ZOO332H1S - Lecture 11
(AJE 2003)

Learning and Memory

(plus parting shot at octopamine, NA and adrenalin, and G Proteins)

Biosynthetic Path for DA, NA, and Adrenalin

(adrenalin = epinephrine; noradrenalin = norepinephrine)

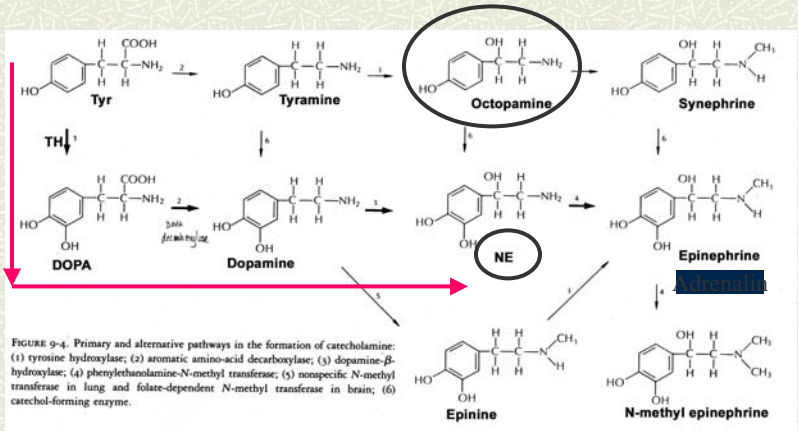


FIGURE 9-4. Primary and alternative pathways in the formation of catecholamine: (1) tyrosine hydroxylase; (2) aromatic amino-acid decarboxylase; (3) dopamine-β-hydroxylase; (4) phenylethanolamine-*N*-methyl transferase; (5) nonspecific *N*-methyl transferase in lung and folate-dependent *N*-methyl transferase in brain; (6) catechol-forming enzyme.

Cooper, Bloom, and Roth (1991)

G-Protein classification

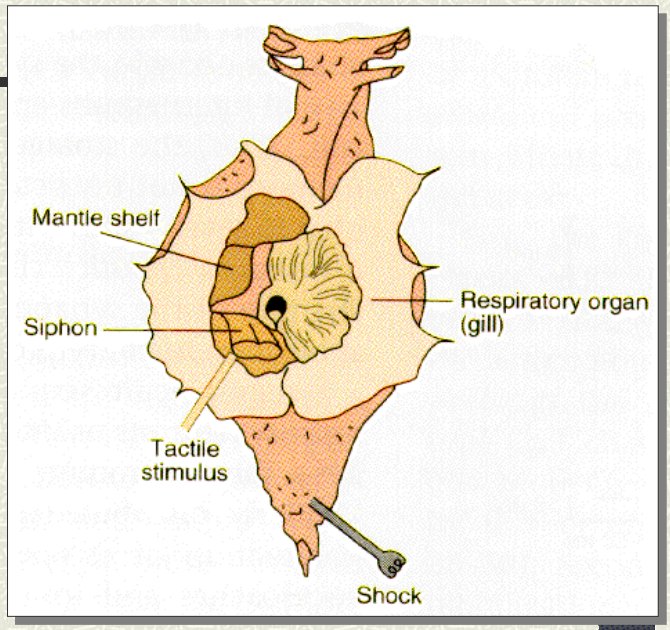
- not integral membrane proteins
- Known: 20 α-subunits, 6 β, and 12 γ subunit
- grouped according to targets recognized by α-subunit
- G_s - activates adenylyl cyclase via α-subunit bound to GTP
- G_i - inactivates adenylyl cyclase (different α-subunit)
  - (three subgroups: G_t activates cGMP-phosphodiesterase (transducin), G_o (two G_o's)- "other" G-proteins – bind to ion channels; mediate activation of guanylyl cyclase, PLA_2, $PLC(G_p)$
- G_q – couples to phospholipase C
- G_{12} – has unknown targets
  - G_{K+} activates K^+-channels;

3

Aplysia, our heroic mollusc (KSJ Ch.36)

4

Aplysia, anatomy

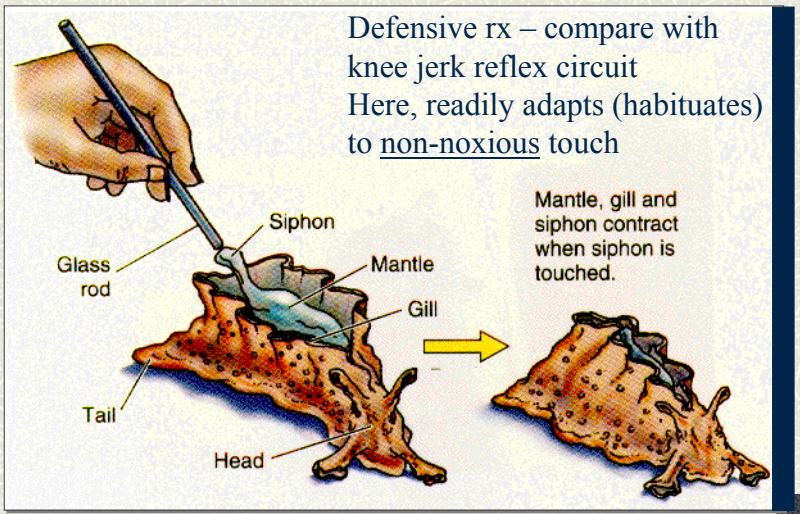


36-1

5

Gill withdrawal reflex

Defensive rx – compare with knee jerk reflex circuit
Here, readily adapts (habituates) to non-noxious touch



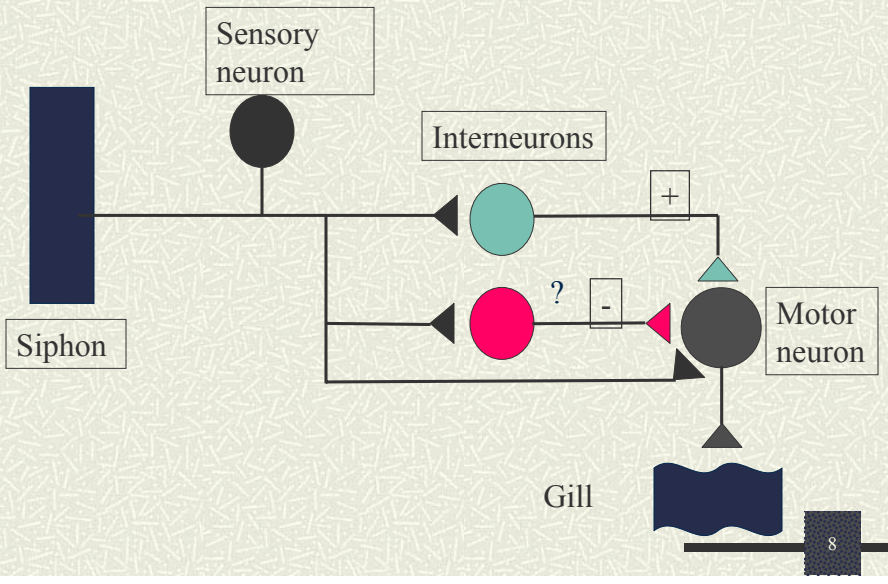
6

Withdrawal reflex examined

- Touch or water jet to siphon
- Reflex withdrawal of gill
- Sensory neurons from siphon synapse with motor neurons, both directly and indirectly via interneurons

