Brain-wide mapping reveals that engrams for a single memory are distributed across multiple brain regions

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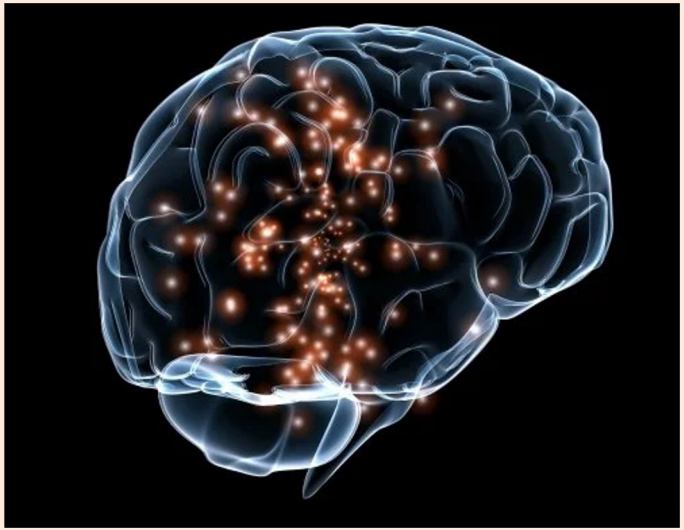


01.

Important Terms

Important Terms

cFOS+

regulate genes that are switched on or repressed depending on influences from external stimuli.

Memory Engram

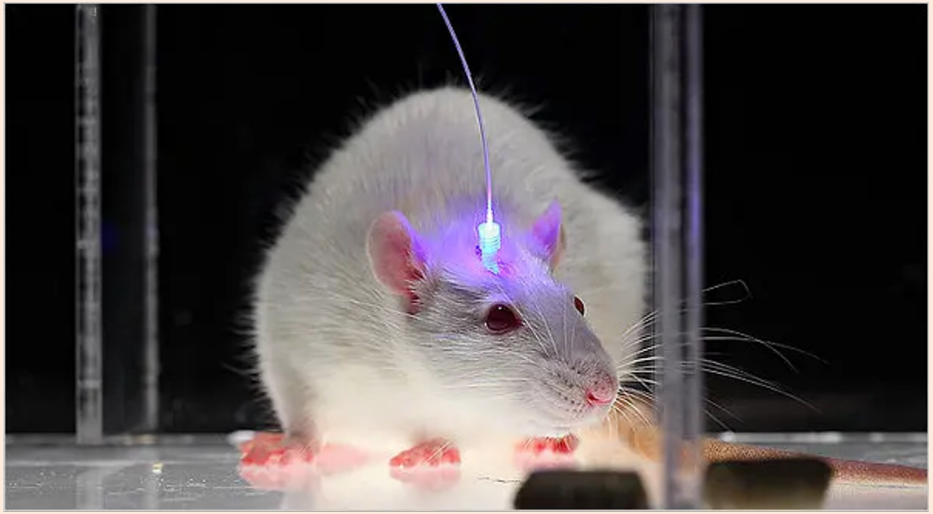
the enduring physical or chemical changes that occur in brain networks upon learning representing acquired memory information

4-hydroxytamoxifen (4-OHT)

4-OHT is a compound that can specific activate these ERT2 receptors (estrogen receptor) and initiate the express of the Cre-dependent tdTomato stain.

Optogenetics

Scientists genetically engineer neurons to produce light-sensitive proteins known as opsins. Opsins permit the transmembrane movement of ions (causing an action potential) so scientists are able to use light to control specific neuron firing.



02.

Previous Research

Previous Studies

Previously Identified Engram Regions

- Hippocampal subfields
- Amygdala subregions
- Retrosplenial cortex
- Prefrontal cortex

“Indeed, early limited studies supported [Semon’s engram concept] for contextual fear memory. While these findings enhanced our understanding of engram-based memory storage, a thorough mapping of a unified engram complex for a specific memory has been a challenging endeavor.”

