Neurofunctional Techniques

Lesson 11

6 November 2024

Optogenetics & memory

Calendar

- M 30 Sept: Course introduction
- W 2 Oct: Functional imaging
- F 4 Oct: Statistics (Cesca)
- M 7 Oct: Functional imaging
- W 9 Oct: Biophysics of diffusion
- F 11 Oct: Statistics (Cesca)
- M 14 Oct: Functional imaging
- W 16 Oct: General introduction to the papers for the presentations
- F 18 Oct: Statistics (Cesca)
- M 21 Oct: Modeling in neuroscience
- W 23 Oct: Molecular approaches in neuroscience
- F 25 Oct: Statistics (Cesca)
- F 25 Oct: Laboratory (14:00- 18:00)
- M 28 Oct: Practical exercises on the first part of the course
- W 30 Oct: Genome editing in neuroscience (Dr. Jaudon)
- M 4 Nov: Optogenetics
- W 6 Nov: Papers assignment to the groups; introductions to the specific papers
- T 12 Nov: X-genetics + Practical exercises on the second part of the course
- W 13 Nov: Introductions to the specific papers
- M 18 Nov: Introductions to the specific papers

- 9, 10, 11 Dic: Paper presentation 15:00-19:00)
- Tue 17 Dic: Test (14:00 - 16:00 Room 3A, Building H2bis)

Monday 9.12.24

Group 3 - Paper 1
Mayia My
Giuseppina Russo
Diomira Elettra Lenti
Saadet Alkan
Matteo Theodule

Group 2 - Paper 2
Martina Merga
Irene Giovani
Carlotta Tiranzoni
Nosiba Yaseen
Jiulija Vodenik

Group 1 - Paper 3
Canonero Marida
Di Filippo Dalila
Nicchiotti Francesca
Pau Alessandra
Russo Martina

Tuesday 10.12.24

Group 5 - Paper 1
Sofia Mosconi
Maria Sole Faeti
Valeria clai
Alessandra guida
Maryamsadat Seyedi

Group 4 - Paper 2
GAMAL AHMED MAHMOUD ABDELAZIM
MOHAMED AL SIYABI
Amanuel Bekaffa
Emmanuel
Yabets Woldegiorgis

Group 9 - Paper 3
Teo Zakula
Danilo Zanghì
Diana Dall'Olio
Noemi Valero
Gulnur Asci
azziza haddad

Wednesday 11.12.3

Group 6 - Paper 1
Rossella Di Pompeo
Elena Lunardon
Beatrice Buso
AnnaMaria Benetti
Tobia De Rosso

Group 8 - Paper 2
Francesca Ronchi
Zahra Lashkari
Lorenzo sieni
Silvia Cassani
Virginia Camporesi

Group 7 - Paper 1
Cristina Baio
Fabiana Cusimano
Angela Marchiano
Rimoun Kaldas

Group 10
Corinna Perone
Davide Pontiggia
Marianna Cis
Sara Abbasigharaei
Delaram Forouzeh

MEMORY

From Mind to Molecules

LARRY R. SQUIRE AND ERIC R. KANDEL

A bit of history

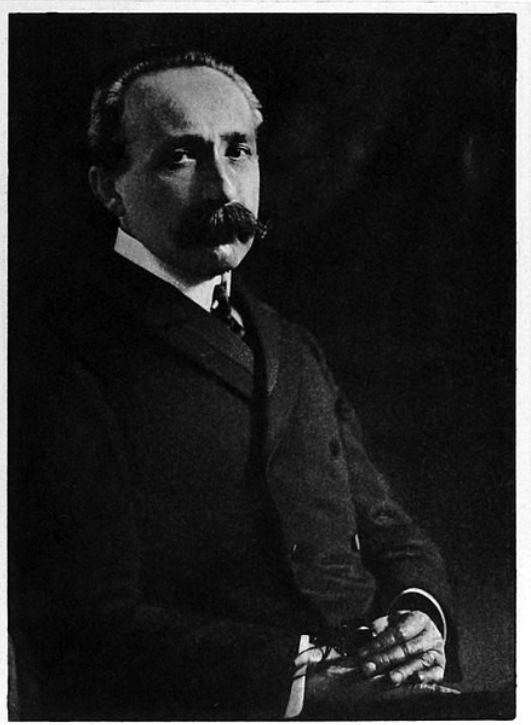
The idea that memory is stored in the brain as physical alterations goes back at least as far as Plato (427-347 BC)

In the Theaetetus, he puts the following words into the mouth of Socrates:

"I would have you imagine, then, that there exists in the mind of man a block of wax, which is of different sizes in different men; harder, moister, and having more or less of purity in one than another, and in some of an intermediate quality. . . Let us say that this tablet is a gift of Memory, the mother of the Muses, and that when we wish to remember anything which we have seen, or heard, or thought in our own minds, we hold the wax to the perceptions and thoughts, and in that receive the impression of them as from the seal of a ring; and that we remember and know what is imprinted as long as the image lasts; but when the image is effaced or cannot be taken, then we forget and do not know."

A bit of history

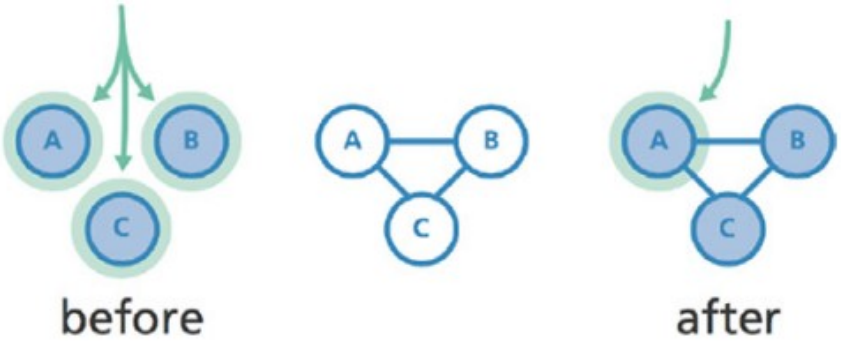
In the first decade of the 20th century, Richard Semon, a German zoologist (1904, 1909; translated into English in 1921), advocated the physical theory of human memory. Semon's contributions were almost completely ignored by mainstream psychologists concerned with memory until the late 1970s and early 1980s.



Semon coined the term engram, which he defined as ''…the enduring though primarily latent modification in the irritable substance produced by a stimulus (from an experience)...''

'Engram' is roughly equivalent to 'memory trace''

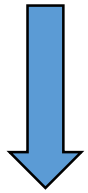
Learning activates a small ensemble of neurons, inducing in these cells persistent physical/chemical changes (memory storage). Reactivation of these cells by relevant (partial) recall cues results in retrieval of the specific memory (memory retrieval).



A bit of history

In the 1920s, Karl Lashley, an American psychologist, pioneered a systemic hunt for engram cells in the rodent brain by introducing lesions of varying sizes into different areas of the cerebral cortex, attempting to find an engram for a maze task.

Lashley found that memory was impaired in many of these lesioned animals, and the severity of the impairments was proportional to the sizes of the lesions.



The engrams for maze-resolving memory are spread throughout the cerebral cortex with no obvious localization (Mass Action Principle) (Lashley 1950).

A bit of history

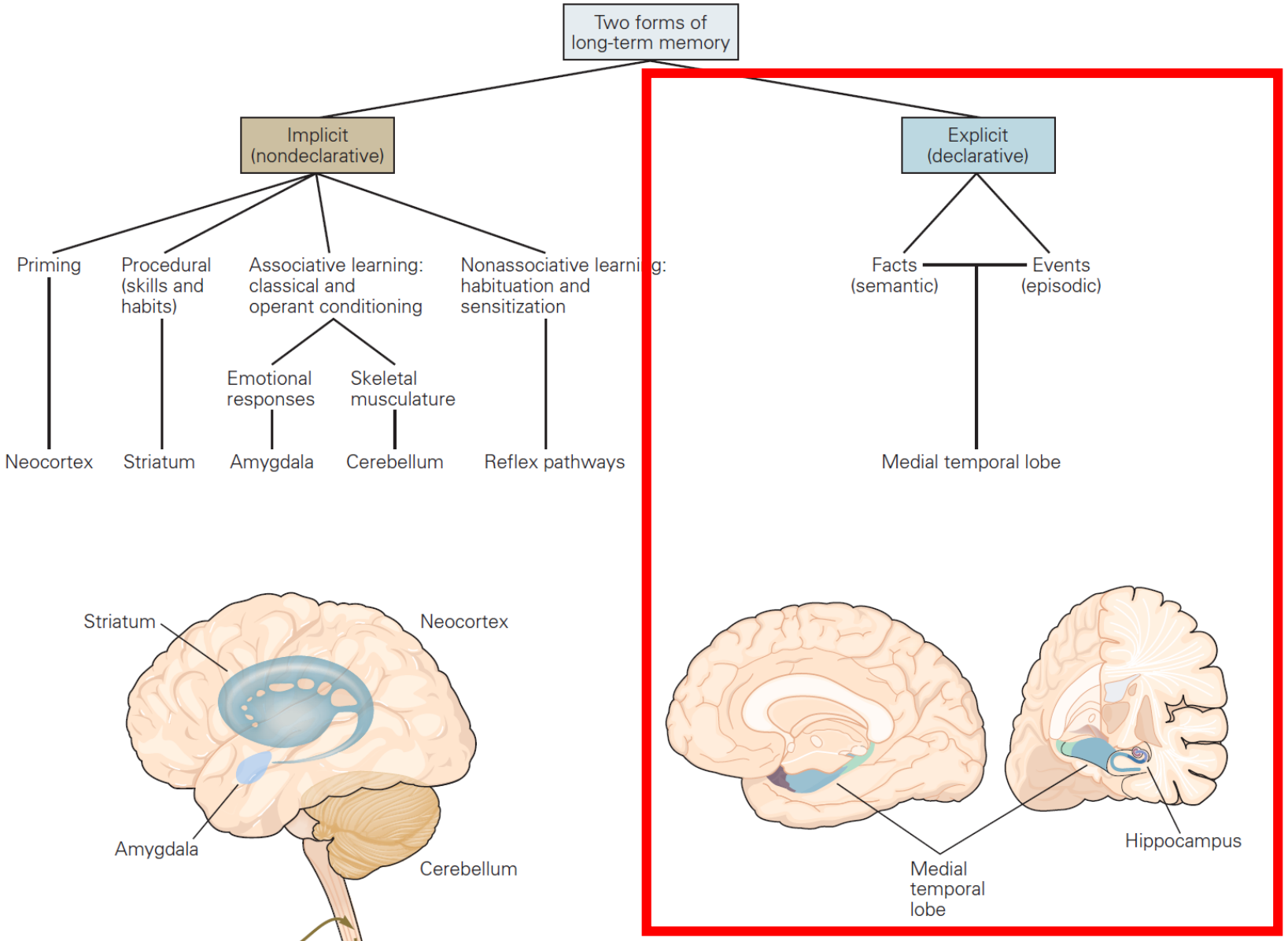
In the 1920s, Karl Lashley, an American psychologist, pioneered a systemic hunt for engram cells in the rodent brain by introducing lesions of varying sizes into different areas of the cerebral cortex, attempting to find an engram for a maze task.

