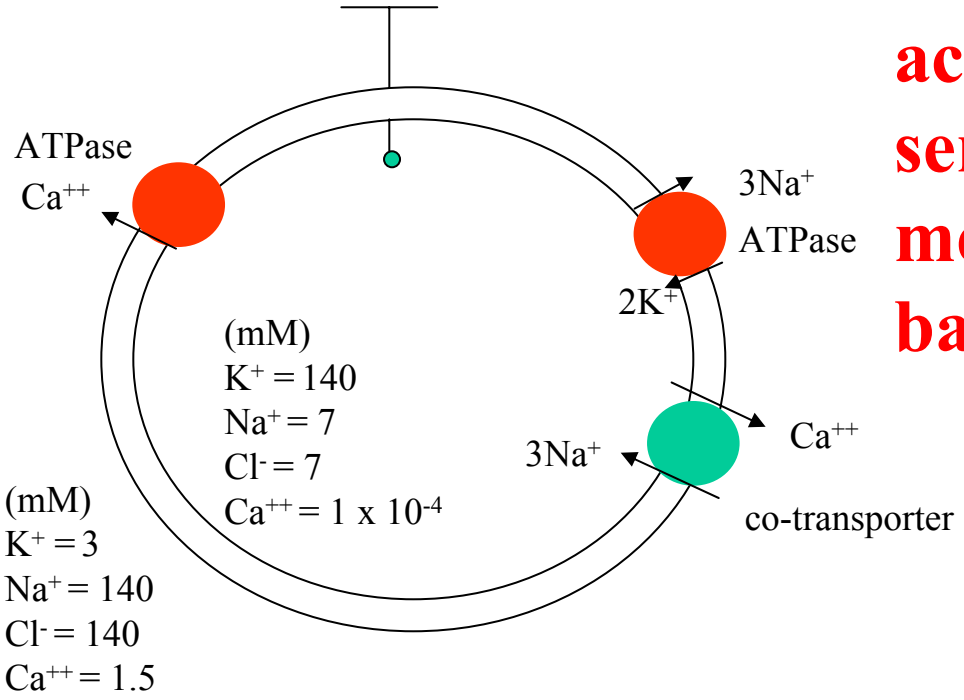
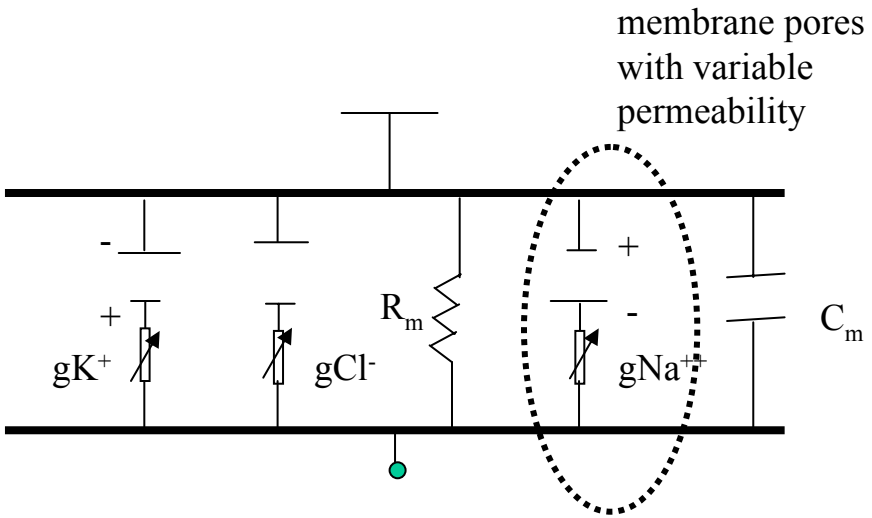
Lecture 1: An Introduction To Plasticity and Cellular Electrophysiology

MCP 2003

Charge separation across a semipermeable membrane is the basis of excitability.





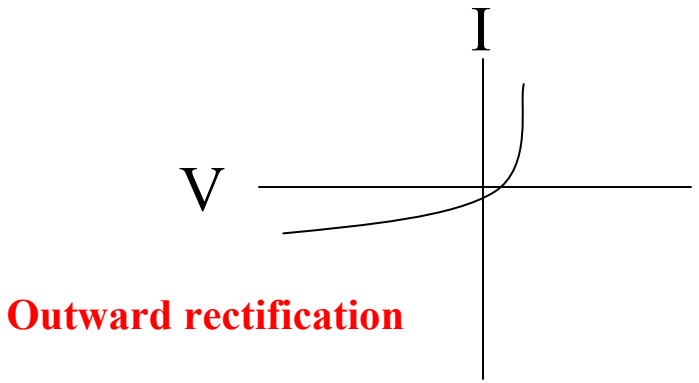
Convention: Current direction is defined by the direction of increasing positive charge.

Na++ flux into a cell is an inward current.
K+ flux out of a cell is an outward current.
Cl- flux into a cell is an outward current.

A depolarizing current is a net influx of + ions or a net efflux of negative ions.

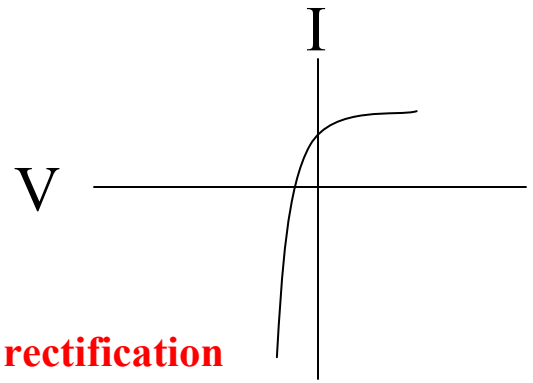
A hyperpolarizing current is a net efflux of + ions or a net influx of negative ions.

Outward rectification: when a membrane allows outward current (net + charge out) to flow more easily than and inward current.



Outward rectification

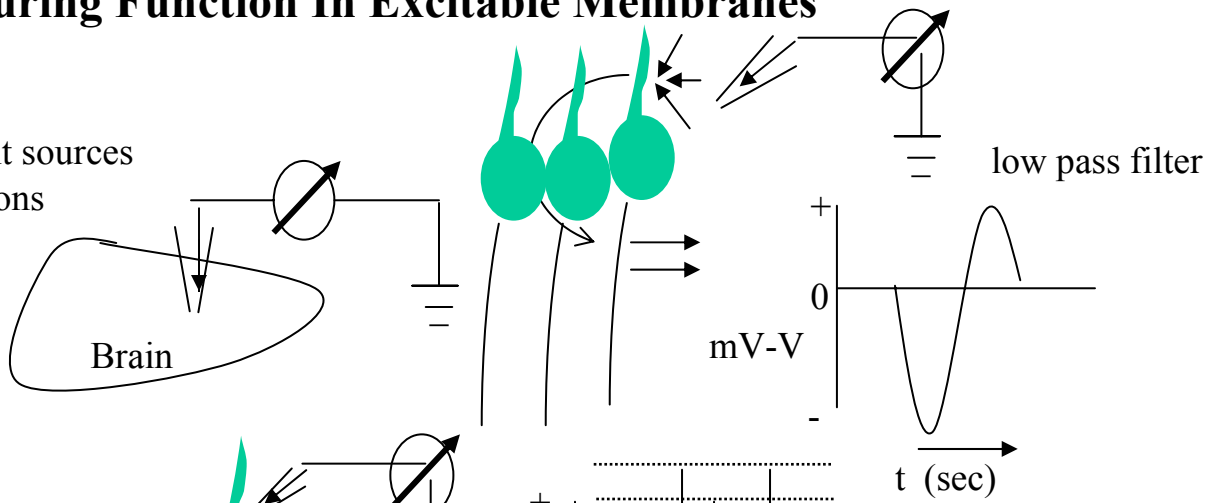
Inward rectification: when a membrane allows inward current to flow more easily than an outward current.



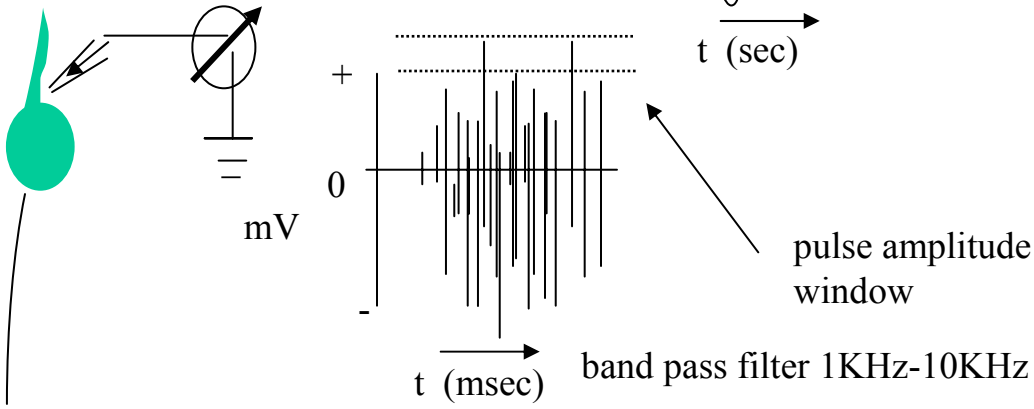
Inward rectification

Methods of Measuring Function In Excitable Membranes

Field potentials: measure current sources and sinks from populations of neurons across the electrode resistance.

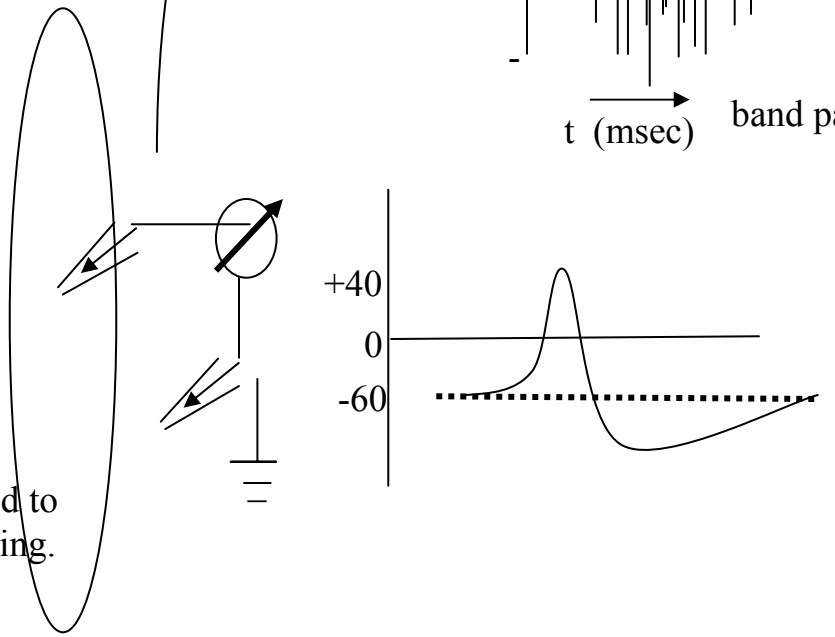


Microelectrode extracellular recording: measures action potentials from a small number of neurons.



Intracellular recording: can measure voltage,

Voltage clamp recording: Can pass current to compensate for voltage change. In this way voltage is held ~ constant and current applied to compensate is a measure of the current flowing. This will give a measure of current.



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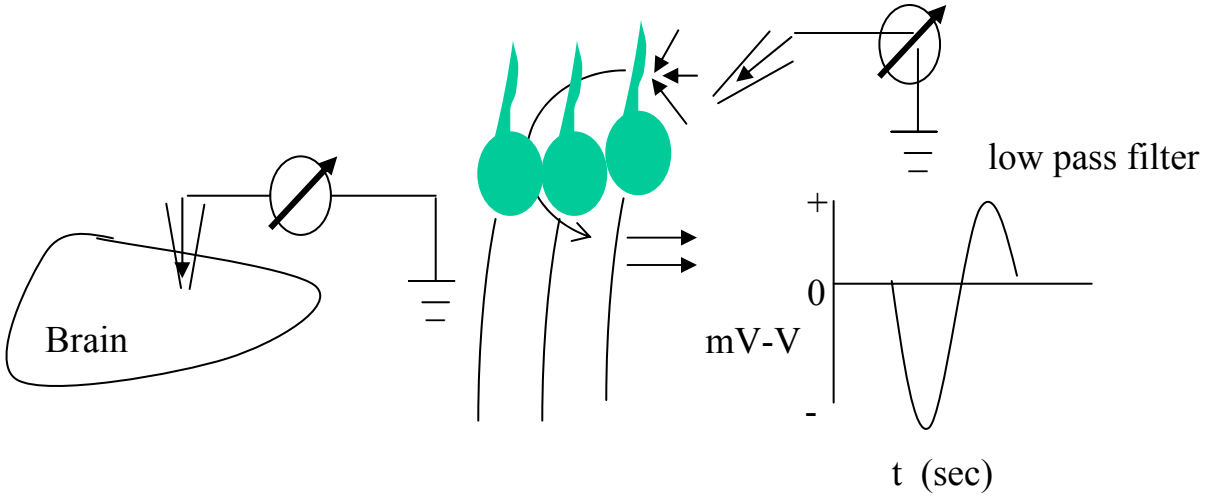

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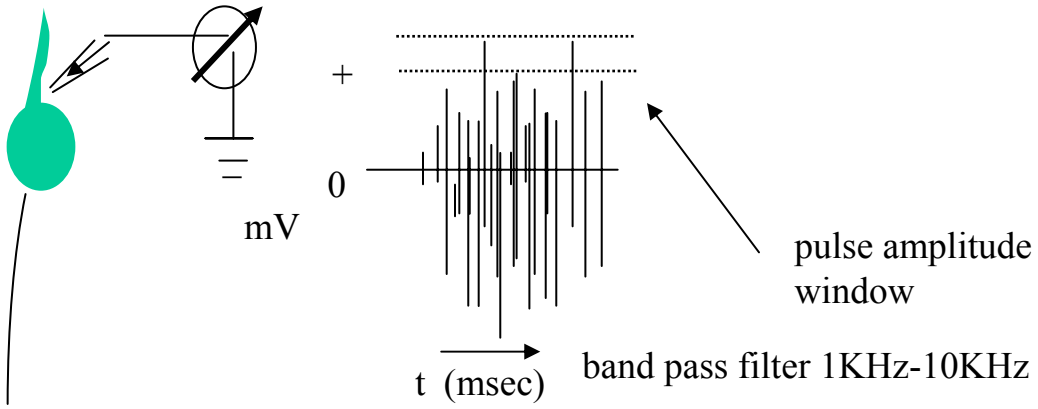

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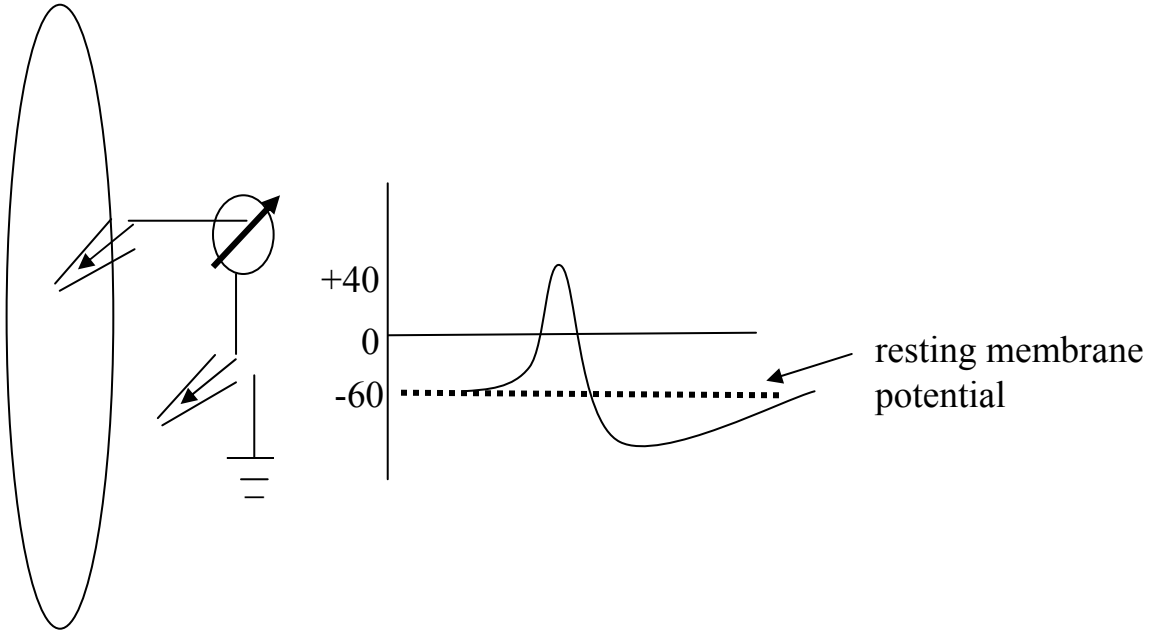


Voltage clamp recording:
Can pass current to compensate for voltage change. In this way voltage is held ~ constant and current applied to compensate is a measure of the current flowing. This will give a measure of current.

Ion movement through channels is governed by their **Electrochemical Equilibrium Potential** also known as their **Reversal Potential** because At E_{rev} the net direction of the ions' flow across the membrane switches direction.

E_{rev} is given by the Nernst Equation:

$$E_{i\,rev} = (RT/zF)\ \ln\ ([ion]_{out}/[ion]_{in}$$

R= the gas constant
T= absolute temperature
z= the valency of the ion
F= the Farady

At 20° C $(RT/zF) = 25$mV

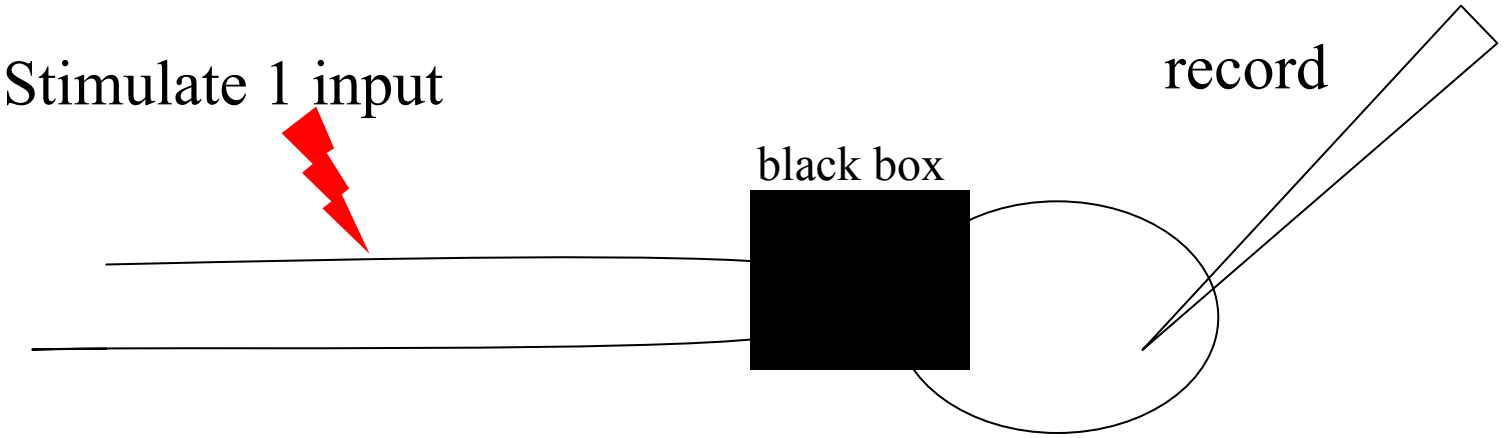
$E_{i\,rev}$ for Na+ at 20 degrees is
58log [140mM]/[7mM]= +75mV

$E_{i\,rev}$ for Ca++ at 20 degrees is
58log [1.5mM]/ [.0001mM]= +129 mV

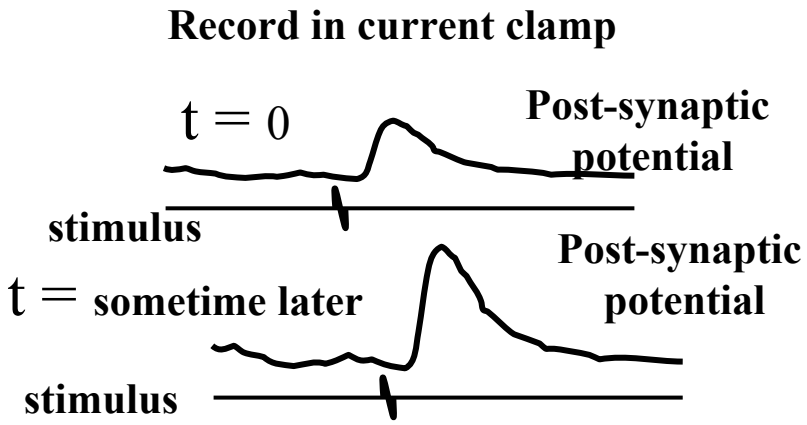
Multiply by 2.3 to convert base 10 log
$(RT/zF) = 58$mV. At 37℃ $(RT/zF) = 68$mV

See page 55 of Hammond, C, (2001) Cellular & Molecular Neuroscience for a presentation of the physics underlying the Nernst equation.

Functional synaptic plasticity is a change in the efficacy of synaptic transmission between at least 2 neurons.



