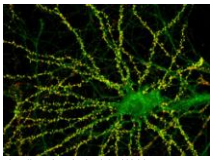
Communication in the CNS: from rapid information transfer to long term synaptic plasticity



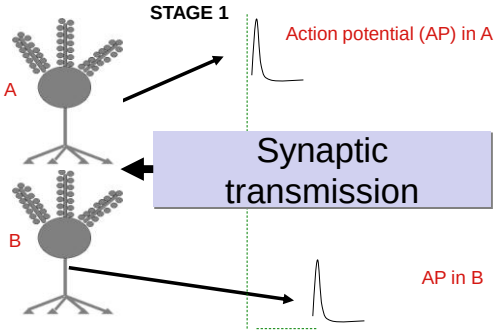
http://www.its.caltech.edu/~mbklab/

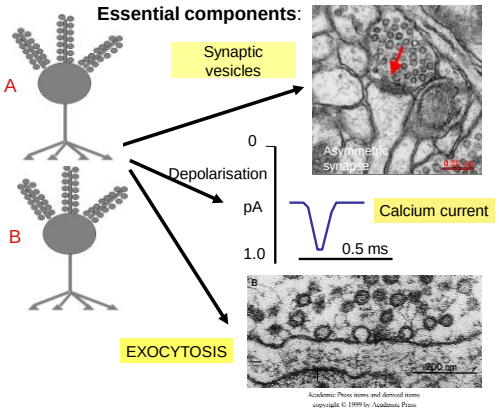
Molecular basis of memory

Synaptic transmission

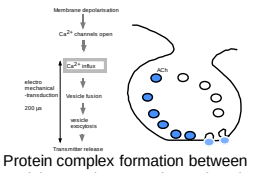
1. Transmitter released from 1st cell
2. Synaptic activation of 2nd cell
3. Signal integration and signal conduction by 2nd cell
4. Pathway activation

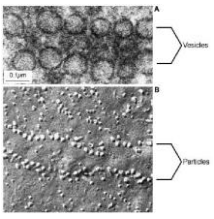
Rapid timescale
Diversity
Adaptability
Plasticity
Learning and memory





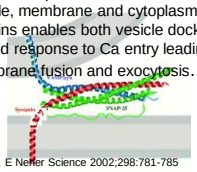
Transmitter release requires Ca^{2+} and requires RAPID transduction

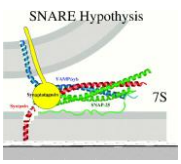




Heuser and Reece 1973

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.





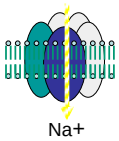
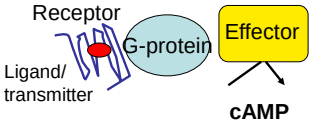
J Rettig, E Neher Science 2002;298:781-785

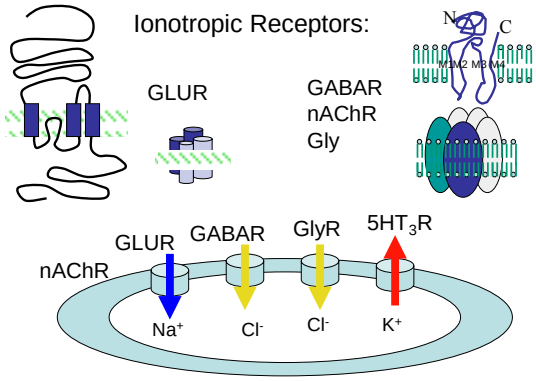
STAGE 2

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	G-protein-coupled receptor
	
Mediate all fast excitatory and inhibitory transmission	Effectors may be enzymes (adenyl cyclase, phospholipase C, cGMP-PDE) or channels (e.g. Ca^{2+} or K^+)
Glutamate, gamma amino butyric acid (GABA), glycine nicotinic receptors	muscarinic receptors, dopamine (DA), noradrenaline (NA), 5-hydroxytryptamine (5HT) and neuropeptides



$GABA_A$ receptors mediate most fast INHIBITORY responses

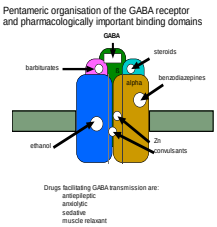
- Activate chloride ion conductance
- Somatic location - profound effect
- Subunits encoded by 17 genes: α (6), β(4), γ(4), delta(1) and rho(2) subtypes
- Each subunit contributes a unique property and exhibits a distinct pattern of distribution: α1 is most abundant, α3 forebrain, α6 cerebellum

