International Core Journal of Engineering Volume 7 Issue 2, 2021
ISSN: 2414-1895 DOI: 10.6919/ICJE.202102_7(2).0044

Inhibiting NMDA Receptor in Targeted CA3 Neurons and Adult-Born Neurons to Prevent Memory Formation and Inspect the Effect on Depression Behavior

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Abstract

Adult hippocampal neurogenesis (AHN) is an intricate and mysterious process that is largely unexplored. Previous studies have shown adult neurogenesis can decrease and alter some levels of depression, however, no studies have shown a complete understanding with a specific example of how AHN actually mitigates and impacts depression-like behavior. This study focuses on the effect of inhibiting memory formation and potentiation in adult-born neurons (ABNs) on depression-like behavior, through the knockout of NMDA receptors (NMDARs). The experiment utilizes gene editing and site-specific recombination, along with optogenetic and fluorescent labeling. Predicted results are the expectancy to knockout NMDARs and prevent long-term potentiation coupled with correct expression of fluorescent. Assuming the mutated mice would perform worse in open-field and tail suspension tests compared with normal AHN mice. This leads to the conclusion that memory formation does help mediate depression-like behavior, which can be further reinforced and studied for future therapies.

Keywords

Adult Hippocampal Neurogenesis; NMDA Receptors; Depression; Gene Recombinase; Arc-CRE.

1. Introduction

Neurogenesis is a process where new neurons are formed in subgranular and subventricular zone [1]. It is a nature process that happens during fetus development and adult aging. It is assumed that AHN can cause memory distortion and reduce depression behavior [2]. Depression or anxiety-like behavior is measured by the passiveness of rodents' activity through tests, including open-field test and tail suspension test. Scientists put effort on studying neurogenesis for finding ways to mitigate depression through learning specific aspects of neurogenesis that reduce depression. In other words, AHN might bring profound impact and advance our understanding on antidepressant drugs.

This study focuses on whether memory formation of ABNs reduces depression. The recall of a memory is accessing some of the neurons which are involved in learning that memory. However, when AHN is blocked, the neurons that are activated in CA3 during learning are not activated when trying to recall that memory [3]. Subsequently, it might be interesting to explore how inhibiting NMDARs in specific CA3 neurons would affect depression. NMDA receptor is a type of ionotropic glutamate receptor that is required for long-term potentiation and synaptic plasticity that forms memory [4].This study employs CRE-recombinase and FLP-recombinase to delete a sequence of genome in rodents. They are tools to target and edit desired parts of chromosomes, which are used to knock out NMDARs in this case. Nestin is an intermediate filament protein, and its gene is found in early stages of neuron development[3]. It is an advantageous way to locate ABNs from other neurons. Optogenetic is later being used to activate specific neurons. It is a technique that uses light to control

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neurons that have been genetically modified to express light sensitive ion channel [5]. Channelrhodopsin-2 is a nonspecific cation light-gated ion channel that can be activated through blue light [6]. It is used to activate and identify ABNs and CA3 neurons.

2. Method

2.1 Knockout NMDA receptor in ABNs

The Nestin gene is expressed solely in immature neurons which control FLP. Hence, in an adult mouse, locating Nestin genes is a way to target ABNs apart from other neurons in dentate gyrus. The Nestin-FLP construct is a recombinant gene inserted into a mouse chromosome. The other recombinant DNA construct is a modification of the NMDA receptor(NMDAR) gene that places FRT sites on opposite sides of the NMDAR gene. FLP and its target site FRT is a recombinase system that functions similar to CRE-recombinase. GFP labeling is added downstream after the NMDAR gene and the FRT, and it acts as a visualiser to ensure the process. If the Nestin promoter is activated, it would trigger FLP to recombine to FRT sites, and delete the NMDAR gene in ABNs(*Fig.1*). After the procedure, potentiation is being recorded by intracellular or extracellular electrodes.

There are two possible controls that can be used: using mutated Nestin promoter mouse, expecting no recombinase to happen, and record long-term potentiation(LTP); using a mouse that carries mutated NMDAR gene between two FRT sites, and also record LTP. These two controls along with GFP labeling should be able to determine the validation of the results.

