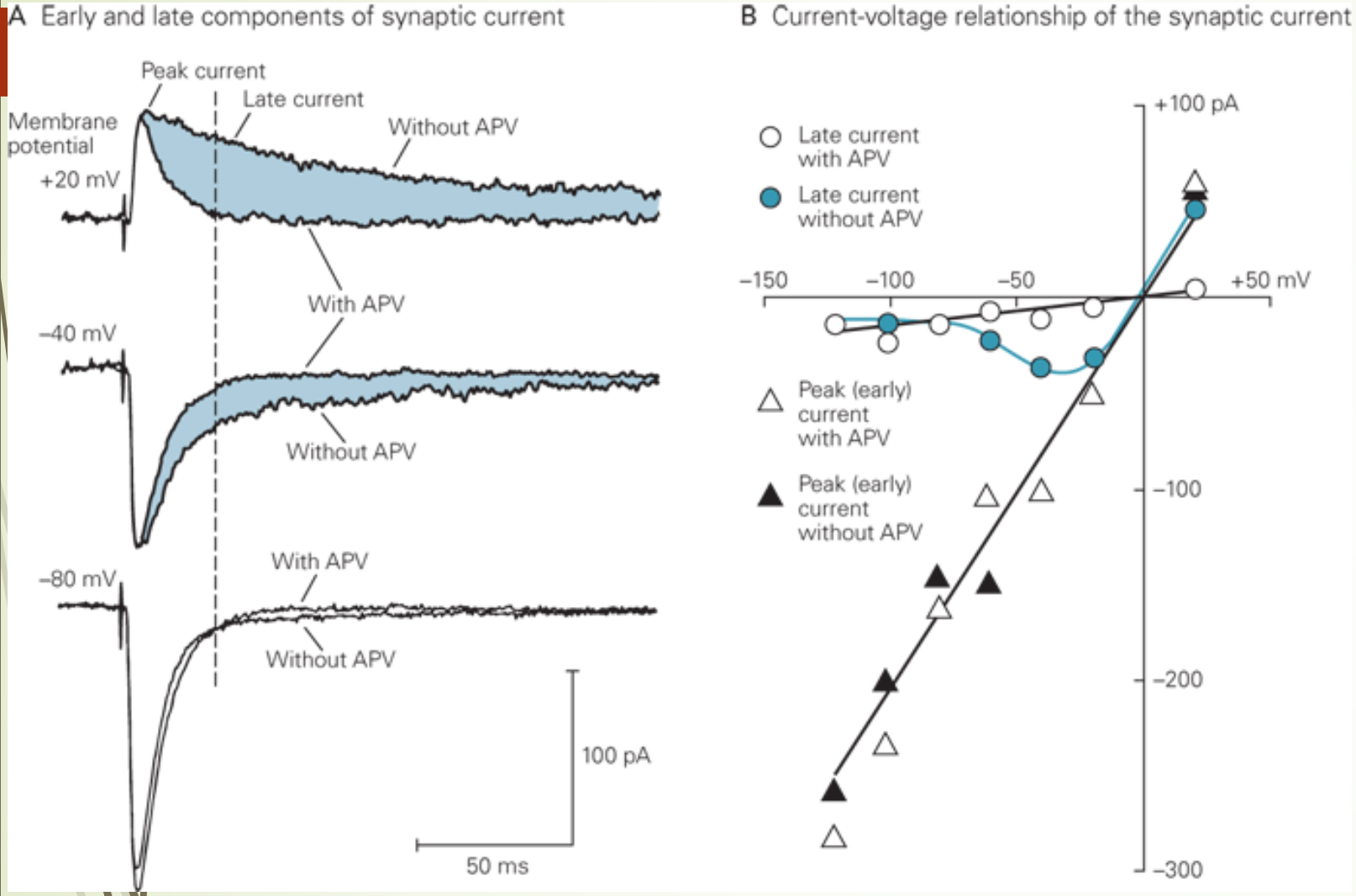
4 NMDA receptor is inhibited by the hallucinogenic drug phencyclidine (PCP, angel dust) and by MK801, both of which bind to a site in the pore of the channel that is distinct from the Mg^{2+} binding site.

Blockade of NMDA receptors produces symptoms that resemble the hallucinations associated with schizophrenia, whereas certain antipsychotic drugs enhance current from NMDA receptors.

This has led to the hypothesis that schizophrenia may involve a defect in NMDA receptor function



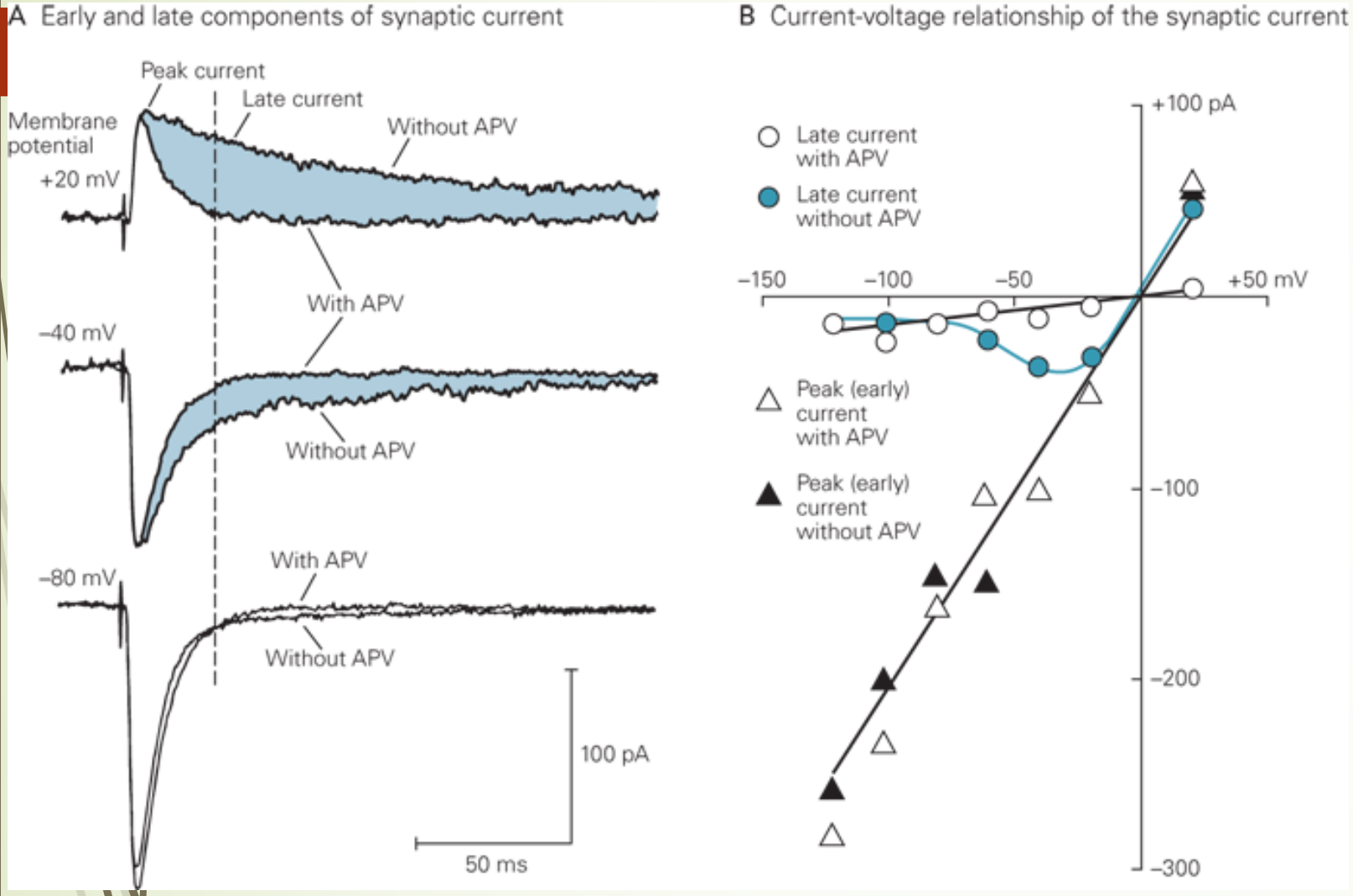
NMDA and non –NMDA receptors coexist on postsynaptic membrane



At the normal resting potential of most neurons, the NMDA receptor-channels are largely inhibited by Mg^{2+}.

EPSC is due by charge flow through the AMPA receptors, which generate a current with a very rapid rising phase and very rapid decay phase.

In few areas (CA3 hippocampus) EPSC is due to KAINATE Receptors



As neuron becomes depolarized, Mg^{2+} is driven out from the NMDA pore and more charge flow through the channels.

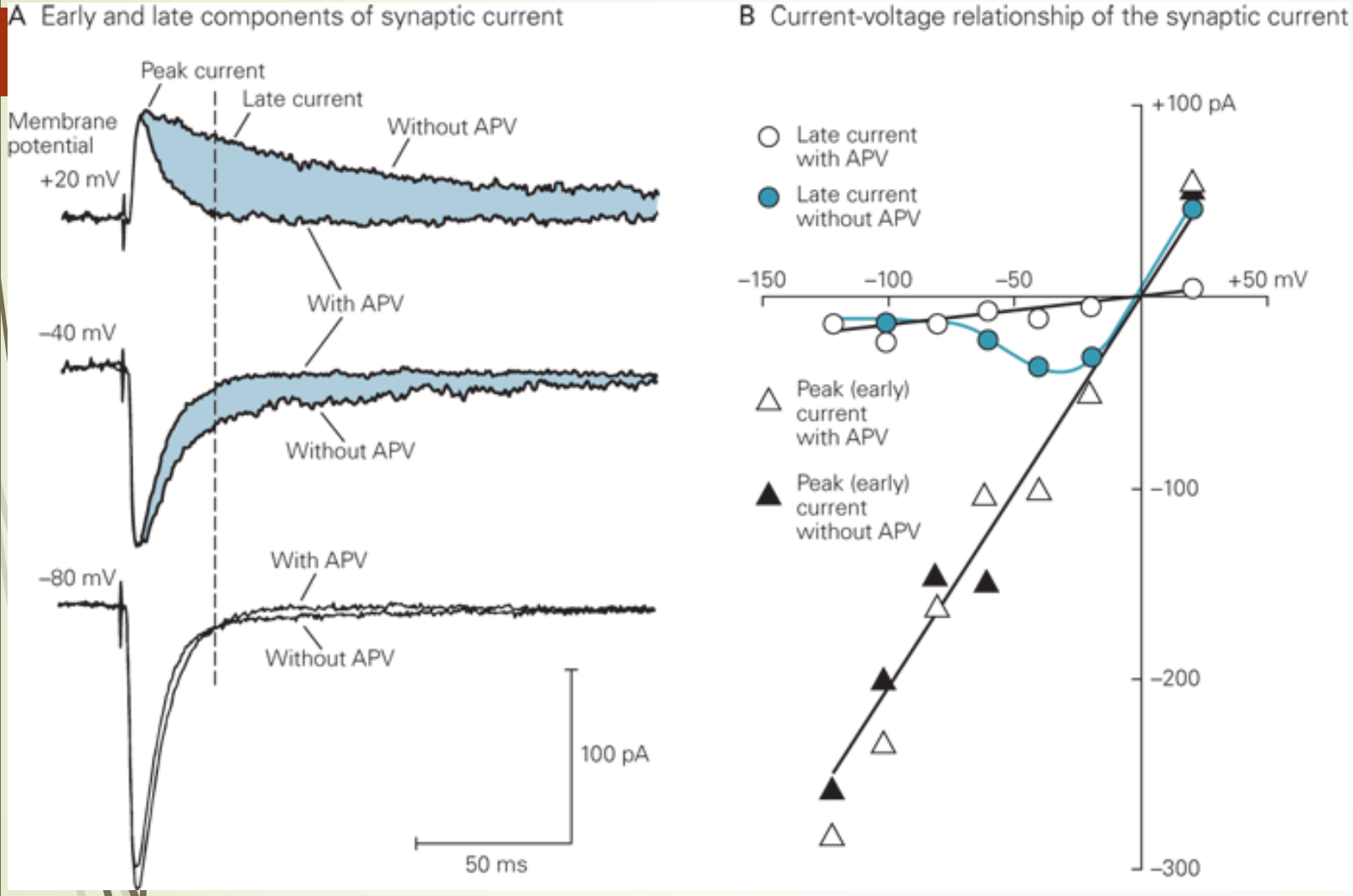
NMDA receptor conduct current maximally when two conditions are met:

Glutamate is present

Cell is depolarized

NMDA act as a **COINCIDENCE RECEPTOR**

Detecting a timing relationship between activation of presynaptic and postsynaptic cells



The current through NMDA receptor rises and decay with a much slower time course than the AMPA receptor current.

NMDA receptors contribute to a late, slow phase of EPSP and EPSC

Most glutamatergic synapses contains AMPA receptors that are capable of triggering action potential

What is the role of NMDA?

Moreover NMDA are blocked by Mg^{2+} at resting potential

When glutamate is paired with depolarization, NMDA conduct Ca^{2+} into the postsynaptic cells.

This lead to $[Ca^{2+}]_i$ that can activate various calcium-dependent signaling cascades, including CaMKII.

Activation of NMDA can translate electrical signals in biochemical ones

Some of these biochemical reactions lead to long lasting changes in synaptic strength, long term synaptic plasticity that are though to be important during synapse development and for regulating neural circuits in the adult brain

In particular NMDA receptors-dependent long term potentiation (LTP) of excitatory synaptic transmission has been implicated in certain form of memory storage.

Downside of Ca^{2+} entry through NMDA receptors:

Excessively high concentrations of glutamate result in an overload of Ca^{2+} in the postsynaptic neurons and therefore cause toxicity: **Glutamate exotoxicity.**

Glutamate toxicity may contribute **to cell damage after stroke**, to the cell death that occurs with episodes of rapidly repeated seizures experienced by patients who have status epilecticus, and to degenerative disease such as Huntington disease. Agent that selectively block NMDA receptor may protect against the toxic effect of glutamate and have been tested clinically.

Unfortunately the hallucinations that accompany NMDA blockade have so far limited the usefulness of such compounds. A further complication of blocking NMDA receptors function is that physiological levels of NMDA receptor activation can actually protect neurons from damage and cell death

NMDA receptors are the only receptors in postsynaptic membrane

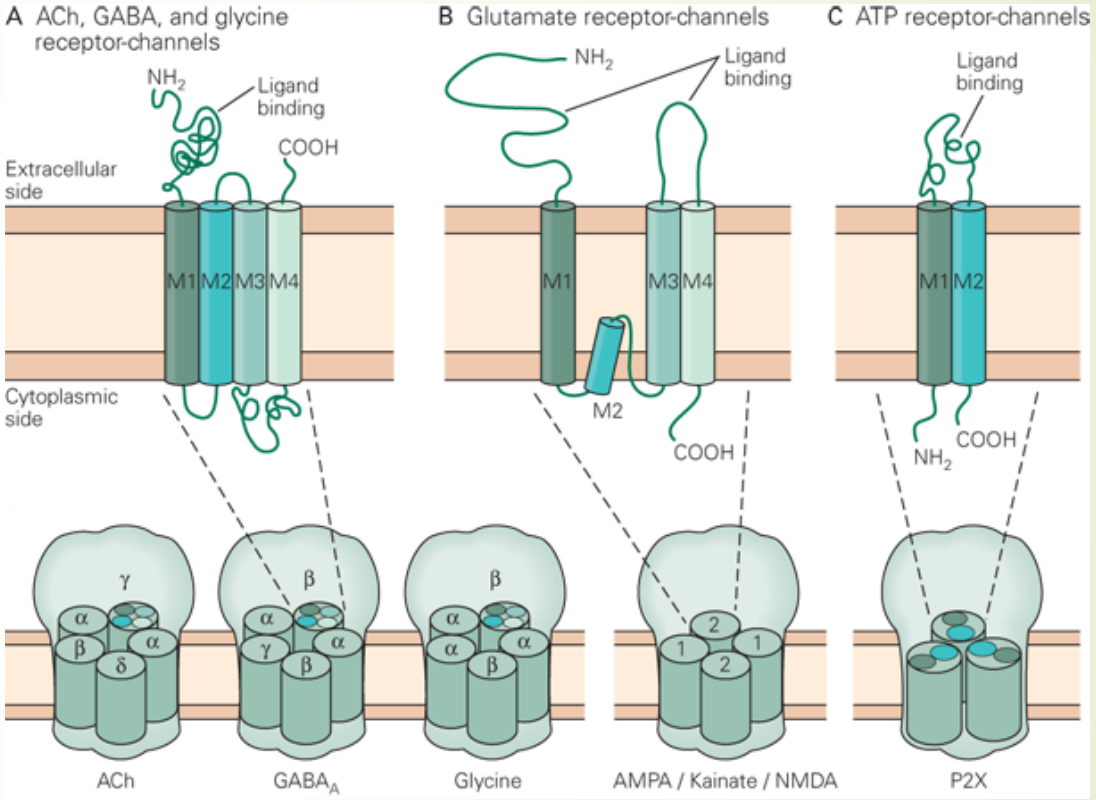
In certain preparations, EPSP in response to glu stimulation, only show one component, the NMDA component. This has led to the assumption that not all NMDA receptor are blocked by Mg^{2+} at resting membrane potential. As an example GluN2A and GluN2B-containing channels are more sensitive to Mg^{2+} block as compared with GluN2C and GluN2D.

The mechanism would be:

When Glu in the synaptic cleft is high enough to activate few NMDA receptors not blocked by Mg^{2+} at resting potential, a small inward current is activated which produces a small depolarization that in turn unbolcks additional NMDA receptors. This triggers a regenerative phenomenon.

Ionotropic glutamate receptors

Unlike pentameric nAChR family, AMPA, Kinate and NMDA receptors are tetrameric proteins with four subunits arranged around a central pore.



Ionotropic glutamate receptors

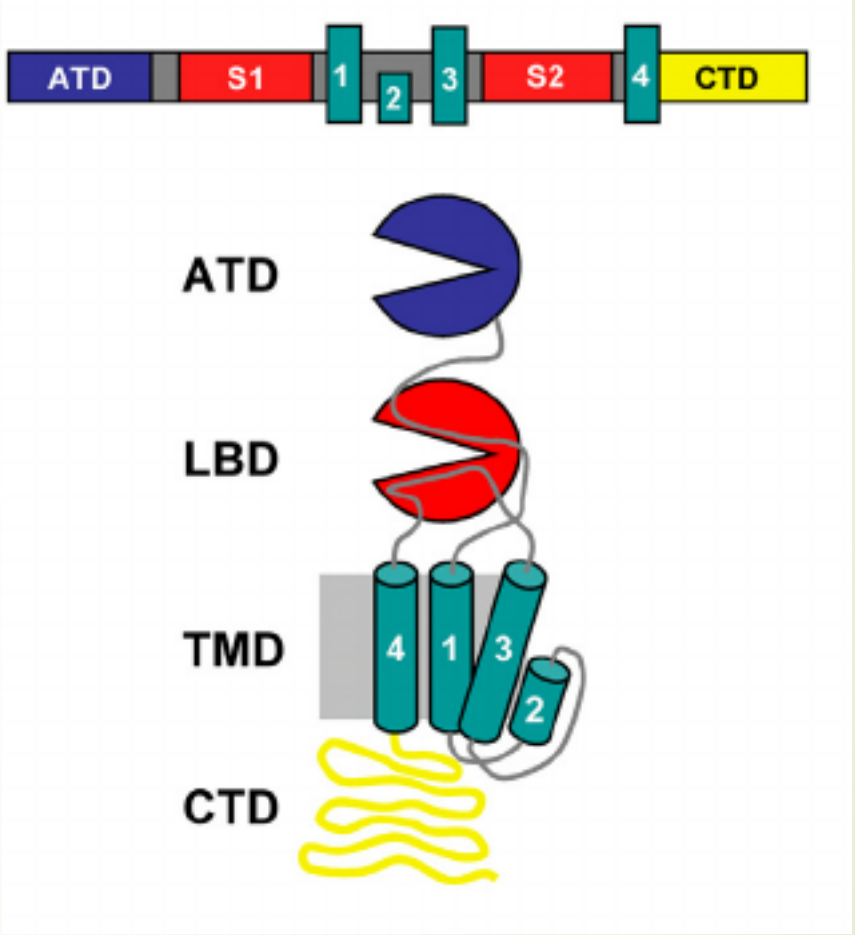
NMDA			AMPA			Kainate		
Old	HUGO	IUPHAR	Old	HUGO	IUPHAR	Old	HUGO	IUPHAR
NR1	*GRIN1*	GluN1	GluR1	*GRIA1*	GluA1	GluR5	*GRIK1*	GluK1
			GluR2	*GRIA2*	GluA2	GluR6	*GRIK2*	GluK2
NR2A	*GRIN2A*	GluN2A	GluR3	*GRIA3*	GluA3	GluR7	*GRIK3*	GluK3
NR2B	*GRIN2B*	GluN2B	GluR4	*GRIA4*	GluA4			
NR2C	*GRIN2C*	GluN2C				KA-1	*GRIK4*	GluK4
NR2D	*GRIN2D*	GluN2D				KA-2	*GRIK5*	GluK5
NR3A	*GRIN3A*	GluN3A						
NR3B	GRIN3B	GluN3B						

Ionotropic glutamate receptors

iGluR subunits have in common a large extracellular N-terminus domain and four hydrophobic segments. Immunocytochemical and biochemical studies have indicated that C-term is intracellular. M2 does not span the membrane but is considered to lie in close proximity to the intracellular surface and to have a hairpin structure (P-loop).

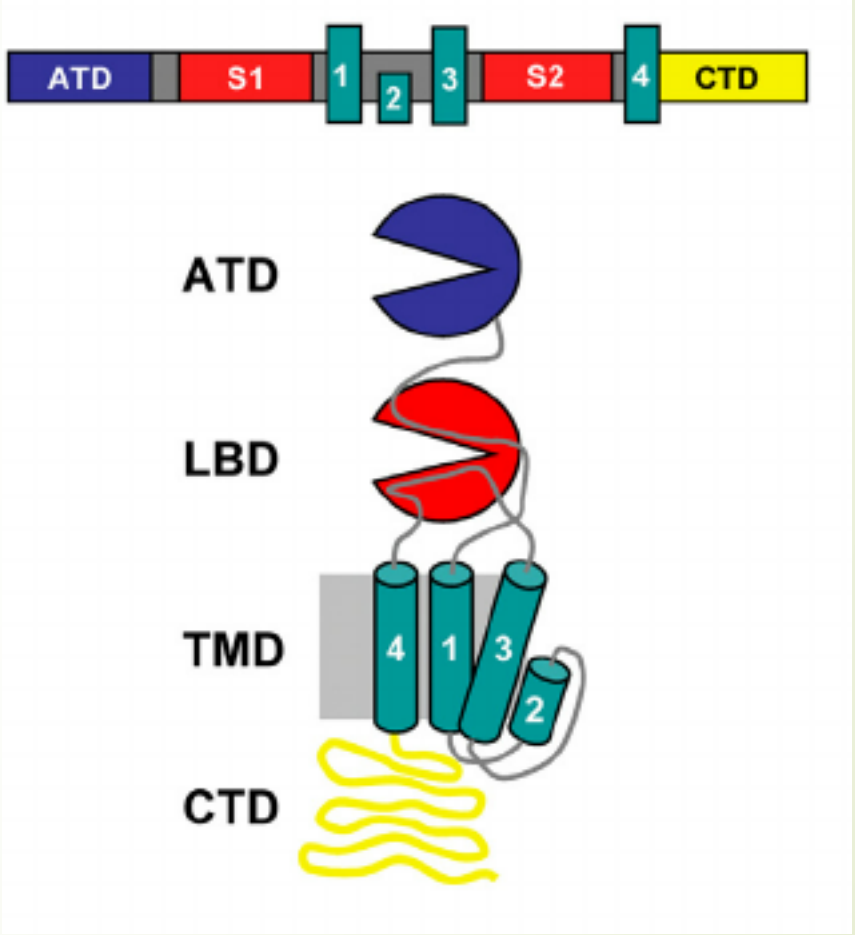
iGluR consist of:

N-term domain **ATD** that participate in subtype-specific receptor assembly, trafficking and modulation. Forming a "clamshell"



Ionotropic glutamate receptors

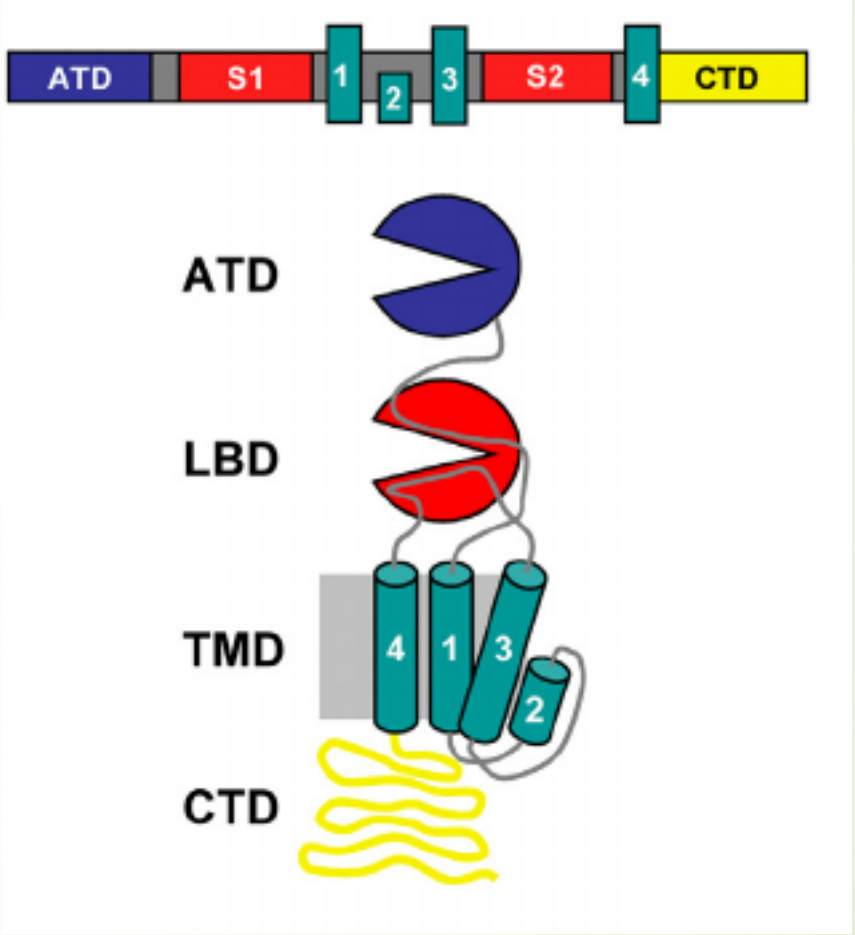
Ligand-binding domain **LBD** central to agonist/competitive antagonist binding and to activation gating. LBD is composed by 2 regions: **one region located from N-term to M1**; the second is formed by the **large extracellular loop between M3 and M4**. The binding of one glutamate to the clamshell triggers the closure of the lobes of the clamshell ("shell"); competitive antagonist bind to the site but fail to trigger the closure. Thus the **conformational change associated with the clamshell closure is though to be coupled to the opening of the ion channel**



Ionotropic glutamate receptors

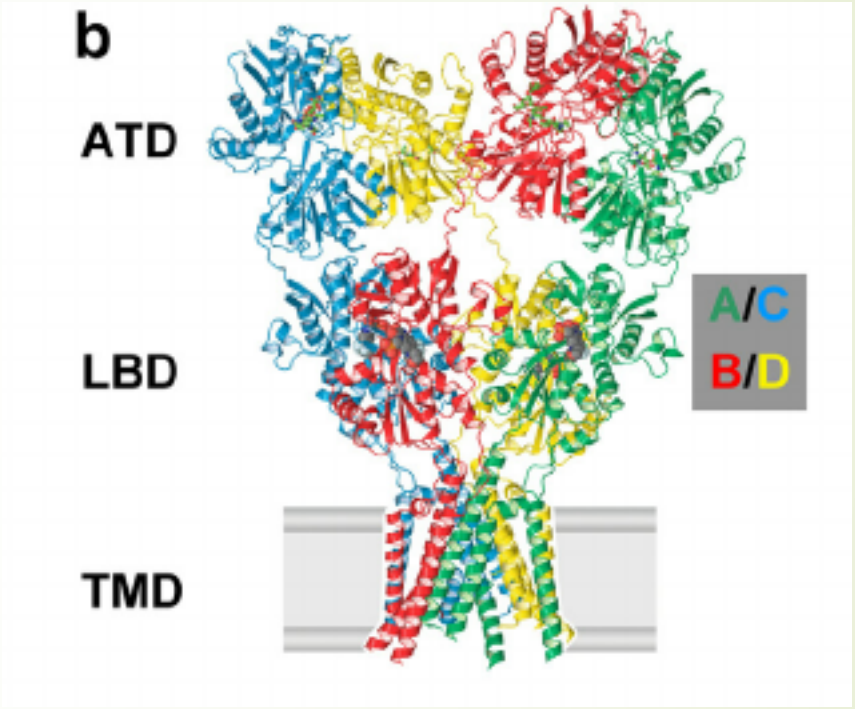
Transmembrane domain **TMD** consisting of M1, M3, M4 and the P-loop M2 that forms the channel

Cytoplasmic domain **CTD** of variable length involved in receptor trafficking and coupling to signaling cascade



Ionotropic glutamate receptors

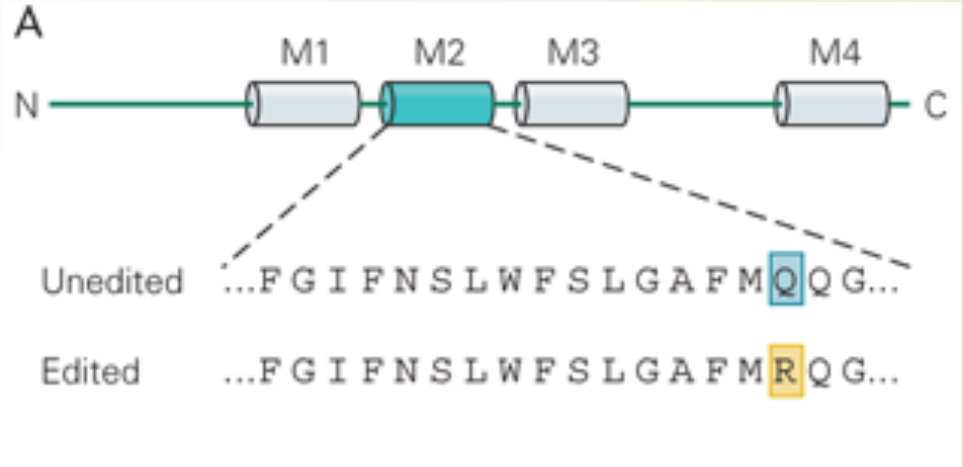
Crystal structure of the homotetrameric GluA2 receptors



NMDA receptors

Inclusion if just 1 **GluA2** subunits in the tetramer causes AMPA receptor channels to have a very low permeability to Ca^{2+}, most likely due to a very strong electrostatic repulsion by arginine **R**.