Intracellular Recording



This change could be due to:

1. **An increase in the amount of transmitter released**
2. **An increase in the rate of transmitter released**
3. **An increase in post-synaptic receptors at each contact.**
4. **An increase in the number of contacts between the stimulated axon and the post-synaptic cell**

How do you analyze where the change in transmission occurs?
All methods depend on several assumptions. These may not be true.

All miniature synaptic currents/potentials arise from spontaneous release of 1 vesicle of neurotransmitter. Not true for all synapses.

At some synapses multiple vesicles can be released from one release site.

All vesicles contain the same amount of a particular transmitter called a quantum. The amount of transmitter loaded in vesicles can be different.

All vesicles release transmitter at the same rate.

Post-synaptic receptors are not saturated. This is not always true.

***pCa* = no. of stimuli / no. of increases in EPSCCaT**
***pr* = aggregate probability of release from any or all release sites at the pre-synaptic terminal apposing the dendrite.**

Question:
Is *p*Ca and accurate measure of *p*r ?

YES

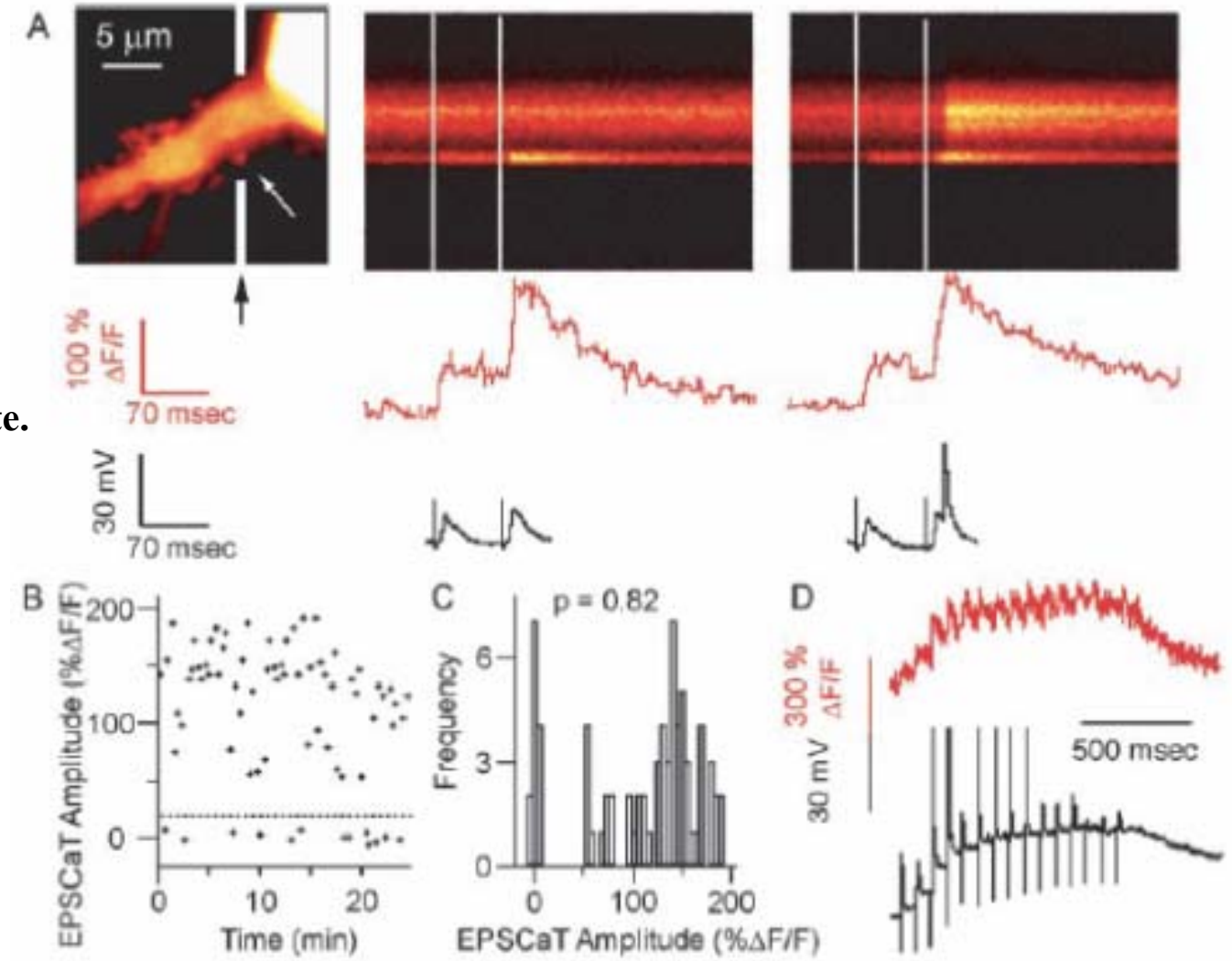
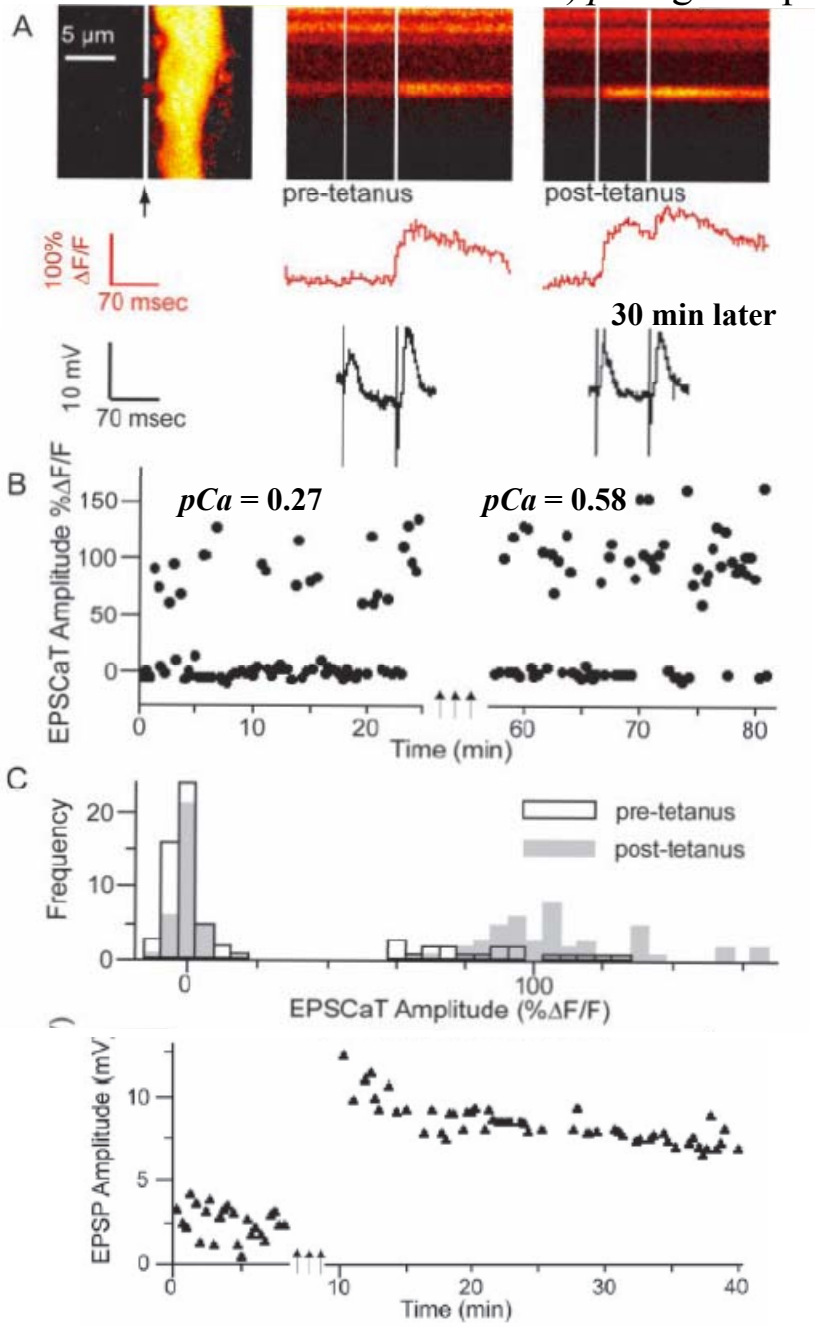


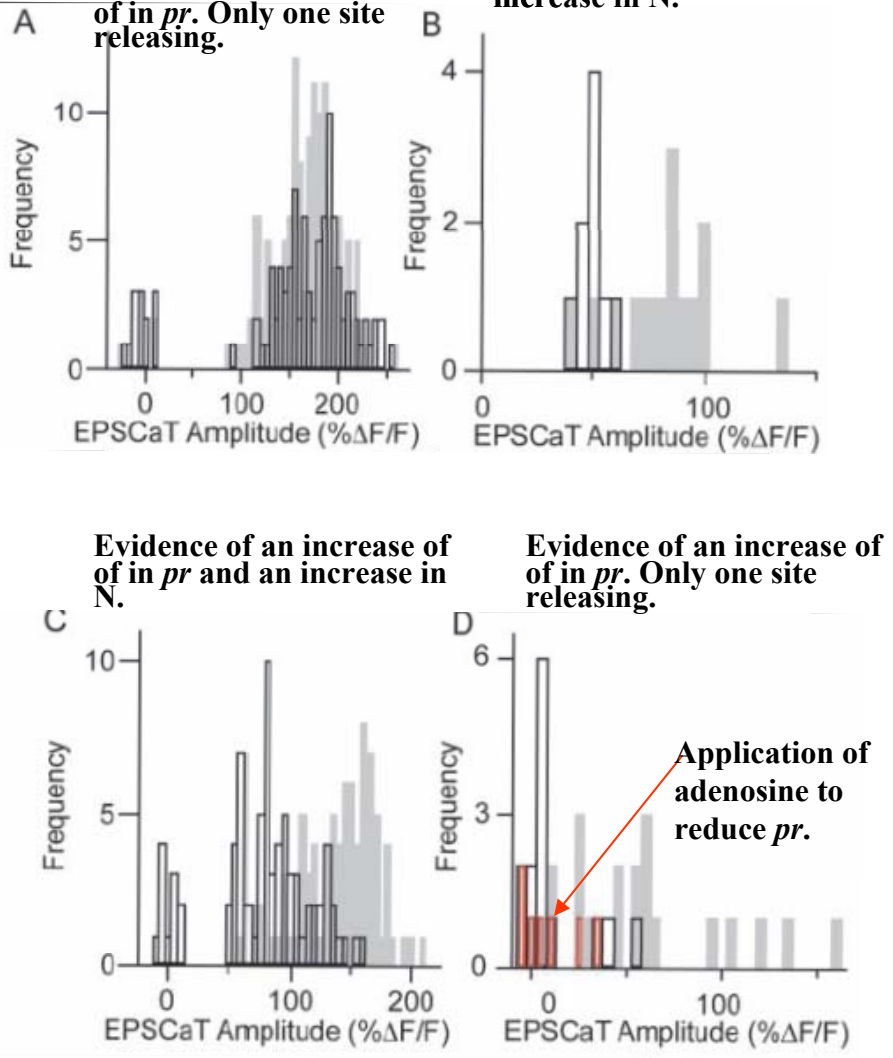
Figure 1. EPSCaTs detect transmitter release at single mossy fiber synapses. *A*, Left, proximal apical dendritic segment of a CA3 pyramidal neuron in a cultured hippocampal slice, filled with calcium indicator (Oregon Green 488 BAPTA-1). The soma is at the top right; the apical dendrite extends distally to the bottom left. Two line-scan images obtained along the trajectory are indicated by the black arrows, and white line segments are shown in the middle and right panels. Directly beneath each line scan is the Ca^{2+} signal (red top traces, expressed as fractional change in fluorescence, $\Delta F/F$) from the large spine (thorny excrescence) at the level

From: Reid et al. (2004) J Neurosci. 3618-3626

Using a potentiating stimulus (3, 1 sec trains of 100Hz stimuli at 20 sec intervals) *pCa* goes up.



Examination of *pCa* in individual spines before and after potentiation show evidence of changes in *pr* at individual release sites and in N, the number of active individual release sites onto that spine.



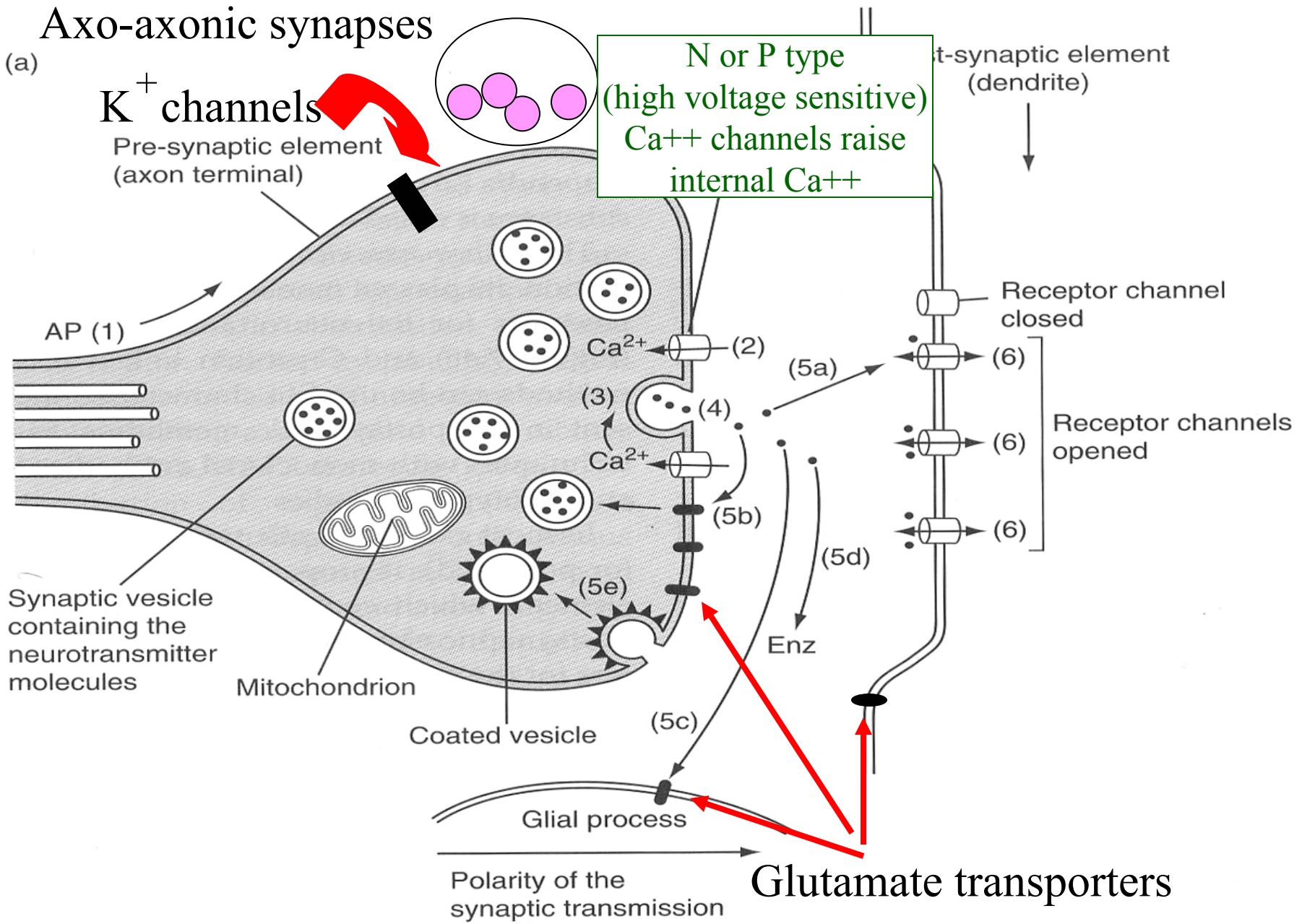
Short term plasticity (minutes):
post-tetanic potentiation, paired-pulse facilitation, paired-pulse depression.

Long -term plasticity (hours to days):
NMDA receptor dependent long-term potentiation (LTP); NMDAR independent LTP; NMDAR dependent LTD; Ca++ sensitive adenylyl cyclase dependent LTP (mossy fiber-CA3 synapse); mGluR, AMPA dependent LTD at parallel fiber to Purkinje cell synapses.

Homosynaptic plasticity:
Plasticity expressed at an activated synapse as a result of the activity of the activated synapse.

Heterosynaptic plasticity:
Plasticity induced by other synapses on the same synaptic relay.

Long-Long Term Potentiation: Involves growth of new contacts and is protein synthesis dependent


Axo-axonic synapses
(a)
K+ channels
N or P type
(high voltage sensitive)
Ca++ channels raise
internal Ca++
Pre-synaptic element
(axon terminal)
t-synaptic element
(dendrite)
AP (1)
Ca2+
(2)
(3)
(4)
Ca2+
(5a)
(5b)
(5c)
(5d)
(5e)
Receptor channel
closed
(6)
(6)
(6)
Receptor channels
opened
Enz
Synaptic vesicle
containing the
neurotransmitter
molecules
Mitochondrion
Coated vesicle
Glial process
Polarity of the
synaptic transmission
Glutamate transporters

Voltage Gated Ca++ Channels

High threshold TYPES. L (long lasting, N (neither L or P) , P (after Purkinje cell where this type was first discovered).
High threshold voltage gated Ca++ channels- means tiggered by large depolarizations (eg from -90 or -80mV to -20 or -10 mV).
Generally presynaptic but P type can generate dendritic Ca++ spikes.

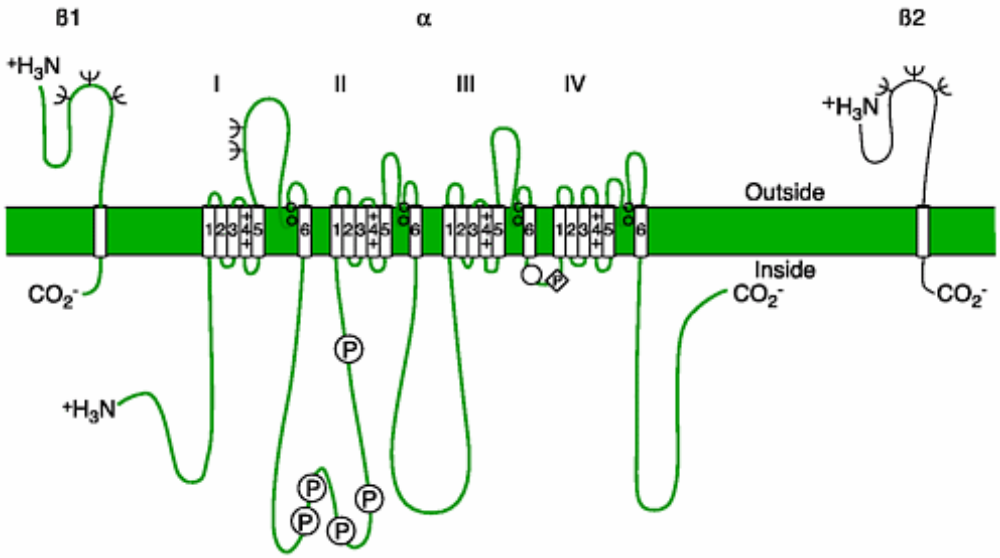
Identification by Pharmacology

L-type channels- Bay K 8644agonist; Nimodopine, antagonist

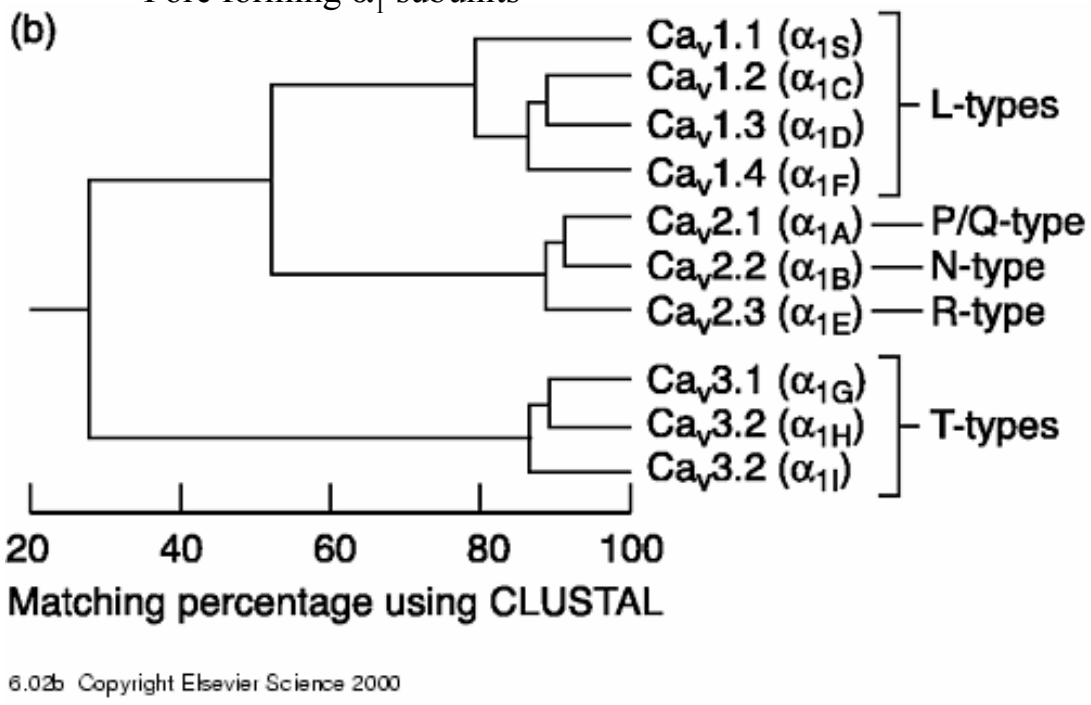
N-type channels- conus toxin ω-conotoxin (antagonist)

