

Exploring the specific relationship between optogenetic activation of SuM-induced behavioural changes in AHNs and microglia for cognitive enhancement in mice

Chaojian Shang

Life Science, Tongji University, Shanghai, 200000, China

lethenesis@qq.com

Abstract. Patients with Alzheimer's disease (AD) exhibit progressive memory loss, depression, and anxiety, accompanied by impaired adult hippocampal neurogenesis (AHN). Recent studies have pointed out that stimulation of the suprachiasmatic nucleus of the hypothalamus (SuM) enhances AHN in two different AD mouse models (53FAD and 33Tg-AD), and additional chemical gene activation of SuM enhances adult new-born neurons (ABNs), restoring memory and emotional deficits in these AD mice. However, the exact cause of the improvement in cognitive performance in mice is not clear, as there is an alteration in the behaviour of microglia during activation of SuM and ABNs. We therefore designed experiments in the hope of experimentally verifying that the cognitive enhancement in mice comes directly from the altered activity of the AHN. And two other experiments were designed to test whether the altered microglia behaviour resulting from activation of SuM would have some effect on cognitive performance. The last experimental design then attempted to test whether the cognitive enhancement resulting from optogenetic activation of SuMs and chemical activation of ABNs was long-term.

Keywords: Alzheimer's Disease, Supra-mammillary Nucleus, Adult-born Neurons, Microglial Activation, Function.

1. Introduction

Alzheimer's disease (AD) is a complex neurodegenerative disorder with two main pathological mechanisms: the accumulation of β -amyloid ($A\beta$) plaques in the brain and the formation of neuroprogenitor fibre tangles (NFTs) due to hyperphosphorylation of tau protein. These pathological processes lead to neuronal dysfunction, especially synaptic dysfunction, which triggers severe cognitive and behavioural impairments. In addition, neuroinflammation and oxidative stress play an important role in the development of the disease. Previous studies have shown that activation of the supraoptic nucleus of the hypothalamus (SuM) significantly enhances the function of adult neurons (ABNs) and improves cognitive and emotional function in animal models. Li et al. showed that optogenetic activation of SuM in an Alzheimer's disease model significantly increased the number and maturation of ABNs thereby improving cognitive and emotional functions. Changes in the physiological characteristics of microglia were detected. And microglia play a key role in clearing amyloid plaques and regulating brain inflammation. However, the specific relationship between activation of SuM, activation of SuM-modified ABNs and altered fractional cognitive abilities, and altered microglia behaviour is not clear.

2. Research Objectives and Hypotheses

2.1. Objectives

This study aims to delve deeper into the mechanisms by which SuM-enhanced ABN activation improves cognitive and emotional functions in AD mice. Specific objectives include:

- 1.To confirm the direct effects of SuM-enhanced ABN activation on cognitive and emotional functions in AD mice.
- 2.To explore the impact of SuM-enhanced ABN activation on microglial cell functions and their role in cognitive and emotional improvements.
- 3.To assess the long-term effects of SuM-enhanced ABN activation

2.2. Hypotheses

Hypothesis 1: Altered cognitive performance in mice was directly caused by AHNs, independent of microglia behaviors.

Hypothesis 2:The improvement in cognitive performance in mice was indirectly due to the modulation of microglia by AHNs.

Hypothesis 3: Optogenetic activation of SuM and chemical activation of SuM-modified ABNs produces long-term effects.

3. Experimental Design and Methods

3.1. Experiment One: Verifying the Effects of ABN and Microglial Cell Activation

3.1.1. Overview

Recent findings suggest that the supramammillary nucleus (SuM) activation enhances the function of ABNs leading to improved cognitive and emotional outcomes in mice. However, the precise mechanisms. Whether these are direct effects of ABN activation or indirectly influenced by changes in microglial cell behavior remain unclear. This experiment aims to dissect the individual contributions of ABNs and microglial cells by selectively inhibiting each and observing the resultant behavioral changes.