Some cells form **AMPA receptor lacking GluA2 subunits**. Such AMPA receptors generate a **significant Ca^{2+} influx**, because their pores lack the positively charged **R** residue



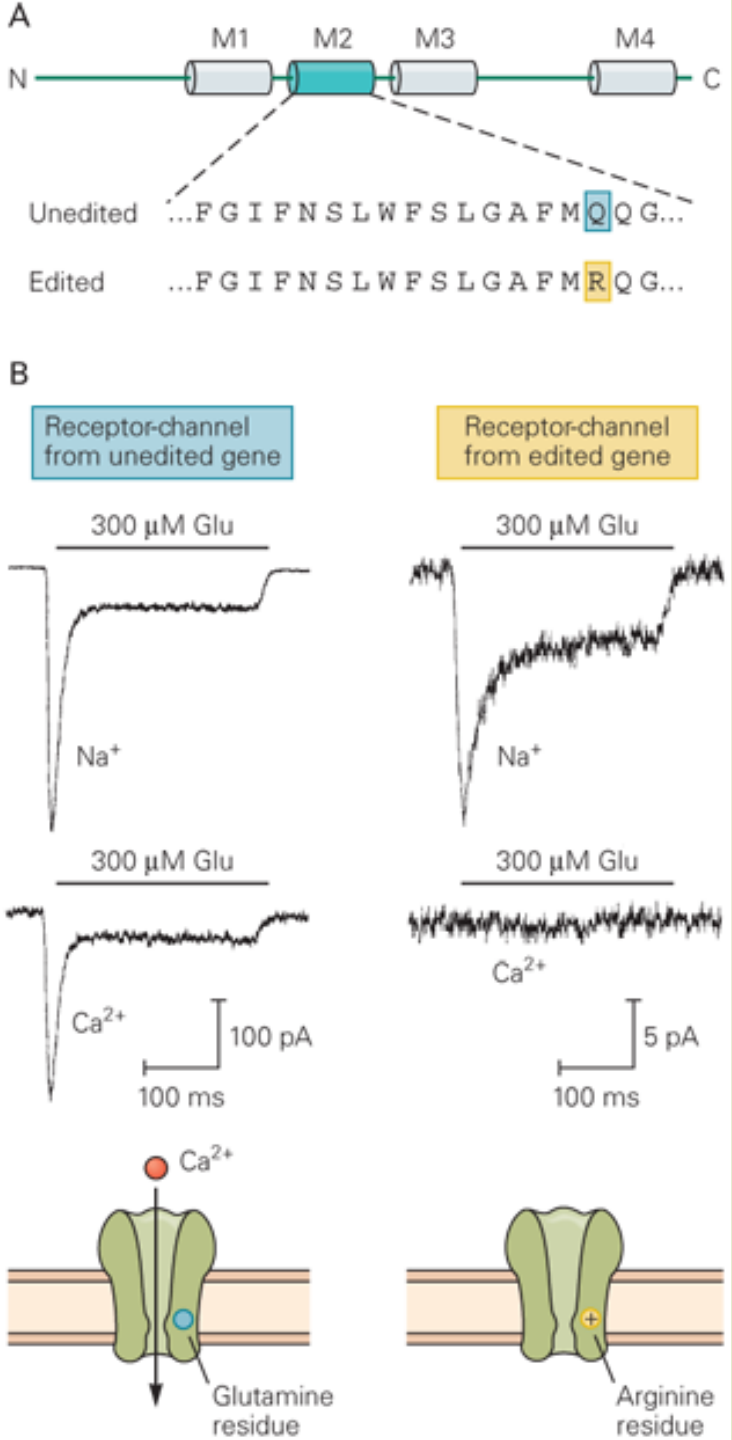
NMDA receptors

DNA of ***GluA2* gene** does not encode arginine R in the M2 loop but rather a glutamine **Q** residue.

After transcription the codon of glutamine in the *GluA2* mRNA is replaced with one of arginine R due to a chemical modification of a single nucleotide base through an enzymatic process (RNA editing).

The importance of this process is underscored by a genetically engineered mouse that is designed to express a *GluA2* gene in which the **Q residues** could **no longer be edited in R.**

These mice develop seizures and die within few weeks after birth, presumably because of an excess of intracellular Ca^{2+} as all AMPA receptors in these mice have a high Ca^{2+} permeability.



NMDA receptors

A residue of serine S in M3 is moreover important to regulate subtype NMDA receptor dependence of selective permeability to Ca^{2+} as well as Mg^{2+} Ic_{50}. GluN2 subunits contribute to the four diheterotrimeric NMDAR subtypes (GluN1/GluN2A - GluN1/GluN2B - GluN1/GluN2C - GluN1/GluN2D)

GluN2A and GluN2B exhibit higher affinity for Mg^{2+} (4-5 folds) and higher single channels conduction than GluN2C and GluN2D

- **Strategic role for Serine S in M3**

M3

GluN2A	KGTTSKIMVSVWAFFAVIFLASYTANLAAFMIQEEF	658
GluN2B	KGTTSKIMVSVWAFFAVIFLASYTANLAAFMIQEEY	659
GluN2C	RGTTSKIMVLVWAFFAVIFLASYTANLAAFMIQEQY	669
GluN2D	RGTTSKIMVLVWAFFAVIFLASYTANLAAFMIQEEY	683

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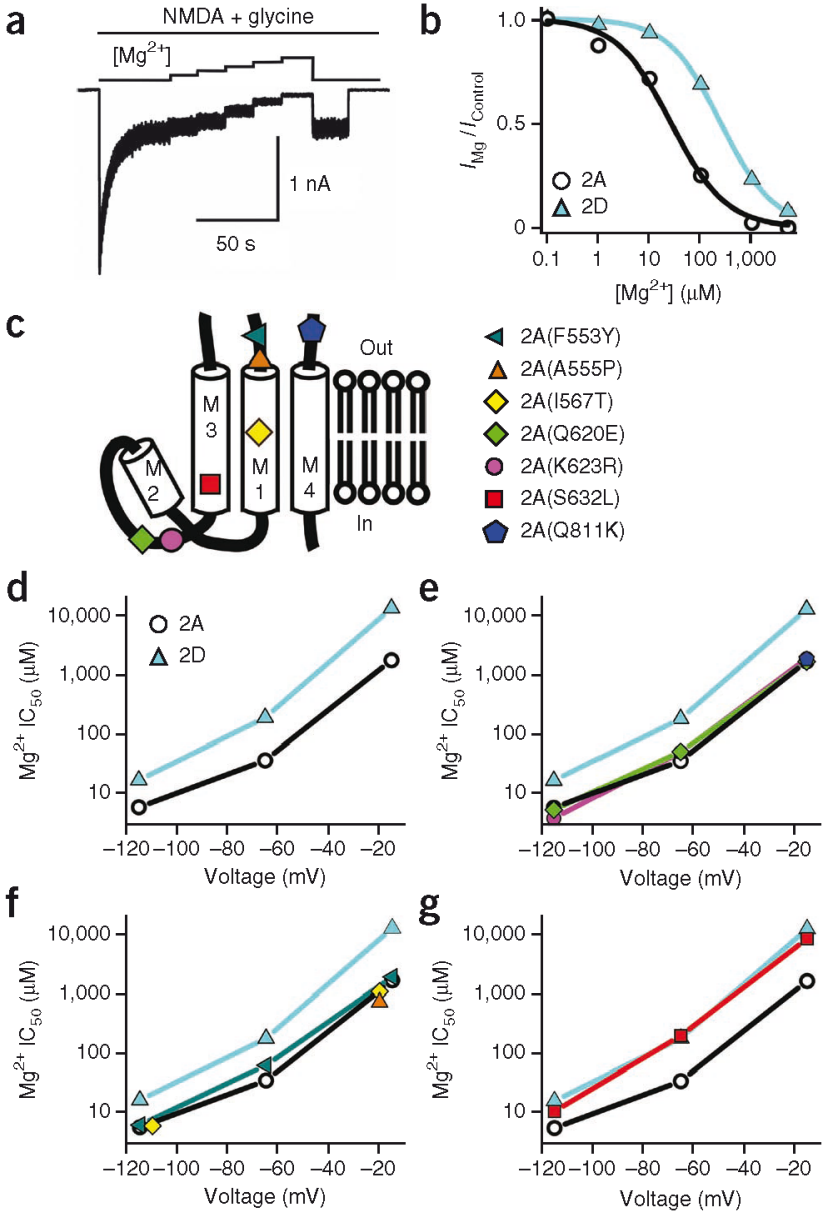
Siegler Retchless B et al, Nat Neurosc. 2012



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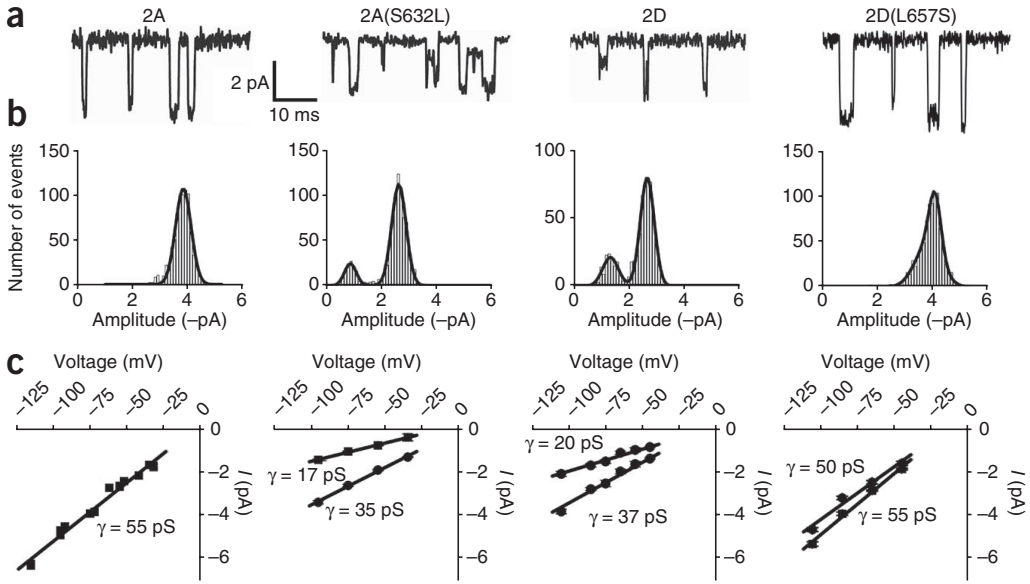
- **Strategic role for Serine S in M3**



M3

GluN2A	KGTTSKIMVSVWAFFAVIFLASYTANLAAFMIQEEF	658
GluN2B	KGTTSKIMVSVWAFFAVIFLASYTANLAAFMIQEEY	659
GluN2C	RGTTSKIMVLVWAFFAVIFLASYTANLAAFMIQEQY	669
GluN2D	RGTTSKIMVLVWAFFAVIFLASYTANLAAFMIQEEY	683

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Siegler Retchless B et al, Nat Neurosc. 2012

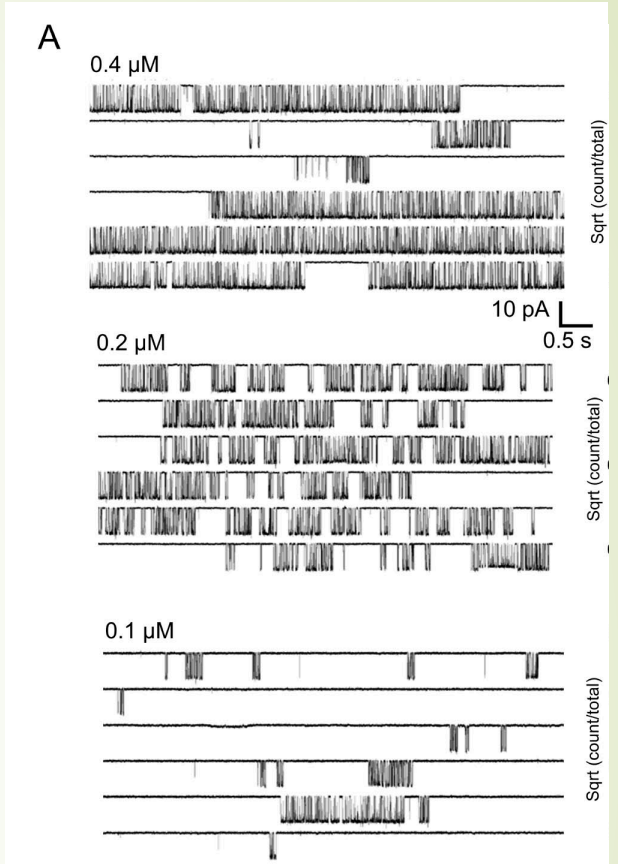
NMDA receptors

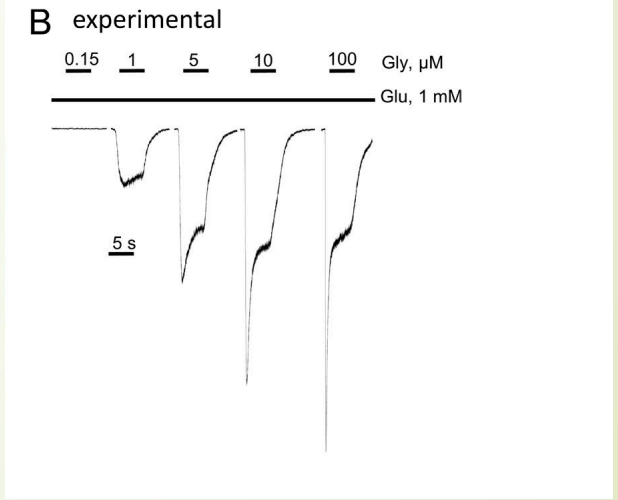
Glycine is a co-agonist of NMDA receptors

Glycine is an amino acid that acts as inhibitory neurotransmitter in several nervous systems synapses in vertebrates. However, this aa also play a positive role in modulating NMDA response. The molecular mechanism of this potentiating effect remain to be determined, but it is unlikely that it involves an intracellular signaling (due to glycine effect on excised patches).

Physiologycal considerations:

Glycine is present at relative high concentrations in the cerebrospinal fluid (several μM) close to the concentrations required to produce its maximum effect. However, high affinity pumps may lower the extracellular level of glycine in proximity of glutamatergic synapses. These transporters can therefore play a significant role in determining NMDA response





Inhibitory synapses

Inhibitory synapses play essential role in the nervous system both by preventing too much excitation and by helping coordinate activity among networks of neurons.

IPSP in spinal motor neuron and most central neurons are generated by the aminoacid neurotransmitters GABA and glycine.

GABA is a **major inhibitory** transmitter in the **brain** and **spinal cord**.

It acts on 2 receptors: $GABA_A$ and $GABA_B$.

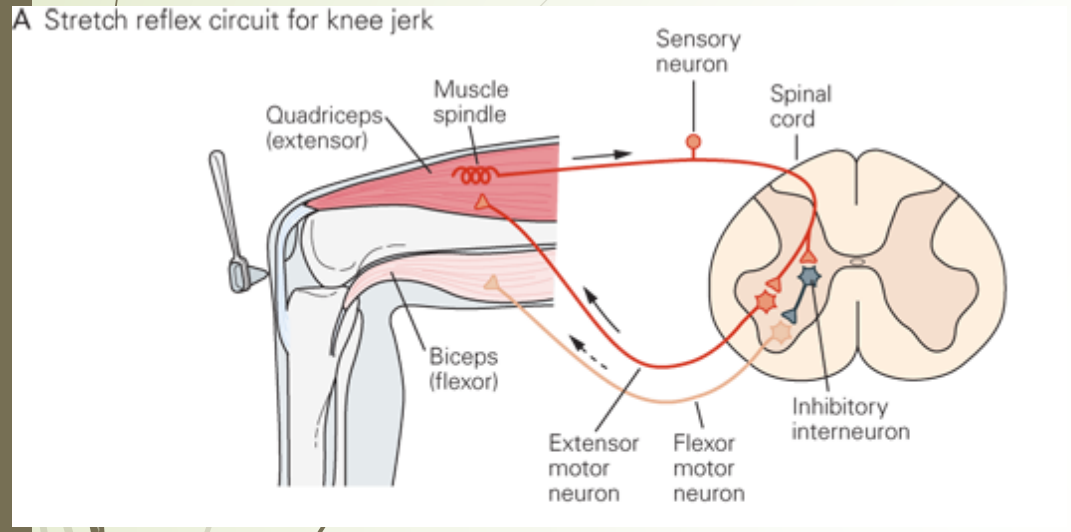
$GABA_A$ is ionotropic receptor that directly opens a Cl- channel.

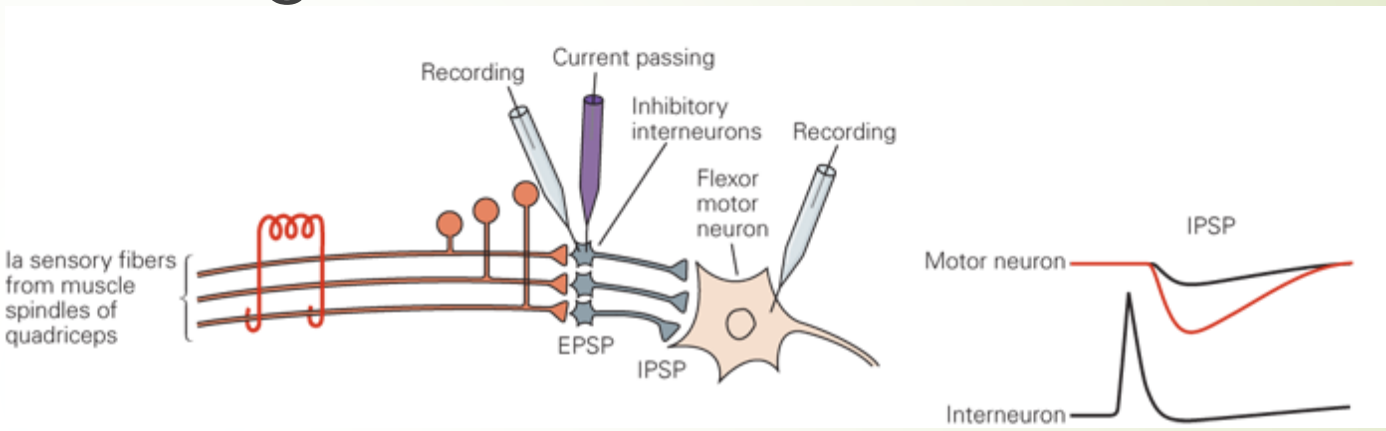
$GABA_B$ is a metabotropic receptor that activates a second messenger cascade which in turn activate a K+ channel

Glycine is a less common transmitter in the brain, also activates ionotropic receptors that directly open a Cl- channel.

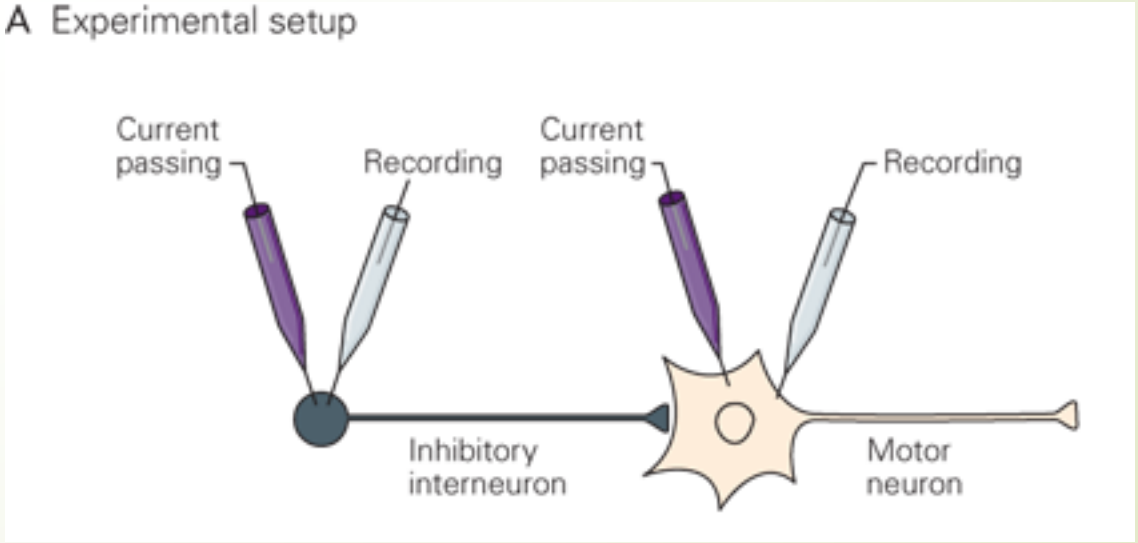
Glycine is the **major transmitter** released in **spinal cord** by interneurons that inhibit antagonist muscle

Glycine is the major transmitter released in spinal cord by interneurons that inhibit antagonist muscle





The ionic mechanism of IPSP can be studied in spinal motor neurons by systematically changing the level of the resting membrane potential in a motor neuron and stimulating presynaptic inhibitory interneuron



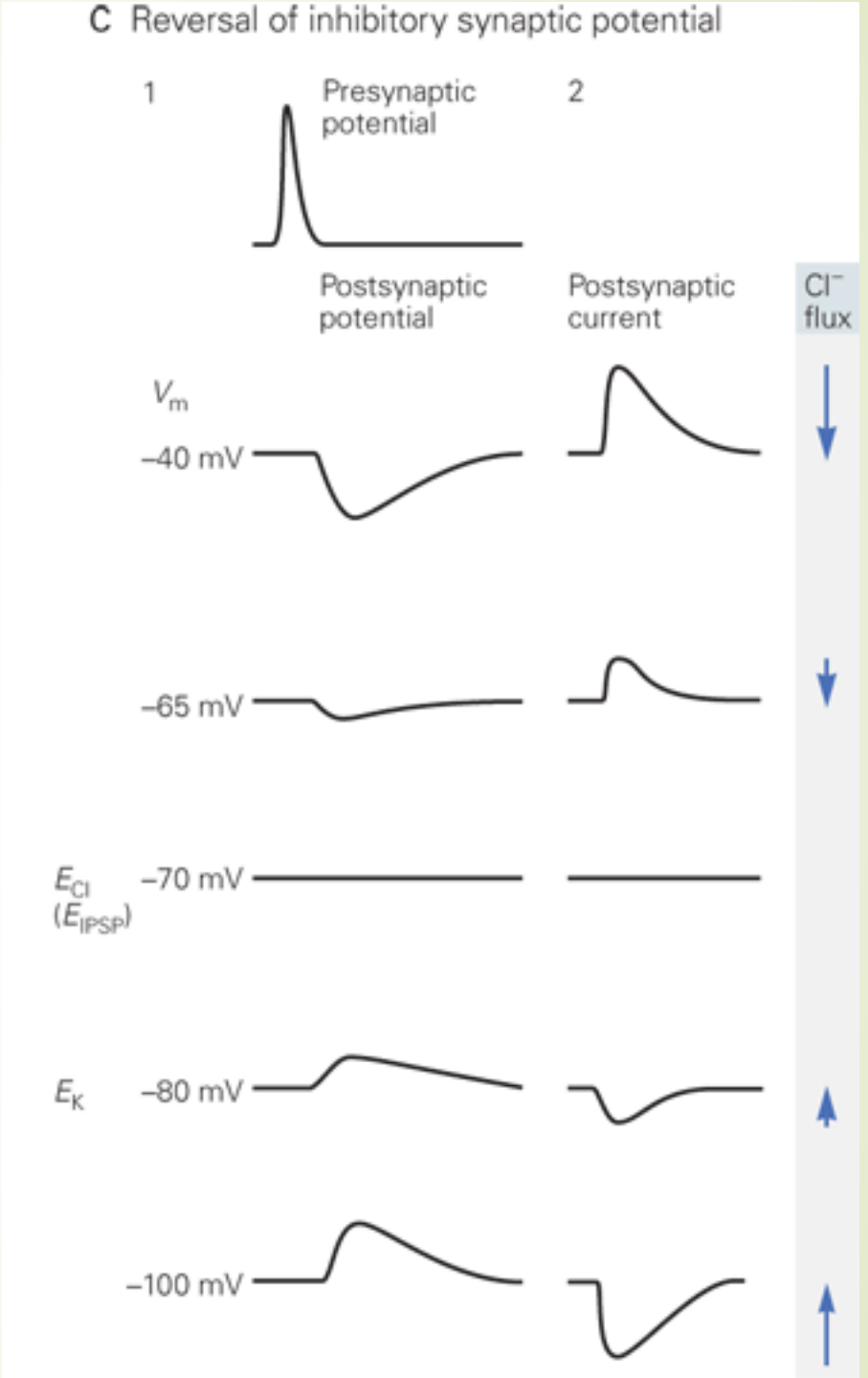
When a motor neuron Vm is held at resting potential (-65mV), a small hyperpolarizing potential is generated when the interneuron is stimulated.

When Vm is held at -70mV, no change in potential is recorded when the interneuron is stimulated.

At Vm < -70mV the motor neuron generates a depolarizing response following stimulation of interneuron.

