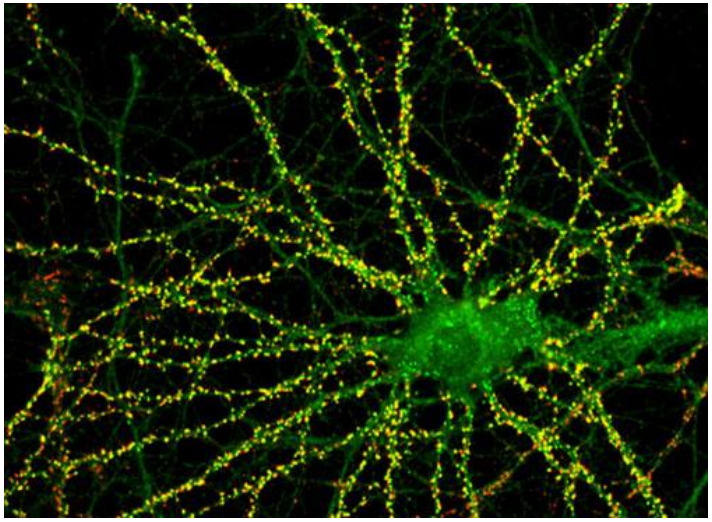
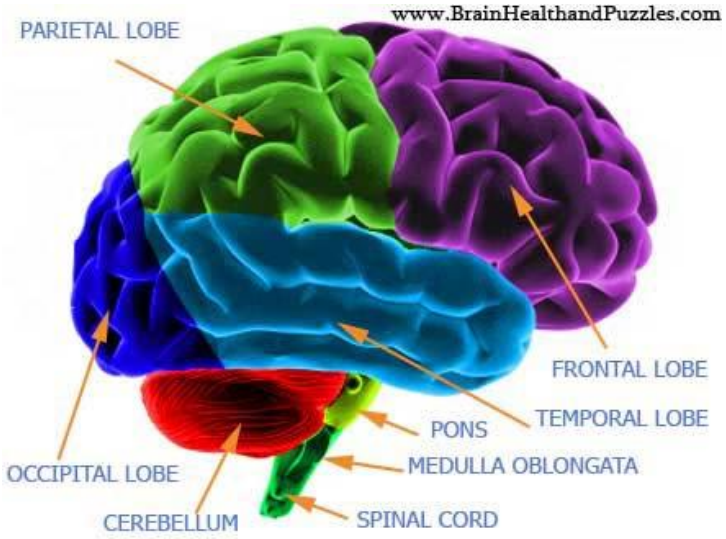
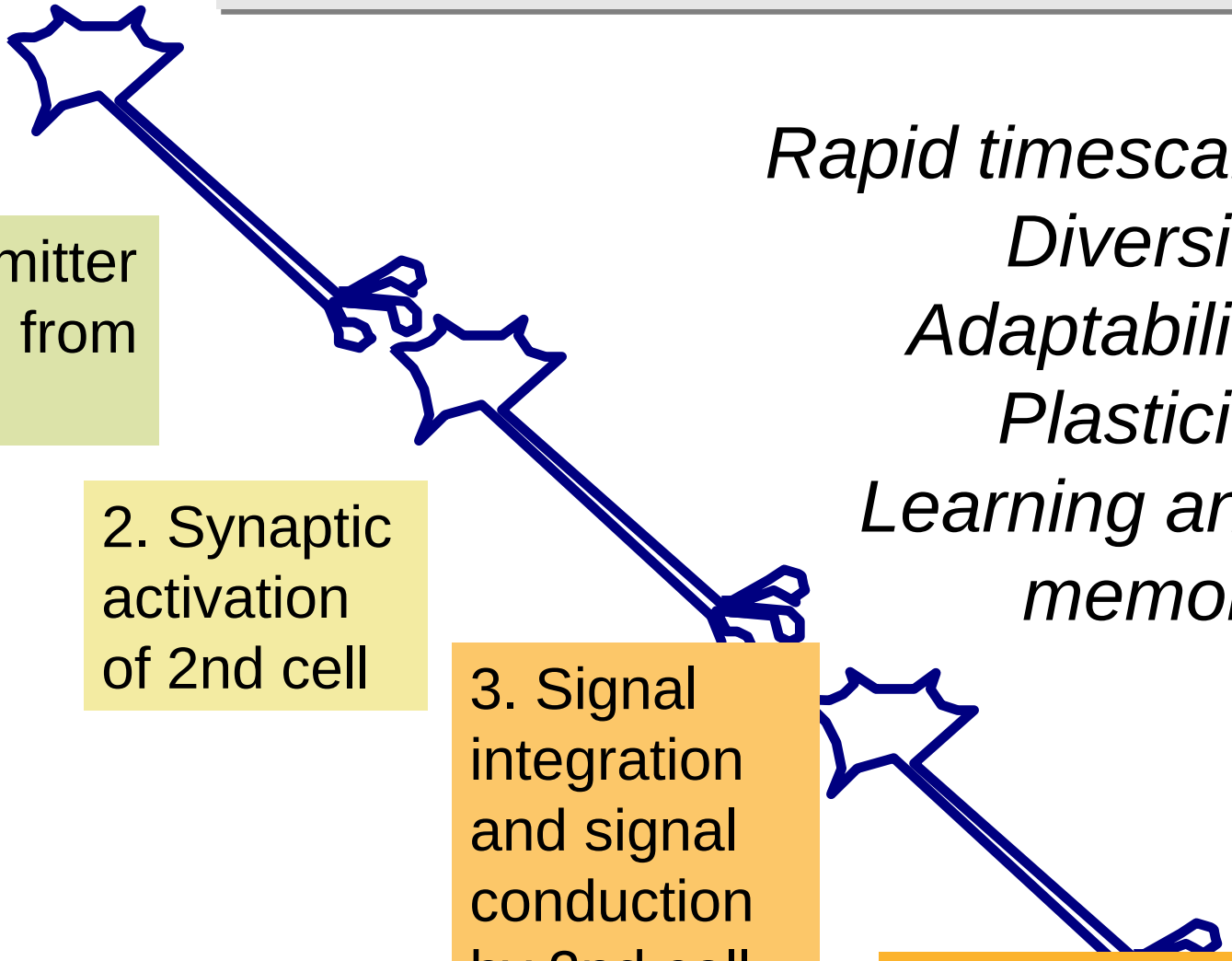
Neurotransmitters in the Brain: from rapid information transfer to long term synaptic plasticity



http://www.its.caltech.edu/~mbklab/



Synaptic transmission



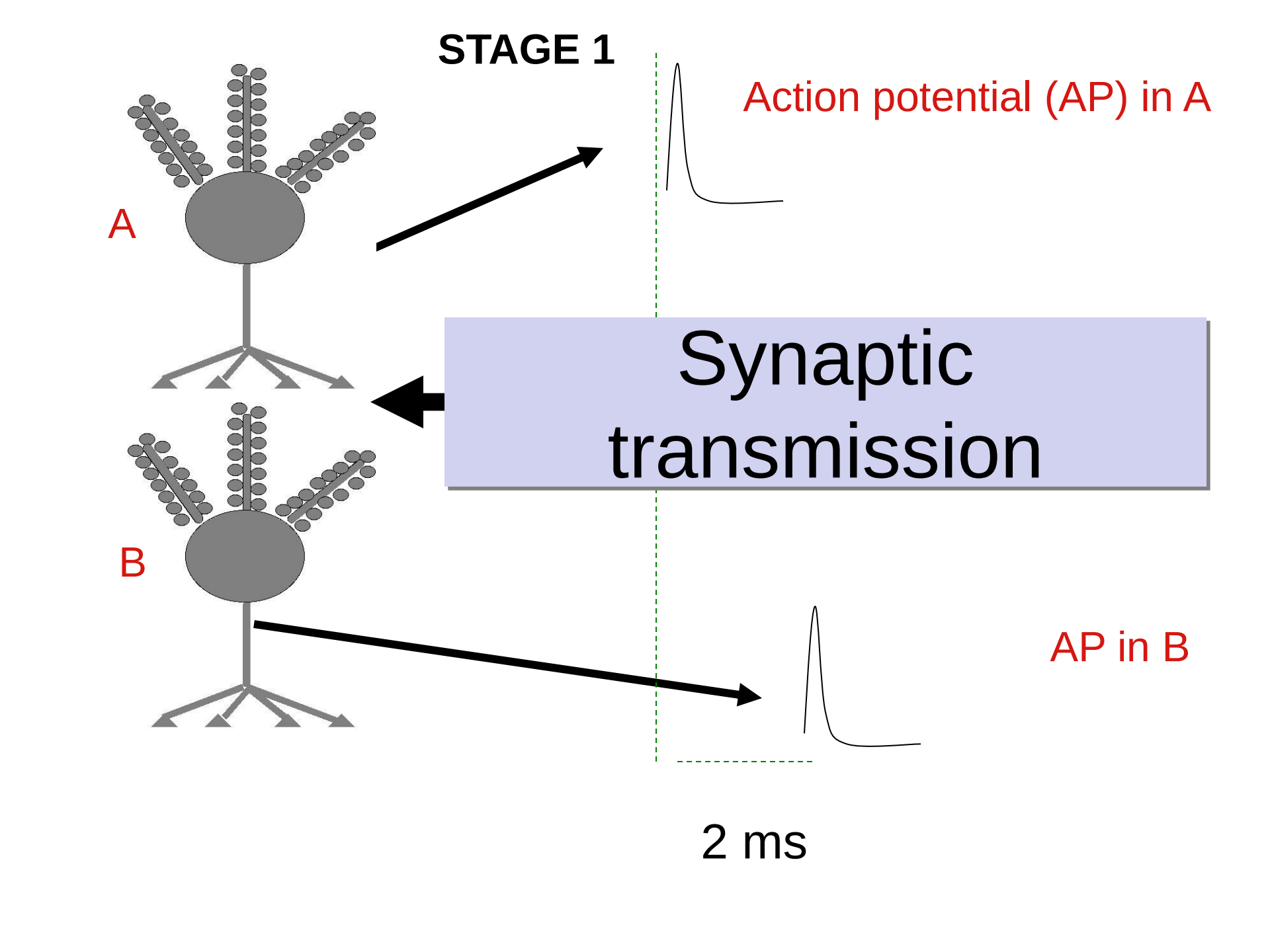
1. Transmitter released from 1st cell

2. Synaptic activation of 2nd cell

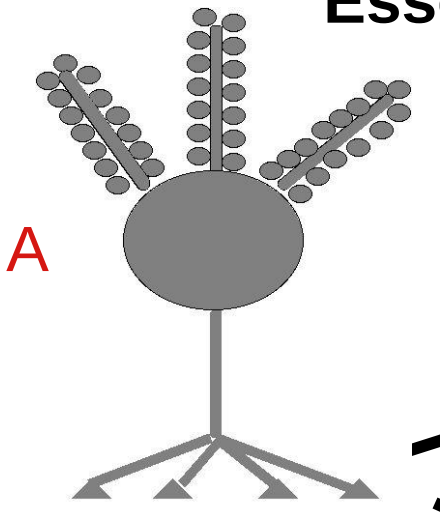
3. Signal integration and signal conduction by 2nd cell

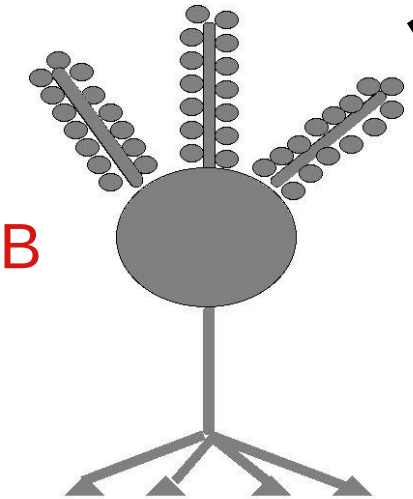
4. Pathway activation

Rapid timescale
Diversity
Adaptability
Plasticity
Learning and memory

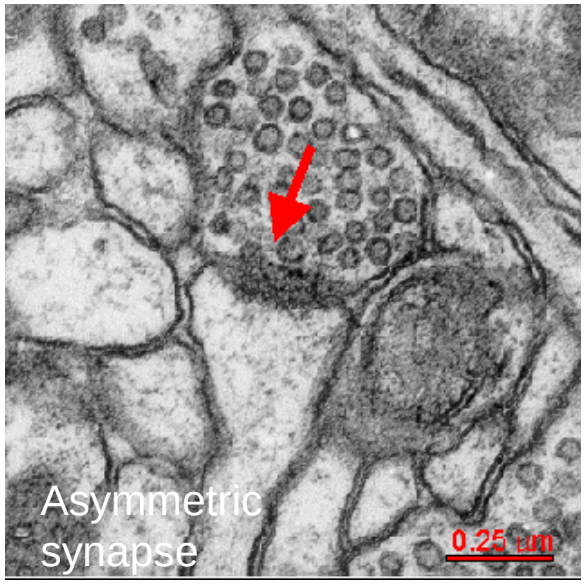

STAGE 1
Action potential (AP) in A
A
Synaptic transmission
B
AP in B
2 ms

Essential components:

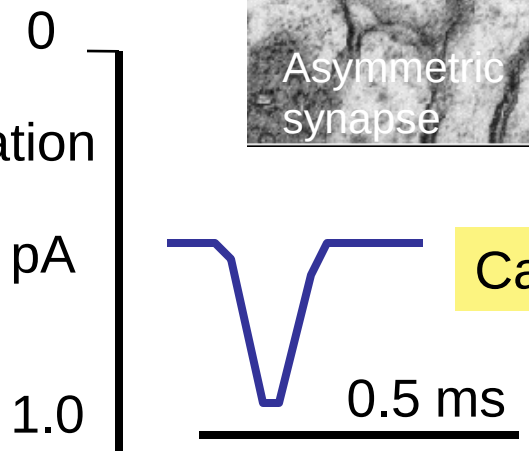




Synaptic vesicles

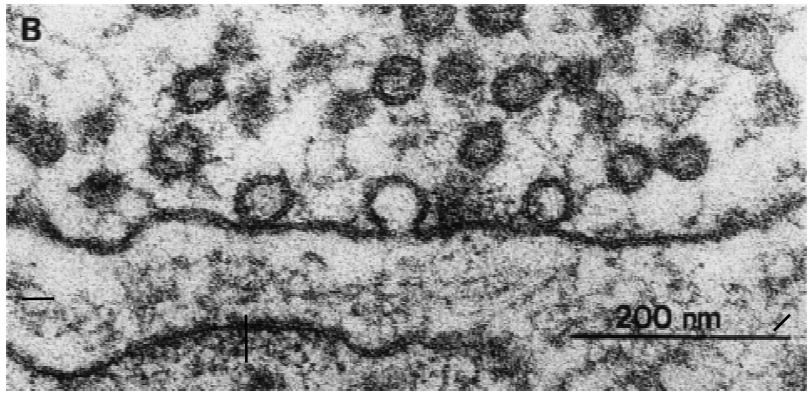


Depolarisation

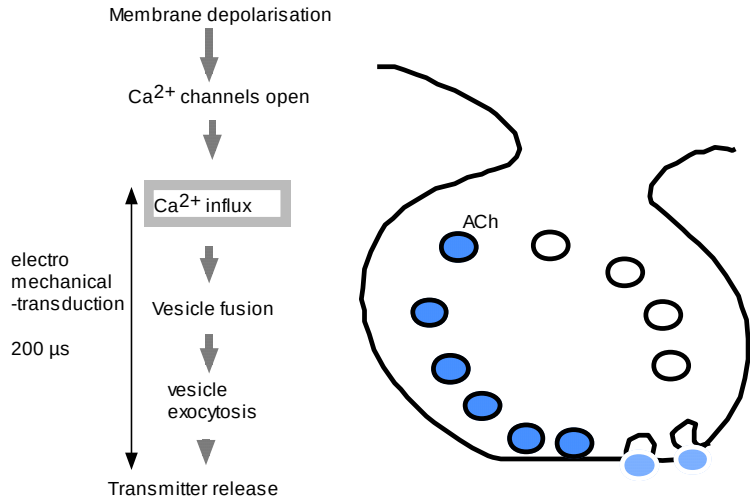


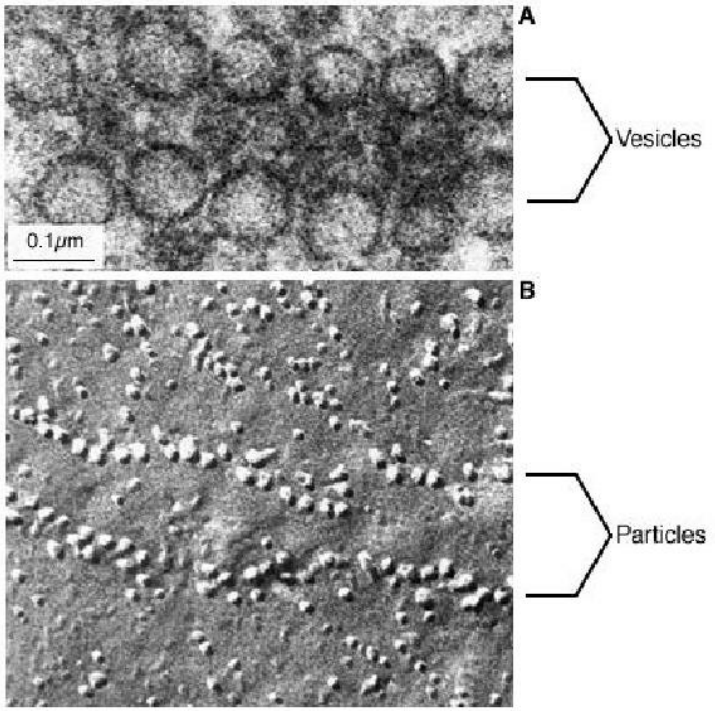
Calcium current

EXOCYTOSIS



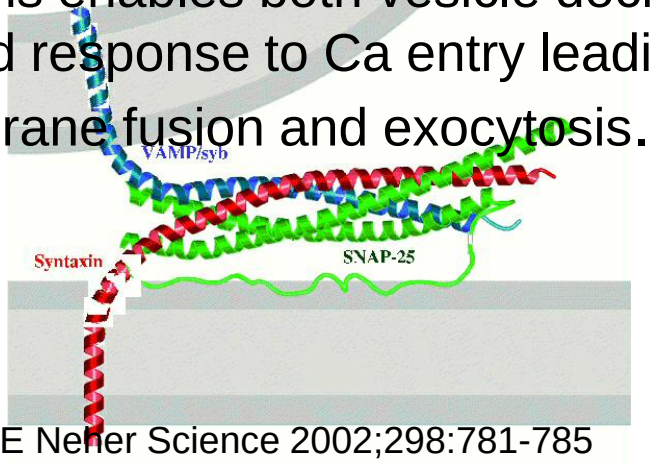
Transmitter release requires Ca^{2+} and requires RAPID transduction

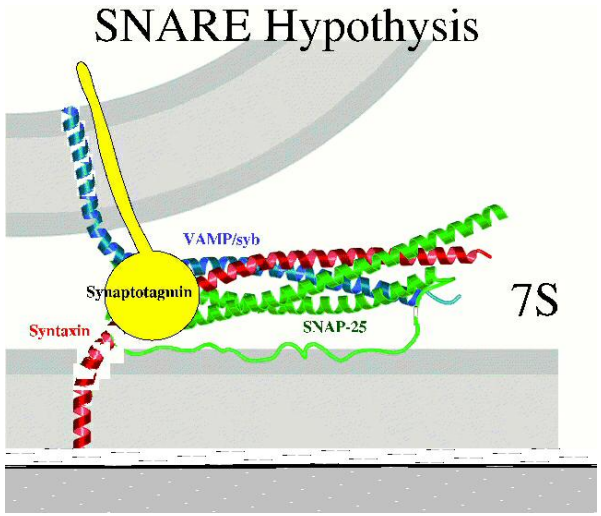




Heuser and Reece 1973

Protein complex formation between vesicle, membrane and cytoplasmic proteins enables both vesicle docking and a rapid response to Ca entry leading to membrane fusion and exocytosis.





J Rettig, E Neher Science 2002;298:781-785

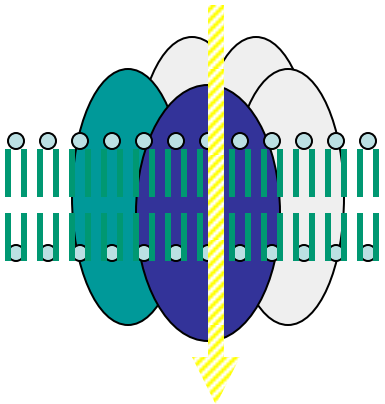
STAGE 2

NEUROTRANSMITTERS

- Provide enormous diversity in variety of transmitters and their receptors.
- **Amino acids** (e.g. glutamate, gamma amino butyric acid [GABA], glycine [gly]), **amines** (e.g. noradrenaline [NA] and dopamine [DA]) and **neuropeptides** (e.g. opioid peptides).
- May mediate rapid (µs - ms) or slower effects (ms)
- Vary in abundance from mM to nM in CNS tissue
- Neurones receive multiple transmitter influences which are integrated to produce diverse responses

Neurotransmitter action is defined by receptor kinetics

Ion channel receptor

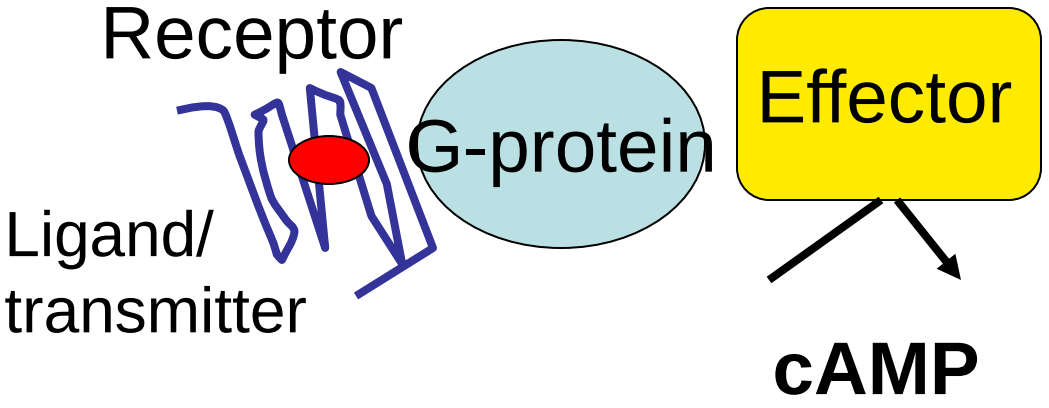


Na+

Mediate all fast excitatory and inhibitory transmission

Glutamate, gamma amino butyric acid (GABA), glycine nicotinic receptors

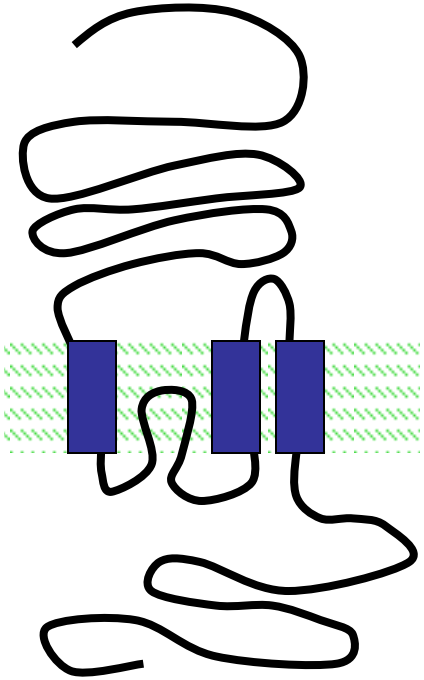
G-protein-coupled receptor



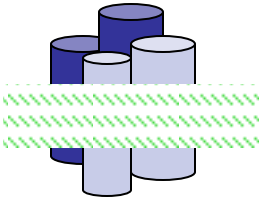
Effectors may be enzymes (adenyl cyclase, phospholipase C, cGMP-PDE) or channels (e.g. Ca^{2+} or K^{+})

muscarinic receptors, dopamine (DA), noradrenaline (NA), 5-hydroxytryptamine (5HT) and neuropeptides