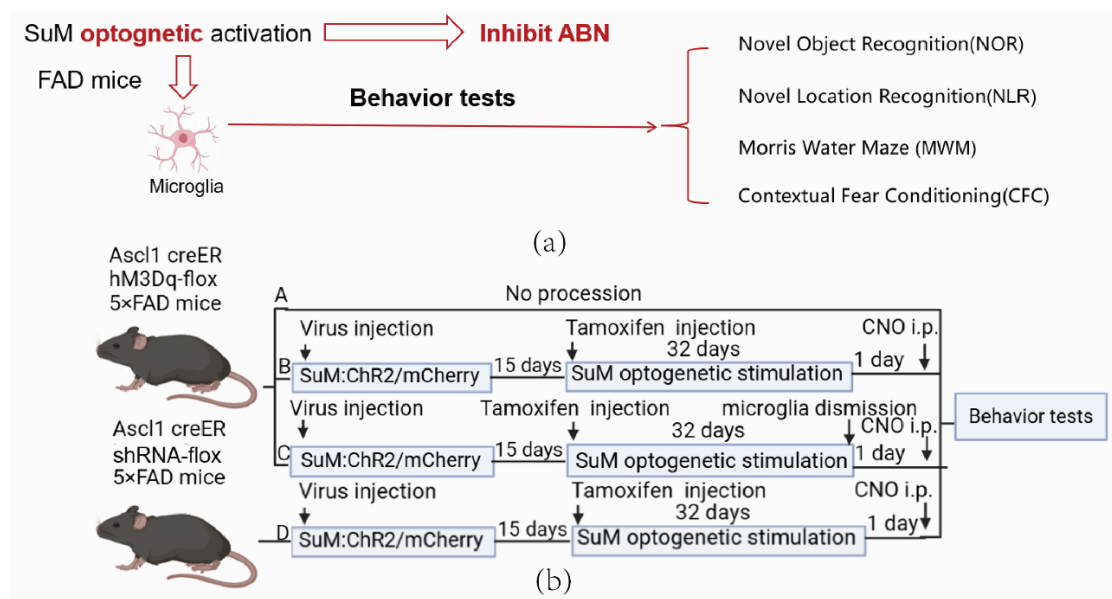


Figure 1. Experimental flow chart

In order to test the validity of the above hypothesis, we did the following experimental design. (a)Experimental Flow Design for Hypothesis 1. (b)Experimental Flow Design for Hypothesis 2.

3.1.2. Model Description

Model Used: The Familial Alzheimer's Disease (FAD) mouse model will be utilized for this series of experiments. This model is genetically predisposed to develop Alzheimer's disease-like symptoms early in life, making it ideal for studying the pathological and behavioral changes associated with AD.

Relevance: Using this model allows for a controlled analysis of therapeutic interventions and mechanistic explorations in a system that closely mimics human disease progression. The FAD mouse model expresses mutations that are directly linked to familial forms of Alzheimer's disease, providing a relevant context for assessing the impact of ABN and microglial manipulations.

3.1.3. Microglial Cell Inhibition

Microglia survival is dependent on colony-stimulating factor 1 receptor (CSF1R) signalling. the CSF1R inhibitor PLX3397 effectively induces microglia elimination in model mice. A 99% inhibition rate can be achieved by precisely controlling the addition of PLX3397 to the feed fed to mice, precluding interference from microglia production.