The V_{rev} = -70mV correspond to the V_{Cl} in spinal motor neurons (extracellular concentration of Cl^- is greater than intracellular)

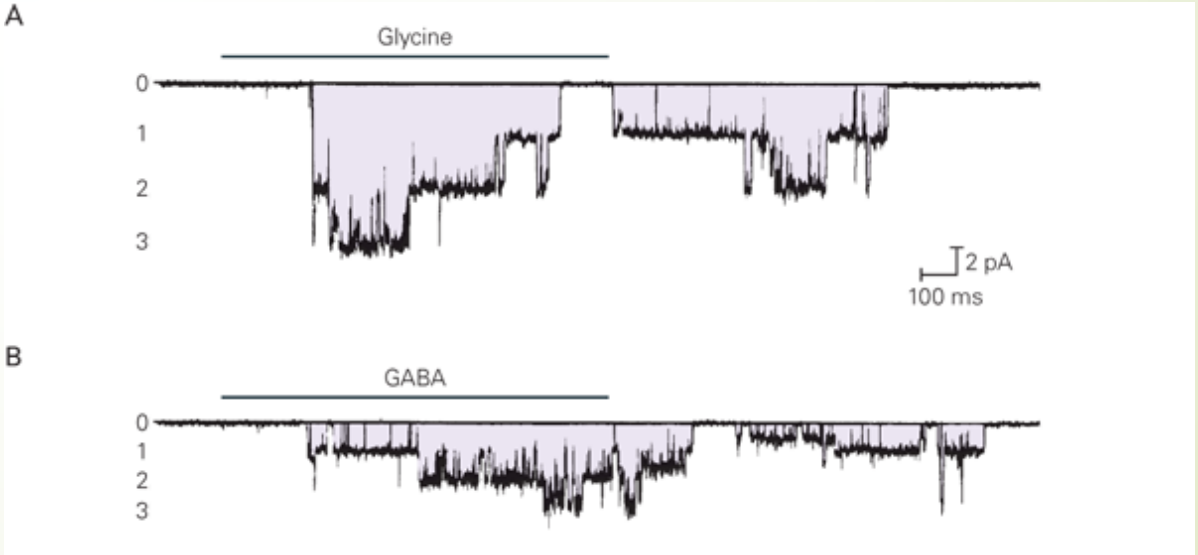
IPSP results from an increase of Cl^- conductance



Currents through GABA and Glycine ionotropic Receptor

Both receptors activate Cl- currents. The single channels conductance of a glycine receptor-channel (46pS) is larger that that of $GABA_A$ receptor-channel (30pS). As a results the unitary current through the glycine-gated channel is somewhat larger that that of $GABA_A$ receptor-channel

This is due to the fact that the diameter of the glycine receptor-channel pore is slightly larger than that of the $GABA_A$ receptor-channel pore



ICl- through $GABA_A$ and glycine receptor-channels normally inhibit the postsynaptic cell

V_m of a typical neuron is -65mV = slightly more than E_{Cl} (-70mV). At this resting potential the chemical force driving Cl^- into the cell is slightly greater than the electrical force opposing Cl^- influx – that is the electrochemical driving force on Cl^- ($V_m - E_{Cl}$) is positive

The opening of Cl^- channels leads to a positive current based on the relation:

$I_{Cl} = g_{Cl} (V_m - E_{Cl})$

Because the charge carrier is the negatively charged Cl^-, the positive current correspond to Cl^- influx into the neuron, down its electrochemical gradient.

This causes a net increase in negative charge on the inside of the membrane

The membrane becomes hyperpolarized

Cl^- through $GABA_A$ and glycine receptor-channels normally inhibit the postsynaptic cell

Some central neurons have a resting $V_m = E_{Cl}$. In such cells an increase in Cl^- conductance does not change the membrane potential - the cell does not become hyperpolarized – because the electrochemical driving force for Cl^- is nearly 0.

However, the opening of Cl^- channels in such cells still inhibits the cell from firing an action potential in response to a near-simultaneous EPSP.

This is because the depolarization produced by an excitatory input depend on a weighted average of the batteries for all types of open channels – excitatory and inhibitory synaptic conductances and resting conductances – with weighting factor equal to the total conductance for a particular type of channels.

I_{Cl} through $GABA_A$ and glycine receptor-channels normally inhibit the postsynaptic cell

Because the battery of Cl^- channels is near the V_m, opening these channels helps hold the membrane near its resting potential during EPSP by increasing the weighting factor for Cl^- battery.

The effect that the opening of Cl^- channels has on the magnitude of a EPSP can also be described in terms of Ohm's law.

$$\Delta V_{EPSP} = I_{EPSP} / g_I$$

ΔV_{EPSP} = amplitude of depolaization during EPSP

I_{EPSP} = excitatory synaptic current

g_I = total conductance of all other channels open in the membrane, including resting channels and any contributions from the transmitter-gated Cl^- channels

Because the opening of Cl^- channels increases the resting conductance, the depolarization during EPSP decreases. This consequence of synaptic inhibition is called the **short-circuiting** or **shunting** effect.

In some cells, such as those with metabotropic receptors $GABA_B$ receptors, inhibition is caused by the opening of K^+ channels. Because the E_K = -80, always negative to the resting potential, opening of K^+ channels inhibits the cell even more profoundly than opening Cl^- channels (assuming a similar-size synaptic conductance). $GABA_B$ responses turn on more slowly and persist for longer time compared with $GABA_A$ responses.

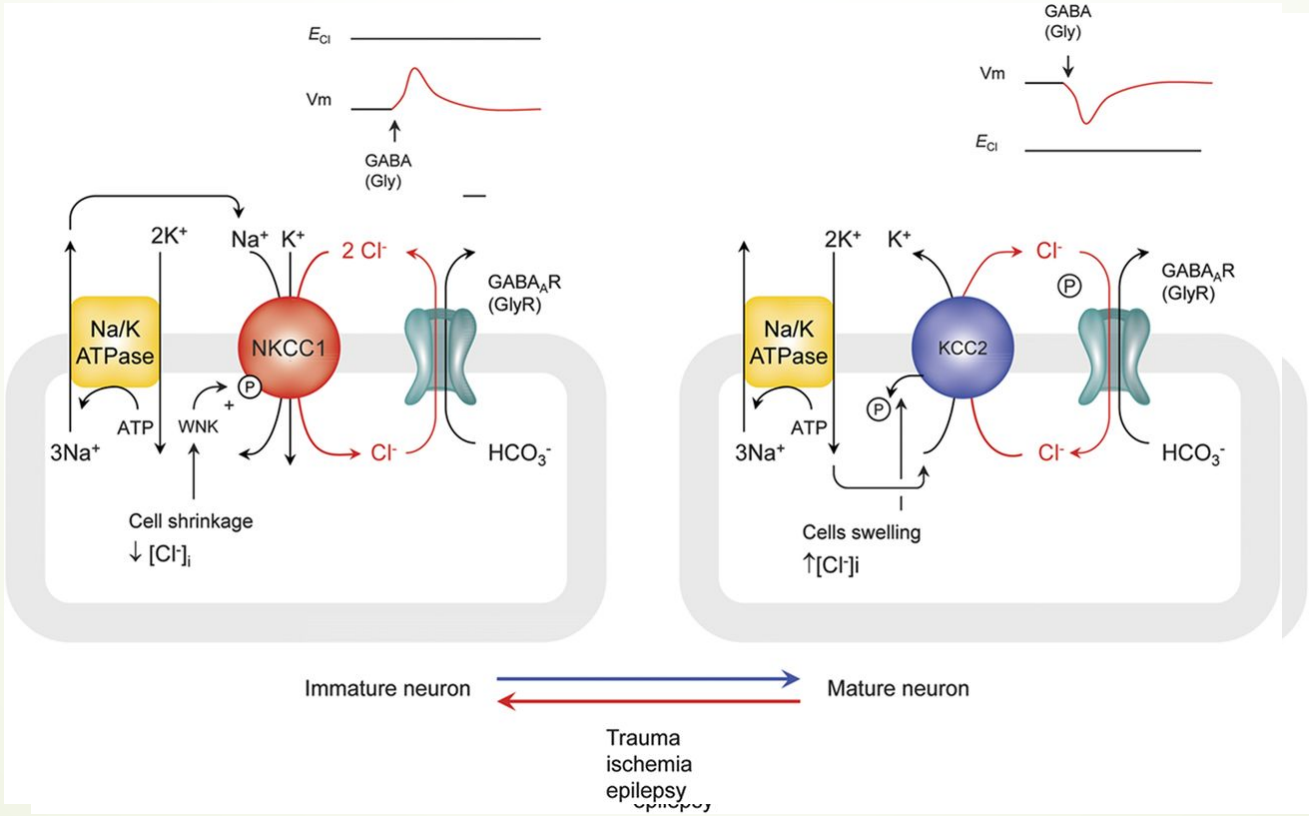
Paradoxically, under some conditions the activation of $GABA_A$ receptors in neurons can cause excitation.

This is because the influx of Cl^- after intense periods of stimulation can be so great that the intracellular Cl^- concentrations increases substantially.

It may even double.

As a result, the E_{Cl} may become more positive than the resting potential. Under these conditions, the opening of Cl^- channels leads to efflux and depolarization of the neuron.

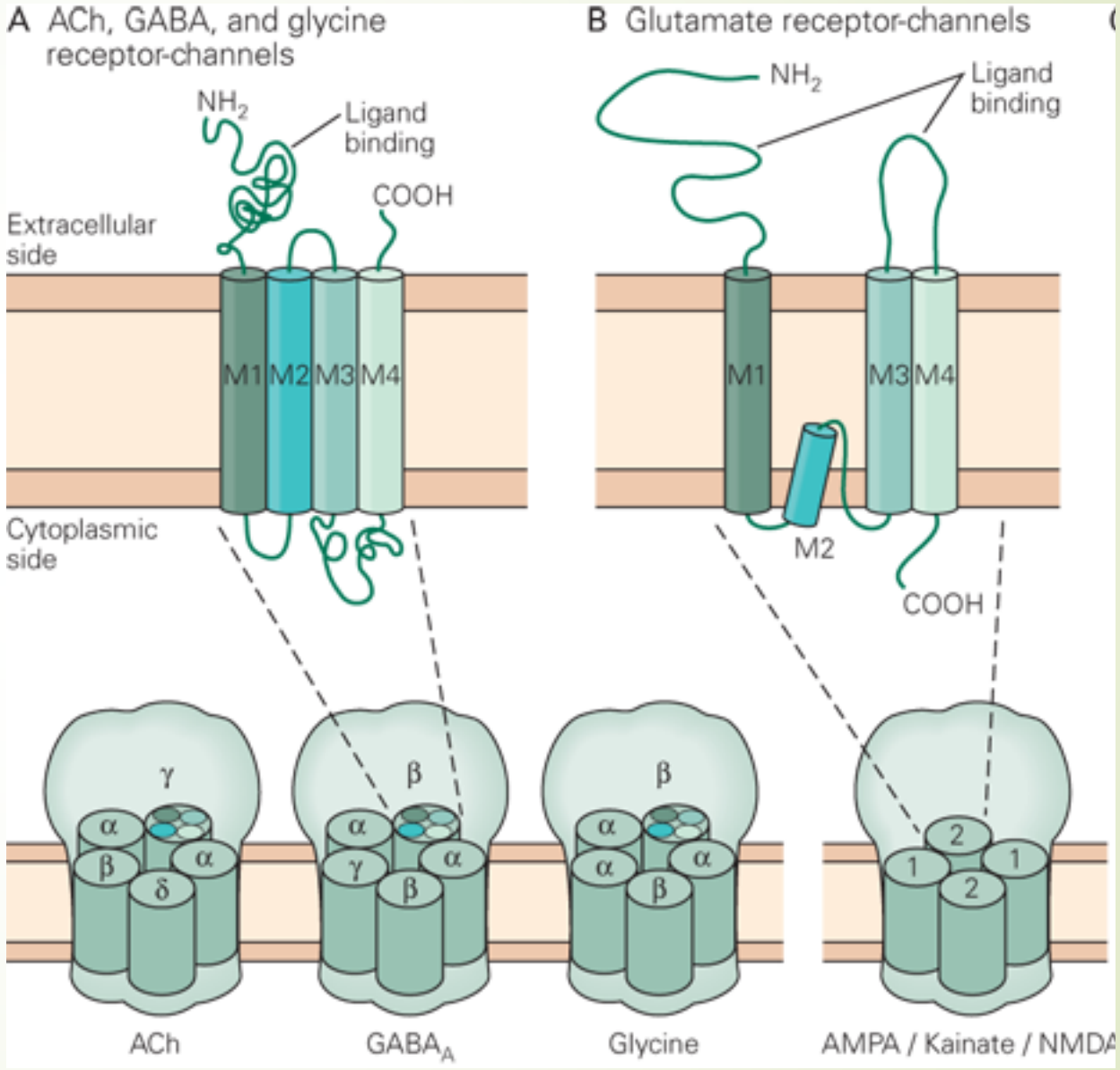
Such depolarizing Cl^- responses occur normally in newborn animals, where $[Cl^-]_i$ tends to be high even at rest. This is because the K^+-Cl^- cotransporters expressed at low levels during early development. Similarly excitatory Cl^- responses by $GABA_A$ may contribute to epileptic discharges in which large, synchronized and depolarizing GABA response are observed



Ionotropic GABA and glycine

$GABA_A$ and glycine receptors are structurally related to nACh receptors even though the latter are selective for cations.

In contrast glutamate receptors evolved form a different class of proteins and thus present a second gene family of ligand-gated channels

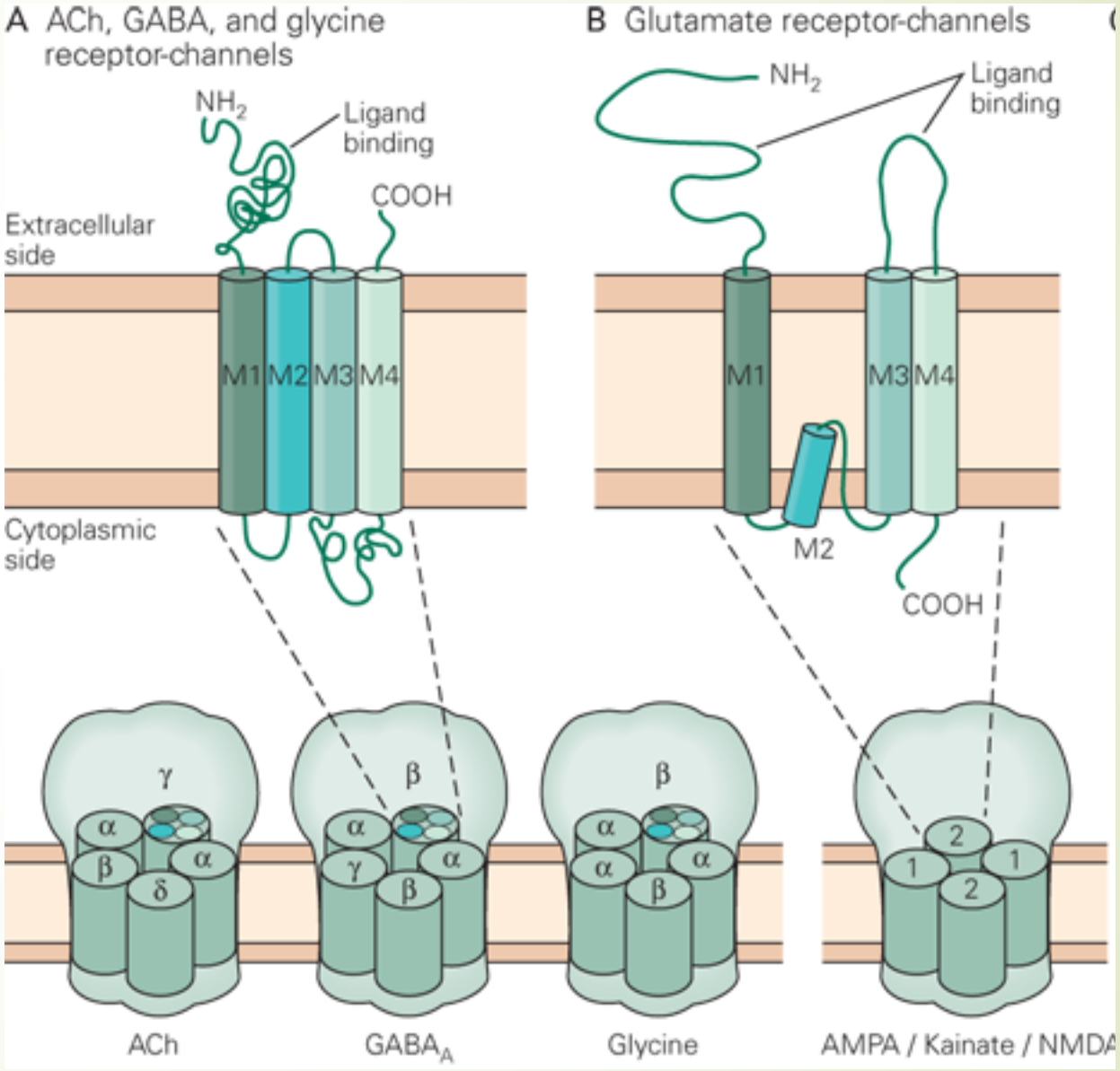


Ionotropic GABA and glycine

$GABA_A$ receptors are pentameric usually composed of 2α, 2β 1γ or 1δ subunit.

Receptors are activated by the binding of 2 molecules of GABA in cleft formed between the α and β subunits

Glycin receptors are formed by 3α and 2β subunits and require the binding for up to 3 molecules of ligand to open.



Ionotropic GABA and glycine

Similarly to nACh receptors, M2 forms the lining of the channel pore.

Differences with nAChR:

nAChR present **rings of negatively charged** aa residues that helps selecting for cations over anions.

GABA and Glycine receptors contain either **neutral or positive charged residues** at the homologous positions, which contribute to the selectivity for anions

Ionotropic GABA and glycine

$GABA_A$ and glycine receptors play important roles in disease and in the action of drugs.

$GABA_A$ receptors are targets for several types of drugs that are clinically important and socially abused, including anesthetics and benzodiazepines, barbiturates and alcohol.

They all act on different sites of the receptor but act similarly to increase P_{open} of the GABA receptor channel.

Example: while GABA binds to a cleft between α and β subunits, benzodiazepines (or barbiturate) bind more strongly to the receptor-channel when GABA is also bound, and this tight binding helps stabilize the channel in the open state. In this manner the various compounds all enhance inhibitory synaptic transmission.

Inotropic GABA and glycine

How come that all these compounds act on the same receptor but promote such different behavioral and psychological effects, for example reducing anxiety versus promoting sleep?

Many of the compounds interacts selectively with specific subunits subtypes, which can be localized in different brain regions.

Example: zolpiden (non-bezodiazepine that promotes sleep) binds selectively to $GABA_A$ receptors containing $\alpha 1$ subunit. In contrast, the anxiolytic effect of benzodiazepines require binding to the $\alpha 2$ and γ subunits.

Other types of ionotropic receptors in CNS

Certain fast excitatory synaptic actions are mediated by the neurotransmitter **serotonin (5-HT) acting at the 5-HT_3** class of ionotropic receptors.

These receptors have four transmembrane domains and are structurally similar to the nicotinic receptors.

Like nACh receptors they are permeable to cations and have a E_{rev} = 0mV.

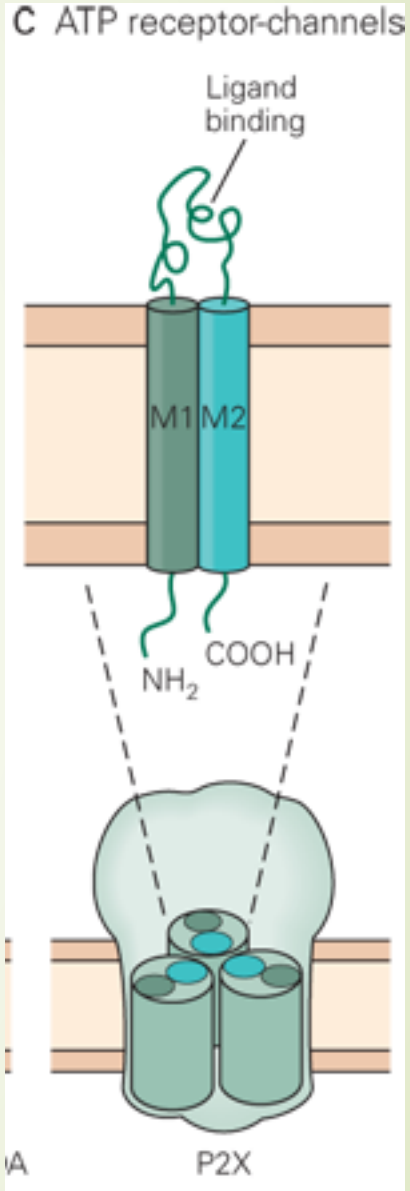
Other types of ionotropic receptors in CNS

Ionotropic receptors for ATP, which serves as excitatory transmitters at selected synapses, constitute a third major family of transmitter-gated channels = **PURINERGIC RECEPTORS.**

These receptors occurs in smooth muscle cells innervated by sympathetic neurons of the autonomic ganglia as well on certain central and peripheral neurons.

These receptors (P2X) are activated by ATP and the channel is permeable to both monovalent cations as well as Ca^{2+}, they present a E_{rev} = 0mV

The structure of P2X receptors reveals that it is organized in three subunits, each containing only two transmembrane domains surrounding a central pore



Integration of Excitatory and inhibitory synapses in CNS

Each neuron in the CNS is constantly bombarded by an array of synaptic inputs from many other neurons.

A single motor neuron for example may be innervated by as many as 10000 different presynaptic endings. Some are excitatory and other inhibitory; some strong other weak.

Some inputs contact the motor neurons on the tips of its apical dendrites, other in proximity of the soma. The different inputs may reinforce or cancel one another.

Integration of Excitatory and inhibitory synapses in CNS

The net effect of the inputs at any individual excitatory or inhibitory synapse will depend on several factors:

Location, size and shape of the synapses, the proximity and relative strength of other synergistic or antagonistic synapses and the resting potential of the cell.

In addition, the response of the postsynaptic neurons is the results of two types of currents:

- Currents across receptor channels evoked by neurotransmitters at the postsynaptic membrane due to the interaction of the transmitter and the receptor and the properties of the receptors
- Current across voltage-gated channels at non synaptic membrane (dendritic, somatic or the initial segment) and are generated by voltage changes resulting from currents of synaptic origin, or from currents generated during the first action potential

Integration of Excitatory and inhibitory synapses in CNS

Inputs are coordinated in the postsynaptic neuron by a process called **neuronal integration.**

This cellular process reflects the task that confronts the nervous system as a whole: making decisions.

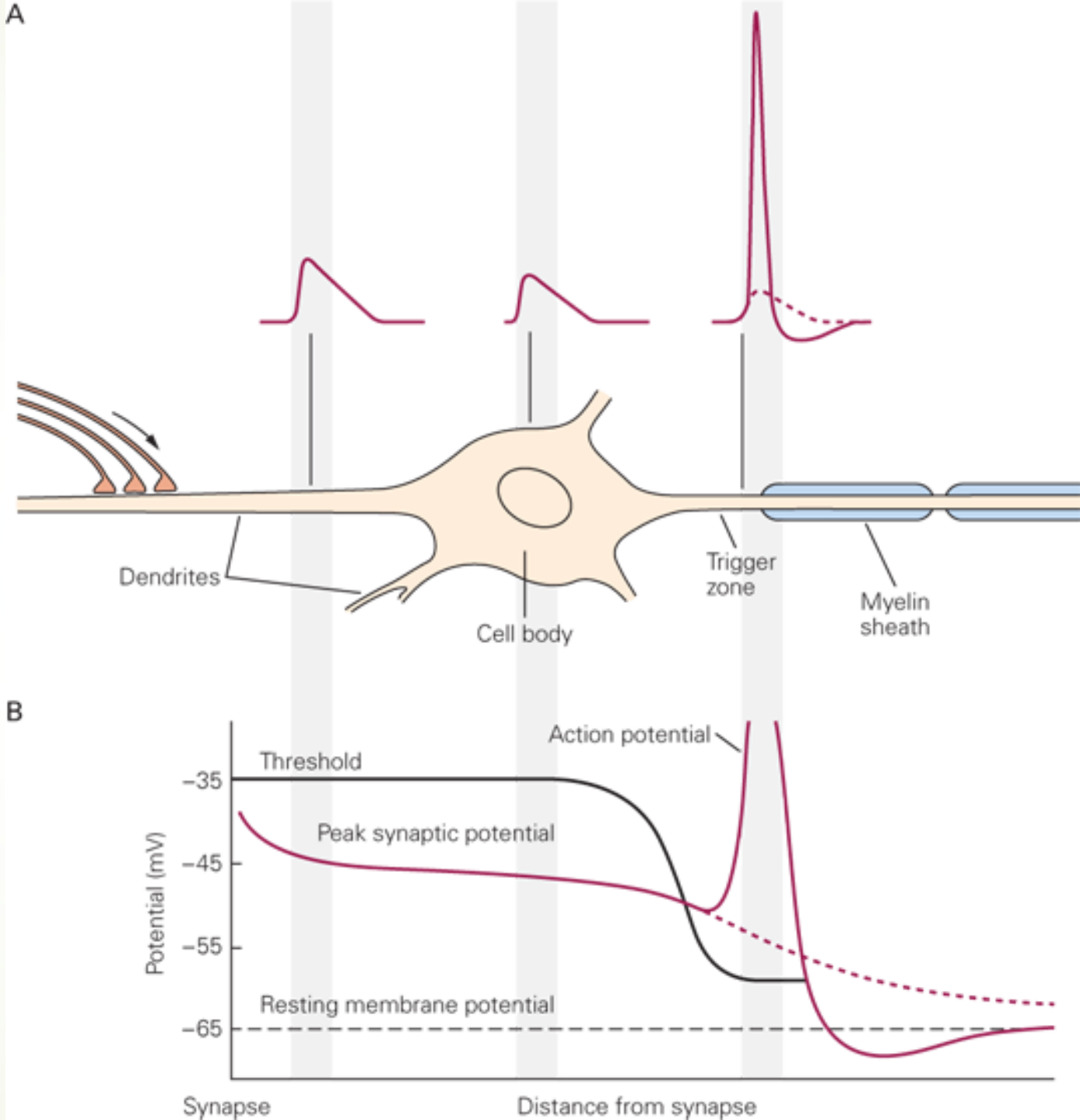
A cell at any moment has 2 options: fire or not fire an action potential. This brain's ability to choose between competing alternatives is called **integrative action of the nervous system**

In most neuron the decision to fire or not is making at one site: the **initial segment** of the axon **(trigger zone)**.

Here the cell has a lower threshold for action potential generation as compared with cell body or dendrites because it has a higher density of HVA Na^+ channels.

With each increment of membrane depolarization, more Nav open thus generating a higher density of inward current at the axon initial segment as compared with other areas.

The action potential triggered at the trigger zone then depolarizes the membrane of the cell body to threshold and at the same time is propagated along the axon.

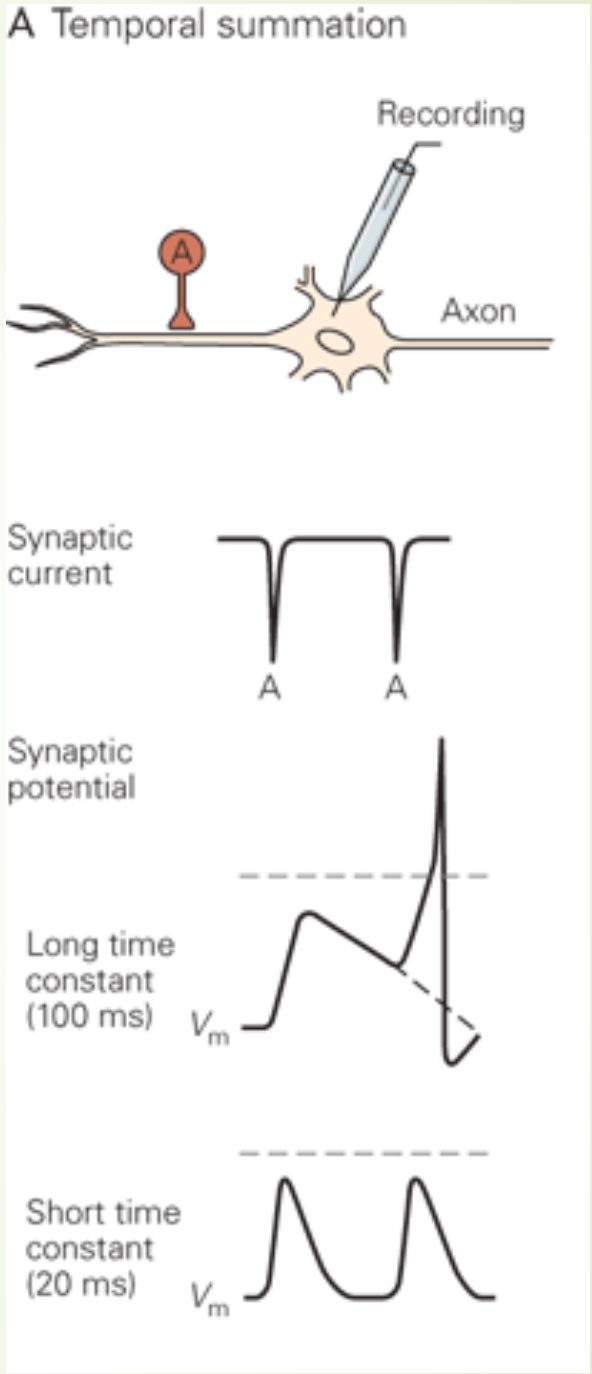


Neuronal integration involves the summation of synaptic potentials that spread to the trigger one, it is clinically affected by two passive membrane properties of the neuron:

- **Membrane time constant** helps determine the time course of the synaptic potential controlling **temporal summation,** the process by which consecutive synaptic potential at the same site are added together in the postsynaptic cell.