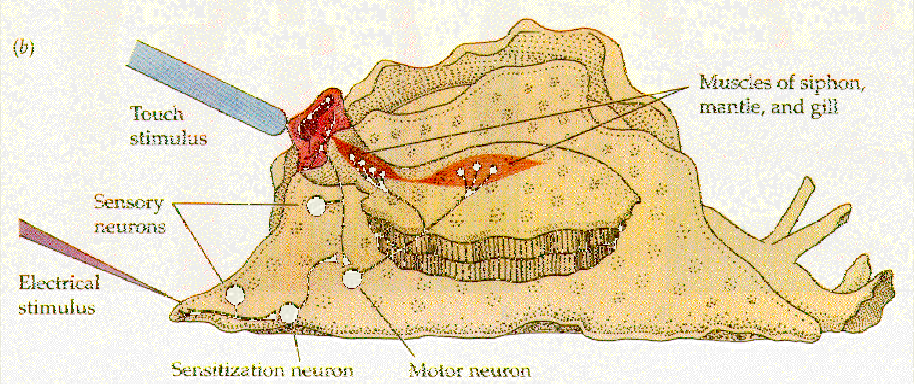
7

Gill-withdrawal circuit



8

Neuron pathways (*simplified*) involved in sensitization of withdrawal reflex



9

Habituation

- Repeated stimulus to siphon causes gill withdrawal to lessen
- Results from lessening of transmitter (glu) output
- Mechanisms (possibilities):
  1. Mobilization of vesicles to docking sites
  2. Inactivation of Ca^{2+} channels
- Called "homosynaptic depression"

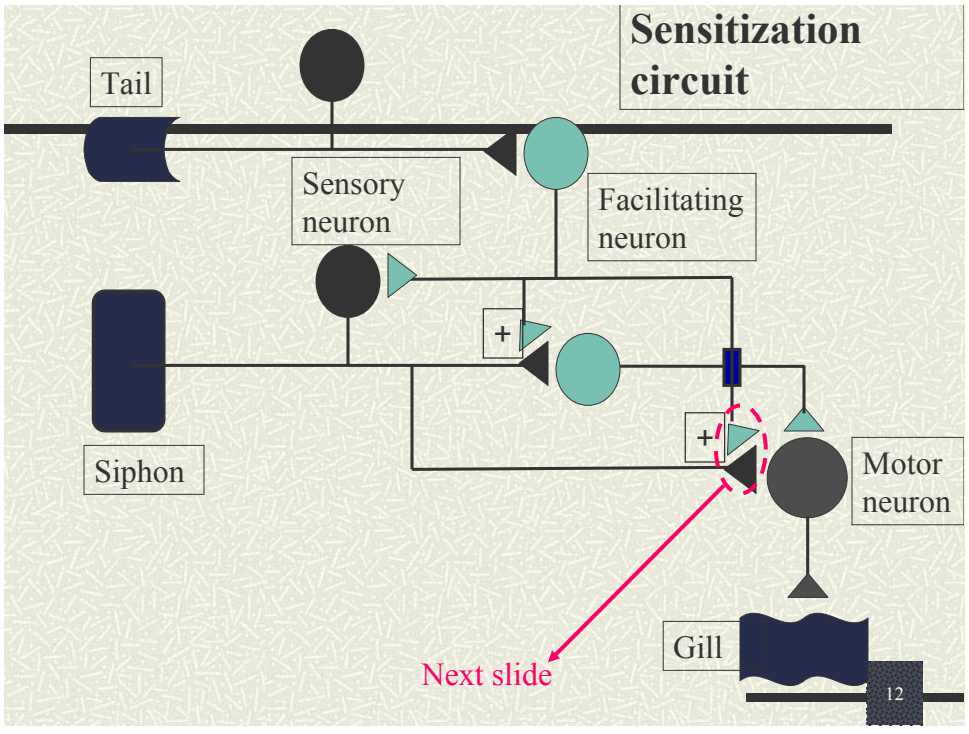
This functional decrease in synaptic effectiveness can last minutes, days, weeks (Sherrington)

10

Sensitization – heightened awareness to environment

- Harmful (noxious) stimulus to tail enhances (sensitizes) withdrawal reflex
- Single shock causes short-term effect: minutes to hours
- Several shocks causes long-term effects: days
- Effect due to increases in NT release from sensory neurons
- (this is *non-associative* because it doesn't specifically couple the two stimuli – see Classical Cond'ing)

11



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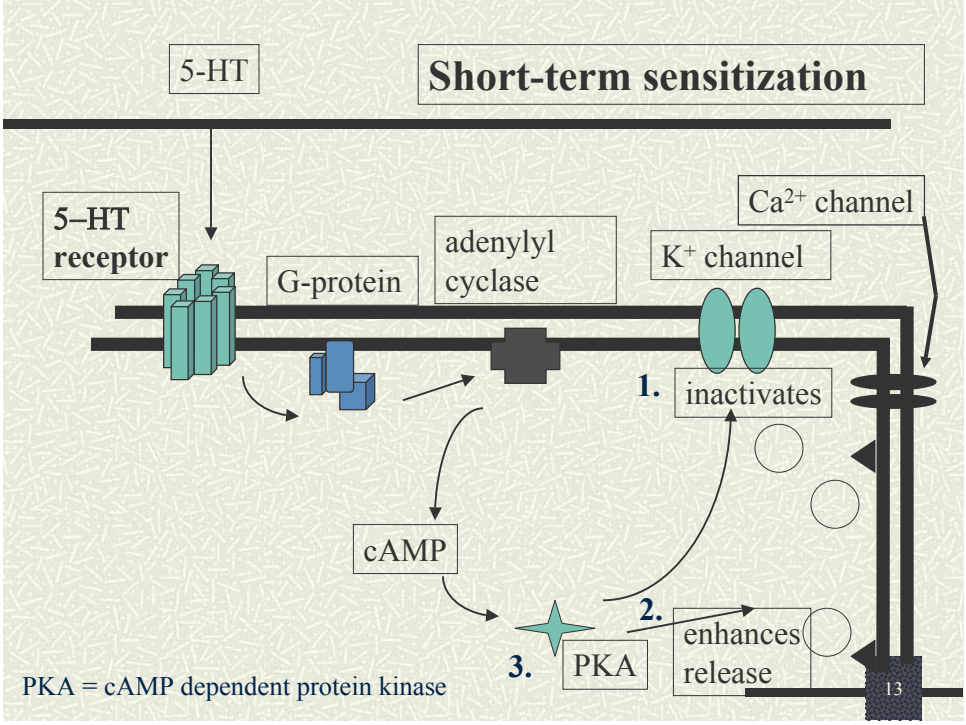


Short-term sensitization

Increase cAMP (via G_s) and (consequently) cAMP-dependent protein kinase:

1. Kinase effect on 2-types K^+ channels (prolong AP, increase influx Ca^{2+} (n-type), enhance NT release)

2. Enhance mobilization of vesicles (μtubules, Ca-independent)

Grey zone between short and long term; overlap> graded process

3. Alters L-type Ca^{2+} channel (remember NA on heart), can also affect mobilization of vesicles

14

Mechanisms of plasticity

Both short- and long-term sensitization appear to occur at same synapses in circuit

Graded effect - recall effect of recurrent training; so, are short- and long-term memory related?