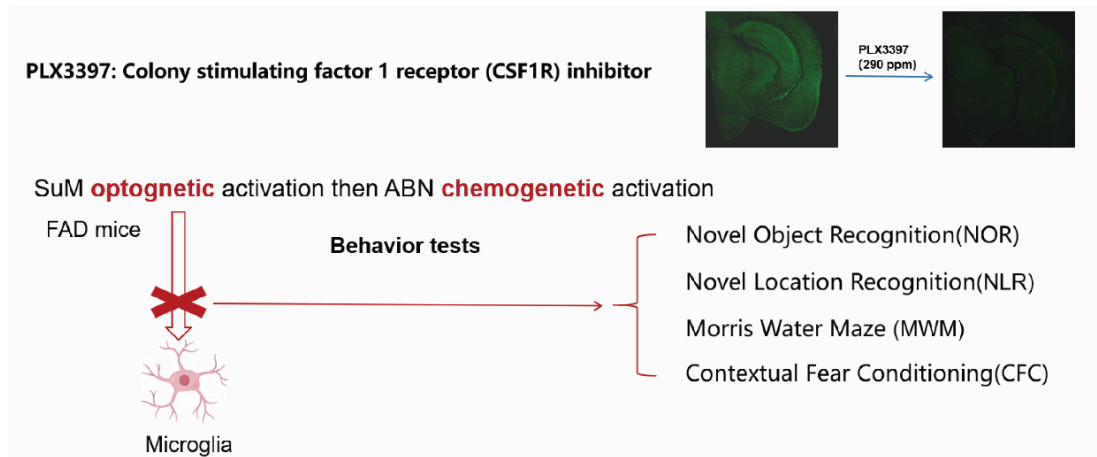


Figure 2. Microglia blocking experimental design

3.1.4. ABN Inhibition

Since all conventional approaches only produce a certain degree of inhibition of AHNs. In order to completely eliminate the possible interference of AHNs, we decided to use the CRE-loxP system, where the unique NSC promoter of neural stem cells is able to express CRE proteins, which are activated after tamoxifen injection into mice, shear loxp sequences, and shRNAs are expressed to achieve the interference of cyclinE.

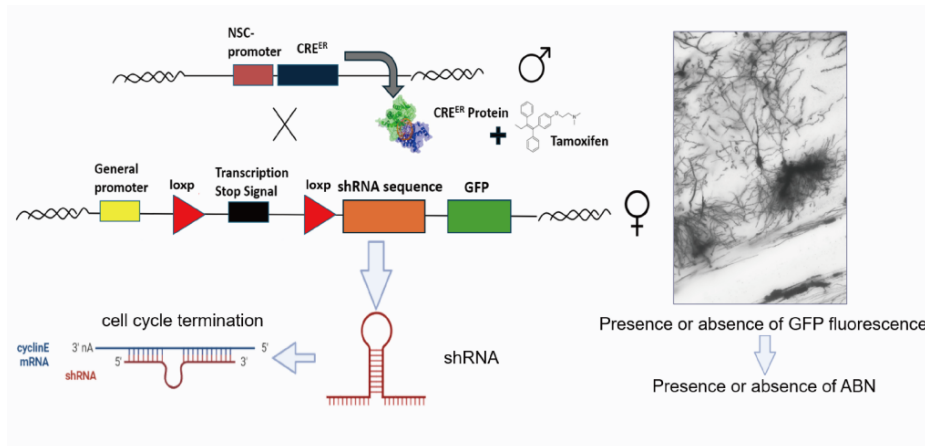


Figure 3. Experimental design for blocking ABN

3.1.5. Experimental Groups

Group A (Control): No treatment to model

Group B (Blocking ABNs): ABNs were blocked to assess the effect of their absence on cognitive and emotional functioning.

Group C (Blocking Microglia): Inhibit microglia to assess the effects of their inactivation

Group D (Positive Control): Both SuM and ABNs stimulated without any blockade to serve as a positive control showing the optimal outcome of ABN and SuM activation.

3.1.6. Behavioral Tests

Tests Used: Novel Object Recognition (NOR), Novel Location Recognition (NLR), Morris Water Maze (MWM), and Contextual Fear Conditioning (CFC).

Expected Outcomes: It is hypothesized that Groups B and C will show differential performances compared to the control groups, with Group B expected to perform poorly in cognitive tests due to the lack of ABN activity, whereas Group C might show intermediate results, suggesting indirect roles of microglia in cognitive and emotional functions.

