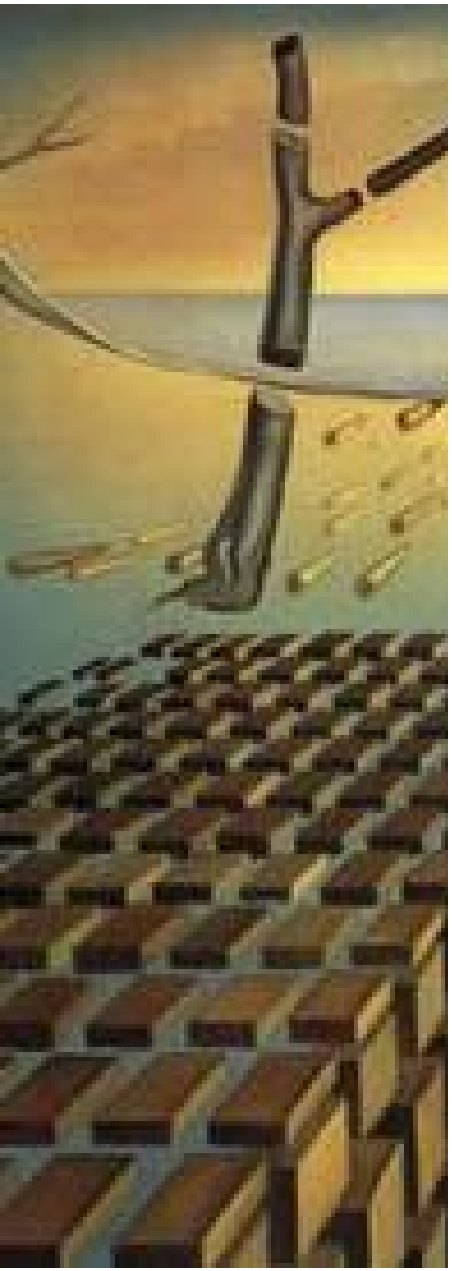


Mechanisms of memory

By Hana Brozka

‘We are like dwarfs sitting on the shoulders of giants. We see more, and things that are more distant, than they did, not because our sight is superior or because we are taller than they, but because they raise us up, and by their great stature add to ours’

John of Salisbury

Outline

- What is memory
- History of memory research
- Synaptic plasticity
  - Short term plasticity
  - LTP and LDP
  - Immediate early genes
  - PKMζ
- Brain regions implicated in memory
  - Hippocampus
- Systems consolidation theory
- Multiple trace memory theory
- Memory ‘index’ theory

What is memory

- Ability to retain knowledge
- 3 components: encoding, storage and retrieval (Brem et al. 2013)
- Engram = memory trace
  - terms for a neural substrate of stored information resulting from a past experience

History of memory research -Semon

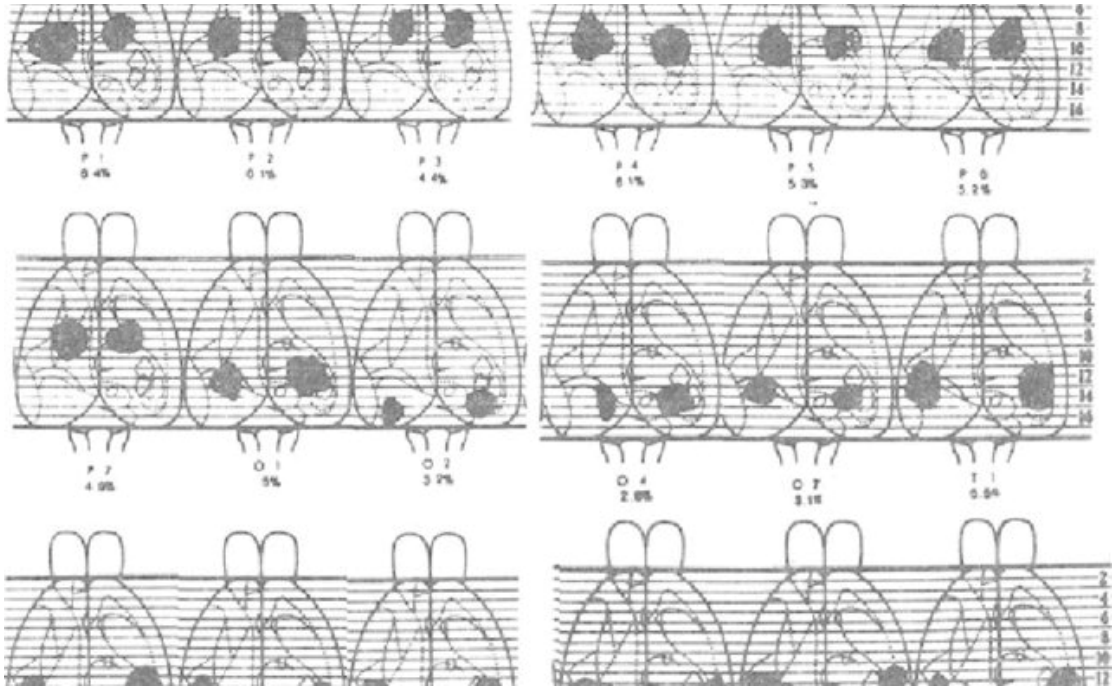
- Richard Wolfgang Semon (1859–1918)
  - Introduced term ‘engram’ as ‘the enduring though primarily latent modification in the irritable substance produced by a stimulus’ - change in a physical substance
  - Learning passed to future generations - dismissed by scientific community (too Lamarckian)

  Contributions:
  - Focus on retrieval not only on learning
  - engram-awakening stimulus need not completely overlap with the original stimulus (present-day ‘pattern completion’ by CA3 area of hippocampus)
  - Awakened engram leads to generation of new engram = memory is not static but changes with use (similar to Multiple trace theory)
  - Distributed engram

Matthew effect in science

- ‘For unto everyone that hath shall be given, and he shall have abundance: but from him that hath not shall be taken even that which he hath.’ — *Matthew 25:29*
- *(*’The rich get richer and the poor get poorer’)
- More famous scientists get taken more seriously for the same results than unknown ones
- heighten the visibility of contributions to science by scientists of acknowledged standing and to reduce the visibility of contributions by authors who are less well known (Merton, 1968)
- Positive feedback