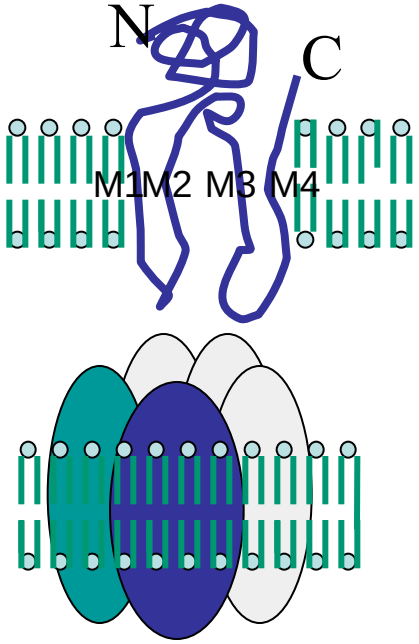
Ionotropic Receptors:

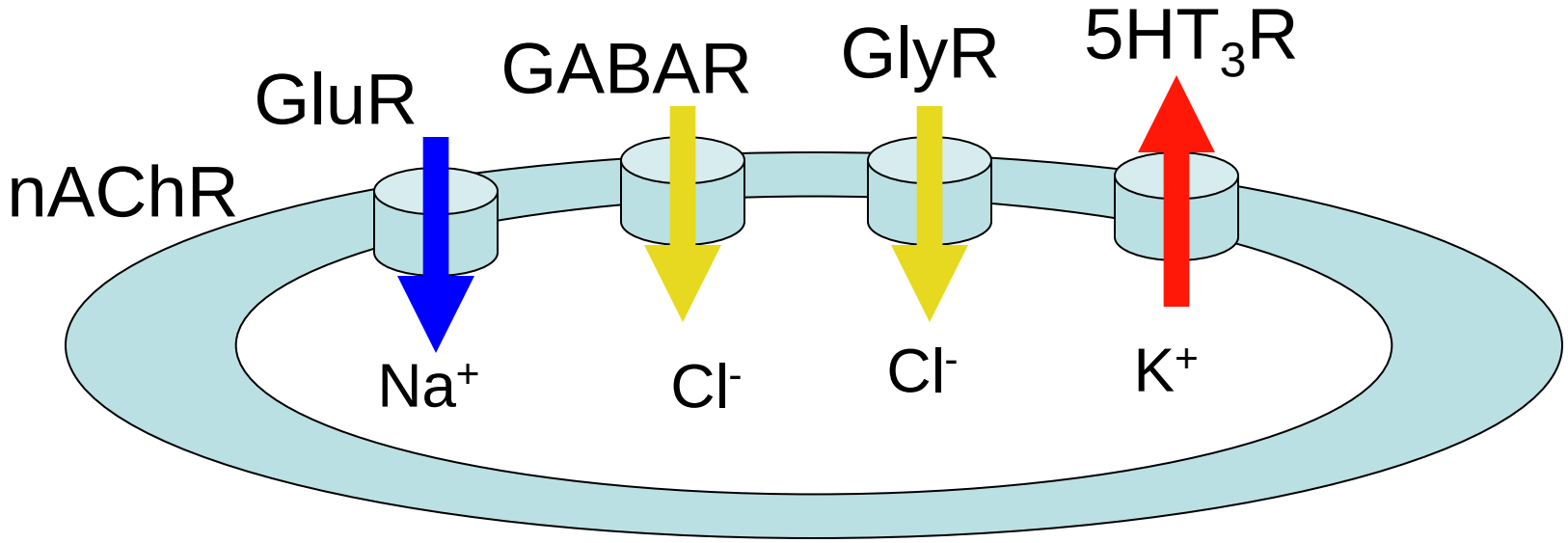


GluR



GABAR
nAChR
Gly





$GABA_A$ receptors mediate most fast INHIBITORY responses

- Activate chloride ion conductance
- Somatic location - profound effect
- Subunits encoded by 19 genes: α (6), β(3), γ(3), δ(1), ε(1), θ(1), η(1) and ρ(3) subtypes
- Each subunit contributes a unique property and exhibits a distinct pattern of distribution: α1 is most abundant, α3 forebrain, α6 cerebellum

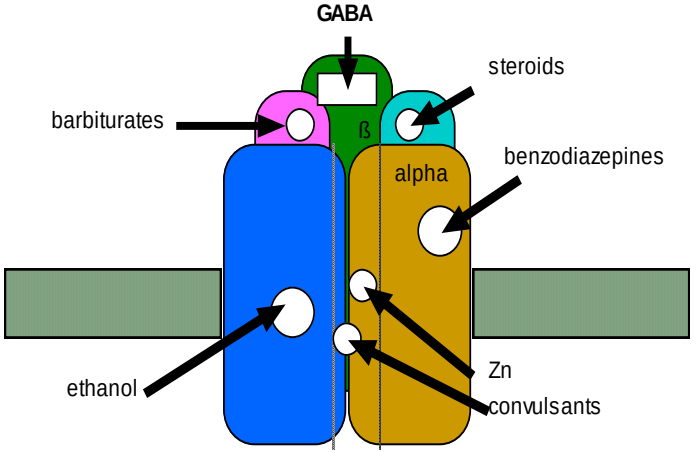
The most common native receptor contains α1, β2 and γ2

$GABA_A$ receptor subunits possess different properties

- GABA affinity (µM to mM) defined by the α subunit
- Each subunit confers a different response to BZ, Barbiturates, ethanol, steroids

- Ethanol modulation most evident in: $\alpha 6\ \beta 2\ \gamma 2_L$ receptors which are localised in cerebellum (motor incoordination)

Pentameric organisation of the GABA receptor and pharmacologically important binding domains

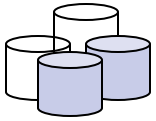


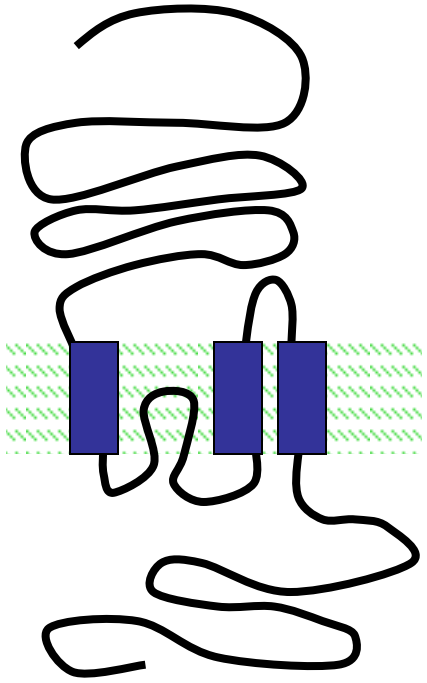
Drugs facilitating GABA transmission are:
- antiepileptic
- anxiolytic
- sedative
- muscle relaxant

GLUTAMATE RECEPTORS

- Excitatory transmission in primarily mediated by Ionotropic glutamate receptors (iGluRs)
- iGluRs mediate basic information processing and underlie changes in synaptic efficacy
  - e.g. learning and memory, developing and maintaining cellular connections,pain perception

Receptors are composed of 4 subunits, each with 3 TMs and a loop

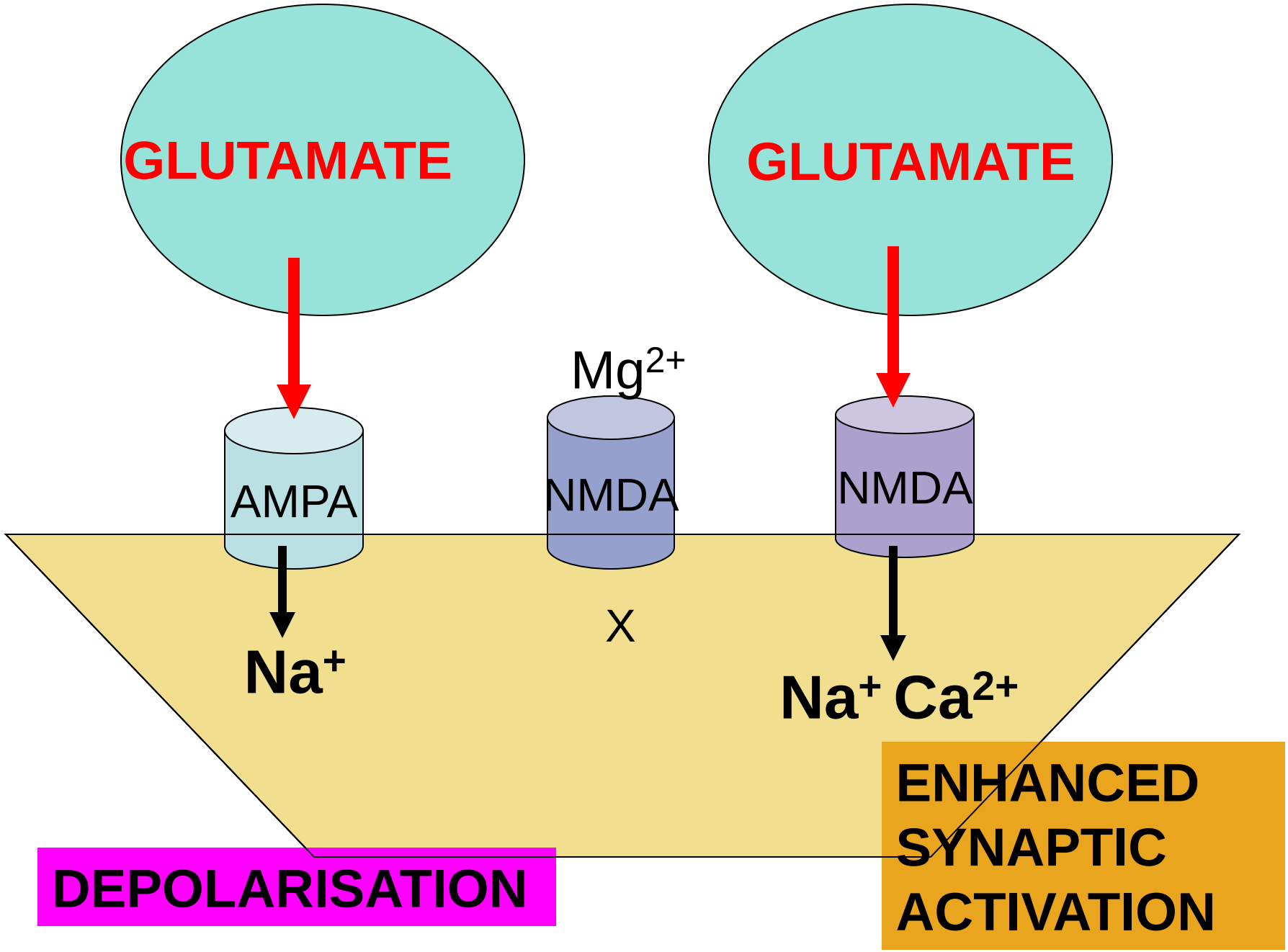




There are 3 main glutamate receptors, each having a unique role

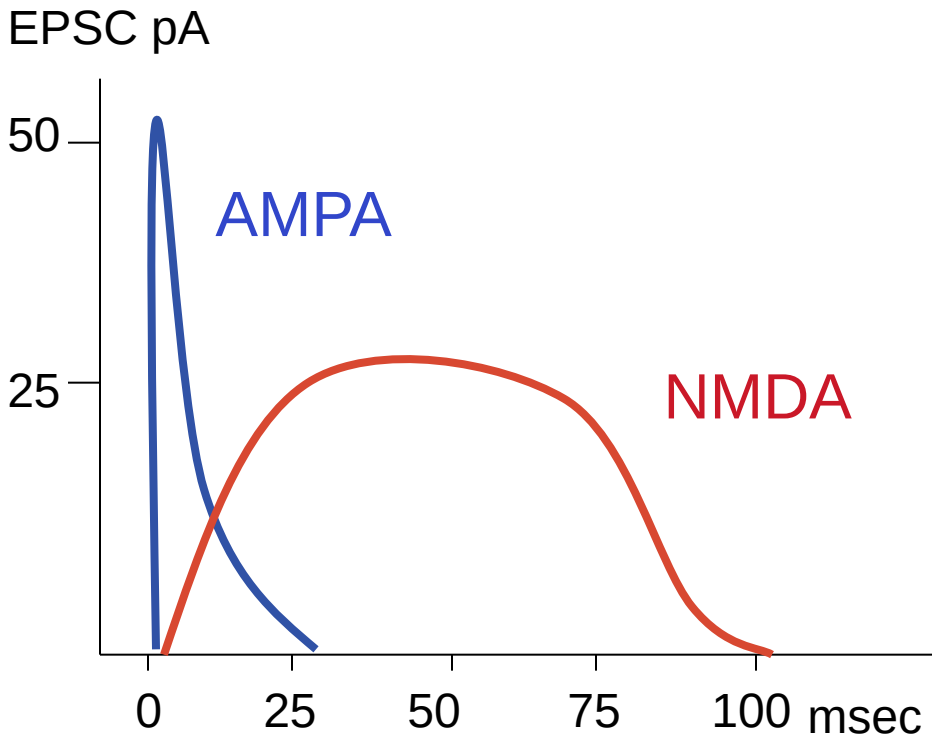
AMPA - main receptor mediating fast CNS transmission (GluR1,2,3,4)
NMDA - coincidence detection and synaptic adaptation (NR1, NR2A, B, C, D, NR3)
Kainate - modulatory role at pre and post synaptic sites (GluR5,6,7, KA1,2). Agonists are potent convulsants and environmental neurotoxins.

