Highlights from 1st MCP lecture

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 valence of the ion
F= the Faraday

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
(58/2)log [1.5mM]/ [.0001mM]= +121 mV

Multiply by 2.3 to convert base 10 log
$(RT/zF) = 58/z$ mV. At 37 Co $(RT/zF) = 68/z$ mV
z is + for a positive ion and - for a negative ion

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

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.

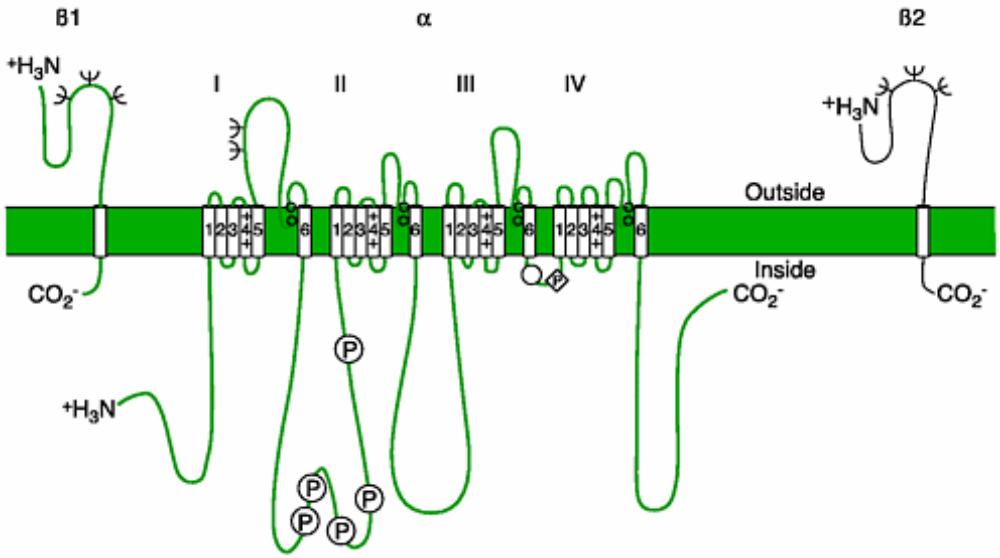
Identification by Pharmacology

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

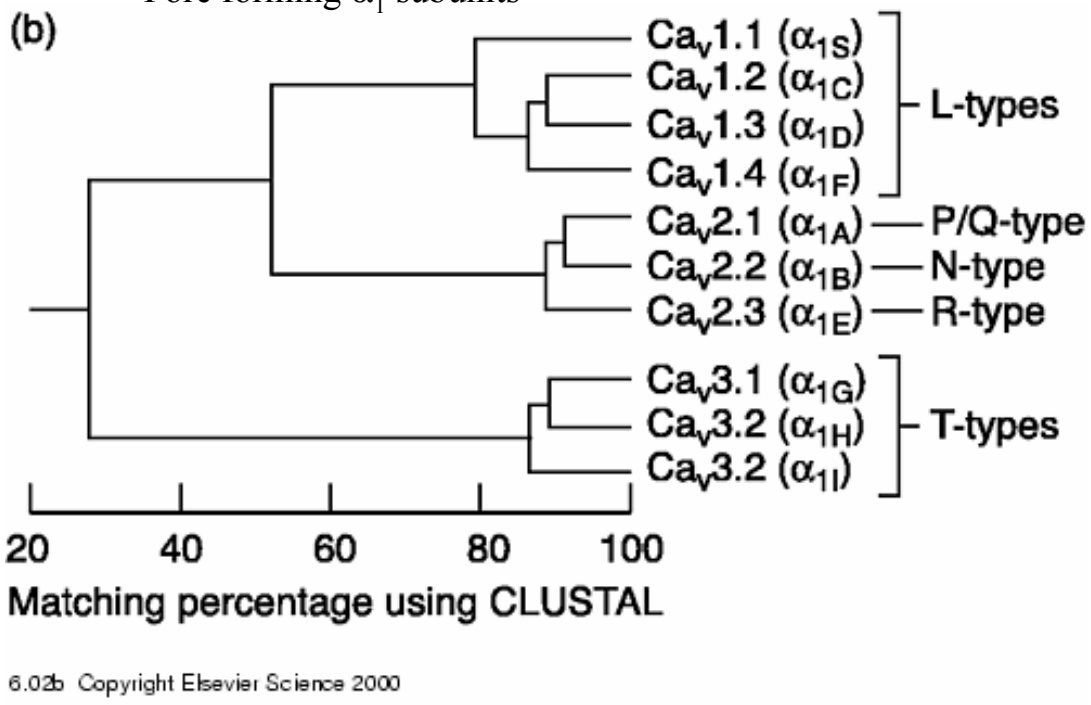
N-type channels- conus toxin ω-conotoxin (antagonist)