1. Happening in same neuronal circuitry
2. Both involve increase in release of NT
3. Application of 5-HT, evokes graded effect (short or long) in facilitation
4. cAMP involved in short and long term changes

BUT - simplified situation

15

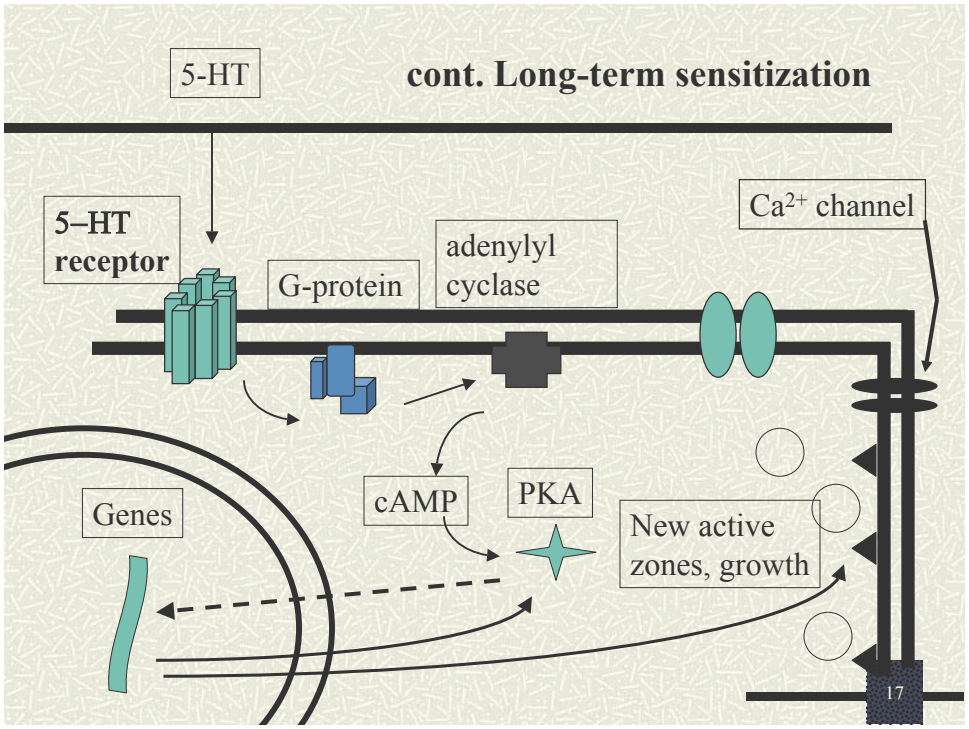
cont...Mechanisms of plasticity

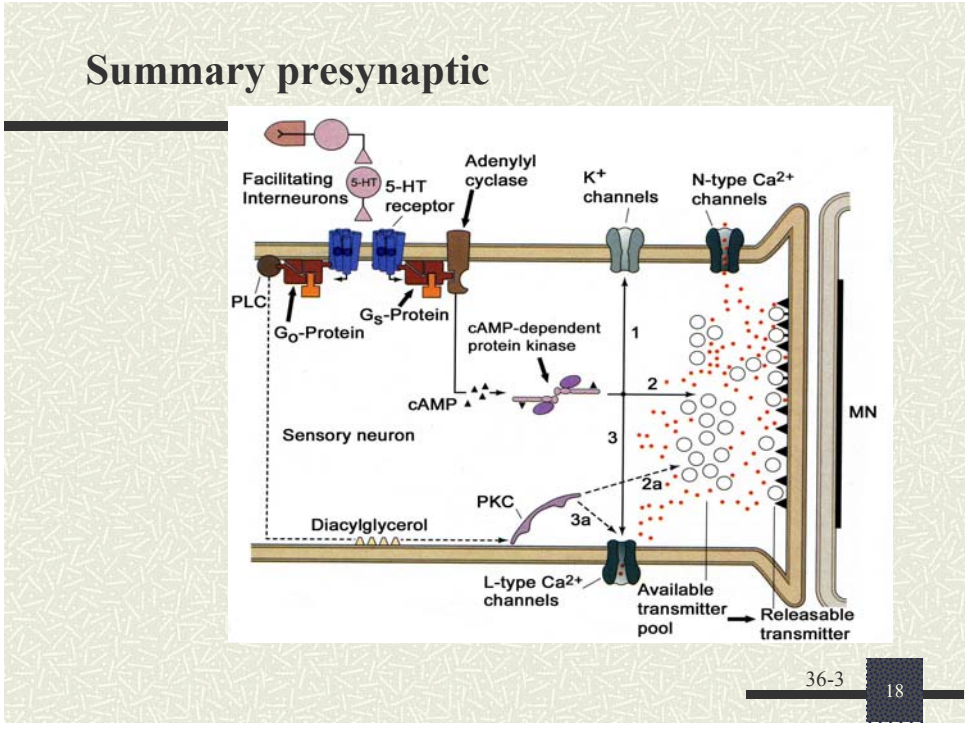
Long-term changes more involved

Changes in synaptic efficacy - requires gene activation and protein synthesis

Inhibitors of protein or mRNA synthesis

16


5-HT
cont. Long-term sensitization
5–HT receptor
G-protein
adenylyl cyclase
Ca2+ channel
Genes
cAMP
PKA
New active zones, growth
17

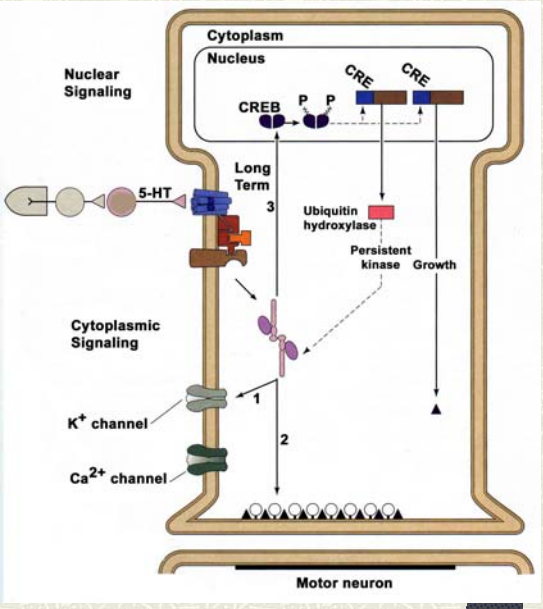

Summary presynaptic
Facilitating Interneurons
5-HT
5-HT receptor
Adenylyl cyclase
K+ channels
N-type Ca2+ channels
PLC
Go-Protein
Gs-Protein
cAMP-dependent protein kinase
cAMP
Sensory neuron
MN
1
2
3
2a
3a
PKC
Diacylglycerol
L-type Ca2+ channels
Available transmitter pool
Releasable transmitter
36-3
18

cont. Presynaptic summary – turning short to long term

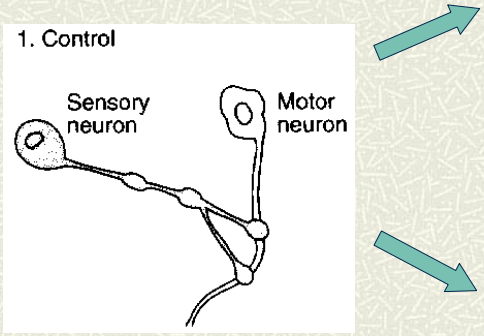
- cAMP levels up
- higher PKA levels
- local and distant effects (on transcription – activate genes)