AMPA = (S)- -Amino-3-hydroxy-5-methyl-4-isoxazole propionic acid,
NMDA = N-methyl-D-aspartate


GLUTAMATE
GLUTAMATE
Mg2+
AMPA
NMDA
NMDA
X
Na+
Na+ Ca2+
DEPOLARISATION
ENHANCED SYNAPTIC ACTIVATION

AMPA

Fast gating kinetics
Desensitise strongly
Poorly permeable to Ca^{2+} typically
Blocked by polyamines



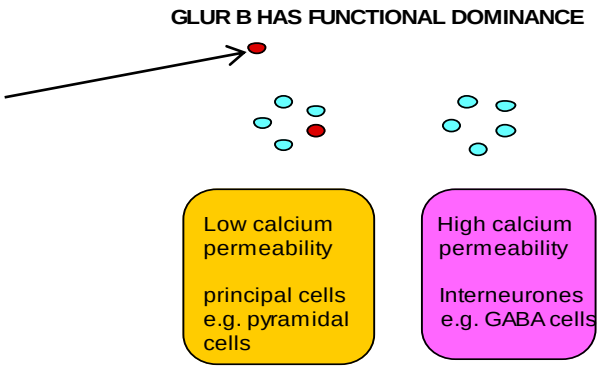
NMDA

Gate much more slowly
Desensitise weakly
Highly calcium permeable
Blocked by extracellular Mg^{2+} in a strongly voltage dependent manner

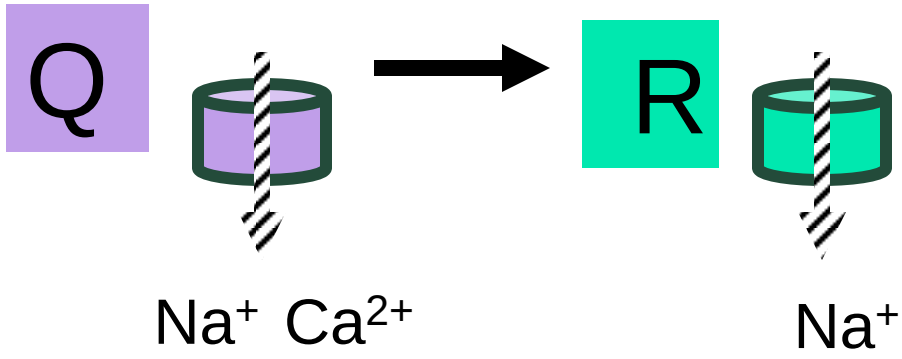
The native **AMPA** receptor is a tetrameric (dimer of dimers) complex formed from GluR1, GluR2, GluR3 and GluR4

- Most receptors contain GluR2 which confers Ca^{2+} impermeability *(typical of AMPA receptors in vivo)*
- Expression of GluR2 with either GluR1 or GluR3 yields a receptor with little divalent Ca^{2+} permeability *(Equivalent to AMPA receptor response in situ in pyramidal cells).*
- Expression of GluR1 or GluR3 alone or in combination yields a Ca^{2+} permeable channel *(unlike the native receptor !)*

GluR2 has functional dominance



RNA EDITING



In GluR2 there is a glutamine residue (Q) present in the primary genomic sequence which is edited to arginine R. This confers the change in property from calcium permeable to calcium impermeable.
GluR2 editing is ~100% in adult mammalian brain

Editing requires:

- formation of double stranded RNA around the critical region with adjacent pairing in the intronic region
- Double stranded RNA adenosine deaminase (CAG to CIG)

GluR2 determines calcium permeability

Q/R

Q/R editing is essential in the adult for mediating physiological excitation

Transgenic mice in which the GluR2 editing site has been mutated

↓

Calcium permeability increased

↓

Severe epileptic seizures

↓

Cell death in hippocampus

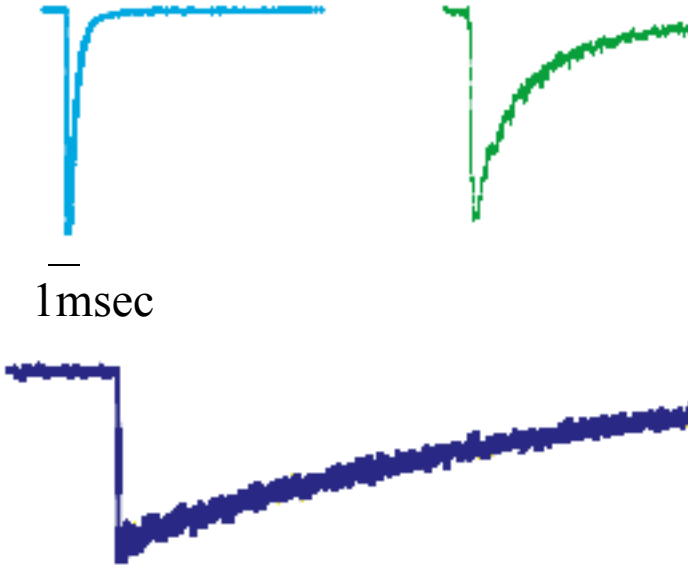
↓

Mice die at 3 weeks

Rapid excitatory transmission: NMDA receptors

- Strong afferent stimulation is needed to produce sufficient depolarisation to remove the Mg^{2+} block and activate N-methyl-D-aspartate receptors causing Ca^{2+} and Na^{+} influx.
- NMDA receptors are critical for the induction of certain forms of synaptic plasticity
- Receptors associate with other signalling molecules e.g. kinases to mediate their postsynaptic response.

NMDA receptors (NR1 + NR2A-D/NR3A-B)



Receptors with NR2B subunits instead of NR2A stay open longer - functional consequences?

NR2B-receptors have a longer phase of memory activation increasing the window during which coincidence detection can occur. Animals with increased expression of the NR2B subunit have an enhanced ability to learn (see later).

The modulatory NR2 subunits have profound effects on receptor kinetics

Cull-Candy & Leszkiewicz (2004)

Ionotropic Glutamate receptors are formed from subunits which are encoded by 18 genes which gives structural DIVERSITY

- due to multiple subunit combinations
- RNA editing
- multiple splice variants
- Regulatory sites
- post-translational modifications

which translates into functional DIVERSITY

- ion channel properties
- regional specificity
- developmental specificity
- adaptation to synaptic activity

Q/R site

NR1 has 8 slice variants

Zn^{2+} inhibition NR2A>>>B>C>D

STAGE 3 Integration of Signals

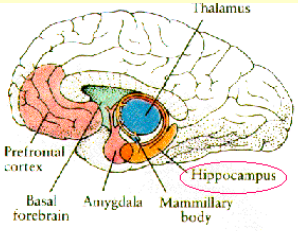
The NMDA synapse provides a "molecular model of associative memory"

If two neurones are excited at the same time then the active synapse between them is strengthened (Hebb's rule).

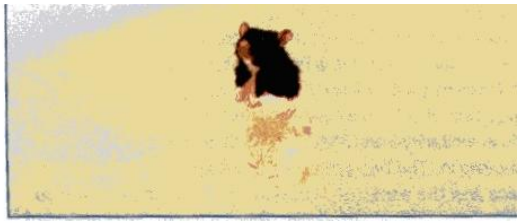
COINCIDENCE DETECTION

- A change in the efficiency of synaptic transmission ("Long term potentiation"- **LTP**.)
- Occurs during development, learning new skills, responding to environmental changes (harmless/noxious)
- Long lasting effects require protein synthesis

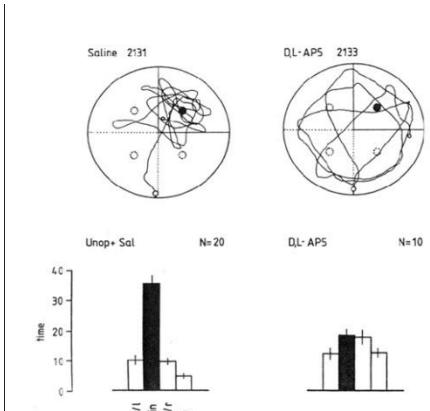
The hippocampus is a major site of learning: consolidation of new long term memory