Previous Studies

- Optogenetic stimulation of hippocampal engram activates fear memory recall.
  - [PMC free article] [PubMed] [Google Scholar]
- Wiring and molecular features of prefrontal ensembles representing distinct experiences.
  - [PMC free article] [PubMed] [Google Scholar]
- Distinct neural circuits for the formation and retrieval of episodic memories.
  - [PMC free article] [PubMed] [Google Scholar]
- Memory retrieval by activating engram cells in mouse models of early Alzheimer's disease.
  - PMC free article] [PubMed] [Google Scholar]
- Bidirectional switch of the valence associated with hippocampal contextual memory engram.
  - [PMC free article] [PubMed] [Google Scholar]

03.

Hypothesis

Hypothesis

A specific memory is stored in functionally connected engram cell ensembles that are widely distributed across multiple brain regions and are activated by natural recall cues. This is not an original hypothesis but part of Richard Semon and Donald Hebb's theory of synaptic plasticity.

Evidence Supporting Hypothesis:

- Gene expression is altered by widespread, behaviorally-defined neural circuits.
- Distributed brain regions involved in memory formation have been identified.
- Engram cells have been identified in hippocampal subfields and the amygdala.

04.

Test Groups

Test Groups

Group 1: Home Cage Group

Mice that received 4-hydroxytamoxifen (4-OHT) and stayed in their home cage

Group 2: CFC Group

Mice that received 4-OHT and contextual fear training and then went back into their home cage

Group 3: Recall group

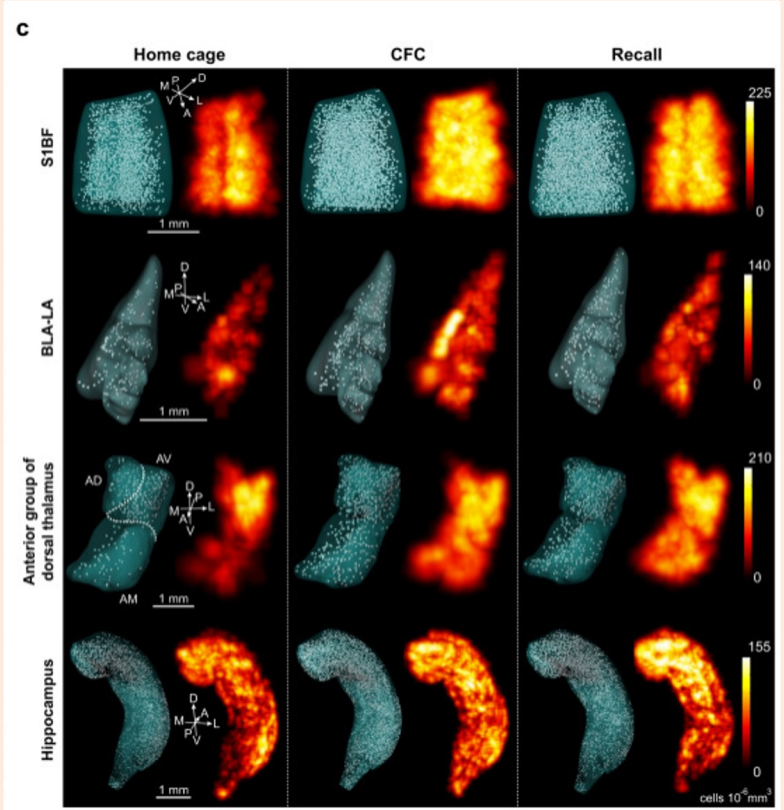
Mice that received 4-OHT and contextual fear training and then after CFC were tested for fear memory recall.

05.