P-type - components of funnel webb spider venom = FTX also a peptide in the venom ω-agatoxin IVA

Subunits of Voltage Gated Ca++ Channels

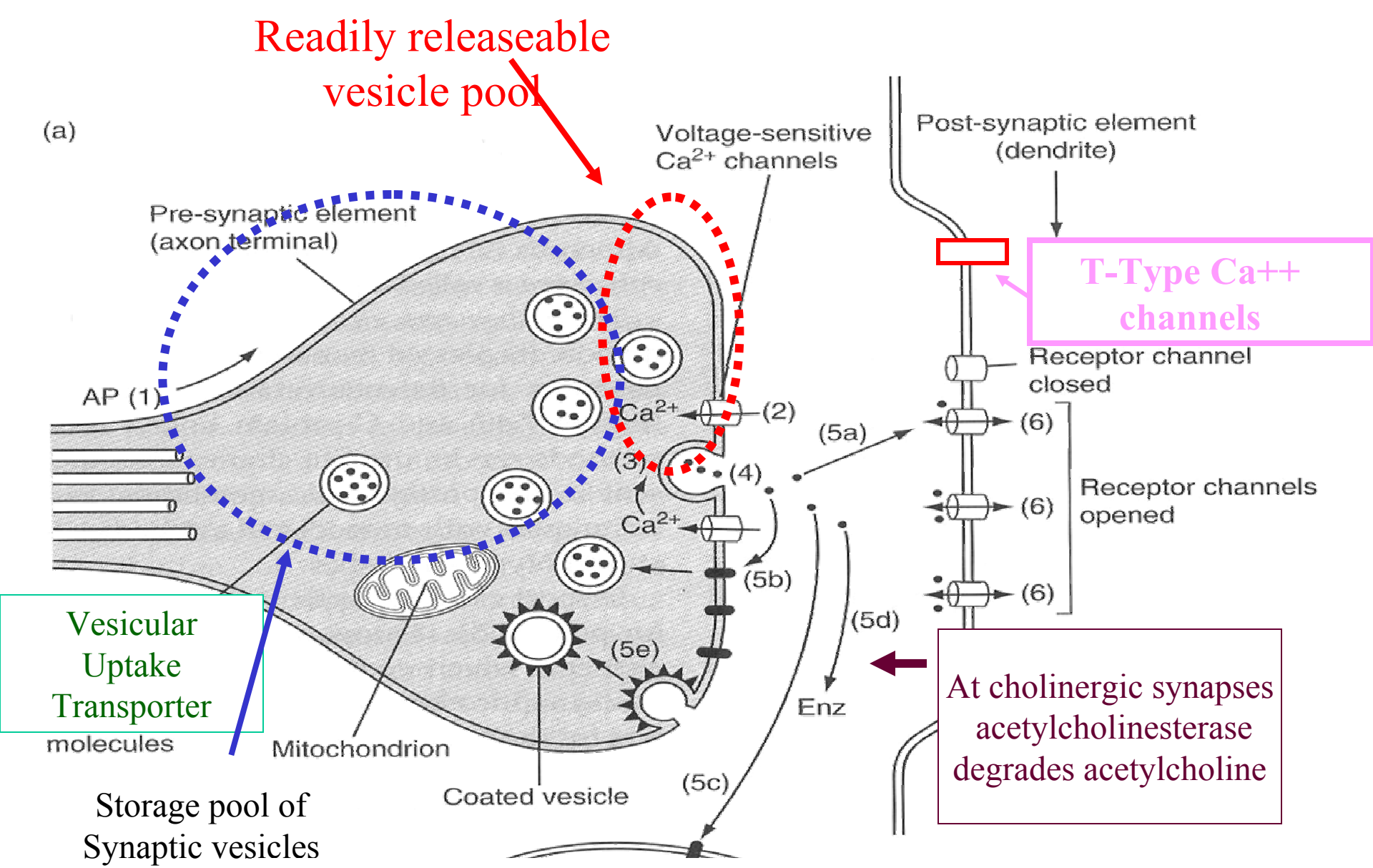


Pore forming α_1 subunits

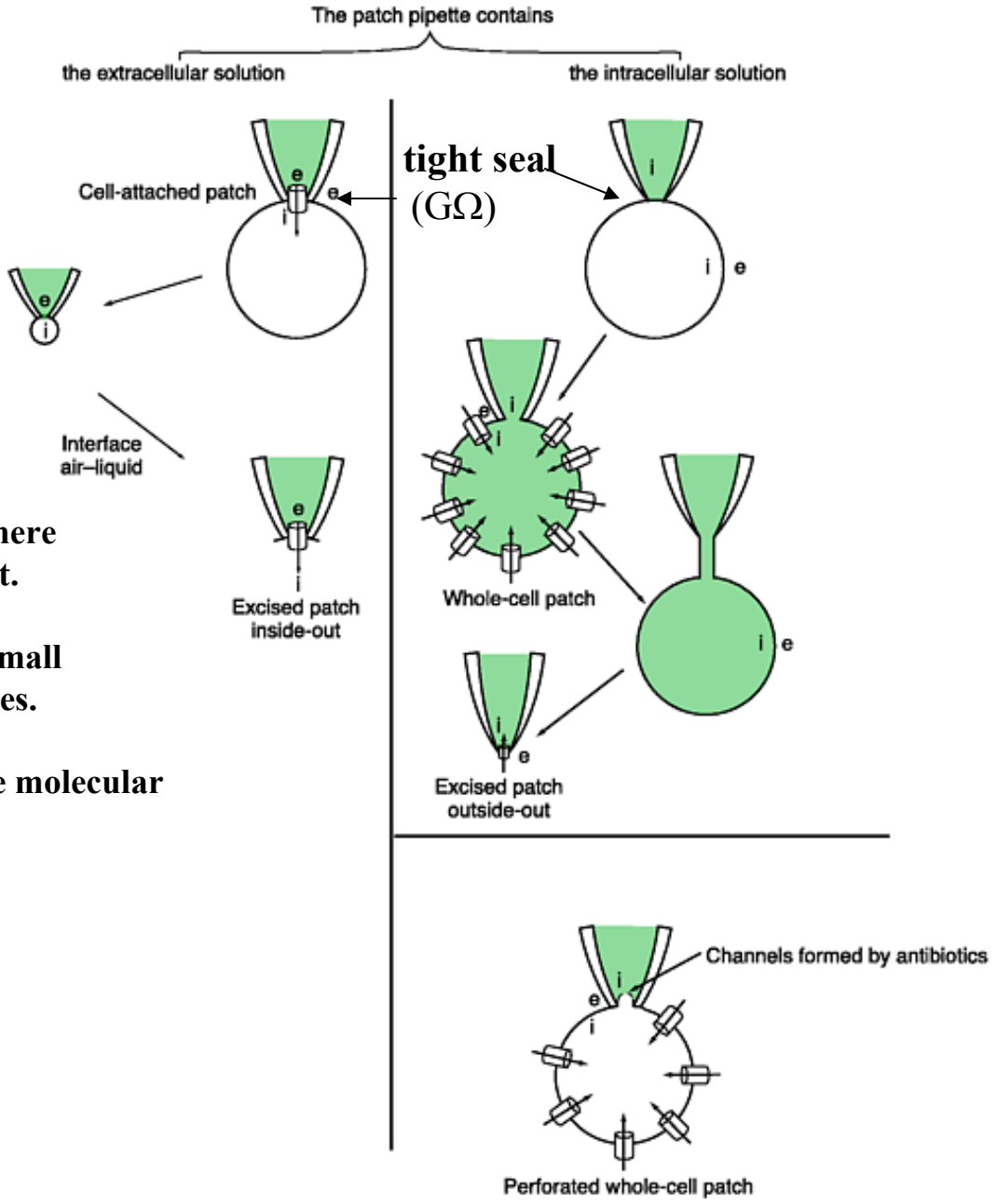


L-type Ca++ channels require phosphorylation by PKA in order to maintain functions

Fig 6.2. From HammondC. *Cell & Molecular Neurobiology* Academic Press 2001


Readily releaseable vesicle pool
(a)
Voltage-sensitive Ca^{2+} channels
Post-synaptic element (dendrite)
Pre-synaptic element (axon terminal)
T-Type Ca++ channels
Receptor channel closed
AP (1)
Ca^{2+}
(2)
(5a)
(6)
(3)
(4)
Receptor channels opened
Ca^{2+}
(6)
(5b)
(6)
(5d)
(5e)
Enz
At cholinergic synapses acetylcholinesterase degrades acetylcholine
Vesicular Uptake Transporter molecules
Mitochondrion
Coated vesicle
(5c)
Storage pool of Synaptic vesicles
Movement of vesicle from storage to readily releasable pool= dependent On phosphorylation of **synapsin**

Patch-Clamp Electrodes* Have Greatly Facilitated the Examination of Synaptic Currents in Neurons



Advantages:

1. **In all but the cell attached patch mode there is access to the intracellular environment.**

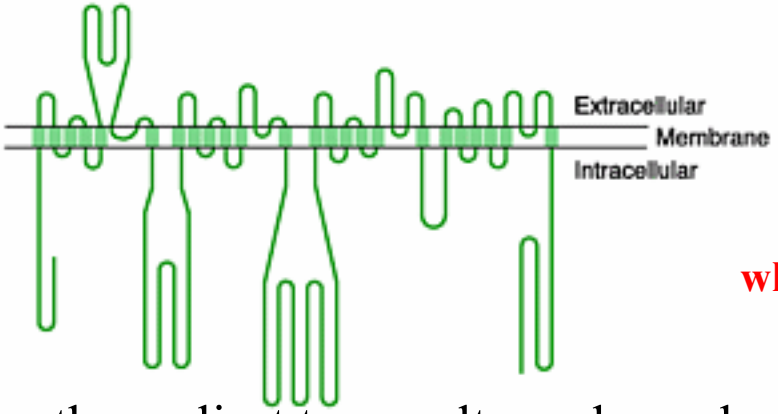
2. **Recordings can be made from cells too small to be implaed with intracellular electrodes.**

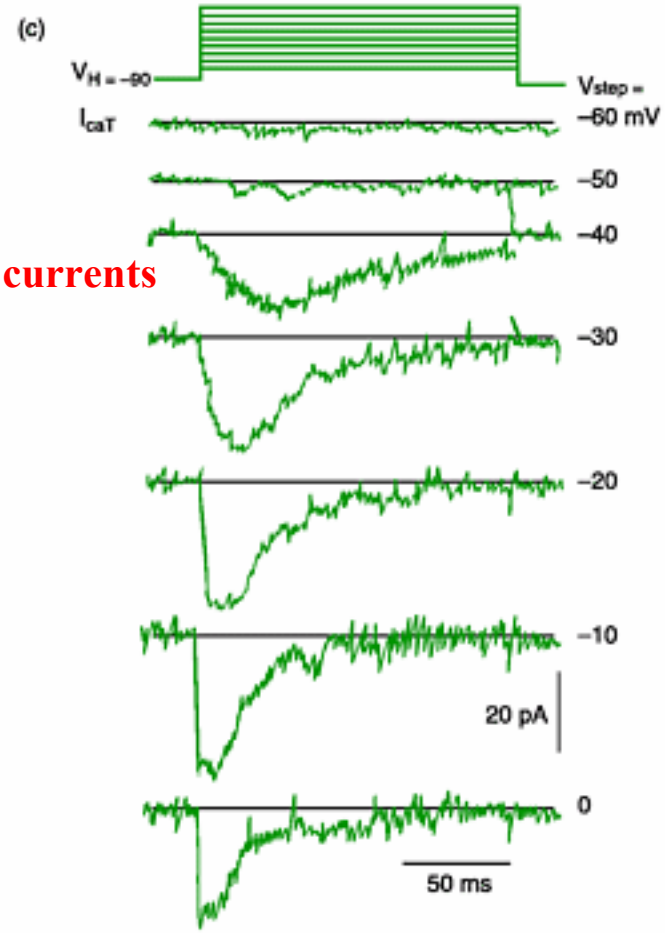
3. **Currents can be recorded through single molecular channels.**

* Neher and Sakmann won a Nobel Prize for developing this technique

FigA5.6 From Hammond C. *Cellular and Molecular Neurobiology.* (2001)Academic Press

T (transient/tiny)- type of low voltage Ca++ channels:Composed of one of three different α subunits, α1G, α1H, α1I.





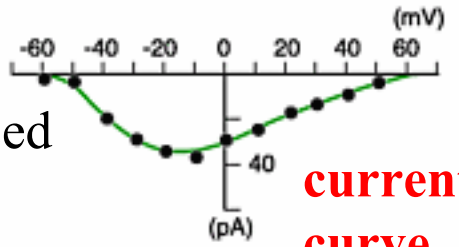
whole cell currents

Can be the earliest type voltage-dependent Ca++ channel expressed. Very small conductance.

T type Ca++ channels enhance excitability <u>after</u> membrane hyperpolarization due to activation /deactivation kinetics of the channel.

Totally inactivated near resting potential.
De-inactivated during a small hyperpolarization.

Therefore readily activated when membrane depolarizes to ~-40V after a hyperpolarization (increased excitability). Fig 6.2. From HammondC. *Cell & Molecular Neurobiology* Academic Press 2001



current/voltage curve

Ion channel opening is a stochastic process

a stimulus greatly increases the probability of channel opening

Na+ channels **K+ channels**

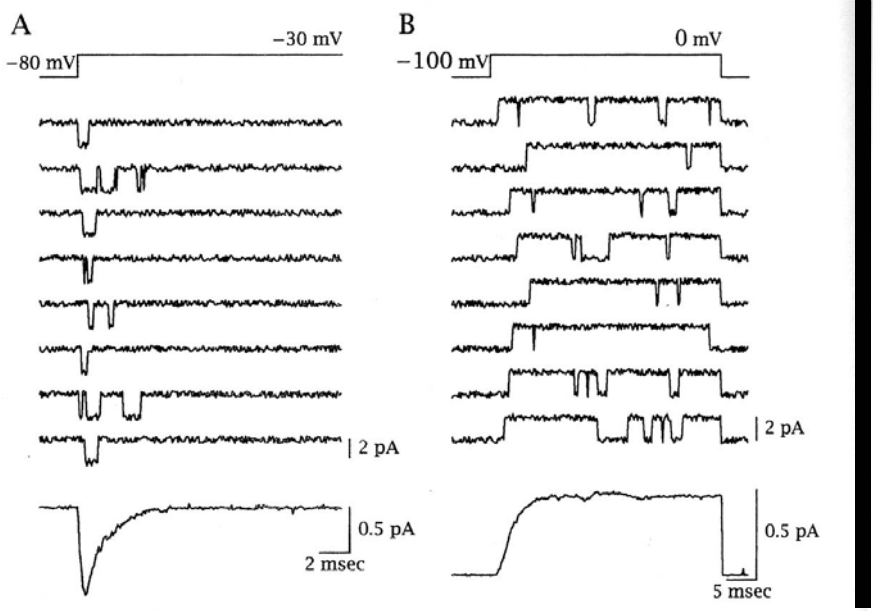


Figure 8.5 Unitary currents (upper 8 traces) and the ensemble average of unitary cur rents (lowest trace) of a Na^+ (A) and K^+ (B) channel. The membrane voltage is steppe from $V_H = -80$ mV to $V_c = -30$ mV for (A) and from $V_H = -100$ mV to 0 mV for (B Records in (A) are simulated after data from Horn and Vandenberg (1984), and records i (B) are simulated with the reaction scheme shown in figure 10.1.

T-type Ca++ channel **L-Type Ca++ channel**

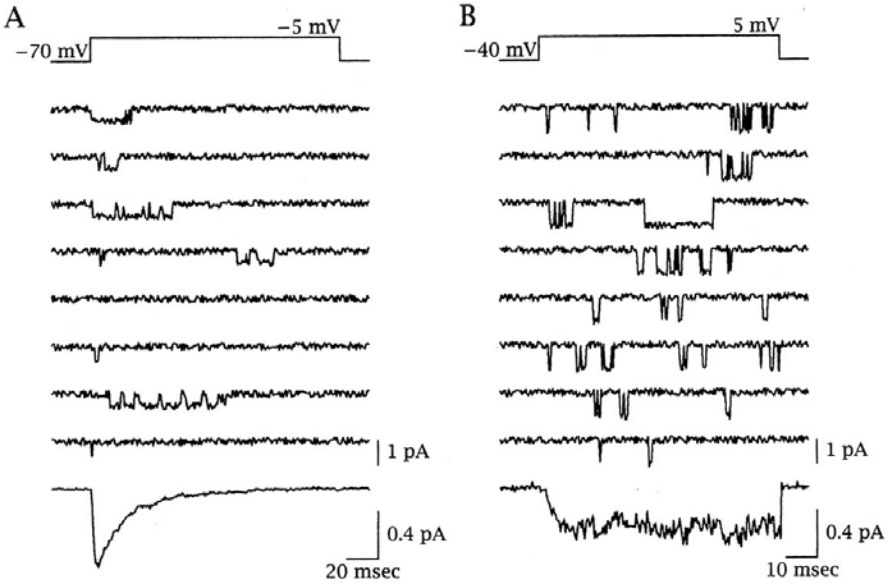


Figure 8.6 Unitary currents (upper 8 traces) and the ensemble average of unitary cur- rents (lowest trace) of a T-type (A) and L-type (B) calcium channel. The membrane voltage is stepped from $V_H = -70$ mV to $V_c = -5$ mV for (A) and from $V_H = -40$ mV to $V_c = 5$ mV for (B). Records are simulated after data from Fisher et al. 1990.

Whole Cell Currents represent the temporal summation of all channel openings initiated by the same stimulus

From: Johnston,D; Wu,SM *Foundations of Cellular Neurophysiology*. MITPress, 2001

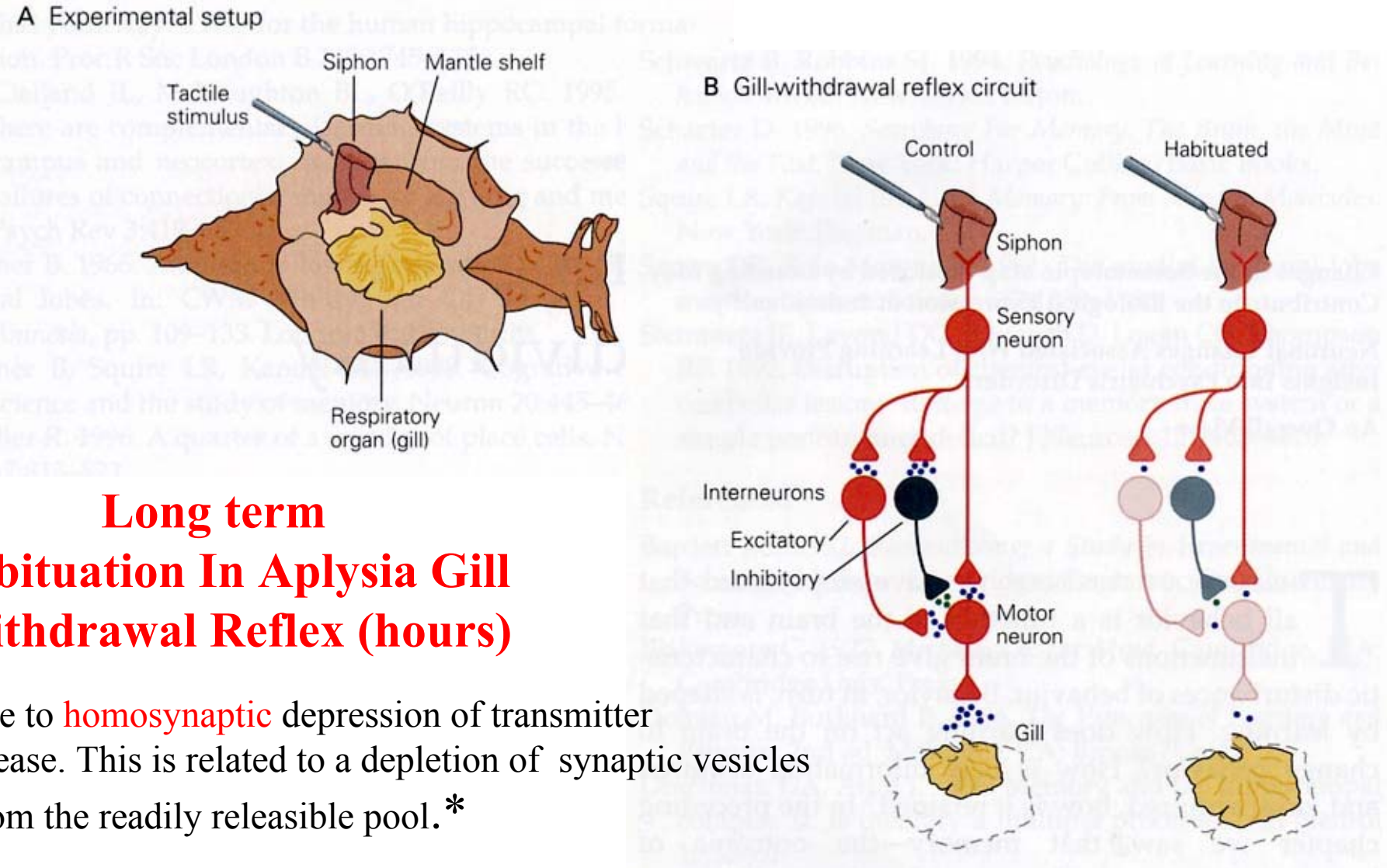


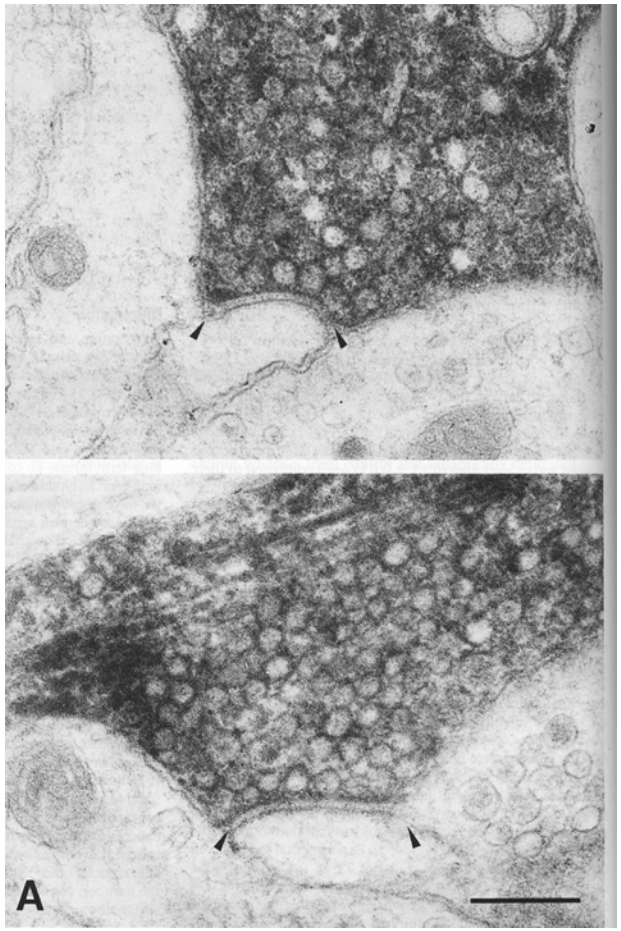
Figure 63-1 The cellular mechanisms of habituation have been investigated in the gill-withdrawal reflex of the marine snail *Aplysia*.