Lashley's notion that engram cells for a specific memory are spread broadly and indiscriminately throughout the brain has not been supported by subsequent studies.

Lashley's failure in identifying localized engram cells might be because the maze tasks he used were too complex and required multiple regions of the cerebral cortex.

Lashley's extreme view was essentially wrong.

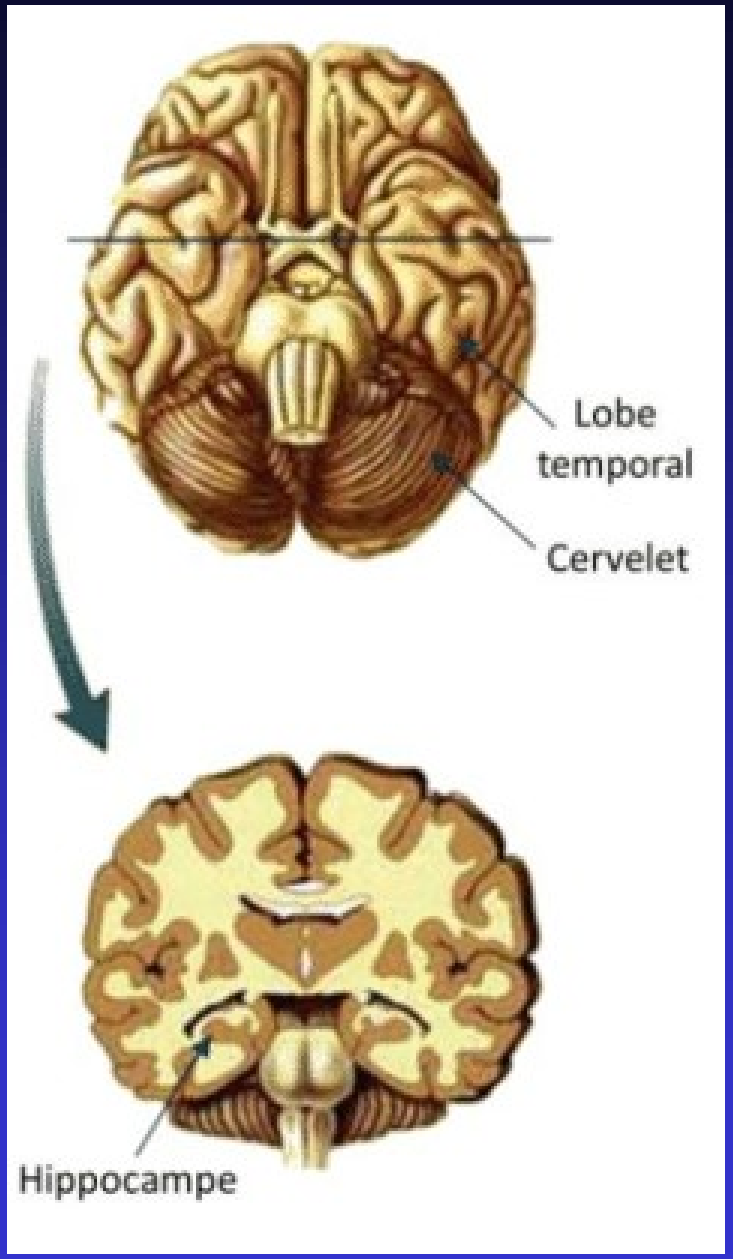
Forms of memory and their localization in the brain



Kandel, 2013

Explicit memory requires the medial temporal lobe and the hippocampus

Hippocampus



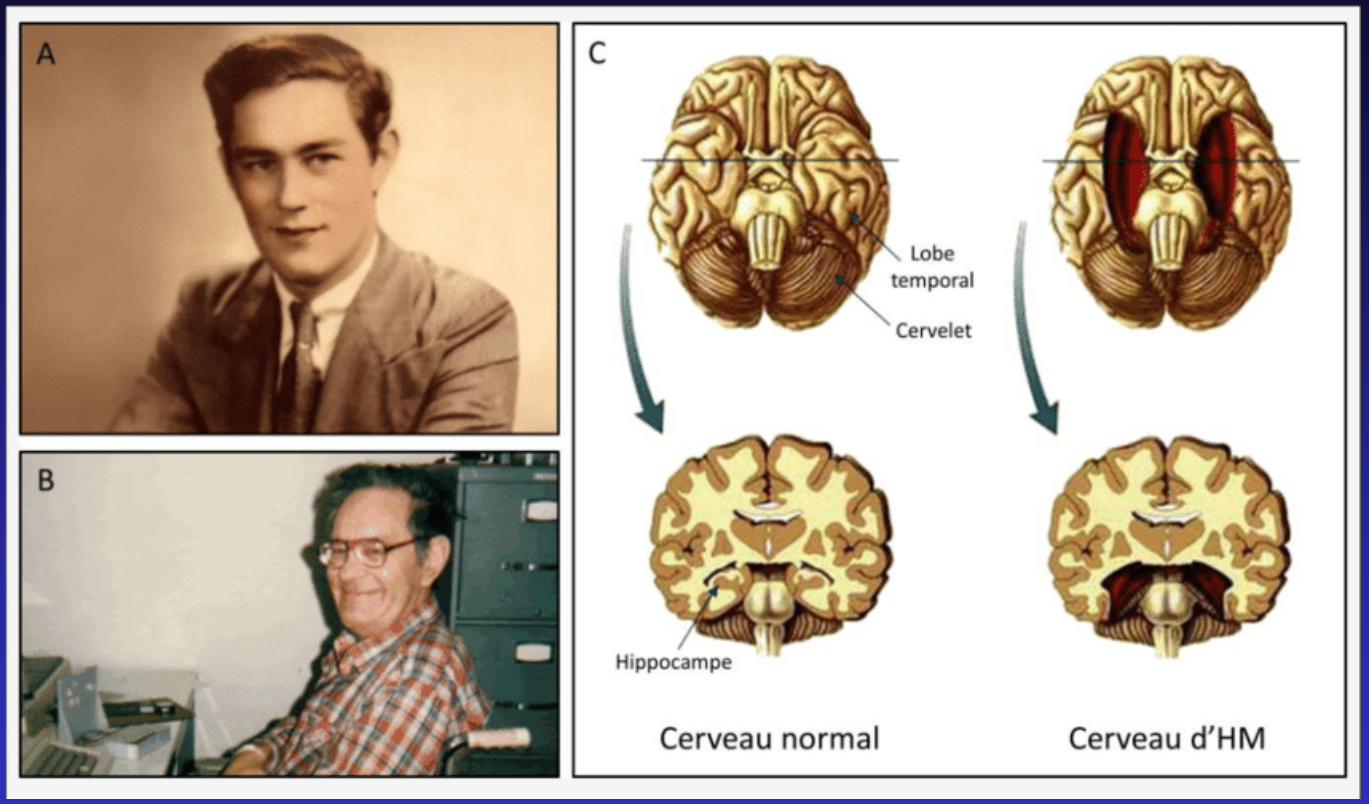
Explicit memory requires the medial temporal lobe and the hippocampus

The neurosurgeon Wilder Penfield was the first to demonstrate, in the 1940s, that human memory is localized in particular regions of the brain.

In ≥1000 awake patients, he introduced electrodes and stimulated different areas of the cortex. Only when stimulating the temporal lobe, he evoked, in 8% of patients, a memory, i.e.. the patient described a coherent memory of a previous experience.

