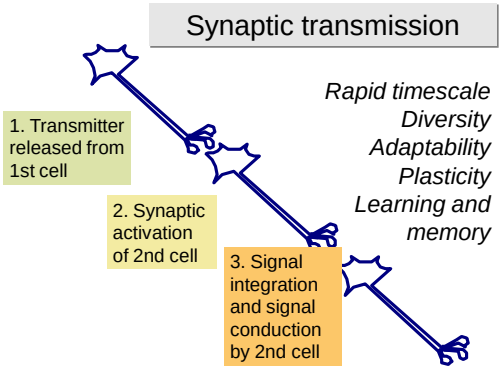
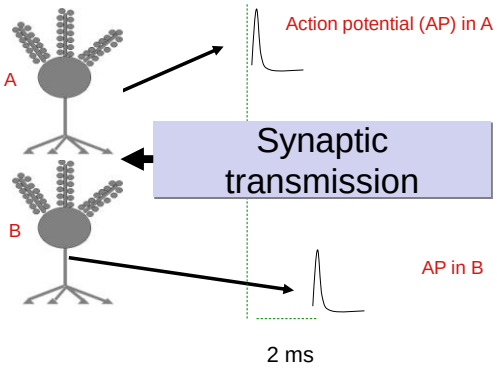
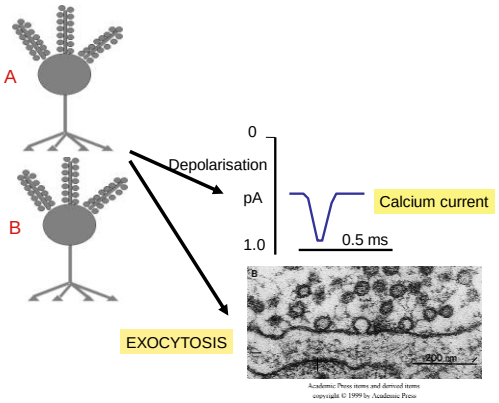
Communication in the CNS: from rapid information transfer to long term synaptic plasticity

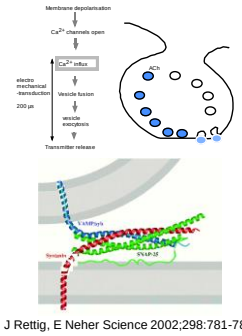
Molecular basis of memory



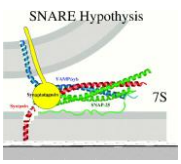




Transmitter release requires Ca^{2+} and requires RAPID transduction

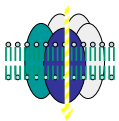
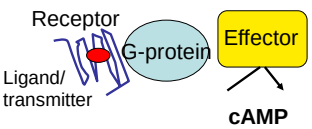


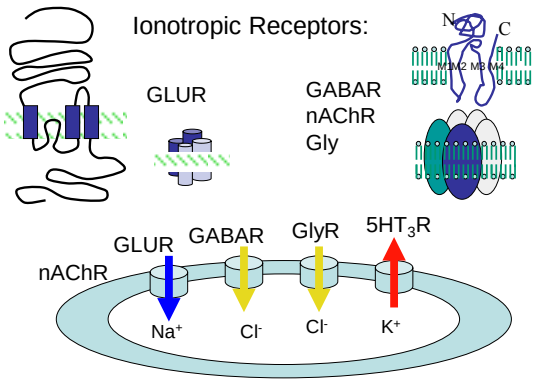
Protein complex formation between vesicle, membrane and cytoplasmic proteins enables both vesicle docking and a rapid response to Ca entry leading to membrane fusion and exocytosis.



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

Neurotransmitter action is defined by receptor kinetics

Ion channel receptor	G-protein-coupled receptor
 Na+	 cAMP
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

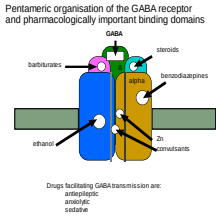
- Activate chloride ion conductance
- Somatic location - profound effect
- Subunits encoded by 17 genes: α (6), β(4), γ(4), delta(1) and rho(2) subtypes
- Each subunit contributes a unique property and exhibits a distinct pattern of distribution: α1 is most abundant, α3 forebrain, α6 cerebellum

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

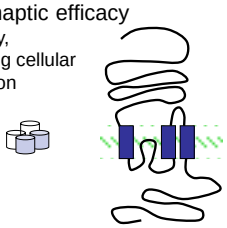
- BZ modulation requires β1
- Ethanol modulation most evident in: α6 β2 $\gamma 2_L$ receptors which are localised in cerebellum (motor incoordination)