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

Subunits of Voltage Gated Ca++ Channels

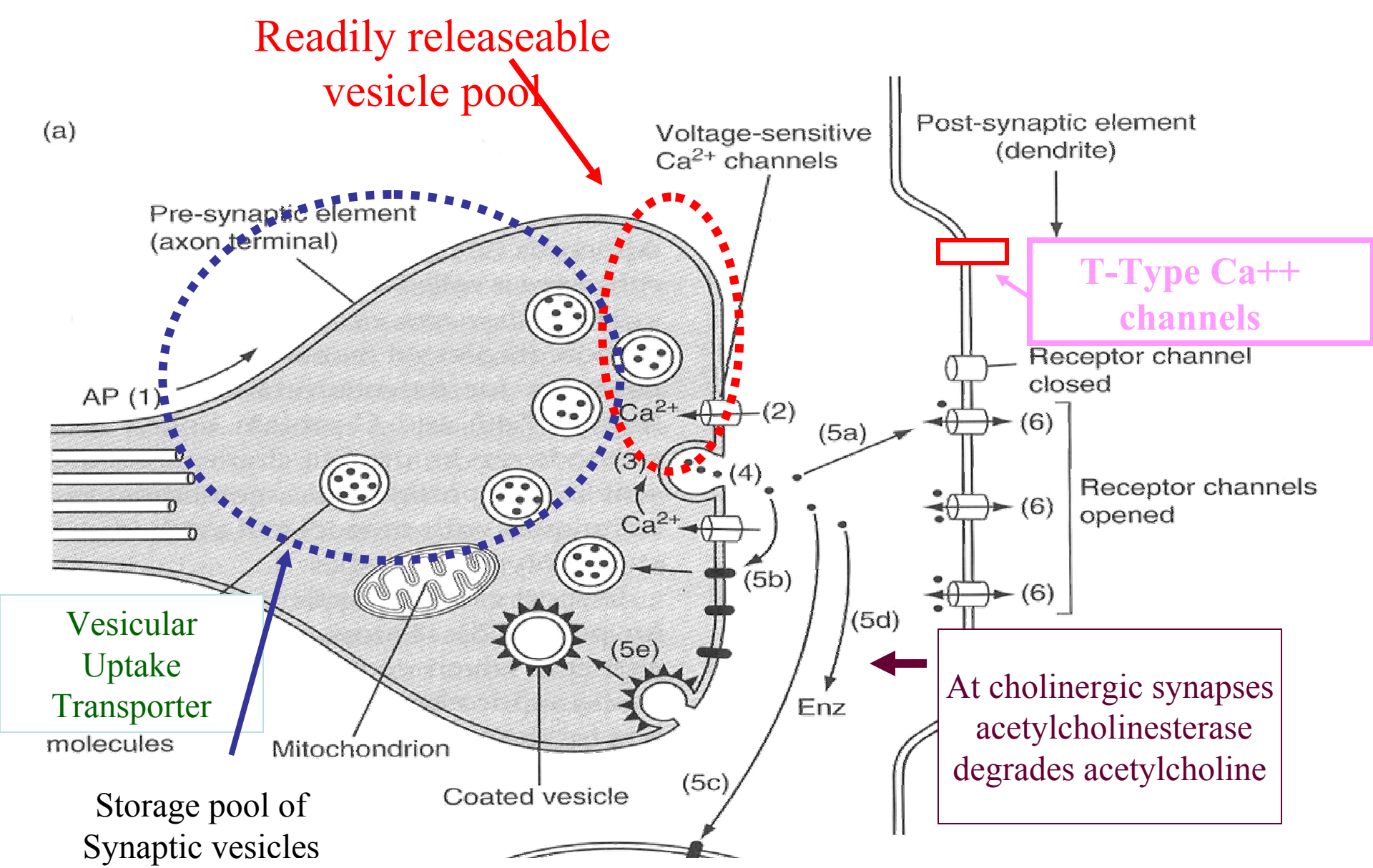


Pore forming α_1 subunits

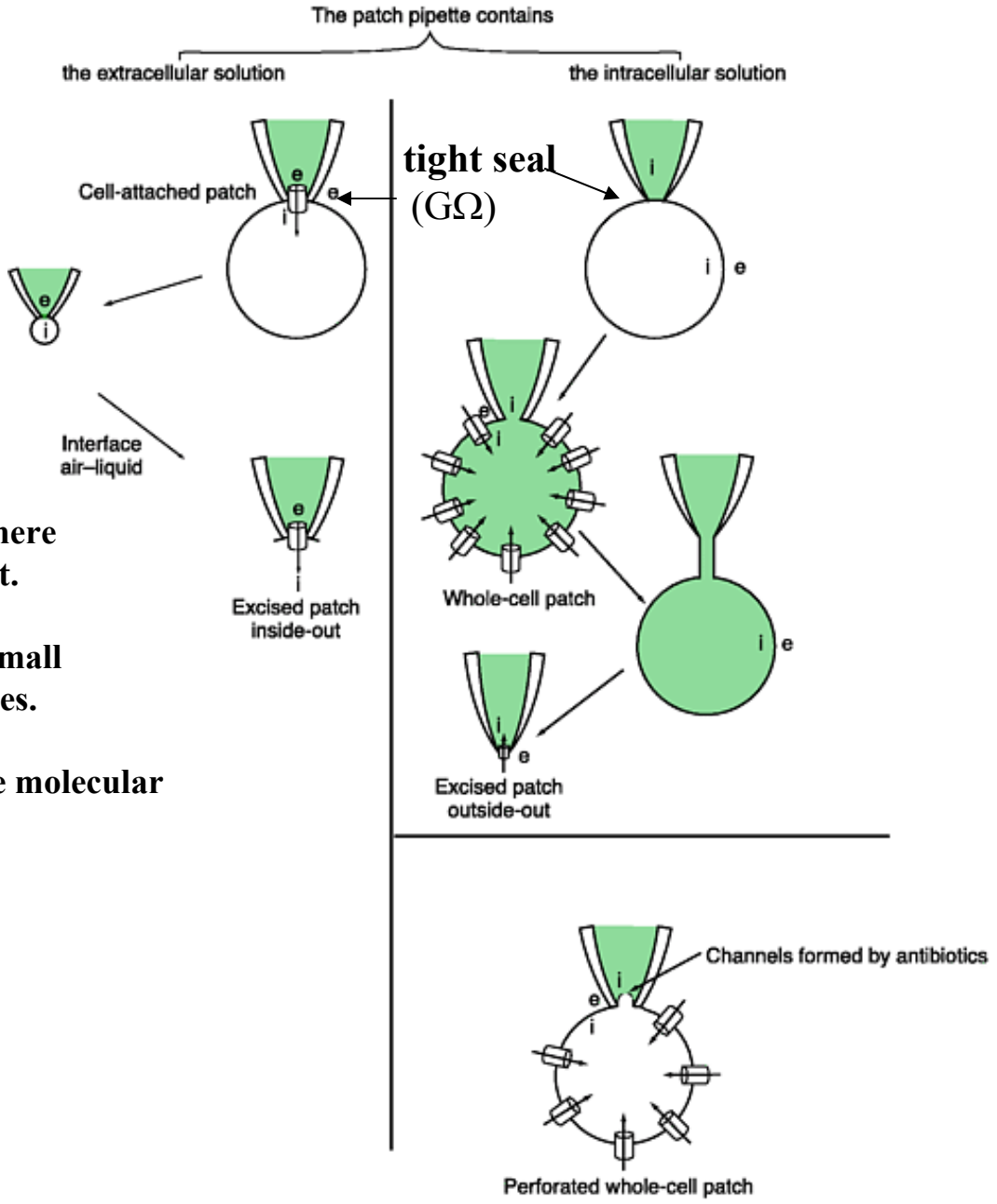


L-type Ca++ channels require phosphorylation by PKA in order to maintain functions

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)
(3)
(4)
(5a)
(6)
Receptor channels opened
Ca^{2+}
(5b)
(5d)
(5e)
Enz
Vesicular Uptake Transporter molecules
Mitochondrion
Coated vesicle
(5c)
At cholinergic synapses acetylcholinesterase degrades acetylcholine
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.**

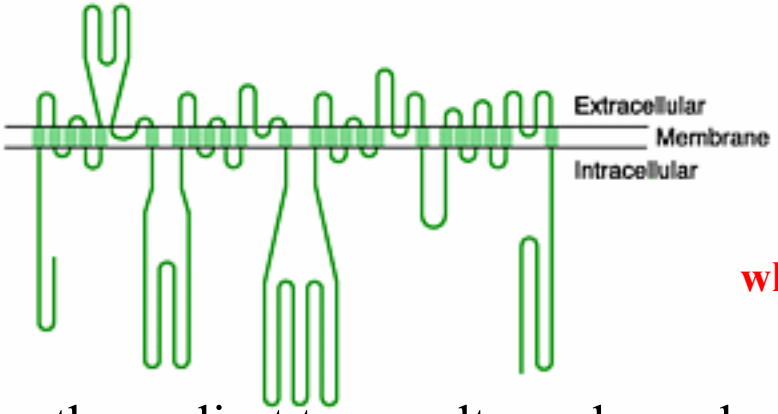
2. **Recordings can be made from cells too small to be implaed with intracellular electrodes.**

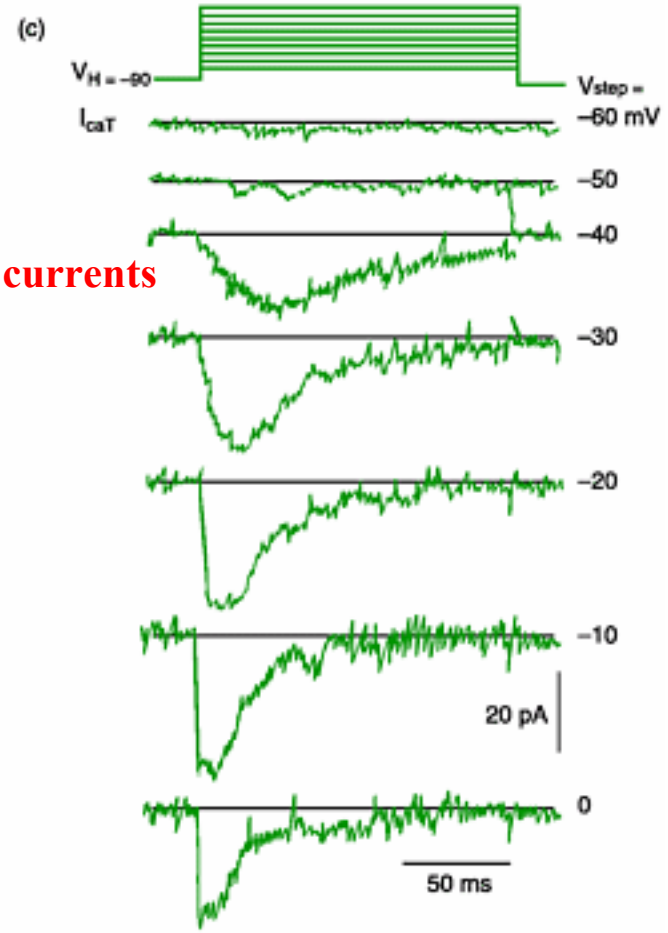
3. **Currents can be recorded through single molecular channels.**

* 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.





