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Hippocampal Synaptic Plasticity Reduction Leads to Decreased Resilience to Depression and Anxiety in the ASCL-Mouse Model

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Abstract:

A substantial body of research has been conducted with the objective of elucidating the role of synaptic plasticity in the aetiology of depression and anxiety. Despite the fact that numerous existing tests have demonstrated a correlation between neuroplasticity and depression, the precise role of synaptic plasticity remains unclear. The objective of this experiment is to elucidate the impact of hippocampal synaptic plasticity on resilience to depression and anxiety. The experiment comprises a preliminary test and a series of main experiments. The preliminary tests are conducted to eliminate the potential effects of apoptosis caused by the BAX gene. The main experiments then assess anxiety and cognitive levels by dividing subjects into multiple groups and administering a battery of tests. In a 40-week period, the mice will be modified and will experience major depression as a result of an 8-week UCMS treatment.

Keywords: Adult Hippocampal Neurons, Major Depression, Synaptic Plasticity

1. Introduction

Major depression (MD) is one of the most prevalent and debilitating personal and public health conditions worldwide. [1] It is less well documented that the impact of depression is not distributed equally among those who experience it, given that MD is not a homogeneous pathology. The condition is typified by a constellation of core symptoms, including anhedonia and depressive mood, which are frequently accompanied by disturbances in sleep and eating, psychomotor alterations, and anxiety. [2] Major depression (MD) is a highly debilitating disorder that affects millions of people worldwide [1] and is thus a main contributor to the global burden of healthcare and the economy. Currently, there are several pharmacological approaches in existence, including selective serotonin reuptake inhibitors (SSRIs), which have been developed with the objective of alleviating symptoms. (Madhukar et al., 2006). Notwithstanding the advent of a plethora of pharmacological interventions designed to assuage symptoms, approximately one-third of patients persist in exhibiting symptoms following treatment, [3] and only 30% achieve remission.

The prevailing hypothesis regarding MD was that it was a disease of monoamine deficiency. This hypothesis posited that low levels of monoamines in the synaptic cleft were the underlying cause of depressive symptoms. [4]

Nevertheless, a novel hypothesis has emerged suggesting that the depression may be linked to neurons rather than monoamine. [5] This proposition postulates that the downstream effects of antidepressants, such as augmented neurogenesis, contribute to enhancements in cognition and mood. [6]

The term “neuroplasticity” is defined as the brain’s capacity to undergo neurobiological alterations in response to external stimuli, such as early-life adversity [7] and chronic exposure to stress. [8] Research has substantiated the assertion that neural plasticity, spanning from the hippocampus to the prefrontal cortex, illuminates the correlations between neural plasticity and depression. [9] The results of Wei’s experiment indicate that hippocampal neural plasticity has a beneficial impact on resilience against depression and anxiety.

However, when it comes to the hippocampal synaptic plasticity, its effectiveness remain unknown. The hippocampus uses a mechanism named Long Term Potentiation to form synapses and make them stronger. In this process, the NMDA receptor plays a vital role which allows the calcium ions to pass. However, when the NMDA receptor was blocked, the cell will not die because of it.In the same time, the AMPA receptor will increase in number. Although, the cell remains alive, the lack of NMDA receptors prevents it from LTP and thus there is no synaptic plasticity.[8]

Given the functional role of hippocampal synaptic plasticity, reducing such plasticity before stress could facili-

tate the promotion of stress-induced impairments on hippocampal functions, which are typically impaired in MD. Accordingly, the hypothesis is that the absence of synaptic plasticity would diminish the mice's overall resilience to depression and anxiety. It is conceivable that cognitive disorders may not be the primary symptoms of MD. However, there are also patients who exhibit memory deficits, decreased flexibility, and impaired inhibitory control, which could also be induced by the absence of such plasticity. [1]