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

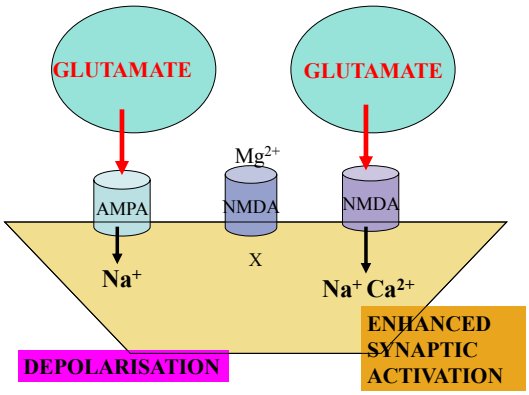
Receptors are composed of 4 subunits, each with 3 TMs and a loop



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

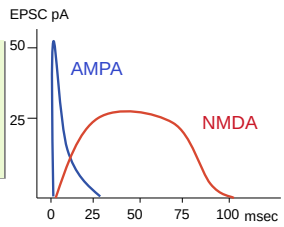
AMPA - main receptor mediating fast CNS transmission (GluR1,2,3,4)
NMDA - coincidence detection and synaptic adaptation (NR1, NR2A, B, C, D, NR3)
Kainate - modulatory role at pre and post synaptic sites (GluR5,6,7, KA1,2). Agonists are potent convulsants and environmental neurotoxins.

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



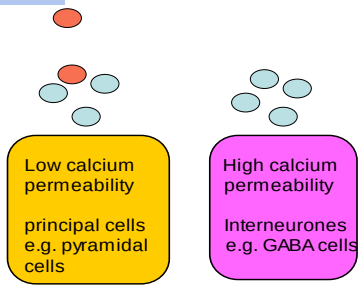
NMDA

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

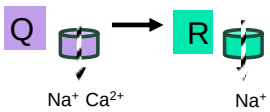
- Most receptors contain GLUR2 which confers calcium impermeability *(typical characteristic of AMPA receptors in vivo)*
- Expression of GLUR2 with either GLUR1 or GLUR3 yields a receptor with no divalent cation (Ca^{2+}) permeability *(Equivalent to AMPA receptor response in situ in pyramidal cells).*
- However, expression of GLUR1 or GLUR3 alone or in combination yields a Ca^{2+} permeable channel unlike the native receptor !

GLUR 2 HAS FUNCTIONAL DOMINANCE



Distinct AMPA receptor subunit compositions

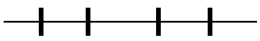
RNA EDITING



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

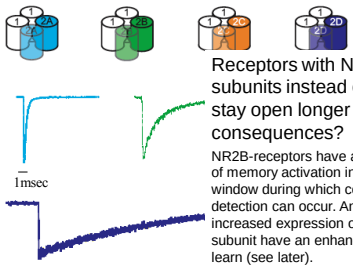
Q/R editing is essential in the adult for mediating physiological excitation

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

"Molecular models of memory"

Long term potentiation

Modification of function by experience and its relevance to learning and memory and development

COINCIDENCE DETECTION

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

Provides the model for associative memory in the hippocampus

What is the molecular mechanism that underlies long term memory ?

Synaptic plasticity

- A change in the efficiency of synaptic transmission
- Occurs during development, learning new skills, responding to environmental changes (harmless and noxious).
- Long term potentiation (LTP) is the most widely used model.

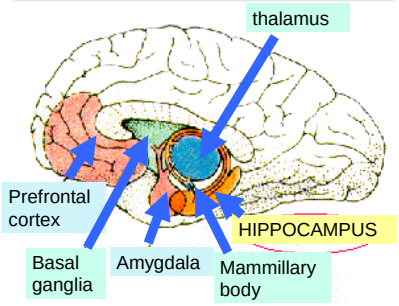
Plasticity is reflected by a modification of synaptic strength

- Occurs only on activated synapses and not on neighbouring synapses even on the same cell
- Involves intracellular signalling cascades and phosphorylation
- Long lasting effects require protein synthesis