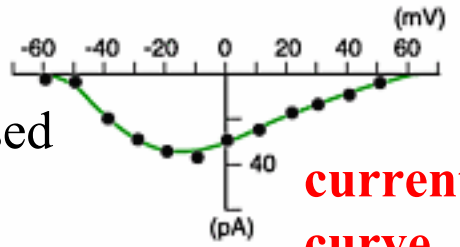
whole cell currents

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**

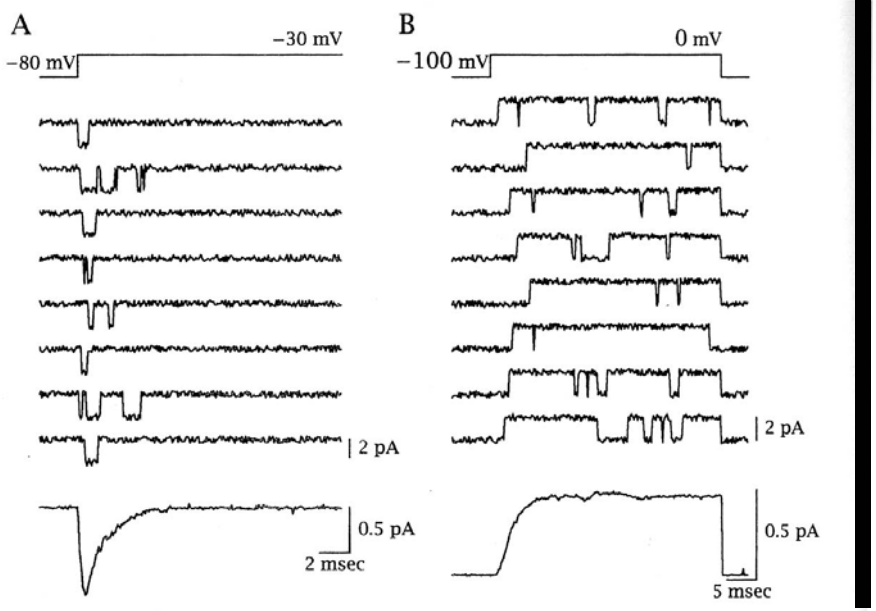


Figure 8.5 Unitary currents (upper 8 traces) and the ensemble average of unitary cur rents (lowest trace) of a Na^+ (A) and K^+ (B) channel. The membrane voltage is steppe from $V_H = -80$ mV to $V_c = -30$ mV for (A) and from $V_H = -100$ mV to 0 mV for (B Records in (A) are simulated after data from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

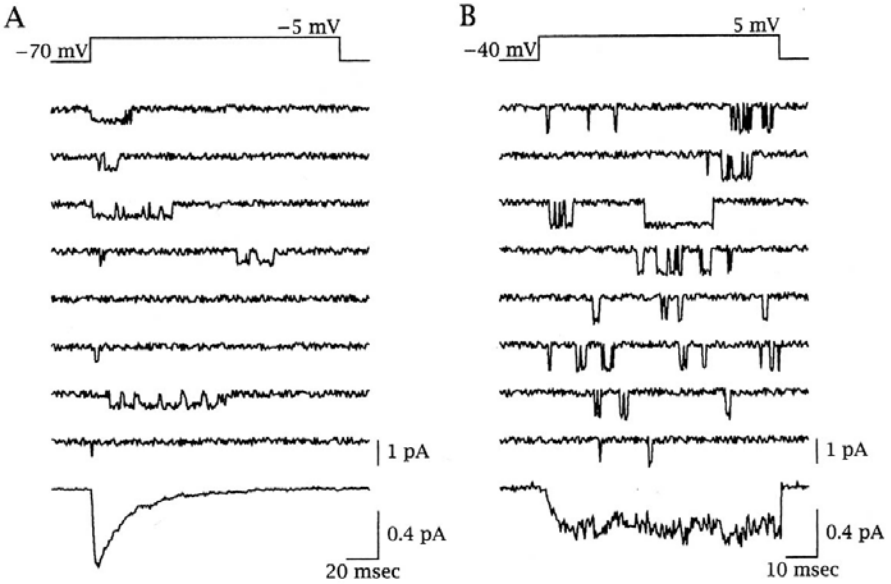


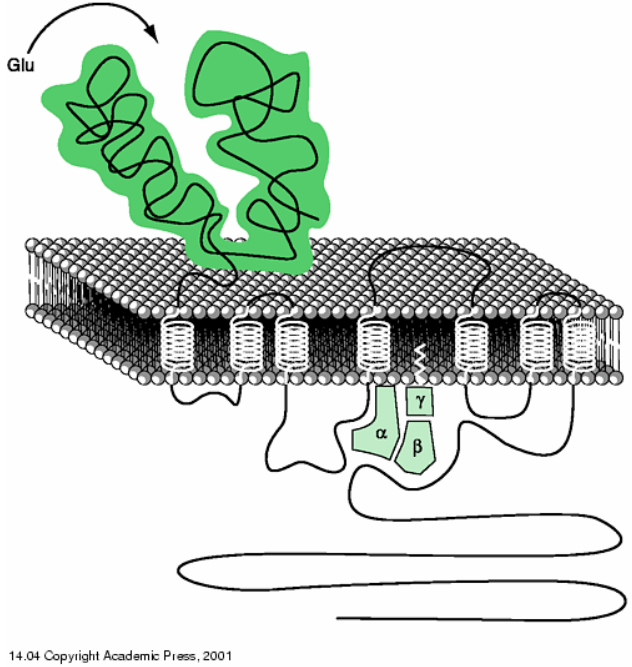
Figure 8.6 Unitary currents (upper 8 traces) and the ensemble average of unitary cur- rents (lowest trace) of a T-type (A) and L-type (B) calcium channel. The membrane voltage is stepped from $V_H = -70$ mV to $V_c = -5$ mV for (A) and from $V_H = -40$ mV to $V_c = 5$ mV for (B). Records are simulated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

Metabotropic Glutamate Receptors

G-protein linked receptors



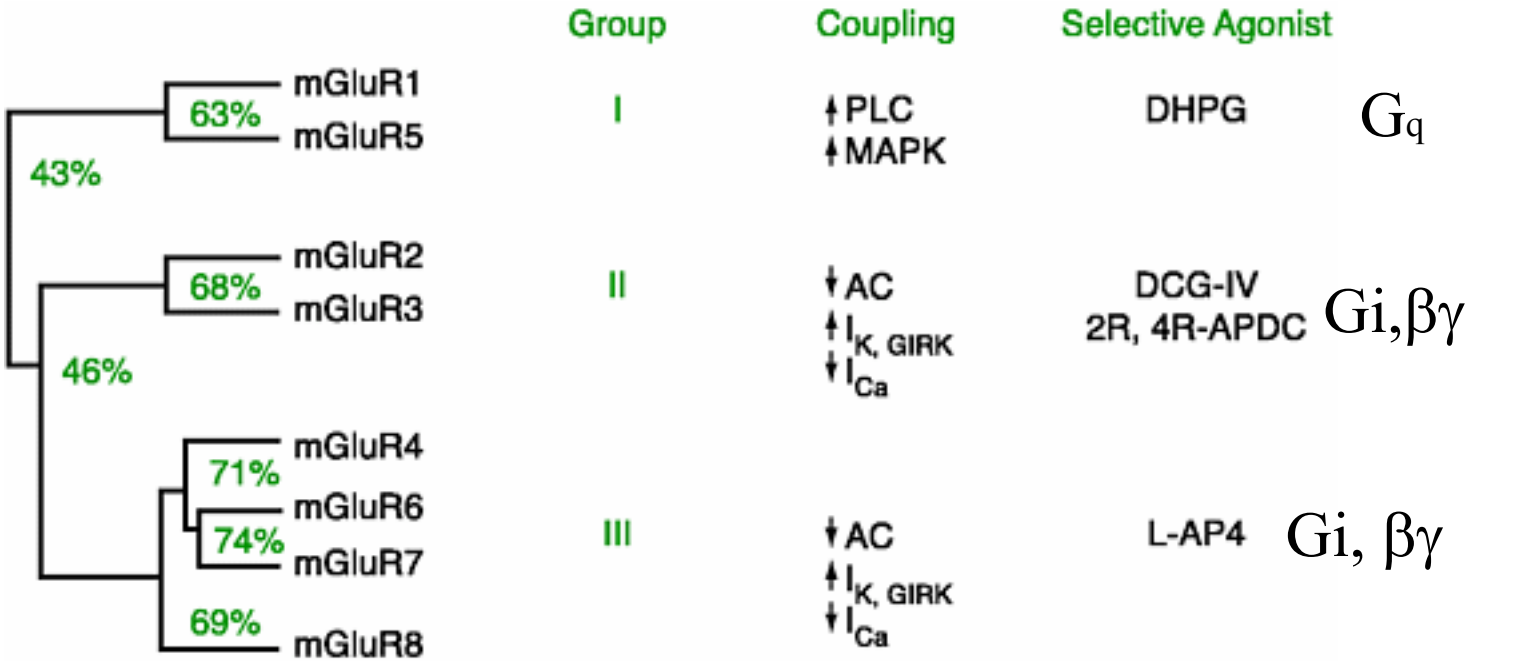


Fig 14.1From HammondC. *Cell & Molecular Neurobiology* Academic Press 2001

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)