Methods

Methods

- The brains of the mice were processed using the SHIELD technique to preserve endogenous tdTomato fluorescence and the tissue structure.
- They created a list of engram-containing regions of the brain. From this data they were able to form an engram index.
- To verify their engram index, the scientists found the overlap between tdTomato and recall-activated cFos+ neurons.
- They used optogenetics (control neurons through light) to test memory recall.
    - Dorsal CA1 and BLA engram cells were used as positive controls because they show significant memory recall when optogenetically activated.



06.

Results

New Engram Regions Discovered

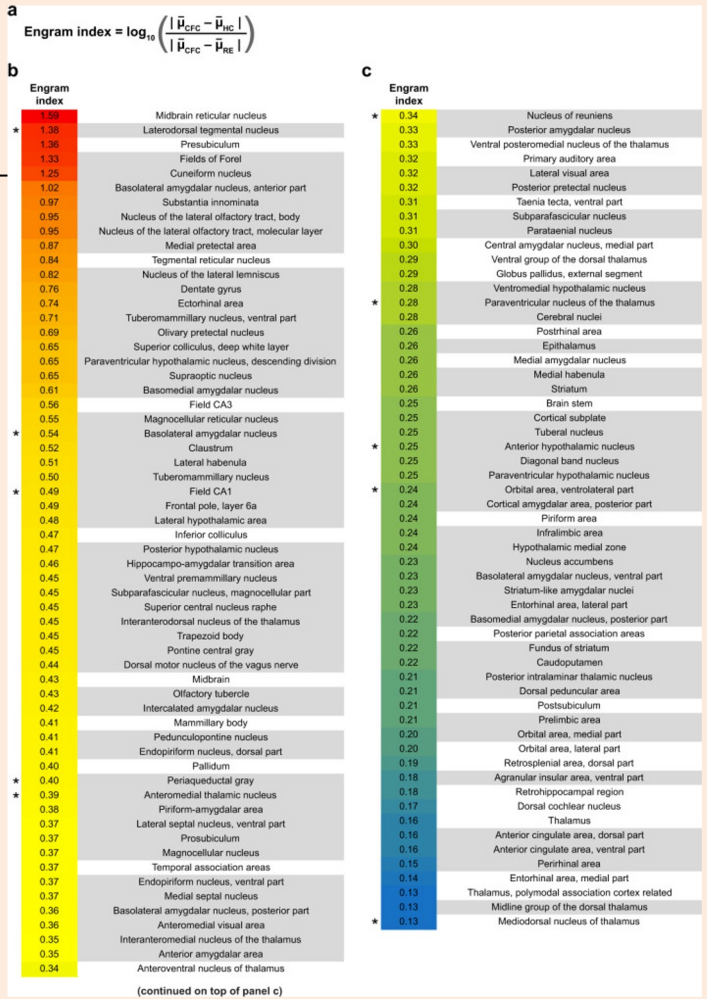
- **LDTg** - emotional arousal under adverse conditions
- **Anterior hypothalamic nucleus (AHN)** - expression of conditioned fear behaviors
- **RE Thalamus** - contextual memories, specifically discerning similar environments (supports the idea that thalamic ensembles don't solely play a passive relay role)
- **Ventrolateral orbital area (VLO)** - long-term memory retention
- **AM thalamus** - context-specific, high valence memories
- **Periaqueductal gray (PAG)** - freezing behavior
- **Paraventricular (PVT) Thalamus** - arousal, stress, emotional memory, and motivation.

*Note: many of these functions are just possibilities and have not been fully confirmed yet.