This study provides a "gain-of-function" or "sufficiency" evidence for the notion that the temporal lobe harbors a biological locus for episodic memory.

Declarative memory:
the startling case of Henry Molaison (H.M.)

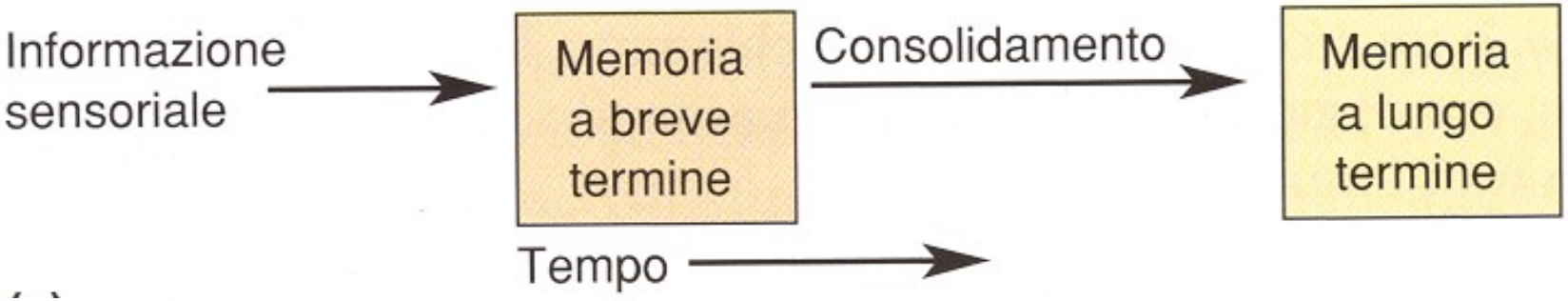


In the early 1950s Brenda Milner studied the case of H.M., who, after undergoing removal of the medial portion of the temporal lobe (including the hippocampus) from both hemispheres because of refractory epilesy, presented a dramatic memory deficit:

Complete inability to form new long-term memory traces; however, IQ, speech, memory related to experiences prior to surgery remained unaffected.

Declarative memory:
the startling case of Henry Molaison (H.M.)

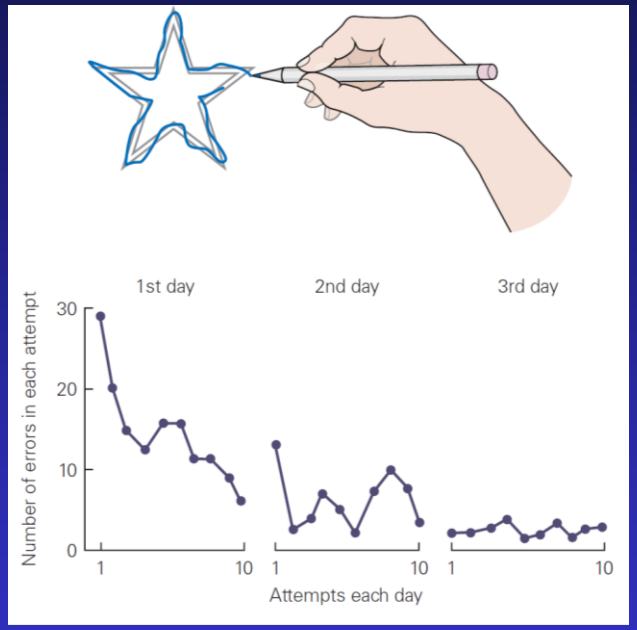
Ablation of the medial temporal lobes (and the hippocampi) eliminated the ability to transfer learned new information from short-term memory (seconds, few minutes) to long-term memory (days, years).



This study provides a "loss-of-function" or "necessity" evidence for the notion that the temporal lobe is required for episodic memory.

Declarative memory:
the startling case of Henry Molaison (H.M.)

HM could learn motor skills in a normal way.



The medial temporal lobe (with the hippocampus) is involved in the transfer of memory from short- to long-term only for those tasks that require the recognition of faces, things, places (explicit memory) while it is not involved in memory related to motor skills (implicit memory)

Why makes optogenetics such a valuable tool for comprehending the memory engram?

Experimental evidence for memory engrams

To pinpoint a biological process as the underlying mechanism for a specific phenomenon, three types of evidence are normally required.

1) **Correlation: recording the parallel occurrence between the phenomenon and the process, which will show an indirect relationship between these two**

2) **Blockade: interrupting the candidate process, and if this also interferes with the phenomenon, then this shows the necessity of the process for the expression of the phenomenon**

3) **Mimicry: to artificially generate the process, and if by doing so one can recreate the phenomenon, then this demonstrates sufficiency**

LETTER

doi:10.1038/nature11028

Optogenetic stimulation of a hippocampal engram activates fear memory recall

Xu Liu[1]*, Steve Ramirez[1]*, Petti T. Pang[1], Corey B. Puryear[1], Arvind Govindarajan[1], Karl Deisseroth[2] & Susumu Tonegawa[1]

A specific memory is thought to be encoded by a sparse population of neurons[1,2]. These neurons can be tagged during learning for subsequent identification[3] and manipulation[4–6]. Moreover, their ablation or inactivation results in reduced memory expression, suggesting their necessity in mnemonic processes. However, the question of sufficiency remains: it is unclear whether it is possible to elicit the behavioural output of a specific memory by directly activating a population of neurons that was active during learning. Here we show in mice that optogenetic reactivation of hippocampal neurons activated during fear conditioning is sufficient to induce freezing behaviour. We labelled a population of hippocampal dentate gyrus neurons activated during fear learning with channelrhodopsin-2 (ChR2)[7,8] and later optically reactivated these neurons in a different context. The mice showed increased freezing only upon light stimulation, indicating light-induced fear memory recall. This freezing was not detected in non-fear-conditioned mice expressing ChR2 in a similar proportion of cells, nor in fear-conditioned mice with cells labelled by enhanced yellow fluorescent protein instead of ChR2. Finally, activation of cells labelled in a context not associated with fear did not evoke freezing in mice that were previously fear-conditioned in a different context, suggesting that light-induced fear memory recall is context-specific. Together, our findings indicate that activating a sparse but specific ensemble of hippocampal neurons that contribute to a memory engram is sufficient for the recall of that memory. Moreover, our experimental approach offers a general method of mapping cellular populations bearing memory engrams.

Susumu Tonegawa. Nobel Prize for Physiology or Medicine in 1987, for his discovery of the genetic mechanism that produces antibody diversity.

1. Immediate early genes

To identify which neurons are active during the formation of a memory, one can rely on the activity-dependent nature of immediate early genes (IEGs). The best characterized IEGs are zif268, c-fos and Arc/Arg3.1.

The proportion of cells expressing Arc and c-fos in DG (2-6%), CA3 (20-40%) and CA1 (40-70%) after exposure to a novel environment resembles the proportion of hippocampal excitatory cells physiologically active in a given environment.

The cellular expression pattern of c-fos and Arc is different for different contexts, but remains stable upon re-exposure to the same context.

Hypothesis: cells expressing c-fos after a training episode are participating in the encoding of the memory for that specific experience. These cells may therefore represent a component of the stored memory engram.

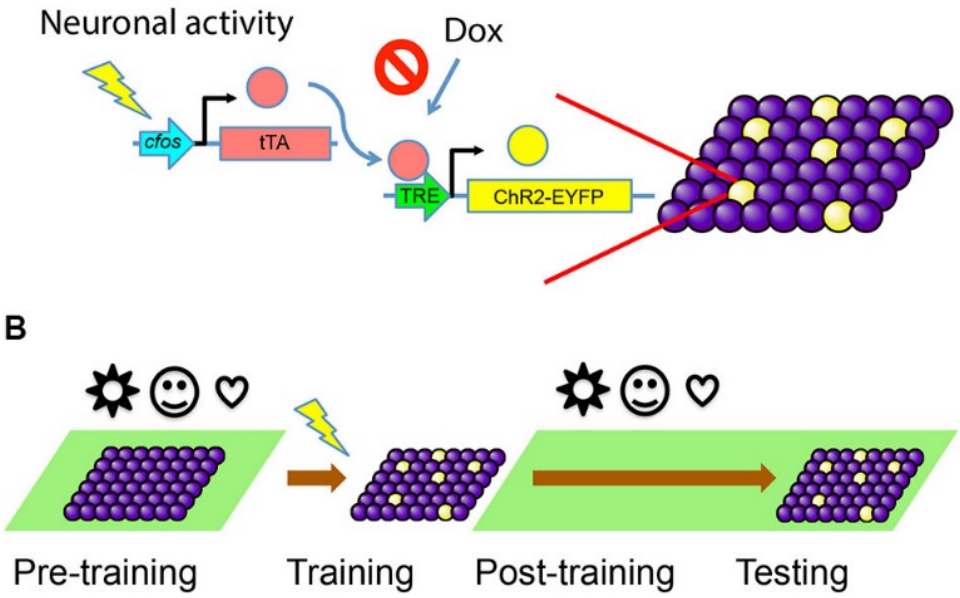
2. The TetTag mouse

Transgenic mouse TetTag: drives the expression of the tetracycline transactivator (tTA) under the control of the c-fos promoter. tTA mimics the expression pattern of endogenous c-fos and appears only transiently in activated neurons.