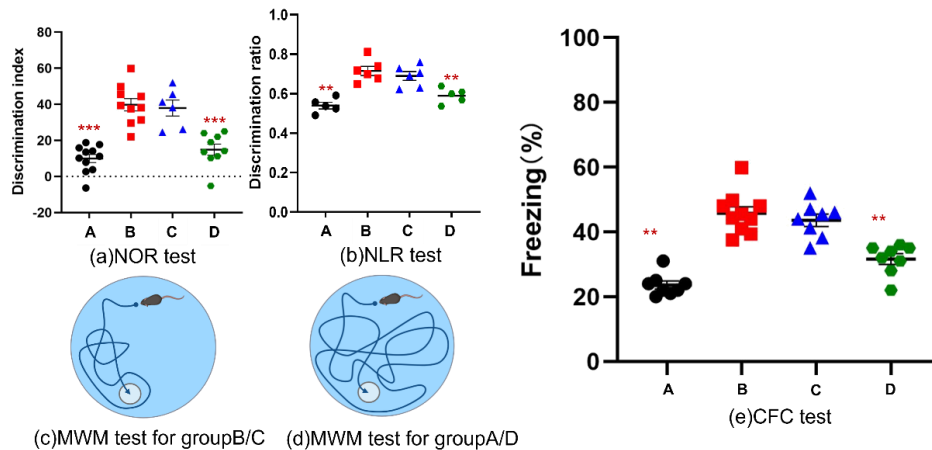


Figure 4. Expected Outcomes

3.1.7. Analysis

Cognitive Assessment: Using Discrimination Index (DI) and Discrimination Ratio (DR) in the NOR test to evaluate cognitive abilities.

Memory and Learning: Performance in the MWM and CFC tests will measure spatial memory and fear-associated learning, respectively, with expectations set for Groups B and C to delineate the contributions of ABNs and microglia.

3.2. Experiment Two: Analysis of Microglial Cell Activation Mechanisms

3.2.1. Overview

The previous research has shown that ABNs can activate microglia and enhance their phagocytic activity. However, studies also demonstrate that in the pathological mechanism of Alzheimer's Disease (AD), on one hand, microglia can be activated by A β plaques and exert their phagocytic activity by clearing A β and damaged cells. Microglia can also induce the clearance of A β by releasing proteases that degrade A β , such as insulin-degrading enzyme and plasminogen. Therefore, this experiment aims to investigate whether microglia are activated by ABN or by A β plaques in the context of SuM activation.

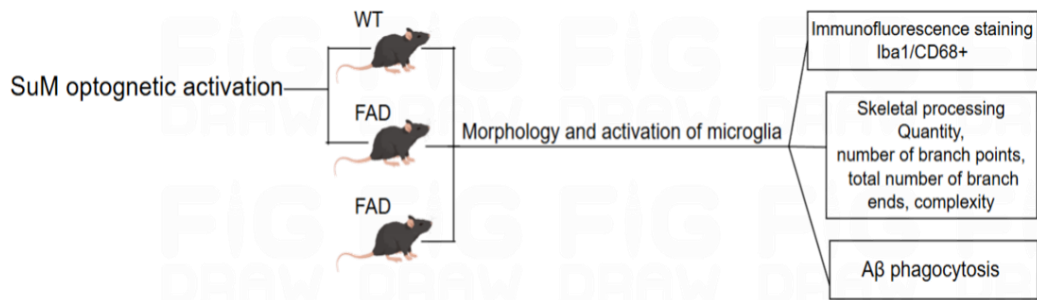


Figure 5. Experimental flow chart

3.2.2. Hypotheses Formulation

Hypothesis 1: Microglia is only activated by Aβ and is not related to the activation of SuM.

Hypothesis 2: Microglia are activated by both Aβ and SuM, and the degree of simultaneous activation of both is higher than that of activation by Aβ alone.

3.2.3. Experimental Setup

The experimental design involves four groups of mice:

Groups A and D (Wild-type mice): Group A undergoes SuM activation, whereas Group D remains inactive.

Groups B and C (FAD mice): Both groups undergo SuM activation. Group B has SuM activation while Group C does not. This setup allows for the assessment of microglial activation states under different experimental conditions.