Long term Habituation In Aplysia Gill Withdrawal Reflex (hours)

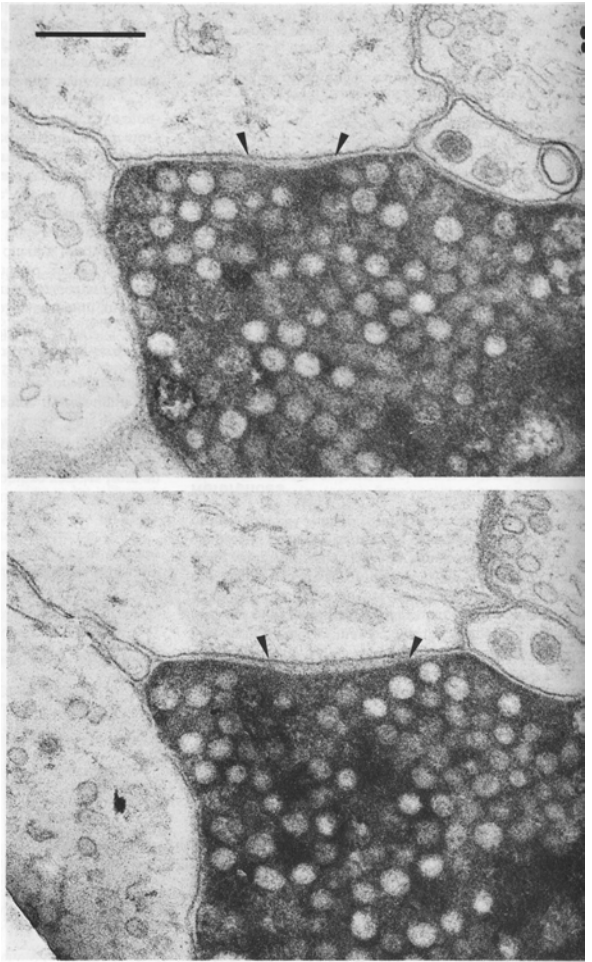
Due to homosynaptic depression of transmitter release. This is related to a depletion of synaptic vesicles From the readily releasible pool.*

*** probable mechanism involves the decreased phosphorylation of synapsins which normally causes them to separate from synaptic vesicles and releases the vesicles to move to the synaptic membrane.**

Before Habituation: Many vesicles in the readily releasable pool of Aplysia sensory motor synapses.



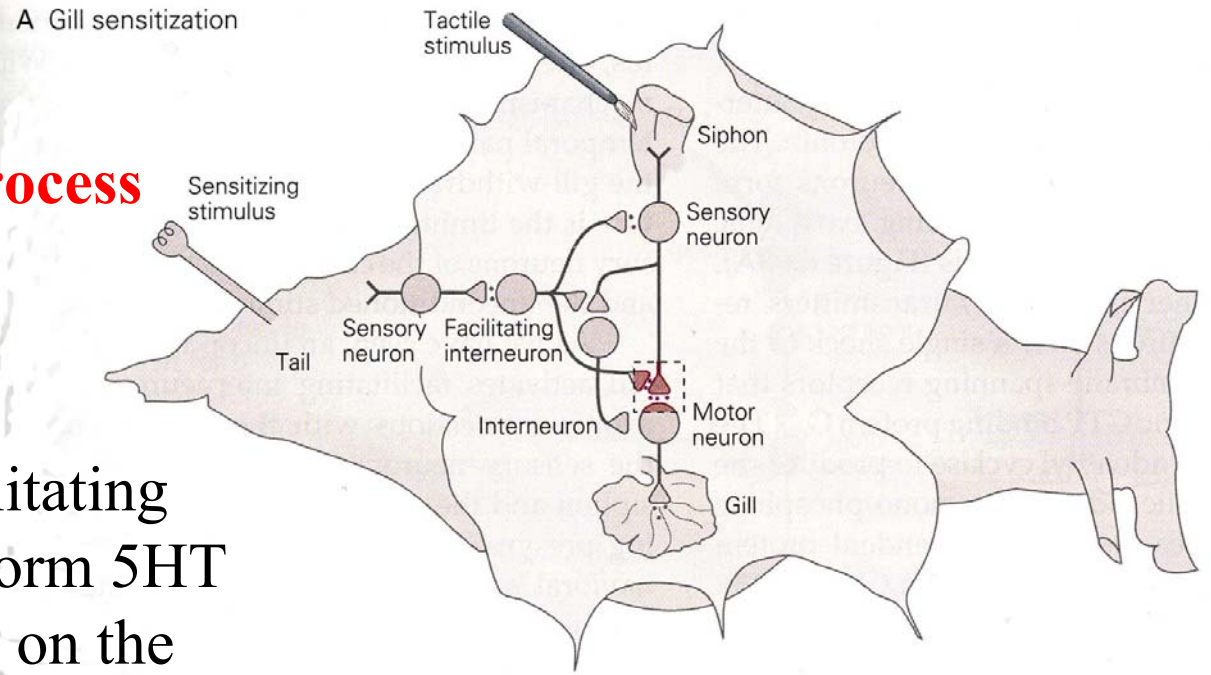
Chen & Baily, 1995



After Habituation:
Readily releaseable pool is depleted

Sensitization of the Siphon Withdrawal Reflex involves application of a noxious stimulus to another part of the body (the tail).

A Heterosynaptic Process



This activates facilitating interneurons that form 5HT mediated synapses on the presynaptic terminals of siphon sensory neurons. Two types of 5HT synapses are activated. One coupled to Gs.
One coupled to Go.

What will be the effect of Gs? **Increase in cyclic AMP and activation of PKA**

What will be the effect of Go ? **Activation of phosphlipase C (PLC) resulting in production of IP3, DAG and an increase in PKC activity.**

PKA inhibitory subunit inactivated by cyclic AMP

1. Decreases K+ channel activity.

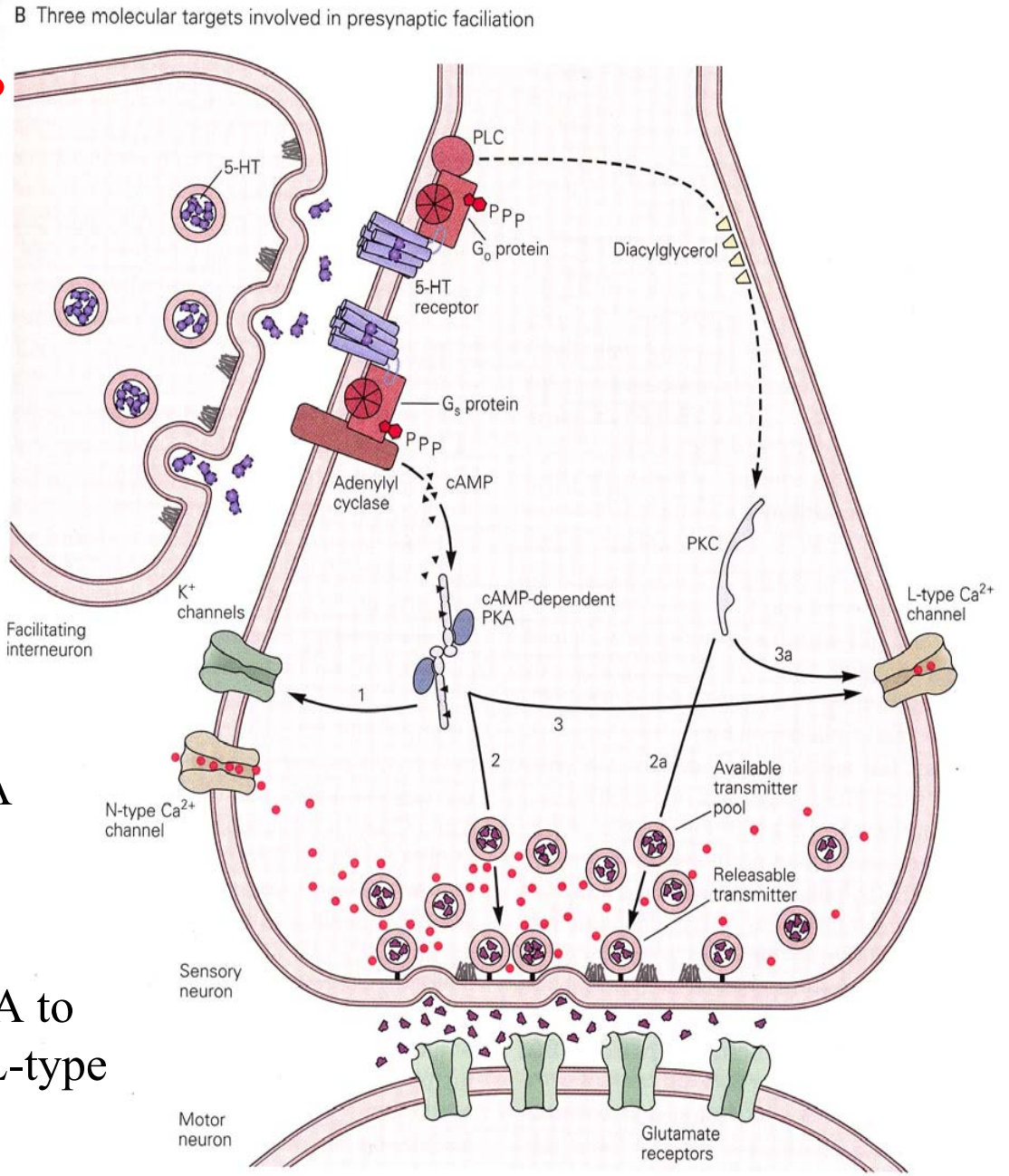
2. Mobilizes vesicles to the readily releasable pool.

3. Opens L-type Ca++ channels.

Phopholipase C activates PKC

1. PKC participates with PKA to mobilize vesicles to the readily releasable pool.

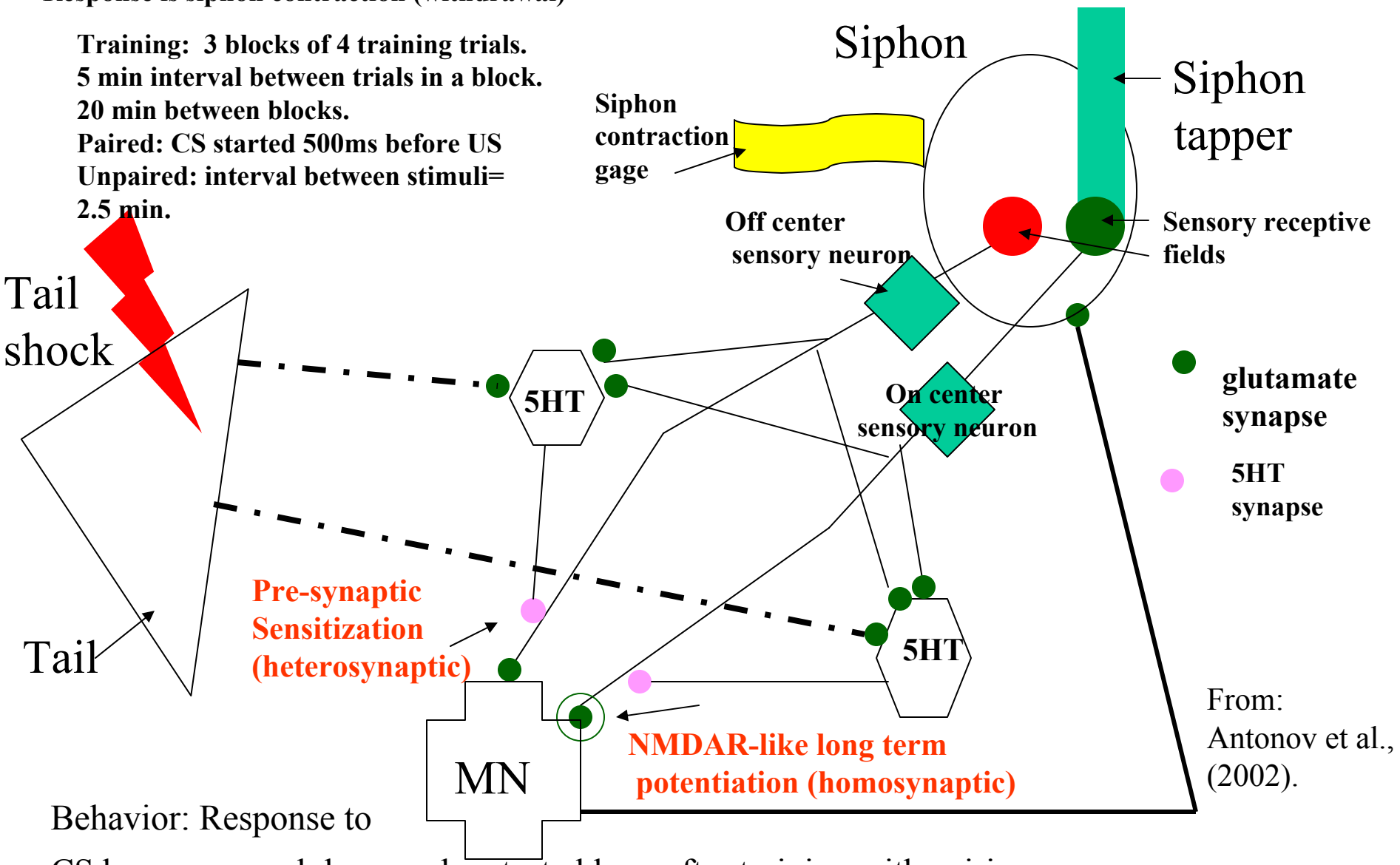
2. PKC participates with PKA to phosphorylate and increase L-type Ca++ channel activity.



Studies Using Siphon withdrawal Reflex of Aplysia:

Conditioned stimulus (CS) is the siphon tap
Unconditioned stimulus (US) tail shock
Response is siphon contraction (withdrawal)

Training: 3 blocks of 4 training trials.
5 min interval between trials in a block.
20 min between blocks.
Paired: CS started 500ms before US
Unpaired: interval between stimuli= 2.5 min.

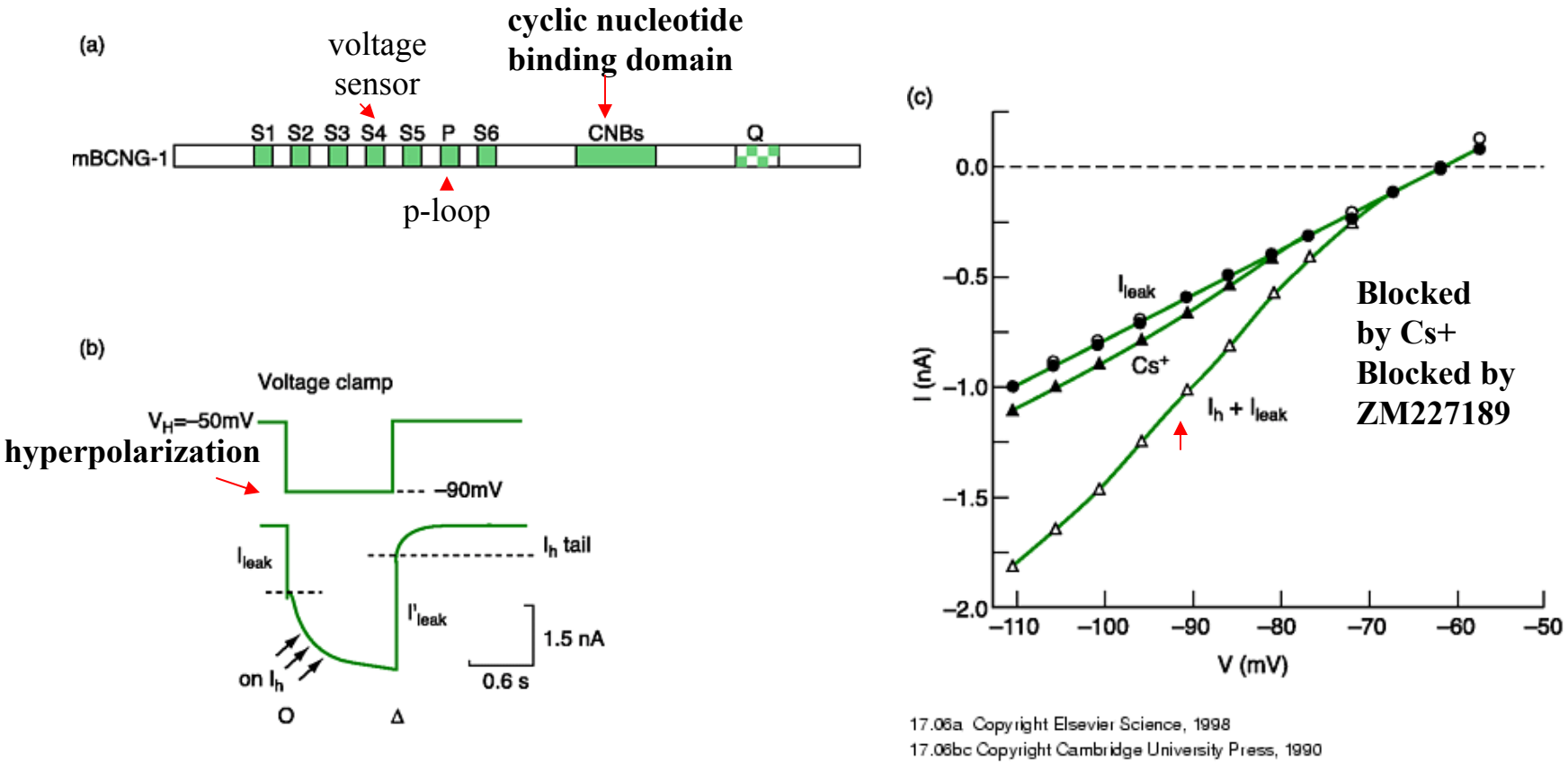


From: Antonov et al., (2002).

Behavior: Response to CS becomes much larger when tested long after training with pairing.