Engram Index

The scientists devised an engram index that ranks the possible engram cell complexes. This index is signified by two parts:

1. Calculation of the average number of cFos+ neurons during memory encoding as compared to home cage.
2. Calculation of difference between activation levels in memory encoding and recall.



a

$$\text{Engram index} = \log_{10}\left(\frac{|\bar{\mu}_{CFC} - \bar{\mu}_{HC}|}{|\bar{\mu}_{CFC} - \bar{\mu}_{RE}|}\right)$$

b

	Engram index	
	1.59	Midbrain reticular nucleus
*	1.38	Laterodorsal tegmental nucleus
	1.36	Presubiculum
	1.33	Fields of Forel
	1.25	Cuneiform nucleus
	1.02	Basolateral amygdalar nucleus, anterior part
	0.97	Substantia innominata
	0.95	Nucleus of the lateral olfactory tract, body
	0.95	Nucleus of the lateral olfactory tract, molecular layer
	0.87	Medial pretectal area
	0.84	Tegmental reticular nucleus
	0.82	Nucleus of the lateral lemniscus
	0.76	Dentate gyrus
	0.74	Ectorhinal area
	0.71	Tuberomammillary nucleus, ventral part
	0.69	Olivary pretectal nucleus
	0.65	Superior colliculus, deep white layer
	0.65	Paraventricular hypothalamic nucleus, descending division
	0.65	Supraoptic nucleus
	0.61	Basomedial amygdalar nucleus
	0.56	Field CA3
	0.55	Magnocellular reticular nucleus
*	0.54	Basolateral amygdalar nucleus
	0.52	Claustrum
	0.51	Lateral habenula
	0.50	Tuberomammillary nucleus
*	0.49	Field CA1
	0.49	Frontal pole, layer 6a
	0.48	Lateral hypothalamic area
	0.47	Inferior colliculus
	0.47	Posterior hypothalamic nucleus
	0.46	Hippocampo-amygdalar transition area
	0.45	Ventral premammillary nucleus
	0.45	Subparafascicular nucleus, magnocellular part
	0.45	Superior central nucleus raphe
	0.45	Interanterodorsal nucleus of the thalamus
	0.45	Trapezoid body
	0.45	Pontine central gray
	0.44	Dorsal motor nucleus of the vagus nerve
	0.43	Midbrain
	0.43	Olfactory tubercle
	0.42	Intercalated amygdalar nucleus
	0.41	Mammillary body
	0.41	Pedunculopontine nucleus
	0.41	Endopiriform nucleus, dorsal part
	0.40	Pallidum
*	0.40	Periaqueductal gray
*	0.39	Anteromedial thalamic nucleus
	0.38	Piriform-amygdalar area
	0.37	Lateral septal nucleus, ventral part
	0.37	Prosubiculum
	0.37	Magnocellular nucleus
	0.37	Temporal association areas
	0.37	Endopiriform nucleus, ventral part
	0.37	Medial septal nucleus
	0.36	Basolateral amygdalar nucleus, posterior part
	0.36	Anteromedial visual area
	0.35	Interanteromedial nucleus of the thalamus
	0.35	Anterior amygdalar area
	0.34	Anteroventral nucleus of thalamus

(continued on top of panel c)