3.2.4. Microglial Activation Analysis

Immunofluorescence Staining: Using IBA1 as a marker for microglia, and CD68 as a marker for microglial activation and phagocytosis. The staining process will capture images of microglia under confocal fluorescence microscopy to analyze their activation and phagocytic states.

Morphological Analysis: The activation of microglia is mainly reflected in their morphological changes from a highly branched resting state to an enlarged, amoeboid activated state. We will use ImageJ software coupled with the NeuroJ plugin to analyze the quantity, number of branch points, total number of branch ends, and the complexity of microglia to determine their activation status.

3.2.5. Phagocytic Capability Assessment

The phagocytic ability of microglia will be evaluated using a method where DAPI is used to determine the range of cytoplasm, and then the range of Aβ plaques is determined. The number of Aβ in each cell is calculated as follows:

$$\text{Number of A}\beta \text{ per cell} = (\text{Average number of Spot} \times \text{Number of cytoplasm}) / \text{DAPI number}.$$

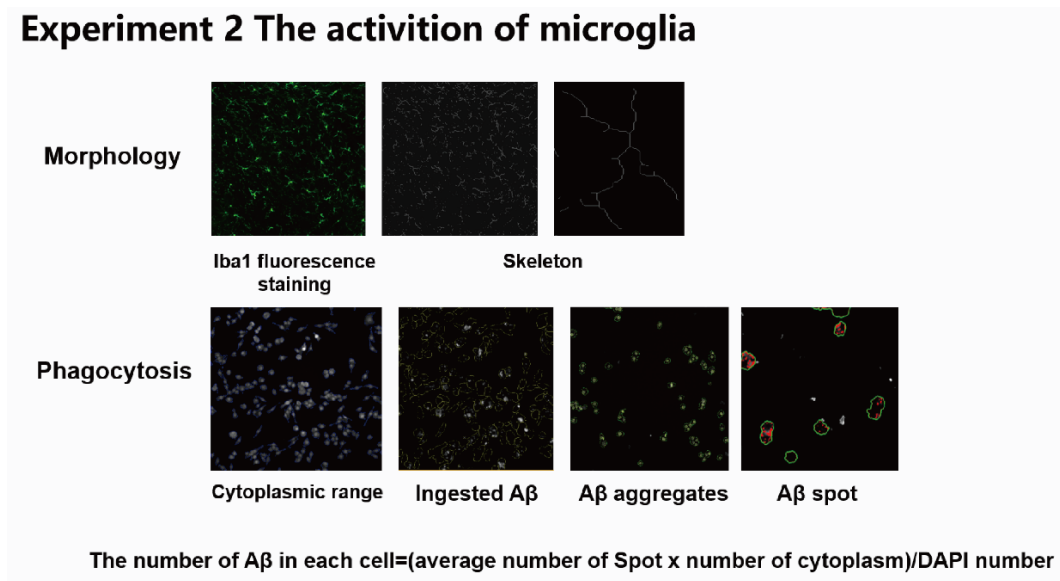


Figure 6. Morphological features of activated microglia.

3.2.6. Expected Results

If the microglia in the WT group (Group A) are not activated after activating SuM, this indicates that only Aβ activates the microglia.

If both WT and FAD groups show microglial activation after SuM activation, this indicates that microglia are activated by both Aβ and SuM.

Comparisons between the activation degree and phagocytic ability of microglia in the FAD mice of Group B and Group C will indicate the relative activation potential of Aβ plaques and SuM on microglia.

Table 1. Expected Results

Expected result	1	2	3
Activation of microglia	WT ×	WT √ FAD√	WT √ FAD√
Activation and phagocytosis	-	BC two groups comparable	Group B stronger than those of Group C
Conclusion	Only activated by Aβ	Both Aβ and SuM	Both Aβ and SuM Activation effect enhanced

3.3. Experiment Three: Assessing Long-Term Effects

3.3.1. Overview

This phase of the study focuses on investigating the long-term effects of SuM-modified ABNs on memory and emotional functions in AD mice. Previous phases have demonstrated that optogenetic activation of SuM followed by chemogenetic activation of ABNs in 5×FAD mice enhances microglial phagocytic activity and improves cognitive and emotional abilities. A critical question remains: Are these improvements long-lasting?