History of memory research - Lashley

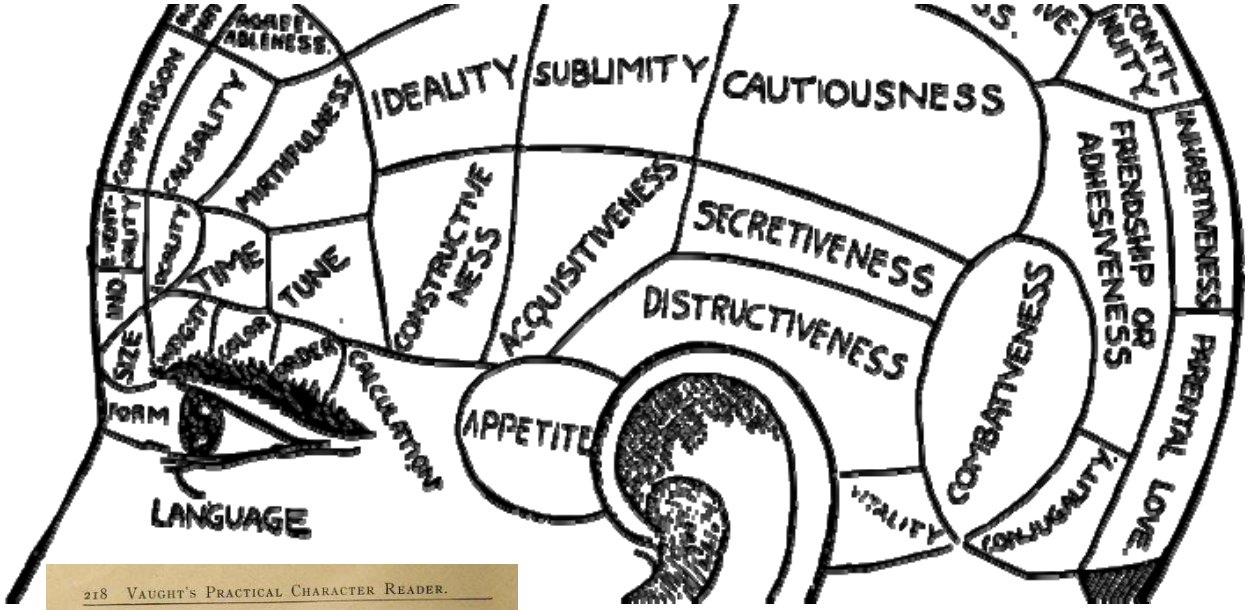


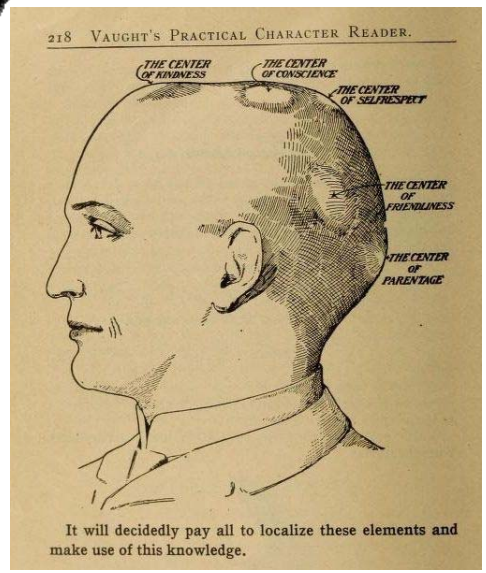
- **Karl Spencer Lashley** (1890–1958)
- 'inspiring teacher who described all teaching as useless' (Beach, 1961)
- Popularized term 'engram'
- Searched for its localization for more than 30 years
- trained rats to navigate a maze than lesioned some part of the cortex
- Size but not location correlated with the memory impairment
  - But extent of damage also correlated with the extent of hippocampal damage - Lashley did not realize this
- Engram was everywhere 'the trick, is not to find where the trace is located, but where it is not' (Orbach 1999)

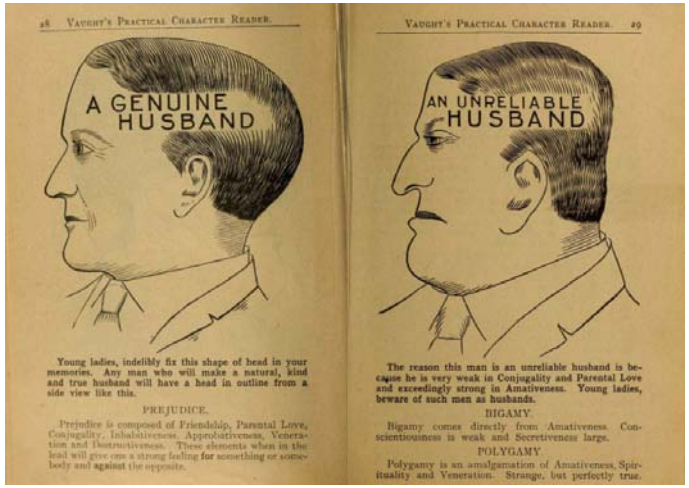
Contributions

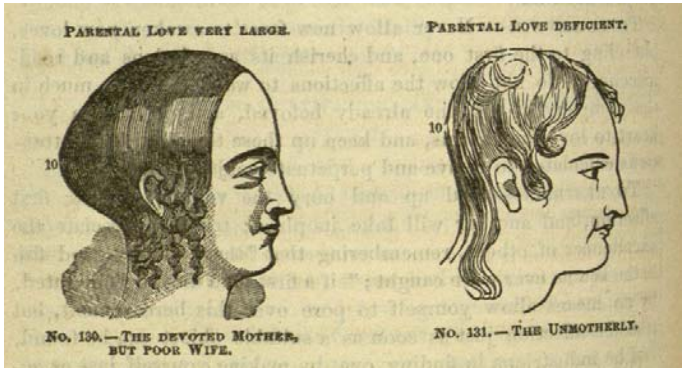
- Current methods are very similar to lesions done but Lashley, only more sophisticated
- Modern concept: distributed processing in contrast to 'new phrenology'

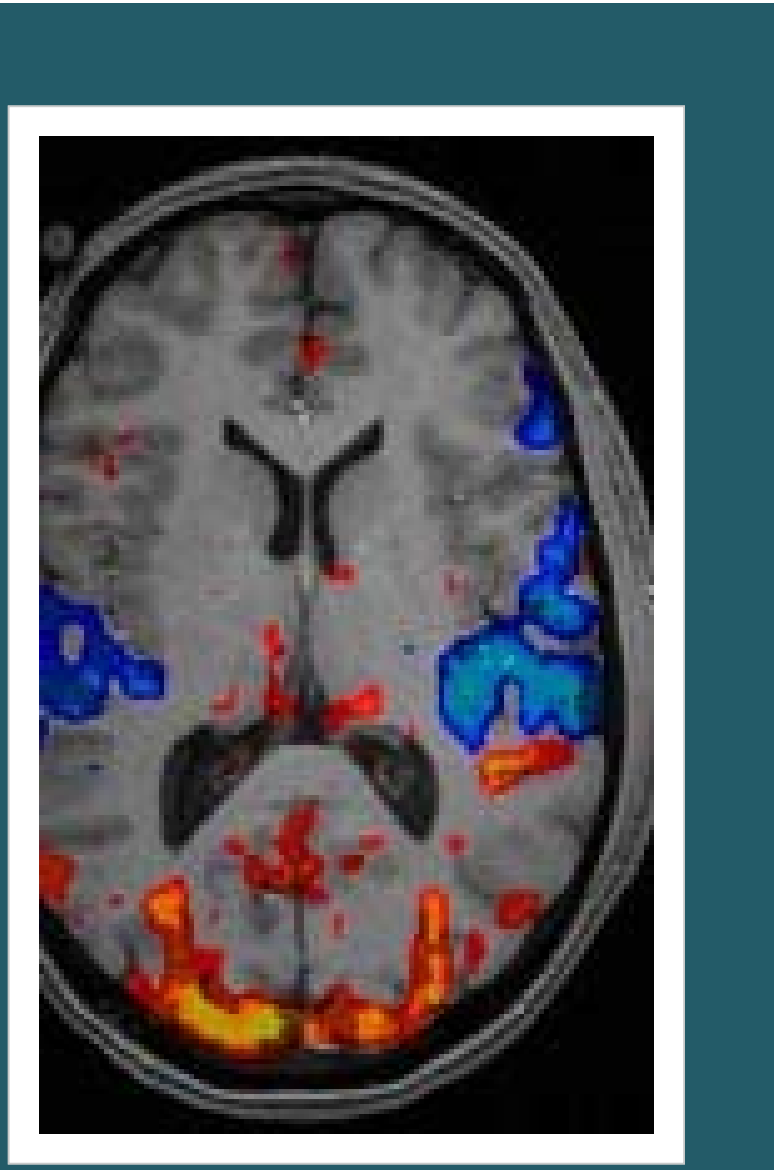
Old Phrenology











New phrenology

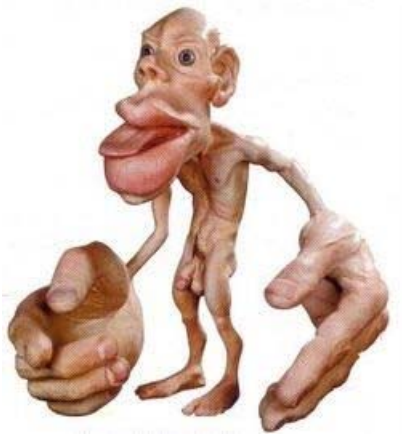
- Modern neurosience boxes: attention, memory, sensation, motivation, emotion, selfhood
- We fit them to different brain regions
  - Hippocampus = memory
  - Amygdala = emotion
  - Insula = selfhood
  - Orbitofrontal cortex = behavioral inhibition
  - dACC = conflict monitoring
  - Prefrontal cortex = planning and motivation
- These boxes were created before brain was studies
- Alternative: distributed processing