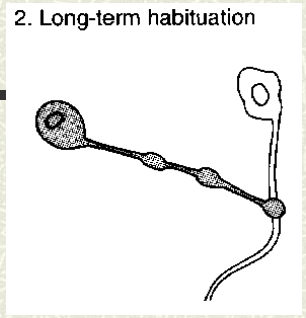
Persistent activation of PKA by:
- level of cAMP (high)
- ratio of regulatory: catalytic subunit
- level of cAMP (low)

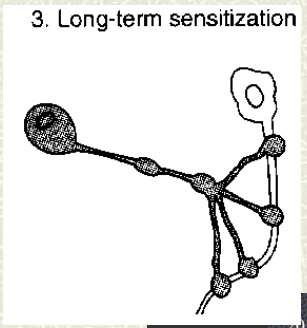
but…



Long-term structural changes







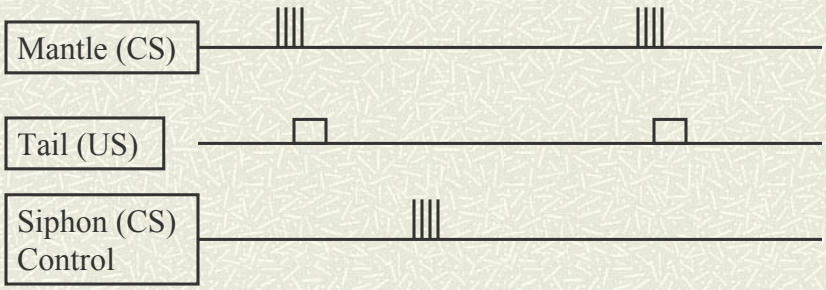
36-6

Classical conditioning of gill reflex

- CS = touch to mantle, which produces weak withdrawal
- US = shock to tail, which sensitizes response to mantle
- If CS (stimulus to mantle) just precedes US (stimulus to tail), sensitization is strengthened
- Facilitated synapses are "primed"

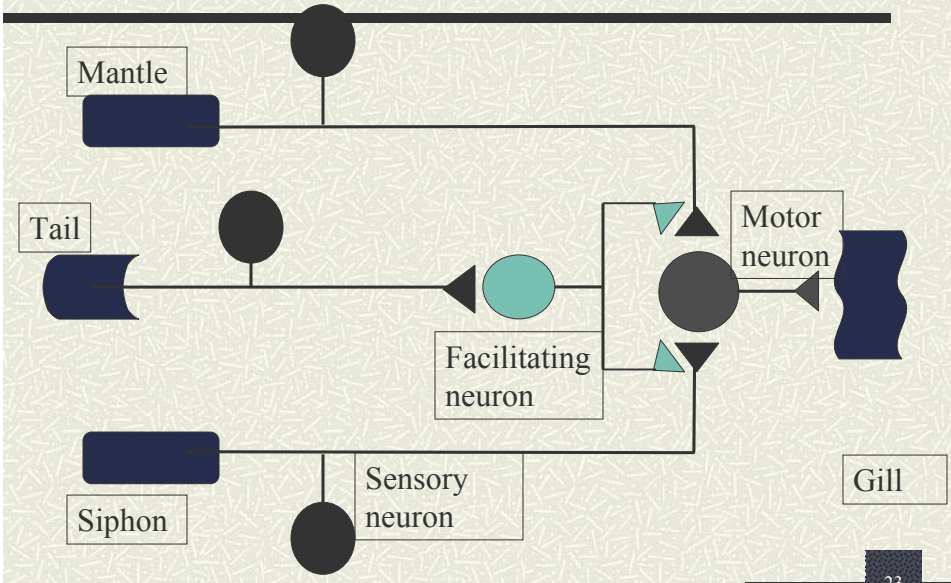
21

Conditioning scheme

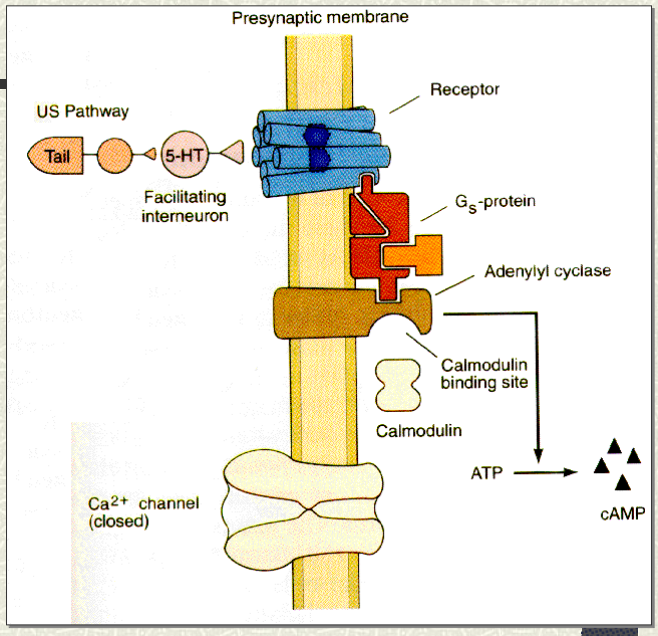


22

Classical conditioning pathways



Mechanism of classical conditioning



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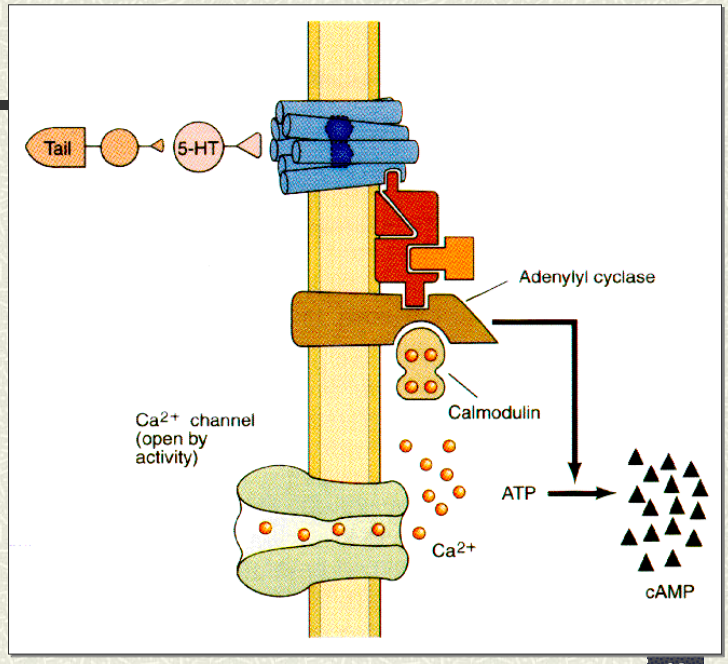
Ca^{2+} enhances adenylyl cyclase activity