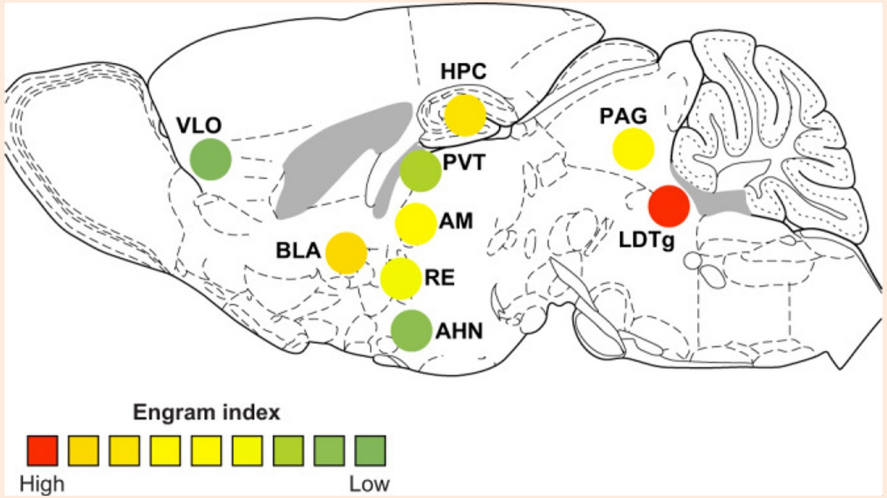
c

	Engram index	
*	0.34	Nucleus of reuniens
	0.33	Posterior amygdalar nucleus
	0.33	Ventral posteromedial nucleus of the thalamus
	0.32	Primary auditory area
	0.32	Lateral visual area
	0.32	Posterior pretectal nucleus
	0.31	Taenia tecta, ventral part
	0.31	Subparafascicular nucleus
	0.31	Parataenial nucleus
	0.30	Central amygdalar nucleus, medial part
	0.29	Ventral group of the dorsal thalamus
	0.29	Globus pallidus, external segment
	0.28	Ventromedial hypothalamic nucleus
*	0.28	Paraventricular nucleus of the thalamus
	0.28	Cerebral nuclei
	0.26	Postrhinal area
	0.26	Epithalamus
	0.26	Medial amygdalar nucleus
	0.26	Medial habenula
	0.26	Striatum
	0.25	Brain stem
	0.25	Cortical subplate
	0.25	Tuberal nucleus
*	0.25	Anterior hypothalamic nucleus
	0.25	Diagonal band nucleus
	0.25	Paraventricular hypothalamic nucleus
*	0.24	Orbital area, ventrolateral part
	0.24	Cortical amygdalar area, posterior part
	0.24	Piriform area
	0.24	Infralimbic area
	0.24	Hypothalamic medial zone
	0.23	Nucleus accumbens
	0.23	Basolateral amygdalar nucleus, ventral part
	0.23	Striatum-like amygdalar nuclei
	0.23	Entorhinal area, lateral part
	0.22	Basomedial amygdalar nucleus, posterior part
	0.22	Posterior parietal association areas
	0.22	Fundus of striatum
	0.22	Caudoputamen
	0.21	Posterior intralaminar thalamic nucleus
	0.21	Dorsal peduncular area
	0.21	Postsubiculum
	0.21	Prelimbic area
	0.20	Orbital area, medial part
	0.20	Orbital area, lateral part
	0.19	Retrosplenial area, dorsal part
	0.18	Agranular insular area, ventral part
	0.18	Retrohippocampal region
	0.17	Dorsal cochlear nucleus
	0.16	Thalamus
	0.16	Anterior cingulate area, dorsal part
	0.16	Anterior cingulate area, ventral part
	0.15	Perirhinal area
	0.14	Entorhinal area, medial part
	0.13	Thalamus, polymodal association cortex related
	0.13	Midline group of the dorsal thalamus
*	0.13	Mediodorsal nucleus of thalamus

Engram Index

Brain regions that have an increase in activation levels during encoding as compared to home cage and don't have much difference between encoding and recall activation levels are placed highest on the engram scale.

To the scientists surprise, they found some regions with a higher recall activation than compared to memory encoding. They believe that this is because of some **unknown recall enhancing process.**



07.

Discussion

Summary

The engram index is based on the concept that engrams are held by neuronal ensembles that are activated by learning and are reactivated to support recall. The rain-wide engram mapping experiments revealed that certain brain regions exhibit higher recall activation as compared to CFC memory encoding. While there have been previous studies on brain mapping, this study permits a more complete map that shows the different roles that different parts of the brain play in memory and provides more support for the hypothesis.

Future Research

Future studies can, using the extensive brain mapping of this study, generate a more extensive map of engram cell ensembles that includes the identification of their functional connectivity as well as the mnemonic functions of individual ensembles.

Although this study provides evidence supporting the concept that a memory is stored in a functionally connected engram ensembles' complex distributed broadly across the brain, consistent with Semon's unified engram complex hypothesis, it would be interesting to see studies that either contradict, or prove a different hypothesis.

As mentioned earlier, the engram index was flawed so future research or replication could rectify that.

Thank You