様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

# 科学研究費助成事業　　研究成果報告書

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研究課題名（和文）Functional elucidation of m6A mRNA modification in spatial map formation in hippocampal CA1 pyramidal cells

研究課題名（英文）Functional elucidation of m6A mRNA modification in spatial map formation in hippocampal CA1 pyramidal cells

研究代表者

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交付決定額（研究期間全体）：（直接経費）　　2,200,000円

研究成果の概要（和文）：m6A機能が欠如した状態で、空間学習による場所細胞形成の細胞メカニズムを解明しました。具体的には、VR線形トラック課題を行うマウスの個々の興奮性ニューロンにおける樹状突起スパインの活動を観察しました。本研究は、VR課題と2pカルシウムイメージングを組み合わせる利点を実証し、特定の目標を達成しました。

研究成果の学術的意義や社会的意義
Our research with a cKO mouse model lacking YTHDF3 revealed key insights into spatial learning and memory. It highlighted m6A methylation's role in synaptic plasticity and suggested therapeutic targets. Integrating VR technology enhances experimental accuracy and innovation.

研究成果の概要（英文）：In this study, we used a conditional knockout (cKO) mouse model lacking the m6A reader protein YTHDF3 in excitatory neurons. An adeno-associated virus (AAV) with GCaMP6f, a green calcium fluorescence sensor, was introduced into the hippocampal CA1 to monitor neuronal activity. Two weeks post-injection, 3-month-old mice underwent optical window implantation. After a one-month recovery, the mice were placed on a Styrofoam treadmill under a two-photon microscope with a secured head plate. They performed virtual reality tasks on a linear track with water rewards. We monitored over 1,000 neurons in the hippocampal CA1 using in vivo two-photon (2p) calcium imaging. Running speed and licking behavior were recorded and matched with visual cues. We identified cellular mechanisms of spatial learning-induced place cell formation without m6A function, observing dendritic spine activity. This study demonstrated the benefits of combining VR tasks with 2p calcium imaging, achieving our research goals.

研究分野：Neurobiology

キーワード：m6A　YTHDF3　YTHDF1　Place cells　Calcium imaging　Two photon microscopy　Spatial memory　Virtual reality

１．研究開始当初の背景

N6-methyladenosine (m6A) is the most prevalent internal chemical modification of mRNA in the hippocampus. YTHDF3 contains a YTH domain that recognizes m6A sites on tagged mRNA. Binding of this reader protein to m6A sites regulates the translation efficiency and stabilization of target mRNA. In 2018, we identified 2,921 m6A-tagged transcripts localized at synapses (Merkurjev et al., Nat. Neurosci, 2018), highlighting m6A's crucial role in spine dynamics in response to behavioral experiences.

２．研究の目的

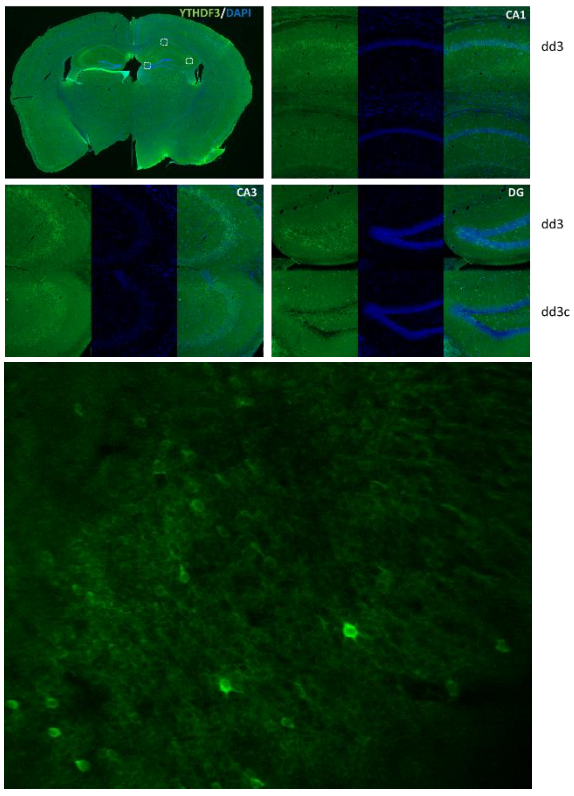
This study aims to elucidate how m6A/YTHDF functions regulate place cell activity induced by spatial learning, potentially impacting spatial mapping and cognitive processes. By understanding these mechanisms, we hope to uncover new therapeutic targets for cognitive disorders and advance our knowledge of m6A's role in synaptic plasticity.