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

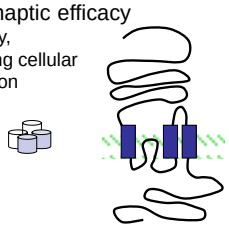
- BZ modulation requires β1
- Ethanol modulation most evident in: α6 β2 $\gamma 2_L$ receptors which are localised in cerebellum (motor incoordination)



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

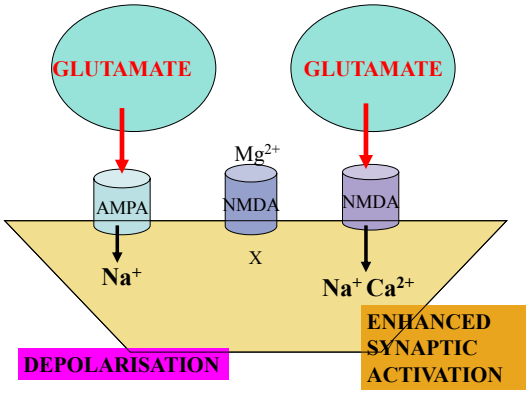
Receptors are composed of 4 subunits, each with 3 TMs and a loop



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

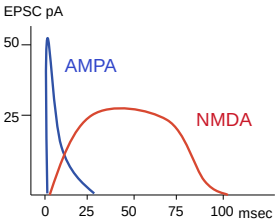
AMPA - main receptor mediating fast CNS transmission (GluR1,2,3,4)
NMDA - coincidence detection and synaptic adaptation (NR1, NR2A, B, C, D, NR3)
Kainate - modulatory role at pre and post synaptic sites (GluR5,6,7, KA1,2). Agonists are potent convulsants and environmental neurotoxins.

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



AMPA

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



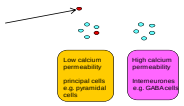
NMDA

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

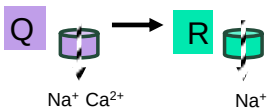
The native **AMPA** receptor is a tetrameric (dimer of dimers) complex formed from GLUR1, GLUR2, GLUR3 and GLUR4

- 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



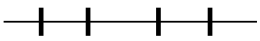
RNA EDITING



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

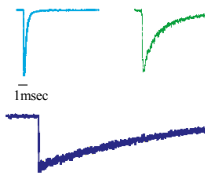
Q/R editing is essential in the adult for mediating physiological excitation

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/NR3)



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
- post-translational modifications

which translates into functional DIVERSITY

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

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).

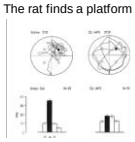
COINCIDENCE DETECTION

- 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

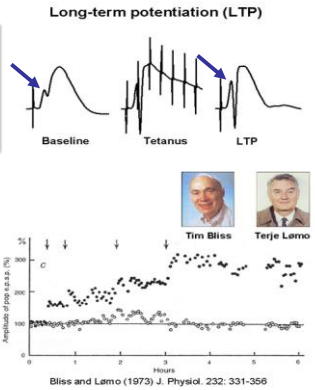
Morris water maze

The rat finds a platform



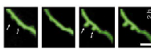
- Spatial navigation learning in rats depends on a hippocampal "map of spatial relations"
- NMDA R antagonists impair learning
- Several forms of ASSOCIATIVE memory are mediated by GLUTAMATE at the NMDA receptor and these are widely used models
- Effects are long lasting and depend on protein synthesis.

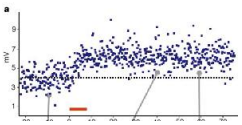
Evoked potentials in the hippocampus

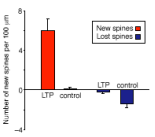


NMDA receptor-mediated Long Term Potentiation in the hippocampus provides a model of learning

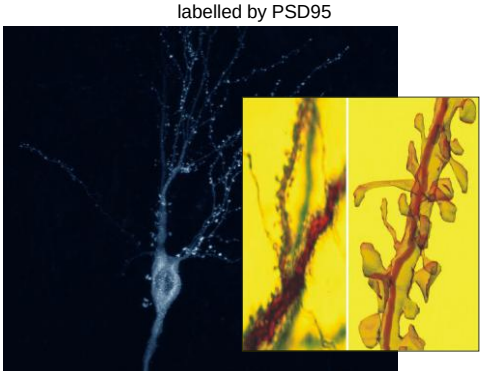
Dendritic spine changes associated with hippocampal long-term synaptic plasticity
Florian Engert & Tobias Bonhoeffer