Morris water maze

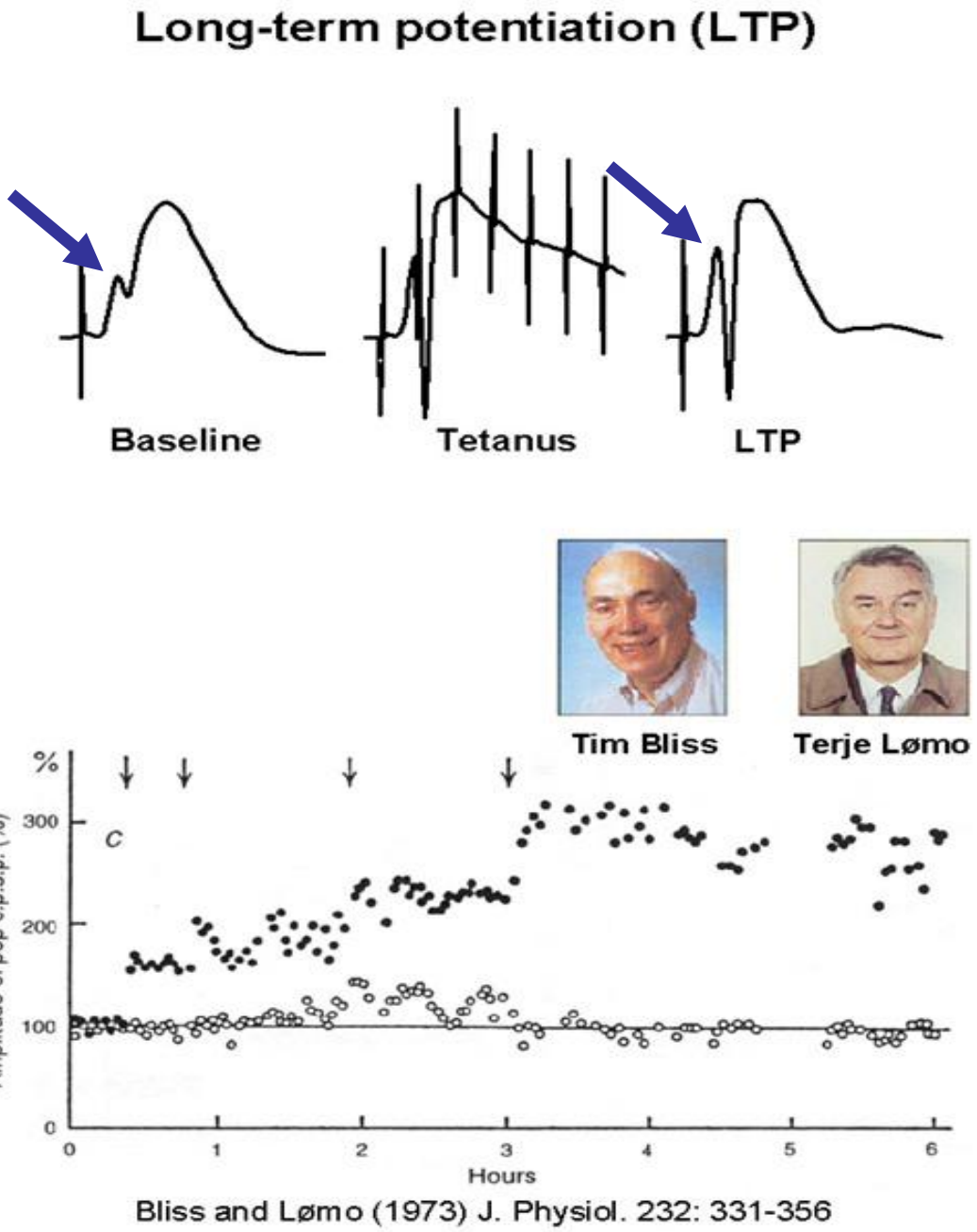


The rat finds a platform



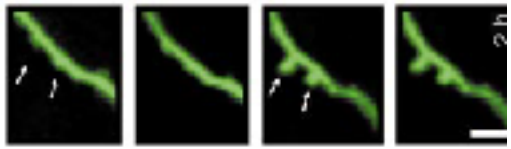
- Spatial navigation learning in rats depends on a hippocampal “map of spatial relations”
- NMDA R antagonists impair learning
- Several forms of ASSOCIATIVE memory are mediated by GLUTAMATE at the NMDA receptor and these are widely used models
- Effects are long lasting and depend on protein synthesis.

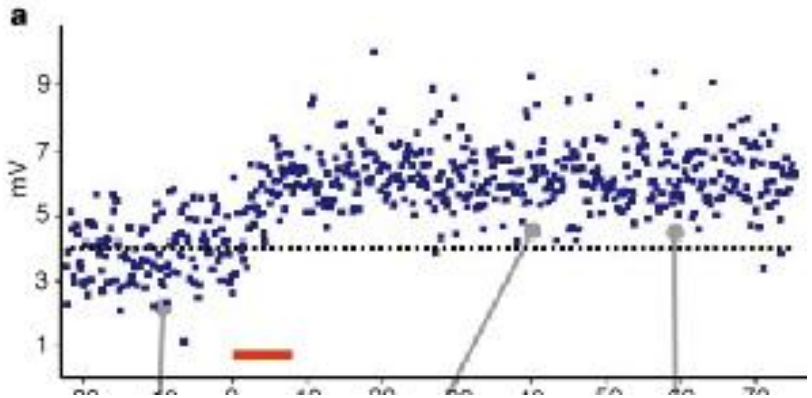
Evoked potentials in the hippocampus

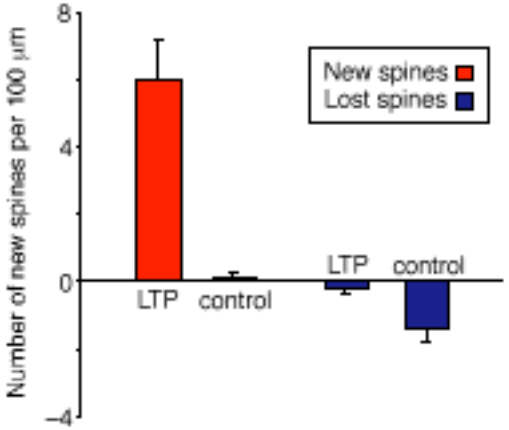


NMDA receptor-mediated Long Term Potentiation in the hippocampus provides a model of learning

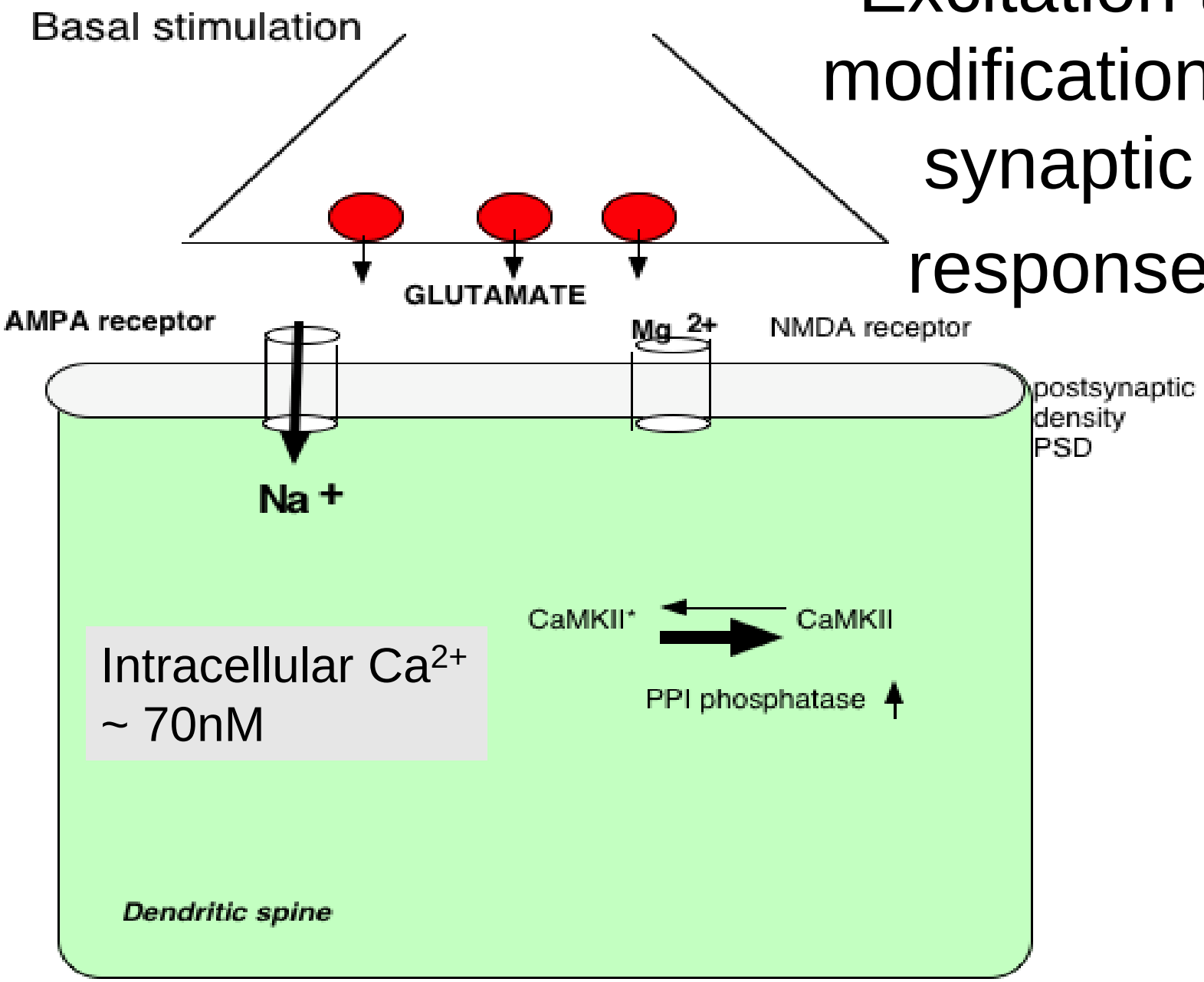
Dendritic spine changes associated with hippocampal long-term synaptic plasticity
Florian Engert & Tobias Bonhoeffer







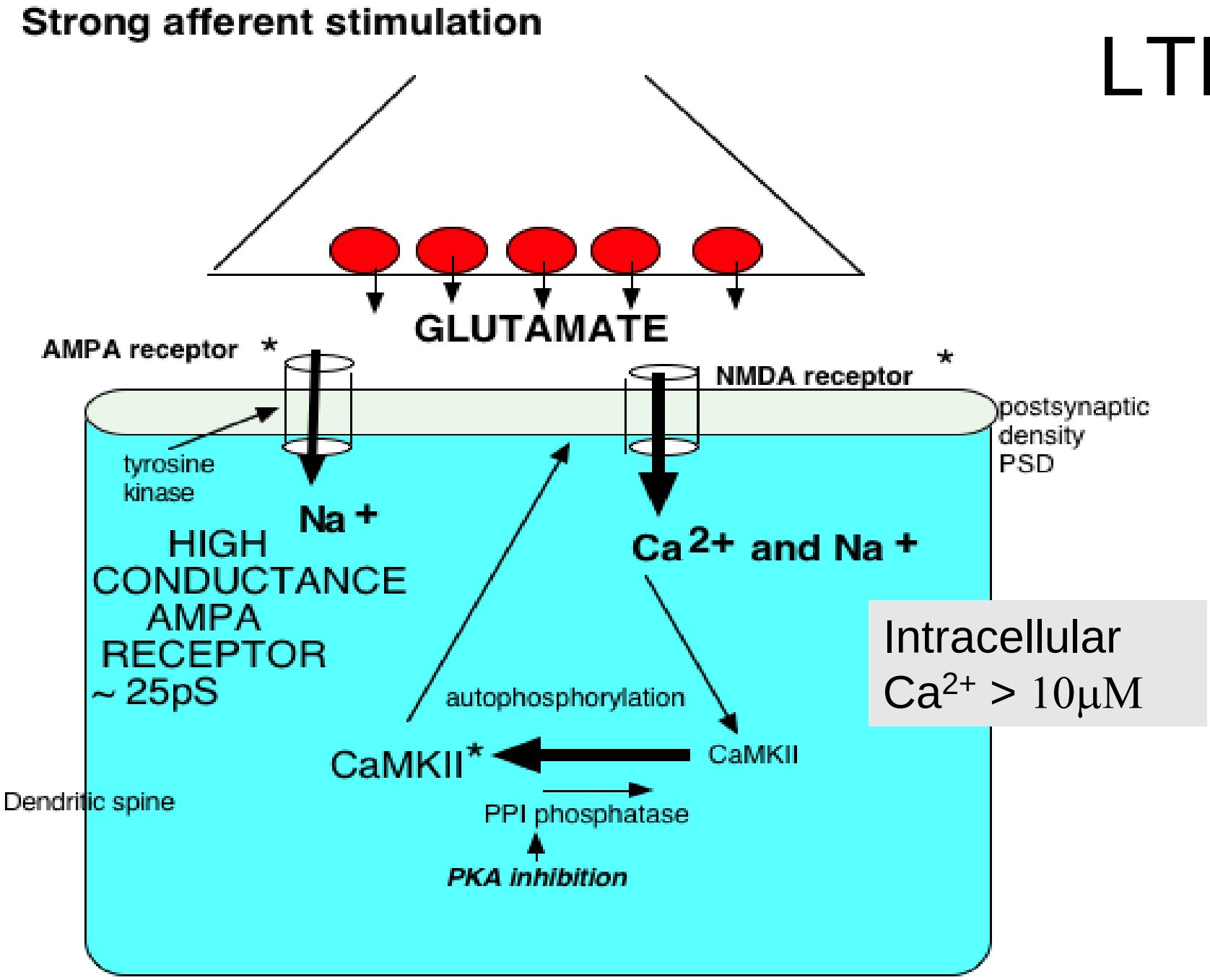
Excitation to modification of synaptic response