History of memory research – Donald Hebb

- **Donald Olding Hebb** (1904–1985)
- Studied English literature and Philosophy
- Lashley's PhD student
- Studied human brain injury on IQ
- Hebb-Williams maze - effect of early life experiences on early cognition: believed in early education
- Returned to Lashley, wrote *The organization of behavior: a neuropsychological theory*
- Hebb's cell assembly theory
  - 'neurons that fire together wire together'
  - Recurrent activity between two neurons strengthens the interconnections between them
  - Proposed 'pattern completion'

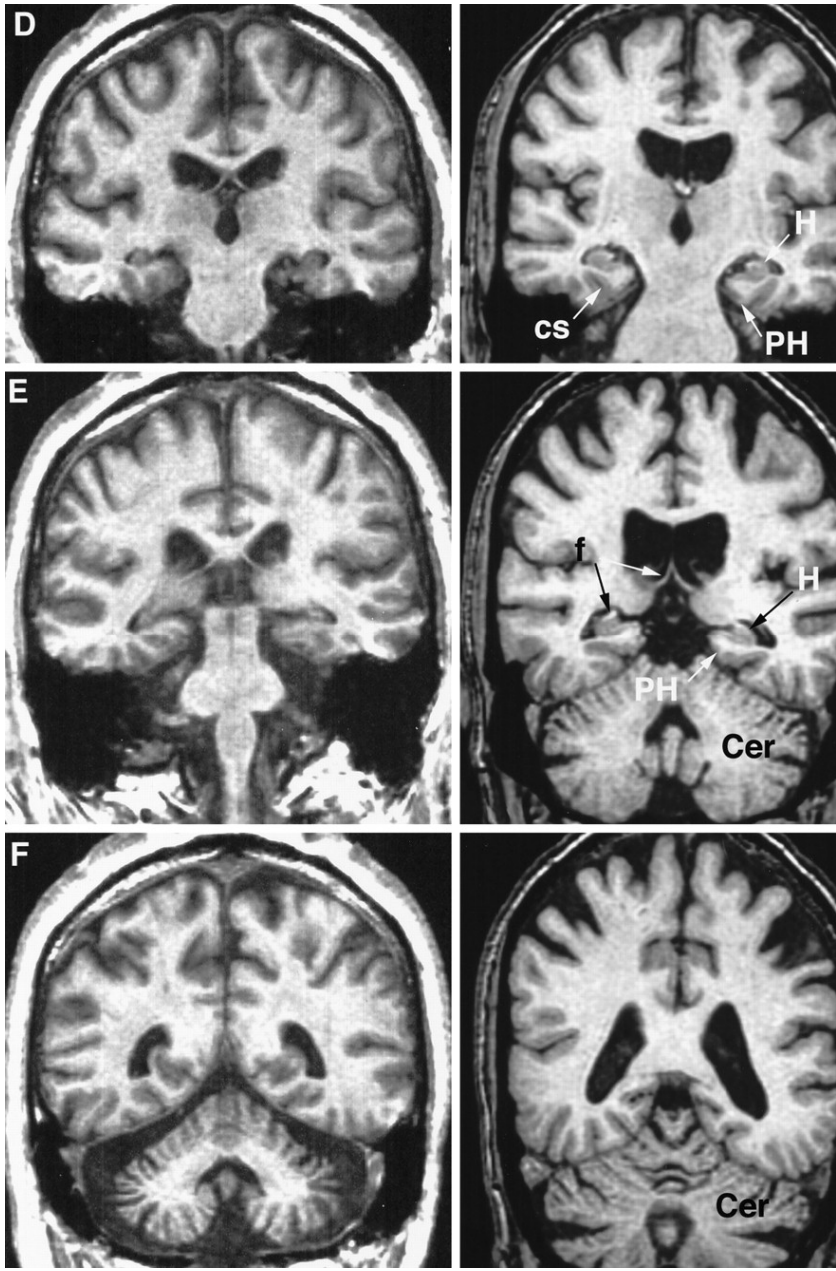
History of memory research – W.G. Penfield

- **Wilder Graves Penfield** (1891–1976)
- Studied Literature, later MD
- Epilepsy neurosurgeon
- Electrically stimulated different brain regions to determine source of epileptic seizures
- Defined **homunculus** (Penfield and Boldfrey, 1937)
- Stimulating temporal lobe elicited experiences identified as previously experienced
  - Very memory-like
  - one patient (Case 3, RW) reported: “My mother is telling my brother he has got his coat on backwards. I can just hear them.” When asked if this event actually occurred, RW reported, “Oh yes, just before I came here” (Penfield and Perot 1963)
- Inspired modern engram awakening methods: optogenetic and chemogenetic
- Electrical activity alone can ‘awaken’ engram
- Started a tradition of memory research on epilepsy patients



History of memory research – Brenda Milner

- **Brenda Milner** (1918-)
- Studied math, then experimental psychology
- Did her PhD under Hebb
- Studied memory impairment in hippocampal damage patients
- Penfield invited her to study Henry Molaison (H.M.) - patient that had both hippocampi removed
- First to show evidence that engram was in fact localized - in the hippocampus
- First to show that there is not 'one' memory (e.g. procedural vs declarative)
- First to show that older memories are spared (older than 3 years) – systems consolidation (However, she said that when transferred, memories lose their episodic nature - became more semantic)