The tTA protein bind to the tetracycline-responsive element (TRE) to trigger the expression of ChR2-EYFP. However, the binding of tTA to TRE is blocked by Dox, which can be administered through an animal's diet.

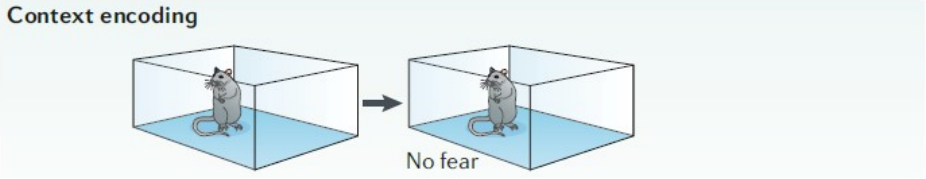
If Dox is removed from the food, a temporal window for activity-dependent labeling is opened and tTA can bind to TRE to turn on the expression of ChR2-EYFP.

ChR2-EYFP is injected in the dentate gyrus via rAAVs.



3. Fear conditioning

Contextual encoding: exposing an animal to a novel environment results in a memory of that context.



Context conditioning: If that context is paired with an adverse stimulus (electric footshock), it will yield an association between context and shock.

Context conditioning occurs with either a signaled shock in which a conditional stimulus (CS, e.g. a sound) is paired with the shock (the unconditioned stimulus, US) or with unsignaled shock.

Contextual retrieval: a CS is paired with the US in one context but not in an another. Subsequently, the context serves to retrieve the meaning of the CS

LETTER

doi:10.1038/nature11028

Optogenetic stimulation of a hippocampal engram activates fear memory recall

Xu Liu[1]*, Steve Ramirez[1]*, Petti T. Pang[1], Corey B. Puryear[1], Arvind Govindarajan[1], Karl Deisseroth[2] & Susumu Tonegawa[1]

A specific memory is thought to be encoded by a sparse population of neurons[1,2]. These neurons can be tagged during learning for subsequent identification[3] and manipulation[4–6]. Moreover, their ablation or inactivation results in reduced memory expression, suggesting their necessity in mnemonic processes. However, the question of sufficiency remains: it is unclear whether it is possible to elicit the behavioural output of a specific memory by directly activating a population of neurons that was active during learning. Here we show in mice that optogenetic reactivation of hippocampal neurons activated during fear conditioning is sufficient to induce freezing behaviour. We labelled a population of hippocampal dentate gyrus neurons activated during fear learning with channelrhodopsin-2 (ChR2)[7,8] and later optically reactivated these neurons in a different context. The mice showed increased freezing only upon light stimulation, indicating light-induced fear memory recall. This freezing was not detected in non-fear-conditioned mice expressing ChR2 in a similar proportion of cells, nor in fear-conditioned mice with cells labelled by enhanced yellow fluorescent protein instead of ChR2. Finally, activation of cells labelled in a context not associated with fear did not evoke freezing in mice that were previously fear-conditioned in a different context, suggesting that light-induced fear memory recall is context-specific. Together, our findings indicate that activating a sparse but specific ensemble of hippocampal neurons that contribute to a memory engram is sufficient for the recall of that memory. Moreover, our experimental approach offers a general method of mapping cellular populations bearing memory engrams.

An important question in neuroscience is how a distinct memory is formed and stored in the brain. Recent studies indicate that defined populations of neurons correspond to a specific memory trace[1], suggesting a cellular correlate of a memory engram. Selective ablation or inhibition of such neuronal populations erased the fear memory response[5,6], indicating that these cells are necessary for fear memory expression. However, to prove that a cell population is the cellular basis of a specific fear memory engram it is necessary to conduct a mimicry experiment to show that direct activation of such a population is sufficient for inducing the associated behavioural output[9,10].

The hippocampus is thought to be critical in the formation of the contextual component of fear memories[11–14]. Modelling[15] and experimental[16,17] studies have demonstrated an essential role of the dentate gyrus (DG) of the hippocampus in discriminating between similar contexts. Cellular studies of immediate early gene expression showed that sparse populations of DG granule cells (2–4%) are activated in a given context[18]. Moreover, although the same population of DG granule cells is activated repeatedly in the same environment, different environments[19] or different tasks[20] activate different populations of DG granule cells. These lines of evidence point to the DG as an ideal target for the formation of contextual memory engrams that represent discrete environments.

To label and reactivate a subpopulation of DG cells active during the encoding of a memory, we targeted the DG of c-fos-tTA transgenic mice[3] with the AAV_9-TRE-ChR2-EYFP virus and an optical fibre implant (Fig. 1a). This approach directly couples the promoter of *c-fos*, an immediate early gene often used as a marker of recent neuronal activity[21], to the tetracycline transactivator (tTA), a key component of the doxycycline (Dox) system for inducible expression of a gene of interest[22]. In our system, the presence of Dox inhibits *c-fos*-promoter-driven tTA from binding to its target tetracycline-responsive element (TRE) site, which in turn prevents it from driving ChR2–EYFP (enhanced yellow fluorescent protein) expression. In the absence of Dox, training-induced neuronal activity selectively labels active c-Fos-expressing DG neurons with ChR2–EYFP, which can then be reactivated by light stimulation during testing (Fig. 1b, c). We confirmed that our manipulation restricts the expression of ChR2–EYFP largely to the DG area of the hippocampus (Fig. 1d–g).

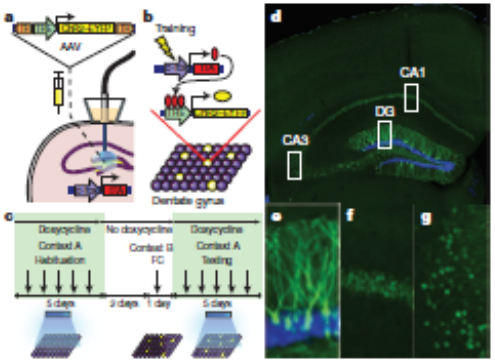


Figure 1 | Basic experimental protocols and selective labelling of DG cells by ChR2–EYFP. a, The c-fos-tTA mouse was injected with AAV_9-TRE-ChR2-EYFP and implanted with an optical fibre targeting the DG. **b**, When off Dox, training induces the expression of tTA, which binds to TRE and drives the expression of ChR2–EYFP, labelling a subpopulation of activated cells (yellow) in the DG. **c**, Basic experimental scheme. Mice were habituated in context A with light stimulation while on Dox for 5 days, then taken off Dox for 2 days and fear-conditioned (FC) in context B. Mice were put back on Dox and tested for 5 days in context A with light stimulation. **d**, Representative image showing the expression of ChR2–EYFP in a mouse that was taken off Dox for 2 days and underwent FC training. **e**–**g**, An image of each rectangular area in **d** is magnified, showing the DG (**e**), CA1 (**f**) and CA3 (**g**). The green signal from ChR2–EYFP in the DG spreads throughout entire granule cells, including dendrites (**e**), whereas the green signal confined to the nuclei in CA1 and CA3 is due to a 2-h half-life EGFP (shEGFP) expression from the *c-fos*-shEGFP construct of the transgenic mouse (**f**, **g**). Blue is nuclear marker 4′,6-diamidino-2-phenylindole (DAPI). Panel **d** is at ×10 magnification and panels **e**–**g** are at ×30 magnification.

[1]RIKEN-MIT Center for Neural Circuit Genetics at the Picower Institute for Learning and Memory, Department of Biology and Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA. [2]Department of Bioengineering and Department of Psychiatry and Behavioral Sciences, Stanford University, Stanford, California 94305, USA.
*These authors contributed equally to this work.