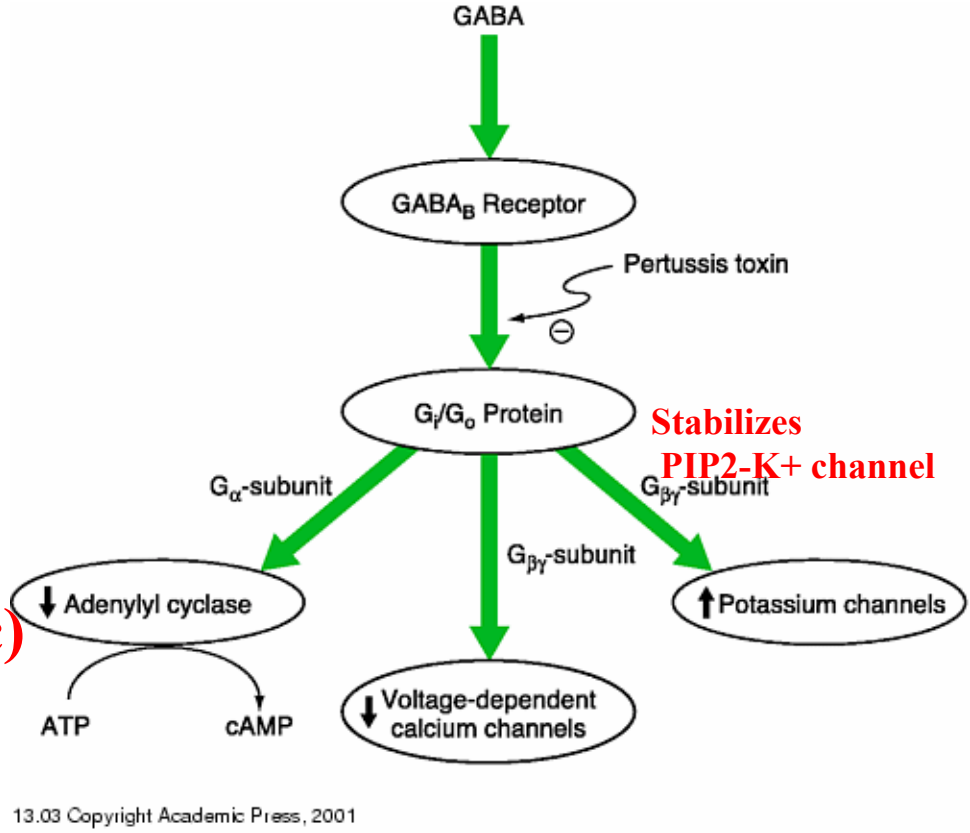
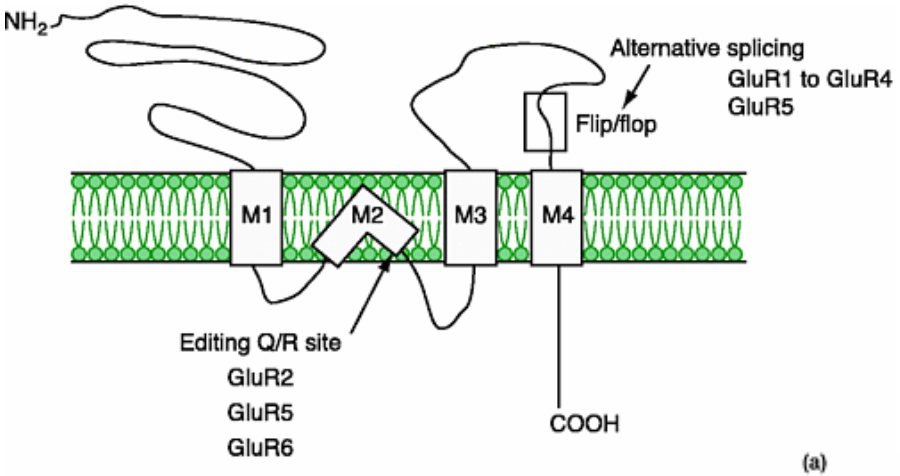
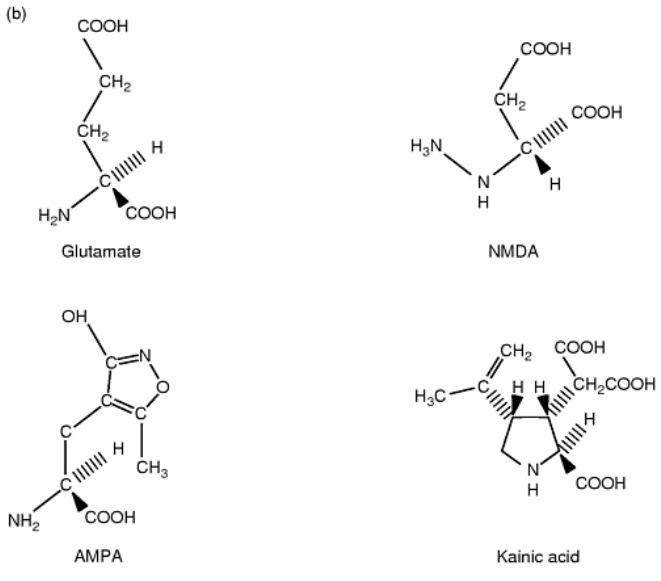
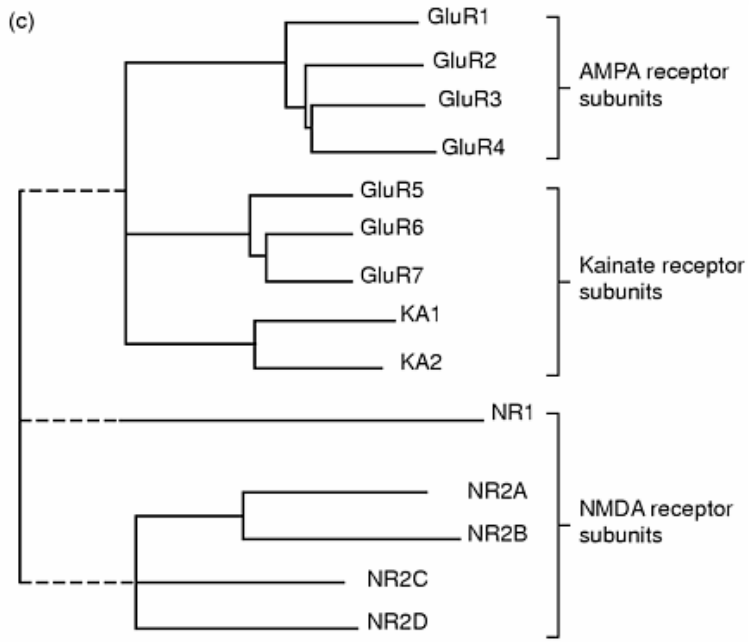


Fig. 13.3 From HammondC. *Cell & Molecular Neurobiology* Academic Press 2001

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.

