Reactivation of Cells in the Mice with Engram-specific Deletion of the de novo DNA Methyltransferase *Dnmt3a*

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Introduction

Memory formation is a fundamental aspect of human cognition, enabling us to learn from past and adapt to future while also shaping our behaviors, defining who we are (Josselyn et al., 2020). In the process of memory formation, DNA methylation - a chemical process that modifies our genetic material – was discovered to have a critical role in memory engram cells, the neurons responsible for storing memories (Yu et al., 2011). However, the specific molecular processes that underpin memory formation and consolidation are still not fully understood.

To shed light on this area, several studies have suggested that DNA methylation is crucial in the regulation of gene activity during memory formation and consolidation, and its impairment can lead to memory deficits. (Liu et al., 2009).

Building on these findings, we seek to explore how deactivating DNA methylation enzymes in neurons activated during fear conditioning (a memory paradigm) affects the formation and retrieval of fear memories.

Moreover, we are focusing on the basolateral amygdala complex (BLA), one of the critical sites along with hippocampus that are potentially storing fear conditioning memory, which are also named regions that contain memory engram cells. As the role of DNA methylation in the BLA for storing fear conditioning memory is unclear compared to the hippocampus. Our study here is examining the role of DNA methylation in learning and memory, specifically examining the BLA.

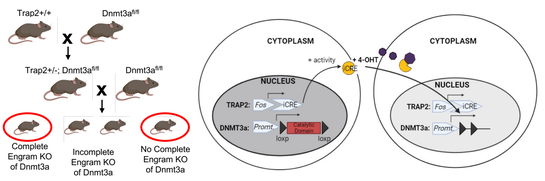
Hypotheses

Previously we performed Dnmt3a knockout in memory engram cells by combining mice strains Trap2 and Dnmt3a$^{fl/fl}$. From this experiment, we demonstrated that mice with memory engram-specific knockout of Dnmt3a showed decreased freezing in both auditory and contextual fear conditioning memory retrieval tests extending from a week to a month. This indicates that learning-activated Dnmt3a expression is required for formation and consolidation of fear conditioning memory.

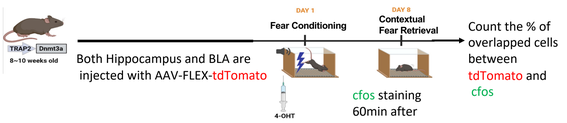
Because Denny et al. 2016 showed that performance in memory retrieval is correlated with the number of reactivated cells during the retrieval, we hypothesize that the impaired memory retrieval performance in engram-specific Dnmt3a knockout mice is due to reduced overlap between cells activated during both fear conditioning learning and fear memory retrieval.

Methods

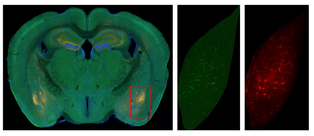
Conditional knockouts of Dnmt3a by cross breeding Trap2 mice (containing tamoxifen-inducible Cre recombinase) and Dnmt3a$^{fl/fl}$ to delete Dnmt3a in neurons activated during learning.



Fear Conditioning and Cell Labeling

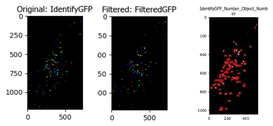


Data Analysis: Cell Counting Pipeline



a. Crop regions of interests for each mouse in Fiji

b. Train for cell identification via pixel classification in ilastik



c. Using ilastik output to perform Cell count for cfos+/tdT+ cells in CellProfiler

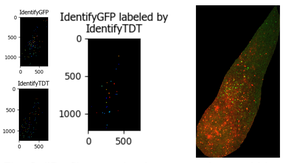
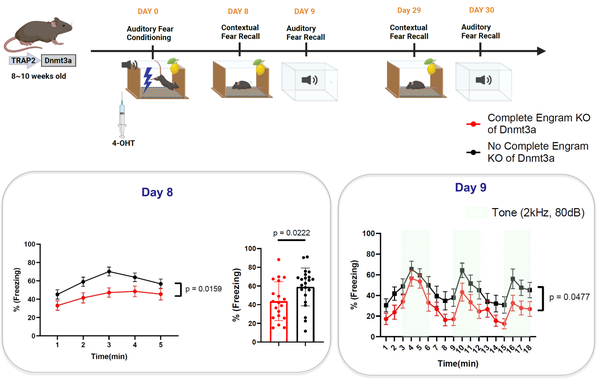


Fig. CellProfiler, a trained automated centroid-based program determining cells in cFos+ and tdT+ images then overlapping the two.

d. Use customized code to extract cell counts from CellProfiler output

Results

Previous results showing impaired memory retrieval in mice with engram-specific knockouts of Dnmt3a:



Current results on counting percentage of fear memory reactivated cells:

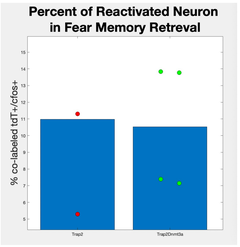


Fig. The average number of cells retained in individual mice, and two bars comparing the averages of cells retained in mice with the Trap2 gene and Trap2Dnm3a gene.

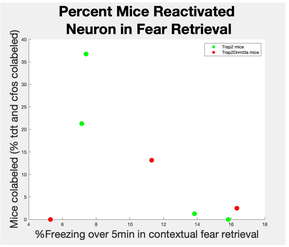


Fig. Correlation graph of % mice freezing during fear retrieval and cells activated

- The study compares the control mice (Trap2, without Dnmt3a conditional knockout in fear conditioning-active cells) and the experimental mice (Trap2Dnm3a, with Dnmt3a conditional knockout in fear conditioning-active cells).
- tdT+ represents fear conditioning learning active cells, and cFos+ represents fear memory retrieval active cells.
- The cells that are both cFos+ and tdT+ represent being activated during fear conditioning learning and fear memory retrieval.
- The 3 control mice had an average of 10.980% and ±4.521SD cells that are activated in retrieval that are also activated in learning.
- The 4 experimental mice had an average of 10.536% and ±3.266SD cells that are activated in retrieval that are also activated in learning.
- There is a weak negative correlation between memory retrieval performance and co-labeled cell populations, with a correlation coefficient of -0.63005

Discussions

- The data remains inconclusive due to several factors.
- One factor relates to the mouse strain type. Trap2 has a genomic background of the wild type C57BL strain, which commonly exhibits less freezing behavior in our current fear conditioning paradigm.
- Conversely, Dnmt3a$^{fl/fl}$ mice has a genomic background of 129 strain, which commonly displays higher freezing in the same fear conditioning paradigm. Freezing behavior of the Trap2Dnmt3a mice in this paradigm shows consistently a reduction of freezing, matching our previous data.
- Therefore, due to the low freezing behavior in the Trap2 mice strain, these controls are not proper in comparison to the Trap2Dnmt3a mice.

Next Steps

- Incorporate proper control mice under the same 129 background that have no knockout of Dnmt3a and have Trap2 alleles to have consistent phenotypical behavior, leading to comparable cellular mechanisms.
- Incorporate 3D cellular imaging acquisition and thus counting can measure cell size and spatial distribution, improving the accuracy of cell counting. The current method which examines 2D image sets might result in miscounting because the cells that counted as one might not be on the same plane. .

References

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