Neurons with a large membrane constant have a greater capability for temporal summation than do neurons with a shorter time constant

The longer the time constant of the membrane, the **greater the likelihood** that **two consecutive inputs from an excitatory presynaptic neuron will summate** to bring the cell membrane to its threshold for action potential

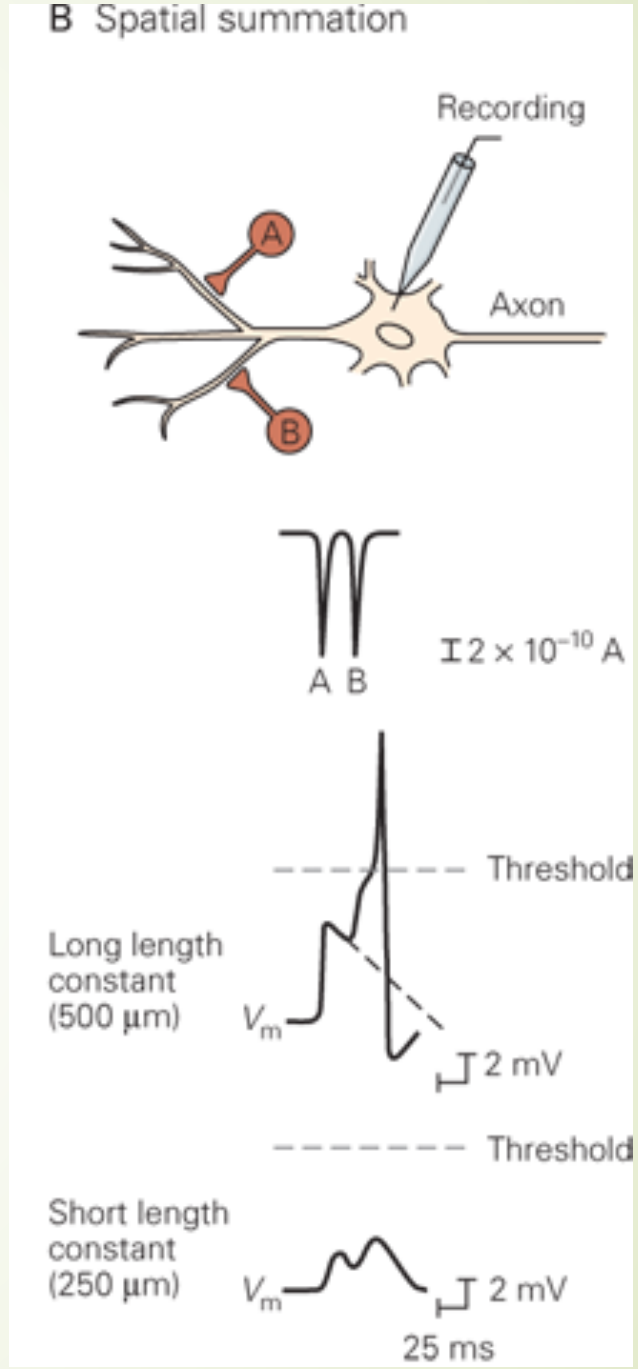


- **Length constant** of the cell determines the degree to which a local depolarization decreases as it spreads passively from a synapse along the length of the dendrite.

In cells with longer length constant, signals spread to trigger zone with minimal decrement; in cells with a short length constant, the signal decay rapidly with distance

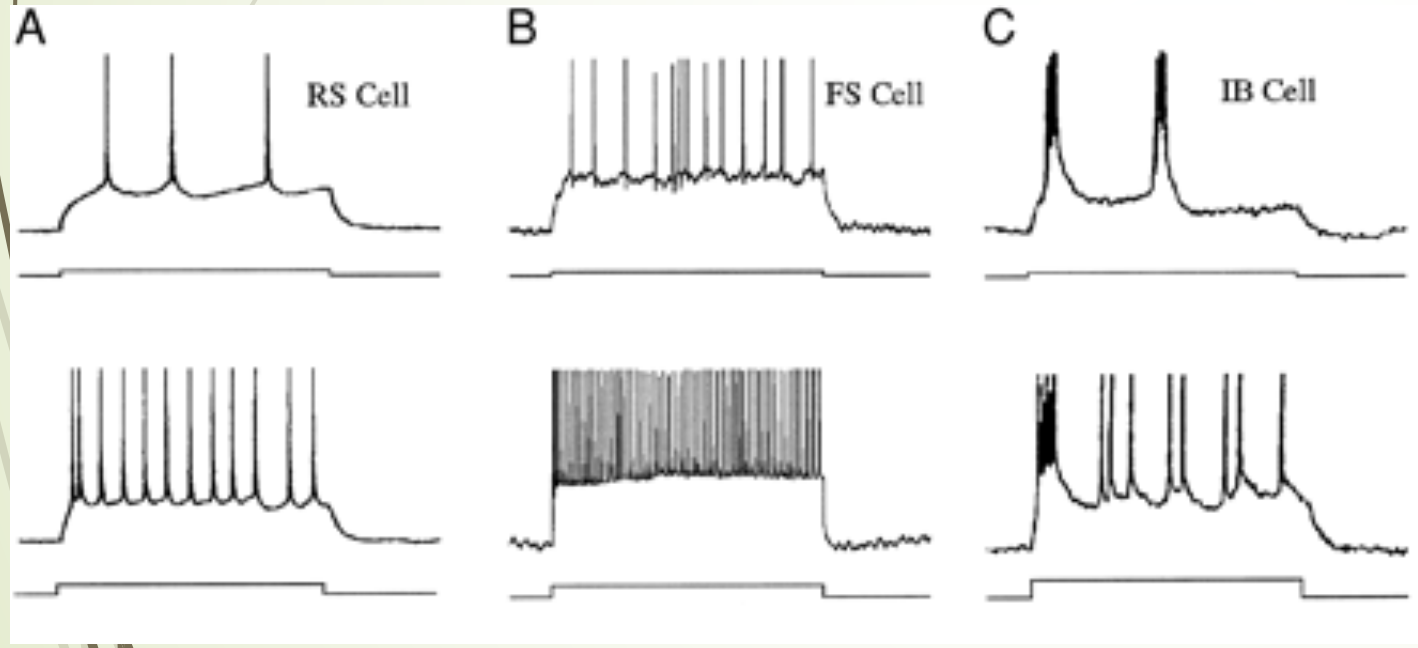
Inputs from many presynaptic neurons acting at different sites of the postsynaptic neuron must be added together: **spatial summation.**

Neurons with larger length constant are more likely to be brought to threshold by inputs arising from different sites than are neurons with a short length constant



Subliminal voltage-gated currents of the somato-dendritic membranes

- Not all neurons respond with the same way when they are activated by a depolarizing current pulse or when the hyperpolarized: they have **different patterns of firing**



Zhu J and Connors BW, Journal of Neurophysiology, 1999

Firing patterns of a regular-spiking (RS) cell (A), a fast-spiking (FS) cell (B), and an intrinsically burst-spiking (IB) cell (C). Top and middle: characteristic response patterns to depolarizing current injection of 2 different intensities. Suprathreshold spiking patterns reveal their physiological identity. Resting membrane potentials of these cells were −65 mV (A), −72 mV (B), and −63 mV (C). Note that fast action potential amplitudes are truncated by the digitization.

Subliminal voltage-gated currents of the somato-dendritic membranes

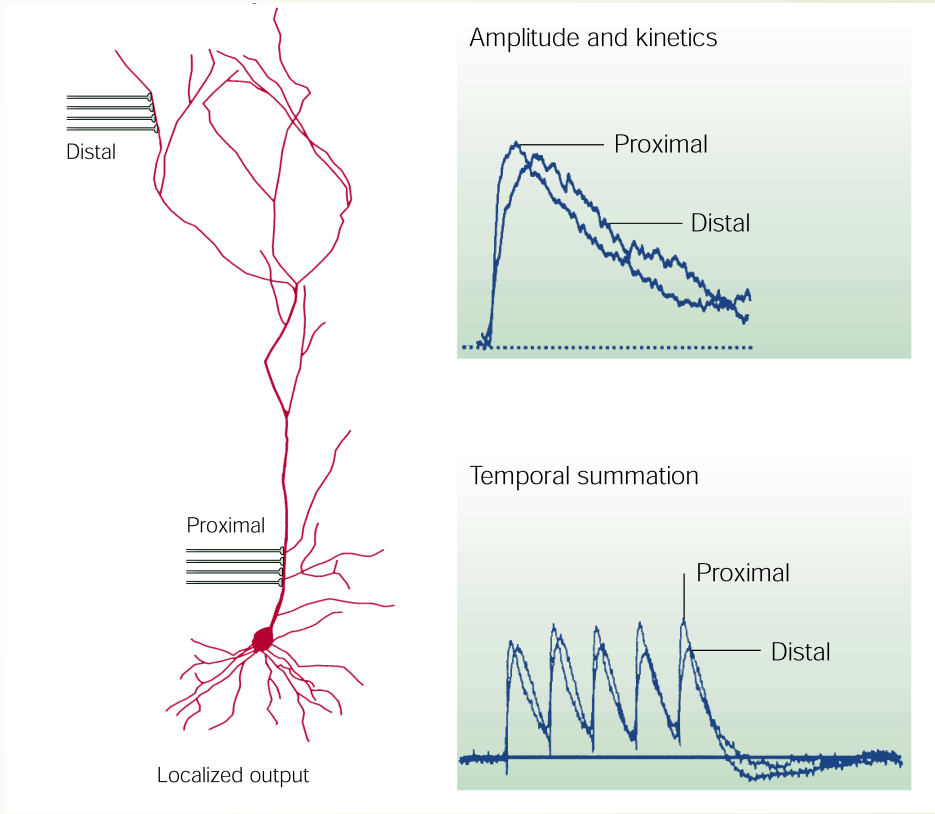
- Neurons are not interchangeable.
- The activity of a neuronal network is related not only to the excitatory and inhibitory interactions among them but also to their **intrinsic electrical properties**.
- The personality of a neuron depend by the **input** (depolarization or hyperpolarization) **and output** (firing) characteristics.
- Input and output are the results of a rich repertoire of ionic currents other than those of the action potential and neurotransmitter activated channels. These currents are called **subliminal voltage-gated currents** because they are activated at voltages subthreshold to that of action potential.
- They are located either in the dendritic or somatic membranes or both

Subliminal voltage-gated currents of the somato-dendritic membranes

- A dendritic tree currents propagate passively, EPSP are attenuated in amplitude and slowed down in time course as they spread into the soma due to passive properties of the dendrite.
- Accordingly the influence of EPSP on neuronal output, which relies on the ability to depolarize the axon, depend upon the initial size and shape of the synaptic response, as well as the cable properties of the dendritic tree filters the response as it spreads from the synapse to the site of the action potential generation.

Subliminal voltage-gated currents of the somato-dendritic membranes

- As a consequence, excitatory and inhibitory synapses located on distal dendrites should be less efficient in depolarizing or hyperpolarizing the soma-initial segment region
- Although theoretical analyses have predicted such a clear location-dependent variability of synaptic input, **there is now considerable evidence indicating that the shape of the EPSPs may be relatively independent of synapse location** (see as an example pyramidal neurons of the CA1 region of the hippocampus)



Magee JC, Nat. Neurosc Rev, 2000

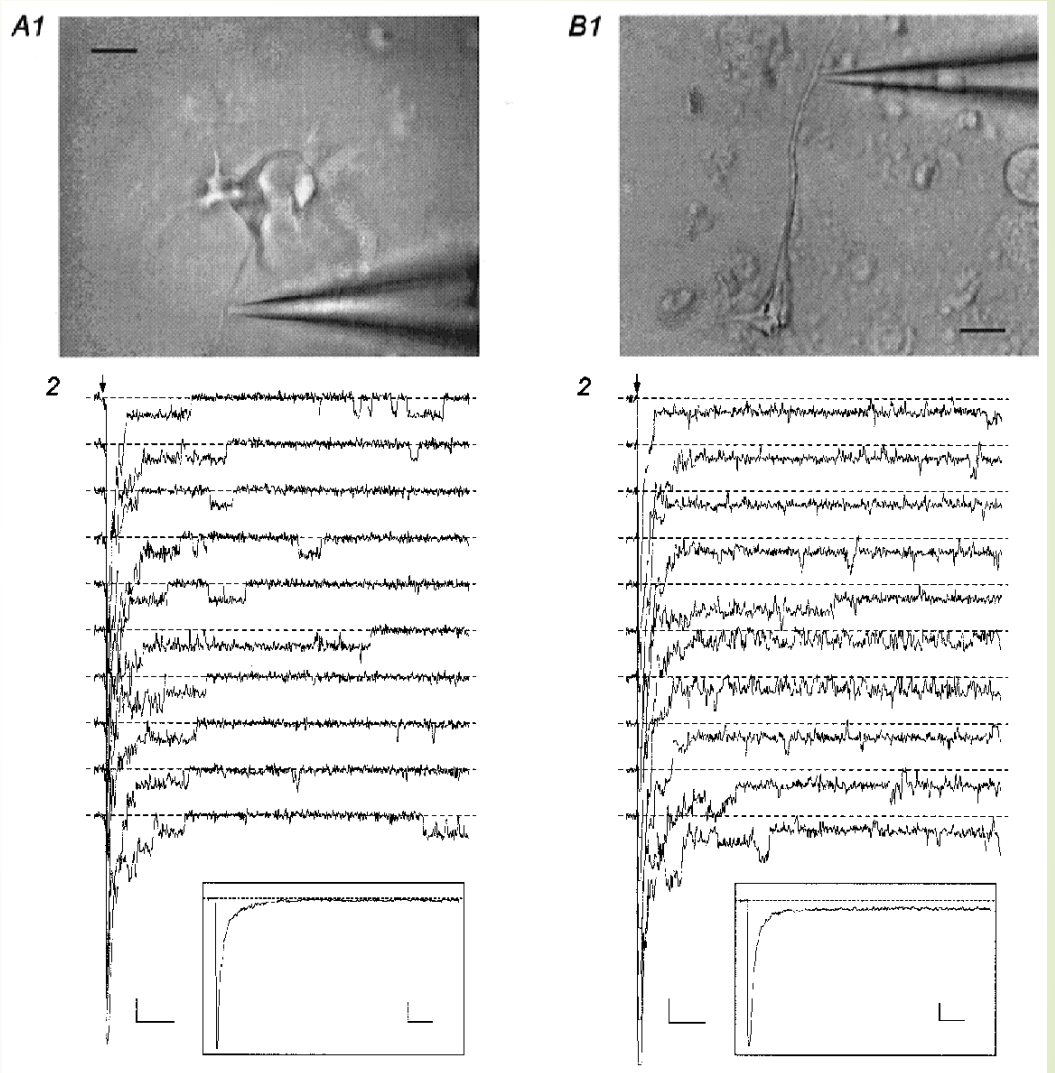
- If certain dendrites do not behave as simple cables, is it because their membranes express subliminal voltage-gated currents?
- In order to answer this questions the best experimental models are brain regions organized in layers such as neocortex or hippocampus. In these regions, dendritic recordings are much easier because the dendritic layer is easily recognizable from the somatic layer

Persistent Na+ channels

- **Persistent Na current I_{NaP}** is a TTX sensitive Na^+ current that activate below spike threshold. It is slowly inactivating (P stand for persistent) in comparison with I_{Na} of the action potential that is transient. Nav 1.6 have been suggested as the channels responsible.

Persistent Na+ channels

Figure 1. Persistent Na^+ channel activity in patches from **stellate** and **pyramidal cell dendrites**. Columns A and B refer to a representative stellate neuron (patch D8716) and a representative pyramidal neuron (patch A8717), respectively. Panels A1 and B1 are microphotographs of the two cells in question (scale bars, 15 um; note the positions of the patch pipettes on the dendritic processes). Panels A2 and B2 illustrate Na^+ channel currents evoked by 50 ms depolarising pulses at −20 mV (A2) and −10 mV (B 2) starting from the time points marked by the arrows. The current traces shown are consecutive sweeps from families of 20. Scale bars: 2 pA and 5 ms. Insets, ensemble average currents obtained from the corresponding 20trace families. Scale bars in insets: 2·5 pA and 5 ms.

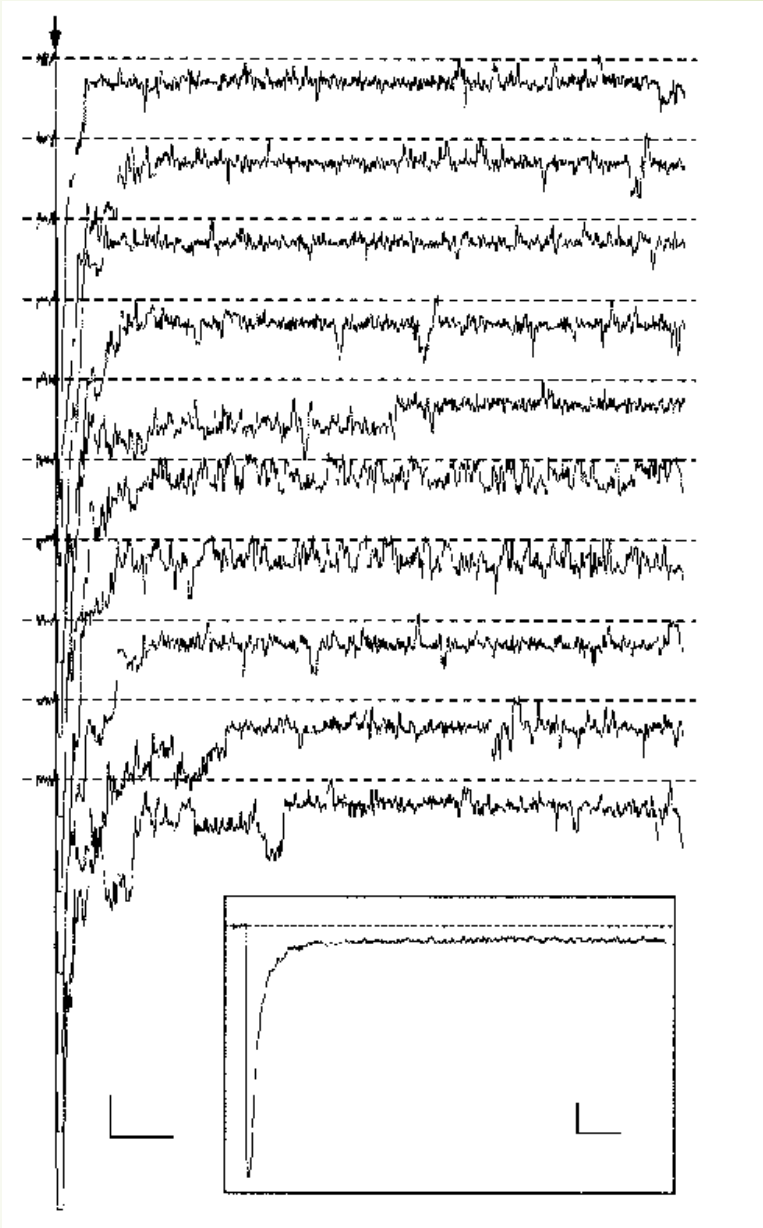


Magistretti J et al, J Physiol 1999

Persistent Na+ channels

Single channel is recorded in patch clamp from dendrite of isolated pyramidal neuron in current clamp.

Depolarization steps (from -100mV to -20mV) evoke a Na+-channel opening in all recorded patches.



Persistent Na+ channels boost EPSPs

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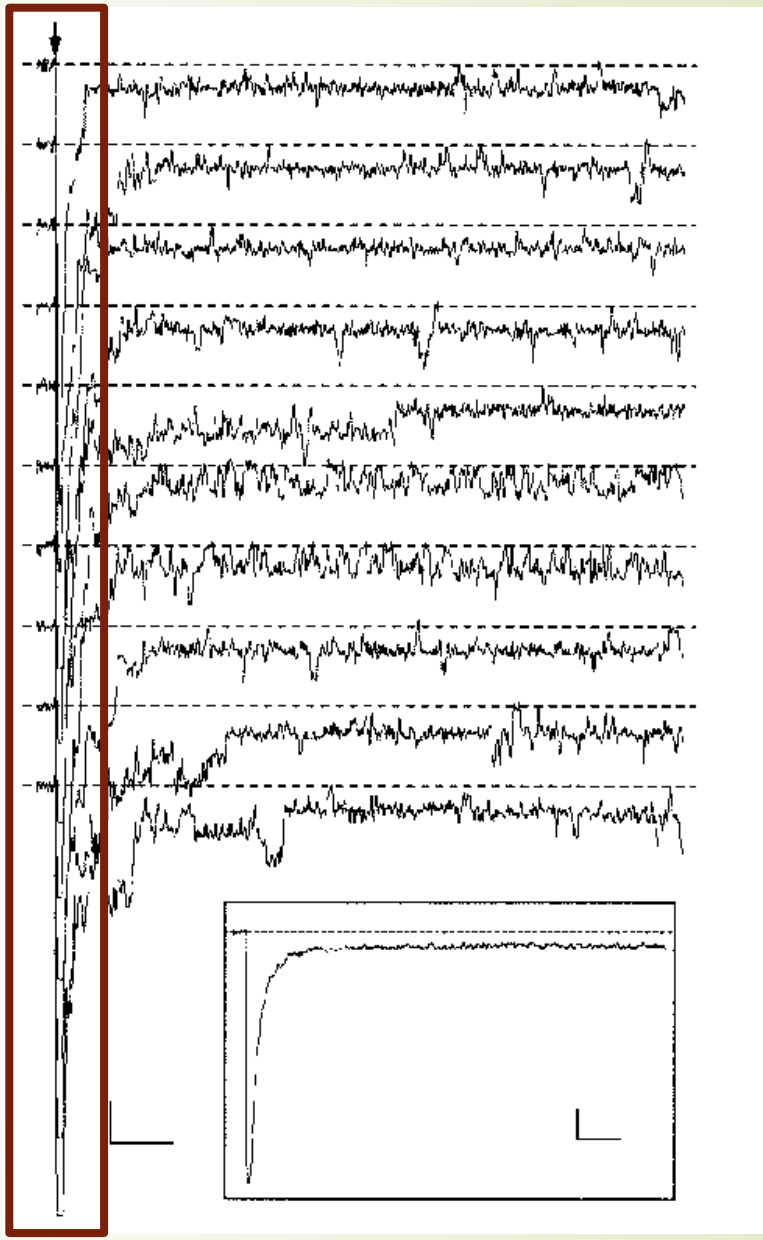

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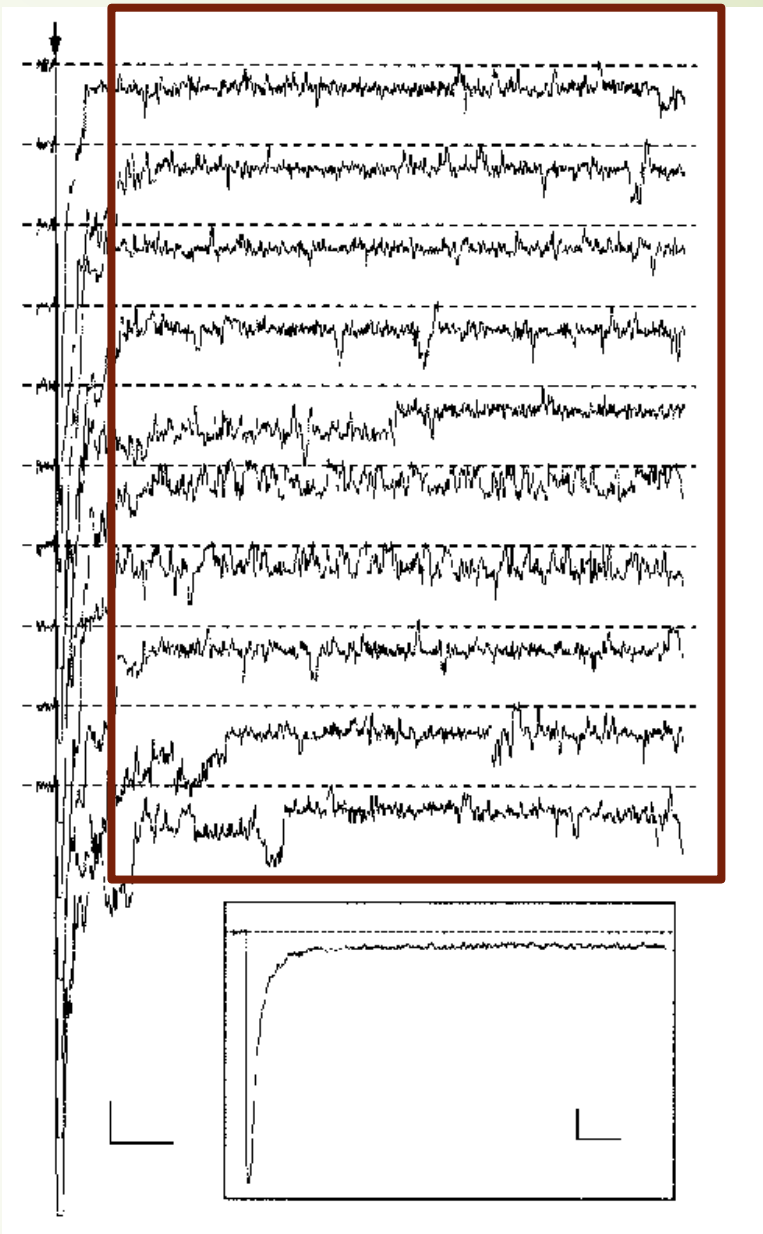
In addition, a different Na-channel activity consisting of prolonged or late openings is recorded that correspond to I_{NaP}.



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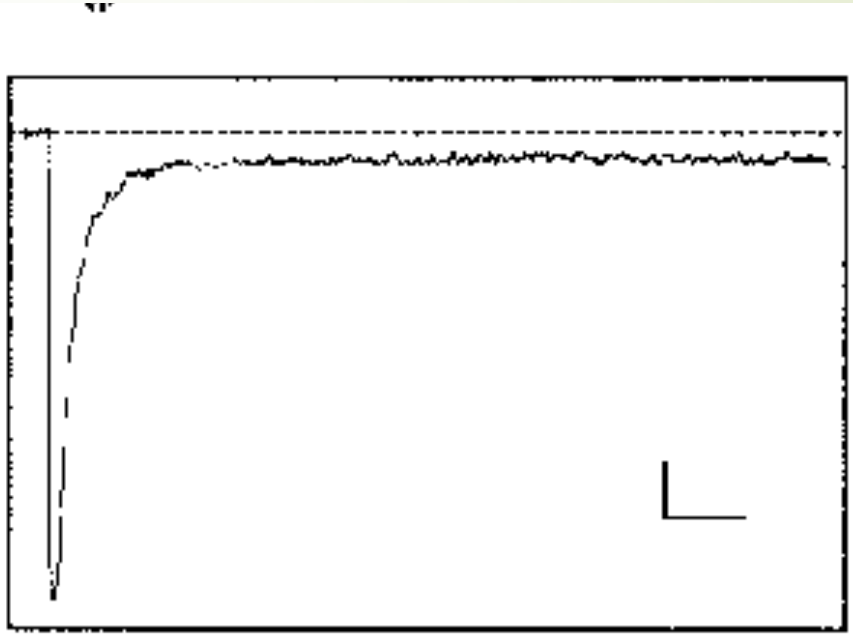
When averaging many traces, this persistent current is able to produce sizable net inward current even for 500ms.



Are Persistent Na^+ channels activated by EPSPs?

Activation of I_{NaP} by local synaptic inputs is tested by simultaneous whole cell dendritic and somatic recordings (in current clamp) made from the same neuron.

Dendritic EPSP are evoked by intradendritic current injection (simulated EPSP).

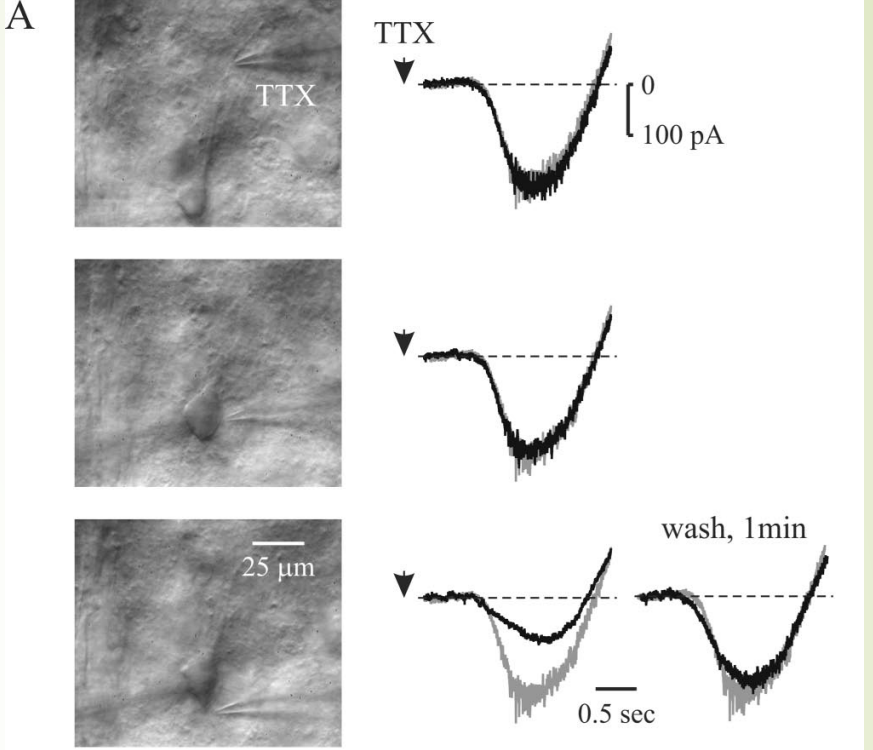
The EPSP must be under threshold in order to evoke only the subliminal, TTX sensitive I_{NaP} and not voltage-gated Na^+ current of the action potentials.