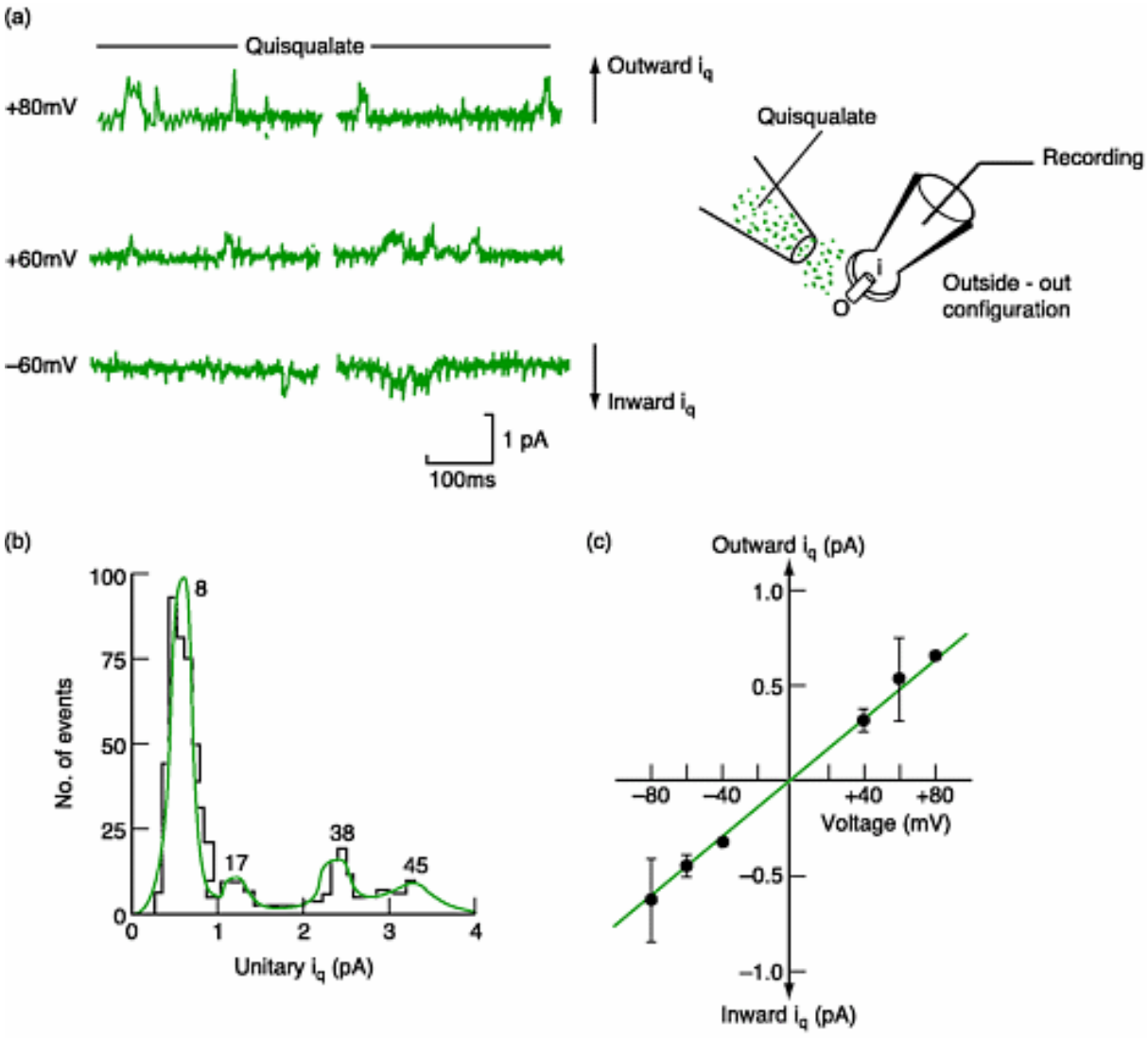
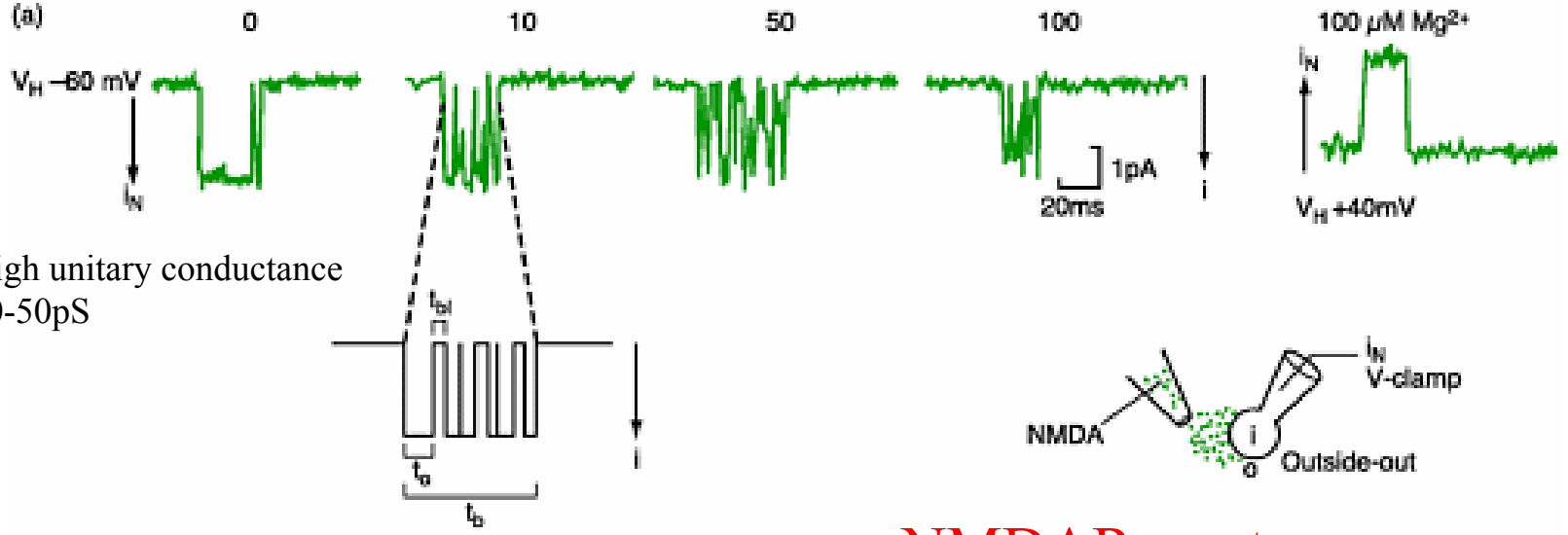
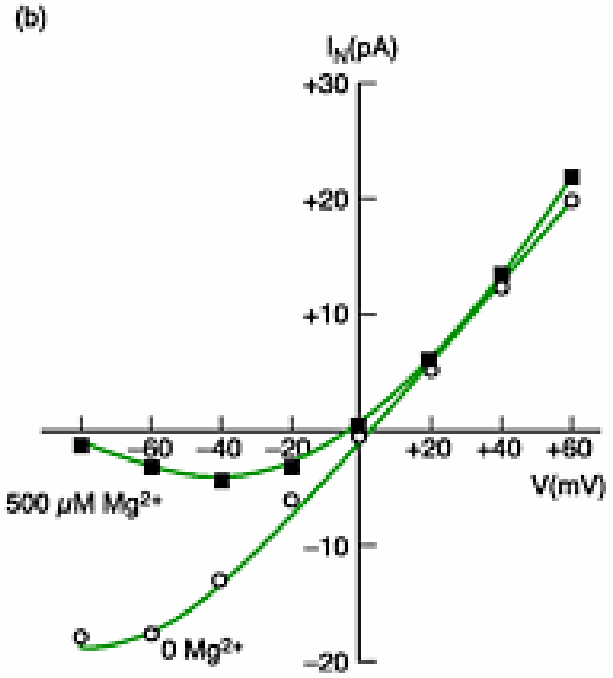


Fig 11.4 From HammondC. *Cell & Molecular Neurobiology* Academic Press 2001



High unitary conductance
40-50pS



NMDAReceptors

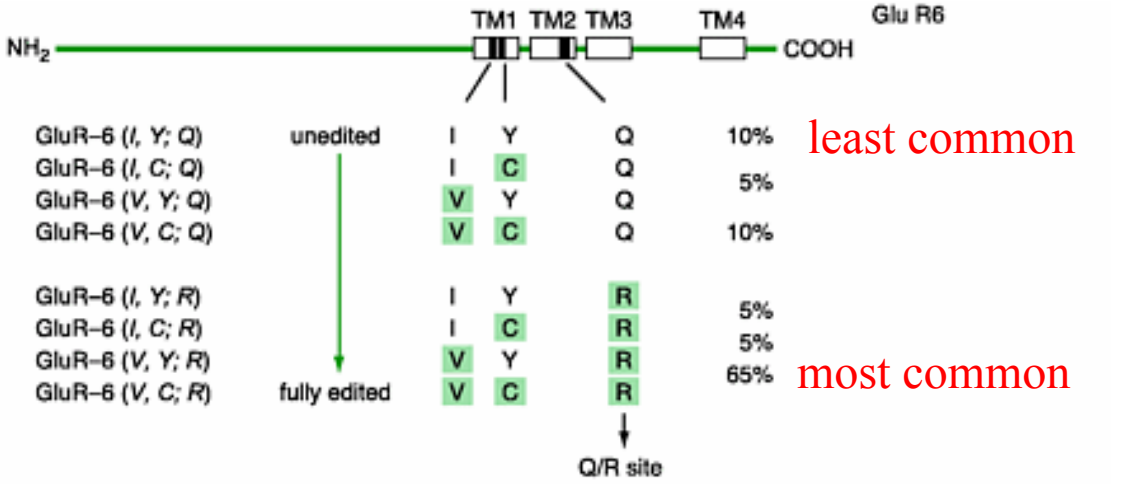
1. Both ligand and voltage-gated.
2. Channel blocked by Mg++ at ~ -40-80mV.
3. Therefore depolarization will relieve the Mg++block.
4. Require glycine as a co-agonist.
5. Have slow kinetics.
6. Have >> greater affinity for glutamate than either AMPAR, KainateRs or mGluRs.

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???