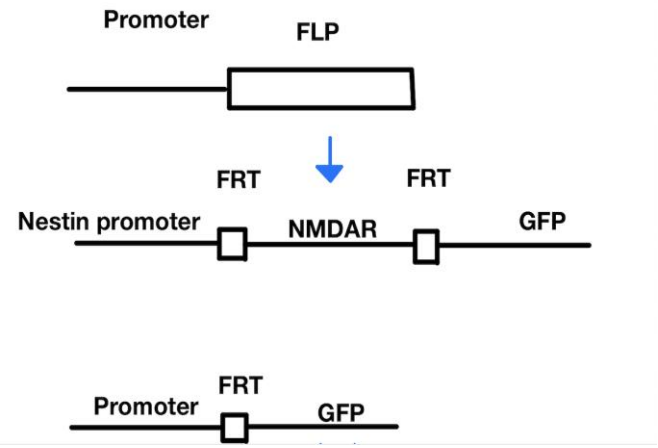

Figure 1 Knockout NMDAR in ABNs through FLP recombinase.

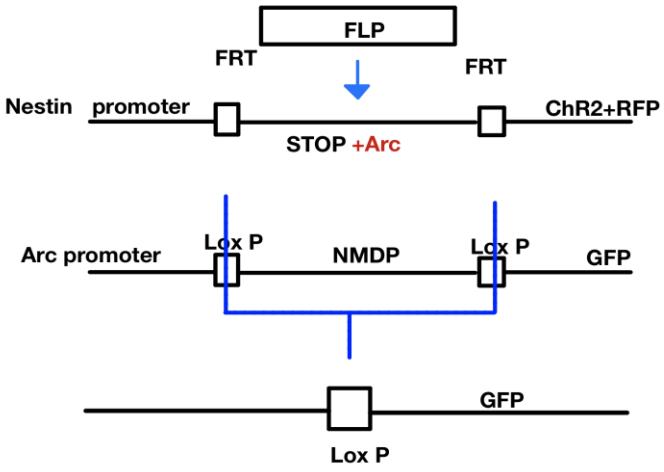

Figure 2 Knockout NMDAR in CA3 neurons. Activate Chr2 using FLP recombinase. Activate Arc-CRE to remove NMDAR gene. Add Arc in ABNs to prevent Arc-CRE recombination ABNs.

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2.2 Knockout NMDA receptor in CA3 neurons that are connected to ABNs by synapses

The idea is to express channelrhodopsin (ChR2) in ABNs, and then use light to trigger action potentials in ABNs. The ABNs will stimulate CA3 neurons, which carry Arc-CRE[3]. The Arc promoter responds to neural activity, thus CRE is expressed and then recombines out the NMDAR gene that is placed between two loxP sites.

The first step is to induce ChR2 in ABNs, using site-specific recombination, to identify which neurons in CA3 are connected to ABNs in DG. As previously mentioned, the Nestin gene controls FLP. Using FLP recombinase should be able to recombine out a 'RNA polymerase Stop signal' that blocks formation of the mRNA for ChR2, and thus express the ChR2 gene. A downstream red fluorescent protein (RFP) is added to confirm and visualise the recombination.

With the expression of ChR2, activating these neurons through a fiber optic light guide would turn on Arc-CRE promoter and cause CRE-recombinase reaction in CA3 neurons that are connected to those ABNs. This would recombine out the NMDAR gene, and thus inhibit potentiation and memory formation(*Fig. 2*). The same trick to put GFP downstream to label them can be applied again. Through the employment of two different recombinase systems, NMDAR genes in CA3 targeted neurons should be knocked out.

While everything seems to be working, there might be a logical fallacy in this methodology. Through the activation of neurons by light using ChR2, it might also cause CRE-recombination in ABNs by prompting the expression of Arc-CRE, which then knocks out NMDAR in ABNs. A possible solution to address this is to put Arc-CRE between the FRT sites(*Fig. 2*). When the Nestin-FLP causes recombination that turns on ChR2, it also removes Arc-CRE. Consequently, the ABNs lose their Arc-CRE gene and cannot recombine out the NMDAR when triggered by light.

2.3 Test

The treated mice in both experiments are tested and recorded through open-field test and tail suspension test, compared with normal ABNs mice. Mice without AHN act as a positive control, and its AHN can be inhibited by artifical x-ray[3].

3. Results

In the first experiment, mice should end up without NMDAR gene with no potentiation, tested by extracellular record. The mice should also express GFP labels. For the two controls, the first control where mice's Nestin promoter is removed, mice are expected to not have FLP-recombination, and not remove their NMDAR, presuming there is still LTP. The second control where mice's NMDAR gene is missing between the two FRT sites, mice are expected to have NMDAR and assuming there is also LTP.