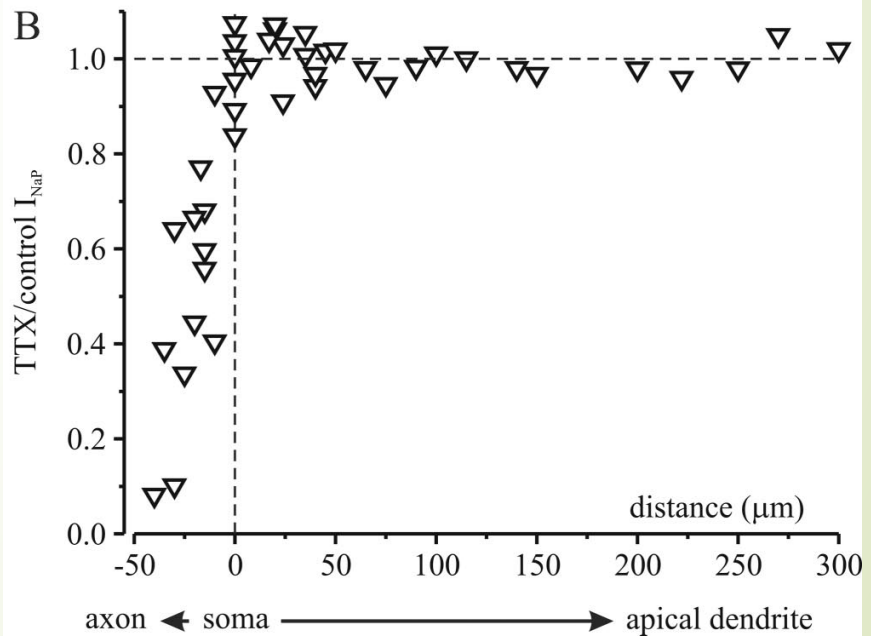
The role of I_{NaP} on these EPSP is then deduced by studying the effect of TTX on the amplitude and duration of EPSPs.

Are Persistent Na^+ channels activated by EPSPs?

Localized application of TTX on layer V pyramidal neuron.

I_{NaP} is activated by subthreshold ramp of voltage applied through the recording electrode in the soma.





Astman N et al., J Neruosc 2006

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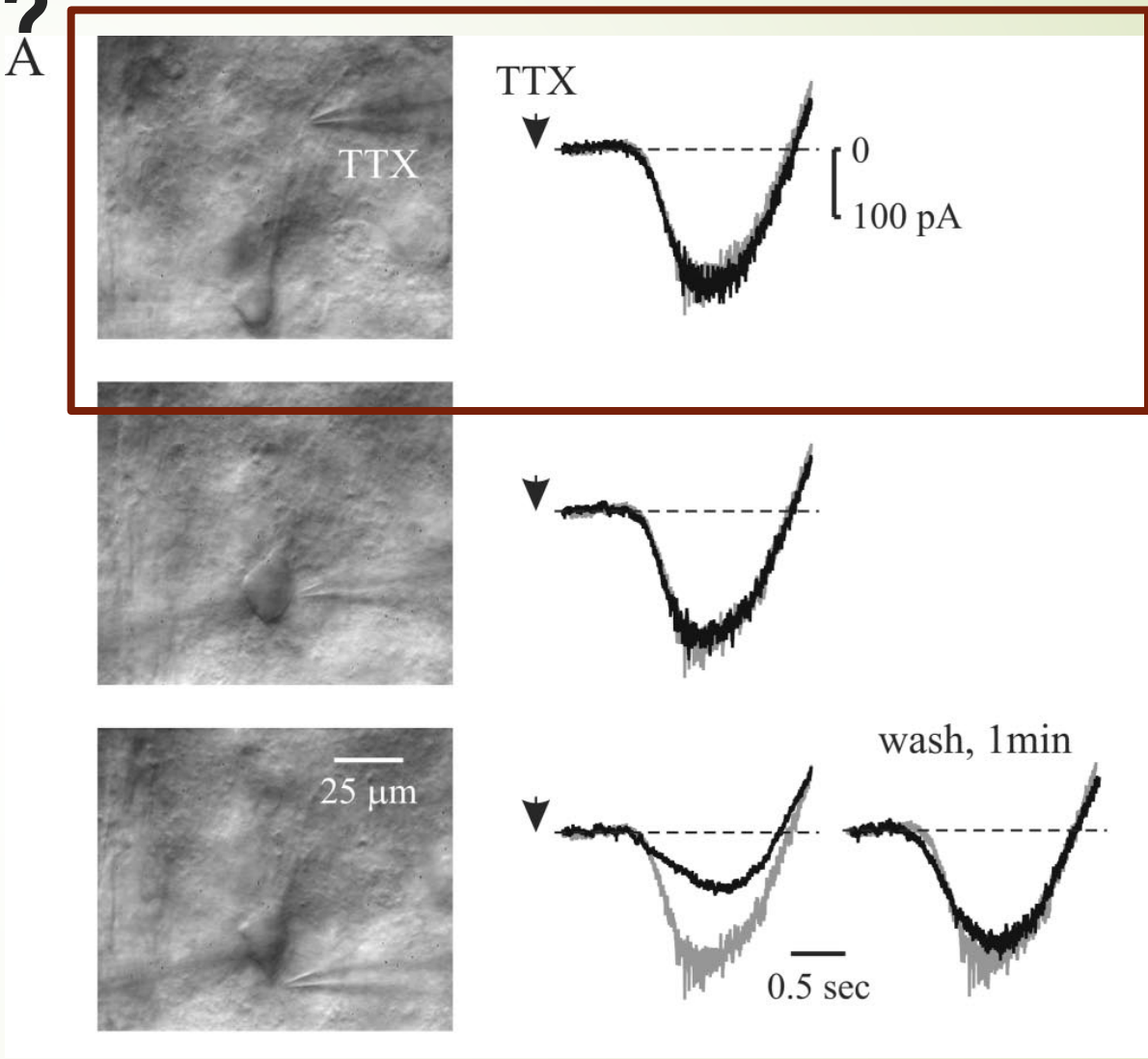

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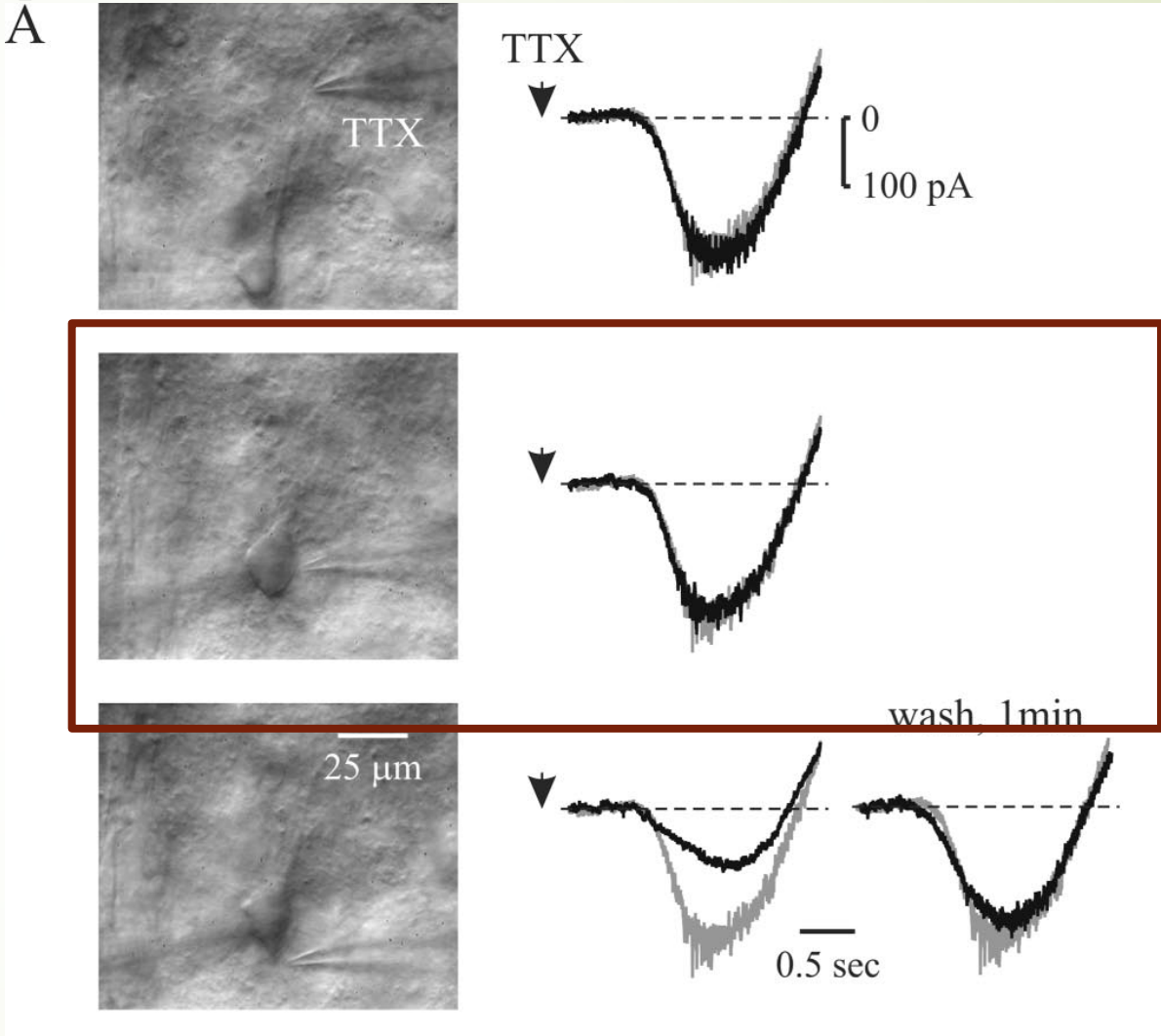
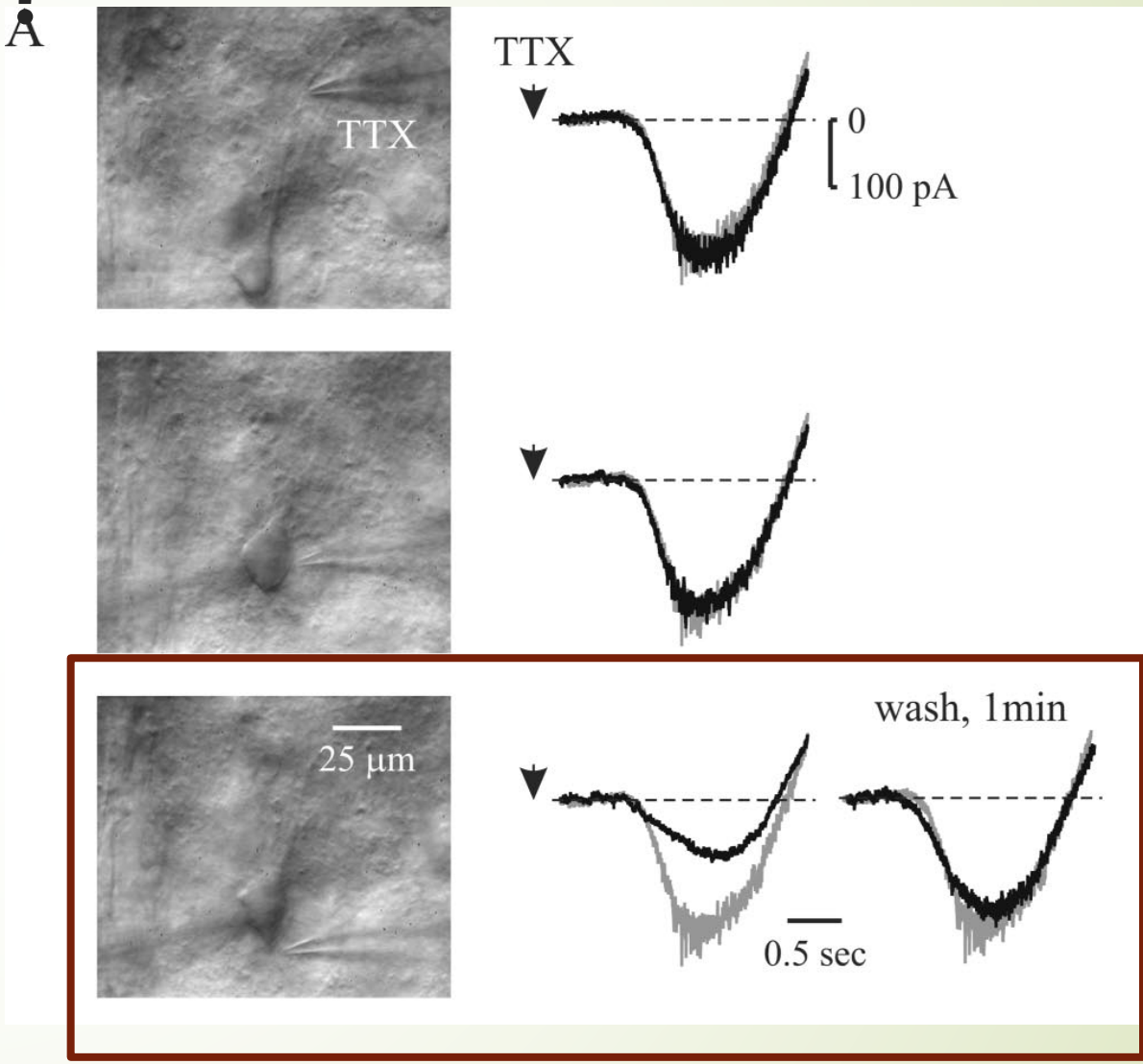


Astman N et al., J Neruosc 2006

Are Persistent Na^+ channels activated by EPSPs?

- Localized application of TTX on layer V pyramidal neuron.
- I_{NaP} is activated by subthreshold ramp of voltage applied through the recording electrode in the soma.
- A low TTX concentration (100nM) was applied locally to the apical dendrite or the soma.
- Application of TTX on the axon promoted a large reduction in the amplitude of I_{NaP}.



Astman N et al., J Neruosc 2006

Are Persistent Na^+ channels activated by EPSPs?

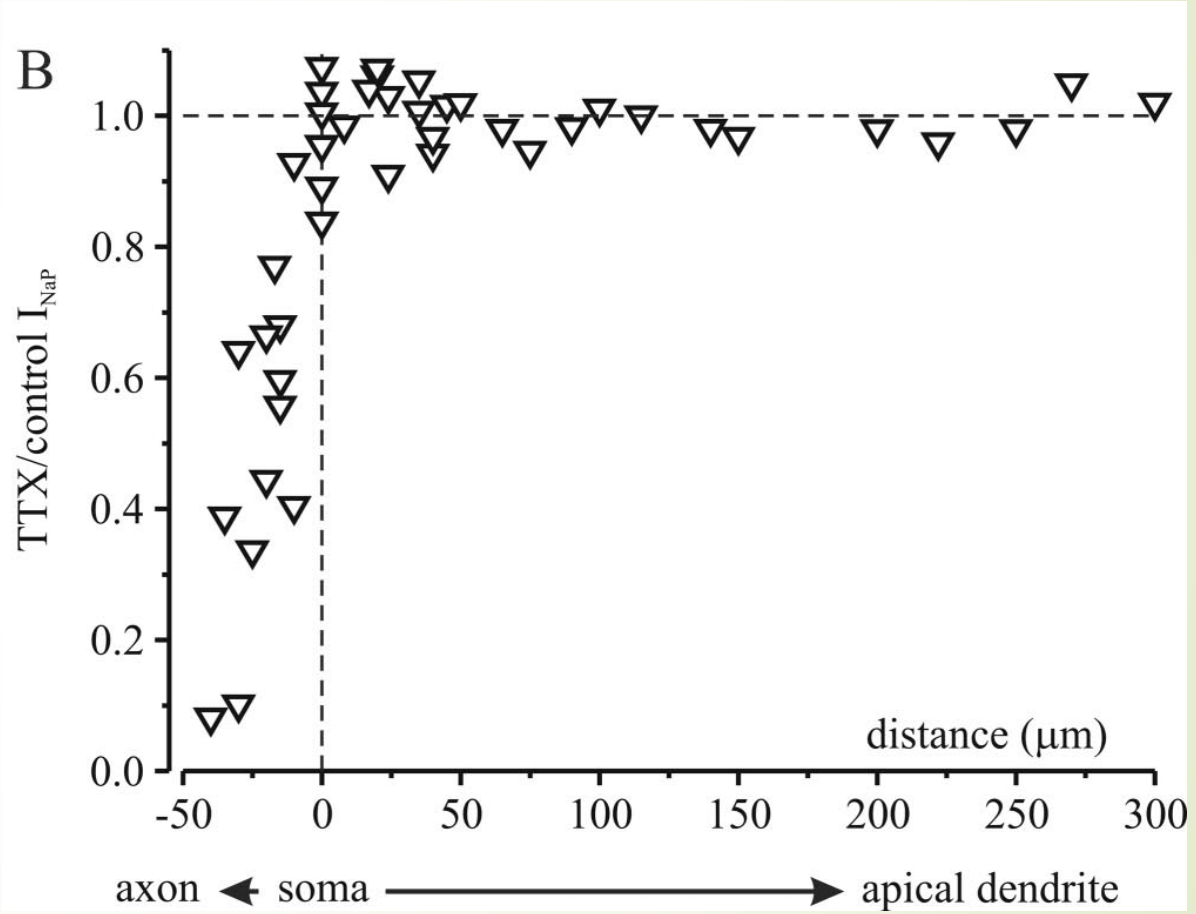
The graph expressing the effect of TTX as a function of the distance of application in the dendrite, soma and axon, show a reduction in the amplitude of I_{NaP} only when TTX is applied to the axon.

In the neocortex **EPSP activate I_{NaP}** and in turn,

The data indicate that the primary source of I_{NaP} is in the spike initiation zone in the proximal axon.

I_{NaP} boosts EPSPs in amplitude and duration. This **amplification mainly occurs in the axo-somatic region.**

Assuming a resting membrane potential of -65/-70mV, to activate I_{NaP}, EPSP must depolarize the membrane by 5-15mV. Only summed EPSP can reach this amplitude



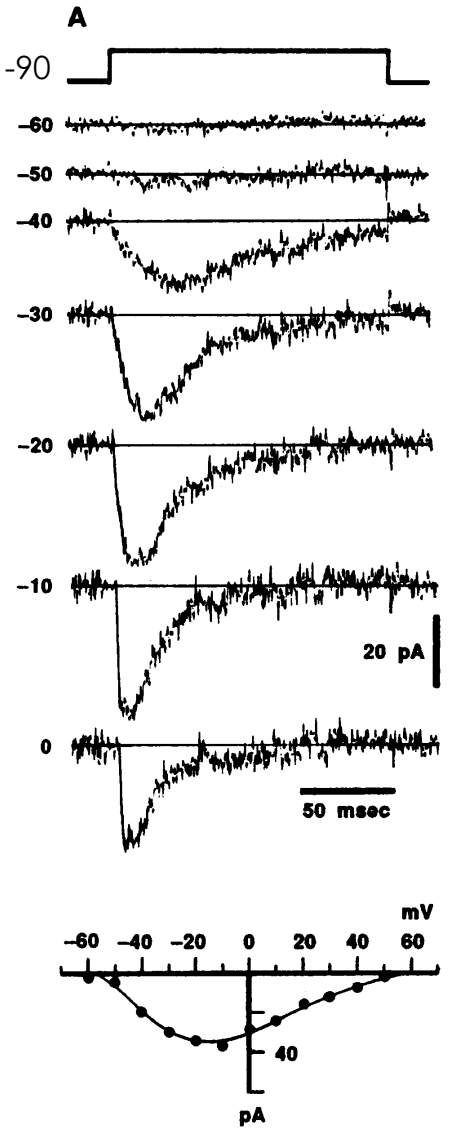
Astman N et al., J Neruosc 2006

T-Type Ca^{2+} channels

T-type Ca^{2+} current is an amiloride and Ni^{2+}-sensitive Ca^{2+} current which activates below spike threshold (low voltage-activated or LVA). The activation threshold is around the resting membrane potential (-50 to -60mV) 10-20mV negative to spike threshold.

T stands for Transient but also Tiny (small single channels conductance).

I/V curve of the whole cell T current from a freshly isolated dissociated hippocampal neuron recorded in 10mM external Ca^{2+}

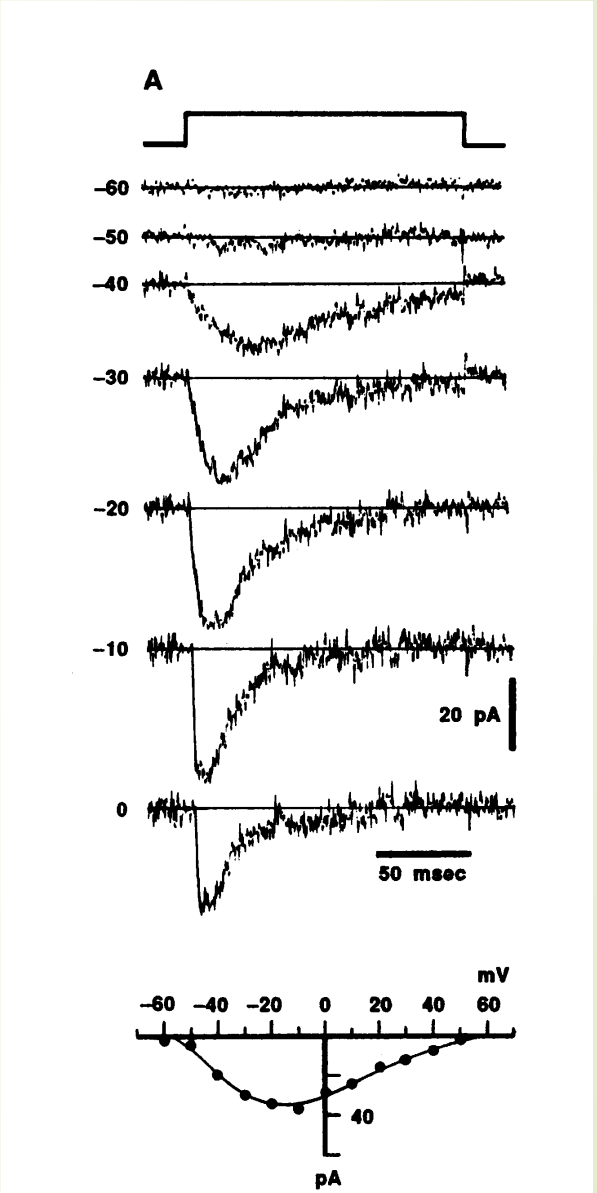


Yaari y et al, Science 1987

T-Type Ca^{2+} channels

It is totally inactivated at potential close to the resting membrane potential and is de-inactivated during a transient hyperpolarization.

It is therefore fully activated by a depolarization only when the membrane potential has been previously maintained at a more hyperpolarized potential



Yaari y et al, Science 1987

Voltage gated Ca^{2+} channels

Table 1. Subunit composition and function of Ca^{2+} channel types

Ca^{2+} current type	α1 Subunits	Specific blocker	Principal physiological functions	Inherited diseases
L	$Ca_v1.1$	DHPs	Excitation-contraction coupling in skeletal muscle, regulation of transcription	Hypokalemic periodic paralysis
	$Ca_v1.2$	DHPs	Excitation-contraction coupling in cardiac and smooth muscle, endocrine secretion, neuronal Ca^{2+} transients in cell bodies and dendrites, regulation of enzyme activity, regulation of transcription	Timothy syndrome: cardiac arrhythmia with developmental abnormalites and autism spectrum disorders
	$Ca_v1.3$	DHPs	Endocrine secretion, cardiac pacemaking, neuronal Ca^{2+} transients in cell bodies and dendrites, auditory transduction	
	$Ca_v1.4$	DHPs	Visual transduction	Stationary night blindness
N	$Ca_v2.1$	ω-CTx-GVIA	Neurotransmitter release, Dendritic Ca^{2+} transients	
P/Q	$Ca_v2.2$	ω-Agatoxin	Neurotransmitter release, Dendritic Ca^{2+} transients	Familial hemiplegic migraine, cerebellar ataxia
R	$Ca_v2.3$	SNX-482	Neurotransmitter release, Dendritic Ca^{2+} transients	
T	$Ca_v3.1$	None	Pacemaking and repetitive firing	
	$Ca_v3.2$		Pacemaking and repetitive firing	Absence seizures
	$Ca_v3.3$			

Abbreviations: DHP, dihydropyridine; ω-CTx-GVIA, ω-conotoxin GVIA from the cone snail *Conus geographus*; SNX-482, a synthetic version of a peptide toxin from the tarantula *Hysterocrates gigas*.

Catterall W, CSH persp, 2011

T-Type Ca^{2+} channels are present in dendrites of cortical neurons

In order to record Ca^{2+} current in isolation, the solution bathing the extracellular face of the patch contains Ba^{2+} (100-120mM) as charge carrier and TEA and TTX to block K^+ and Na^+ currents respectively.

T-type current is identified by inward polarity, unitary current characteristics, sensitivity to Ni^{2+} or amiloride and insensitivity to dihydropyridines (L-type blockers), ω-conotoxins or funnel web toxin (N-type and P-type blockers).

All the pharmacological tools are however NOT sufficient to finally identify T-type channels: those experiments do not exclude some partial contributions of a dendritic R-type current (I_{CaR}) which is also sensitive to Ni^{2+}.

However since I_{CaR} is a high threshold-activated current- in the following experiments it has been considered that the Ca^{2+} current activated in dendrites is I_{CaT}

T-Type Ca^{2+} channels are present in dendrites of cortical neurons

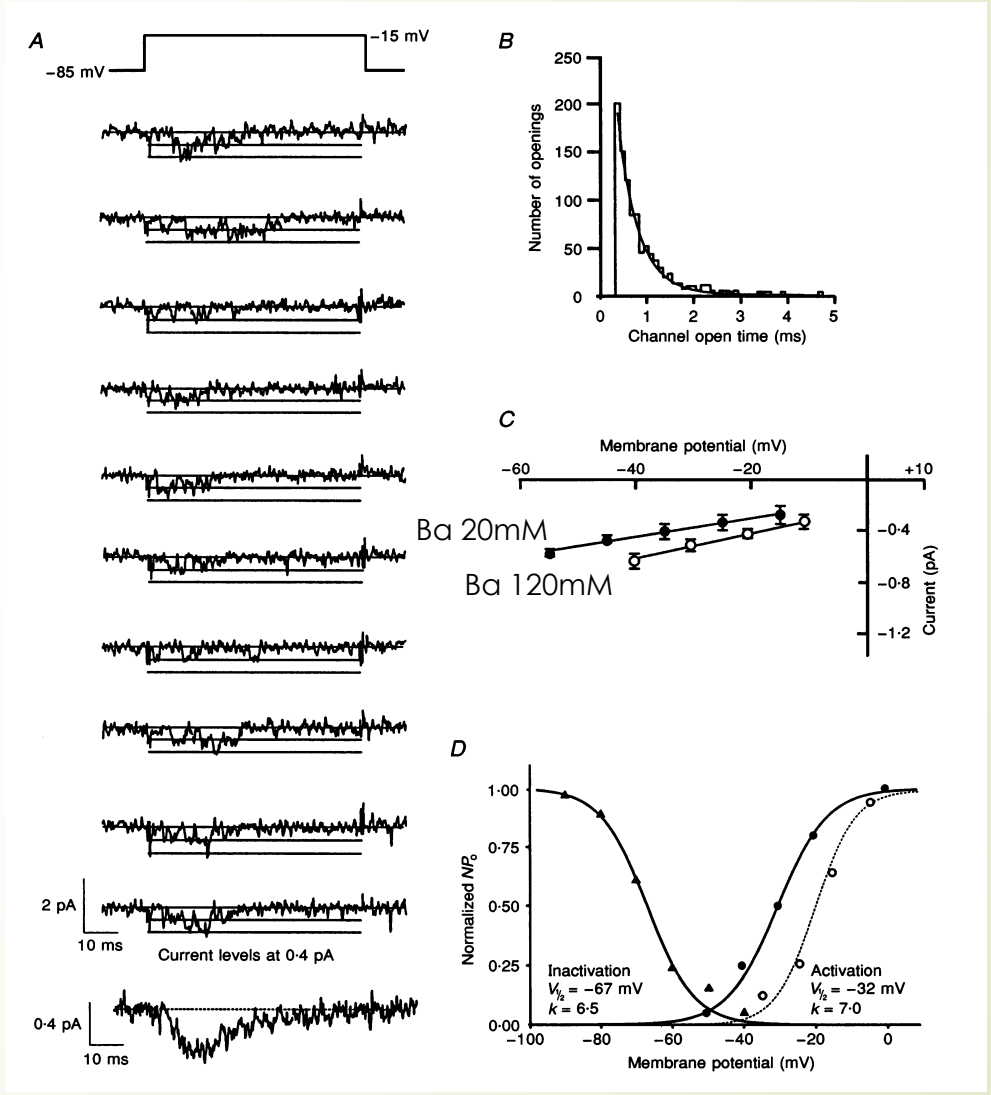
The activity of an apical dendritic membrane is recorded in the dendrite-attached configuration (voltage-clamp).