©2012 Macmillan Publishers Limited. All rights reserved

Fig. 1 - Introduction to the experimental approach

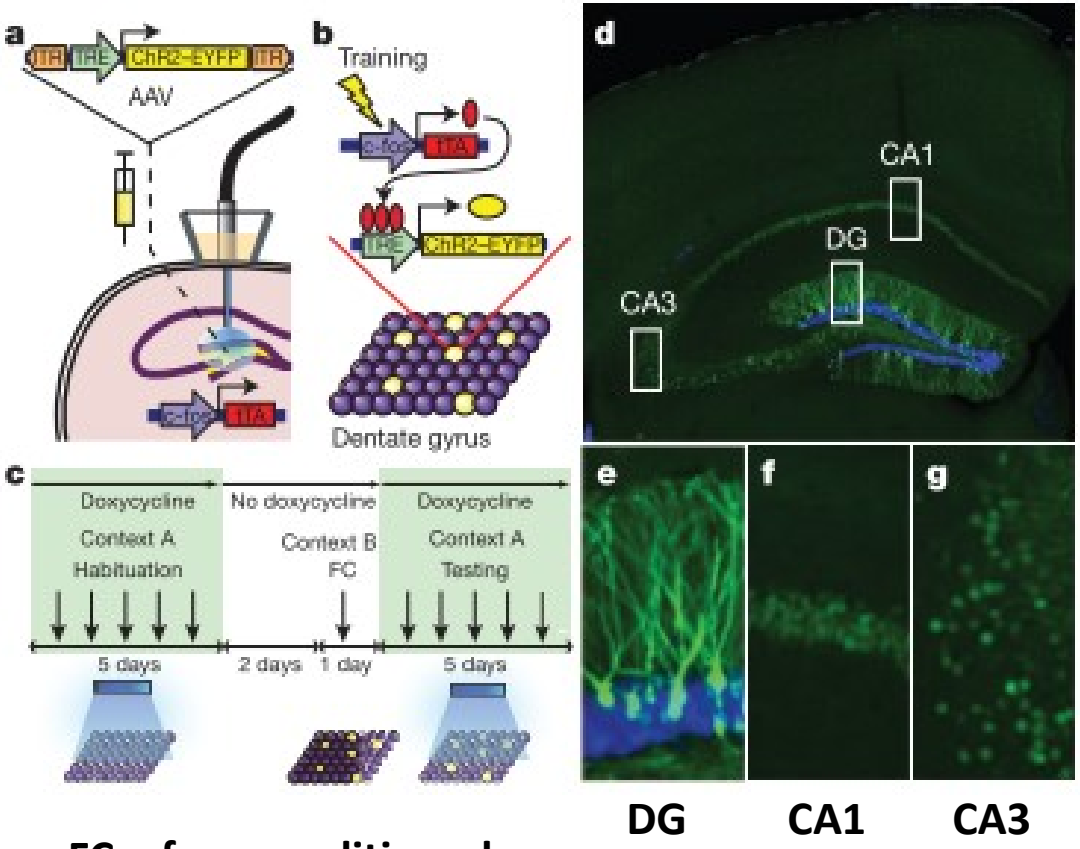
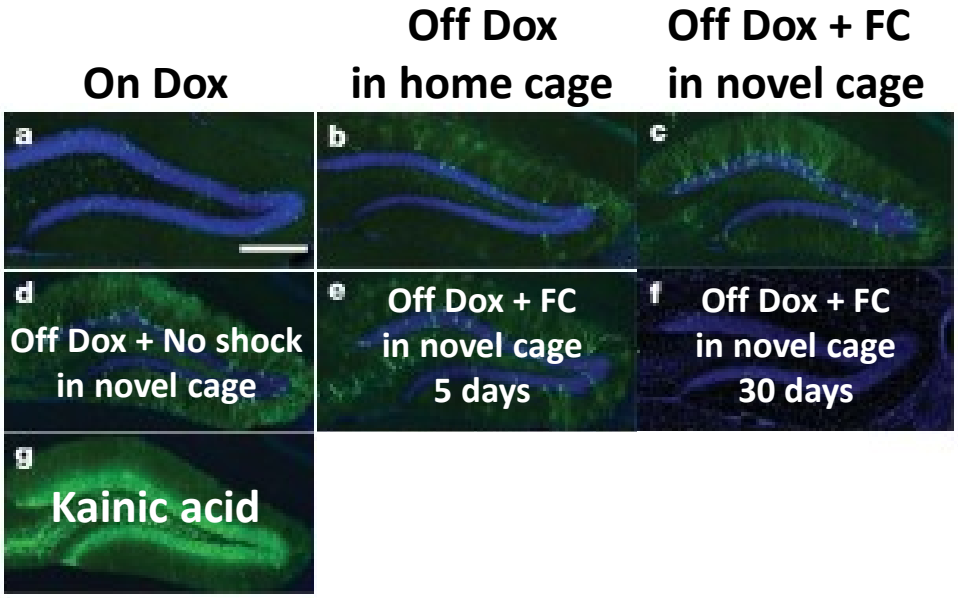


Figure 1 | Basic experimental protocols and selective labelling of DG cells by ChR2–EYFP. **a**, The c-fos-tTA mouse was injected with AAV_9-TRE-ChR2-EYFP and implanted with an optical fibre targeting the DG. **b**, When off Dox, training induces the expression of tTA, which binds to TRE and drives the expression of ChR2–EYFP, labelling a subpopulation of activated cells (yellow) in the DG. **c**, Basic experimental scheme. Mice were habituated in context A with light stimulation while on Dox for 5 days, then taken off Dox for 2 days and fear-conditioned (FC) in context B. Mice were put back on Dox and tested for 5 days in context A with light stimulation. **d**, Representative image showing the expression of ChR2–EYFP in a mouse that was taken off Dox for 2 days and underwent FC training. **e–g**, An image of each rectangular area in **d** is magnified, showing the DG (**e**), CA1 (**f**) and CA3 (**g**). The green signal from ChR2–EYFP in the DG spreads throughout entire granule cells, including dendrites (**e**), whereas the green signal confined to the nuclei in CA1 and CA3 is due to a 2-h half-life EGFP (shEGFP) expression from the c-fos-shEGFP construct of the transgenic mouse (**f**, **g**). Blue is nuclear marker 4′,6-diamidino-2-phenylindole (DAPI). Panel **d** is at ×10 magnification and panels **e–g** are at ×50 magnification.

FC = fear-conditioned

Fig. 2 - Validation of the experimental approach



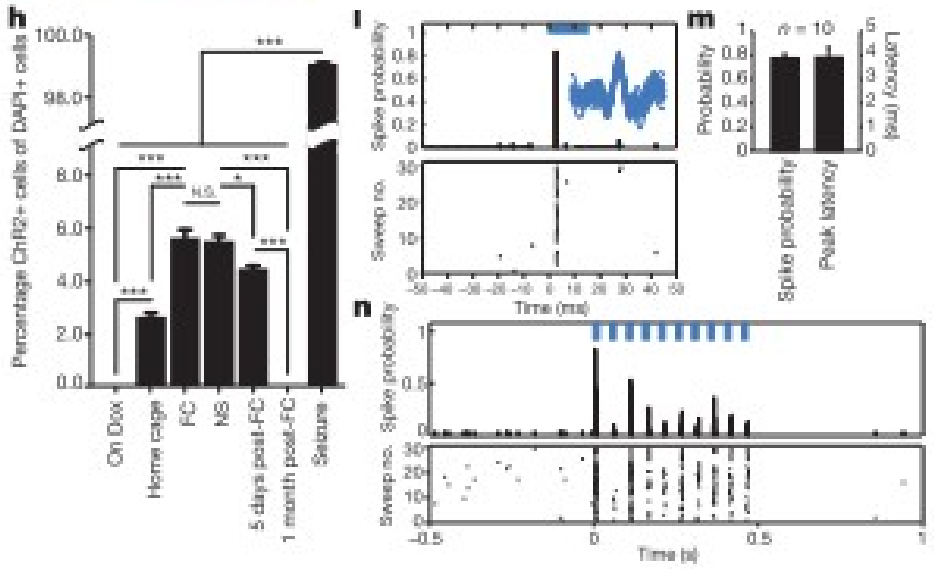


Figure 2 | Activity-dependent expression and stimulation of ChR2–EYFP. **a–g**, Representative images of the DG from c-fos-tTA mice injected with AAV_9-TRE-ChR2-EYFP and killed after the following treatments: on Dox (**a**); off Dox for 2 days in home cage (**b**); same as **b** followed by FC (**c**); same as **c** except no shock was delivered (NS; **d**); same as **c**, 5 days after training (**e**); same as **c**, 30 days after training (**f**); same as **b** followed by kainic acid injection to induce seizure (**g**). Residual green signal in **a** and **f** is from nuclear-localized c-fos-shEGFP (see Fig. 1 legend). **h**, Percentage of ChR2–EYFP-positive cells after various treatments represented by **a–g** ($n = 5$ subjects each; $F_{6,28} = 94.43$, $^*P < 0.05$; $^{***}P < 0.001$). N.S., not significant. **i**, **j**, Representative DG cells after light stimulation in c-fos-tTA mice injected with AAV_9-TRE-ChR2-EYFP (**i**) or AAV_9-TRE-EYFP (**j**). **k**, Percentage of c-Fos-positive cells among ChR2–EYFP-positive cells or EYFP-positive cells after light stimulation ($n = 3$ subjects each; $^{***}P < 0.001$). **l**, Light-evoked single unit activity of a DG neuron from a c-fos-tTA mouse injected with AAV_9-TRE-ChR2-EYFP. Peri-event histogram (top) and raster plot (bottom) show reliable and precisely time-locked spiking relative to the onset of 15 ms light pulses (blue bar). Inset shows an overlay of waveforms for all the spikes during light stimulation. **m**, Spike probability and peak latency for all the light-responsive cells ($n = 10$) recorded as in **l**. **n**, Multi-unit activity in the DG from a c-fos-tTA mouse injected with AAV_9-TRE-ChR2-EYFP in response to trains of 10 light pulses (15 ms; blue bars) at 20 Hz. Scale bar in **a**, 250 μm. Panels **a–g** are at ×10 magnification and panels **i**, **j** are at ×80 magnification. Error bars show mean ± s.e.m.

Fig. 3 - Is optogenetic reactivation of DG neurons active during fear conditioning sufficient to induce freezing behavior?

