Mechanisms that link memories across time
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Types of Associations

Temporally overlapping stimuli: Hebbian mechanisms

Stimuli hours/days apart: *Unknown mechanisms*
The Allocate to Link Hypothesis

Summary

- Part I: Summary CREB, CCR5, Excitability and Memory Allocation
- Part II: Allocation and Linking of Memories
- Part III: Memory linking and aging
CREB is required for memory

- modulates transcription during learning
- stabilizes plasticity
- stabilizes representations (i.e. place cells)
- stabilizes memory

CREB modulates memory allocation

- Increases in CREB enhance excitability and the probability that a given cell will be involved in memory
- Decreases in CREB have the opposite effect

Han et al. (2007). Science 316(5823): 457-460
Increases in CREB enhance memory allocation and therefore may open the window of memory linking: IEG studies

Sheena Josselyn

Increases in CREB enhance memory allocation and therefore may open the window of memory linking: **Inactivation studies**

* Silva et al, Science, 2009
Increases in CREB enhance memory allocation and therefore may open the window of memory linking: **Activation studies**

**ChR2 Activation**

![Chart showing freezing percentages](chart.png)

**CS then US**

*Silva et al, Science, 2009*
*Rogerson, et al., Plos One 2016*
Increases in CREB enhance memory allocation and therefore may open the window of memory linking: Activation studies

ChR2 Activation

![Bar chart showing freezing percentages for different conditions]

- **CS then US**
- **US then CS**

*Silva et al, Science, 2009*
*Rogerson, et al., Plos One 2016*
CCR5 negatively regulates CREB activation

Transgenic Expression

KO Mouse

Miou Zhou
Delayed increases in the expression of CCR5 and its ligand CCL5 following learning may close the window for memory linking.

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**CCL5**

![CCL5 mRNA expression graph](image)

**CCR5**

![CCR5 mRNA expression graph](image)
CCR5 modulates memory allocation and therefore may also modulate memory linking: IEG studies
The Allocate to Link Hypothesis: Excitability

CREB-dependent increases in neuronal excitability enhance memory allocation and may open the window for memory linking.
Increases in neuronal excitability open the window of memory allocation

Volvox ChR1 Activation

SFO Activation
Before Training

Balaji Jayaprakash and Thomas Rogerson
Increases in neuronal excitability open the window of memory allocation.
Linking memories across time

Denise Cai  Daniel Aharoni  Tristan Shuman  Justin Shobe
Memory Allocation ➡ Memory Linking

• **Cellular imaging** of co-allocation of memories close in time

• **Behavioral evidence** of linking of memories close in time

• **Aging** decreases CREB, increases CCR5, decreases neuronal excitability, disrupts co-allocation and consequently linking of memories

• **Rescuing experiment**: increases in excitability and decreases in CCR5 rescue linking of memories in aged mice

Increased CREB activation in CA1 5h, but not 7 days after spatial exploration.
Increase in excitability in CA1 activated cells after spatial exploration
Imaging linked CA1 ensembles

Miniscopes - detect calcium transients in freely behaving animals

TetTag system - allows “snapshots” of active cells at 2 time points
Long-term dynamics of CA1 hippocampal place codes

Yaniv Ziv\textsuperscript{1,5}, Laurie D Burns\textsuperscript{1,5}, Eric D Cocker\textsuperscript{1}, Elizabeth O Hamel\textsuperscript{1}, Kunal K Ghosh\textsuperscript{2}, Lacey J Kitch\textsuperscript{1}, Abbas El Gamal\textsuperscript{2} & Mark J Schnitzer\textsuperscript{1,3,4}
In vivo calcium imaging with Miniscopes

miniscope.org

Daniel Aharoni
miniscope.org: an open wiki source platform

Main Page

UCLA MINIScope Resource
Welcome to the MINIScope Wiki site. We are currently in the process of updating all the content of the site to the newest version of our system. If you are interested in gaining access please leave your information here and we will update you when the site becomes open (by January 1st, 2016).