In response to depolarizing steps to -15mV from an hyperpolarized potential (-85mV to de-inactivate T channels), channel openings are recorded.

The current occurs at the beginning of the stimulus, are small in unitary conductance (7-11pS).

They are sensitive to Ni^{2+} and amiloride.

These data together with the activation-inactivation characteristics, reveal the presence of T-type Ca^{2+} channels in dendrites of pyramidal neurons in hippocampus



Magee JC and Johnston D, J Physiol 1995

T-Type Ca^{2+} channels are present in dendrites of cortical neurons

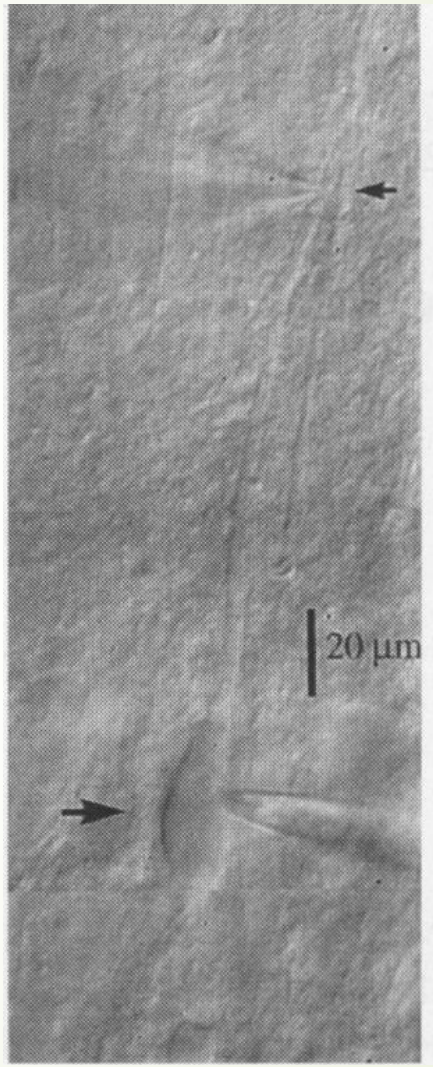
Activation of dendritic I_{CaT} by local synaptic inputs is tested in dendrite-attached and whole-cell somatic recordings from the same pyramidal neuron of CA1 region.

Sub-threshold EPSPs are evoked by threshold Schaffer collateral stimulation. These EPSPs must be sub-threshold (they must trigger Na^+ action potentials), in order to evoke only the low-threshold, Ni^{2+}-sensitive, I_{CaT}.

EPSPs are recorded from the soma (in current clamp mode) after propagation in the dendritic tree.

Single-channel T-type Ca^{2+} currents are recorded from the patch of dendritic membrane (in voltage clamp).

If channels openings only occur during EPSPs, they are considered to have been triggered by it.



Dendrite attached recordings of ICaT (voltage clamp)

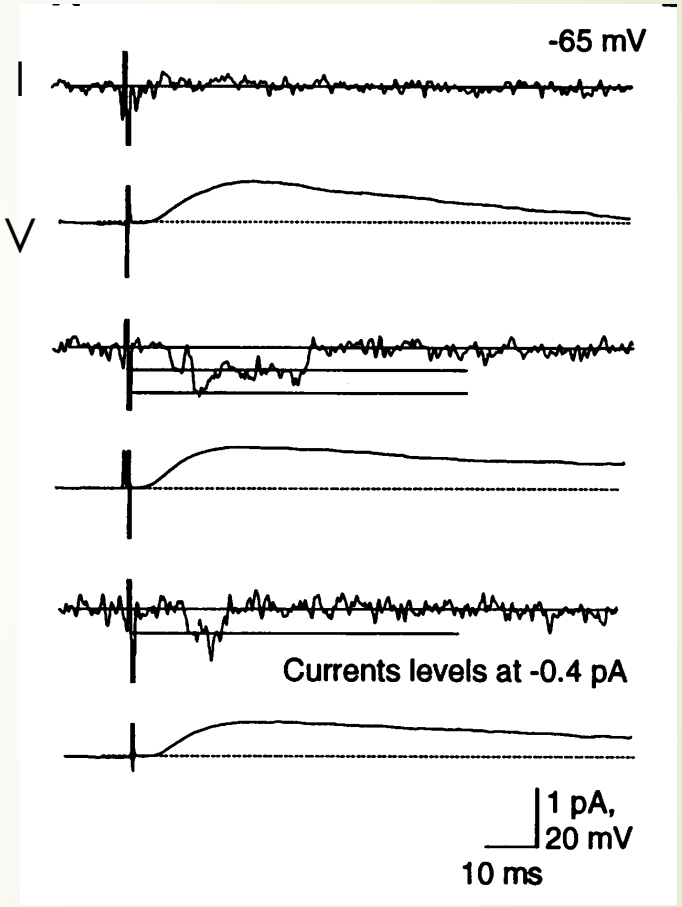
Whole cell recordings of EPSP (current camp)

Magee JC and Johnston D, Science, 1995

T-Type Ca^{2+} channels are present in dendrites of cortical neurons

In response to Schaffer stimulation, the activity of single Ca^{2+} channels is recorded. Single channels openings are most often observed near the peak or falling phases of the EPSP.

EPSP with a peak amplitude of 10m at the somatic recording site are necessary for activation of T-type Ca^{2+} channels



Magee JC and Johnston D, Science, 1995

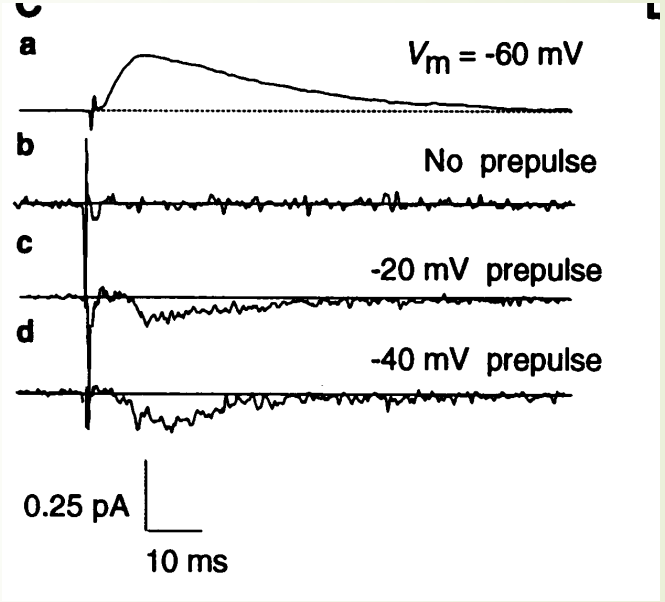
T-Type Ca^{2+} channels are present in dendrites of cortical neurons

When 4s hyperpolarizing prepulse is applied 400ms before synaptic stimulation, P_o of T-type Ca^{2+} channels is increased in a voltage dependent manner.

This suggest that a large proportion of T-type channels population is inactivated at resting potential.

Membrane hyperpolarization (by IPSP), is necessary for maximal channel activation after EPSP

The contribution of T-type Ca^{2+} channels would be particularly enhanced for EPSPs occurring after hyperpolarizing IPSPs



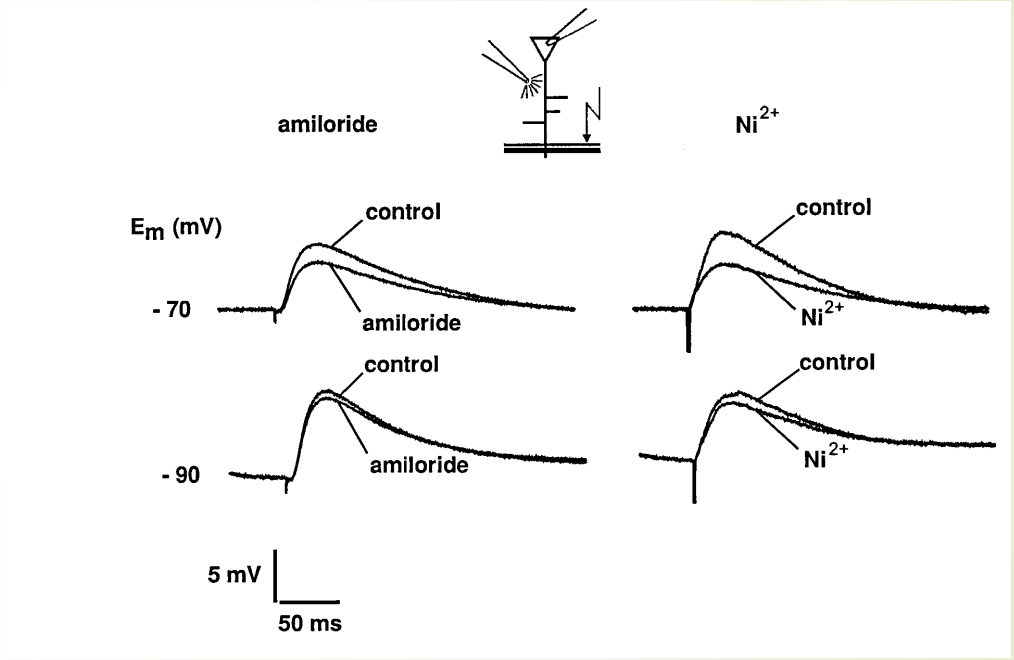
Magee JC and Johnston D, Science, 1995

T-Type Ca^{2+} channels are present in dendrites of cortical neurons

What are the consequences of this local intradendritic Ca^{2+} increase? Does dendritic I_{CaT} boost EPSP?

To address this question, EPSP are evoked far out on the apical dendrite and their shape is recorded at the soma with the dendritic I_{CaT} active or partially inhibited by Ni^{2+} or amiloride.

EPSP are evoked by afferent fiber stimulation (2Hz) and are recorded at the level of the soma (whole cell). To visulalize the approximate spread of Ni^{2+} (5uM) or amiloride (50uM), both drugs are dissolved in food color solutions.



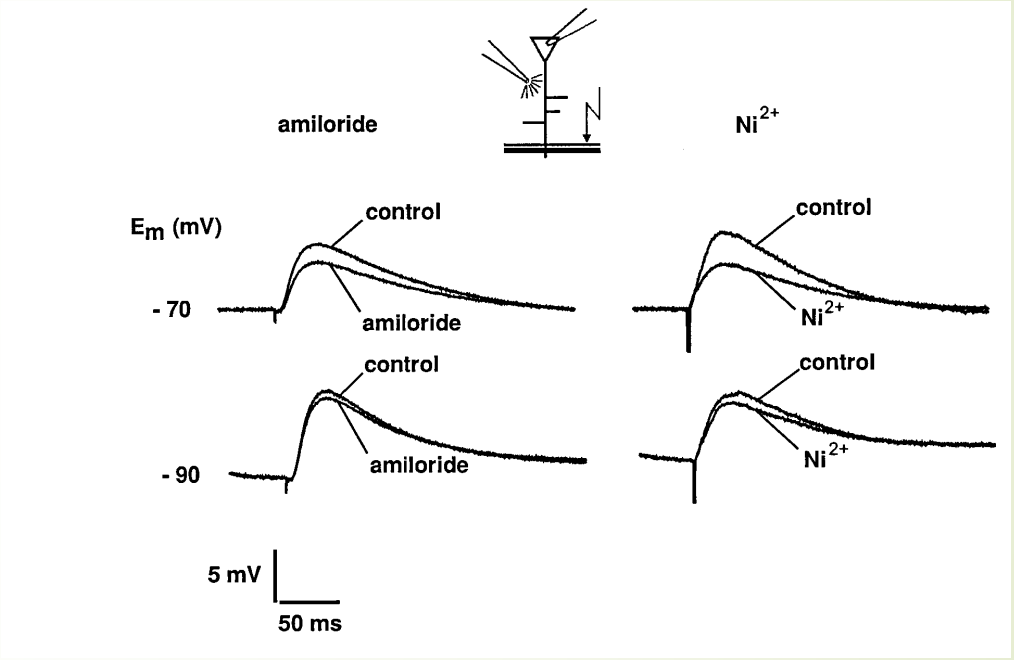
Gillessen T& Alzheimer C, J Neurophysiol 1997

T-Type Ca^{2+} channels are present in dendrites of cortical neurons

To study the role of I_{CaT} in dendrites and have minumum contamination form the soma, the Vm in the soma is set at -70mV and EPSP is adjusted to obtain EPSP peak amplitude of about 7mV in the soma. In these conditions, somatic EPSP should be too low to activate LVA channels.

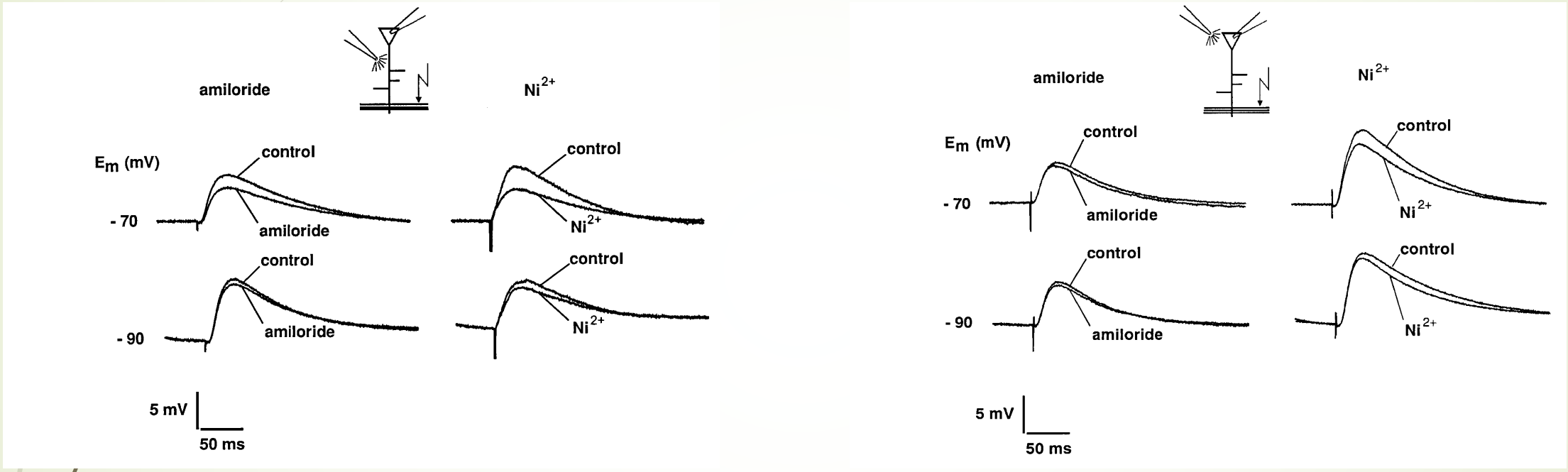
Dendritic amiloride and Ni^{2+} application reduces EPSP amplitude by 27% or 33% respectively. The effect of both reverse within 15-20min wash out.

Hyperpolarization of membrane to -90mV attenuates the effect of both antagonists



Gillessen T& Alzheimer C, J Neurophysiol 1997

T-Type Ca^{2+} channels are present in dendrites of cortical neurons



Gillessen T& Alzheimer C, J Neurophysiol 1997

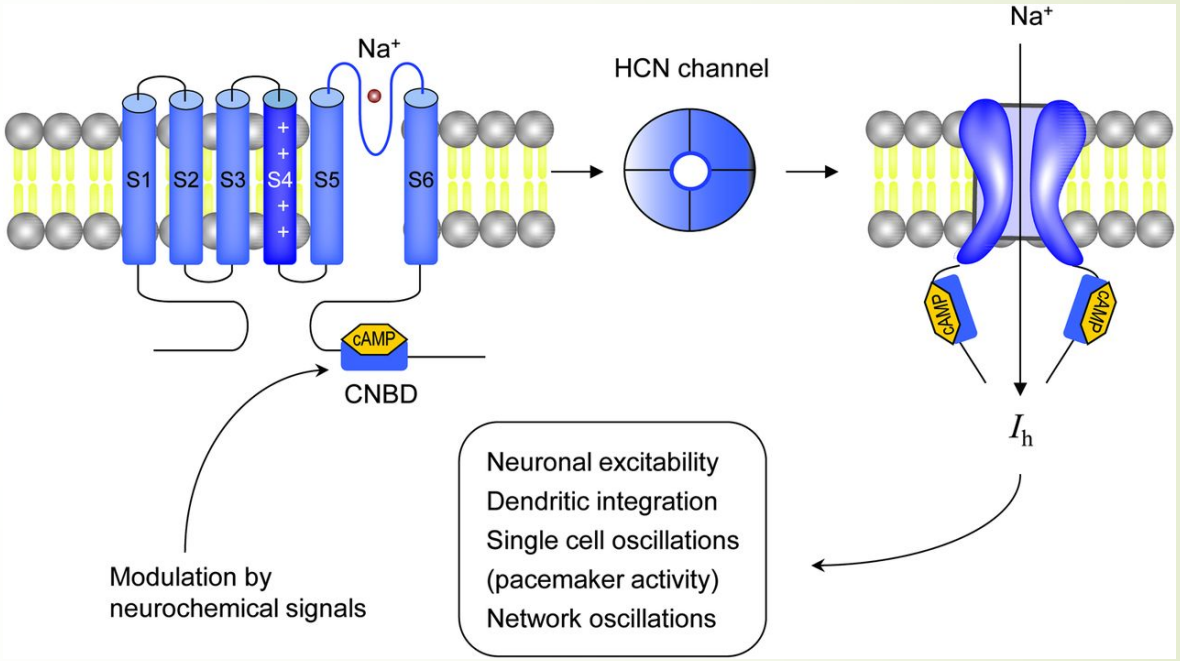
In CA1 pyramidal neurons, **EPSP activate T-type Ca2+ currents that can indeed alter the weight of EPSP. This amplification occurs at the dendritic regions.**

As for persistent Na^+ channels, assuming a resting potential of -65/-70mV, to activate I_{CaT}, EPSP must depolarize the membrane by 5-10mV in order to activate I_{CaT}. Only summed EPSP can reach this amplitude

Hyperpolarization cationic current I_h in dendrites

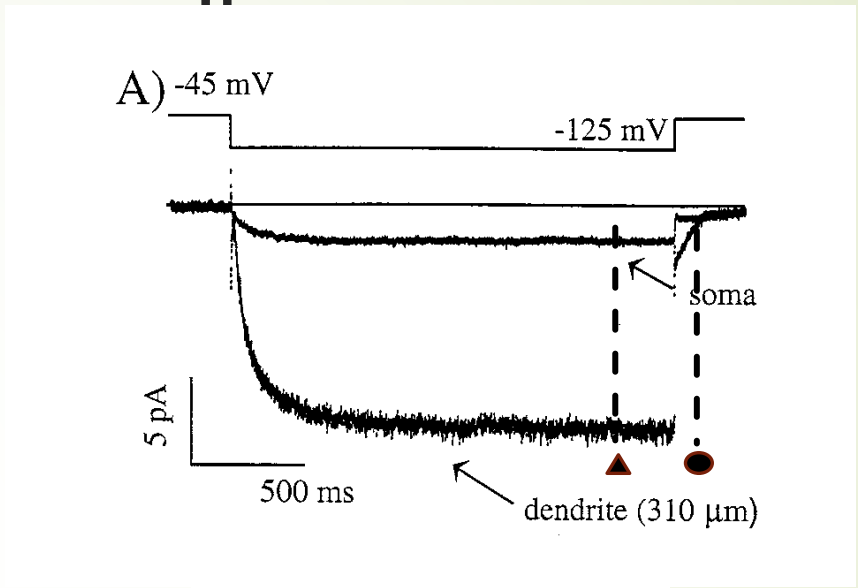
I_h is a cationic current (Na^+, K^+) activated by hyperpolarization beyond resting Vm.

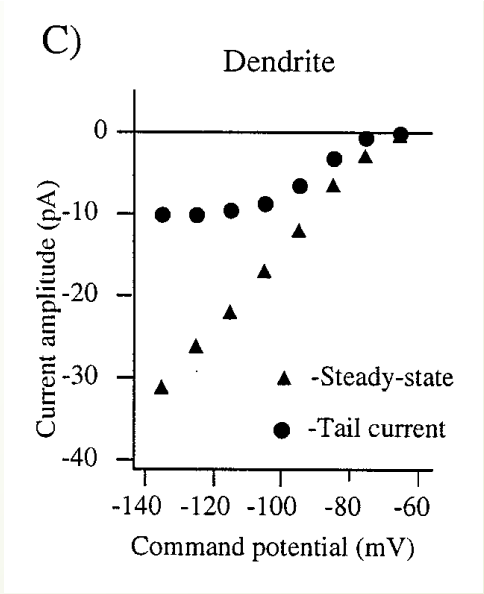
The I_h channel is a family of channels whose name is HCN: **h**yperpolarization, **c**yclic **n**ucleotide-modulated channels. There are 4 known HCN isoforms that combines to form a tetrameric channel. It belongs to the family of K^+ channels. They contain the conserved motive of K^+-voltage gated channels, including S1-S6 segments, a charged S4 voltage sensor and a pore-lining P-loop. In addition all family members contain a conserved cyclic nucleotide-binding (CNB) domain in the C-term. This is homologous to the CNB of the CNG channels



Hyperpolarization cationic current I_h in dendrites

Currents begin to activate at -60mV and steady state current amplitude increase in an approximately linear manner with membrane hyperpolarization up to -140mV.



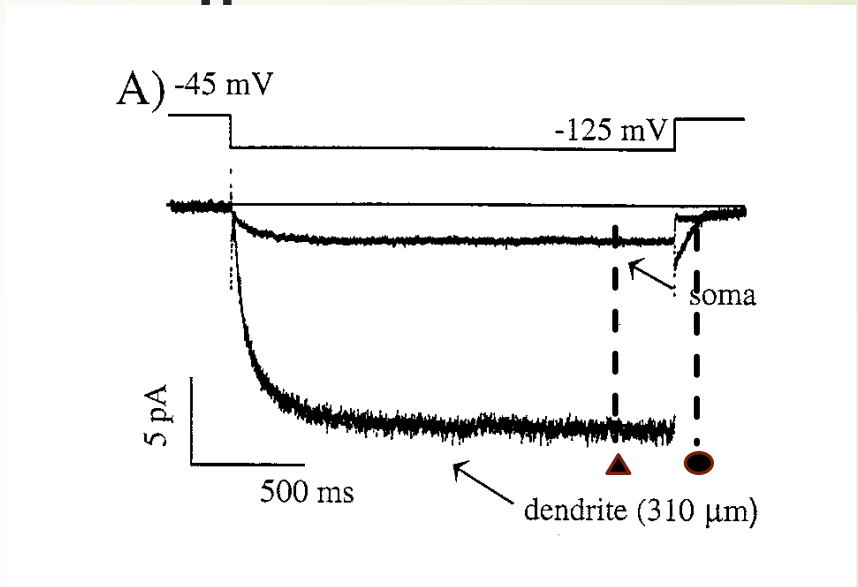


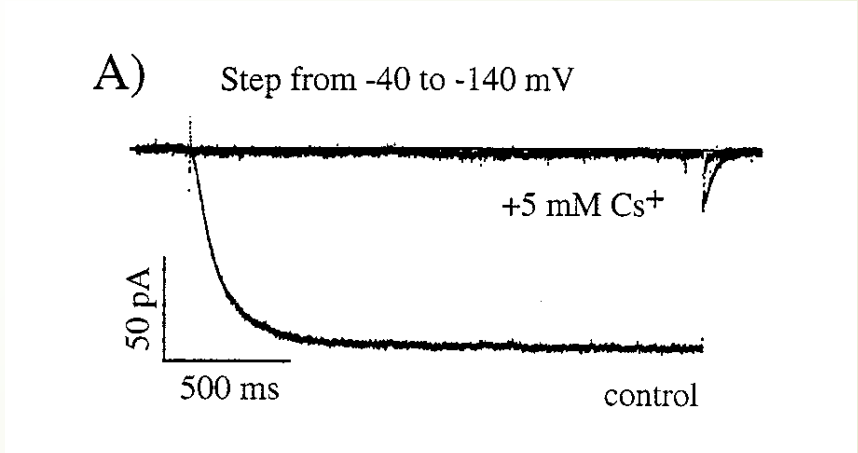
Magee J, J Neurosci 1998

Hyperpolarization cationic current I_h in dendrites

Currents begin to activate at -60mV and steady state current amplitude increase in an approximately linear manner with membrane hyperpolarization up to -140mV.

Inclusion of 5mM Cs^+ in the external recording solution totally blocks the current, thus showing that it is an I_h current.





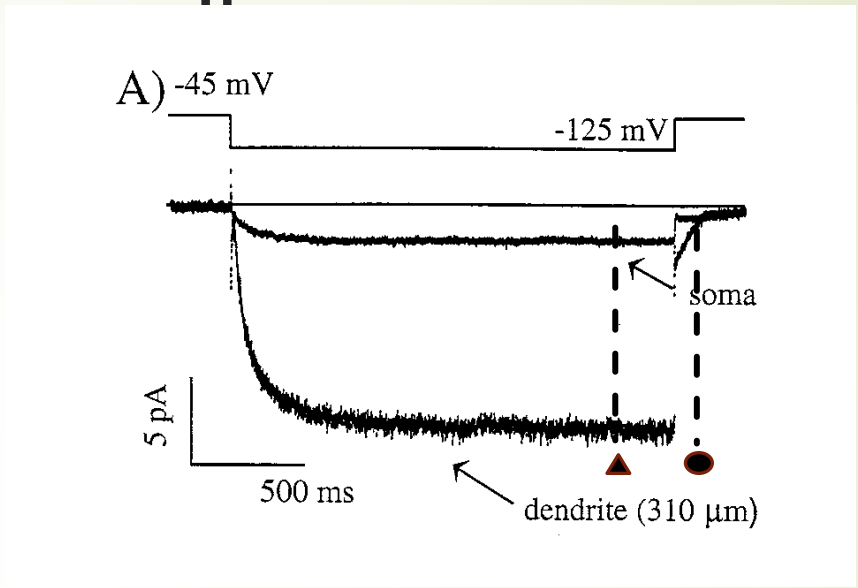
Magee J, J Neurosci 1998

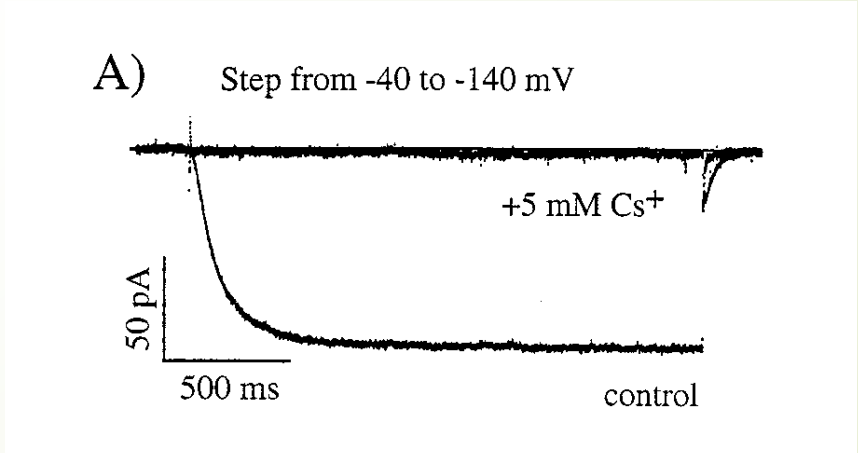
Hyperpolarization cationic current I_h in dendrites

Currents begin to activate at -60mV and steady state current amplitude increase in an approximately linear manner with membrane hyperpolarization up to -140mV.

Inclusion of 5mM Cs+ in the external recording solution totally blocks the current, thus showing that it is an I_h current.