The key experiment

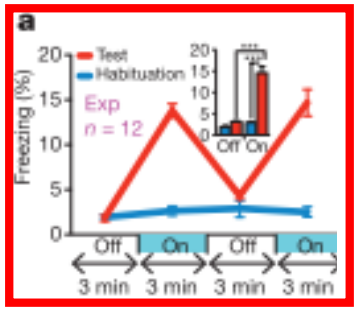
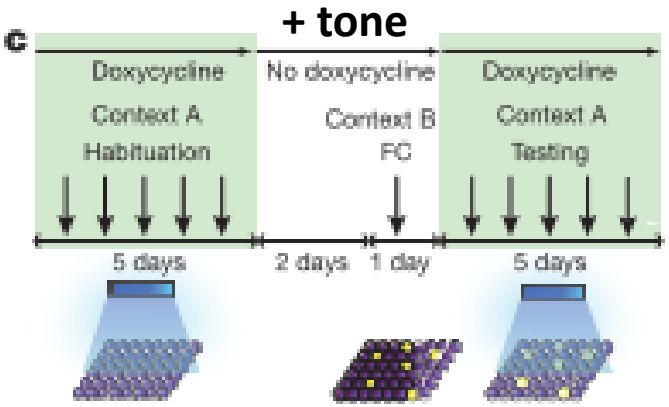
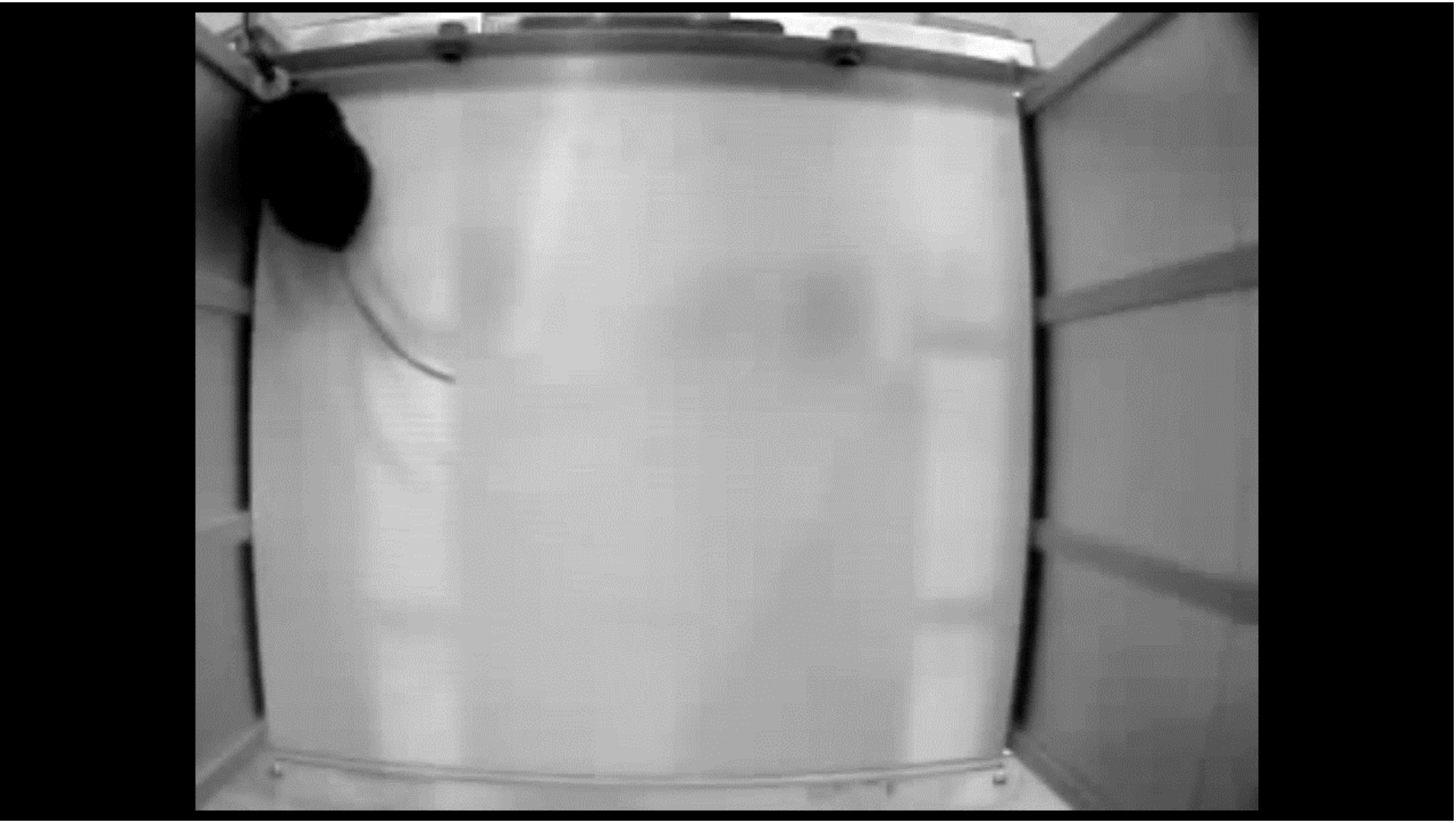


Figure 3 | Optical stimulation of engram-bearing cells induces post-training freezing. **a**, c-fos-tTA mice injected with AAV_9-TRE-ChR2-EYFP and trained with FC (Exp group) showed increased freezing during 3-min light-on epochs. Freezing for each epoch represents 5-day average (Supplementary Fig. 5a, g). Freezing levels for the two light-off and light-on epochs are further averaged in the inset ($n = 12$, $F_{1,22} = 37.98$, $^{***}P < 0.001$). **b**, Mice trained similarly to the conditions in **a** but without foot shock (NS group) did not show increased light-induced freezing ($n = 12$). N.S., not significant. **c**, Mice injected with AAV_9-TRE-EYFP and trained with FC (EYFP group) did not show increased light-induced freezing ($n = 12$). **d**, Mice trained similarly to the conditions in **a** but kept off Dox for 1 day before FC training (Exp-1day group) showed greater freezing during test light-on epochs compared to Exp group ($n = 5$, $F_{1,8} = 38.26$, $^{***}P < 0.001$). **e**, Mice trained similarly to the conditions in **a** but bilaterally injected with AAV_9-TRE-ChR2-EYFP and implanted with optical fibres (Exp-Bi group) showed even higher levels of freezing during test light-on epochs ($n = 6$, $F_{1,10} = 85.14$, $^{***}P < 0.001$). **f**, Summary of freezing levels of the five groups during test light-on epochs ($F_{4,42} = 37.62$, $^{*}P < 0.05$; $^{***}P < 0.001$). Error bars show mean ± s.e.m.



Pre-training



Test session post-training

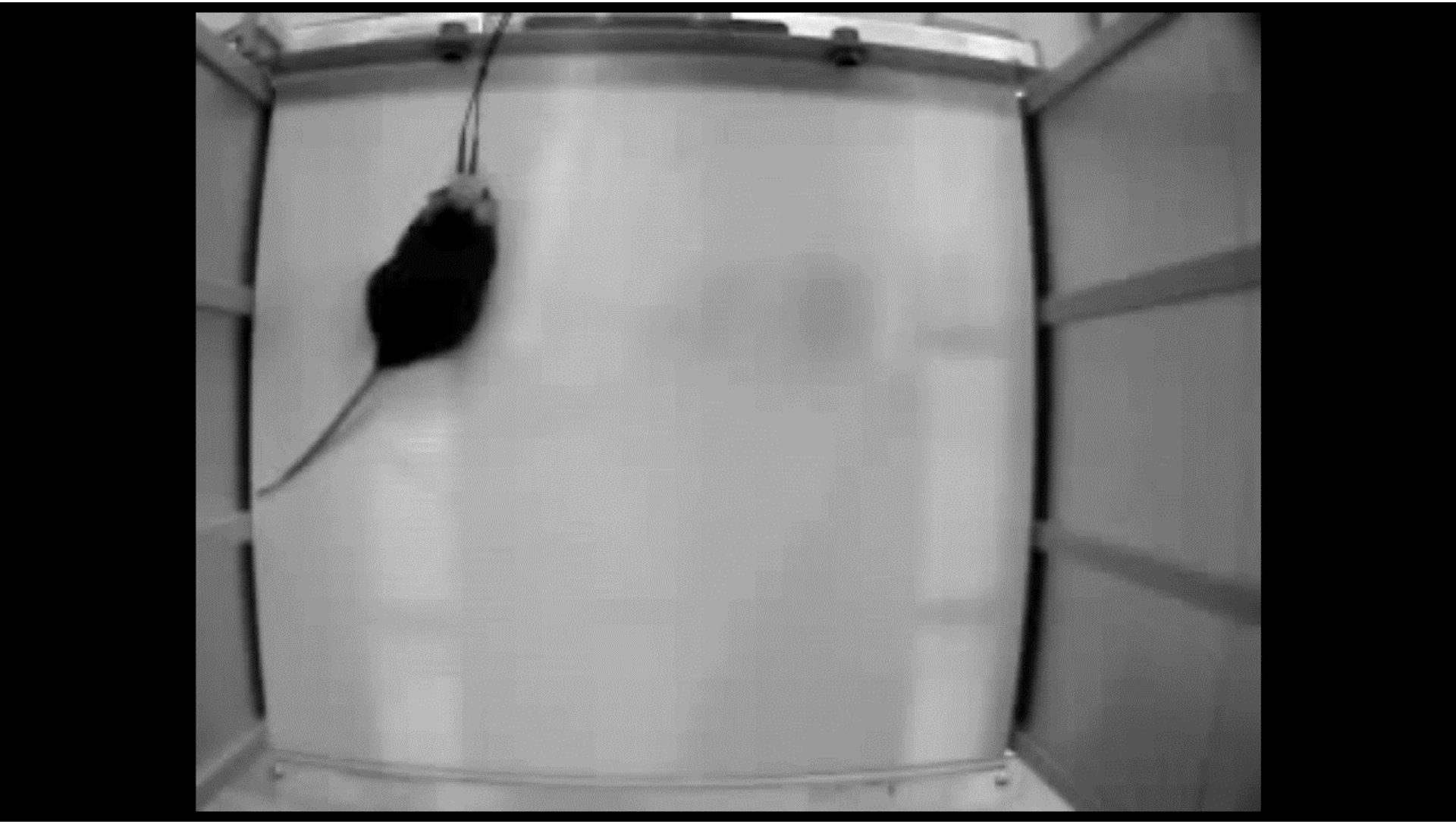
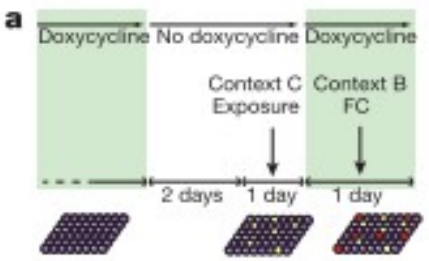


Fig. 4 - Is light-induced fear memory recall context-specific?