36-8b

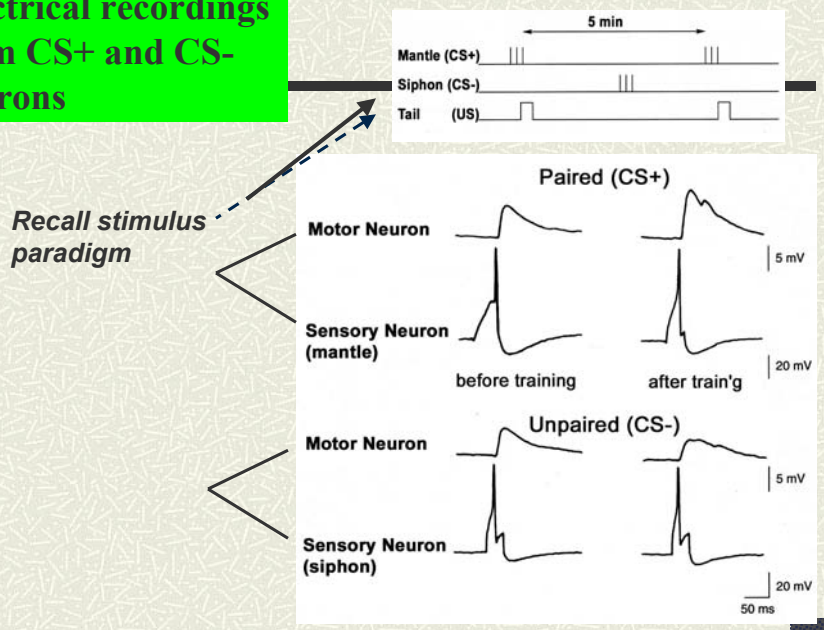
25

Timing critical

- Ca^{2+} enhances activity of adenylyl cyclase via calmodulin
- Raised [Ca^{2+}] only lasts for short time after APs in sensory neuron
- So enhanced sensitization only when sensory neurons active just before facilitating neurons active

26

Electrical recordings from CS+ and CS- neurons



27

Evidence which supports signaling pathways for "learning"

Genetic analysis of *Drosophila* mutants

- mutations which effect cAMP pathway alter fly's ability to learn first year physics (and sensitization & CC)

dunce - lacks phosphodiesterase

rutabaga - defect in Ca^{2+}/calmodulin-dependent adenylyl cyclase

amnesiac - lacks control over activity of adenylyl cyclase

28

Summarizing….

- classical conditioning more effective and longer lasting than sensitization
- CC goes one step further in facilitation of the sensory neuron
- sensory pathways from mantle and siphon are independent and thus one can be conditioned and the other left alone
- recall properties of convergence (and divergence) - timing is critical because of convergence of input to sensory neuron at the motor neuron
- axo-axonic synapses typically modulatory (>> neural activity >>> behaviour)
- works by recruiting Ca^{2+}/calmodulin to cyclase, enhances activity of cyclase and thus production of cAMP

29

Cont…summary notes

• a simple representation of learning and memory

• well established system, fairly well understood - cellular, biochemical, behavioural, electrical and genetic

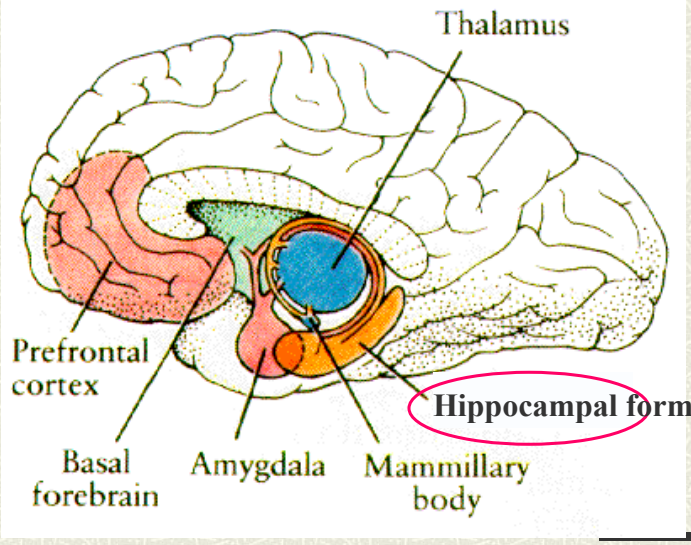
• graded appearance of facilitation (sensitization, CC), coincident with long term effects ("memory")

• cAMP - not the only 2nd messenger involved in synaptic plasticity, and in learning and memory

FOOTNOTE: Eric Kandel shared the Nobel Prize (with Paul Greengard and Arvid Carlsson) in Physiology or Medicine in the year 2000 for their work on signal transduction in the nervous system.

30

Putative Mechanisms of Learning and Memory in Mammals



31

Cont….Learning in mammals

Remember that experimental conditions are not "real" (single inputs to a synapse)

More typically constant streams of APs invade terminals with varying frequency and duration of bursts

Effects on efficacy of synaptic transmission: 1,2,3 – peripheral synapses; 4,5 - CNS

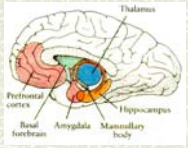
(1) synaptic facilitation

(2) synaptic depression

(3) posttetanic potentiation (PTP)

(4) long term depression (LTP)

(5) long term potentiation (LTP)



32

Notes on Facilitation, Synaptic depression, and PTP

Facilitation: when a brief train of stimuli is applied to a presynaptic nerve, during the train the amplitude of the resulting postsynaptic potentials may either increase (facil) or decrease (depression). Such changes continue after the activity itself has ended` and they have been classified according to the duration over which they persist.

Facilitation: appears instantly, persists during train and for couple 100 ms after train.(slower longer lasting phase called augmentation).

Depression: recovery over seconds after train stops. Tetanus usually results in depression. After recover get phase of PTP (10s of min duration).

LTP and LTD more persistent changes.

Facilitation: at many synapses most immediate effect of repetitive stimulation (*eg*. Frog NMJ); train of stimuli with increasing amplitude as continue in train. Effect outlasts train of stimuli

two components: (1) largest one decays with a time constant of about 50ms; a smaller component has a time constant of decay of about 250ms.

Due to an increase in mean number of quanta NT released by presyn terminal (due to increase in probability of release rather than number of quanta available).(increase by 2x)

Synaptic depression: of end plate potential is presynaptic in origin. Mechanism not completely clear. Depletion of vesicles one possibility. Autoregulation of transmitter release by co-release of ATP, degraded to adenosine, feedback on presynaptic terminal to reduce quantal content of release.