The steady state current amplitude at -130mV progressively increases with distance away from the soma

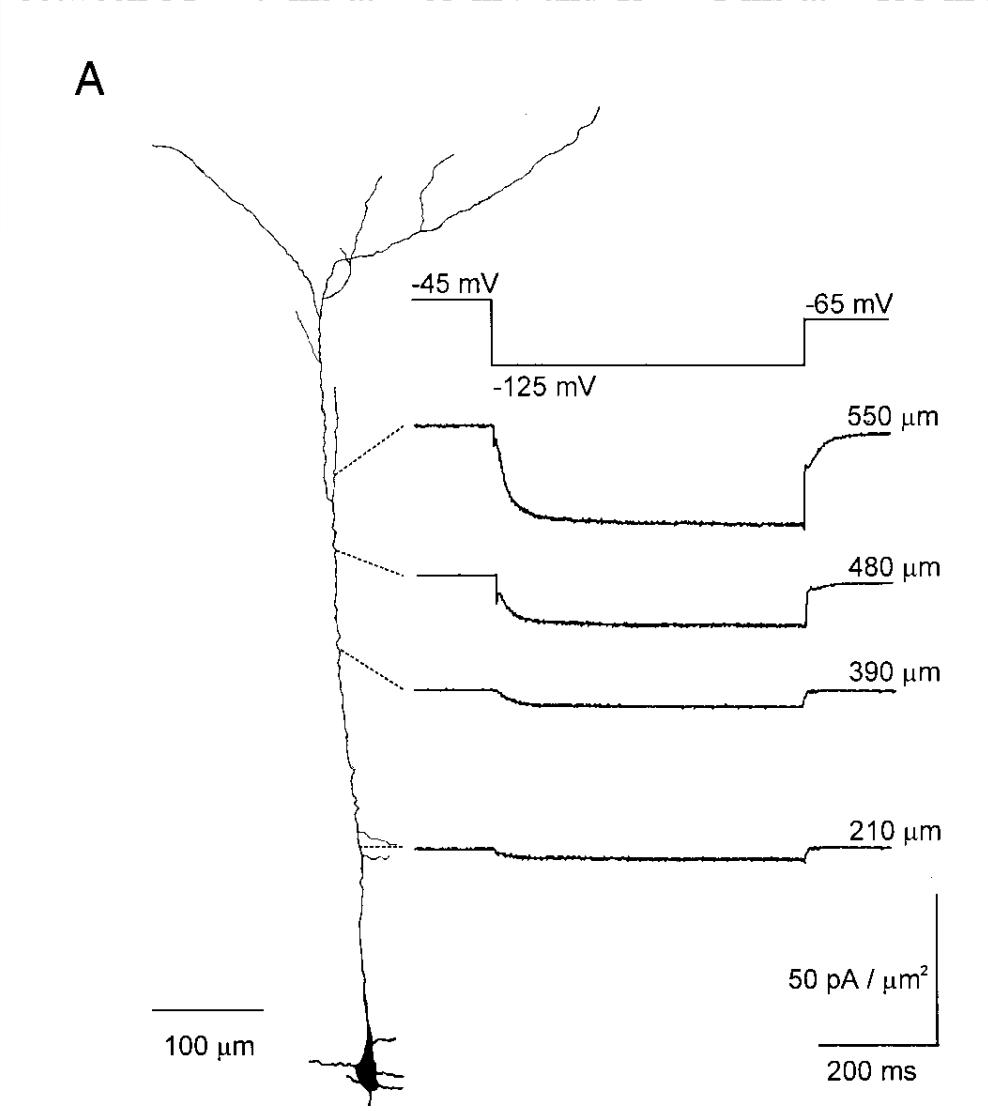


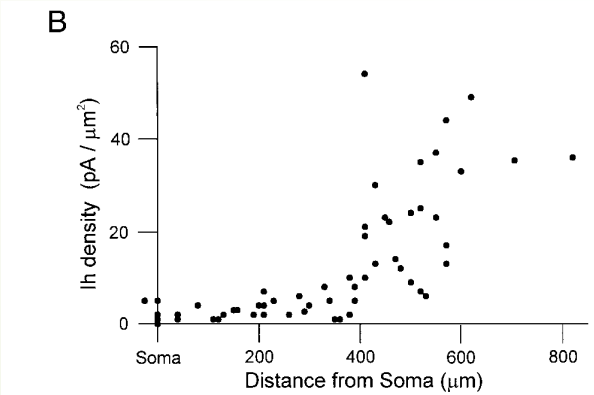


Magee J, J Neurosci 1998

Hyperpolarization cationic current I_h in dendrites

In pyramidal cell of the cortex, where recordings have been performed up to 800um from the soma, this density increases more than 10 times.



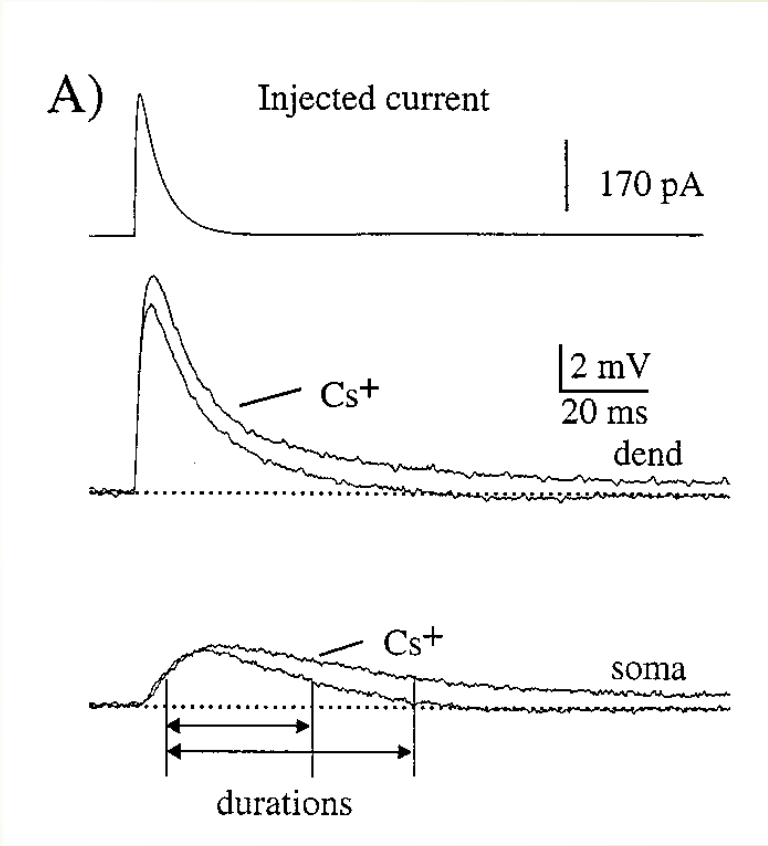


Berger T et al, J Neurophysiol 2001

Dendritic H-type channels are activated by IPSP and I_h decreases EPSP amplitude

The impact if I_h channels on the shape and propagation of sub-threshold voltage signals is determined by using simultaneous whole cell current clamp recordings from both soma and dendrites.

EPSP are simulated by dendritic current injection. Under **control conditions**, current injections in the dendritic compartment result in **EPSP-shaped voltage transients**, the amplitude and kinetics of which are **filtered significantly** as they propagate from the dendritic injection site to the recording somatic region.

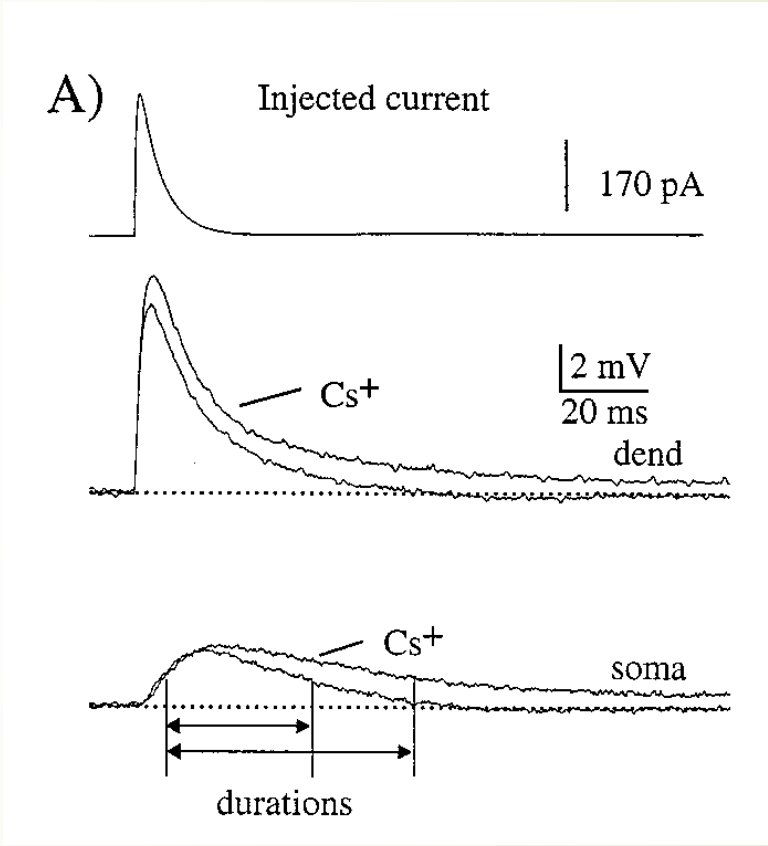


Magee J, J Neurosci 1998

Dendritic H-type channels are activated by IPSP and I_h decreases EPSP amplitude

When **amplitude** of the simulated EPSP is 8mV in the dendritic recording site, it attenuates to 3mV at somatic site.

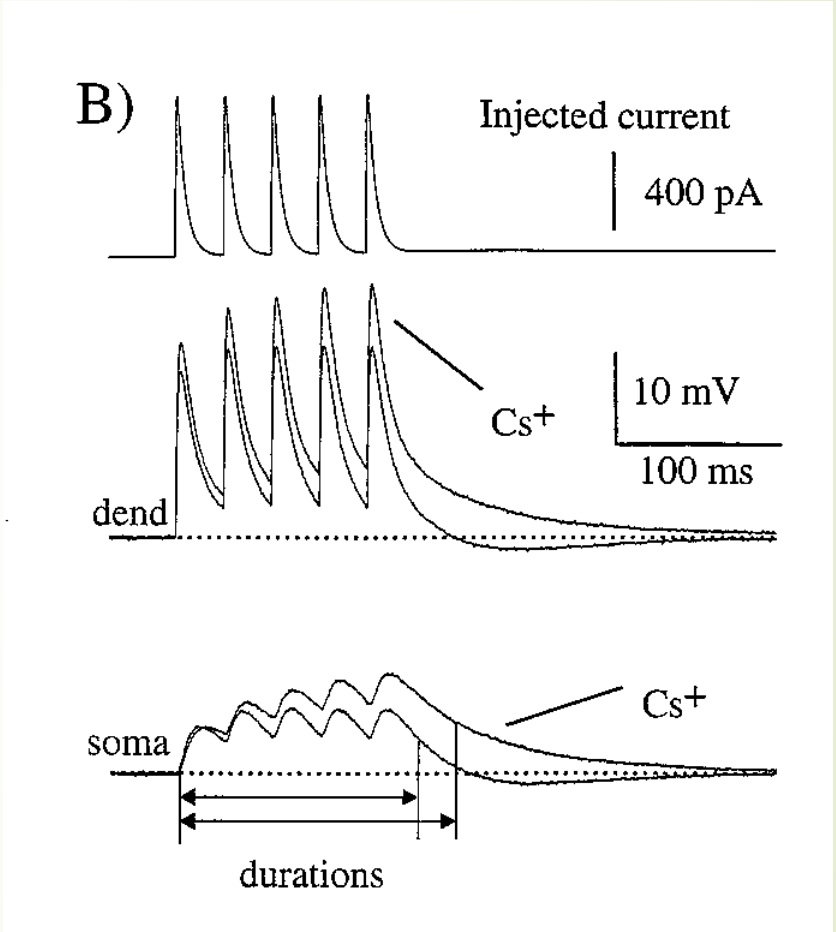
When EPSP **duration** is 15ms at dendritic site, it extends to 39ms at somatic site



Magee J, J Neurosci 1998

Dendritic H-type channels are activated by IPSP and I_h decreases EPSP amplitude

Repetitive dendritic current injections are also given to mimic repetitive synaptic inputs. These events are filtered similarly by dendritic arborization.

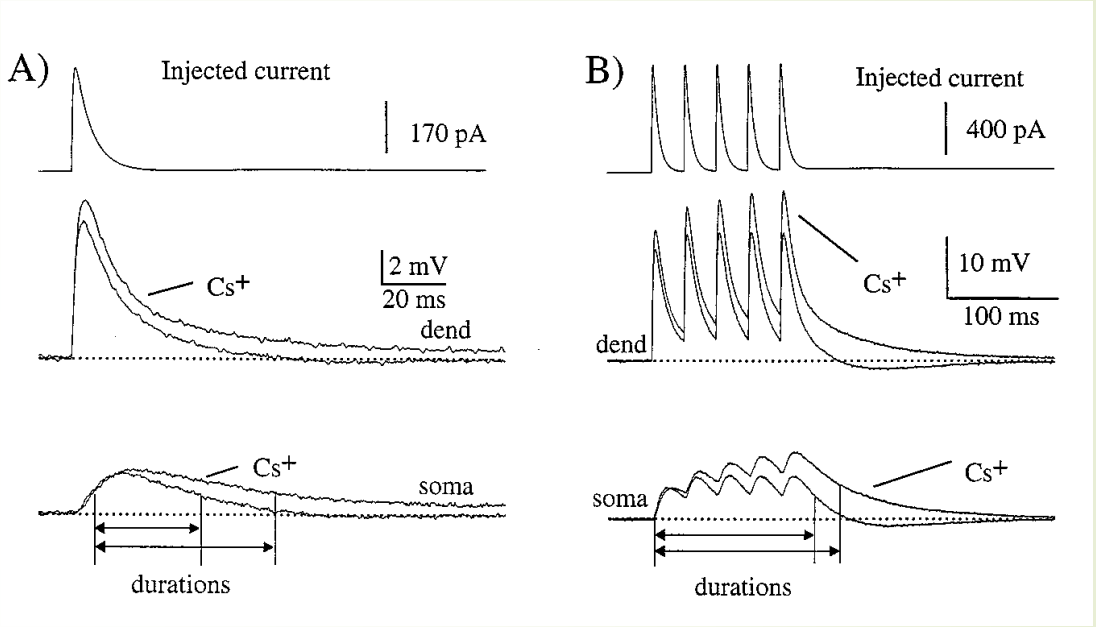


Magee J, J Neurosci 1998

Dendritic H-type channels are activated by IPSP and I_h decreases EPSP amplitude

H channels blockade with external Cs^+ **increases single** EPSP amplitude and duration.

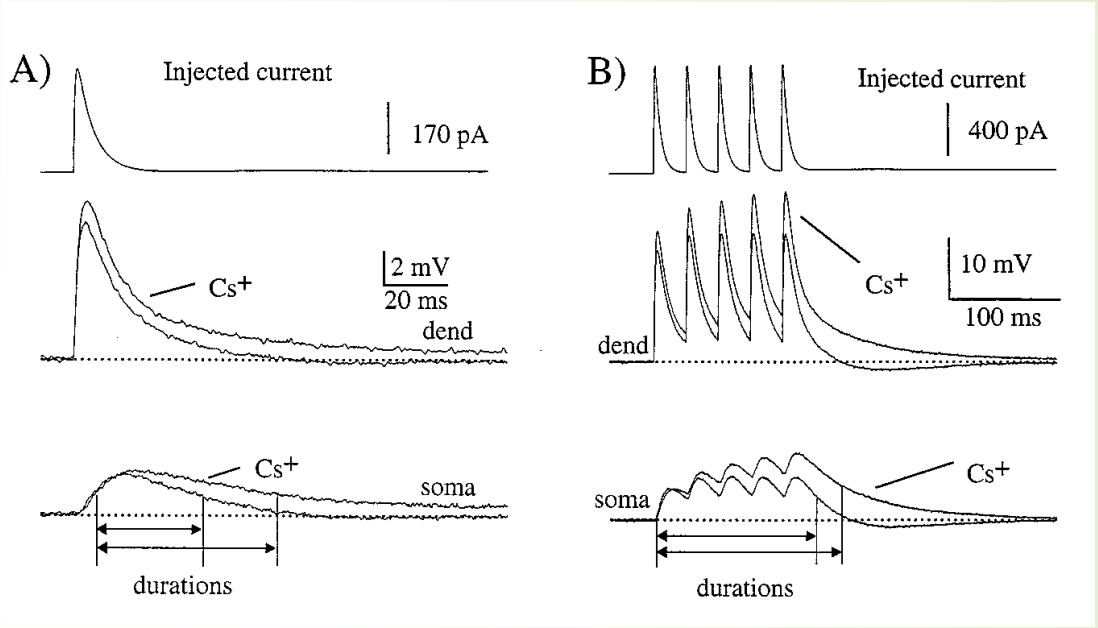
However, the attenuation of amplitude between dendrites and soma is the same in the presence or absence of I_h



Magee J, J Neurosci 1998

Dendritic H-type channels are activated by IPSP and I_h decreases EPSP amplitude

For **repetitive EPSP**, the presence of Cs^+ increases amplitude and duration in both dendritic sites and soma but the amount of increase is double in somatic areas as compared to dendritic areas.



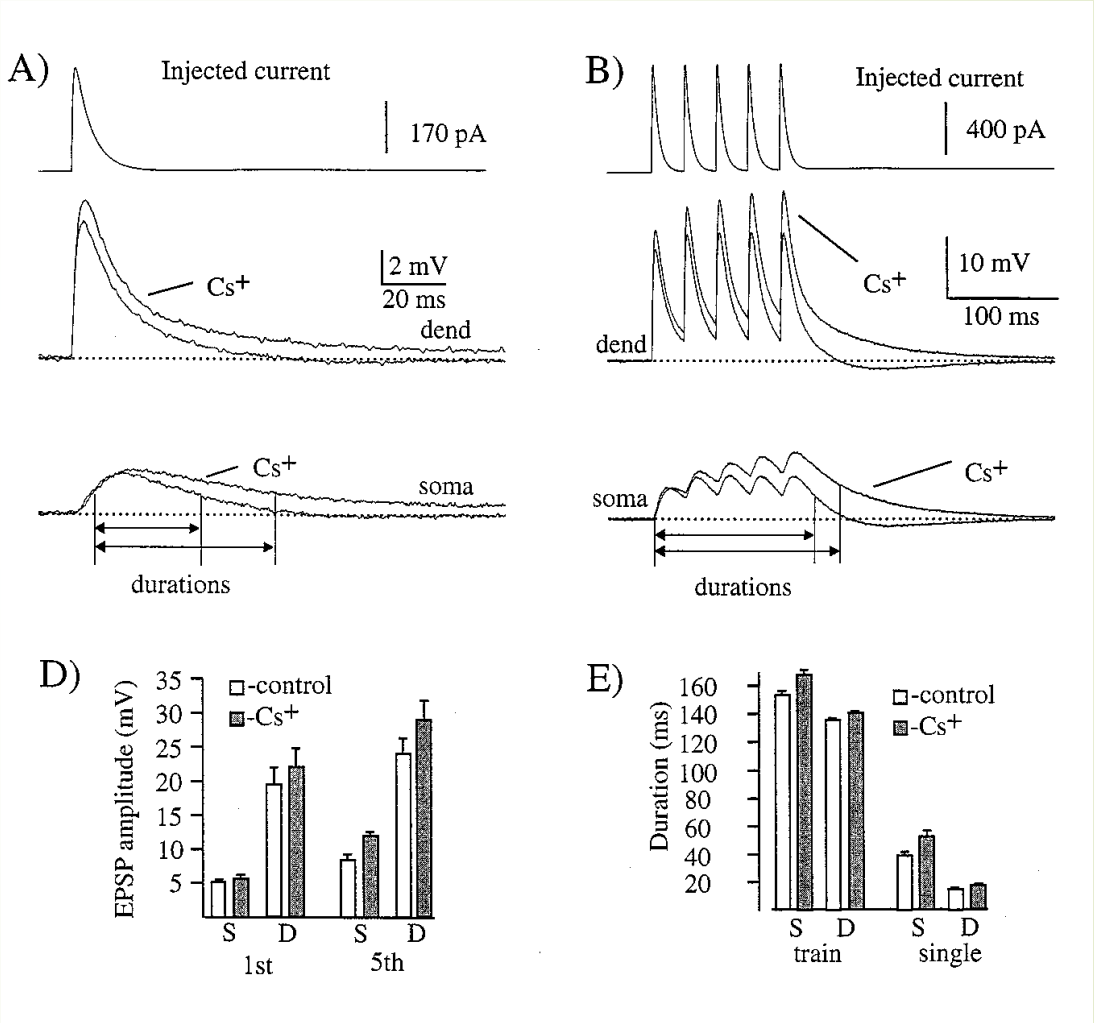
- For repetitive EPSP, I_h reduces the peak amplitude reached during the train by a factor 2

Magee J, J Neurosci 1998

Dendritic H-type channels are activated by IPSP and I_h decreases EPSP amplitude

How does I_h attenuate EPSP summation?

This is due to the deactivation of the I_h during the summation of the dendritic EPSPs because the depolarization produced by EPSP reaches the membrane potential when H channels close. The closure of the channels exerts a hyperpolarizing influence and counterbalance the depolarization produced by EPSP.



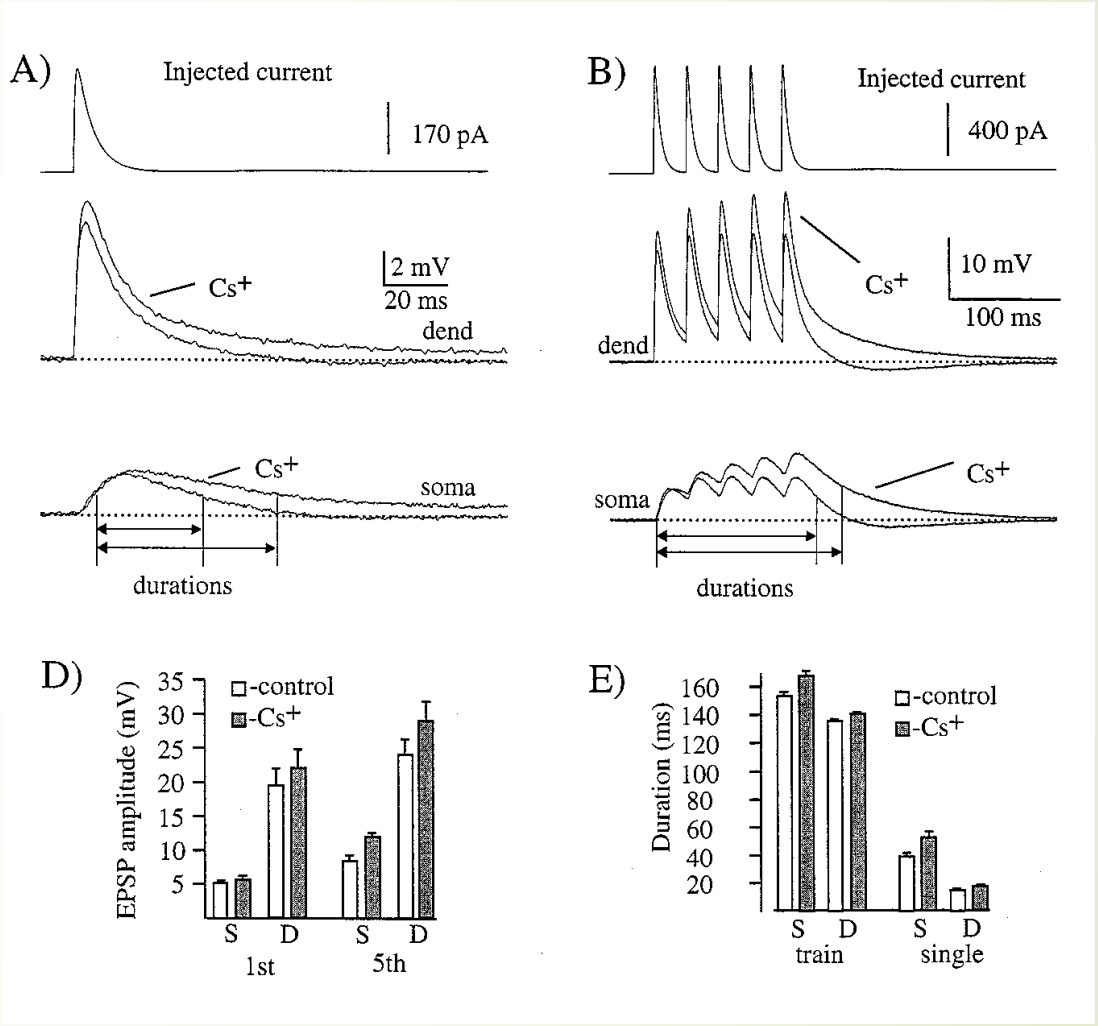
Magee J, J Neurosci 1998

Dendritic H-type channels are activated by IPSP and I_h decreases EPSP amplitude

This influence will be stronger at distal sites because of the large number of H channels at this level.

It also depend on the frequency of stimulation because of the slow kinetics of H channels deactivation. Thus the EPSP must have a an amplitude and duration sufficient to deactivate I_h.

At very high frequency of stimulation, dendritic I_h has less effect on temporal integration because the summation of EPSPs overcome the inhibitory effect of I_h deactivation.

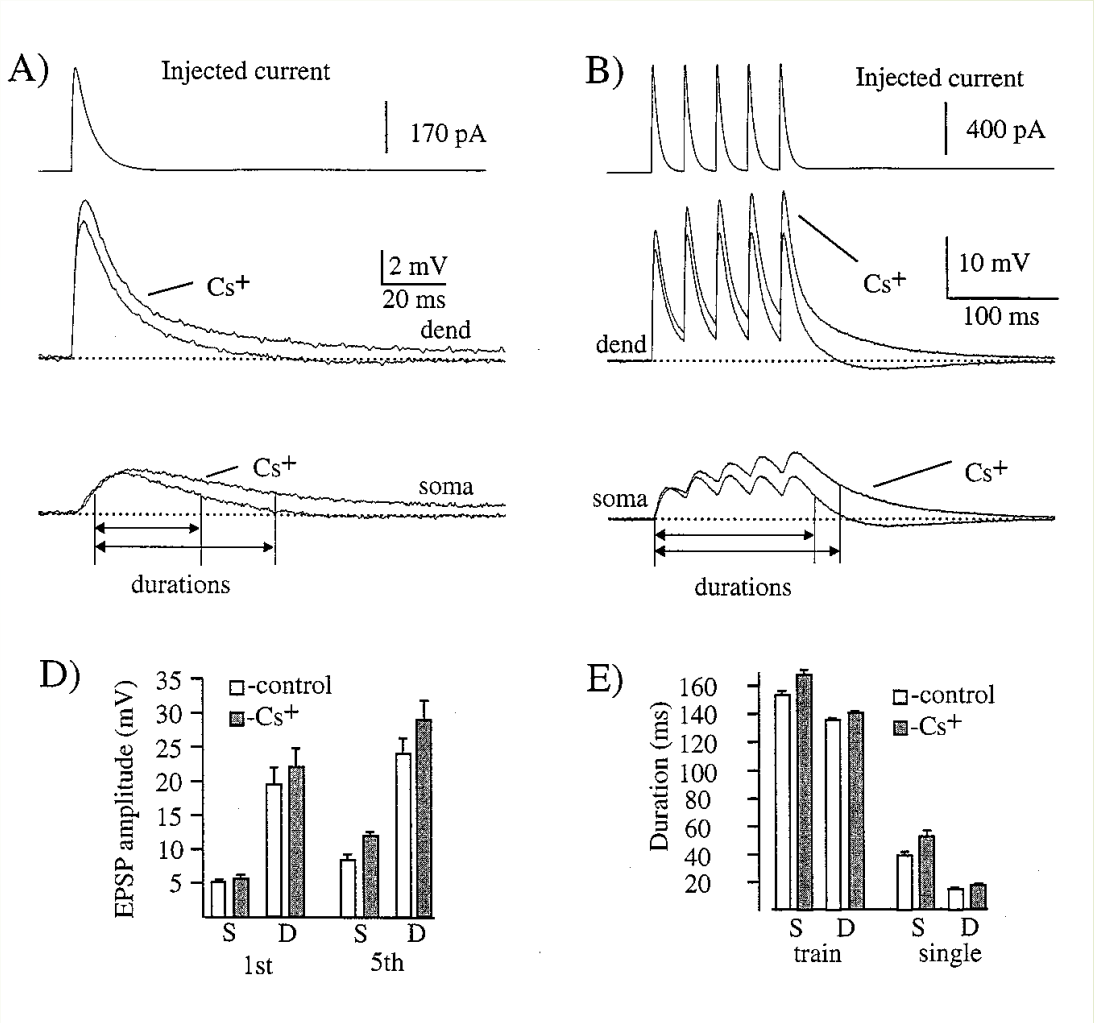


Magee J, J Neurosci 1998

Dendritic H-type channels are activated by IPSP and I_h decreases EPSP amplitude

Dendritic I_h decreases the amount of current transmitted from dendrites to soma in particular for summed EPSP.