2. Methodology

2.1 Experiment Design

The objective of the experiment is to qualitatively ascertain the impact of hippocampal synaptic plasticity on the resilience of depression and anxiety. Consequently, the long-term potentiation (LTP) process will be terminated in order to eliminate the plasticity. Additionally, evidence indicates that the AHN is conducive to the promotion of resilience. Accordingly, it is necessary to avoid the effects of AHN on the experiment. To achieve both objectives, it is possible to rely on the blocking of the NMDA receptor. The blockade of the NMDA receptor will prevent the formation of a new synapse, as the receptor is required for long-term potentiation (LTP). The creation of a hippocampus devoid of synaptic plasticity will allow for a comparison of its depression and anxiety levels with those of the control group, thereby elucidating the impact of plasticity on depression resilience.

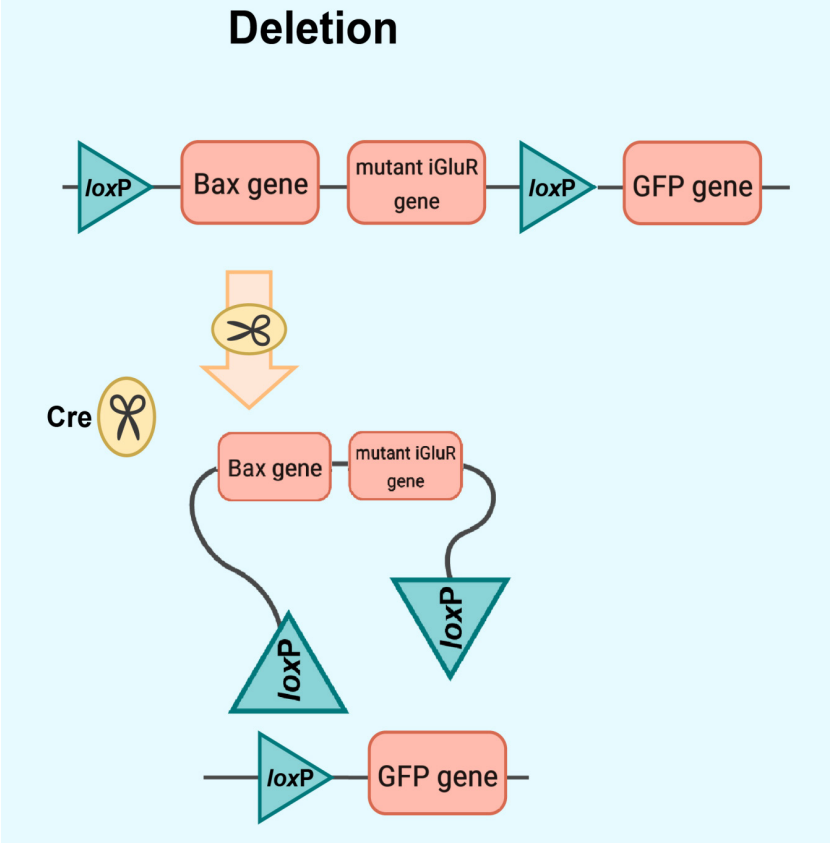

Fig.1 Deletion of BAX gene and GFP expression

2.2 Preliminary Experiment

It can be postulated that cells generated by AHN that have no synapses will die by an apoptotic mechanism that is dominated by the BAX gene. [10] However, an issue may arise during the apoptotic process. Given that both the older-generated neurons and the new AHN were unable to form robust synapses with other neurons, it is unclear whether the older-generated neurons will undergo apoptosis. To address this issue, a preliminary experiment is necessary.