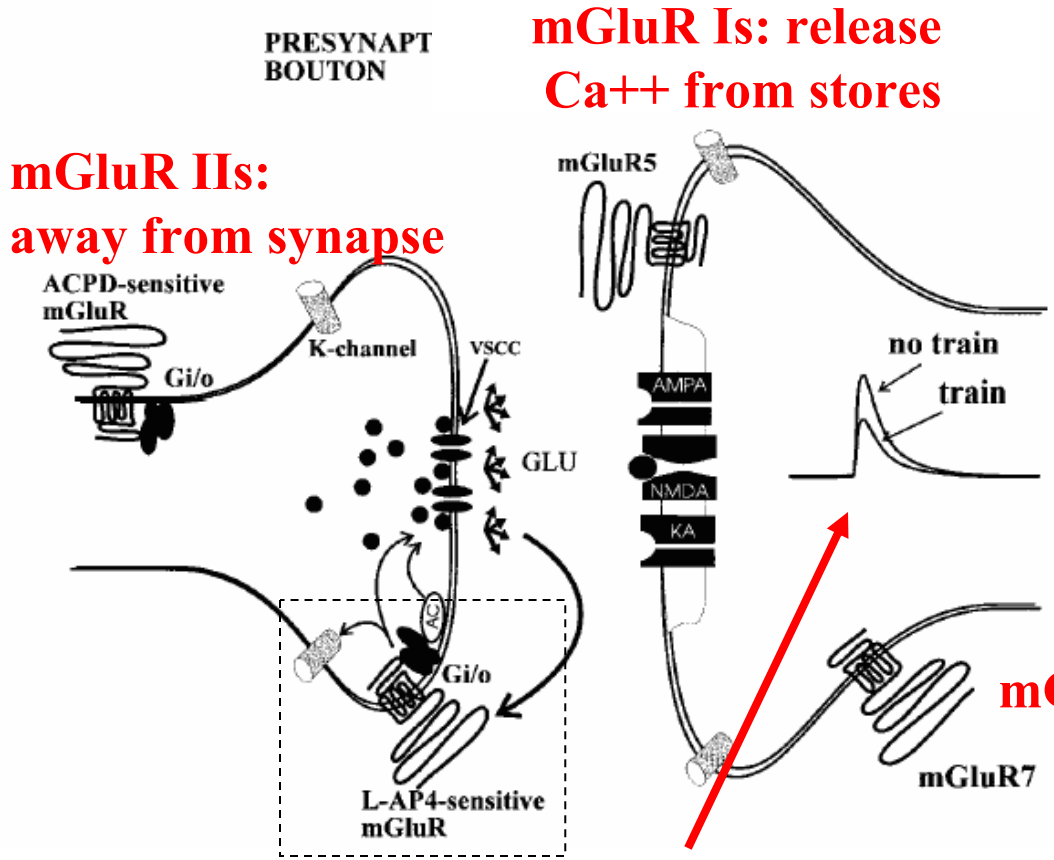
Hyperpolarization activated cationic channel (Ih, If, Iq)

(hyperpolarization-activated, cyclic nucleotide-gated channels, HCN)



Activated by hyperpolarization -produces an inward, depolarizing current that functions in rhythmically active neurons, in heart and to enhance transmitter release (Beaumont & Zucker, 2000).

Fig. 17.6 From HammondC. *Cell & Molecular Neurobiology* Academic Press 2001



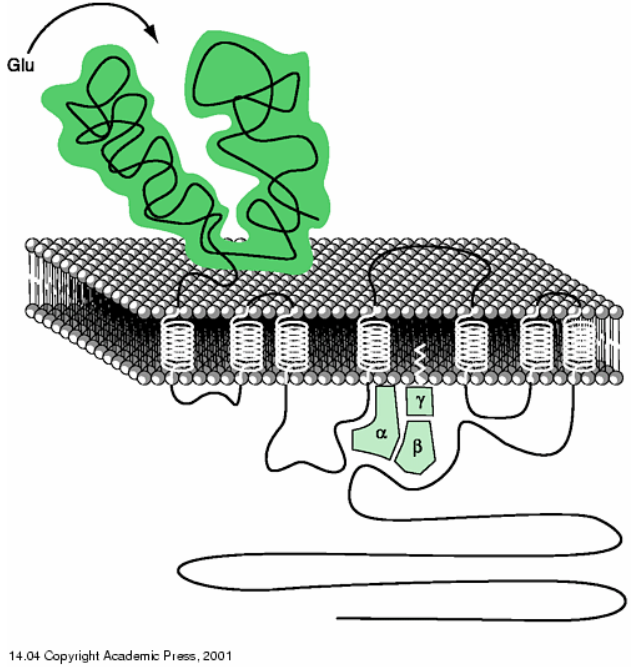
Group III metabotropic glutamate receptors at locus coeruleus synapses mediate activity-dependent depression to high frequency trains of stimuli. High affinity glutamate uptake blockers potentiate this effect.
Function of group II at this synapse remains unknown.

FIG. 7. Possible scenario explaining the mGluR group III (but not group II)–sensitive inhibition of EPSPs after sustained activity at LC synapses. LC spines are innervated by presynaptic terminals expressing receptors for both L-AP4/MAP4 (group III) and *t*-APCD/EGLU (group II) mGluRs. Receptors are localized to different sites on the presynaptic axon: group III mGluRs are close to (possibly in) the synaptic cleft and are activated by the accumulated glutamate in the cleft, thus reducing subsequent release of glutamate. Group II mGluRs are also found to presynaptically depress EPSPs after bath application of specific agonists but, based on the present study, are not found to participate in the activity-dependent depression of EPSPs. Thus, in the present scenario, this receptor is located on the presynaptic terminal but away from the synaptic cleft, making it less susceptible to activation after accumulation of synaptically released glutamate. VSCC, voltage-sensitive calcium channels; $G_{i/o}$, G-protein i/o subtype.

Dube & Marshall (2000) J.Neurophysiol. 83:1141-1149.

Metabotropic Glutamate Receptors

G-protein linked receptors



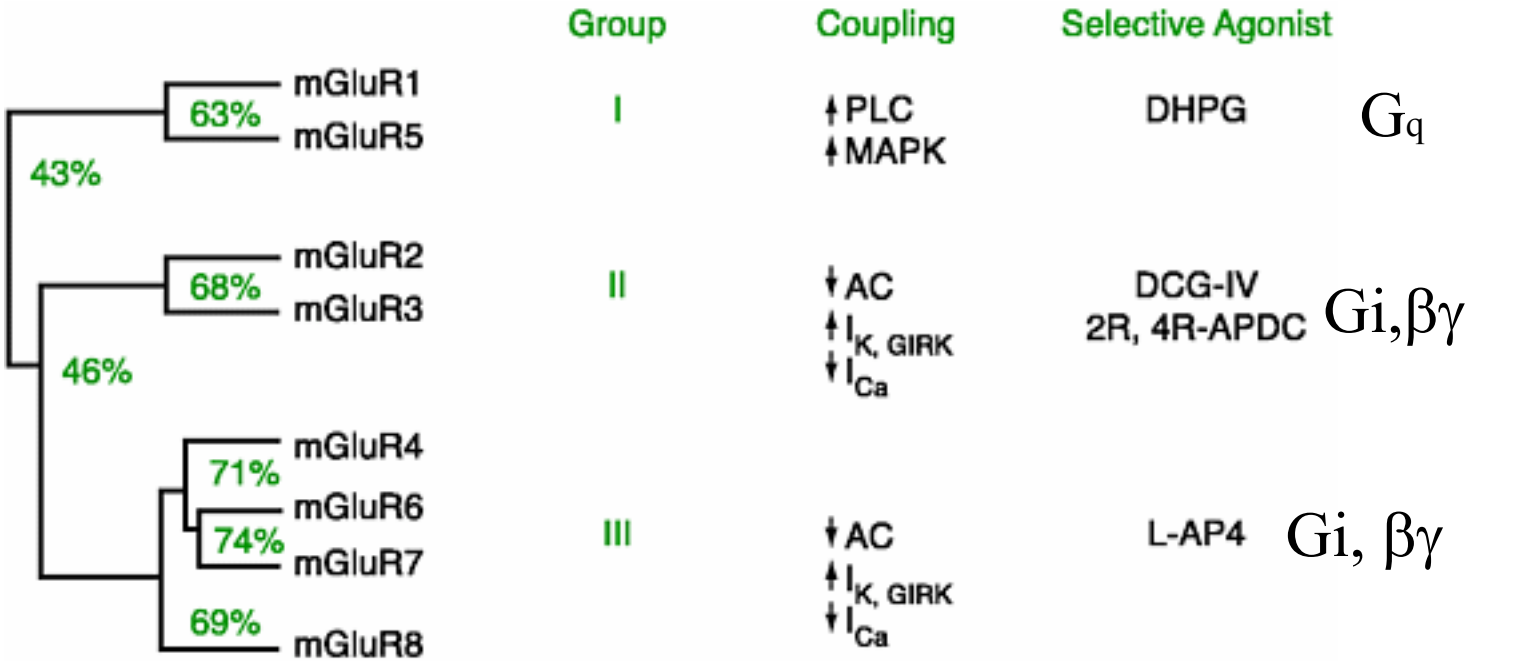


Fig 14.1From HammondC. *Cell & Molecular Neurobiology* Academic Press 2001

Other metabtropic receptors

$GABA_B$ Receptors Pre- or post-synaptic localization

agonists, GABA, Baclofen; antagonists, phaclophen etc.

require dimerization of two 7 pass transmembrane membrane proteins to produce a functional receptor

linked to an inhibitory G protein (G_i/G_o)

Presynaptic Function

decreases transmitter release

Post-synaptic function

Long-latency (20-50 msec); slow rise, slow decay (400-13000 msec)

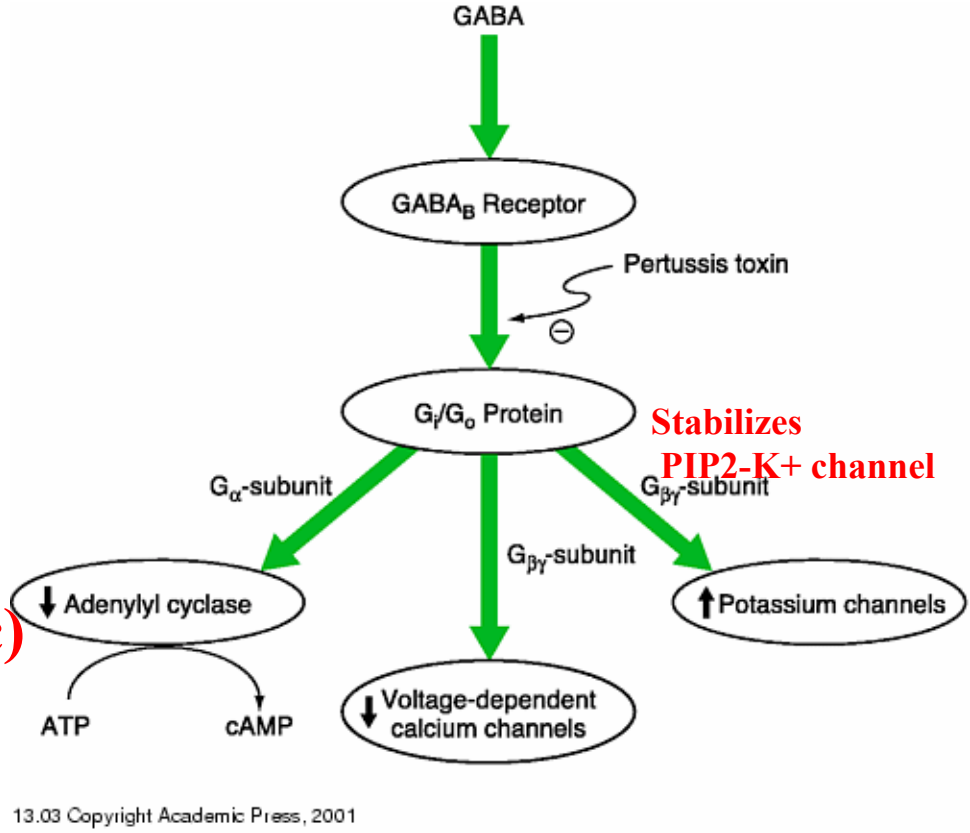
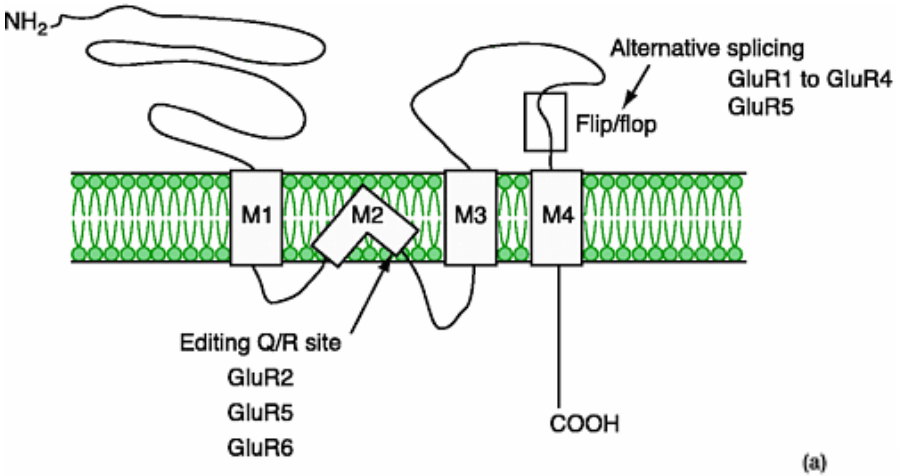
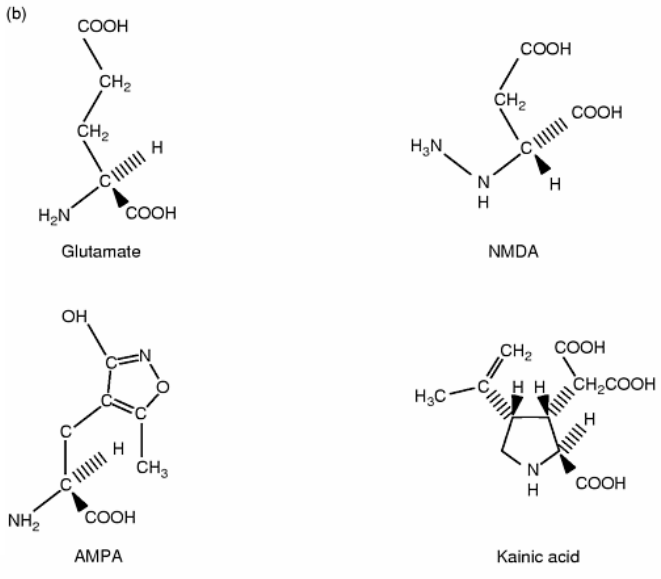
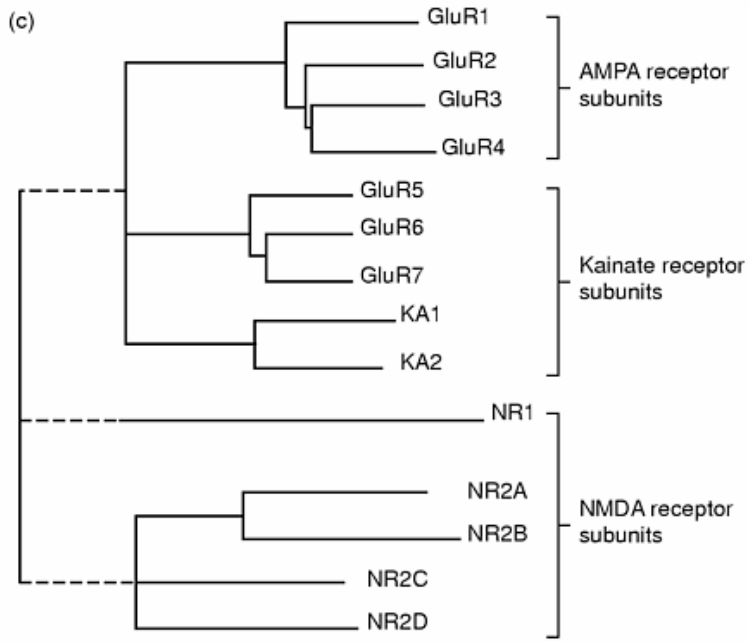


Fig. 13.3 From HammondC. *Cell & Molecular Neurobiology* Academic Press 2001

Ionotropic Glutamate Receptors







(a)

iGluRs	***Non-NMDA receptors***		***NMDA receptors***	
	AMPA R	***Kainate R***	*Glu site*	*Gly site*
Selective agonists		ATPA (GluR5) SYM2081	NMDA	Glycine D-serine
Non-selective agonists	Glutamate AMPA Quisqualate*Kainate		Glutamate	–
Selective antagonists	GYKI 53655 NBQX (1 μM) SYM2206	LY 293558 (GluR5)	D-APV (or AP5)	5,7-dichloro-kynurenate
Selective antagonists	CNQX DNQX NBQX		–	–
Channel blockers	–	–	MK 801 ketamine	–

Figs. 11.2,.1,.3 From HammondC. *Cell & Molecular Neurobiology* Academic Press 2001

* also acts on some mGluRs

AMPA Receptors

Unitary conductance of ~8pS

$$g = I/Vm\text{-}Erev$$

Are permeable to Na+, K+ and, if the GluR2 subunit is present and unedited also to Ca++.

AMPARs can trigger Ca++ influx through VSCC's

AMPARs desensitize rapidly.

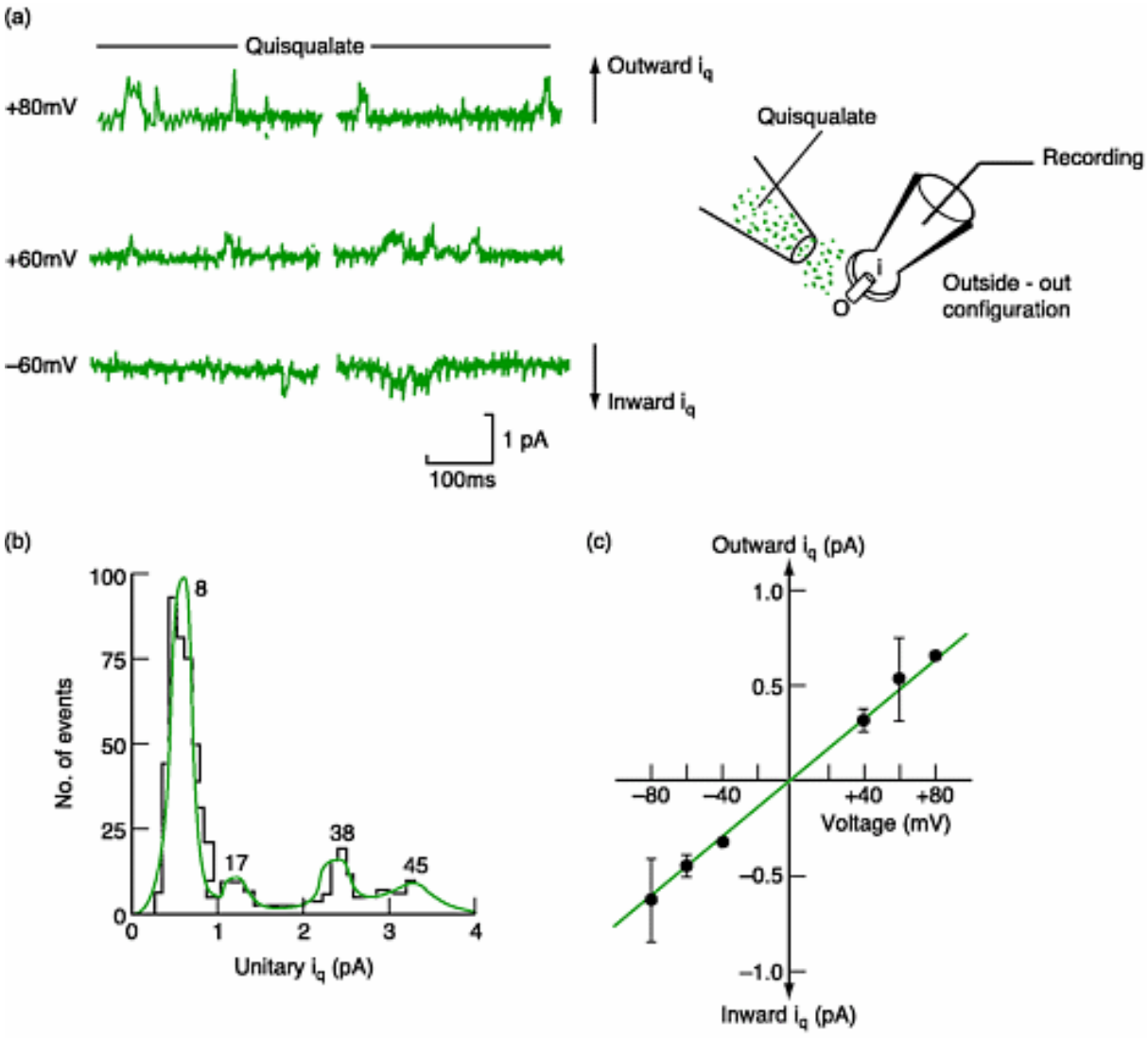
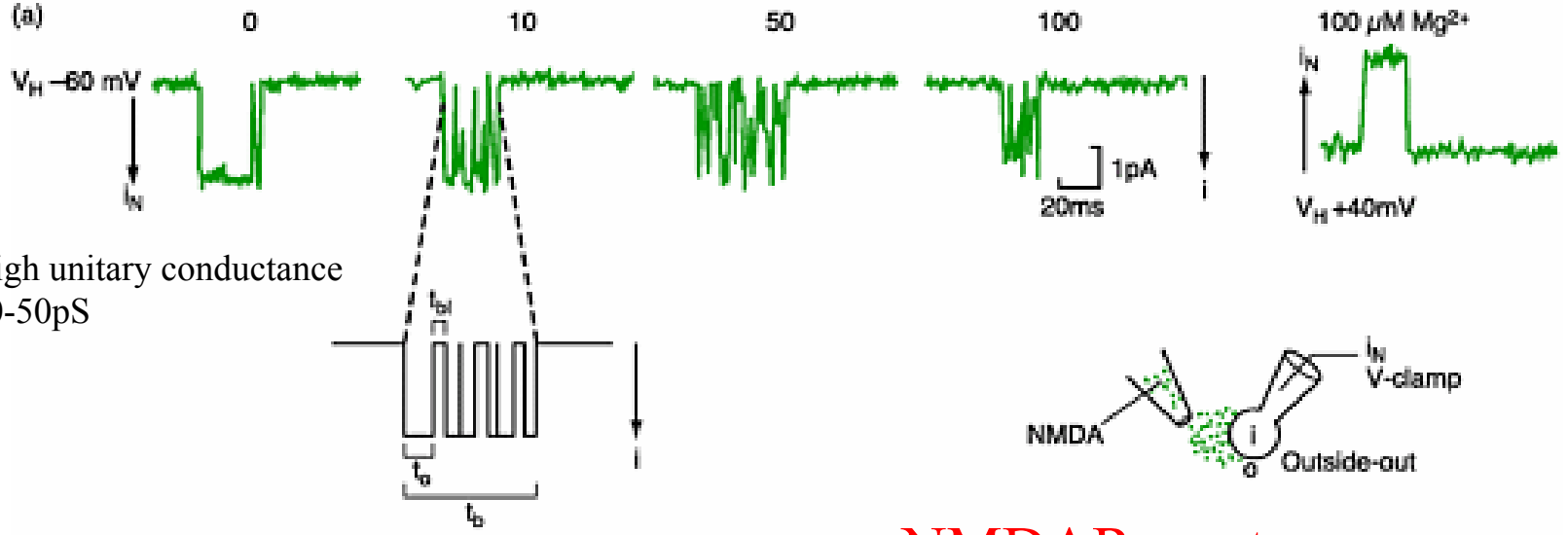
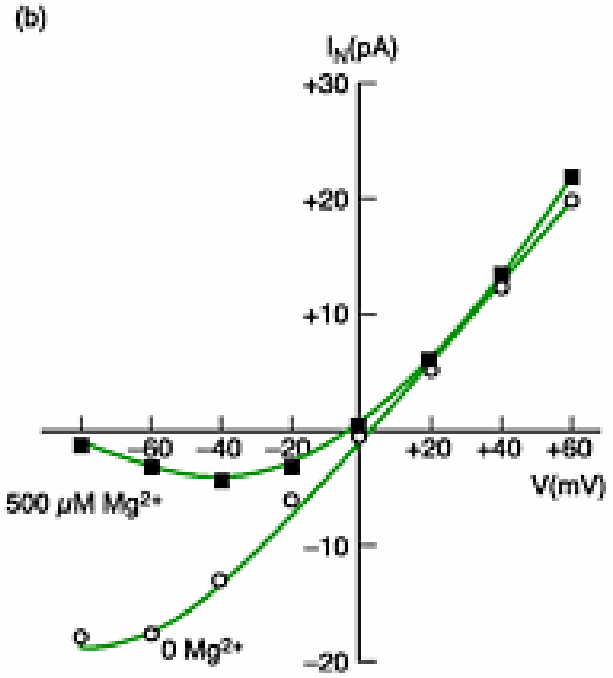