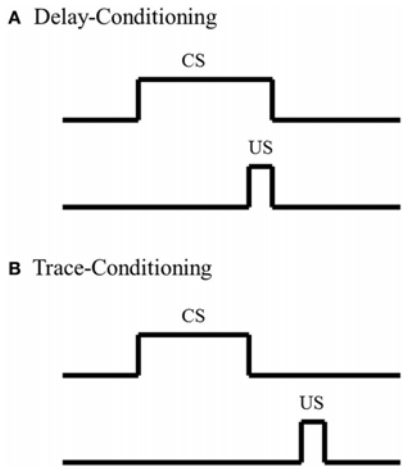


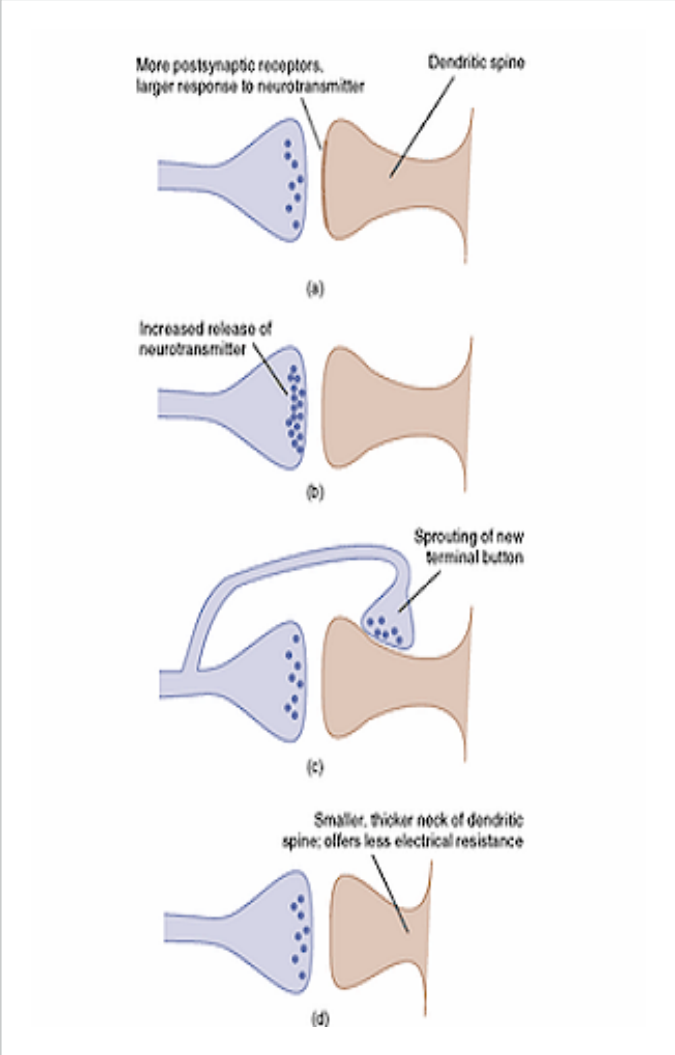
History of memory research – J.V. McConnell

- **James Vernon McConnell** (1925–1990)
- ‘it seems to me that anyone who takes himself, or his work, too seriously is in a perilous state of mental health’
- Studied cannibalistic flatworms
- Worms formed association between light and shock. McConnell observed conditioned contraction response
- When he split worm in half both halves of worm retained the memory
- Then he crushed the trained worm and fed it to naive worm – the naïve worm than displayed the conditioned contraction response: claimed to prove that memory is transmitted through RNA
- His work was not replicated at the time. Was poorly designed. He lost prestige and grants
- However, using more automated procedure split worm experiment was replicated (Shomrat and Levin, 2013)
- And indeed, engram appears to be localized in the nucleus and not in synapse - suggesting epigenetic mechanism (i.e. changes in RNA transcription)

History of memory research – R.F. Thompson

- **Richard Frederick Thompson** (1930–2014)
- Influenced by Lashley (took his chair in fact)
- First to find an engram in an animal - in cerebellum (McCormick and Thompson, 1984)
- Delay eye-blink conditioning in rabbits
- Two criteria to show that a region is critical for engram
  - Changes in electrophysiological activity in the area during learning
  - Lesion of area causes memory damage
- First, Thompson targeted hippocampus - but no, lesion of hippocampus did not impair delay eye-blink CR (it impairs only trace and contextual conditioning)
- But cerebellum fulfilled both criteria
- He used electrophysiological recordings and lesions: a format copied to present day





Neuronal Plasticity

1. Synaptic
    - Short term
        - Paired pulse facilitation
        - Paired pulse depression
        - Post tetanic potentiation
        - Post tetanic depression
    - Long term
        - Long term potentiation (LTP)
        - Long term depression (LTD)
2. Extrasynaptic

Synaptic plasticity - short term

- **Postsynaptic** - desensitization of ligand gated receptors
- **Presynaptic** - changes in the probability of transmitter release (p)

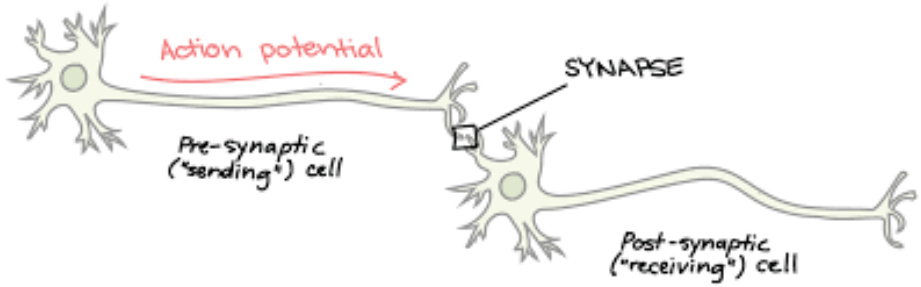
Paired pulse facilitation (20ms-500ms)
- Ca^{2+} from previous stimulus contributes to second response
- Protein kinase activation (eg. synapsin)

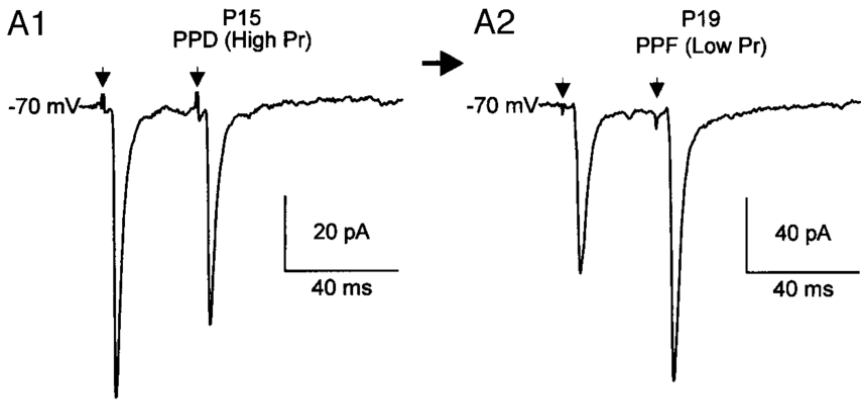
Paired-pulse depression (<20ms)
- Inactivation of Ca^{2+} ana Na^{+} channels
- Depletion of vesicle pool

Tetanic stimulation 200ms-5s, 10-200Hz of presynaptic neurons

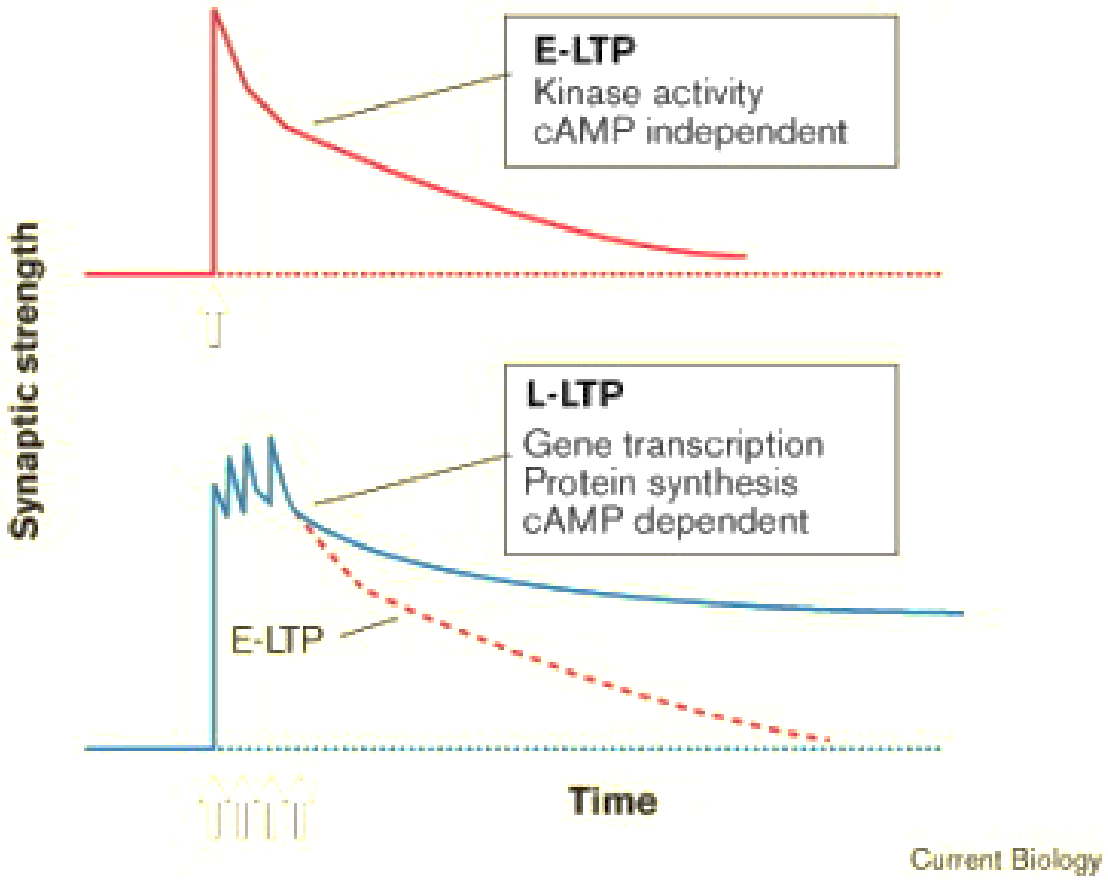
post-tetanic potentiation (PTP)
- Build-up of presynaptic Ca^{2+}
- Seconds to minutes