High frequency stimulation leads to Ca^{2+} gating through the NMDA receptor

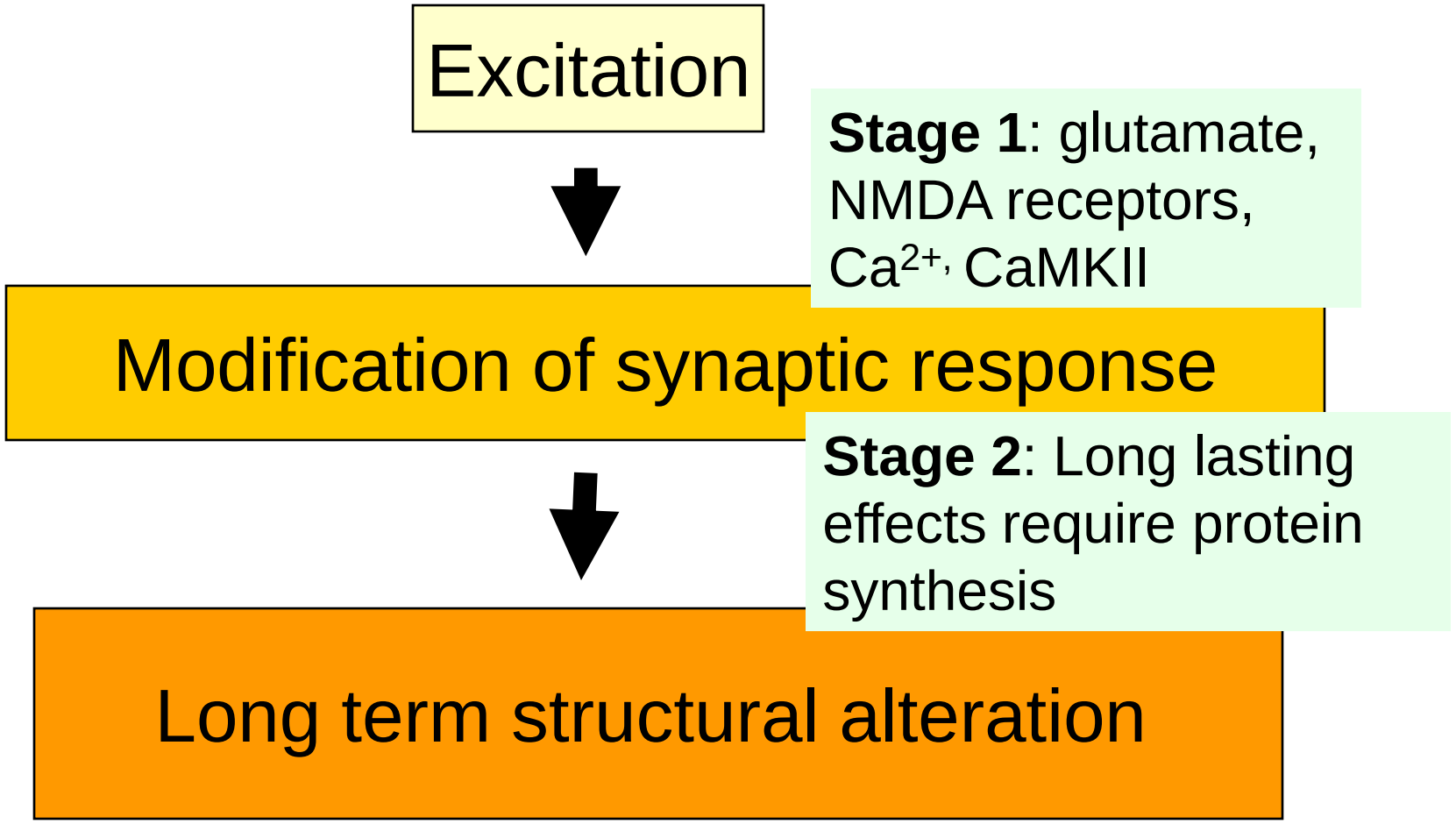
- CaMKII (calcium and calmodulin dependent kinase) is activated by Ca^{2+}
- CaMKII is autophosphorylated (Thr286) and translocates to the subsynaptic region. Point mutation inhibits LTP and memory formation
- Transient Ca^{2+} signal prolongs kinase activity until dephosphorylated by protein phosphatase

LTP



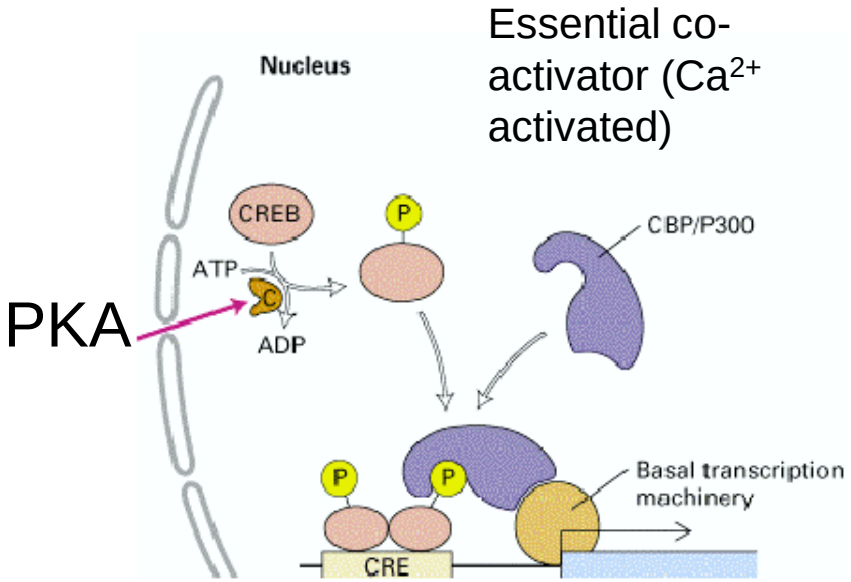
Intracellular $Ca^{2+} > 10\mu M$

What is the molecular process ?



Consolidation of changes in synaptic strength requires transcriptional activation

cAMP-Ca^{2+} responsive element binding protein (CREB) is phosphorylated by CaMKIV and PKA and binds to a CRE sequence present in the promoter region of many genes and regulates transcription

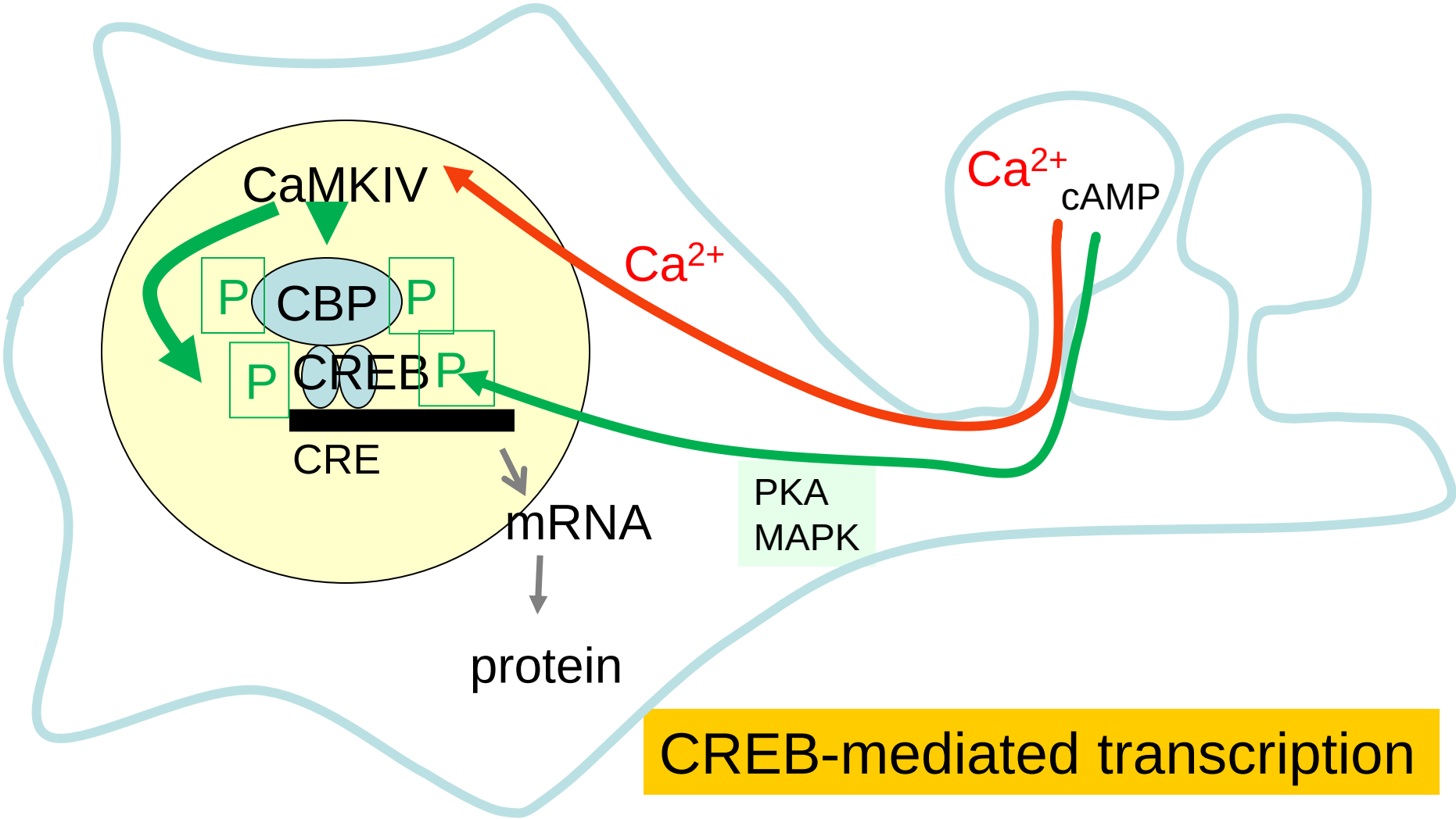


Molecular Cell Biology
Lodish et al (Freeman)

- PKA deficient mice have normal early phase LTP but lack late phase LTP.

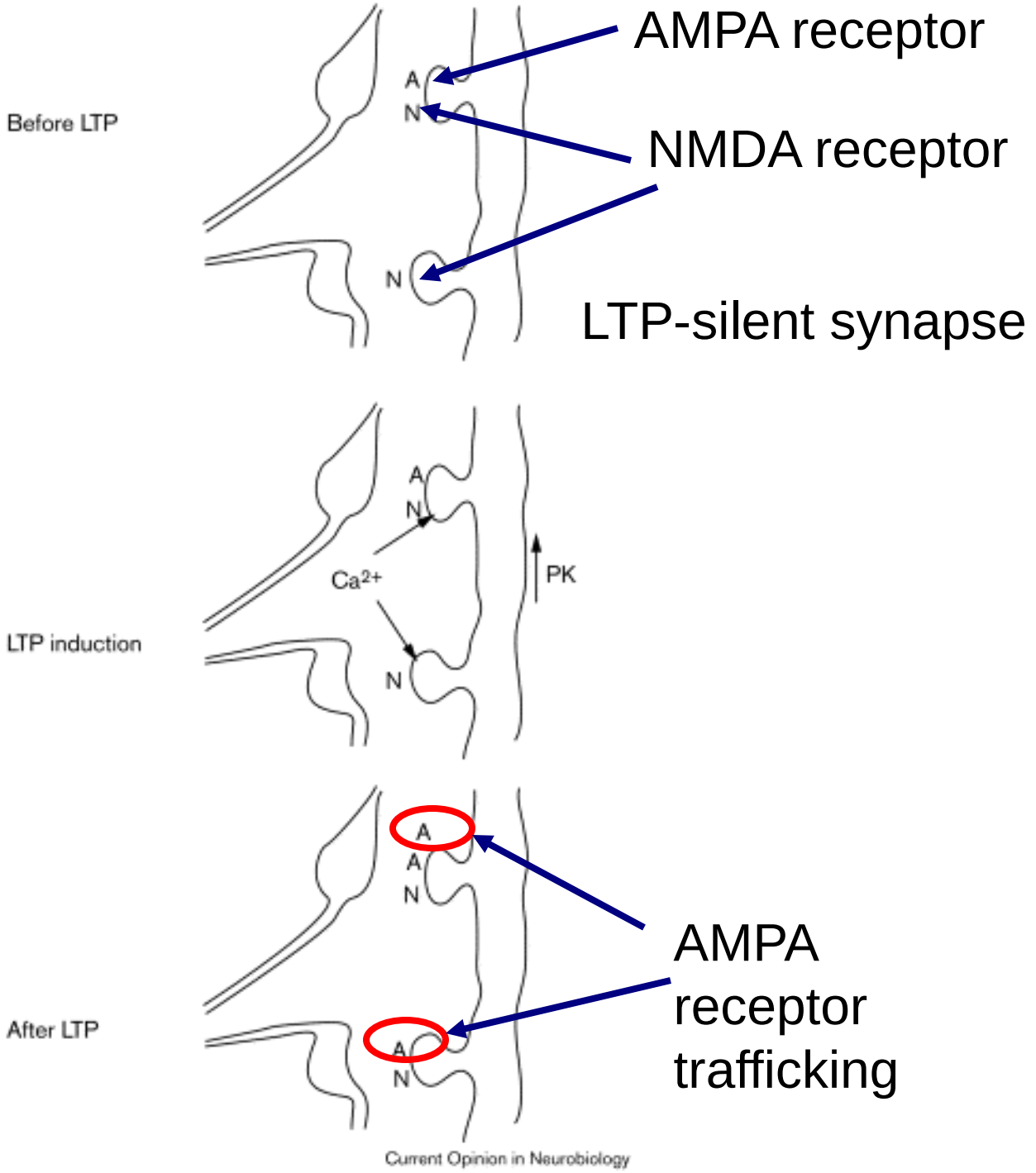
Late phase synaptic plasticity

CaMKIV-dominant negative mutants have normal E-LTP but impaired L-LTP and impaired memory consolidation. Nuclear calmodulin inhibition impairs LTP and LTM