(can decrease to 20% of original …)

Katz and Miledi (1968) – residual calcium from first AP partly responsible for facilitation. Increase in AP size or duration (2^{nd}….) not occur; cultured leech neurons not get increase of calcium entry with subsequent APs in train.

PTP similar to facilitation as it refers to increase in transmitter release from the presynaptic nerve terminal due to prior stimulation. I t is different in that its onset is considerably delayed, reaches maximum several seconds after stimulation has ceased, and it lasts for 10's of minutes.

33

Cont…notes on Facilitation , Synaptic depression, and PTP

Eg., ciliary ganglion chick – curarized,stimulated (100Hz, 15seconds); initial response = depression, but then with subsequent stimuli get increased size of EPSP.

PTP presynaptic origin – increase release of quanta, due to increase of intracellular calcium. Exact mechanism = obscure but exps with NMJ shows depends on calcium entry into the nerve terminal during conditioning train.

Evidence: remove external calcium, no PTP; sodium NOT necessary (TTX & v/c)); however, at rat NMJ not necessary for potentiation but if block Na/K exchange then potentiation is prolonged. Higher Na inside, longer duration of PTP probably due to Calcium being around longer since block Na/Ca exchange ….PTP reduced in magnitude and duration at crayfish NMJ, by interfere with exchange of calcium between cytoplasm and intracell stores (eg., mitoch). Exps suggest that calcium influx during tetanus results in rapid calcium loading of intracellular compartments. Accumulated calcium is then released slowly into cytoplasm during the posttetanic period, thereby maintaining an elevated cytoplasmic concentration.

34

Hippocampus and Dentate Gyrus

Bliss and Lomo (1973) at glutamatergic synapses in hippocampal formation (hippocampus and dentate gyrus (DG))– located in temporal lobe of brain

Hippocampus acts as temporary storage site for long-term memory, later (days>weeks) transferred to other areas (putative sites in cerebral cortex for "permanent" storage); Actually, not clear whether hippocampus acts as temporary storage site or as a facilitator to higher cortical memory storage areas

Major input pathway to hippocampus from entorhinal cortex to DG via the perforant pathway

Orderly arrangement of cells and input pathways – reproducible placement of electrodes for recording and stimulation

3 main pathways: (1) perforant fibre pathway (from entorhinal cortex to granule cells of DG);

(2) Mossy fibre pathway (DG granule cells to CA3)

(3) Schaffer collateral-commissural pathway to pyramidal cells in CA1 (CA3 to CA1)

High frequency stimulation of inputs to cells in DG (perforant pathway) produces a subsequent increase in amplitude of EPSPs that lasts for hours or days (homosynaptic LTP)

Repetitive activity (Schaffer collateral to CA1) at one synaptic input to a cell could influence whether or not another input to the same cell was potentiated by repetitive activity – associative LTP

35

Cont…Hippocampus and Dentate Gyrus

Mechanisms of LTP Induction

• Not complete story but general acceptance that calcium influx and concentration in postsynaptic cell NB factor

• In CA1 pyramidal cells increase calcium by NMDA receptor activation (recall dual regulation)

• Most glutamate-sensitive cells express both non-NMDA and NMDA receptors (both activated by Glu)

• If EPSPs not sufficiently large then NMDA receptors not become unblocked and no LTP; activation of collateral pathway accompanies weaker input, NMDA receptors unblock and LTP invoked

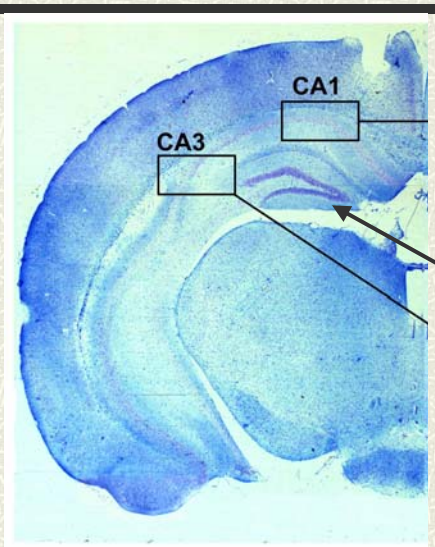
• Evidence LTP induced by postsynaptic [calcium] increase: (1) shown $[Ca2+]_i$ increases during stimulation, prevent LTP by calcium buffer; (2) elevation of calcium by injection or other means evokes long lasting increase in EPSP amplitude

• Induction of two intracellular 2nd messenger pathways particularly NB in induction of LTP: calcium/calmodulin-dependent protein kinase II (CaMKII) and cAMP dependent protein kinase

• CaMKII found in high [ ] and inhibitors of CaMKII block LTP production; genetically, transgenic mouse deficient in critical CaMKII subunit; inhibition of cAMP-depPK reduces LTP

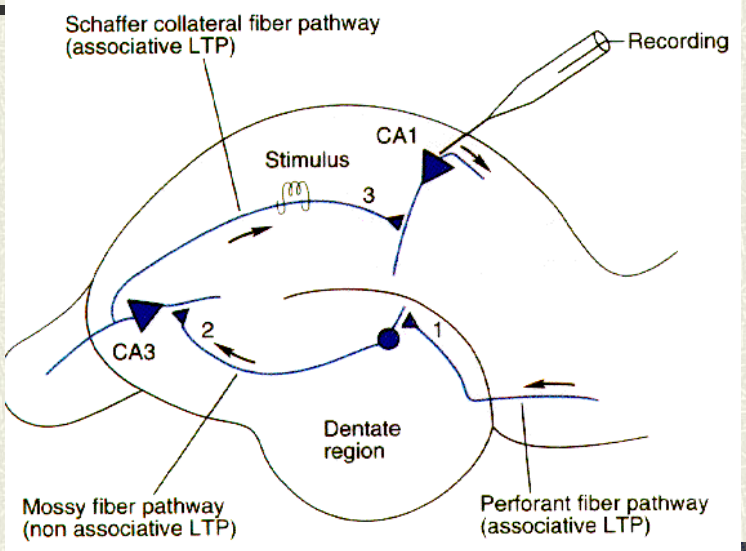
36

Mouse Brain Hippocampus



37

Long-term potentiation



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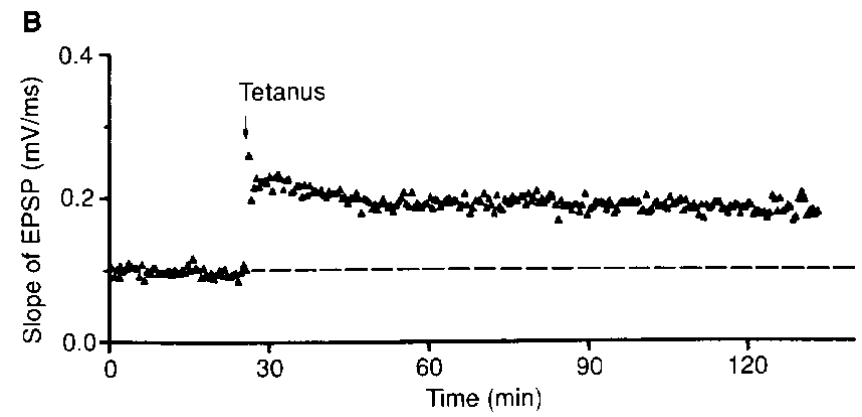
38