Post-tetanic depression (PTD)
- In presynaptic terminals with high probability of neurotransmitter release
- Probably depletion of vesicle pool
- Seconds to minutes





Kumar and Huguenard, 2001



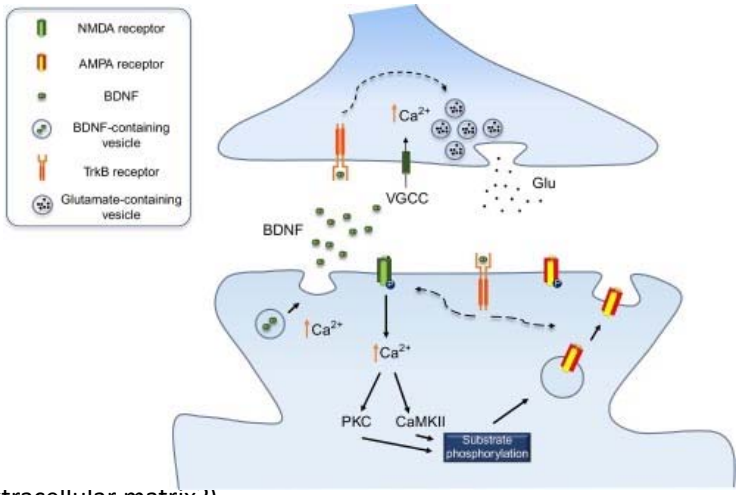
Synaptic plasticity –long term potentiation (LTP) and depression (LTD)

- Basic mechanisms of synaptic plasticity - change of synapse strength
- Presynaptic and postsynaptic mechanisms
- Increase or decrease of excitatory post synaptic potential

- LTP
  - long lasting increase of synaptic transmission between two neurons
  - Cajal (1894) and Hebb (1949), Kandel (1964)
  - directly measured by Bliss and Lømo in 1973 (anesthetized rabbit)
  - 1s, 100Hz
  - Early and late phase (E-LTP, L-LTP)

- LTD
  - Long lasting decrease of synaptic transmission between two neurons
  - 900 stimuli at 1Hz
  - Mechanism of habituation

Synaptic plasticity – <u>early</u> LTP



<u>E-LTP</u> (1-3h)

Not dependent on protein synthesis

Insertion of more AMPA* receptors into the synapse

- Presynaptic:
  - cAMP-PKA signalling
  - increase of transmitter release? (not much studied)
  - Necessity of retrograde signal to presynaptic neuron to induce presynaptic change (Arc maybe? NO? extracellular matrix?)

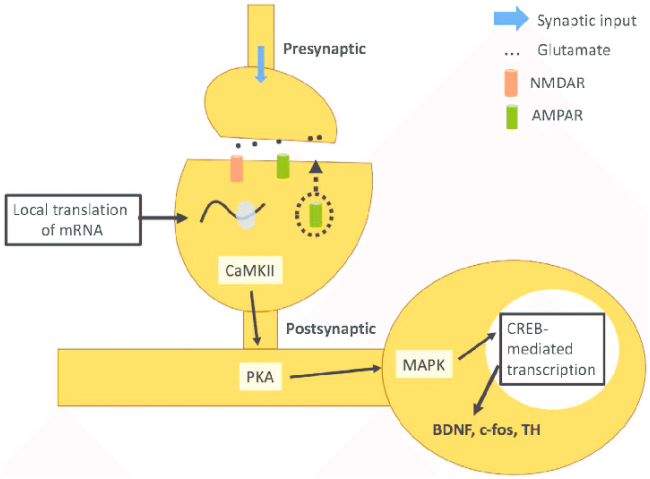
- Postsynaptic:
  - NMDA** dependent (very much studied)
  - Necessity of postsynaptic depolarization with concurrent NMDA activation (NMDA Mg^{2+} block is released)
  - Ca^{2+} enters cels via NMDA receptor - activates intracellular singnaling pathways

  - Ca^{2+}/CaM-dependent protein kinase II (<u>CaMKII</u>) autophosphorylates when Ca^{2+} enters the cell, binds to NMDAr and then phosporilates target proteins including phosphorilating AMPAr (CaMKII – synaptogamins –AMPAr)
  - AMPA is incorporated into the membrane from a ready made pool
  - transmembrane AMPAR regulatory proteins (TARPs) bring AMPA to synapse
  - 'slot proteins' - MAGUKs (membrane-associated guanylate kinases) – PSD-95 for example (interact with TARPs)
  - transmembrane AMPAR regulatory proteins (TARPs) bring AMPA to synapse and interact with MAGUKs

*AMPA = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid specifically activates this glutamate receptor

**NMDA = N-methyl-D-aspartate specifically activates this glutamate receptor

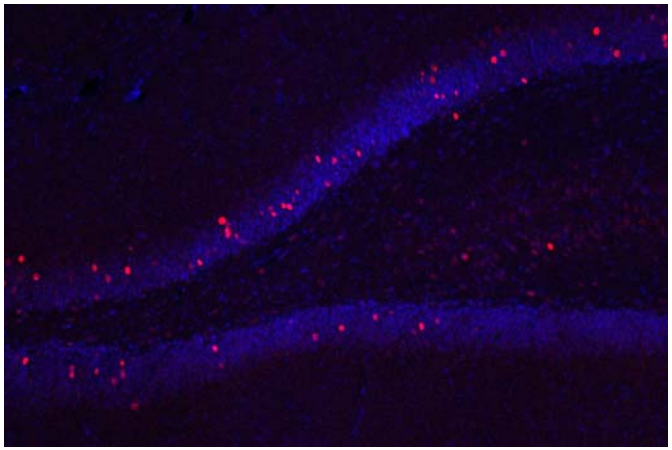
Synaptic plasticity – late LTP



L-LTP up to 24h+ in vitro

- **Changes in gene expression and protein synthesis**
- Ca^{2+} increase activates adenylate cyclase – cAMP.
- cAMP binds to PKA. PKA moves to nucleus and phosphorylates CREB
- CREB is a transcription factor
- PKA or CREB knockouts – E-LTP intact, L-LTP impaired (Bourtchuladze et al., 1994; Abel et al., 1997)
- Alternatively, PKA activates MAPK which phosphorylates CREB, Elk-1
- CREB and Elk-1 are transcription factors for immediate early genes (*cFos Arc*, *Zif268*)