Since the I_h density is 6- to 13-fold higher in distal dendrites, the absolute effectiveness of distal synaptic inputs is reduced by the increasingly large I_h conductance.

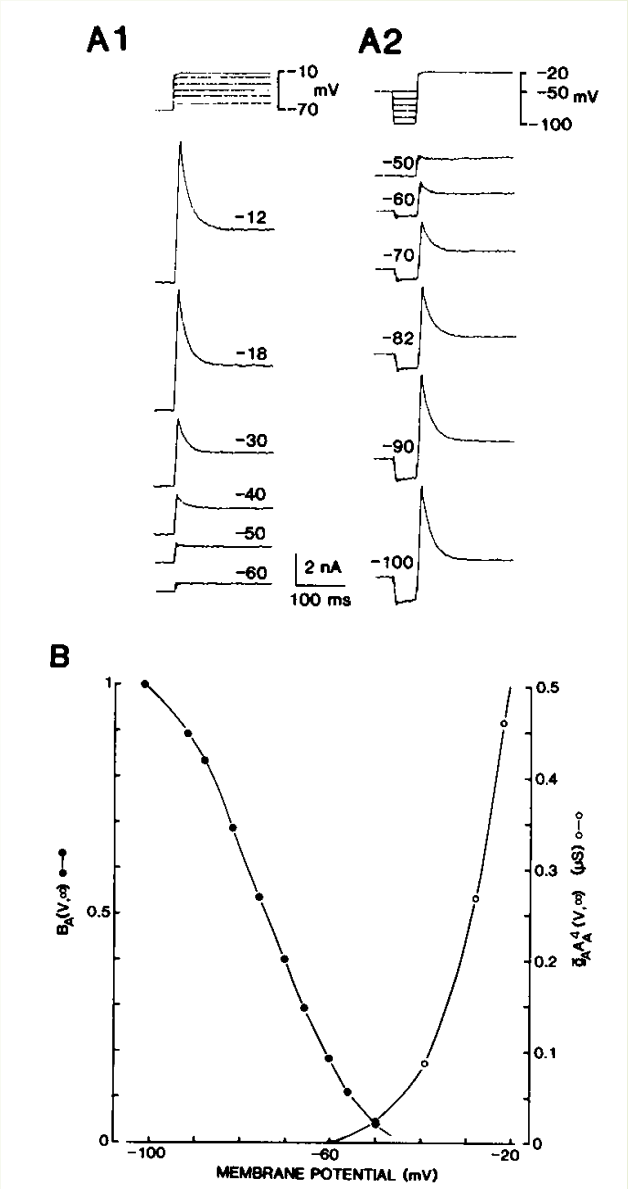


Magee J, J Neurosci 1998

A-type K^+ channels in dendrites

A-type K^+ currents are 4-aminopyridine (4AP)-sensitive **k^+ currents that activates below spikes threshold**. They can be recorded after blocking Na channels with TTX and Ca^{2+} channels with Cd^{2+} and Ni^{2+}.

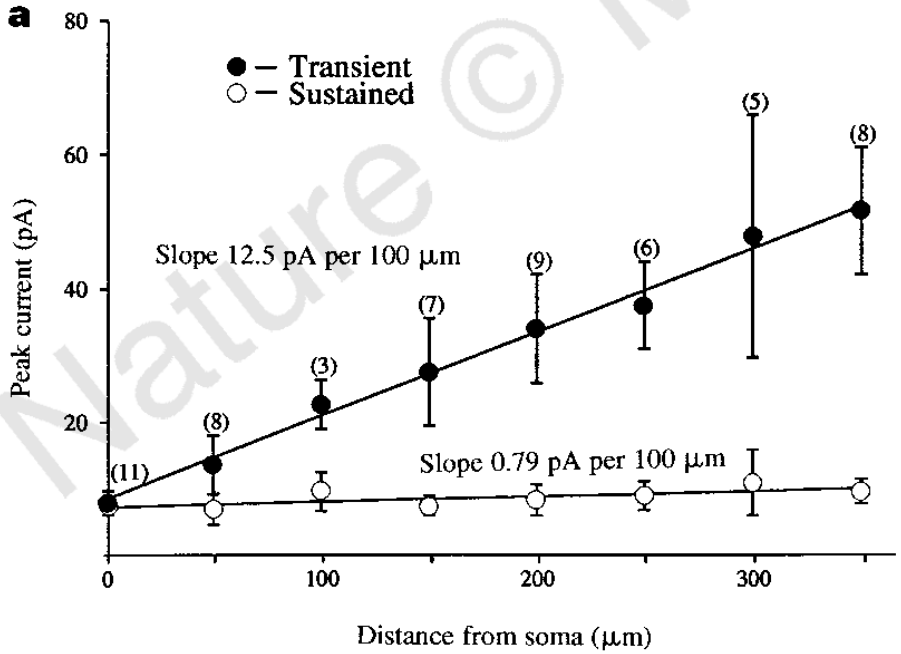
The A-type current is **fast activating K+** current that **inactivates rapidly** and therefore belongs to the family of transient currents

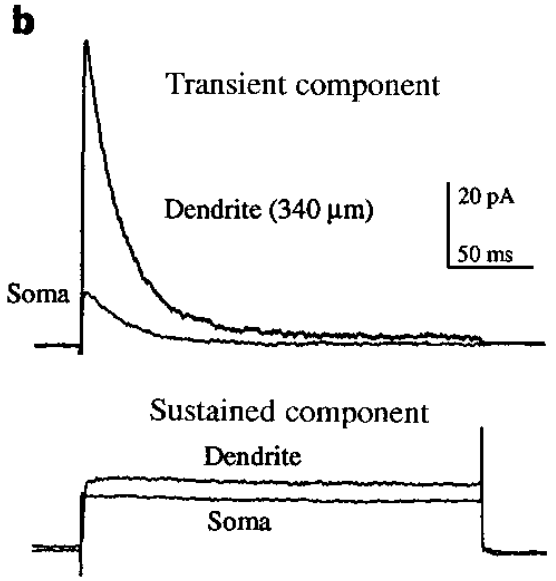


Segal M et al., J Neurosci 1984

A-type K^+ channels in dendrites

The biophysical properties and subcellular distribution of I_A in CA1 pyramidal neurons are investigated in cell-attached configuration, using voltage clamp mode. Depolarizing steps of voltage evoke outward currents. Recordings reveal a high density of outward current composed of two components.





The **first** is **transient that rapidly activates and rapidly inactivates.**

The **second** component is **sustained**.

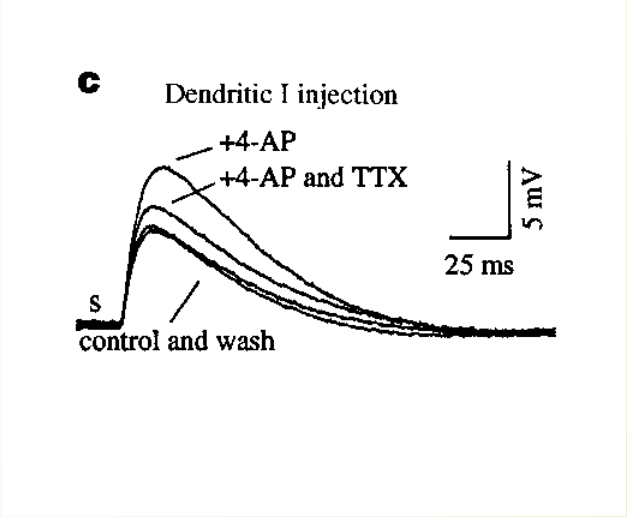
The density of the transient outward current increases linearly with distance while the sustained component remain constant

Hoffman Da et al.,Nature 1997

A-type K^+ channels in dendrites

The high density A-type K^+ current has a substantial effect on sub-threshold synaptic events propagating from the dendrite to the soma. Application of 4AP increases the amplitude and duration of EPSP-shaped voltage transients induced by dendritic current injection

Application of TTX after A-channel blockade reveal that a large fraction of 4AP-induced increase in EPSP is due to Na^+ channels activation.



Hoffman Da et al, Nature 1997

Dendritic A-type channels act to counteract EPSP amplification produced by persistent Na^+ currents

High voltage-gated currents of the somato-dendritic membranes

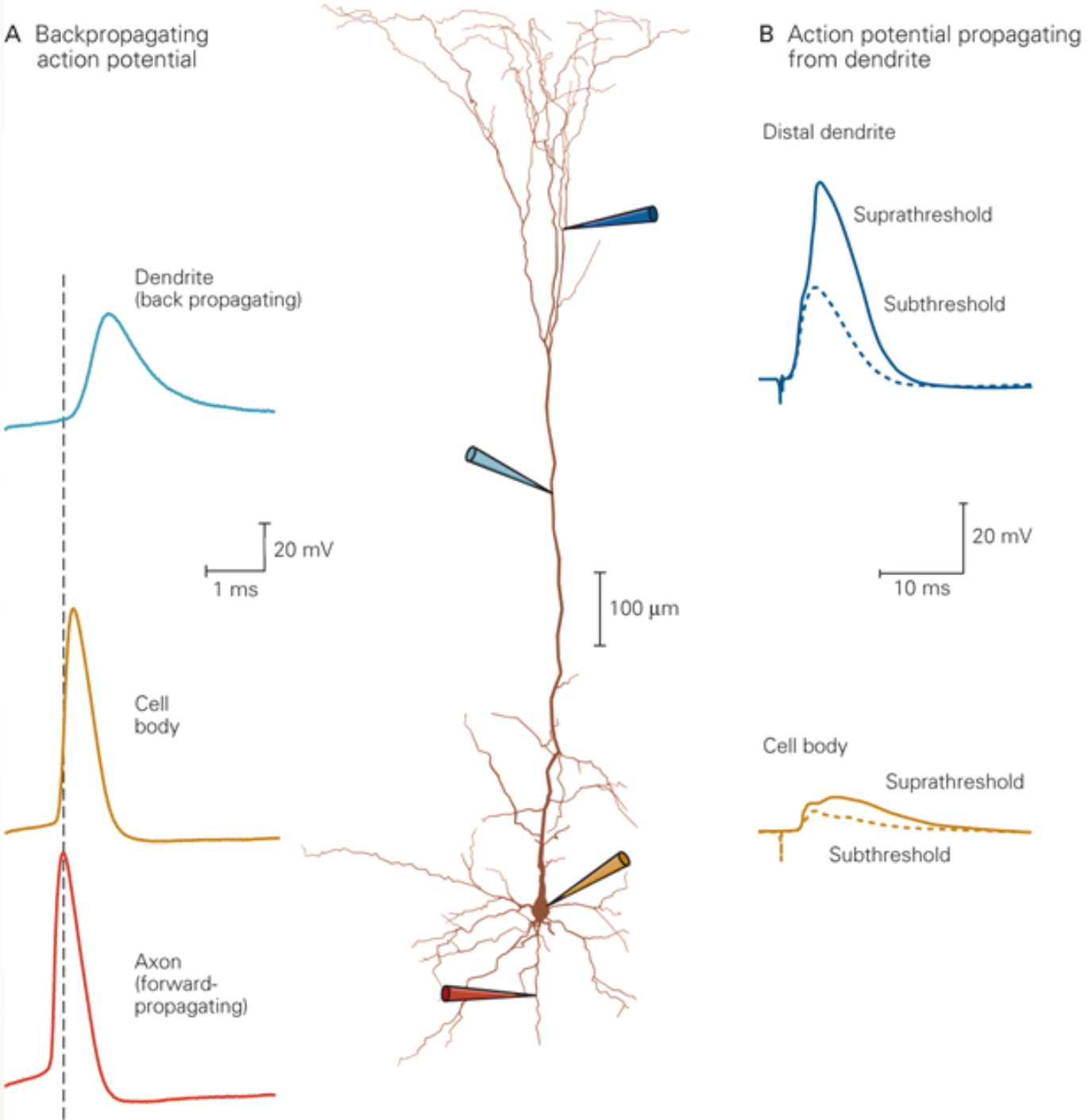
- Propagation of signals in dendrites was originally thought to be purely passive. However intracellular recordings from the cell body and dendrites **demonstrated that dendrites could produce action potential**: they have Na^+, K^+ and Ca^{2+} channels in addition to ligand-gated channels and leakage channels. The rich diversity of dendritic conductances suggest that central neurons rely on a sophisticated repertory of electrophysiological properties to integrate synaptic inputs.
- High density of HVA Na^+ channels are for example present in pyramidal neurons of the neocortex, hippocampus and substantia nigra neurons which represent an exception since most CNS dendrites contain low HVA Na^+ channels

High voltage-gated currents of the somato-dendritic membranes

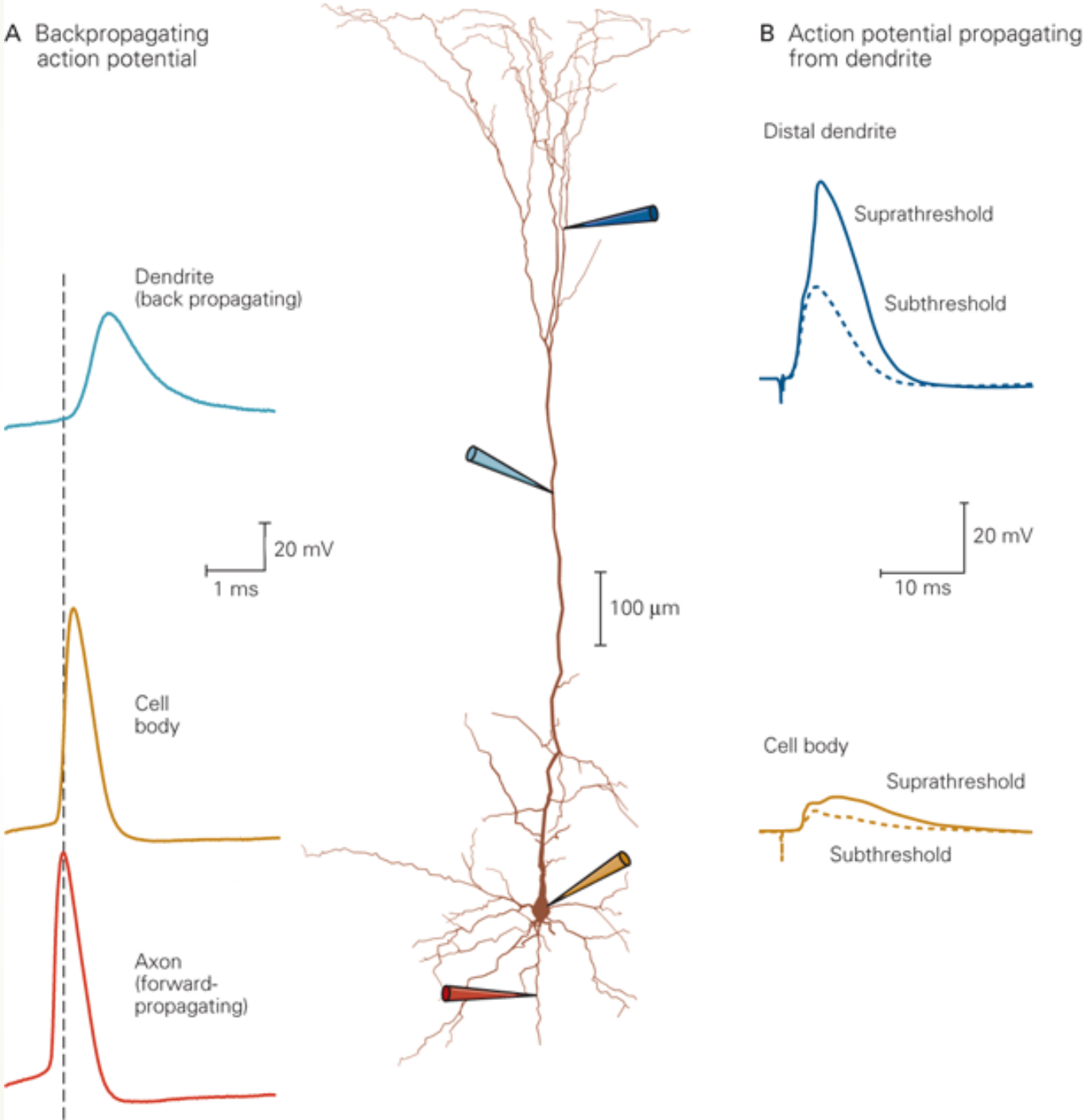
- One function of the voltage-gated Na^+ and Ca^{2+} channels in dendrites may be to **amplify the EPSP**. Some dendrites have enough concentration of voltage-gated channels to serve as local trigger zone. This can further **amplify the signal** input that arrives at remote parts of the dendrites. If the net input is above threshold a **dendritic action potential** may be generated.

- However the number of voltage-gated Na^+ or Ca^{2+} channels is usually not sufficient to support a all or non regenerative propagation of these action potential to the cell body.

Rather action potential generated in the dendrites are local events that propagate electrotonically to the cell body and axon initial segment, where they are integrated with all other input signals in the cell



Dendritic voltage-gated channels also permit action potential generated in the axon initial segment to propagate backward into the dendritic tree: **backpropagating** action potential is largely generated by **Nav**. This was first demonstrated in layer V of pyramidal neuron

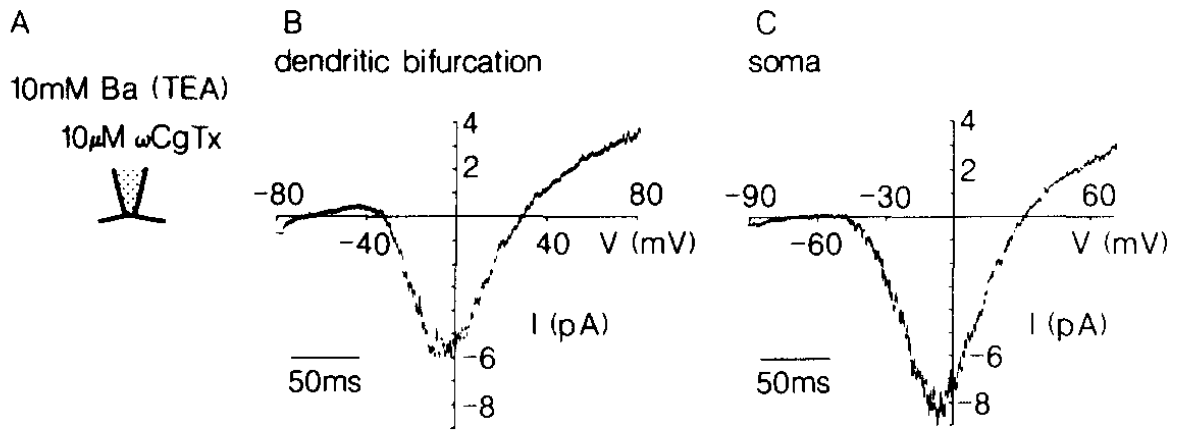


The role of **HVA Ca^{2+}** channels in dendrites can be recorded by adding Ba^{2+} in the extracellular solution as the charge carrier and TEACl and TTX for blocking K^+ and Na^+ currents.

To determine whether dendrites of Purkinje cells contain HVA Ca^{2+} channels, dendrite attached patch recordings are performed in slices.

IV curve carried by 10mM Ba^{2+} was evoked by a voltage ramp -80mV to +80mV. The current activates at -35mV and is maximal at 0mV.

The current is insensitive to GVIA ω-conotoxin (blocker of N-type channels) and to L-type channels blocker (BAY8644)

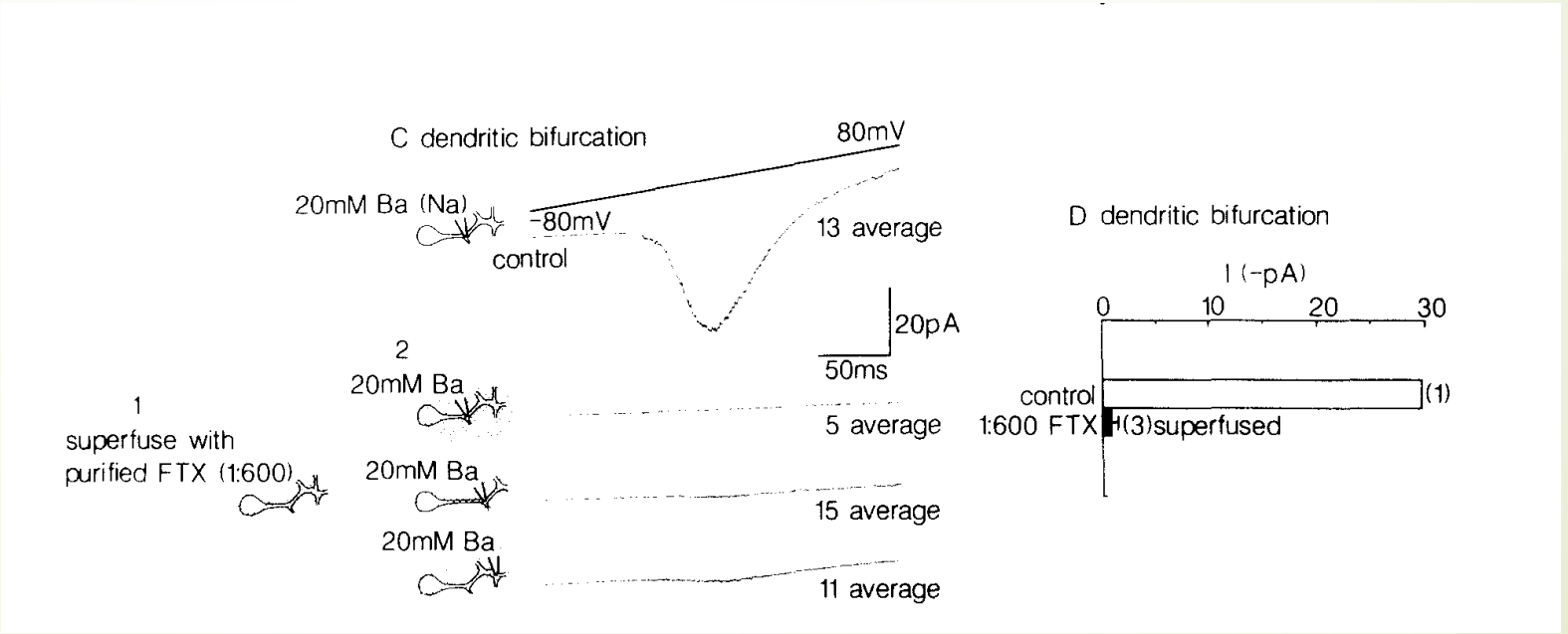


Usowicz mm et al, Neuron 1992

To test whether the currents are due to P/Q type Ca^{2+} currents, a specific blocker funnel web spider toxin (FTX) is tested.

FTX is the only drug that blocks dendrites Ca2+ currents

The dendritic Ba^{2+} curent is carried by P/Q type Ca^{2+} channels



Usowicz mm et al, Neuron 1992

The precise role of these backward propagating signals is unclear.

The only well demonstrated mechanism is the opening of dendritic Ca^{2+} channels and the consequent increase in $[Ca^{2+}]_i$.

In general intradendritic $[Ca^{2+}]_i$ increase will have a postsynaptic effect. Intracellular Ca^{2+} activates biochemical pathways and currents in the membrane

Moreover $[Ca^{2+}]_i$ increase could provide a temporally precise mechanism for regulating current through the NMDA receptor by providing the depolarization necessary to remove Mg2+ block

One particular example for intradendritic $[Ca^{2+}]_i$ increase is found in dopaminergic neurons of substantia nigra.

$[Ca^{2+}]_i$ increase triggers transmitter release from dendrites (a presynaptic effect).

In these cells, clusters of synaptic vesicles containing dopamine are present in dendrites that behave in certain sites as presynaptic elements. Dendritic release of DOPA is Ca^{2+} dependent and TTX sensitive.

Backpropagating action potential may therefore provide the stimulus to trigger DOPA release ad evoke synaptic trasmission for nigral dendrites to postsynaptic sites

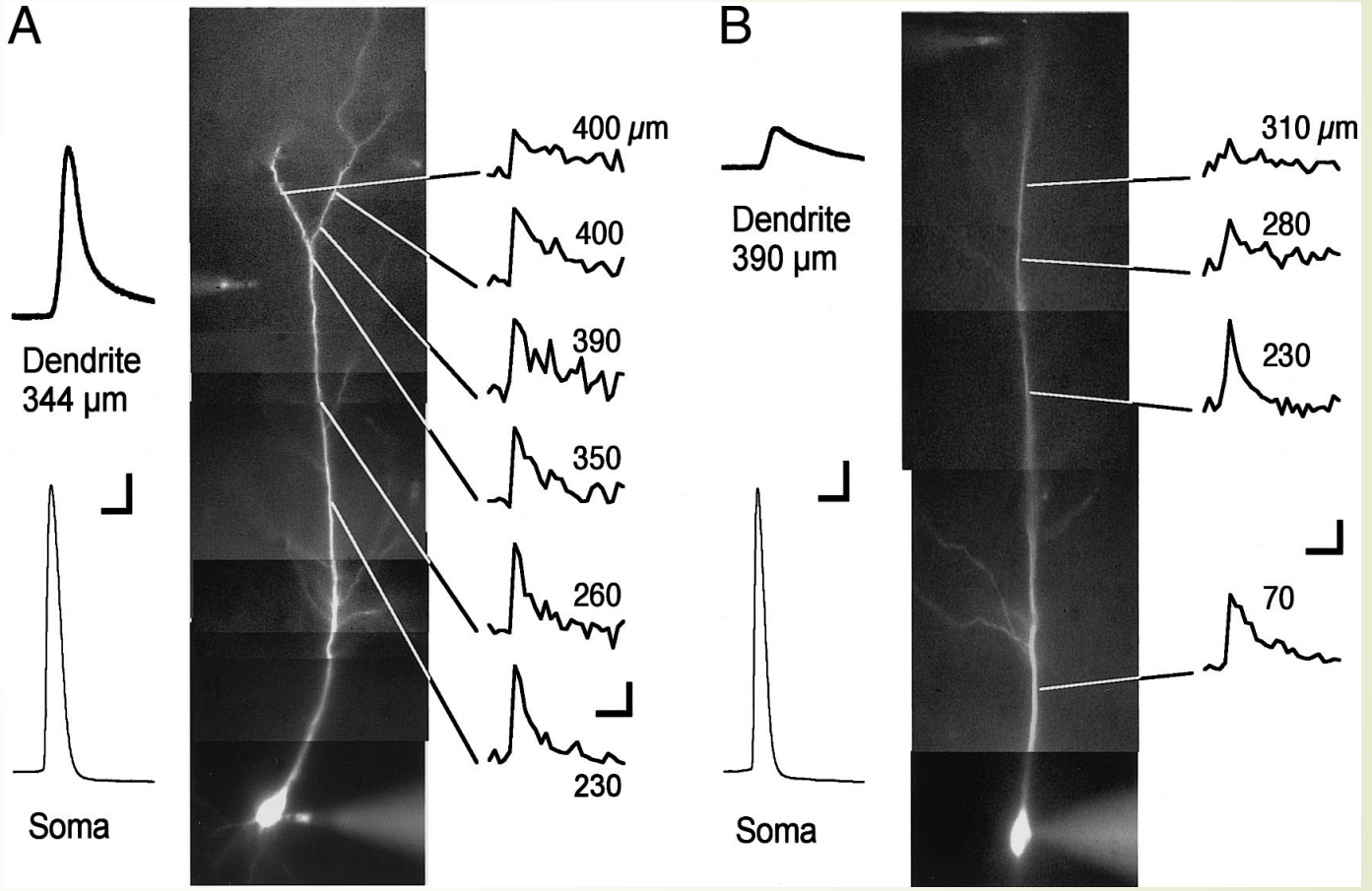
A backward signal that activates dendritic Ca^{2+} channels

In hippocampus, Na^{+} action potential open dendritic Ca^{2+} channels, leading to a widespread influx of Ca^{2+} in the dendrites.

In order to localize and quantify the increase of intradendritic $[Ca^{2+}]_i$ resulting from backpropagating Na^{+} action potential, pyramidal neuron of the hippocampus are loaded with Fura2 and a train of action potential is evoked by somatic depolarization through the whole cell recording electrode.

The changes in $[Ca^{2+}]_i$ are visualized in fluorescence.

The results show that $[Ca^{2+}]_i$ are largest in the proximal dendrites and smaller in more distal dendrites



Golding NL et al, J Neurophysiol 2001