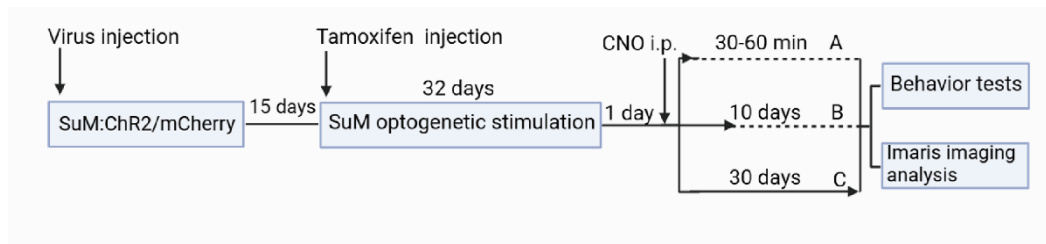


Figure 8. Experimental flow chart

3.3.2. Hypotheses Formulation

To address the longevity of these effects, we propose the following hypotheses:

Hypothesis 1: The function of SuM-modified ABNs on AD mice is temporary.

Hypothesis 2: The function of SuM-modified ABNs on AD mice has a long-term effect.

Hypothesis 3: The function of SuM-modified ABNs on AD mice decreases over time.

3.3.3. Experimental Setup

Model Preparation: We utilize severe *Ascl1 creER hM3Dq-flox 5×FAD* (AM3AD) mice, dividing them into three groups (A, B, and C). Each group is further subdivided, with half receiving ChR2 virus injections into SuM for activation, and the other half receiving mCherry virus injections as controls.

Activation Protocol: 15 days post-injection, all groups receive tamoxifen to activate the hM3Dq gene. SuM optogenetic stimulation is then conducted for 32 consecutive days. To mitigate any acute effects from SuM stimulation, chemogenetic activation via CNO is administered 1 day later, targeting SuM-modified or control ABNs.

3.3.4. Behavioral and Imaging Analysis

Behavioral tests and Imaris imaging analysis are conducted at three intervals post-activation: 30-60 minutes, 10 days, and 30 days. This timeline allows for a comprehensive assessment of both immediate and long-term changes in cognitive and emotional functions.

3.3.5. Expected Results

By comparing cognitive and emotional abilities and microglial phagocytosis improvements in ChR2 AM3AD mice versus mCherry AM3AD mice across the three groups:

If $A = B = C$: Indicates either a long-term effect of SuM-modified ABNs or that one month is too short to observe decline—supporting Hypothesis 1 or 2.

If $A > B > C$: Suggests that the function of SuM-modified ABNs on AD mice decreases over time—supporting Hypothesis 3.

If $A > B = C$ or $A = B > C$: Indicates that the function of SuM-modified ABNs is temporary—confirming Hypothesis 1.

Table 2. Expected Results

result	1	2	3
Cognitive and affective ability	$A=B=C$	$A>B>C$	$A>B=C$ or $A=B>C$
Microglia phagocytosis ability	$A=B=C$	$A>B>C$	$A>B=C$ or $A=B>C$
Conclusion	Hypothesis 1 or 2	Hypothesis 3	Hypothesis 1

Further Investigation: Should the first result pattern emerge, additional experiments will be planned to delay ABN activation to validate whether the function of SuM-modified ABNs on AD mice is indeed permanent.

4. Conclusion

It has been shown that activation of ABN and microglia directly improves learning, memory and

emotion regulation in AD mice. This finding highlights the potential of ABN and microglia as therapeutic targets for cognitive enhancement and mood stabilisation in Alzheimer's disease.

Understanding how ABN and microglia independently or together regulate cognitive function could lead to the discovery of new therapeutic targets. Investigating drugs that activate ABN or microglia offers promising alternatives to current therapies that focus primarily on immune clearance of A β , such as activated phagocytosis using antibodies that bind to A β .