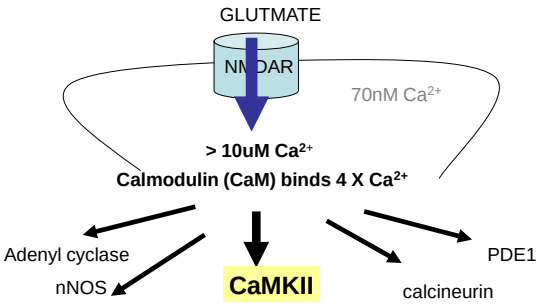


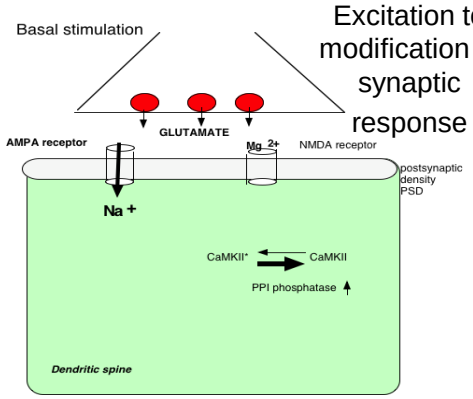


Pyramidal cell in hippocampus showing synapses labelled by PSD95



NMDA receptor and calcium signalling
calcium triggers multiple signals

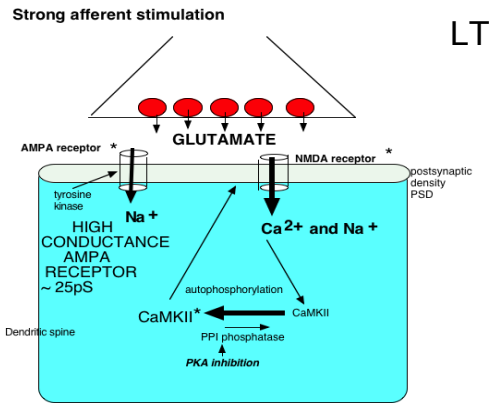




Excitation to modification of synaptic response

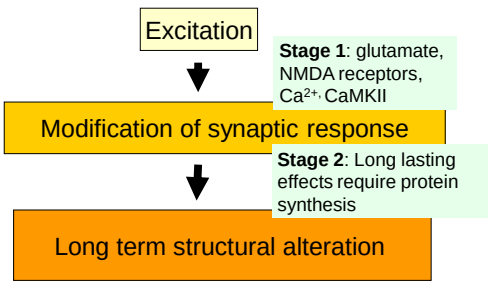
High frequency stimulation leads to Ca^{2+} gating through the NMDA receptor

- 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

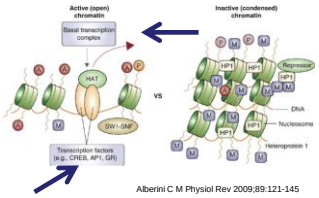
What is the molecular process ?



Consolidation of changes in synaptic strength requires transcriptional activation

- New mRNAs are transcribed after LTP (minutes) **CaMKII**, zif 268 (transcription factor) ARC (activity-dependent cytoskeletal protein)

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



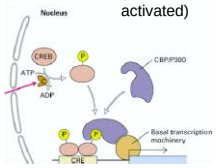
Alberini C M Physiol Rev 2009;89:121-145

CREB plays a major role in long term memory

- CREB phosphorylation occurs at ser133 (CaMKIV and PKA)
- PKA deficient mice have normal early phase LTP but lack late phase LTP.

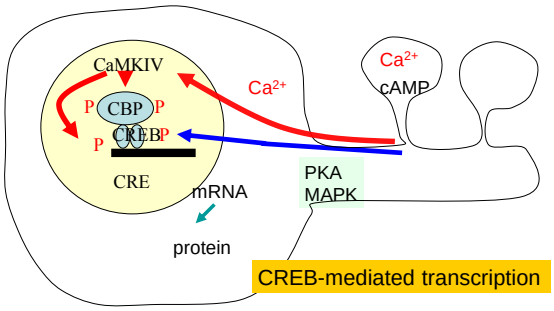
Essential co-activator (Ca^{2+} activated)

PKA

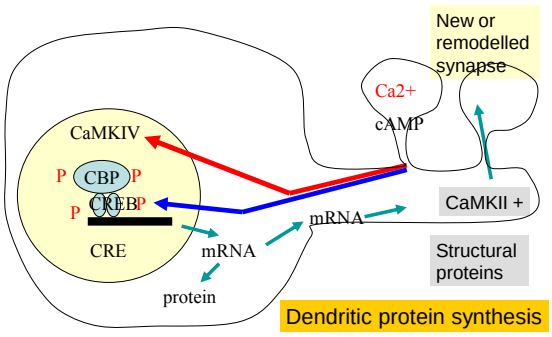


Molecular Cell Biology
Lodish et al (Freeman)