Synaptic Plasticity - Roles of IEGs



- ***cFos***
  - transcription factor
  - Dimerizes with Jun to form transcription factor AP-1
- ***Zif268***
  - Zinc finger transcription factor
- ***Arc***
  - Codes for synaptic protein and a growth factor
  - Arc mRNA is transported to dendrites
  - Arc protein forms a virus-like particle that can enclose RNA and be transferred through the synapse (Pastuzyn et al., 2018)

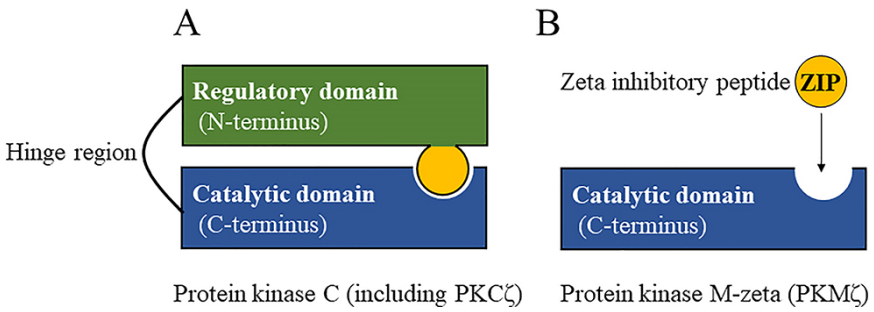
We use IEGs as neural activity markers in studies of behavior and cognition

LTP - long term maintenence

- require *de novo* gene transcription, new protein translation, and synaptic growth at pre- and post-synaptic terminals
- Synaptic enlargement
- AMPA receptor insertion
- Presynaptic mechanisms?

- IEG CCAAT box/enhancer-binding protein (C/EBP) drives the transcription of genes necessary for synaptic growth
- PKMζ
  - Regulates AMPA endocytosis
  - regulating the structure of dendritic spines
- Arc
  - Regulates AMPA endocytosis
  - Arc protein can bind its own mRNA and transport it across the synapse through extracellular vesicle

PKMζ



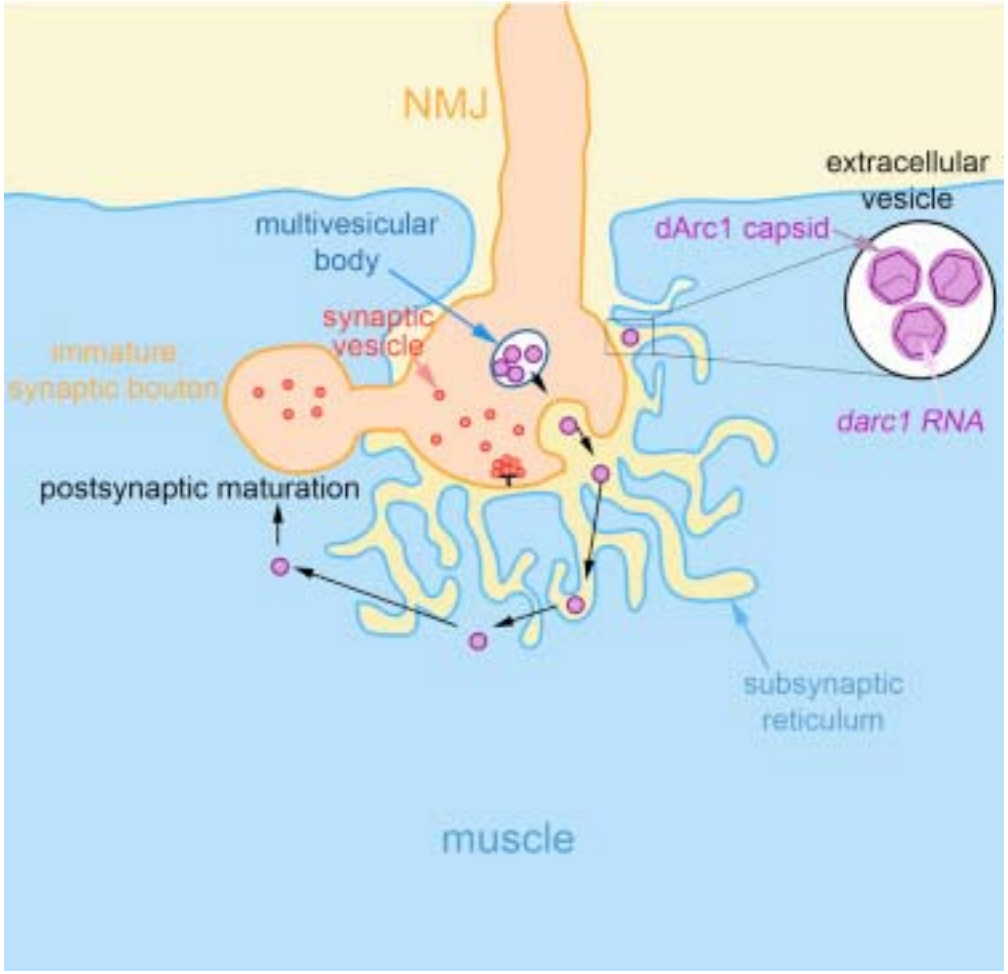
- PKMζ - isoform of PKC
- PKMζ is regulated at three levels:
- Translation, constitutive activity and phosphorylation:
    1. Translation is triggered when MAPK, CaMKII, and PKA all together remove translation block on mRNA that is already present in postsynaptic terminal (Kelly et al., 2007)
    2. PKMζ misses regulatory domain that would otherwise inhibit its own activity
    3. Newly synthetized PKMζ needs to be phosphorylated by phosphoinositide-dependent protein kinase-1 (PDK1)
- Maintains AMPAr in the synapse

PKMζ

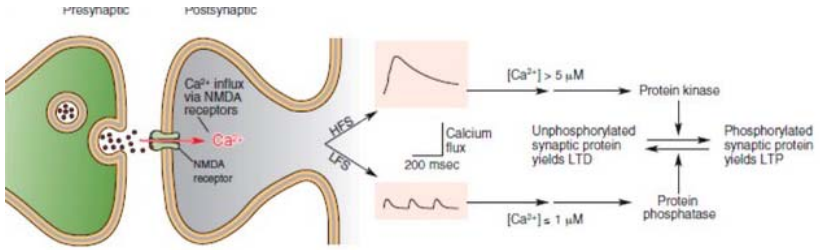
- However, PKMζ knockouts have intact memory (Volk et al., 2013)
- ZIP impaired memory even in knockouts…either toxic or other target
- Alternatively, knockout can develop compensatory mechanisms
- Eg. kinase C iota/lambda (PKCι/λ) is elevated in knockouts (these PKCs are normally responsible for induction of LTP (Tsokas et al., 2016)
- Inhibiting PKCι/λ reverses LTP and impairs established spatial memory in PKMζ knockout mice but not in wild type mice
- specifically inhibiting PKMζ synthesis using RNA oligonucleotides, blocks late-LTP induction and spatial long-term memory in wild-type mice but not PKMζ knockout mice
- PKMζ overexpression improves memory (Schuette et al., 2015)
- Ca^{2+}/CaM-dependent protein kinase II (CaMKII)

Arc

- Following stimulation, Arc is transcribed and transported to activated synapses
- Protein can bind its own mRNA and self-assembles to form a virus-like capsid
- Retroviral-like mechanism