Fig 11.4 From HammondC. *Cell & Molecular Neurobiology* Academic Press 2001



High unitary conductance
40-50pS



NMDAReceptors

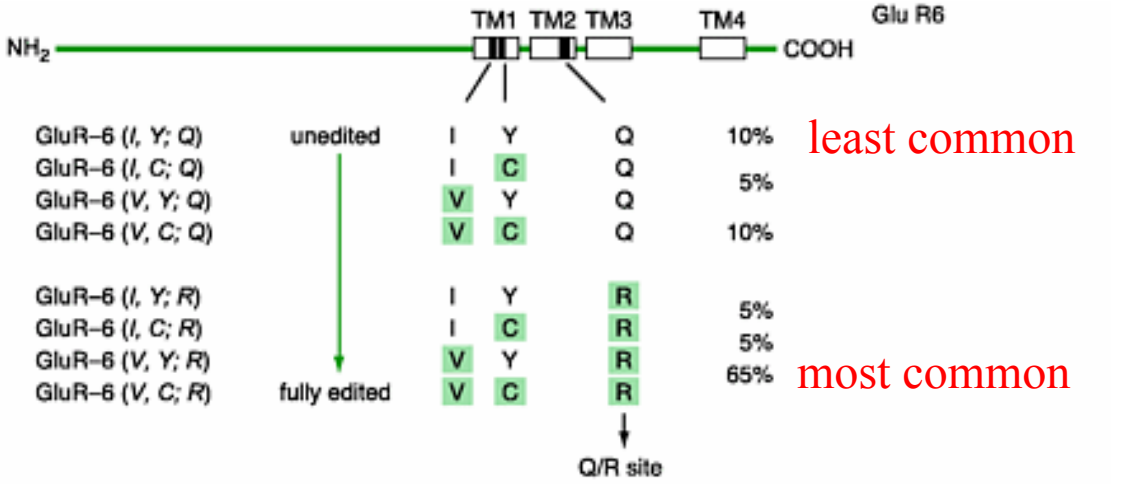
1. Both ligand and voltage-gated.
2. Channel blocked by Mg++ at ~ -40-80mV.
3. Therefore depolarization will relieve the Mg++block.
4. Require glycine as a co-agonist.
5. Have slow kinetics.
6. Have >> greater affinity for glutamate than either AMPAR, KainateRs or mGluRs.

From HammondC. *Cell & Molecular Neurobiology* Academic Press 2001

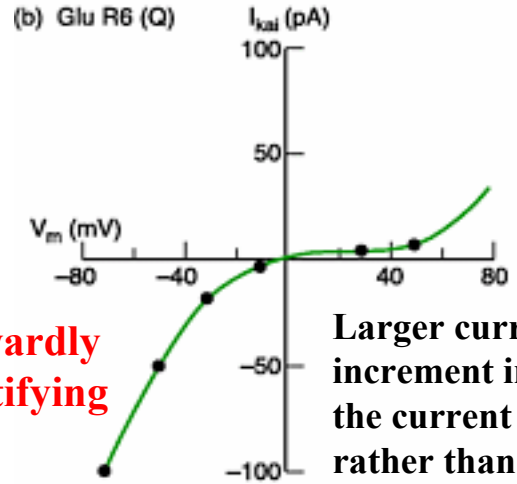
Kainate Currents

Subunits GluR5,6,7, KA1, KA2
Functional receptors are homomers of GluR5 & GluR6 or heteromers of KA2 &GluR5 and GluR6 . Others???

Kainate currents desensitize rapidly

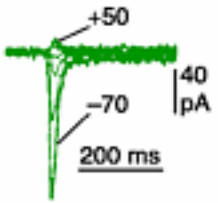


Non-edited



Inwardly rectifying

Larger current change for an increment in voltage when the current is inward rather than outward.



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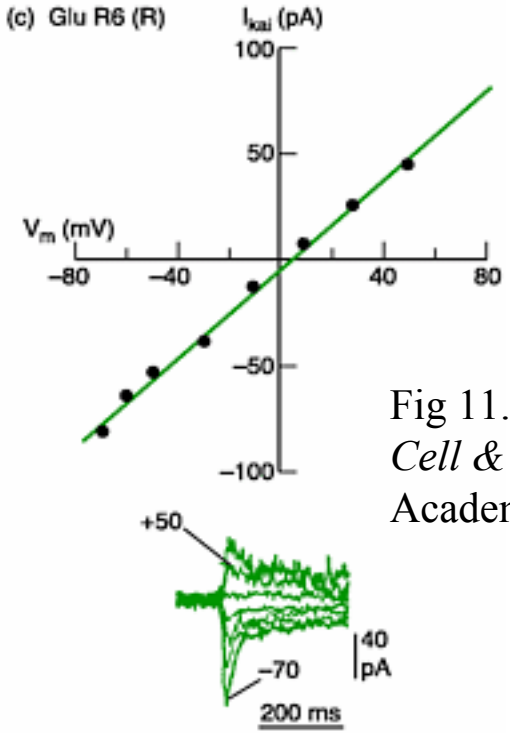
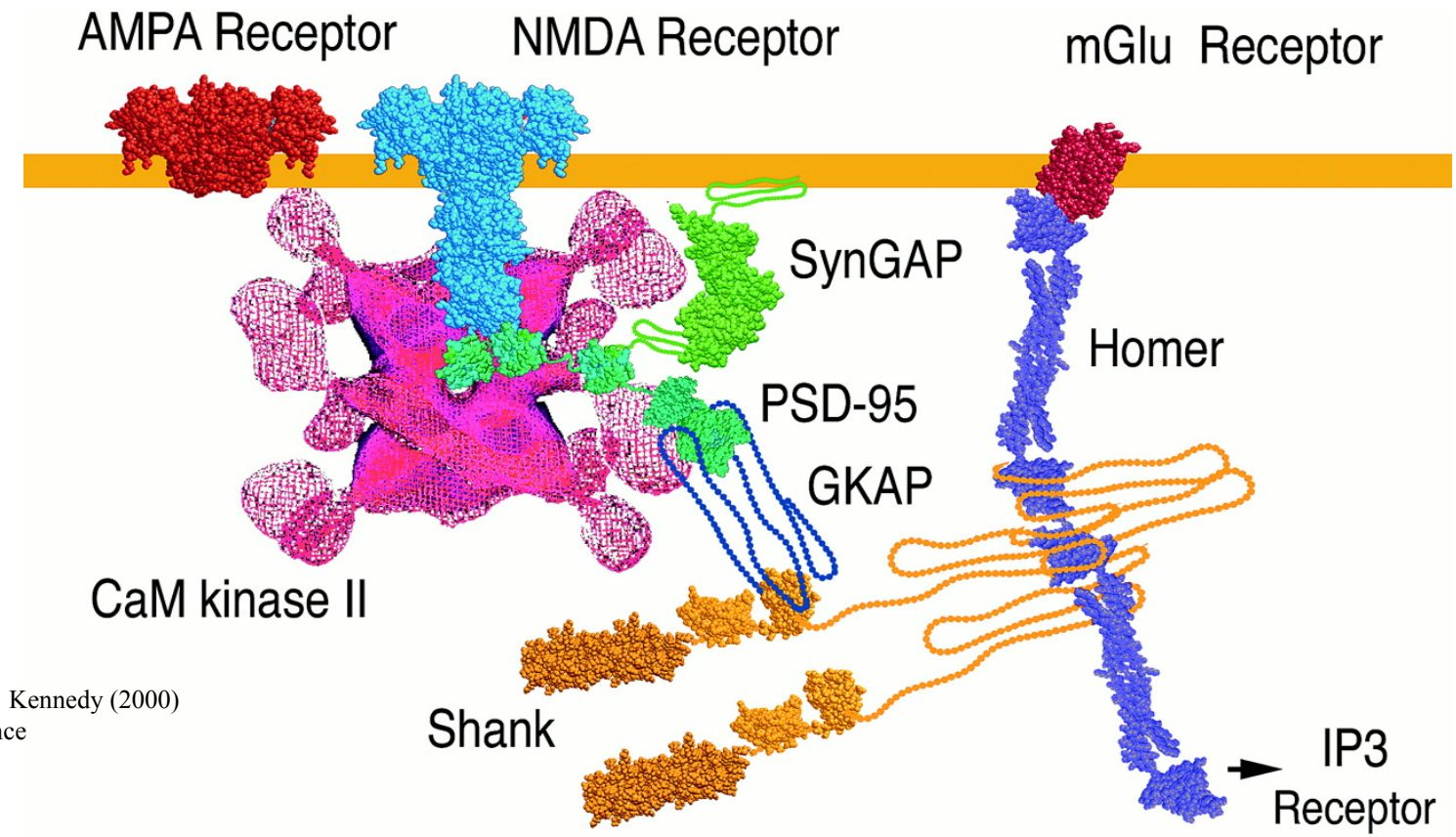


Fig 11.7. From HammondC. *Cell & Molecular Neurobiology* Academic Press 2001

Molecules of the Post-synaptic Density Drawn ~to Scale

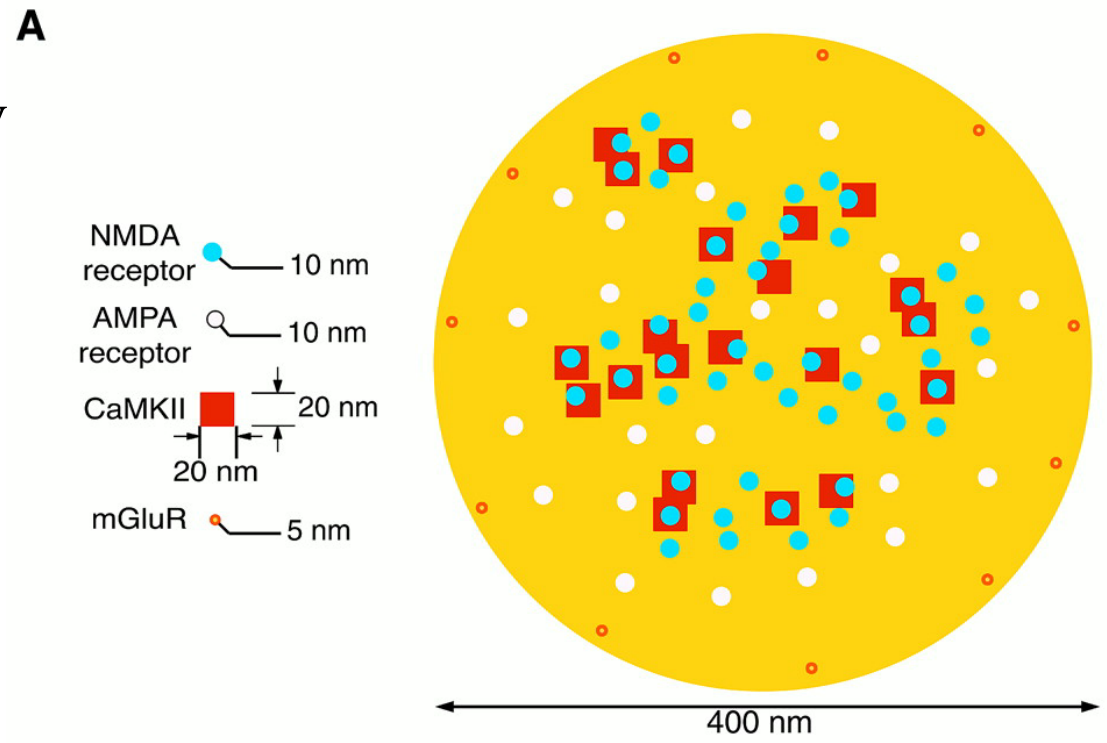


From: Kennedy (2000)
Science

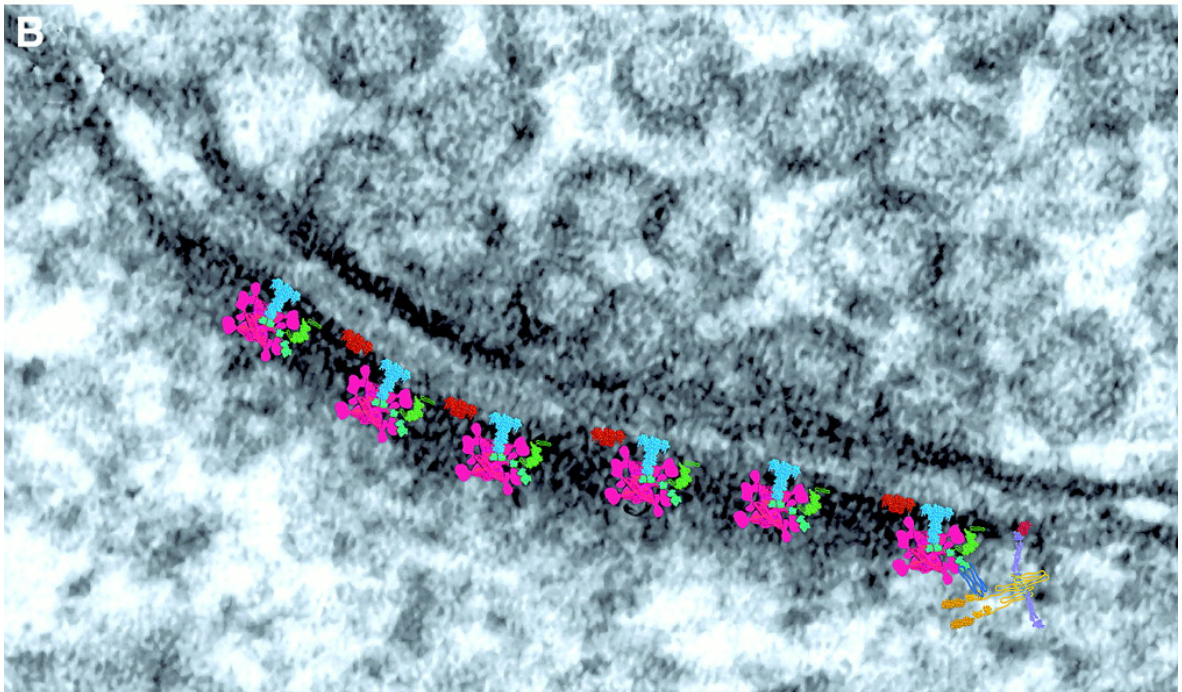
Note: Kainate receptors can also bind to PSD-95

The Post-Synaptic Density At Glutamate Synapse

En face view



Cross-sectional View



Kennedy, M (2000) Science

LECTURE 2:
Vertebrate Systems For Studying Plasticity

MCP’2003

THE HEBB RULE

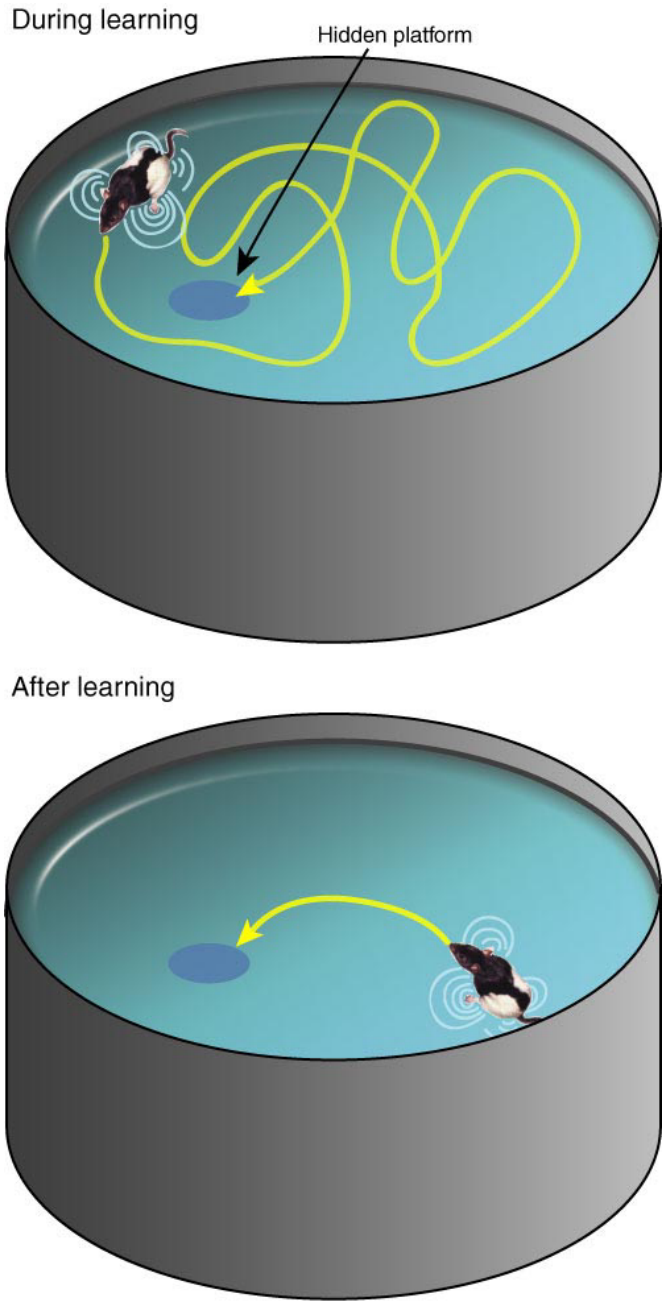
When an axon of cell A is near enough to excite cell B or repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased.