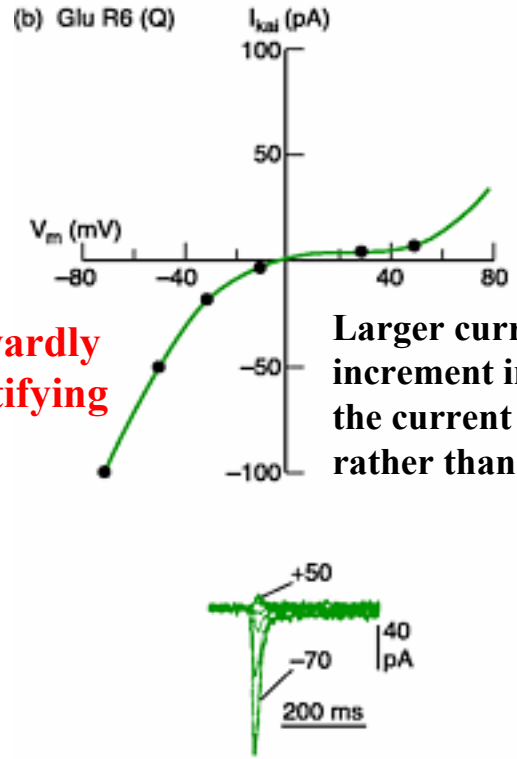
Kainate currents desensitize rapidly



least common

most common

Non-edited



Inwardly rectifying

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

edited

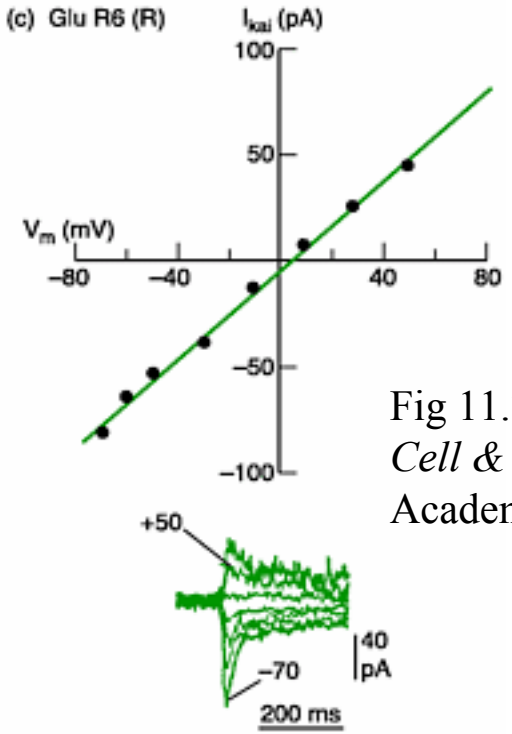


Fig 11.7. From HammondC. *Cell & Molecular Neurobiology* Academic Press 2001

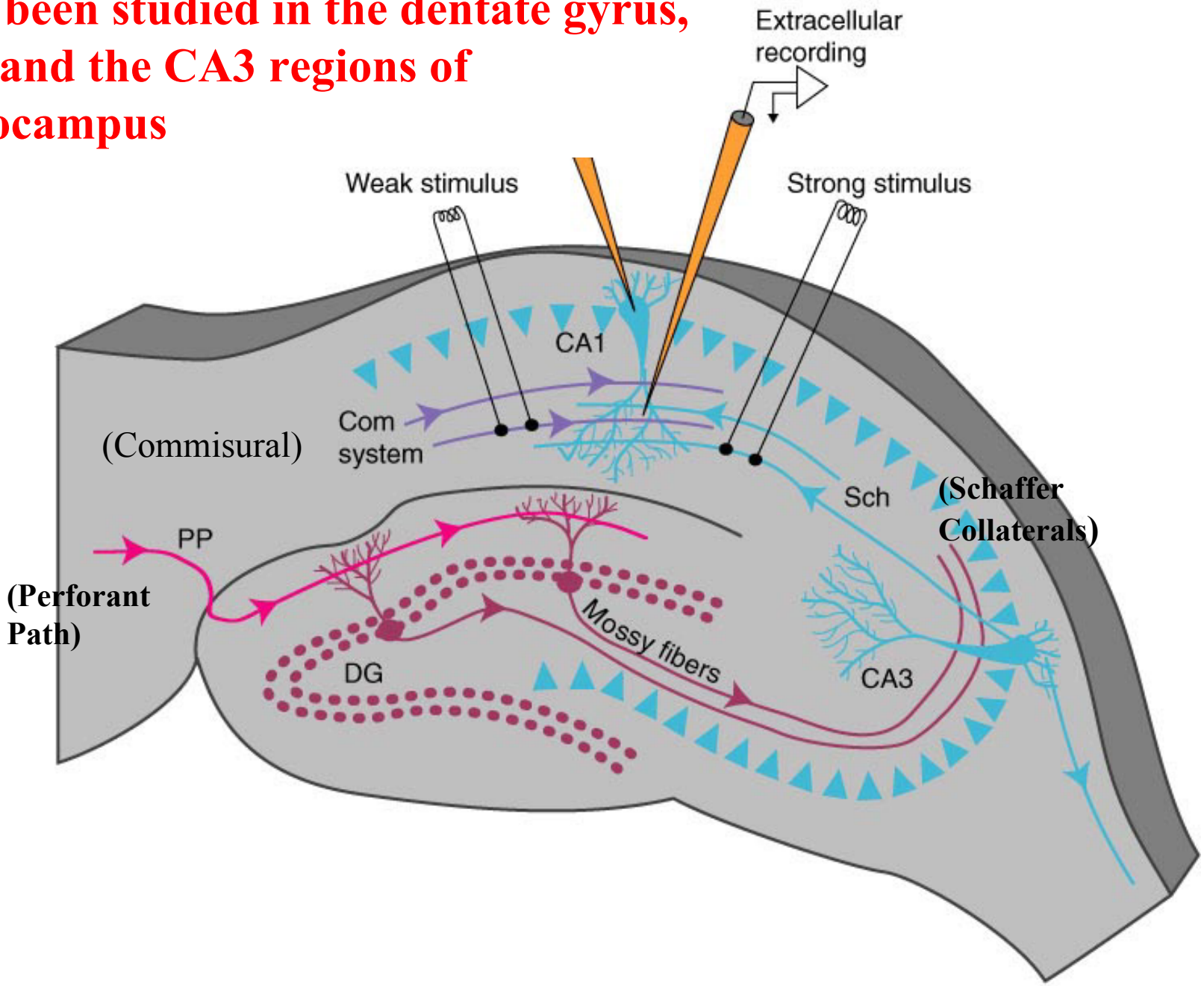
THE HEBB RULE

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

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

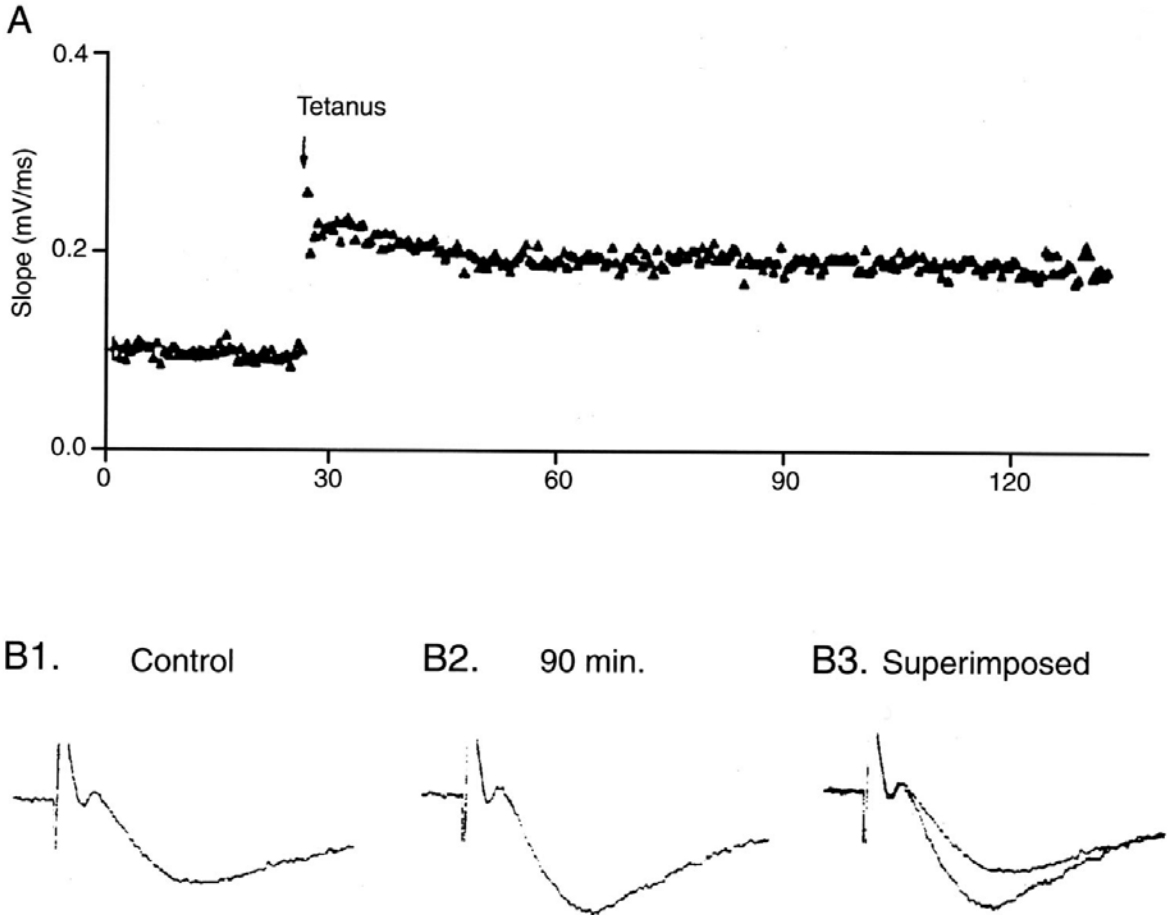


Multi-Input LTP is Measured As A Change in an Extracellularly Recorded Field Potential

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.