Synaptic plasticity - LTD



Scaramozzino, 2017

- dependent on post-synaptic Ca^{2+}
- LTD requires less Ca^{2+}
- (while for LTP surpassing a certain threshold is needed)
- Activation of calcium dependent phosphatases
- Dephosphorylates AMPA - reduces probability of opening - targets for endocytosis
- AMPA is released from PSD-95
- AMPA moves away from synapse and is internalized
- Not enough work on maintenance of LTD

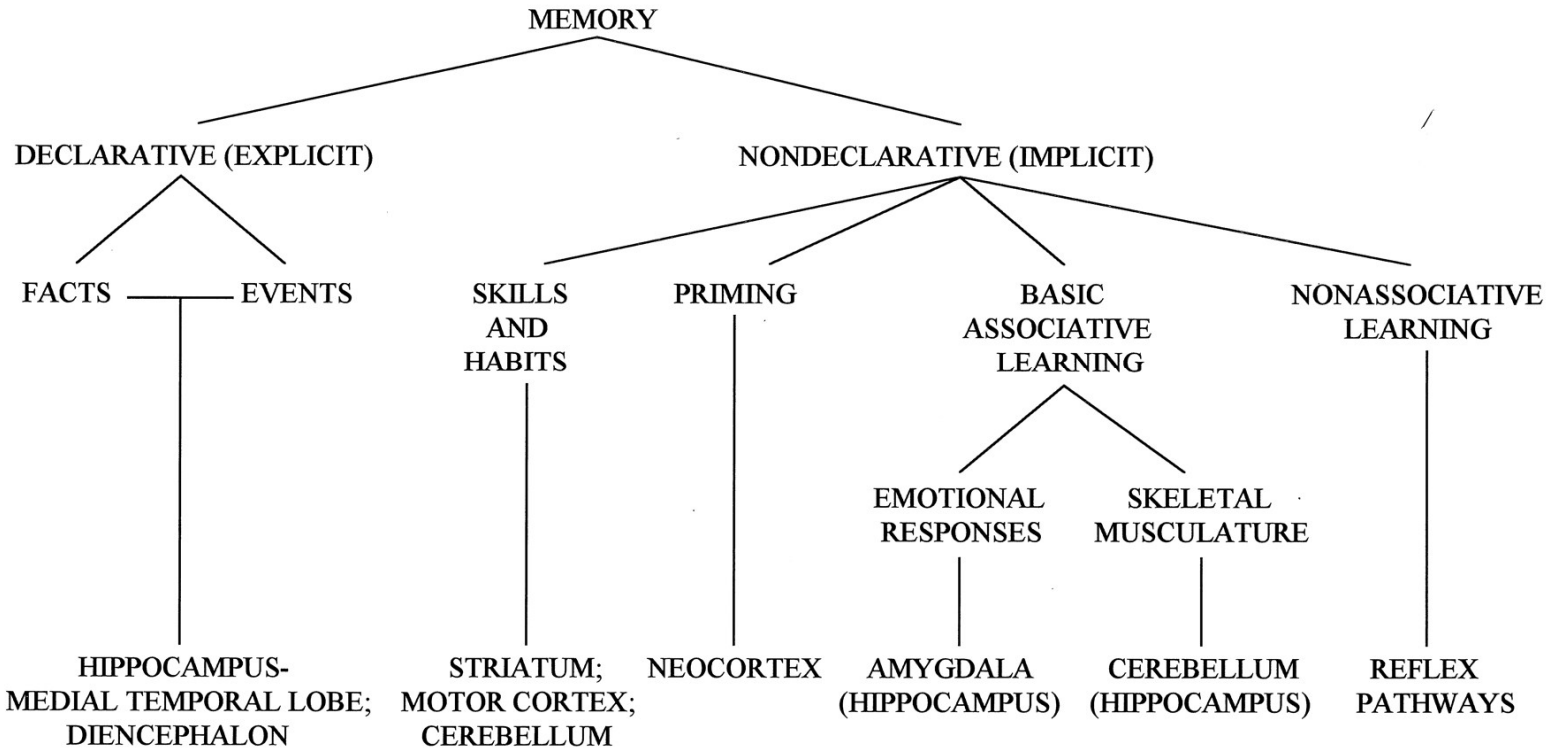
Presynaptic

- postsynapticaly produced 2-arachidonoyl-glycerol + anandamid
- 2-arachidonoyl-glycerol + anandamid retrogradely diffuses to presynaptic membrane activates cannabinoid receptors
- Reduced release of glutamate/GABA from presynaptic membrane

Synaptic plasticity - take home message

- Short term plasticity:
  - mostly presynaptic
  - depends on Ca^{2+} levels and avaliable synaptic neurotransmitter pools
- E-LTP:
  - Mostly postsynaptic
  - NMDA-dependent, Ca^{2+} induces AMPA receptor incorporation into the synapse
- L-LTP:
  - Mostly postsynaptic
  - changes in gene expression and requires protein synthesis
  - NMDA $\rightarrow$ Ca^{2+} $\rightarrow$ adelynat cyclase $\rightarrow$ cAMP $\rightarrow$ PKA $\rightarrow$ (MAPK) $\rightarrow$ CREB/Elk-1 $\rightarrow$ IEGs
- LTD:
  - Mostly postsynaptic but also presynaptic (via canabinoid receptors)
  - NMDA dependent,
  - Lower levels of Ca^{2+} activates phosphatases which dephosphorilates AMPA

Brain regions implicated in memory



Thompson and Kim, 1996

Brain regions implicated in memory - hippocampus

- https://www.youtube.com/watch?v=k_P7Y0-wgos

- 8:22

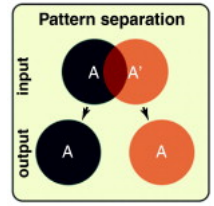
Hippocampus

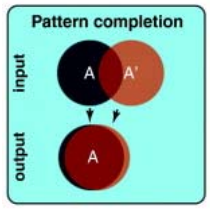
- Pattern separation
- Pattern completion
- Contextual binding
- Temporal binding
- ‘Index’ cortical activity