The NMDA antagonist selected for further investigation is MK 801. To ensure that the ASCL mice will be resistant to MK 801 when the drug is administered, the mice will be genetically modified to be resistant to the antagonist. Once the mice have reached adulthood, the Cre-LoxP system will target the gene encoding the MK 801 binding site

iGluR-related gene. One of the LoxP sequences should be located between the GFP and the iGluR target gene. As the ASCL starter will be triggered exclusively in the AHN, the remainder of the mouse will remain unaltered and should survive. Following the deletion of the gene, the mice were maintained for a brief period, after which MK 801 was administered. Subsequently, the TdT-mediated dUTP Nick-End labeling (TUNEL) assay, which produces red fluorescence, shall be introduced. The quantitative determination of red and green fluorescence will be conducted in the final immunohistochemical analysis. In the event that minimal red fluorescence is observed under the microscope, the BAX gene shall be deleted to guarantee the survival of neurons with synapses. In the event that the BAX deletion process is not required, it will not be necessary to proceed with it.

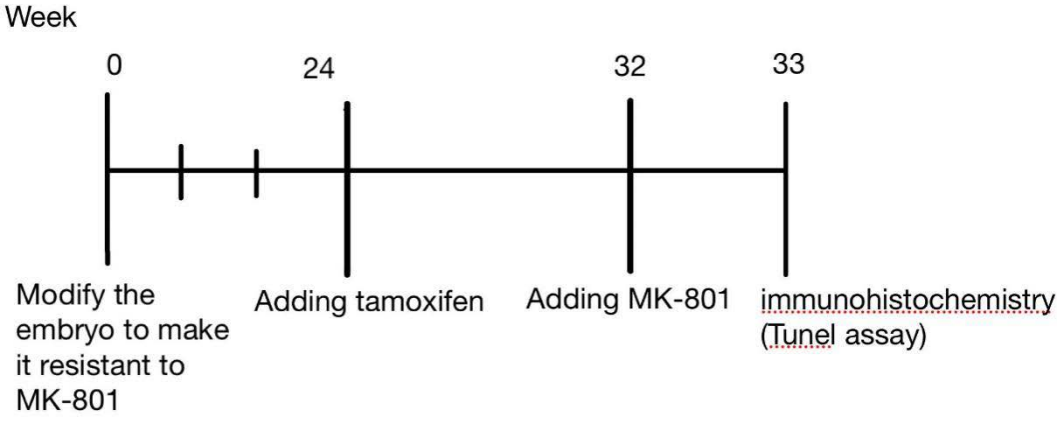

Fig.2 Schedule of preliminary tests

2.3 Variables and groupings

Following the conclusion of the preliminary tests, the result will determine whether the BAX gene will be deleted or retained. Regardless of the outcome, the remaining mice were divided into four groups based on two variables. The first variable is depression. To gain further insight into the potential association between AHN and stress resilience, we stimulated AHN in an animal model of unpredictable chronic mild stress (UCMS), which is a naturalistic model of MD. [10] Therefore, the UCMS and Non-Stressed (NS) groups will serve as the primary variable. The UCMS group will be subjected to the regimen for a period of eight weeks to ensure its efficacy in the murine subjects. The second variable is whether the mice will be administered tamoxifen or not. The group that received tamoxifen was able to successfully delete the target gene, thereby removing the resistance to MK 801, while the vehicle group remained resistant.

2.4 Nest building tests:

Prior to the commencement of the experiment, the animals were placed in larger individual cages for a period to allow for habituation. A single square piece of pressed cotton was placed in each cage at a certain time. The quality of the nest was then assessed at two time points: after 5 h and after 24 h, according to the previously described 1–5 rating protocol. [11] Subsequently, the mice were returned to their home cages.