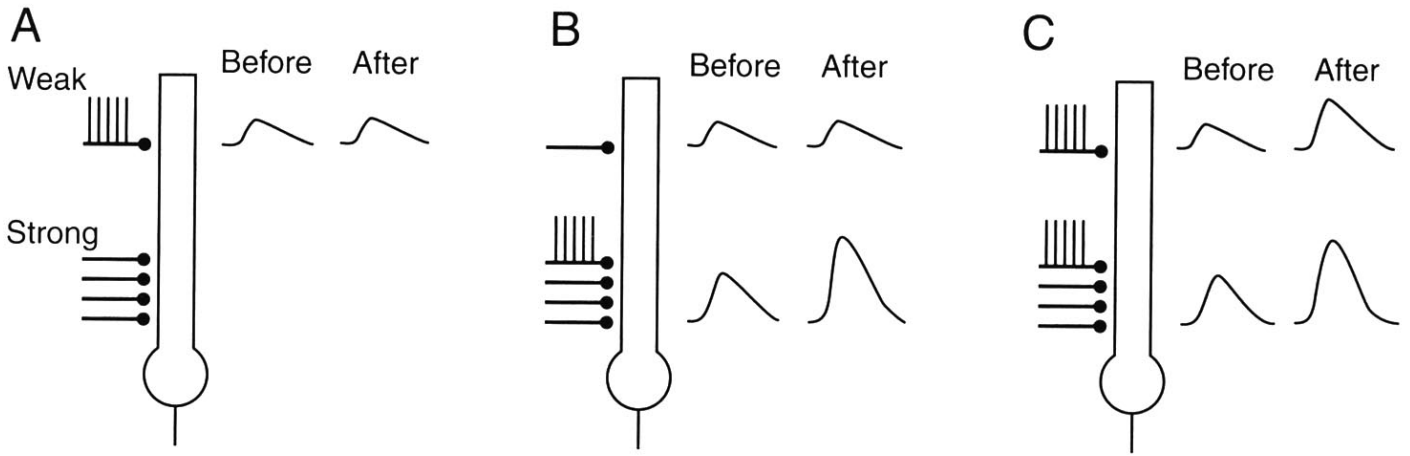


FIGURE 50.10 Features of LTP at CA3–CA1 synapses in the hippocampus. A single hippocampal pyramidal cell is shown receiving a weak and strong synaptic input. (A) Tetanic stimulation of the weak input alone does not cause LTP in that pathway (compare the EPSP before and after the tetanus). (B) Tetanic 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

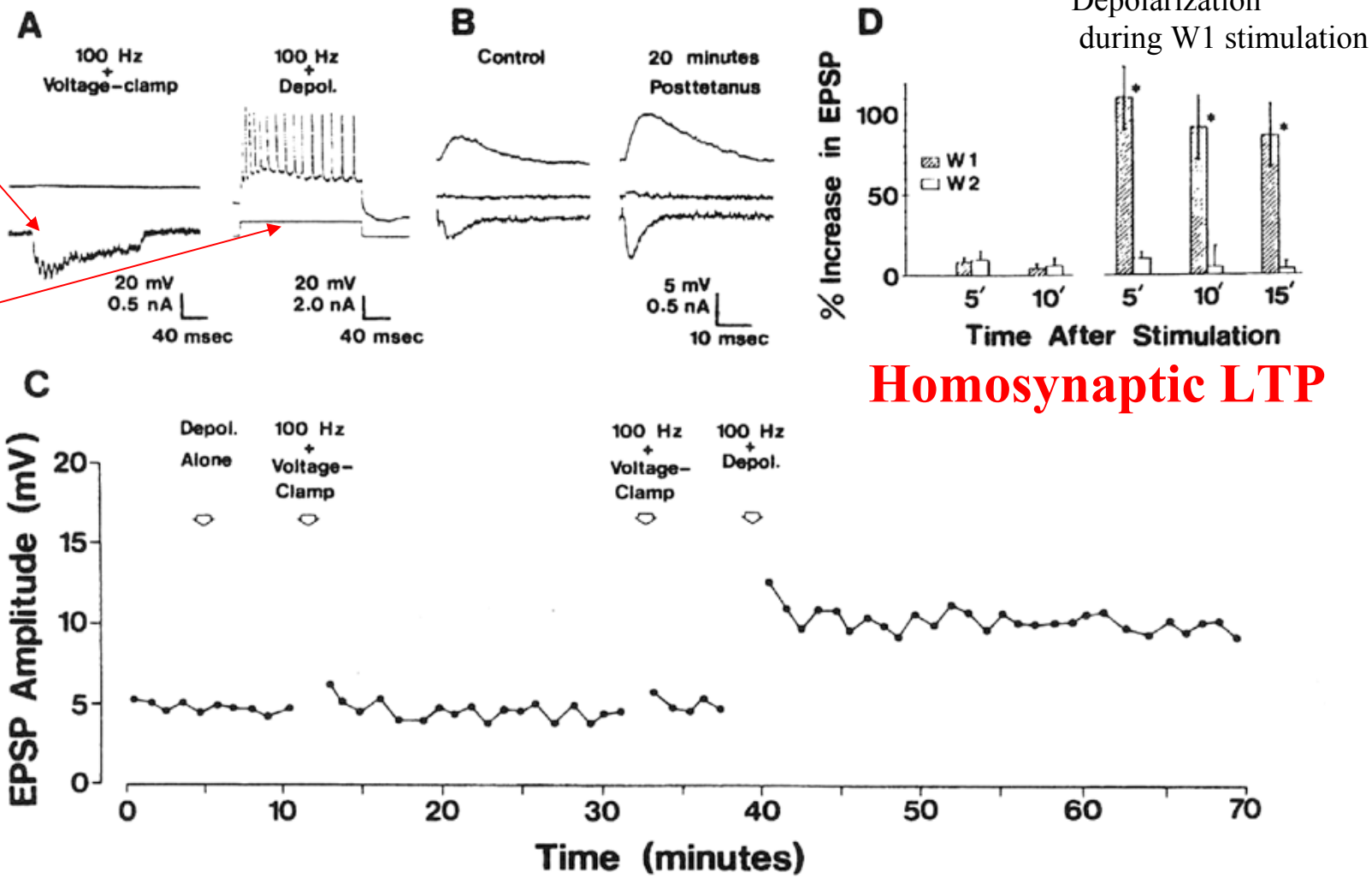
This is a depolarizing current measured intracellularly

voltage

current

This is a depolarizing current injection.