D.O. Hebb, 1948

NEURONS THAT FIRE TOGETHER WIRE TOGETHER

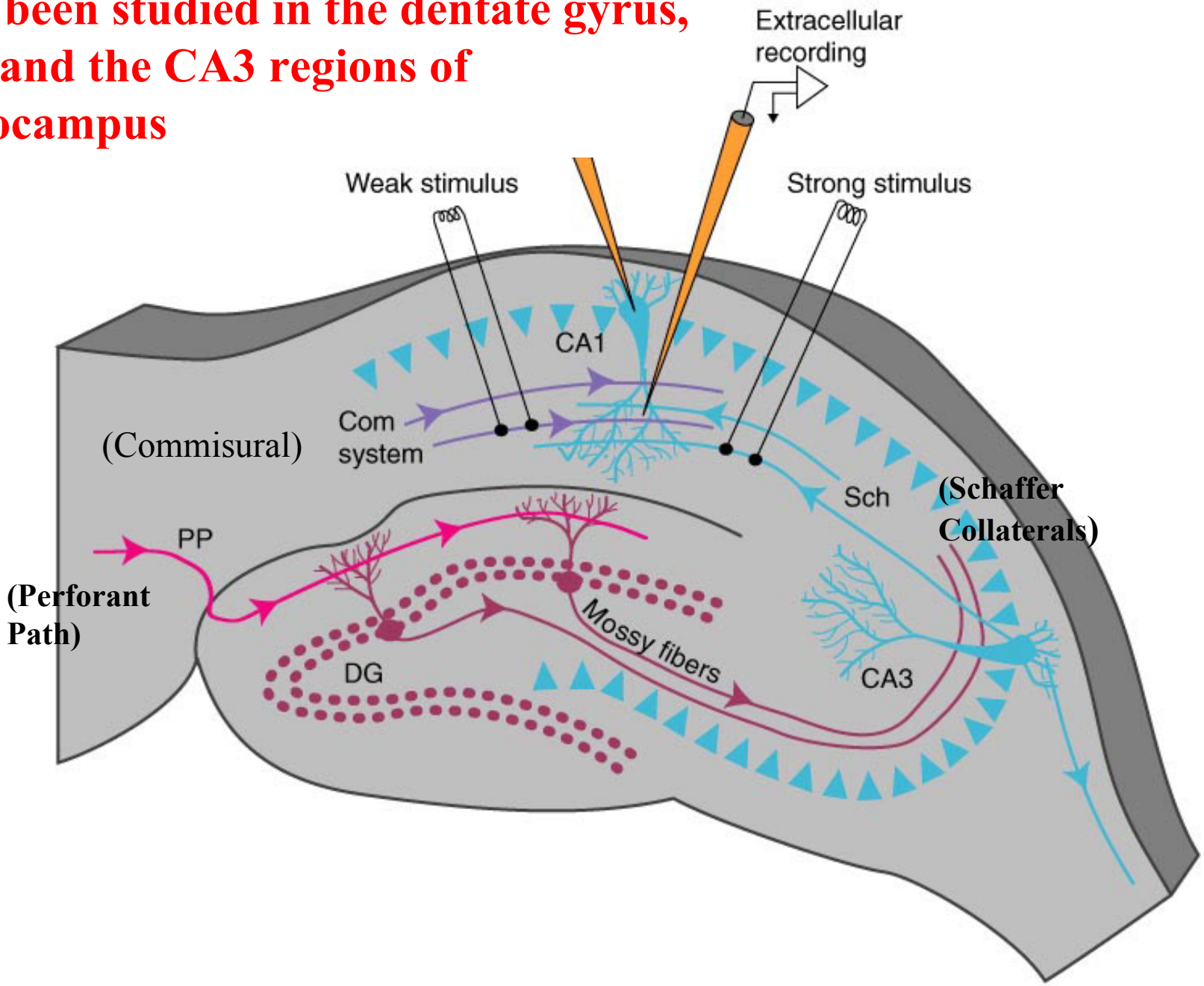
Mice and rats learn to associate the position of a hidden platform in a tank with landmarks in the surround.

Spatial learning is initiated in the Hippocampus.



From: Squire et al 2003,*Fundamental Neuroscience*

LTP has been studied in the dentate gyrus, the CA1 and the CA3 regions of the hippocampus

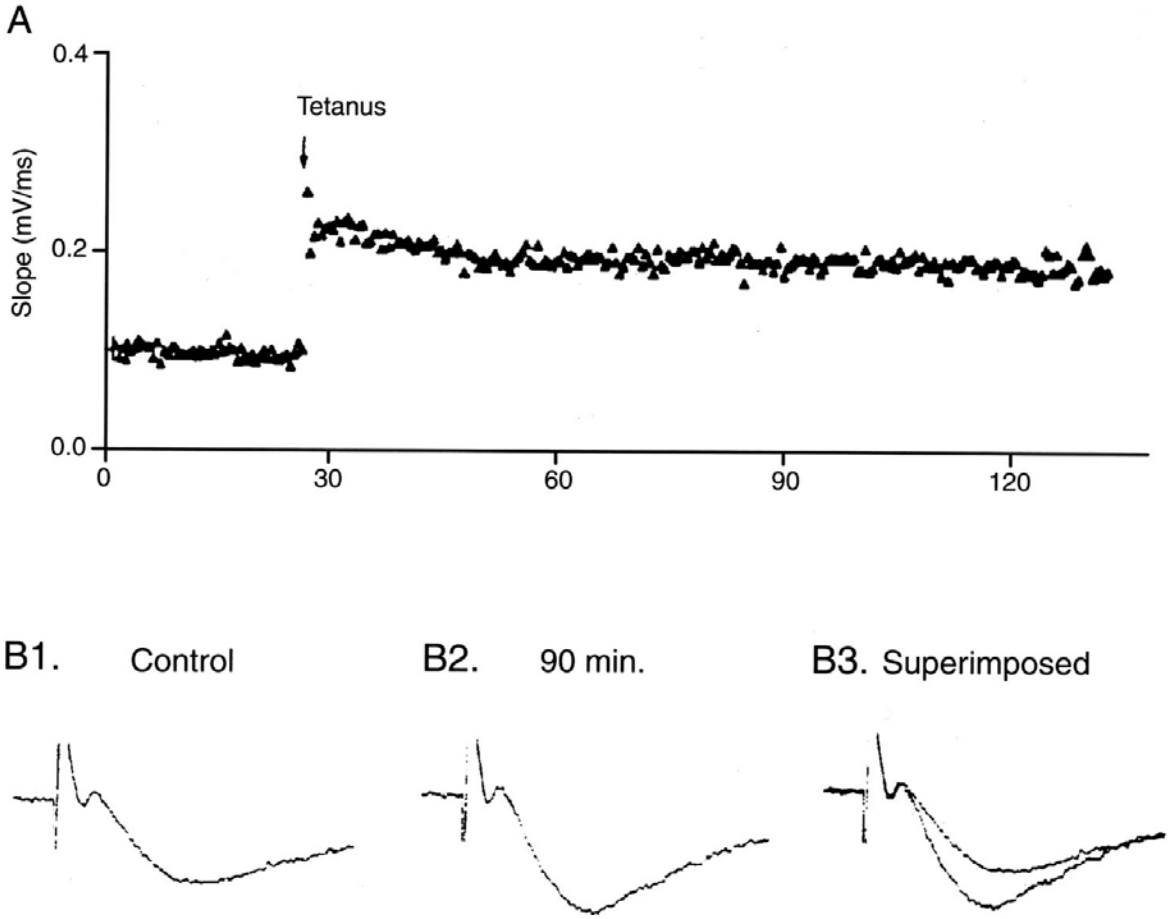


Multi-Input LTP is Measured As A Change in an Extracellularly Recorded Field Potential

Give high frequency stimulation.

Record field potential.

Measure change as as an increase in the slope of the field potential.



Long Term Potentiation Can be Associative. This is a Heterosynaptic Interaction.

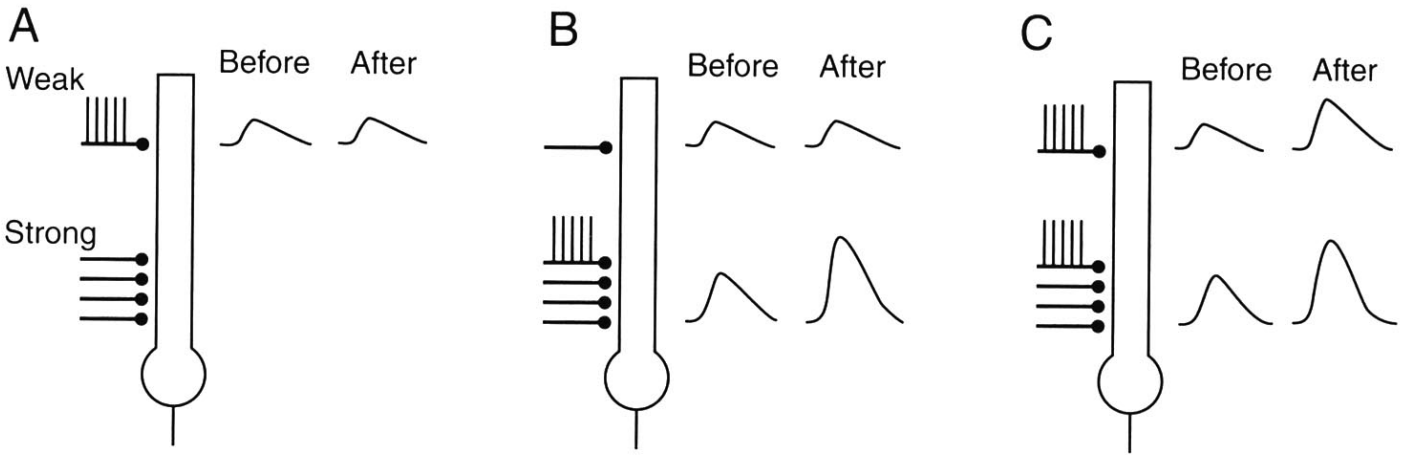


FIGURE 50.10 Features of LTP at CA3–CA1 synapses in the hippocampus. A single hippocampal pyramidal cell is shown receiving a weak and strong synaptic input. (A) Tetanic stimulation of the weak input alone does not cause LTP in that pathway (compare the EPSP before and after the tetanus). (B) Tetanic stimulus of the strong input alone causes LTP in the strong pathway, but not in the weak pathway. (C) Tetanic stimulation of both the weak and the strong pathway together causes LTP in both the weak and the strong pathway. Modified from Nicoll *et al.* (1998).

1.NMDAR dependent
2. Lasts hours in vitro
3. Requires post-synaptic Ca++ increase

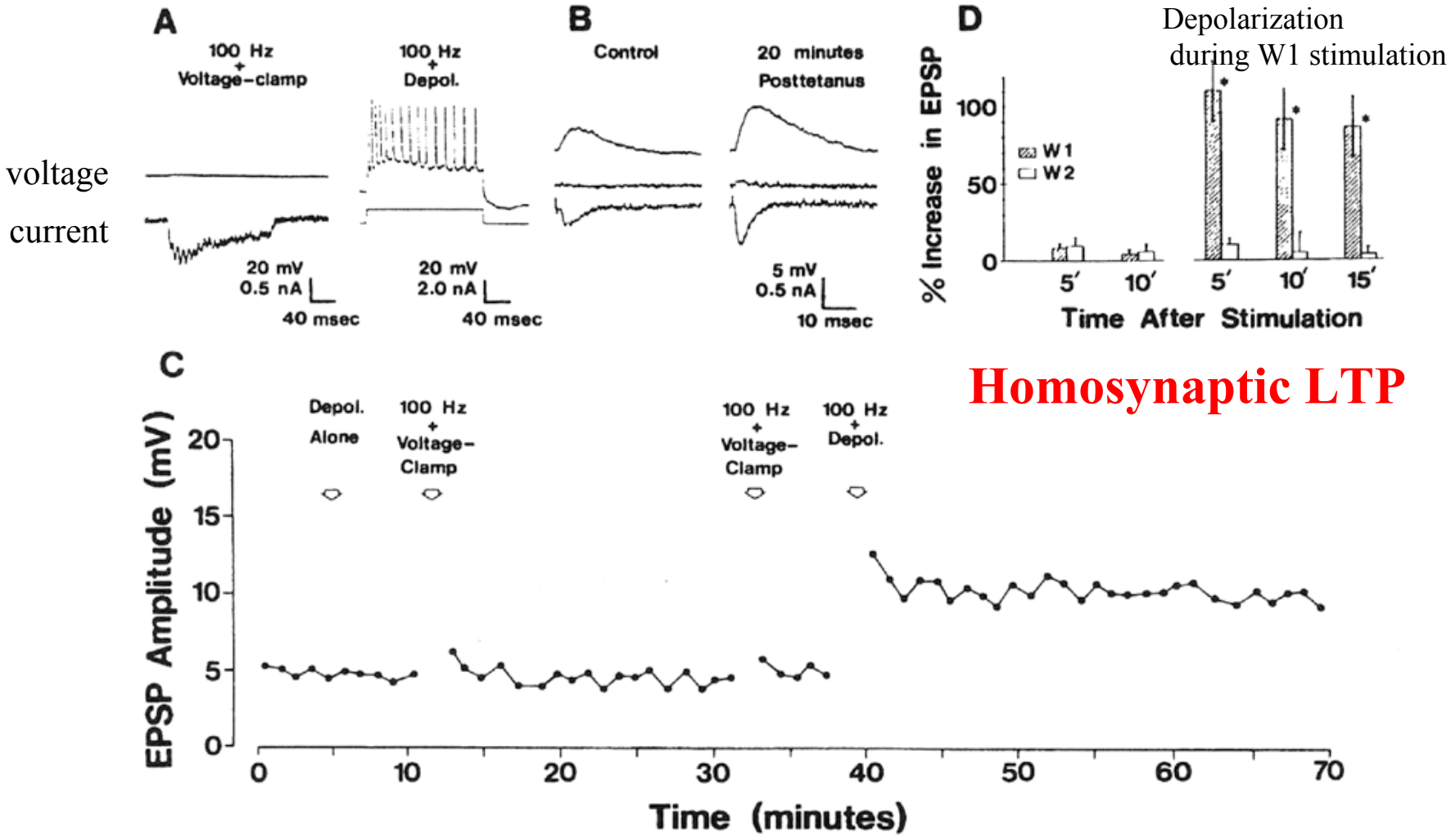
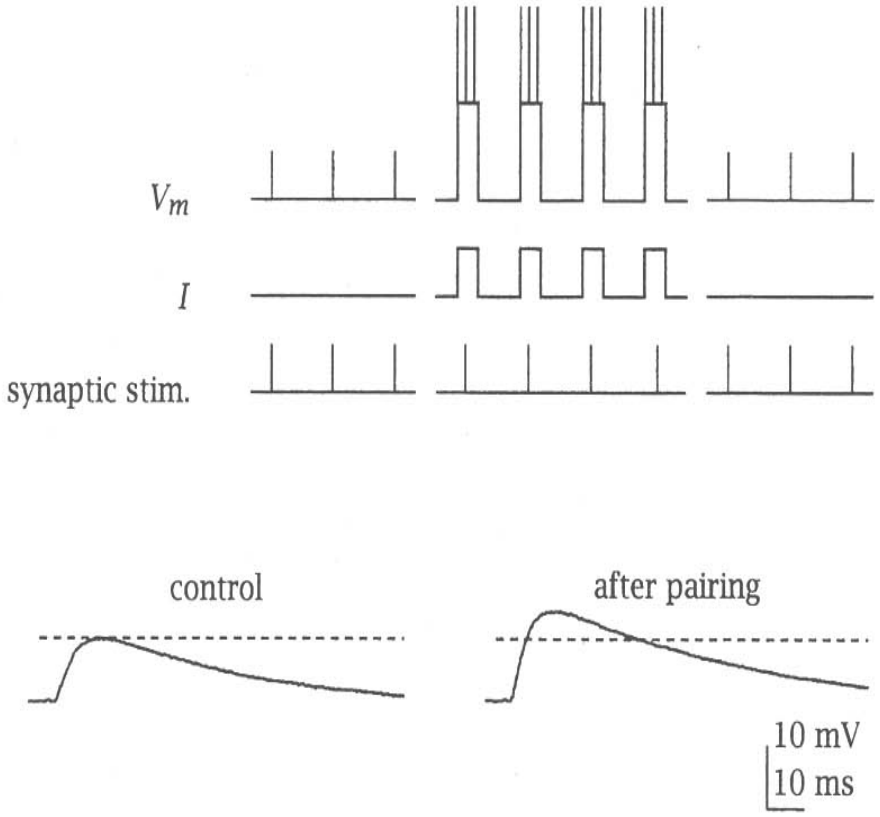


Figure 15.10 Experiment that tested the Hebbian rule for synaptic plasticity. (A) HFS (100 Hz) given under voltage clamp (left) and HFS given with a depolarizing current injection (right). (B) The EPSPs and EPSCs before and 20 min post tetanus are indicated. LTP was elicited when HFS was given in conjunction with postsynaptic depolarization. (C) EPSP amplitude is plotted as a function of time for the different stimulus protocols. (D) Summary data are shown from two pathways (W1 and W2) at different times following HFS given separately to each pathway. On the left the mean increase in the EPSP when a voltage clamp was applied to the postsynaptic cell during HFS is indicated. On the right is the same experiment except that a depolarizing current pulse was given to the cell during the HFS to W1. (From Kelso et al. 1986.)

REQUIREMENTS FOR LTP: DEPOLARIZATION PLUS SUPERIMPOSED EPSP'S

This is often used on young synapses.

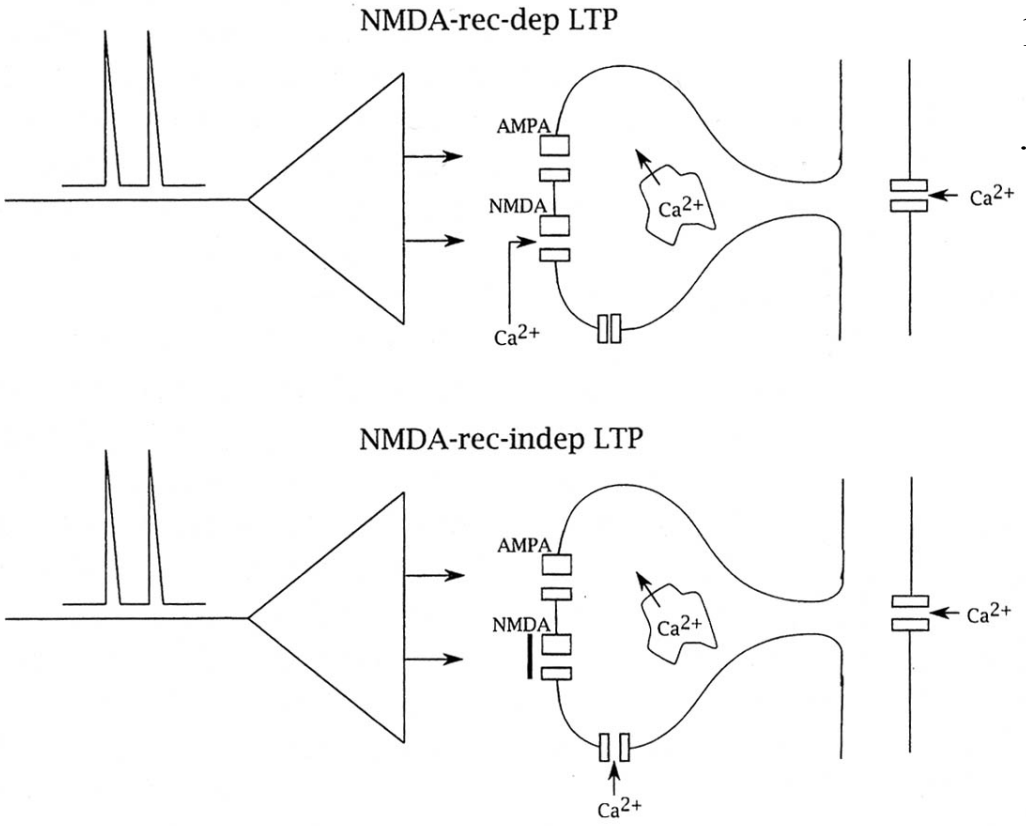


At young synapses afferents cannot follow high frequency bursts of activity.

Frequently used to convert silent synapses to active synapses.

Figure 15.11 Pairing postsynaptic depolarization with a single weak stimulus (with the pairing repeated every 20–30 sec) induces LTP at some synapses.

LTP at Glutamate Synapses can be NMDA receptor dependent or NMDA receptor independent



NMDAR dependent LTP

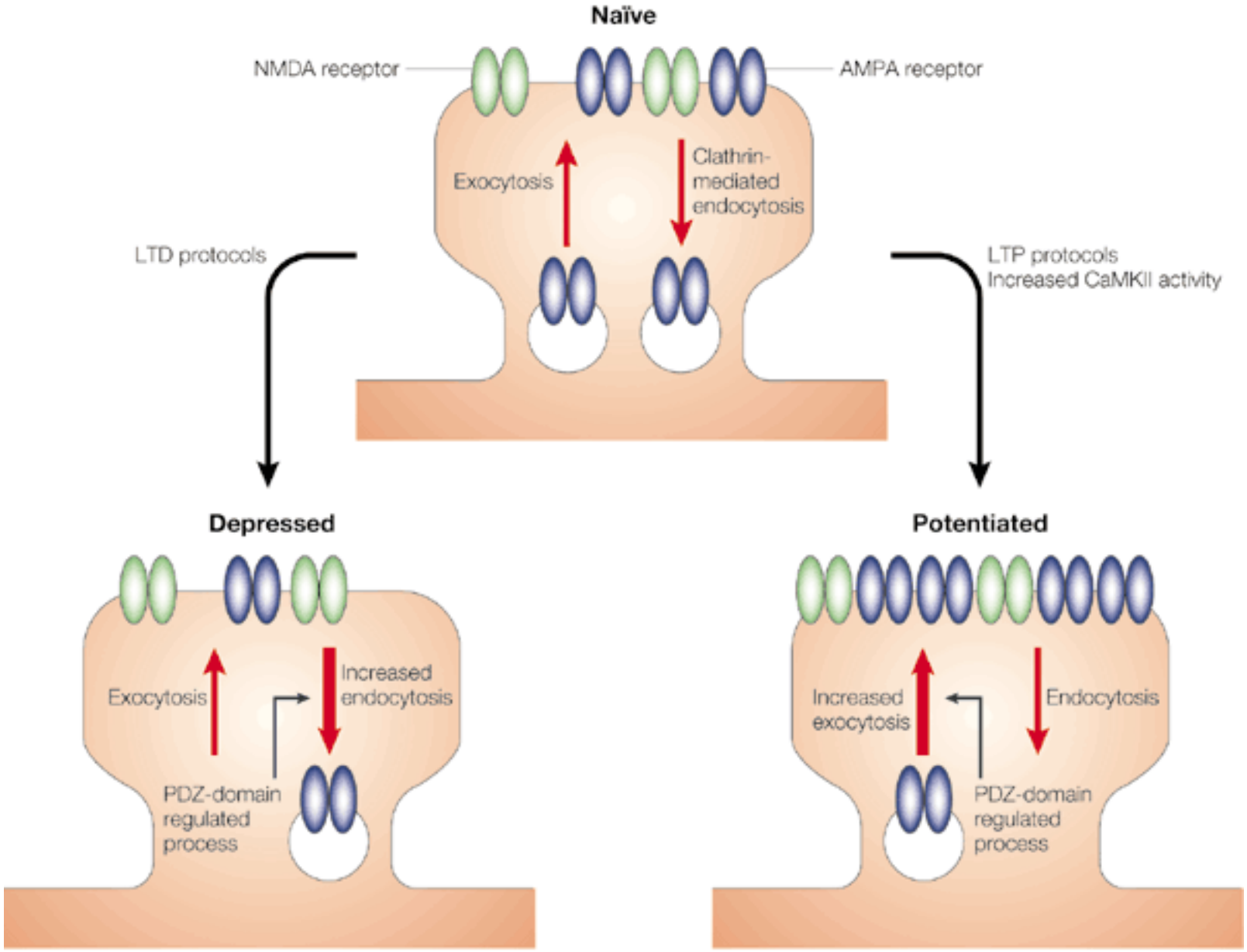
1.Increases in AMPAR function.