Procedure

- Record EPSP, measure slope (depends on amount of depolarizing current)
- Test every 10 seconds
- Stimulate Schaffer collaterals tetanically, (record in CA1): 100 Hz for 1 second, twice in 20 seconds
- Continue testing synaptic efficacy

39

LTP effect



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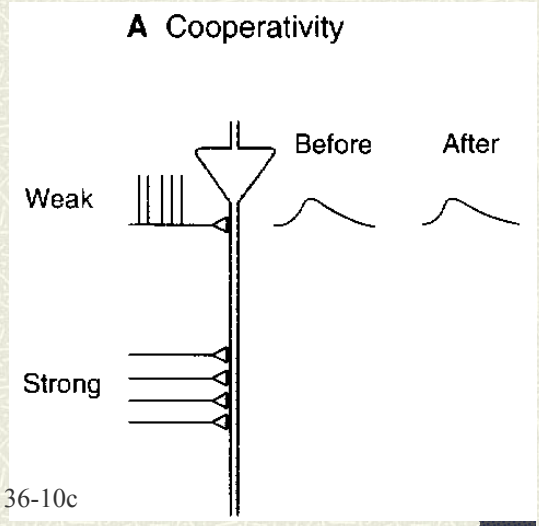
Stimulating two sets of input fibres – individually or in 'pairs'

- Only to weak pathway: no LTP
- Needs both: co-operativity (activate several afferent fibres simultaneously)



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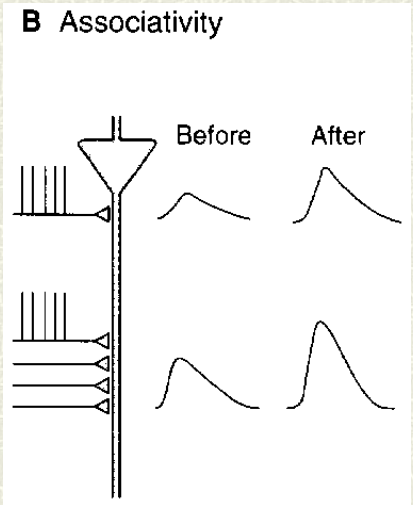
41

Strong and weak pathways together

- LTP in both
- Has to be at same time: "associativity"

(response to weaker input is potentiated only if associated with a stronger input)



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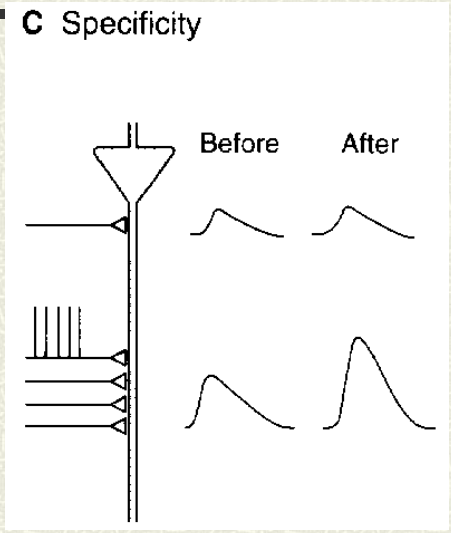
42

Strong only

- LTP in strong, not in weak
- "Specificity"

(LTP generated at one input not carry over to other synapses – LTP is specific to synapse where generated)



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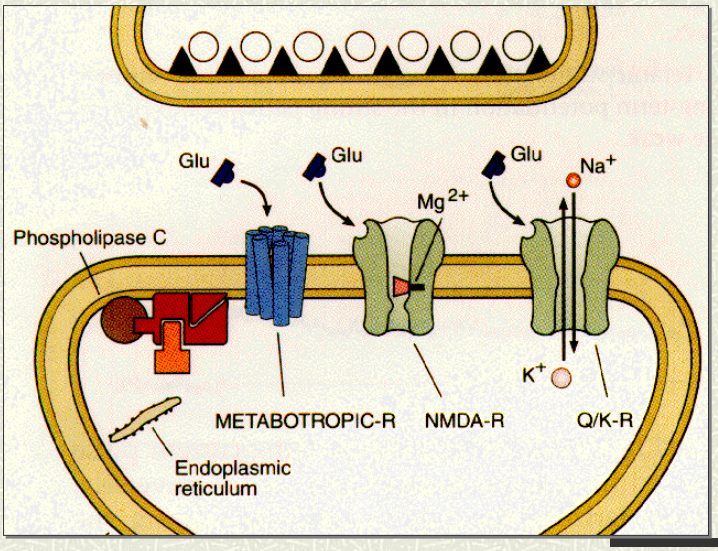
43

Low level of activity (weak input) opens non-NMDA channels only



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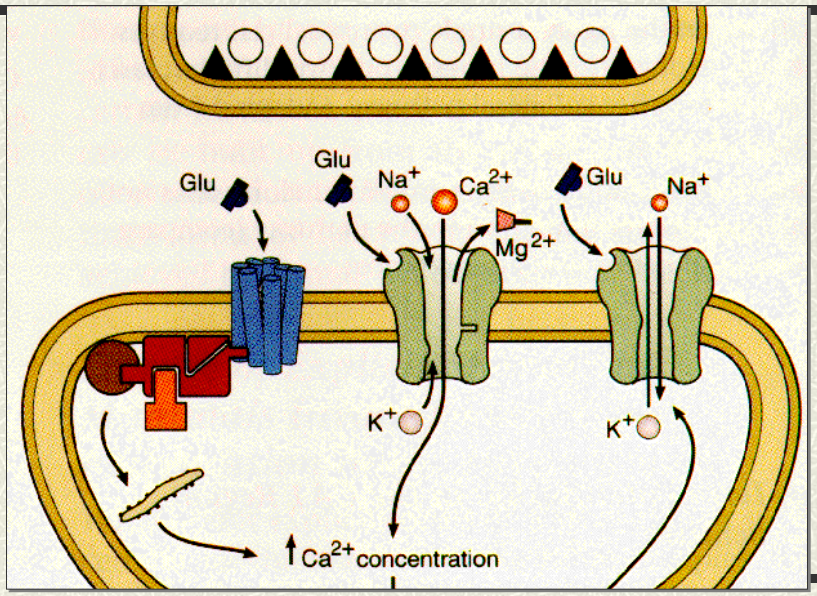
44