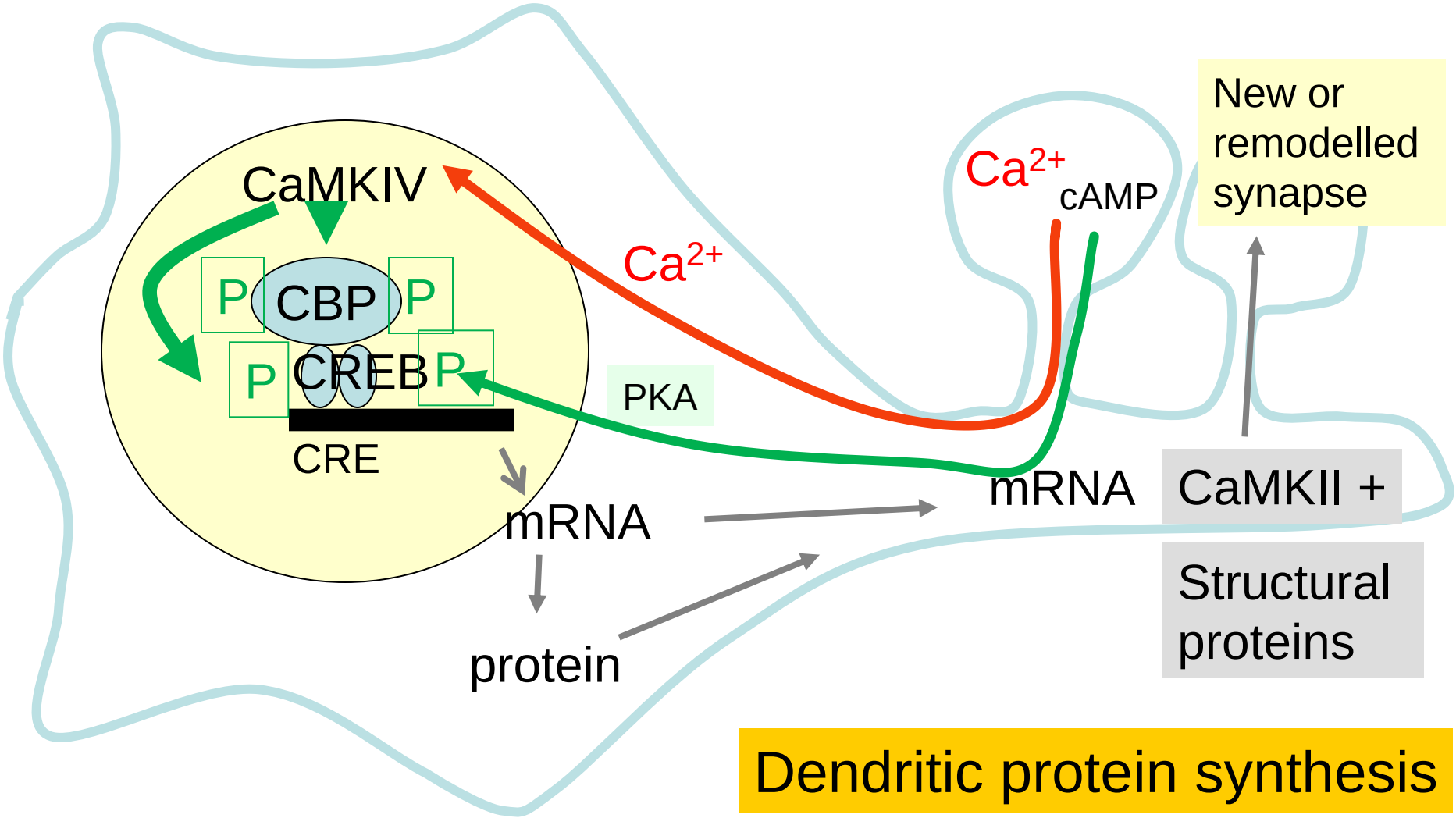


CREB-mediated transcription

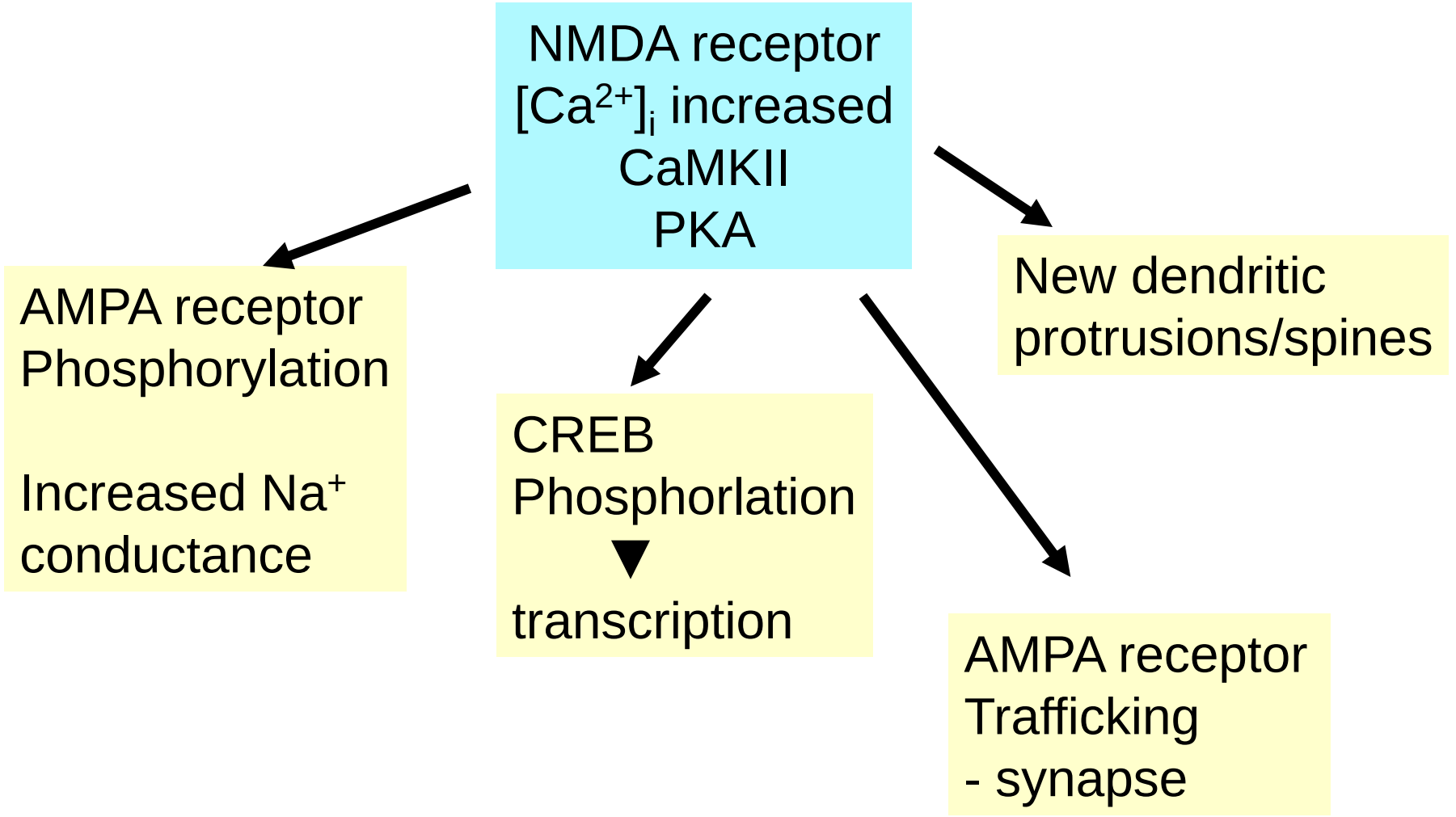
AMPA receptor Trafficking and the Insertion of AMPA receptors at the synapse is essential for LTP activating “silent synapses”



Late phase synaptic plasticity

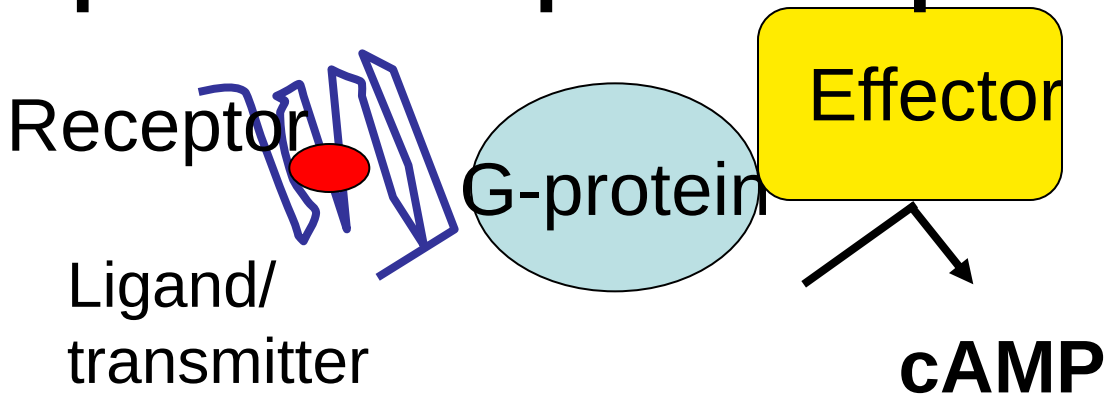


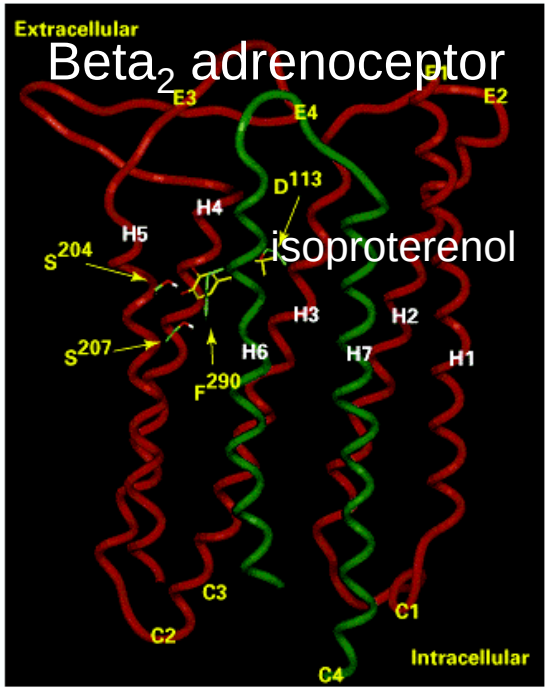
LTP stimulus



Stage 4: Pathway activation

G-protein coupled receptors





Molecular Cell Biology
Lodish et al (Freeman)