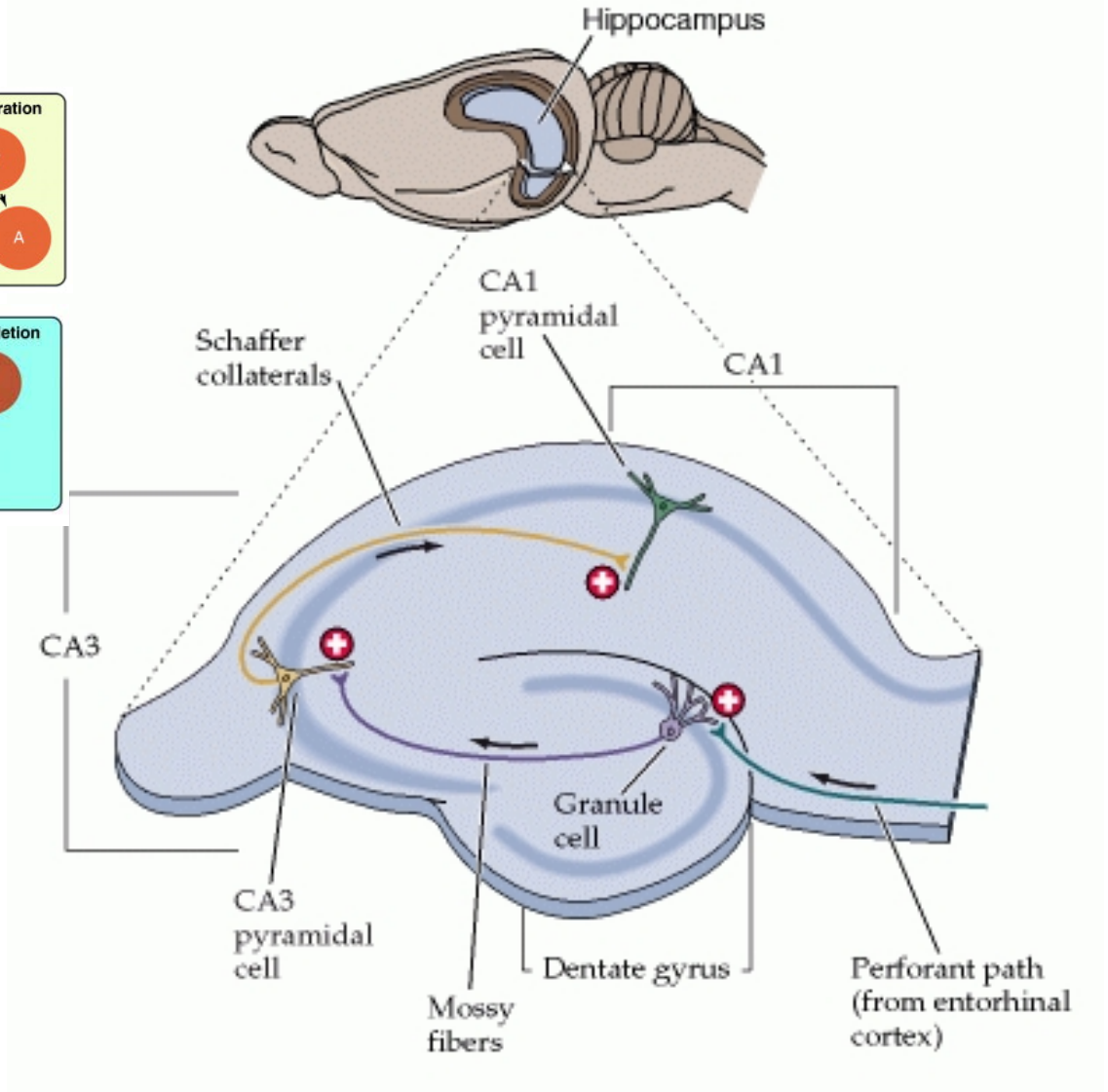
↓

Imagining future and past

- Imaging situation that is not in the present
- symmetrical







Theories of brain-wide memory formation

Systems consolidation theory

- Based on H.M.’s ability to retrieve old memories.
- Memories are only transiently dependent on hippocampus
- Memories will be forgotten if they are not fully represented in neocortex
- Extra-hippocampal sites mature and interact to retrieve a memory hippocampaus is no longer needed
- Notion that hippocampus has a limited capacity
- Time course of memory transfere was not specified
- During sleep- replay of events by hippocampus to neocortex
- Milner - what is remembered is probably coded by neocortex but is of more general (less episodic nature)

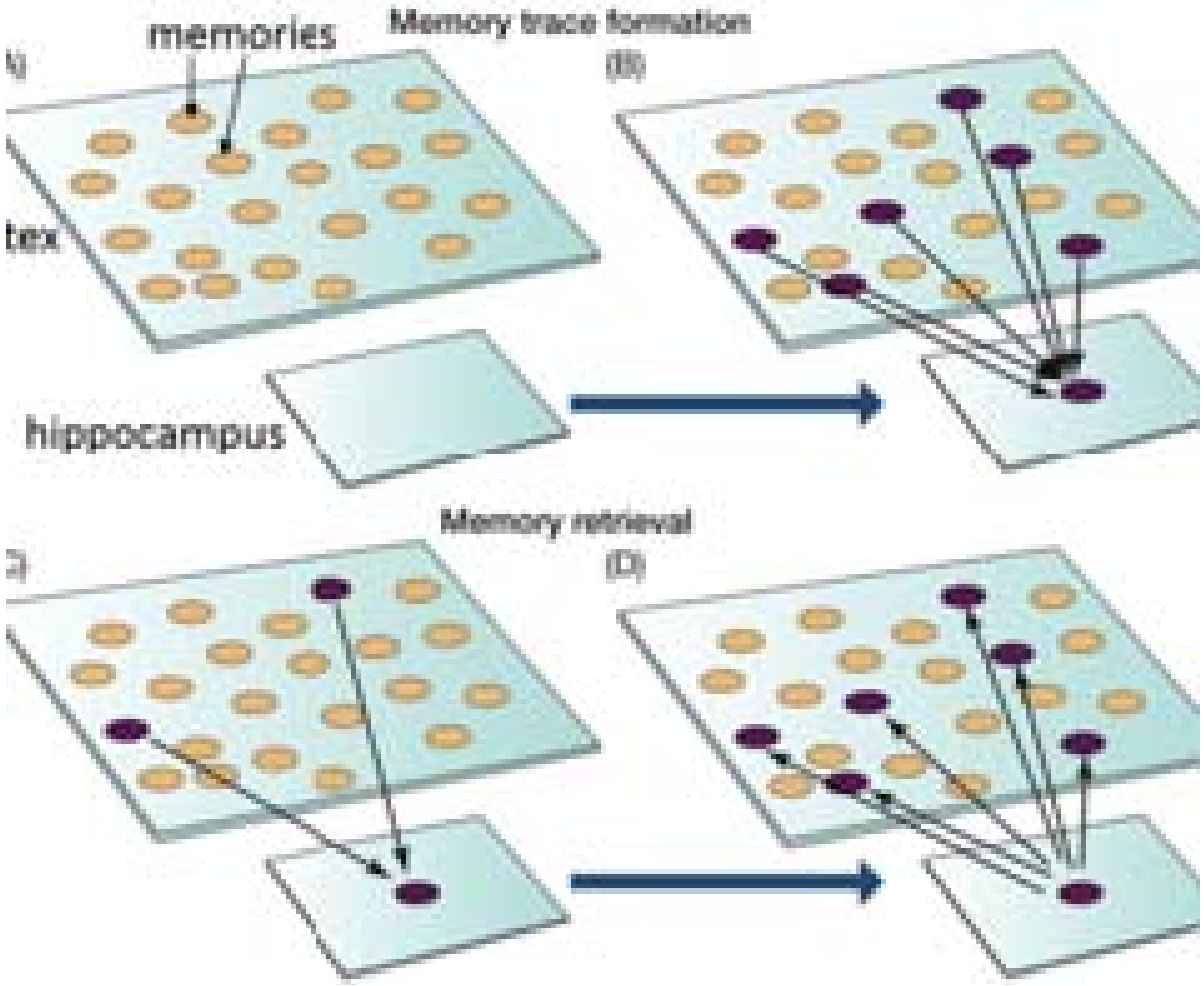
Criticism of systems consolidation

Consolidation in animal studies takes place in hours/days, while in humans it takes years

No mechanism for transfer of memories from hippocampus to neocortex was proposed

‘Alternative’ to systems consolidation

- **Multiple trace theory** (Nadel & Moscovitch, 1997)
  - Older memories are experienced more often and each new recollection eaves new memory trace
  - Predictions
  - Full damage of hippocampaus will lead to full retrograde amnesia
  - Partial hippocampal damage will spare older memories because they are ‘overrepresented’ in hippocampus



Memory ‘index’theory

- Teyler and DiScenna,1986
- Hippocampus does not store memories but creates an ‘index’ of cortical activity
- Reciprocal connections of hippocampus with most neocortical areas
- During memory retrieval hippocampus reproduces activity in neocortex that was present during encoding

Thank you for your attention!

See you on my next presentations

- Neurotransmitters and behavior
- Neurobiology of behavior