Overview [edit]
Our miniature fluorescence microscope uses wide-field fluorescence imaging to record neural activity and network structure in awake, freely moving mice. Overview of system

Links to information on miniscope subsystems [edit]
- Head Mounted Scope
- Data Acquisition Box
- Data Acquisition Software
- Analysis Package

Guides and Tutorials [edit]
We designed our miniscope system to be easy to build and use. The 3 guides below will walk you through component procurement, scope assembly, and software installation.

1. Overview of System Components
2. Part Procurement
3. System Assembly
4. Software and Firmware Setup

Once you have built your system. This tutorial will explain how to use the different features of the scope.

Using Your Miniscope System For The First Time
New Miniscopes

- Wireless miniscope
- Liquid lenses (electrowetting) miniscope
- Optogenetic miniscope
- Light Field miniscope (with Vaziri lab)
- Silicon Probe miniscope (With Masmanidis Lab)
- Field Programmable Gate Array Miniscope (Cong lab)
Example CA1 place fields (GCAMP6f)
Co-allocation of memory ensembles acquired close in time may underlie memory linking: miniscopes

Inject Virus

Implant GRIN lens

Explore A

Explore B

Explore C

7d

5h

Overlapping Ensemble

Ensemble A \cap C

Ensemble B \cap C

Co-allocation of memory ensembles acquired close in time may underlie memory linking: miniscopes

Co-allocation of memory ensembles acquired close in time may underlie memory linking: miniscopes

Order Counterbalanced

Explore A  7d  Explore B  5h  Explore C  2d  Image C  2d  Image B  2d  Image A  2d

<table>
<thead>
<tr>
<th>Overlapping Ensemble (%) reactivated</th>
<th>5h</th>
<th>7d</th>
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<tbody>
<tr>
<td></td>
<td>20</td>
<td>10</td>
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</table>

*
Constant number of active cells across recording sessions

Explore A → 7d → Explore B → 5h → Explore C

Active Ensemble (#active cells/mouse)

A: 600 ± 100
B: 600 ± 100
C: 500 ± 100
Cell activity inclusion criteria does not alter analyses of cell ensemble overlap.
Imaging Co-Allocation With Mayford’s TetTag System

- **c-fos** → tTA
  - **On Dox**: TetO → HiGFP
  - **Off Dox**: TetO

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Expression Level

- **On Dox**: HiGFP
- **Off Dox**: ZIF

- **On Dox**: HiGFP
- **Off Dox**: ZIF

Legend:
- HiGFP
- ZIF (IHC)
Imaging Co-Allocation With Mayford’s TetTag System

<table>
<thead>
<tr>
<th>On Dox</th>
<th>Off Dox</th>
<th>On Dox</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="active_cells_a.png" alt="Explore A" /></td>
<td><img src="active_cells_a.png" alt="Explore A" /></td>
<td><img src="active_cells_b.png" alt="Explore B" /></td>
</tr>
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<td><img src="active_cells_a.png" alt="Explore A" /></td>
<td><img src="active_cells_a.png" alt="Explore A" /></td>
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<td><img src="active_cells_b.png" alt="Explore B" /></td>
</tr>
</tbody>
</table>

Active Cells in A (HiGFP)
Active Cells in B (ZIF)
Total Cells (DAPI)

overlapping cells = co-allocation
Co-allocation of memory ensembles acquired close in time

Neural Ensemble A & B (HiGFP & ZIF)
Similar CA1 cell activation in the 5 hr and 7 day experiments.
Parallel time course of increases in CCR5 expression and decreases in ensemble overlap.
CCR5 KO extends the window of co-allocation of CA1 memory ensembles
CCR5 KO extends the window of coallocation of CA1 memory ensembles

Explore A

Explore B

Explore C

Order Counterbalanced

Co-allocation Index (% Reactivated)

5 hr WT

5 hr CCR5 KO

7 d

2d

2d

2d

2d

2d

Image A

Image B

Image C
The Allocate to Link Hypothesis

Behavioral evidence that two memories can be linked when separated by 5 h, but not by 7 days.
Behavioral evidence that two memories can be linked when separated by 5 h, but not by 7 days.
Behavioral evidence that two memories can be linked when separated by 5 h, but not by 7 days.
Behavioral evidence that two memories can be linked when separated by 5 h, but not by 7 days.
CCR5 extends the window for memory linking.
Parallel time course of memory ensembles overlap and memory linking.
Parallel time course of increases in CCR5 expression and decreases in memory linking.