., LLTP increases in frequency of mini AMPAR currents

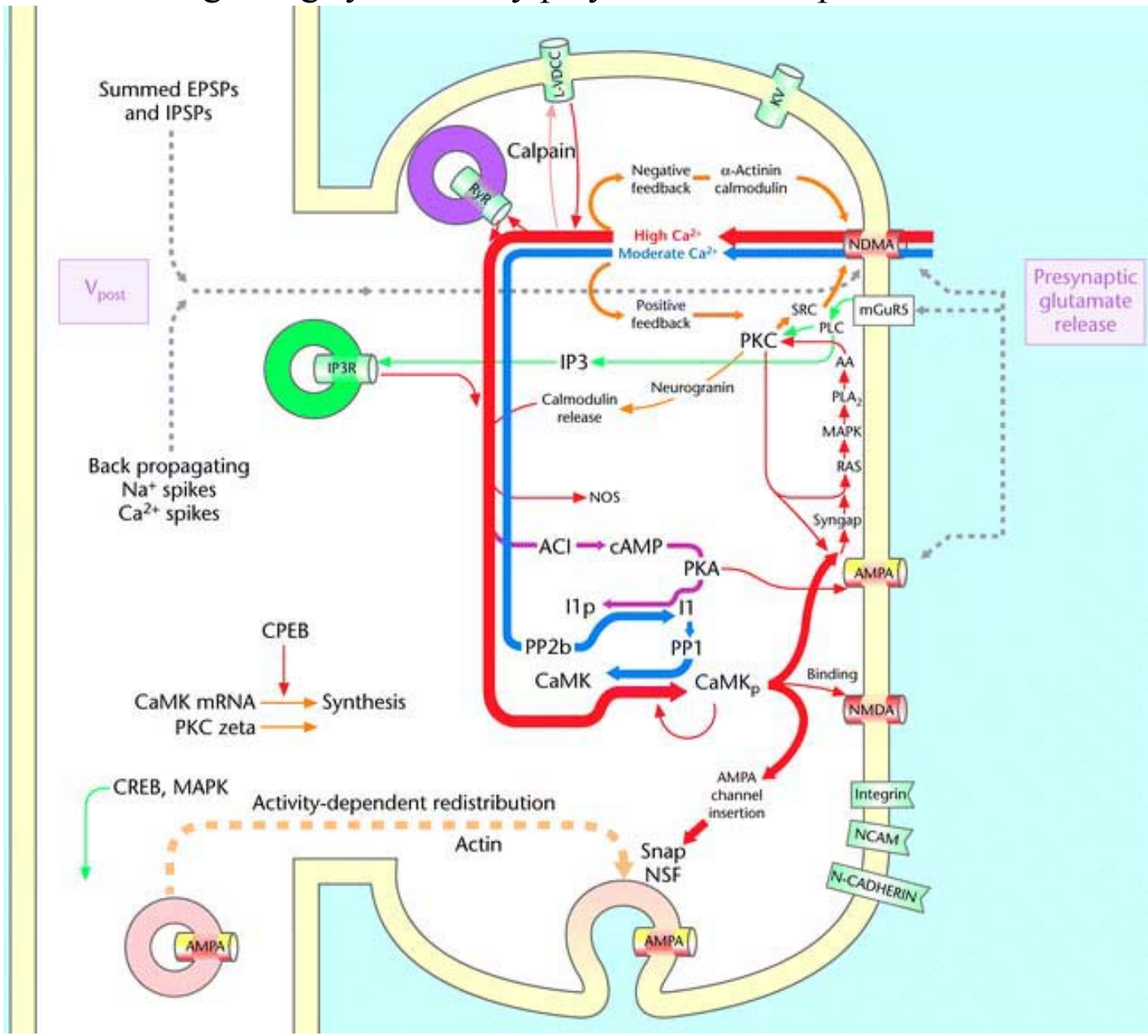
3. Decrease in probability of failure when 1 input is stimulated.

Or, as likely in mossy fiber to CA3 dendrite LTP, the effect can be pre-synaptic effected by a Ca++ sensitive adenylyl cyclase that activates PKA and facilitates synaptic vesicle recycling thereby increasing synaptic release.

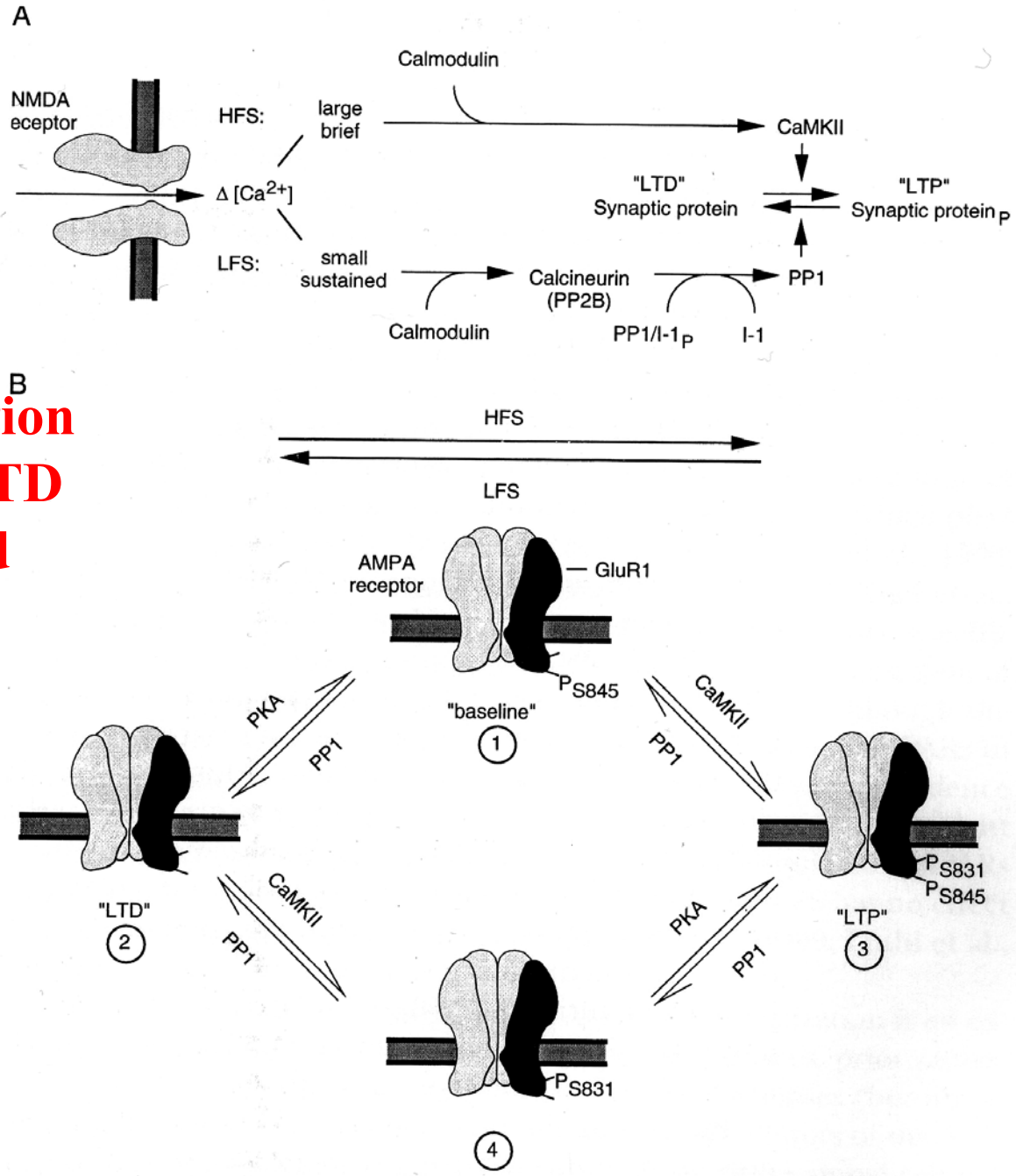
NMDAReceptors Control AMPA receptor expression and internalization



Nothing in a neuron is simple. At different synapses showing
NMDA dependent LTP different signaling systems may play more or less prominent roles.



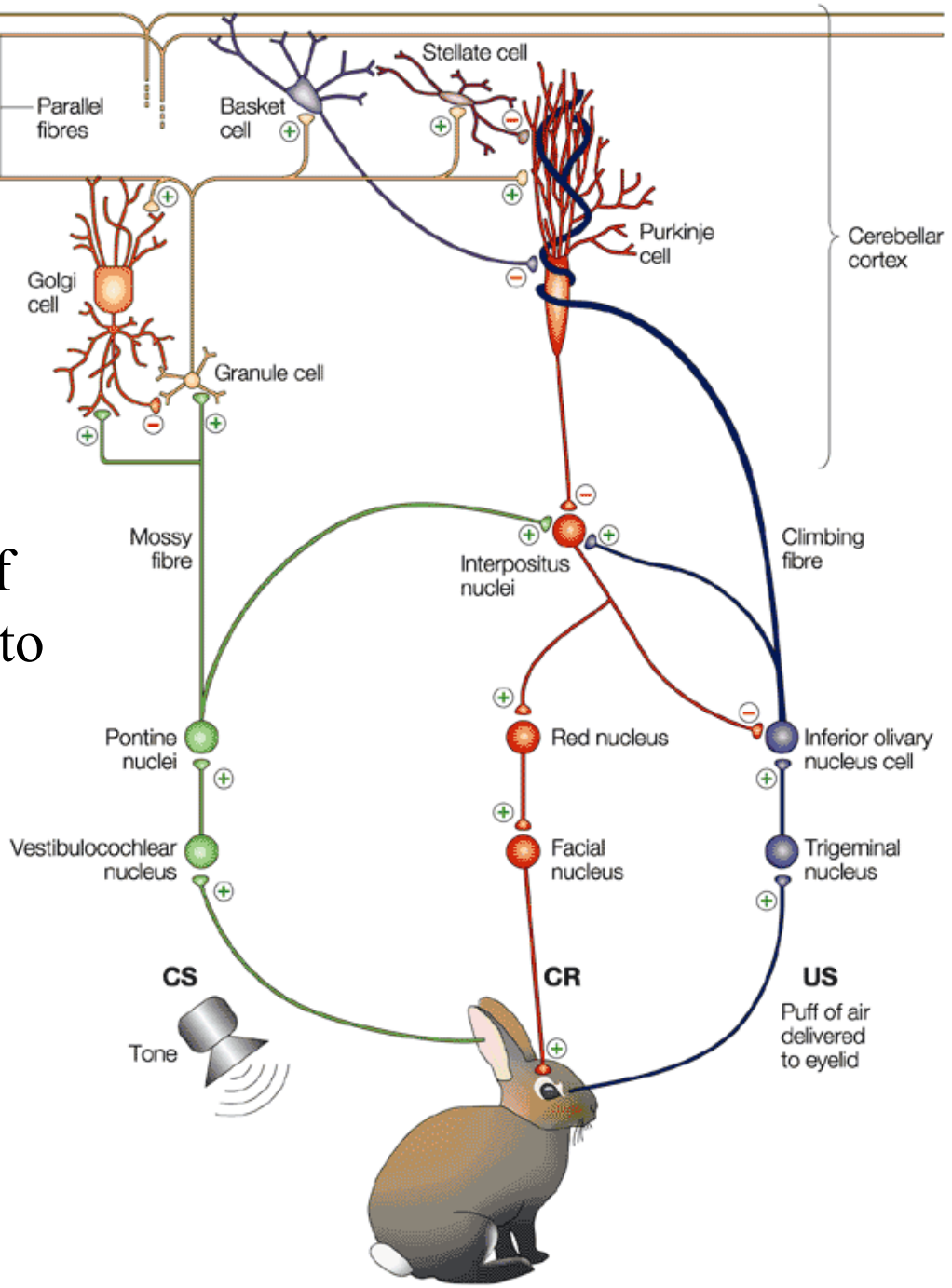
Proposed Phosphorylation Model For LTP and LTD In Hippocampus and Cortex



Eye-Blink Conditioning
(motor learning)

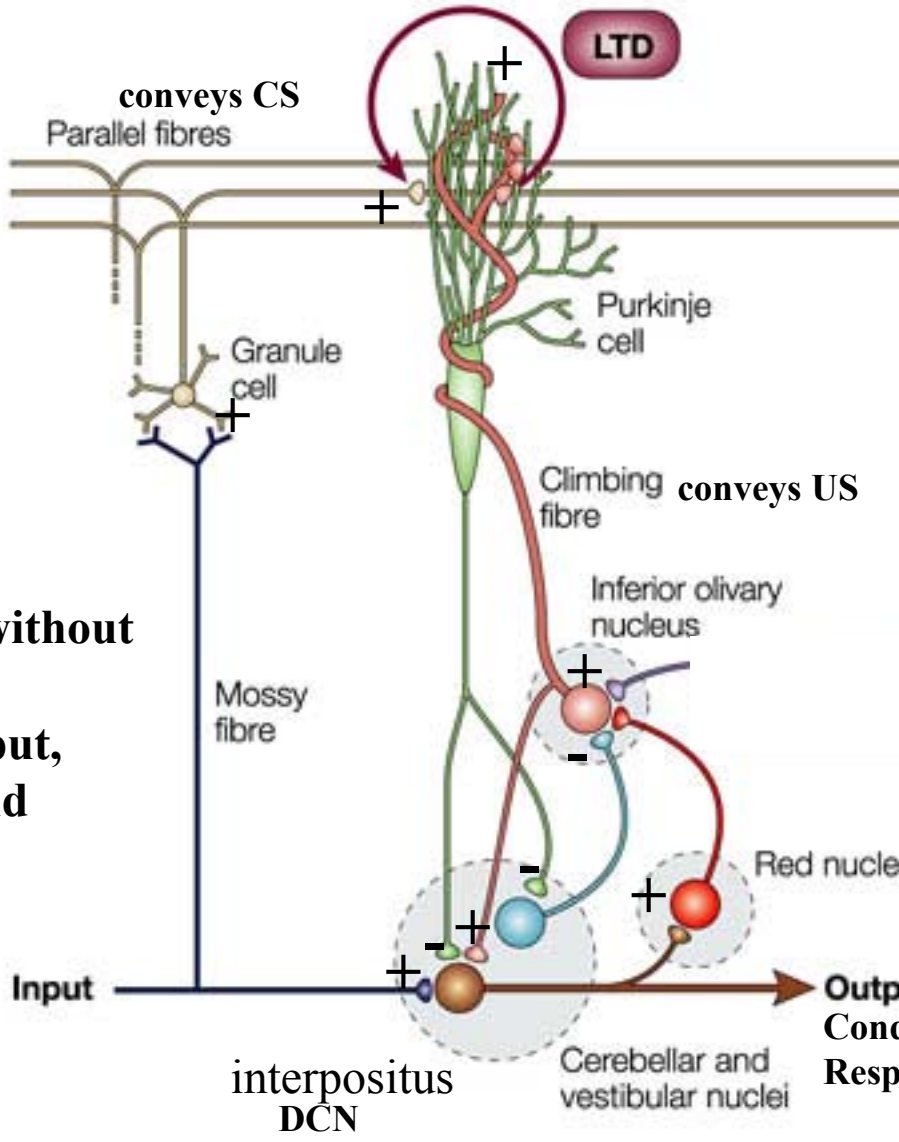
A puff of air to the cornea causes a reflexive eye-blink.

Pairing a tone to the air puff soon entrains the eye-blink to the tone alone.



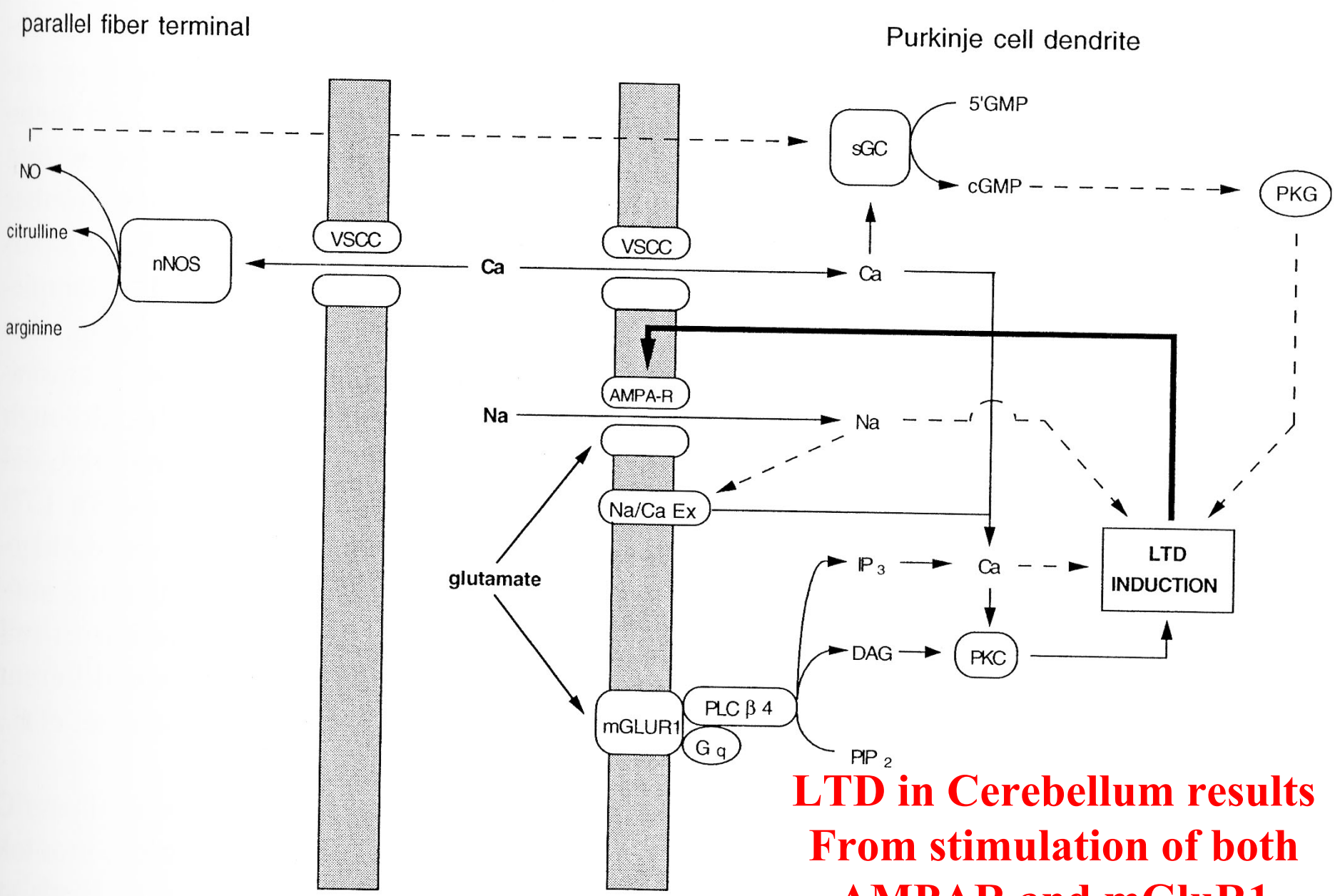
PF and CF activated together at low frequency (~ 4Hz) cause Long Term Depression at PF-PC synapse. This reduces output of the PC causing decreased inhibition of the conditioned response in that nucleus and increases output of the conditioned response.

If the CS were continually stimulated without the US the PF to PC synapse would potentiate leading to increased PC output, increased PC inhibition in the DCN, and extinction of the conditioned response.



1,400 synapses from 1CF / PC;(1 CF innervates ~ 10 PC)

200,000 different granule cell PF synapses/PC



LTD in Cerebellum results From stimulation of both AMPAR and mGluR1 Receptors

Long, long, term changes in synaptic strength require protein synthesis and probably reflect the development of new synapses.

Activity can trigger transcription cascades and lead to activation of immediate early genes (cfos,cjun,zif, ZENK).

Many activity sensitive genes have cyclic AMP responsive elements in their regulatory regions.

Synaptic Ca++ influx causes the phosphorylation of cyclic AMP responsive element binding protein (CREB) in the nucleus permitting it to dimerize, interact with with both the CRE element and CREB binding protein (CBP) and initiate transcription.