The hippocampus is essential for certain types of learning and memory

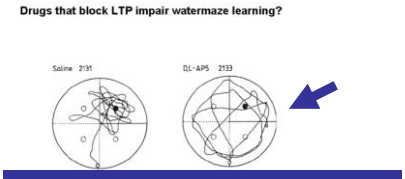
- Unilateral and bilateral damage of the hippocampus occurs in human epilepsy (temporal lobe epilepsy) causing severe memory impairment.
- Bilateral stroke affecting hippocampus (CA1) causes anteriograde amnesia.
- Historic memory is unimpaired but consolidation of new long term memory is affected EPISODIC MEMORY

Brain regions involved in learning



The hippocampus provides a model of learning and memory

- Spatial navigation learning in rats depends on a hippocampal “map of spatial relations”
- Some forms of ASSOCIATIVE memory are mediated by GLUTAMATE at the NMDA receptor and these are widely used models.
- Similar mechanisms are mediated by other receptors.

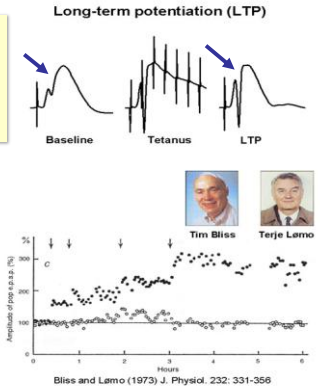


NMDA receptor antagonists impair learning

Morris water maze:
spatial navigation test:
Hippocampal lesions increase time taken to find platform from 10 secs to 1 min.

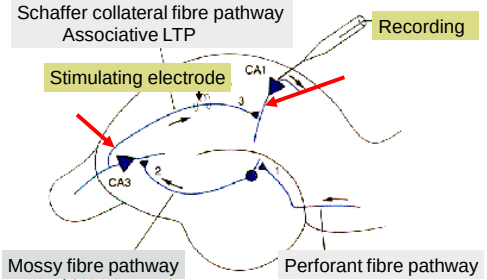
Morris (1989) J. Neurosci. 9: 3040-3057

Evoked potentials in the hippocampus



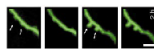
Bliss and Lømo (1973) J. Physiol. 232: 331-356

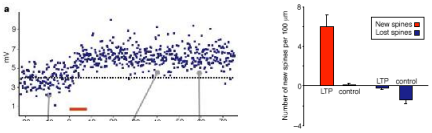
LONG TERM POTENTIATION



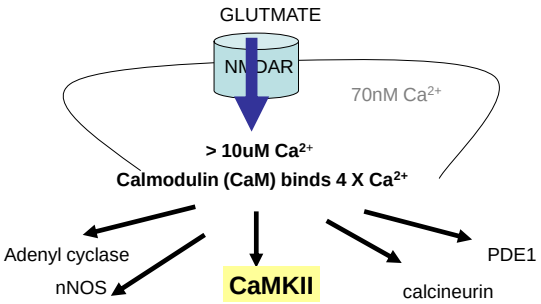
NMDA receptor-mediated Long Term Potentiation in the hippocampus provides a model of learning

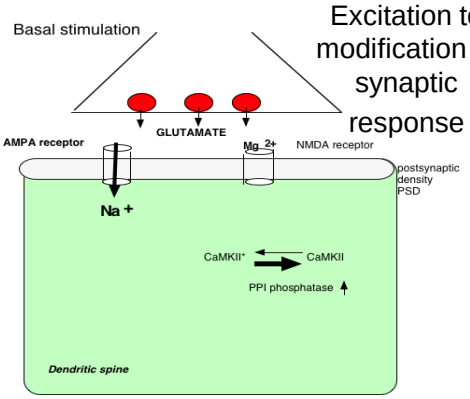
Dendritic spine changes associated with hippocampal long-term synaptic plasticity
Florian Engert & Tobias Bonhoeffer