３．研究の方法

In this study, the following techniques were employed:

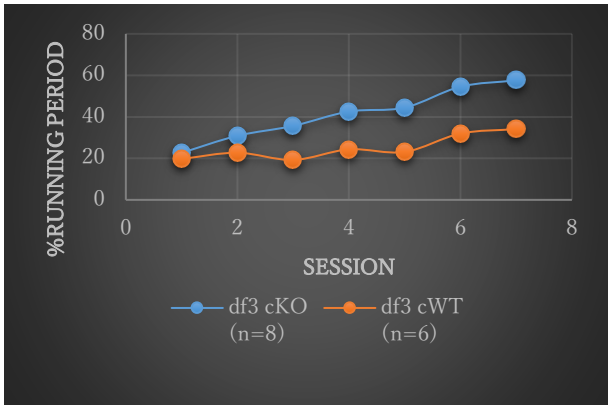
1. Conditional Knockout (cKO) Mouse Model: We generated a cKO mouse model where the m6A reader protein, YTHDF3, was deleted from excitatory neurons.
2. Adeno-Associated Virus (AAV) Delivery: AAV containing GCaMP6f, a green calcium fluorescence sensor, was introduced into the hippocampal CA1 region to monitor neuronal activity.
3. Optical Window Implantation: Two weeks post-injection, 3-month-old mice underwent optical window implantation to expose the hippocampus, followed by a one-month recovery period.
4. Head-Fixing and Virtual Reality (VR) Environment: After recovery, the mice were placed on a Styrofoam wheeled treadmill under a two-photon microscope. A head plate, implanted one day prior to window implantation, was secured to stands for head-fixing. The mice then performed VR environment tasks.
5. In Vivo Two-Photon (2p) Calcium Imaging: During VR task training sessions, the activity of more than 1,000 neurons in the hippocampal CA1 was observed using in vivo 2p calcium imaging.
6. Behavioral Task Monitoring: The VR task involved a linear track with water drops as a reward. Running speed and licking behavior at the reward port were measured and matched with visual cues at different locations on the track.
7. Analysis of Cellular Mechanisms: We delineated the cellular mechanisms underlying spatial learning-induced place cell formation in the absence of m6A function. This included observing dendritic spine activity in individual excitatory neurons during VR tasks.

４．研究成果




**Examples of YTHDF3 expressions between wild- type and conditional knockout mice.** By crossing flow mice with CAMKIIa-cre line, we successfully knocked out YTHDF3 from excitatory neurons.