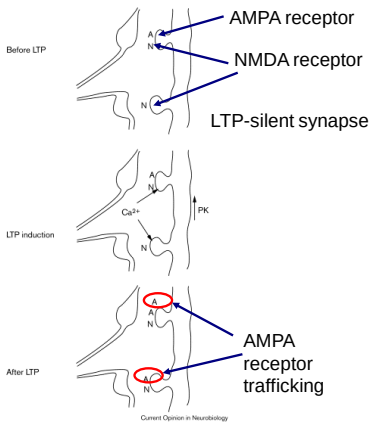
CaMKIV-dominant negative mutants have normal E-LTP but impaired L-LTP and impaired memory consolidation
Nuclear calmodulin inhibition impairs LTP and LTM



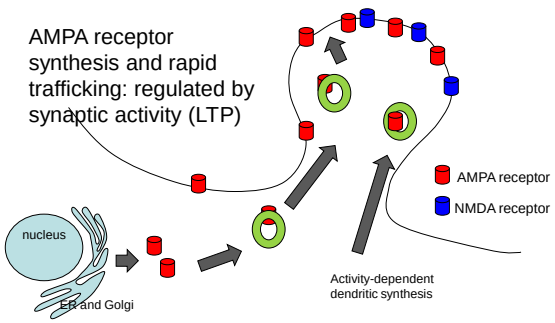
Late phase synaptic plasticity



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



AMPA receptor synthesis and rapid trafficking: regulated by synaptic activity (LTP)

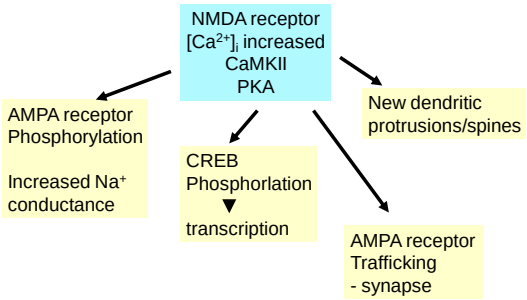


CaMKII-dependent GluR1 phosphorylation and incorporation into endosomes for transport and PKA –dependent insertion into synapse

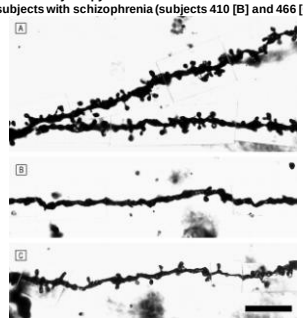
Transmembrane AMPA receptor regulatory proteins (TARPs) such as stargazin, an auxillary subunit, are phosphorylated by CaMKII and stabilise the synaptic receptor through interaction with PSD95

Stargazer mouse mutant: accesory protein affecting AMPA receptor trafficking
stargazin results in absence epilepsy, cerebellar ataxia, and a characteristic abnormal motor syndrome

LTP stimulus



Brightfield photomicrographs illustrating Golgi-impregnated basilar dendrites and spines on dorsolateral prefrontal cortex layer 3 pyramidal neurons from normal control subject 390 (A) and 2 subjects with schizophrenia (subjects 410 [B] and 466 [C])



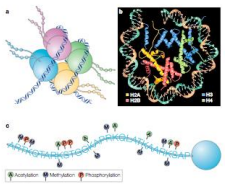
Glantz, L. A. et al. Arch Gen Psychiatry 2000;57:65-73.

ARCHIVES OF GENERAL PSYCHIATRY

The laying down of a stable memory trace in long term memory (LTM) is an experience dependent process which results in long lasting changes in synaptic strength and remodeling as seen in LTP

Recognition memory (distinguishing between familiar and novel objects), cued and contextual fear associations (e.g. addiction)

Recently the importance of histone modification in regulating transcription has been shown in LTP and LTM as well as long-term neuronal responses underlying psychiatric conditions such as drug addiction, stress, epilepsy, and depression



Levenson and Sweatt

Long Term memory (LTM) – defines who we are

Labile ⟶ sensitive to interference ⟶ Consolidation – stable- resistant

Synaptic consolidation: new dendritic spines, protein synthesis, receptor trafficking

Histones form a substrate for LT changes in gene expression

Acquisition	Ongoing consolidation	Maximal performance
STM Day 1 (spatial learning)	Day 3 (hippocampus)	Day 5
	INCREASED	
	CBP, HAT, acetylated histones, c-fos, fos B, zif 268, BDNF	

CBP mutations, HAT inhibition cause a memory defect, impaired performance
HDAC inhbitors restore memory