NMDA receptor and calcium signalling
calcium triggers multiple signals

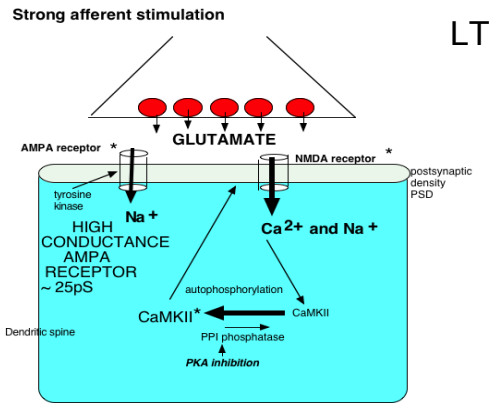




Excitation to modification of synaptic response

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

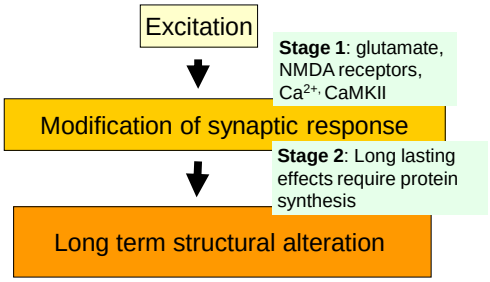


LTP

AMPA receptor subunits are direct targets of protein kinases and phosphatases

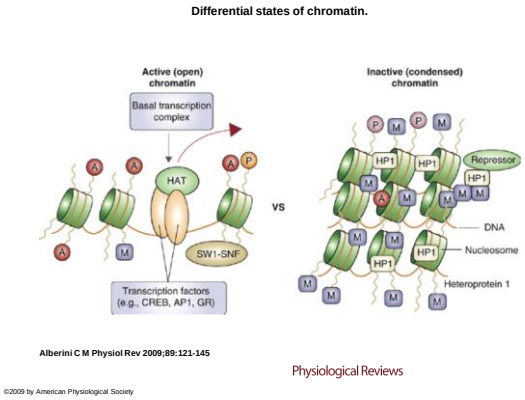
- Phosphorylation of the AMPA receptor regulates the intrinsic channel properties of the receptor causing increased single channel conductance.
- In LTP, GLUR1 is phosphorylated by CaMKII at ser831. Homologous serines are not found in GLUR2-4.

What is the molecular process ?



Consolidation of changes in synaptic strength requires transcriptional activation

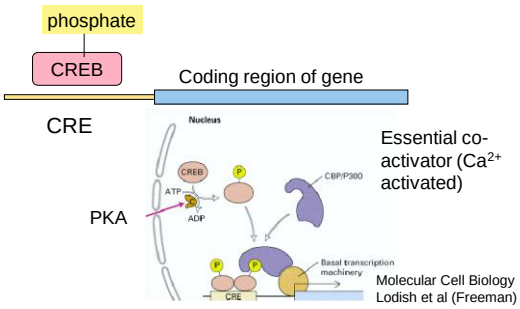
- New mRNAs are transcribed as early as 2 mins after LTP CaMKII, zif 268 (transcription factor) ARC (activity-dependent cytoskeletal protein)
- RNA synthesis inhibitors are only effective against L-LTP if given prior to or at time of LTP



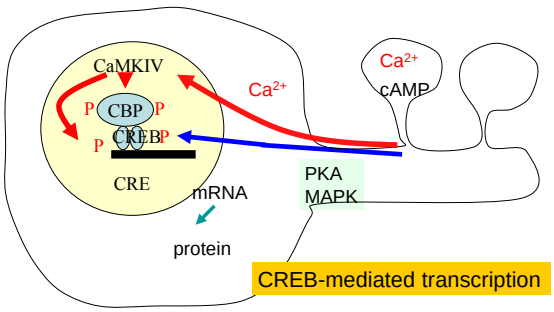
CREB activates transcription

- The transcription factor CREB plays a major role in long term memory
- Phosphorylation of CREB occurs at ser133 (CaMKIV and PKA)
- PKA deficient mice have normal early phase LTP but lack late phase LTP.

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



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



Protein synthesis in LTP
continued

- Protein synthesis occurs in cell body
- Unexpectedly protein synthesis shown in dendrites
- tRNAs and polyribosomes found in dendrites
- mRNAs (esp CaMKII mRNA) located at synapses/dendrites are translationally regulated by neural activity

LTP and protein synthesis

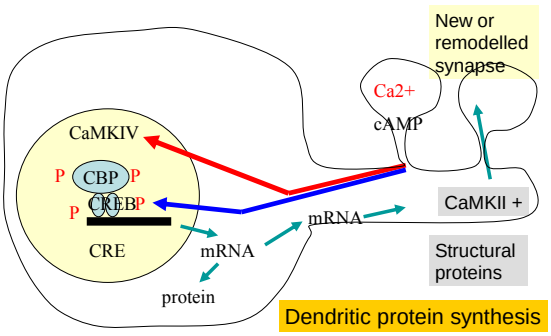
- protein synthesis > 1-2 hours
- Occurs in the SOMA and DENDRITES

PROTEIN KINASE-dependent phosphorylation of nuclear transcription factors

Cytosolic CaMKII phosphorylates polyadenylation element (CPE) activating translation locally in the dendrites