**Transduction of CAMKIIa-GCAMP6 into the CA1 subregion**

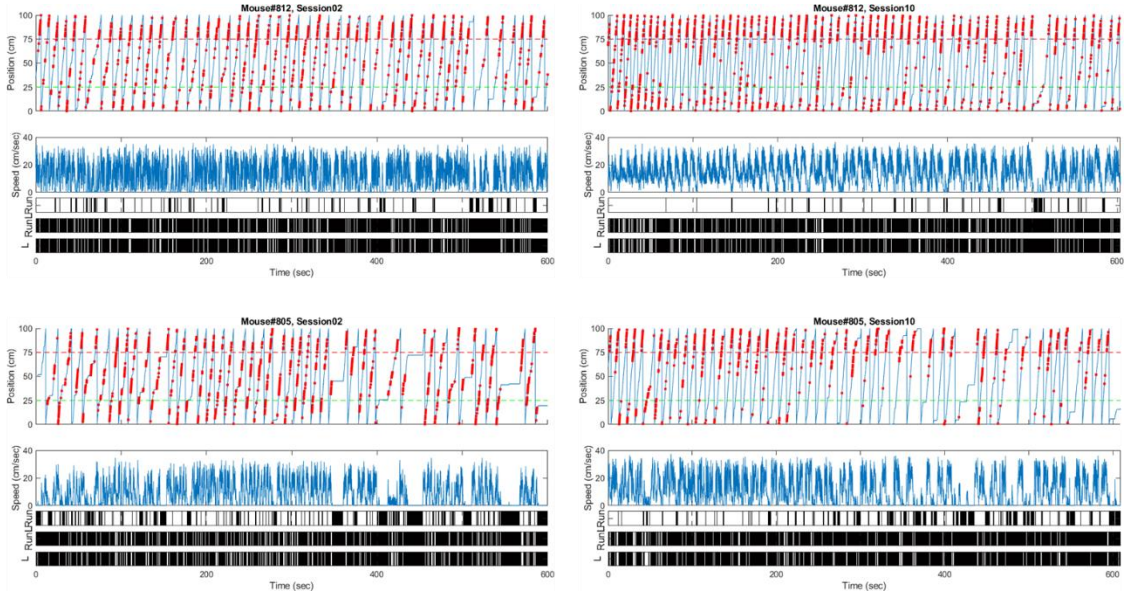



**The effect of *Ythdf3* depletion on running period during habituation.** Here we found that cKO mice exhibited a lower percentage of running period in comparison to WT during habituation session.

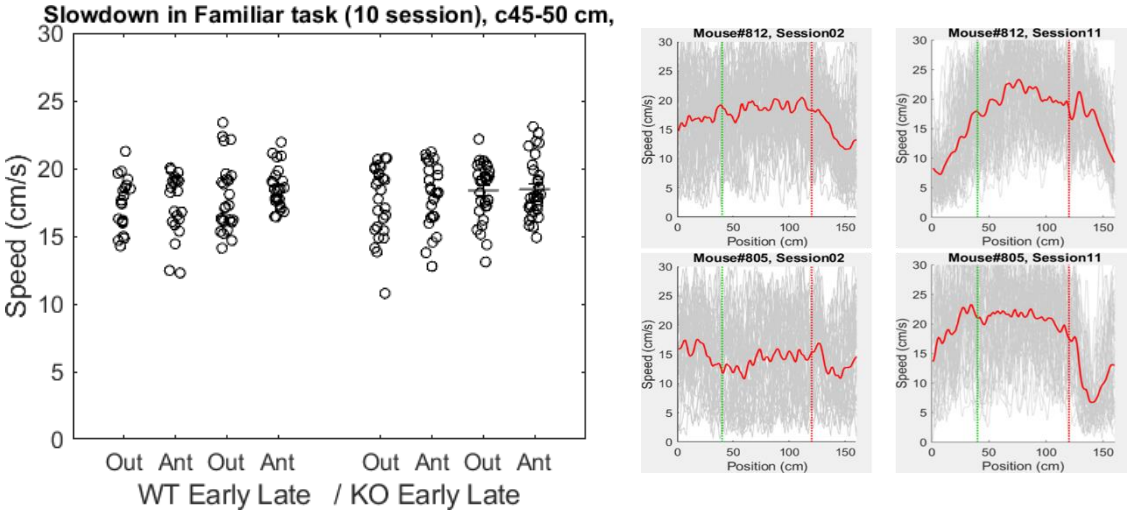
**Licking behavior of the cKO mice.** There is no difference in the pattern of licking behavior between the two genotypes.

WT

cKO




**The effect of YTHDF3 deletion on task engagement and reward anticipation during familiar VR.** cKO performed the task as good as WT control.




In conclusion, the depletion of YTHDF3 in excitatory neurons affects the learning ability during acclimatization. However, it does not interfere with the spatial memory formation. Since we only knocked out YTHDF3 from excitatory neurons, testing different cell types might help better understand the role of YTHDF3 in spatial learning and memory.

５．主な発表論文等

〔雑誌論文〕　計0件

〔学会発表〕　計1件（うち招待講演　0件／うち国際学会　1件）

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| １．発表者名<br>Sarayut Phasuk |
| ２．発表標題<br>2-photon Calcium Imaging of Hippocampal Neuronal Circuits in Awake Head-Fixed Mice Navigating a Virtual Reality Environment |
| ３．学会等名<br>Functional Genomic in Future（国際学会） |
| ４．発表年<br>2022年 |

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

| | 氏名<br>（ローマ字氏名）<br>（研究者番号） | 所属研究機関・部局・職<br>（機関番号） | 備考 |
|---|---|---|---|

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

| 共同研究相手国 | 相手方研究機関 |
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