Depolarization during W1 stimulation

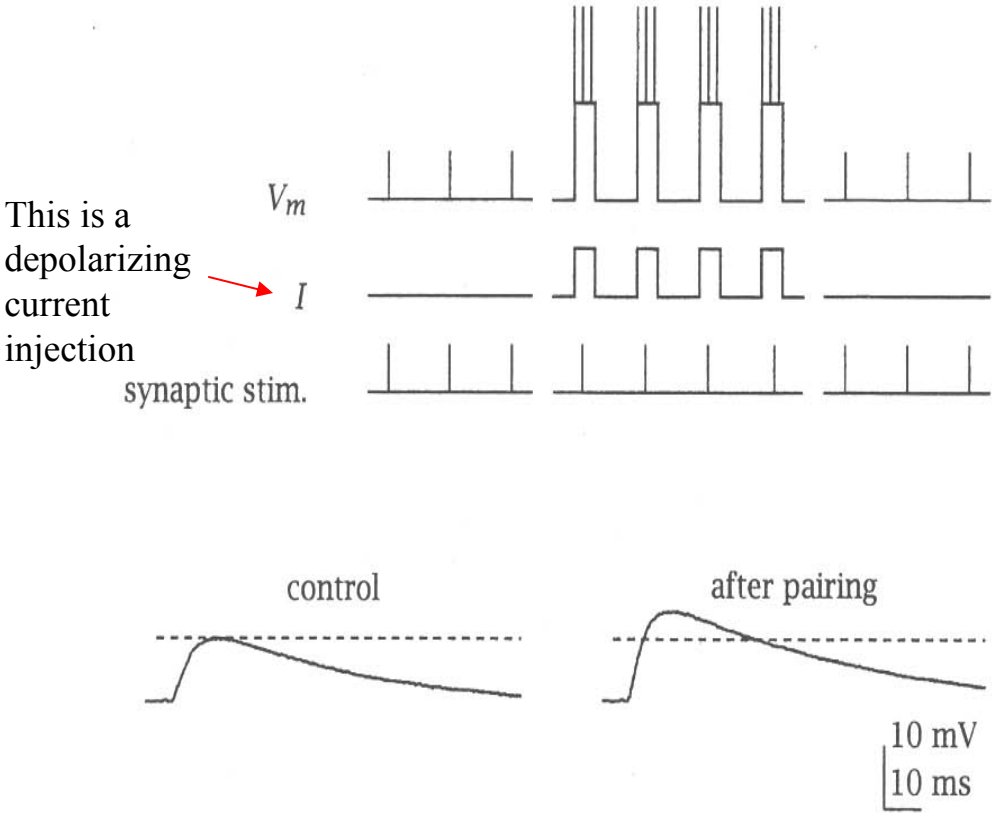


Homosynaptic LTP

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.

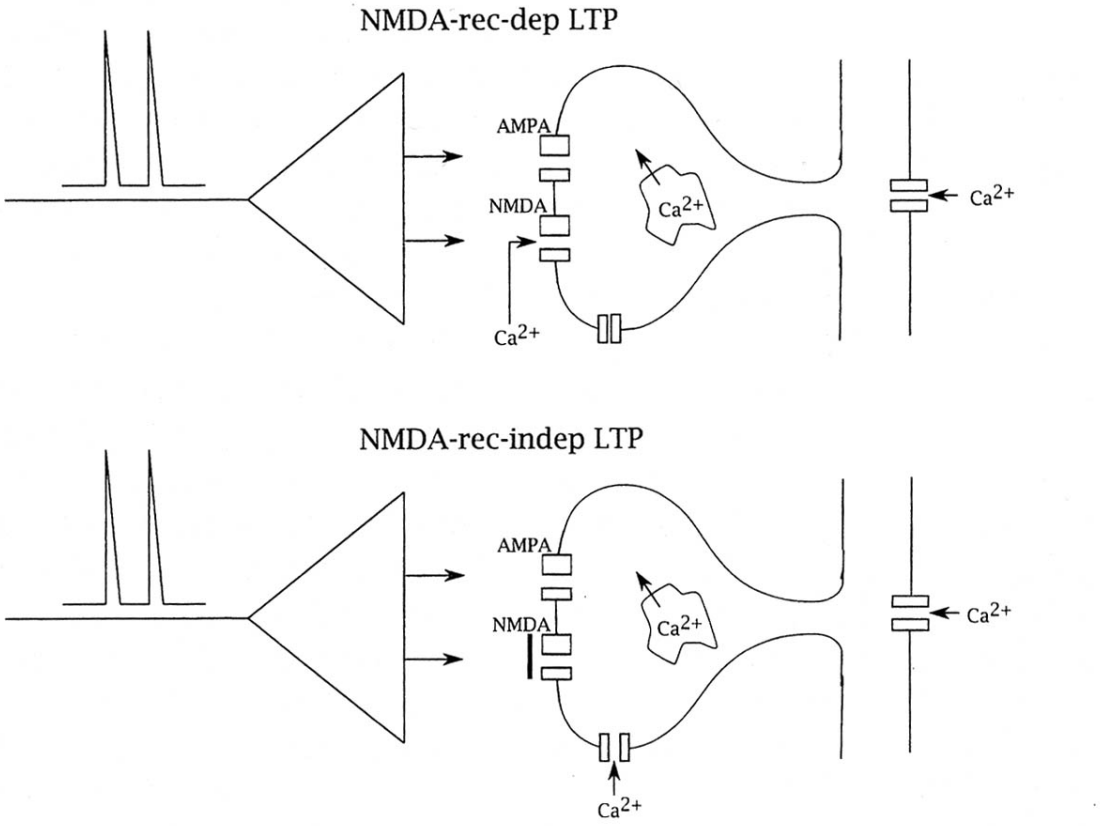


At young synapses afferents cannot follow high frequency bursts of activity.

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.

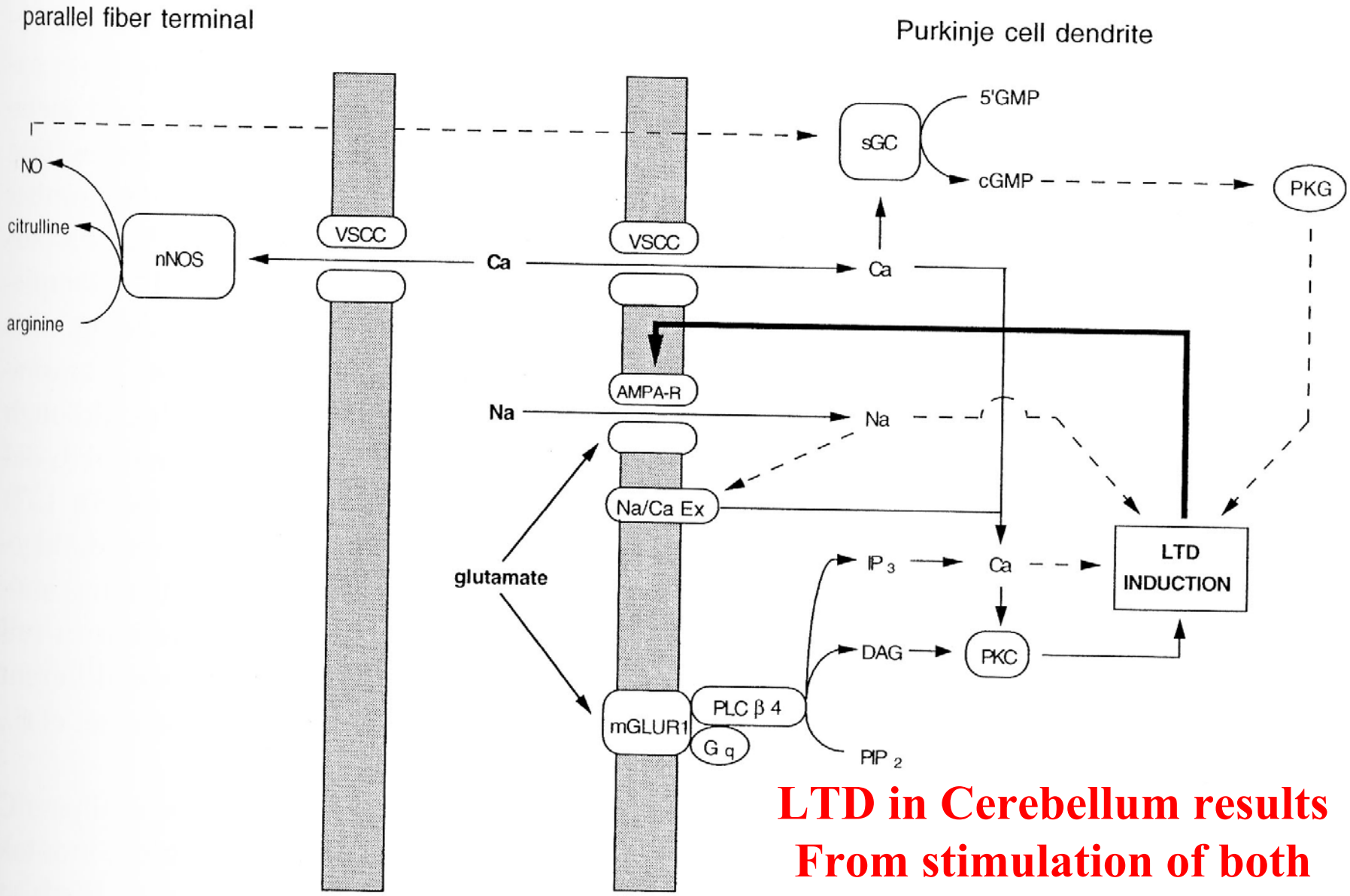
LTP at Glutamate Synapses can be NMDA receptor dependent or NMDA receptor independent



NMDAR dependent LTP

1. Increases in AMPAR function.
2. 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.



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