ChR2-EYFP

Endogenous c-Fos

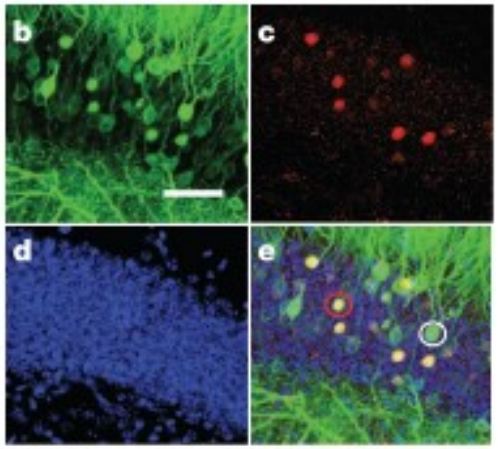


Figure 4 | Labelling and stimulation of independent DG cell populations. **a**, c-fos-tTA mice injected with AAV_9-TRE-ChR2-EYFP were taken off Dox and exposed to context C to label activated cells with ChR2–EYFP (yellow), then put back on Dox and trained with FC in context B to activate endogenous c-Fos (red). **b**–**e**, Representative images of DG from these mice are shown. **b**, ChR2–EYFP-labelled cells activated in context C. **c**, c-Fos-labelled cells activated in context B. **d**, Nuclear marker DAPI. **e**, Merge. The white and red circles show examples of ChR2–EYFP-positive and c-Fos-positive cells, respectively. The c-Fos-positive cells in **e** appear yellow because they express both endogenous c-Fos (red) and the nuclear-localized c-fos-shEGFP (green) (see Fig. 1 legend). **f**, Percentage of ChR2–EYFP-positive, endogenous c-Fos-positive, and double-positive cells among total cells (DAPI+) ($n = 5$). **g**, The observed percentage of double-positive cells is the same as what would be expected if the two cell populations were independent (that is, a product of the observed percentage of ChR2–EYFP single-positive and c-Fos single-positive cells). **h**, Behaviour setup for mice exposed to an open field in context C while off Dox and subsequently fear-conditioned in context B while on Dox (OF-FC). **i**, OF-FC mice ($n = 5$) do not show increased light-induced freezing. N.S., not significant. Panels **b**–**e** are at ×80 magnification. Scale bar in **b**, 10 µm. Error bars show mean ± s.e.m.

Creating a False Memory in the Hippocampus

Steve Ramirez,[1]* Xu Liu,[1,2]* Pei-Ann Lin,[1] Junghyup Suh,[1] Michele Pignatelli,[1] Roger L. Redondo,[1,2] Tomás J. Ryan,[1,2] Susumu Tonegawa[1,2]†

Memories can be unreliable. We created a false memory in mice by optogenetically manipulating memory engram–bearing cells in the hippocampus. Dentate gyrus (DG) or CA1 neurons activated by exposure to a particular context were labeled with channelrhodopsin-2. These neurons were later optically reactivated during fear conditioning in a different context. The DG experimental group showed increased freezing in the original context, in which a foot shock was never delivered. The recall of this false memory was context-specific, activated similar downstream regions engaged during natural fear memory recall, and was also capable of driving an active fear response. Our data demonstrate that it is possible to generate an internally represented and behaviorally expressed fear memory via artificial means.

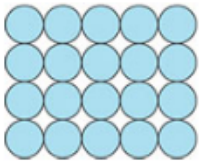
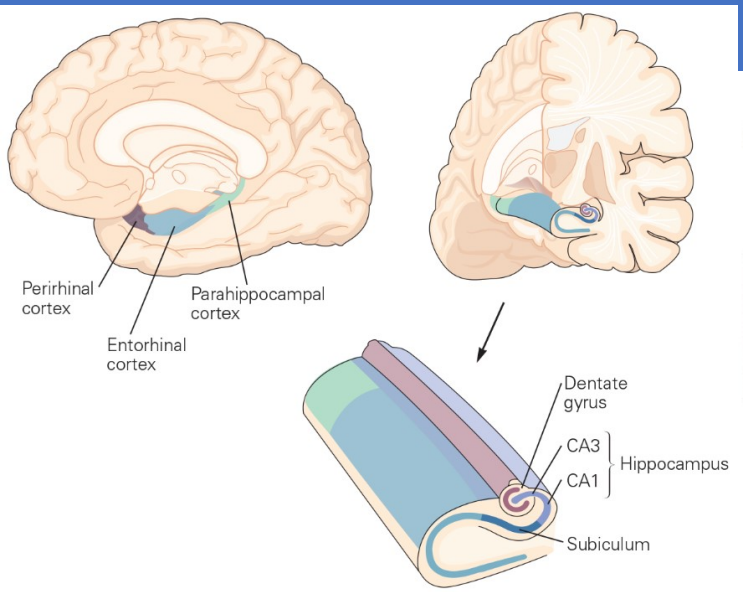


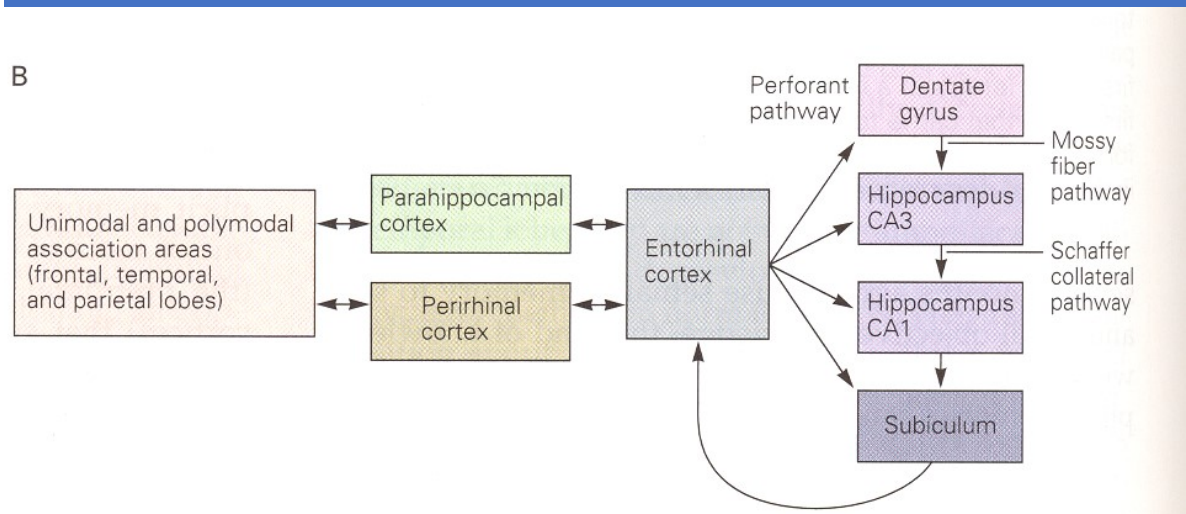
FIGURE 2 | Inception of a false fear memory. *Top*: The behavior paradigm for the experimental animals. Animals were kept on Dox post-surgery (green background), then taken off Dox and allowed to explore context A to label active cells with ChR2. Then they were put back on Dox and fear conditioned (lightening symbol) in context B while receiving light stimulation (blue shower symbol) to activate cells representing context A. When they were put back to context A, they showed a false fear memory for A (freezing indicated by wavy lines) where they were never actually shocked. They showed no fear memory for a control context C and a genuine fear memory for context B where they were shocked. *Bottom*: Cellular activity. Red, gray, and white circles indicate neurons representing contexts A, B, and C respectively. Asterisks indicate neurons activated either naturally by contextual exposure or artificially by light stimulation.