G-protein-coupled receptors mediate the action of many neurotransmitters

Amines: Dopamine, noradrenaline, 5-hydroxy-tryptamine (5-HT), muscarinic cholinergic mACh
Amino acids: $GABA_B$ metabotropic glutamate mGLUR
Neuropeptides: somatostatin, substance P, enkephalin

1. RECEPTOR

- ~1000 G-protein coupled receptors in the genome (many are 'orphan') - All have 7 alpha helices

- G-proteins have 3 subunits

16 alpha subunits define transduction system, 5 beta, 11 gamma subunits

- Effectors: Adenyl cyclase, Phospholipase C (PLC), cGMP phosphodiesterase, Phopholipase A2, K^+ or Ca^{2+} channels

2. G protein

G_S G_i G_q G_t

3. EFFECTOR

enzyme channel

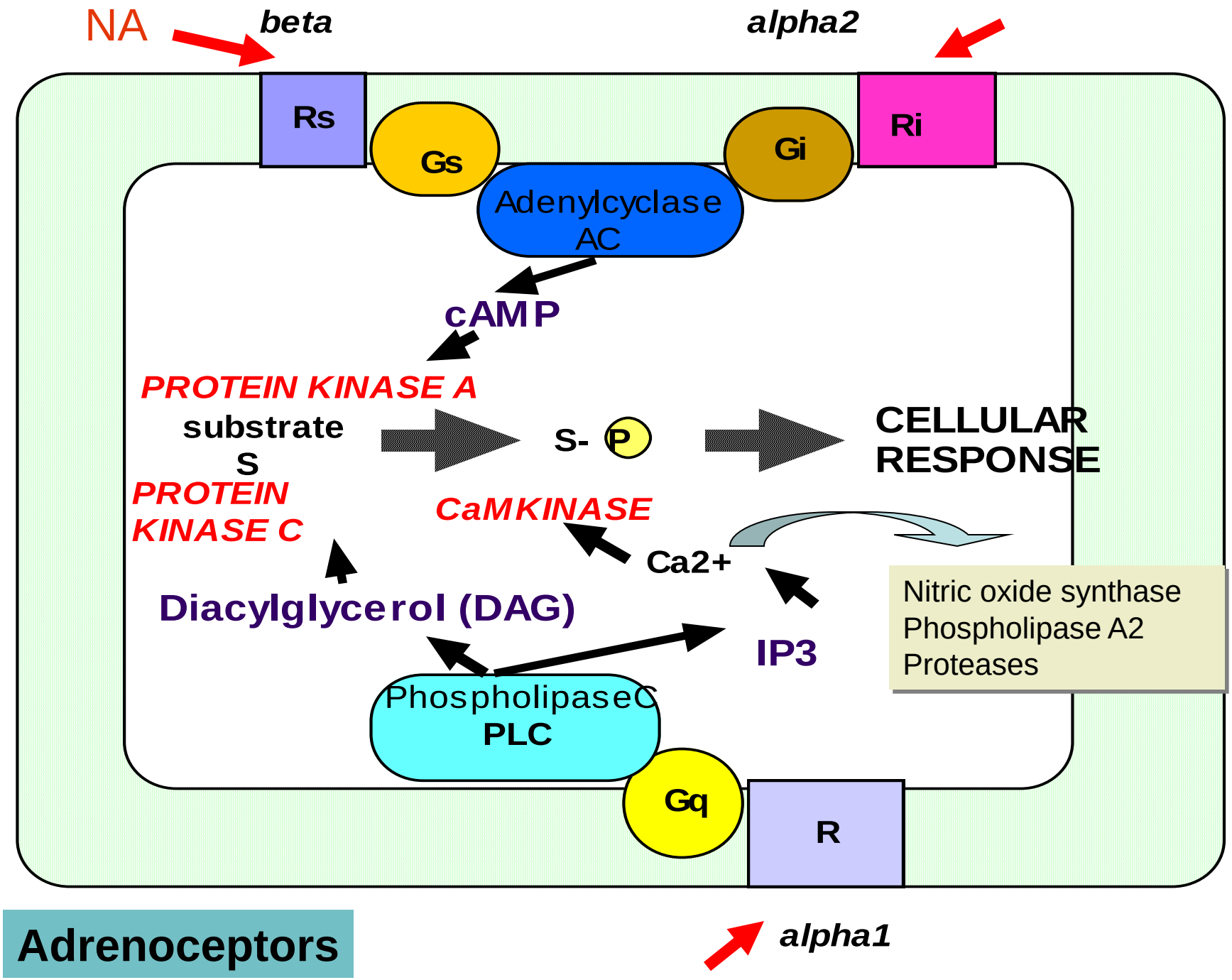
4. Intracellular messenger

cAMP
cGMP

Ca^{2+}

IP_3

Many GPCR actions are nervous system specific



Adrenoceptors

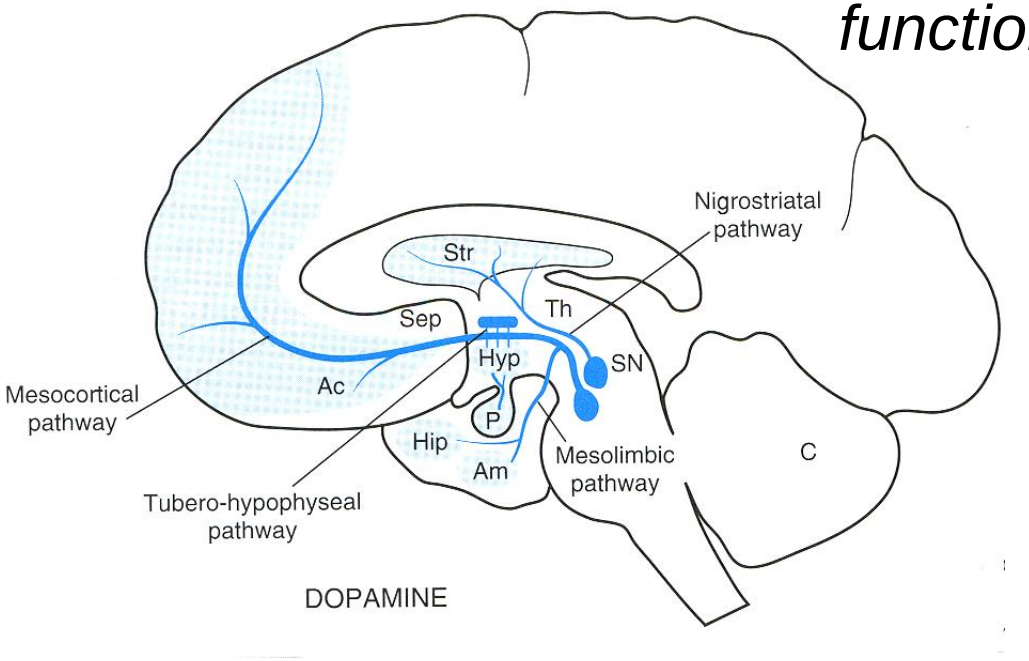
Targets for phosphorylation

- Ion channels * *cell excitability, increases (activation) or decreases (inactivates) open probability*
- Enzymes *affects transmitter availability*
- Receptors *facilitate or desensitise transmitter response*
- Transcription factors (e.g.CREB) *cause long term effects on protein synthesis*

* Direct gating of ion channels by G-proteins also occurs.

Dopamine pathways in the brain

Limbic and motor function



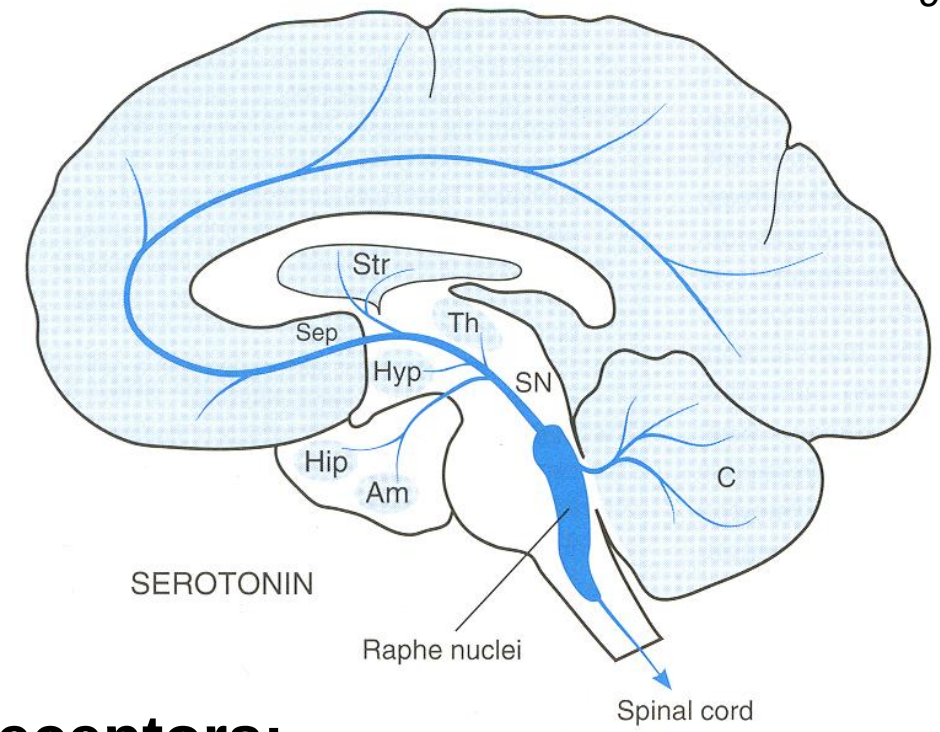
DA receptors:

D1 and D5 (D1-type) stimulate cAMP

D2, D3 and D5 (D2-type) inhibit cAMP/ increase IP3

5-hydroxytryptamine pathways in the brain

sleep, feeding, anxiety thermoregulation (1A)
cerebral vasoconstriction (1C)
CSF secretion (2C)
emesis, anxiety (3)

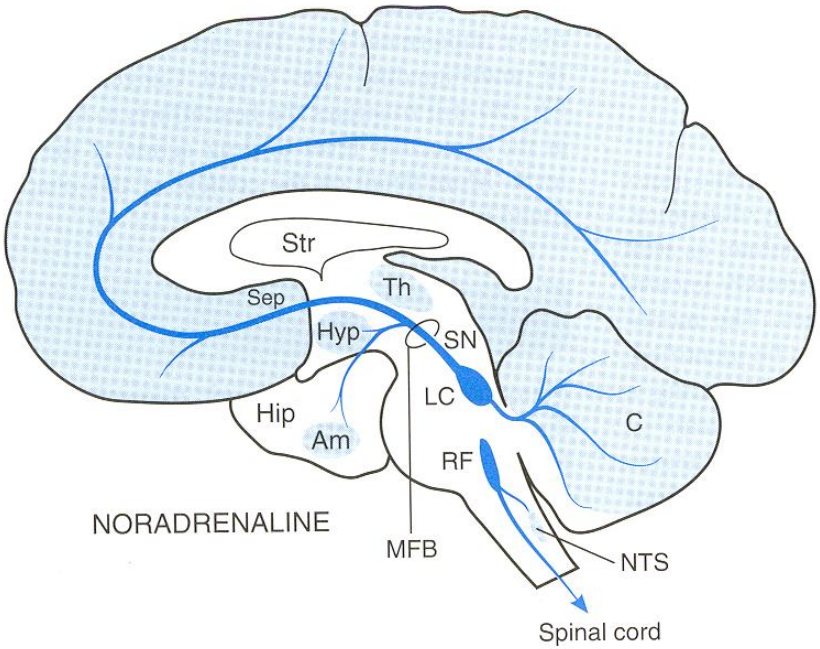


5-HT receptors:

$5HT_{1A, 1B, 1C}$ decrease cAMP
$5HT_{2A, 2B, 2C}$ increase IP_3/DAG
$5HT_3$ is an ion channel

$5HT_{4,7}$ increase cAMP
$5HT_{5, 6}$ CNS effects are less well characterised.

Noradrenergic pathways in the brain



Adrenoceptors:

Beta receptors stimulate cAMP (G_S)
$Alpha_2$ inhibit cAMP ($G_{i/o}$)
$Alpha_1$ increase IP3 (G_q)