Adult Neurogenesis: Does Knockout of NMDA Receptors in Newborn Neurons Reduce the Antidepressant Effects of Fluoxetine?

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Abstract

Patients with Major depressive disorder (MDD) all present different degrees of funcion deficit in their hippocampus. As the hippocampus primarily involves memory and learning function, the patients with MDD always experience decline in learning abilities. The glutamate in the hippocampus-NMDA receptor, has proved to involve learning and memory function in the hippocampus. Thus, we hypothesize that the presence of NMDA receptors can affect depression. On the basis of this, we propose that the knockout of NMDA receptors can reduce the effects of antidepressant drugs. And the NMDA receptors in newborn neurons play a more important role in controlling depression than those in old neurons.

Keywords

Neurogenesis, NMDA receptors, Antidepressant effect.

1. Introduction

According to the World Health Organization, major depressive disorder (MDD) affects about 121 million people worldwide and is the leading cause of disability as measured by years lived with disability (YLDs) [1]. Researchers observe that people with depression usually have functional deficits in their hippocampus, and antidepressant treatment increases neurogenesis, a process of generating functional neurons from existing precursor cells in the dentate gyrus area [2]. Researchers also prove the casual relationship between adult neurogenesis and depression [3]. There is evidence shown that the newborn neuron's activity can affect an individual's depressive behavior [4], but the specific neuronal activities have not been determined. Because newborn neurons play an important role in memory and learning function [5], this paper intends to explore the relationship between learning ability of new neurons and depression.

As NMDA receptors, a glutamate receptor, have been proved to play an important role in learning and depression can affect the capacity of learning, the causal relationship between NMDA receptors and depression is tested by knocking out NMDA receptors in neurons. In the work, three experimental groups are designed to prove that mice with reduced NMDA receptors show less improvement than mice with a normal level of NMDA receptors in anxiety and depression behavior tests after taking fluoxetine. By proving that NMDA receptors activities of newborn neurons are able to increase depression level rather than that of old neurons, the results further show the causality between neurogenesis and depression and introduce a new perspective for medicine production: antidepressant treatment that increases NMDA receptor activities.
2. Results

2.1 Three comparison experiment groups help define the relation of NMDA receptors with depression and anxiety in hippocampus

The experiment uses healthy 8-10 week-old C57BL/6 male and female mice and treats them with electric shock in order to make them depressed. Then, tail suspension test, elevated zero maze, and open field test are introduced to evaluate their depression level, recording their level of anxiety and depression before the experiment. Mice are divided into three experimental groups -- mice with old neurons that lack NMDA receptors, mice with new neurons that lack NMDA receptors and mice with old neurons which have normal levels of NMDA receptors. The experiment lasts for two months and all mice are given the same amount of fluoxetine at the beginning of the experiment.

Then, Ascl1-CreER\textsuperscript{TM} technique is introduced to knock out the NMDA receptors. Group one receives the tamoxifen, a drug that helps knock out NMDA receptors, on the first day of the experiment, inducing the NMDA receptors genes to be silenced. The second group receives the tamoxifen after one month and two weeks while the third group remains for two months without being injected with any additional drug. At the end of two months, all mice are sent to have the three anxiety tests again, the same as at the beginning. Comparisons are made between these results and the ones came out from the initial tests to evaluate the improvements three groups had made.

2.2 Analysis of two experiment outcomes

Three groups help to prove that the knockout of NMDA receptors would exert a negative effect on antidepressant improvement caused by fluoxetine and the newborn neurons could cause a greater effect on antidepressant improvement compared with the old neurons. Specifically, the only difference between group one and group three is that mice in group one have been knocked out NMDA receptors while mice in group three haven't. If the experiment results comply with our hypothesis, the mice in group three would show a greater improvement in the final anxiety tests as the presence of NMDA receptors help the mice behave better. If the test results show that group one and group three have the same improvement or even group one has a greater improvement then group three, the hypothesis may not be valid and more experiments are needed to validate the results. Mice in both group one and group two are knocked out of NMDA receptors but mice in group one have old neurons which lack NMDA receptors while mice in group two have newborn neurons which do not have NMDA receptors when entering into the final anxiety tests. If the experiment results comply with the hypothesis, the group one would have a greater improvement in anxiety behavior tests as old neurons don’t involve in dealing with depression as much as the newborn neurons do. Newborn neurons without NMDA receptors place a greater negative effect on improvement than old neurons without NMDA receptors do. However, if the experiment results show that group one has the same improvement as group two does or even has less improvement, they may not support our hypothesis. Consequently, more experiments are needed to further justify our hypothesis.

2.3 Method

Ascl1-CreER\textsuperscript{TM} is an important technique we used in the experiment to knock out NMDA receptors. Ascl1 is a specific promoter in dentate gyrus neurons that involve neuron differentiation. And it only targets the NMDA receptors in newborn neurons.

3. Conclusion

In the work, three experimental groups are designed to testify our hypothesis that the knockout of NMDA receptors in neurons would cause a negative change on antidepressant effect and the knock out of NMDA receptors in newborn neurons would cause a greater negative change on antidepressant effect than in old neurons. In the experiment, three groups of mice are first made depressed and then treated with fluoxetine in the two months’ experimental period. Tamoxifen is injected to group 1 mice on the first day and group 2 after 1 month and 2 weeks mice to reduce NMDA receptors in old and new neurons, respectively, while group 3 has NMDA receptors in all neurons. The mice’s initial and
final levels of depression and anxiety level are compared. If group three has a greater improvement than group one does, it would validate the first part of the hypothesis. And if group one has a greater improvement than group two does, it would validate the second part of our hypothesis. However, if the results don’t comply with what we expected, different mice and more trials are needed to be applied to verify the hypothesis.

There are also some limitations that exist during the experiment process which may affect the results. Considering the individual’s biological difference, mice may show differences in improvement after receiving the same amount of fluoxetine. Thus, repeating trials of experiments with larger numbers of mice are needed to verify an accurate result. Additionally, in group one, before the newborn neurons without NMDA receptors become old neurons, the newborn neurons without NMDA receptors might make the mice more depressed which would negatively affect the results. More advanced technology may be needed to examine the activities of these neurons in the future. With the impact of COVID-19 and more frequent usage of technology, the number of people who experience depression continues to increase. When most weaknesses are solved, if the results still comply with our hypothesis, the result is helpful to the development of antidepressant drugs in the future as scientists can target the NMDA receptors to mediate the major depressive disorder.

References