Current research:

1) Variability of memory engrams, e.g. hippocampal vs. cortical engrams

Memory traces are initially elaborated in one or more association areas of the cortex that receive and/or elaborate visual, auditory, somatic… information. From here information is transferred through the entorhinal cortex to the hippocampus from where it returns to entorhinal cortex and associative cortices (long-long-term storage)





Current research:

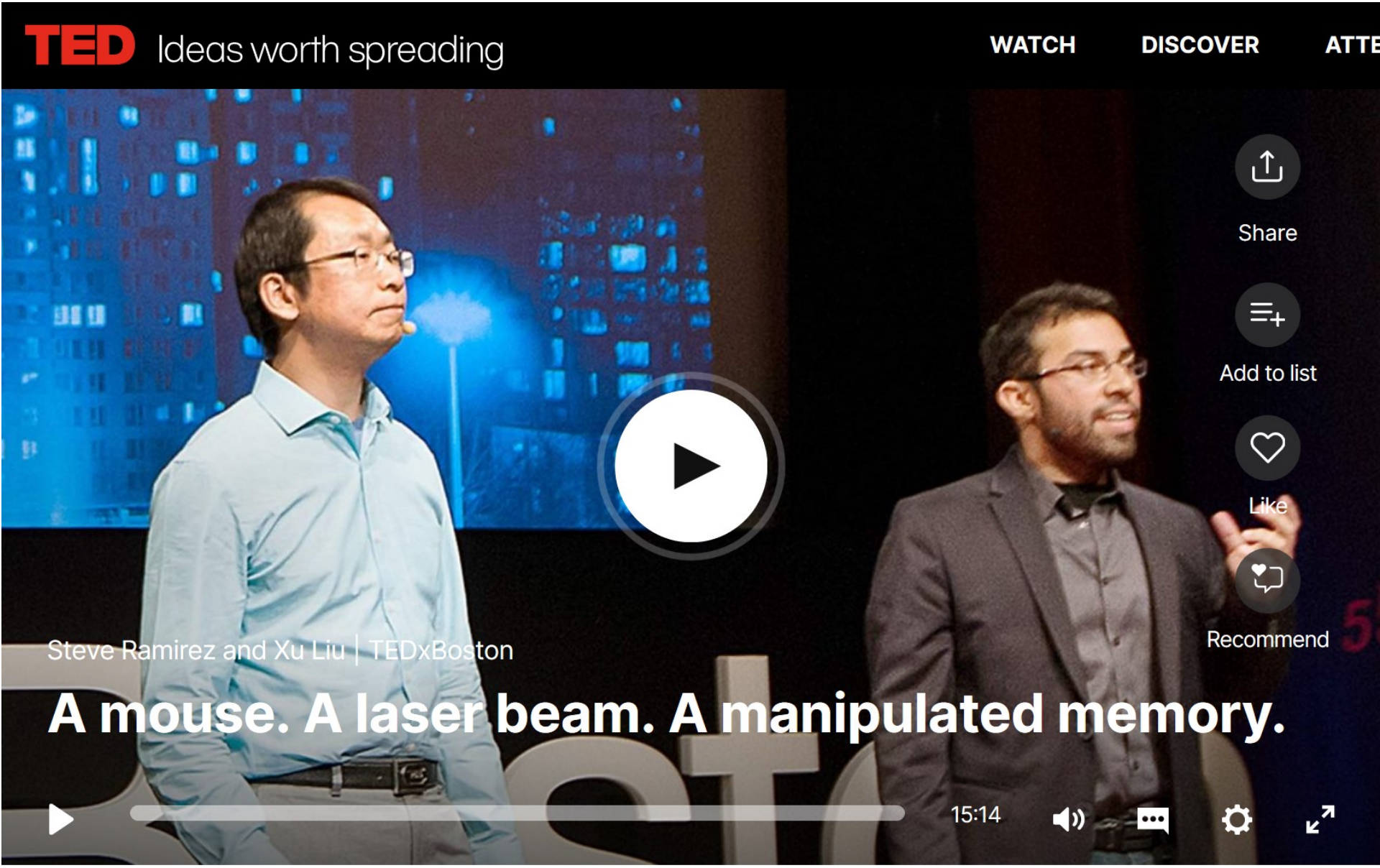
2) How Hebbian plasticity (e.g. LTP; neurons that fire together wire together) relates to the engram?

Several properties of LTP suggest this phenomenon as a possible cellular mechanism underlying memory:

LTP requires a strong and coincident pre- and postsynaptic activity (due to **NMDARs** acting as **coincidence detectors**: they are activated by glutamate released from the pre-synaptic neuron and by post-synaptic depolarization).

The need for a coincident pre- and postsynaptic activity was first postulated by **Donald Hebb** (The organization of behavior; 1949).

When an axon of cell A is near enough to excite cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased.


TED Ideas worth spreading
WATCH
DISCOVER
ATTE
Share
Add to list
Like
Recommend
Steve Ramirez and Xu Liu | TEDxBoston
A mouse. A laser beam. A manipulated memory.
15:14

REVIEWS

The role of engram cells in the systems consolidation of memory

Susumu Tonegawa[1,2], Mark D. Morrissey[1]* and Takashi Kitamura[3]**

Abstract | What happens to memories as days, weeks and years go by has long been a fundamental question in neuroscience and psychology. For decades, researchers have attempted to identify the brain regions in which memory is formed and to follow its changes across time. The theory of systems consolidation of memory (SCM) suggests that changes in circuitry and brain networks are required for the maintenance of a memory with time. Various mechanisms by which such changes may take place have been hypothesized. Recently, several studies have provided insight into the brain networks driving SCM through the characterization of memory engram cells, their biochemical and physiological changes and the circuits in which they operate. In this Review, we place these findings in the context of the field and describe how they have led to a revamped understanding of SCM in the brain.

Finding the engram

Sheena A. Josselyn[1–4], Stefan Köhler[5,6] and Paul W. Frankland[1–4]

Abstract | Many attempts have been made to localize the physical trace of a memory, or engram, in the brain. However, until recently, engrams have remained largely elusive. In this Review, we develop four defining criteria that enable us to critically assess the recent progress that has been made towards finding the engram. Recent 'capture' studies use novel approaches to tag populations of neurons that are active during memory encoding, thereby allowing these engram-associated neurons to be manipulated at later times. We propose that findings from these capture studies represent considerable progress in allowing us to observe, erase and express the engram.

CellPress

Neuron

Perspective

Memory Engram Cells Have Come of Age

Susumu Tonegawa,[1,2,*] Xu Liu,[1,2,3] Steve Ramirez,[1,*] and Roger Redondo[1,2]
[1]RIKEN–MIT Center for Neural Circuit Genetics at the Picower Institute for Learning and Memory, Department of Biology and Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
[2]Howard Hughes Medical Institute, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
[3]We dedicate this article to Xu Liu, who passed away in February, 2015
*Correspondence: tonegawa@mit.edu (S.T.), sramirez@mit.edu (S.R.)
http://dx.doi.org/10.1016/j.neuron.2015.08.002

The idea that memory is stored in the brain as physical alterations goes back at least as far as Plato, but further conceptualization of this idea had to wait until the 20th century when two guiding theories were presented: the "engram theory" of Richard Semon and Donald Hebb's "synaptic plasticity theory." While a large number of studies have been conducted since, each supporting some aspect of each of these theories, until recently integrative evidence for the existence of engram cells and circuits as defined by the theories was lacking. In the past few years, the combination of transgenics, optogenetics, and other technologies has allowed neuroscientists to begin identifying memory engram cells by detecting specific populations of cells activated during specific learning epochs and by engineering them not only to evoke recall of the original memory, but also to alter the content of the memory.

frontiers in
BEHAVIORAL NEUROSCIENCE

REVIEW ARTICLE
published: 17 January 2014
doi: 10.3389/fnbeh.2013.00226

Identification and optogenetic manipulation of memory engrams in the hippocampus

Steve Ramirez[1], Susumu Tonegawa[1,2] and Xu Liu[1,2]*

[1] *Department of Biology and Department of Brain and Cognitive Sciences, RIKEN-MIT Center for Neural Circuit Genetics at the Picower Institute for Learning and Memory, Massachusetts Institute of Technology, Cambridge, MA, USA*
[2] *Howard Hughes Medical Institute, Massachusetts Institute of Technology, Cambridge, MA, USA*

Edited by:
Anton Ilango, National Institutes of Health, USA

Reviewed by:
Mazahir T. Hasan, Charité-Universitätsmedizin, Germany
Yu Zhou, Medical College of Qingdao University, China

****Correspondence:***
Xu Liu, Department of Biology and Department of Brain and Cognitive Sciences, RIKEN-MIT Center for Neural Circuit Genetics at the Picower Institute for Learning and Memory and Howard Hughes Medical Institute, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Building 46-5261, Cambridge, MA 02139, USA
e-mail: xuliu@mit.edu

With the accumulation of our knowledge about how memories are formed, consolidated, retrieved, and updated, neuroscience is now reaching a point where discrete memories can be identified and manipulated at rapid timescales. Here, we start with historical studies that lead to the modern memory engram theory. Then, we will review recent advances in memory engram research that combine transgenic and optogenetic approaches to reveal the underlying neuronal substrates sufficient for activating mnemonic processes. We will focus on three concepts: (1) isolating memory engrams at the level of single cells to tag them for subsequent manipulation; (2) testing the sufficiency of these engrams for memory recall by artificially activating them; and (3) presenting new stimuli during the artificial activation of these engrams to induce an association between the two to form a false memory. We propose that hippocampal cells that show activity-dependent changes during learning construct a cellular basis for contextual memory engrams.

Keywords: optogenetics, memory engram, IEG, ChR2, false memory