In the second experiment, mice should end up with some CA3 neurons missing their NMDARs. These mutated neurons in CA3 region are those that are connected to ABNs in dentate gyrus, and those ABNs that are connected should have their NMDARs. These ABNs are expected to be red fluorescently labeled with their RNA stop signal and Arc-CRE removed, and ChR2 are expressed(*Fig.3*). Then, those CA3 neurons are green fluorescently labeled with their NMDAR gene removed by Arc-CRE recombinase. That is triggered by the activation of ChR2 through blue light(*Fig.3*).

There should be a reduction of time of treated mice to be immobile in tail suspension test, and less activity in open field test compared to normal mice, after the inhibition of NMDARs and memory formation. The positive control group of mice where they do not experience AHN should be expected to exhibit no reduction of depression-like behavior, since AHN would reduce some level of depression based on previous literature[1]. Therefore, this control group presumably has similar results with treated mice and revealed there is a correlation between AHN and depression. This hypothetical result would imply memory formation of ABNs is essential for the reduction of depression. Apart from that interpretation, it is safe to speculate that mice knockout NMDARs in

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ABNs might exhibit a higher level of depression than mice knockout NMDARs in some CA3 neurons. Based on traditional conception, changes at a source could lead to a bigger overall effect. Thus, the knockout of ABNs' NMDARs might stimulate a higher level of depression than that of neurons in CA3.

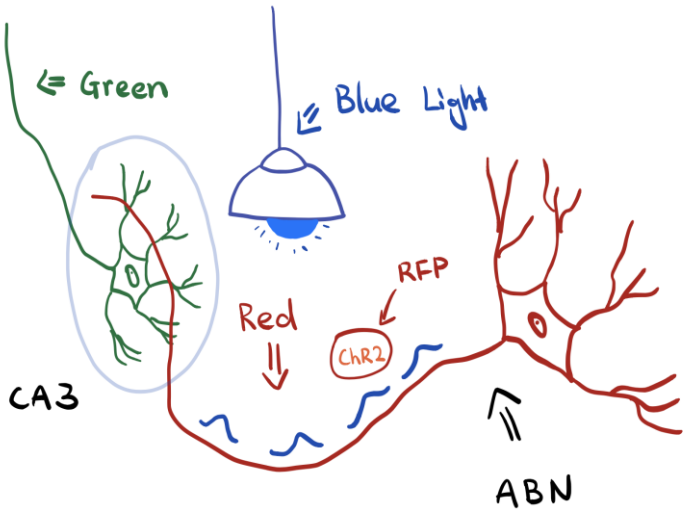

Figure 3 Diagram of expected results for second experiment. ABNs are red fluorescently labeled. Action potential activated by blue light marked connected CA3 neurons through synapses. Arc-CRE removes NMDARs in CA3, making it fluorescently labeled green.

4. Conclusion

The proposed methodology and speculated results incline that plasticity and memory formation during AHN is vital for the reduction of depression. It is believed that NMDA receptors can be knocked out through recombination and stops plasticity. It is assumed by removing potentiation, it would stop memory formation in rodents' brains. This would answer the paper's question, that memory formation of ABNs would reduce depression. Nevertheless, memory formation and memory storage is a rather complicated mechanism. There are other ways to form memory apart from potentiation and plasticity, including neuron circuits. Potentiation and plasticity might only be an important but not decisive aspect of memory. The artificial knockout of NMDA receptors might not be able to fully prevent formation of memory, or the recall of memory. We could change the physical environment of mice to prevent new memory formation. For example, put mice in a complete dark environment and record levels of depression after a certain period. If the knockout of NMDA receptors do lead to a higher level of depression compared to normal AHN ones, new drugs and therapies can be developed to mitigate depression and anxiety.

Nonetheless, what if the reduction of depression by neurogenesis has nothing to do with memory formation in ABNs? Then, all of the afore-mentioned predicated conclusions might be inaccurate. If that is the case, there could be other aspects or directions of neurogenesis that induce this relationship with depression, for instance, the disruption of neuron circuits. Of course, there are many places that can be improved in our experiment. We can use western blot to confirm the recombination process, instead of using fluorescent. There can be additional controls for the second experiment to ensure each step is successful.

Overall, this hypothetical study might establish a connection between how neurogenesis reduces depression, but more importantly, this paper encourages more attention and studies to be drawn to this topic. Adult hippocampal neurogenesis is an intriguing while challenging process. Depression on the other hand is a pain for many people. We hope there can be more antidepressant drugs available

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in the future, and we want to conduct research on neurogenesis as it could be a potentially powerful direction. Our understanding is still superficial at this point and there are far more areas that we did not cover yet. Our study is rather an introduction to this topic, and it raised some thoughts for the future.

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