Exposure of dark reared rats to light for 30min leads to an activity dependent reorganisation of visual cortex with increased CaMKII mRNA polyadenylation

Late phase synaptic plasticity



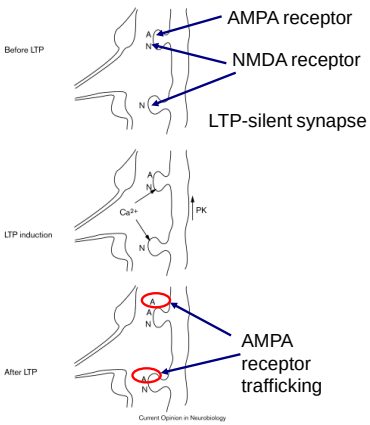
Receptor synthesis and trafficking to the synapse

AMPA receptor number, synaptic localisation and subunit composition are regulated by activity and history of activity

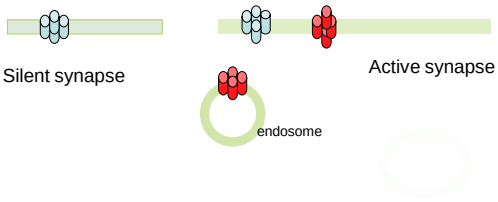
AMPARs are highly mobile proteins

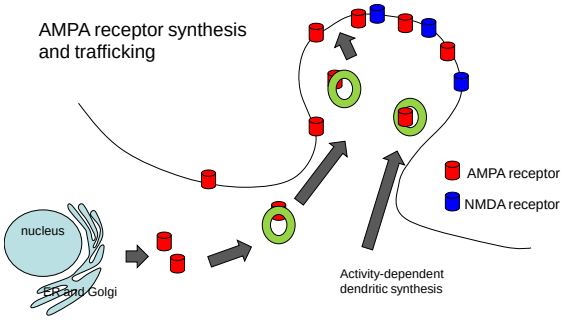
Changes in AMPAR formation (delivery and properties) are involved in LTP.

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



AMPA receptor trafficking





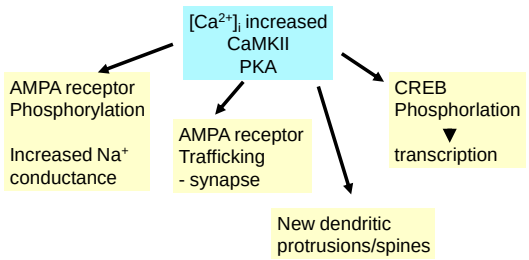
AMPA receptor trafficking

NMDA receptor
Ca^{2+}
↓
CaMKII-dependent GluR1 phosphorylation
↓
Transfer of phosphorylated GluR1 containing endosomes from dendritic shaft to spine in LTP via the adaptor protein Rab11 involves myosin Va.
↓
On LTP GluRs undergo PKA-dependent insertion at perisynaptic sites and then are translocated to the synapse for full expression in LTP (PKC-dependent)
↓
Regulation though endocytosis and degradation though phosphorylation and dephosphorylation

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

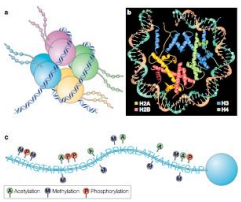


Are the processes occurring in LTP relevant to laying down memory and memory read-out?

The laying down of a stable memory trace is an experience dependent process which results in long lasting changes in synaptic strength and remodeling

Expression of long term memories has common features with LTP e.g. Recognition memory (distinguishing between familiar and novel objects), cued and contextual fear associations (e.g. addiction)

Histone modification has a profound effect on transcription

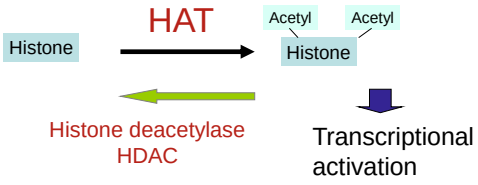


Levenson and Sweatt

Transcriptional cascades are integral to long-term synaptic plasticity and long-term memory formation. Recent studies have now focused on the chromatin changes that are key to this process and have shown that chromatin remodeling is involved in learning and memory, as well as long-term neuronal responses underlying psychiatric conditions such as drug addiction, stress, epilepsy, and depression

Histone acetyl transferase (HAT)

- Acetyl transferases activate transcription by adding acetyl groups to histones
- Histone deacetylases reduce transcription



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) INCREASED	Day 5

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