**CCR5 expression**

- Graph showing CCR5 mRNA expression (fold of naive) over time (naive, 3h, 6h, 12h, 24h, 48h, 7d).

**Memory linking**

- Graph showing freezing percentage over time (5h, 8h, 1d, 2d, and 7d).
Aging, CCL5/CCR5, CREB, excitability, and memory linking

- Aging increases CCL5/CCR5 levels, decreases cAMP/CREB signaling and neuronal excitability in CA1
- Therefore, aging should disrupt memory linking. Decreases in CCR5 and increases in excitability could reverse the linking deficits
Coallocation of CA1 memory ensembles in young but not older mice: miniscopes

Coallocation of CA1 memory ensembles in young but not older mice: TetTag approach

Behavioral evidence of linking of memories in young but not in older mice


Denise Cai
Behavioral evidence of linking of memories in young but not in older mice

CCL5 (CCR5 ligand) expression is dramatically increased in older mice.
CCR5 mutation *rescues* memory linking deficits in older mice
CCR5 mutation **rescues** memory linking deficits in older mice

Miou Zhou
“Rescuing” excitability with DREADD rescues memory linking in older mice

Artificial activation with the DREADD system
“Rescuing” excitability with DREADD rescues memory linking in older mice

Competition between engrams influences fear memory formation and recall

Asim J. Rashid,1,2,3,4 Chen Yan,1,2,3,4 Valentina Mercaldo,1,2,3,4 Hwa-Lin (Liz) Hsiang,1,2,3,4 Sungmo Park,1,2,3,4 Christina J. Cole,1,2,3,4 Antonietta De Cristofaro,1 Julia Yu,1 Charu Ramakrishnan,5 Soo Yeun Lee,5 Karl Deisseroth,5 Paul W. Frankland,1,2,3,4* Sheena A. Josselyn1,2,3,4*

Collections of cells called engrams are thought to represent memories. Although there has been progress in identifying and manipulating single engrams, little is known about how multiple engrams interact to influence memory. In lateral amygdala (LA), neurons with increased excitability during training outcompete their neighbors for allocation to an engram. We examined whether competition based on neuronal excitability also governs the interaction between engrams. Mice received two distinct fear conditioning events separated by different intervals. LA neuron excitability was optogenetically manipulated and revealed a transient competitive process that integrates memories for events occurring closely in time (coallocating overlapping populations of neurons to both engrams) and separates memories for events occurring at distal times (disallocating nonoverlapping populations to each engram).
Memories are not stored in isolation from other memories but are integrated into associative networks. However, the mechanisms underlying memory association remain elusive. Using two amygdala-dependent behavioral paradigms—conditioned taste aversion (CTA) and auditory-cued fear conditioning (AFC)—in mice, we found that presenting the conditioned stimulus used for the CTA task triggered the conditioned response of the AFC task after natural coreactivation of the memories. This was accompanied through an increase in the overlapping neuronal ensemble in the basolateral amygdala. Silencing of the overlapping ensemble suppressed CTA retrieval-induced freezing. However, retrieval of the original CTA or AFC memory was not affected. A small population of coshared neurons thus mediates the link between memories. They are not necessary for recalling individual memories.
The Allocate to Link Hypothesis

The Allocate to Link Hypothesis: **open questions**

- What is the critical cell type for CCR5’s role in memory linking? Neurons? Microglia?
- Is there a role for CCR5 in microglia in memory linking?
- What other mechanisms modulate the open and closure of the memory linking window?
- etc., etc. etc.
Collaborators

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UCSD
Jeremy Biane
Mark Tuszyński

NIMH, NIA, NINDS, Adelson Medical Research Foundation
CNO does not increase anxiety in aged mice