Future research directions: We aim to investigate whether microglial activation can inversely affect the function of ABNs, providing a synergistic approach to improving neuroplasticity and cognitive function in AD models.

In addition, we plan to determine the specific effects of ABN maturation, number and activity on cognitive and emotional improvements. Analysis of neuronal dendritic spine density and number of synaptic branches by Golgi staining will serve as an indicator to assess whether activation of microglia can enhance the function of ABNs.

References

- [1] Selkoe, D.J. (2001). Alzheimer's disease: genes, proteins, and therapy. *Physiological Reviews*, 81, 741–766. <https://doi.org/10.1152/physrev.2001.81.2.741>
- [2] Selkoe, D.J. (2002). Alzheimer's disease is a synaptic failure. *Science*, 298, 789–791. <https://doi.org/10.1126/science.1074069>
- [3] Palmer, K., Berger, A.K., Monastero, R., Winblad, B., Bäckman, L., and Fratiglioni, L. (2007). Predictors of progression from mild cognitive impairment to Alzheimer disease. *Neurology*, 68, 1596–1602. <https://doi.org/10.1212/01.wnl.0000260968.92345.3f>
- [4] Moreno-Jiménez, E.P., Flor-García, M., Terreros-Roncal, J., Rábano, A., Cafini, F., Pallas-Bazarra, N., Avila, J., and Llorens-Martín, M. (2019). Adult hippocampal neurogenesis is abundant in neurologically healthy subjects and drops sharply in patients with Alzheimer's disease. *Nature Medicine*, 25, 554–560. <https://doi.org/10.1038/s41591-019-0375-9>
- [5] Tobin, M.K., Musaraca, K., Disouky, A., Shetti, A., Bheri, A., Honer, W.G., Kim, N., Dawe, R.J., Bennett, D.A., Arfanakis, K., and Lazarov, O. (2019). Human hippocampal neurogenesis persists in aged adults and Alzheimer's disease patients. *Cell Stem Cell*, 24, 974–982.e3. <https://doi.org/10.1016/j.stem.2019.05.003>
- [6] Terreros-Roncal, J., Moreno-Jiménez, E.P., Flor-García, M., Rodríguez-Moreno, C.B., Trincherro, M.F., Cafini, F., Rábano, A., and Llorens-Martín, M. (2021). Impact of neurodegenerative diseases on human adult hippocampal neurogenesis. *Science*, 374, 1106–1113. <https://doi.org/10.1126/science.abl5163>
- [7] Zhou, Y., Su, Y., Li, S., Kennedy, B.C., Zhang, D.Y., Bond, A.M., Sun, Y., Jacob, F., Lu, L., Hu, P., et al. (2022). Molecular landscapes of human hippocampal immature neurons across lifespan. *Nature*, 607, 527–533. <https://doi.org/10.1038/s41586-022-04912-w>
- [8] Ammothumkandy, A., Ravina, K., Wolseley, V., Tartt, A.N., Yu, P.N., Corona, L., Zhang, N., Nune, G., Kalayjian, L., Mann, J.J., et al. (2022). Altered adult neurogenesis and gliogenesis in patients with mesial temporal lobe epilepsy. *Nature Neuroscience*, 25, 493–503. <https://doi.org/10.1038/s41593-022-01044-2>
- [9] Spalding, K.L., Bergmann, O., Alkass, K., Bernard, S., Salehpour, M., Huttner, H.B., Bostroöm, E., Westerlund, I., Vial, C., Buchholz, B.A., et al. (2013). Dynamics of hippocampal neurogenesis in adult humans. *Cell*, 153, 1219–1227. <https://doi.org/10.1016/j.cell.2013.05.002>
- [10] Wang, W., Wang, M., Yang, M., Zeng, B., Qiu, W., Ma, Q., Jing, X., Zhang, Q., Wang, B., Yin, C., et al. (2022). Transcriptome dynamics of hippocampal neurogenesis in macaques across the lifespan and aged humans. *Cell Research*, 32, 729–743. <https://doi.org/10.1038/s41422-022-00678-y>