Large depolarisation (strong input) also opens NMDA channels



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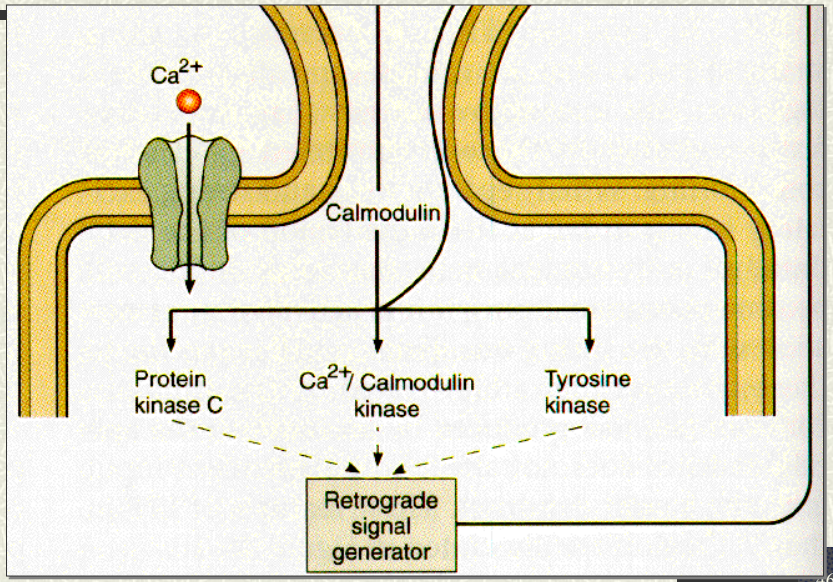
45

The increased [Ca^{2+}] induces 2nd messengers



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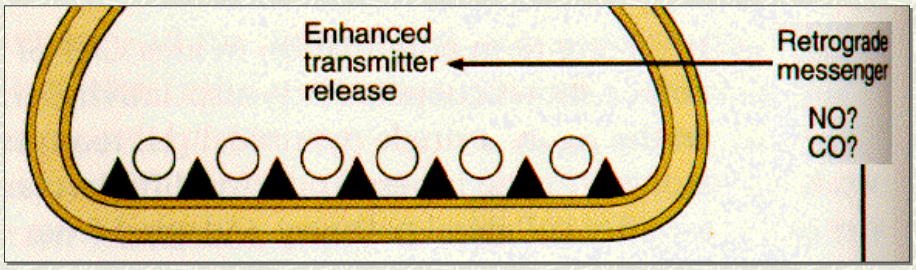
46

Which, via retrograde messengers such as NO, can have a long-lasting effect on NT release (and potentially on neighbouring cells)



36-11

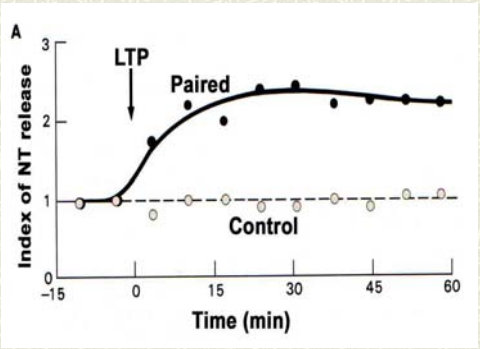
47

Evidence for increased presynaptic NT release

1. LTP occurs with an increase of Glu release

2. Quantal analysis of LTP in CA1 region; increased probability of NT release (and increased sensitivity of non-NMDA receptors)

3. Induction of LTP in neighbouring cells

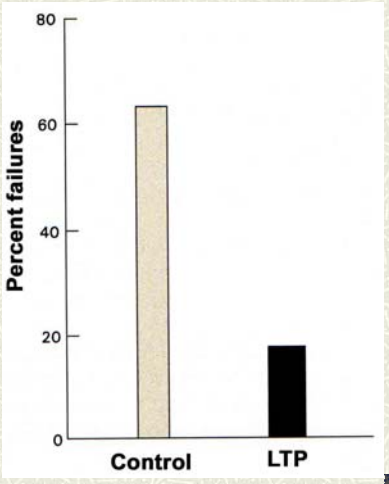


48

cont...Evidence for increased presynaptic NT release

In control situation, normally record about 60% failures when try to evoke release of NT. In a pathway where LTP has been invoked the rate of failures of presynaptic NT release is much lower.

Therefore, there must be some feedback mechanism (diffusible retrograde 2nd messenger) that sends information (positive feedback) back to the presynaptic cell



49

Other evidence indicates increased responsiveness postsynaptically without the need for an increase in NT release (bottom line: a combo of both, ~ depends on synapse)

Easy to imagine LTP to be caused by increase in quantum content (amount of NT released per synaptic response) – *i.e.*, a presynaptic mechanism whereby more vesicles released per input (recall facilitation and PTP)

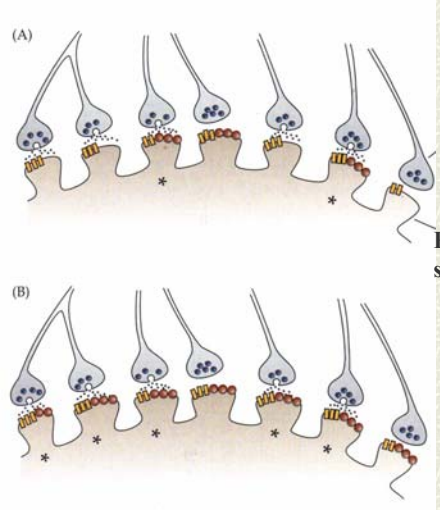
Other experiments and statistical analyses indicated not need to invoke increase in NT release as a mechanism, sufficient to have postsynaptic effect (larger postsynaptic response to each quantum of NT released)

Malenka and Nicoll (1999) – silent synapses – must think of postsynaptic membrane as dynamic (not static). Suggest some presynaptic excitatory boutons overlie postsynaptic areas on dendritic spines that have only a few functional AMPA receptors. Induction of LTP up-regulates number of AMPA receptors and synapse becomes functional and quantum content of the response would increase

50

LTP - Postsynaptic Mechanisms – Upregulation of AMPA Receptors

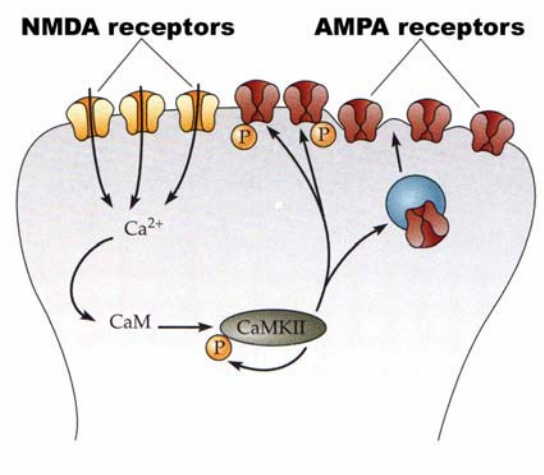
- NT release not effective at some spines (lack AMPA receptors (circles) – silent synapses)
- Quantum content = 2 even though 5 released
- During LTP – AMPA receptors inserted, quantum content of response is now 5, with no change in quanta released



NMWF12-8 (Malenka and Nicoll (1999) – silent synapses)

Cont…LTP – Postsynaptic Mechanisms

- Ca^{2+} entry through NMDA receptors;
- CaMKII activated by Ca^{2+} influx retains it's active (phosphorylated) state by autophosphorylation
- CaMKII phosphorylates AMPA receptors present and facilitates insertion of new receptors from reserve pool



NMWF12-8 (Malenka and Nicoll (1999) – silent synapses)