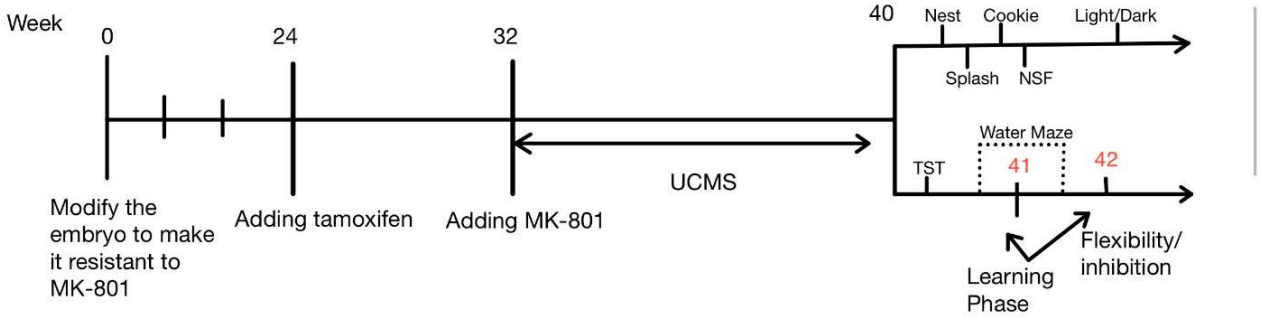

Fig.3 Schedule for main experiments after preliminary tests

2.5 Light/Dark Boxes

The apparatus was a light/dark box comprising a lightbox with transparent sides and a dark box connected to it with tunnels. The animals were placed within the lightbox, and once the animal entered the tunnel, the test commenced and continued for a fixed period of time . The number of

entries and the total time spent in the dark box were documented. Since the mice will avoid being in well lit areas, the anxiety-like behavior can be indicated. [12]

2.6 Novelty-Suppressed Feeding Test (NSF)

Mice would be placed in a box which is illuminated by red light. Food reward is located in the center of the box. The tests will ask mice to choose between food and open space. Once the mice were situated within the apparatus, the latency to explore and the total consumption of food will be recorded. [13]

2.7 Splash Test

The test was conducted in accordance with the methodology previously described in reference. [10] The dorsal coats of the mice were treated with a 10% sucrose solution in the cages under red light conditions. The palatability of the solution was intended to stimulate grooming behaviors. The time of grooming will be determined [14]

2.8 Tunnel Test

The objective of the test is to ascertain whether the subject displays anhedonic traits. The mice will be placed in a tunnel comprising three rooms, the first of which is connected to the second, which is connected to the third. Two gates are positioned adjacent to one another. The mice will be placed in the initial chamber while the cookie reward is situated in the third chamber. In order to obtain the reward, the subject must traverse the entirety of the tunnel and reach the opposite end. Subsequently, upon the mice's entry into the second room, the gate between the first and second rooms is closed. The interval of time during which the subject exhibited indecision and the final consumption of the cookie.

2.9 Tail Suspension Test

The assessment of stress-coping behaviors was conducted through the utilization of the tail suspension test. This test entails the suspension of mice above the ground by their tails with the aid of tape, for a period of six minutes, without the possibility of escape or the ability to grasp onto any surface [15]. The immobilization time, as reflected by this test, serves as an indicator of the mice's resignation and subsequently, their depressive-like behaviors.

2.1 0. Water Maze Tests

The objective is to assess two aspects of executive functions, namely cognitive flexibility and inhibitory control. Executive functions represent a set of cognitive processes that facilitate the elaboration and control of adaptive or complicated responses. [5]

The objective of flexibility tests is to ascertain the subject's capacity to alter their objective. Inhibition entails a conversion of the goal in accordance with the circumstances, thereby facilitating the mice's capacity to relinquish their established strategy and pursue a goal-driven approach.

The device is a maze with four arms (N, S, W, E), and the water temperature and light intensity are fixed in the center. In any context or task, the N-side arm will be illuminated by an additional light source.

The variables in question are the lenses and the location of the reward. Two contexts will be used to determine the sense in question. Context A will result in the establishment of a sense, whereas Context B will not. In Task 1, the objective is to place the goal on the E-side arm and N-side arm in Task 2. Consequently, four distinct combinations have been identified: A-1, A-2, B-1, and B-2. The experiment will utilize these four compounds in three distinct stages of the test.

The initial stage of the process is devoted to learning. The initial stage of the experiment will last for four days, during which the mice will be exposed to the A-1 and B-2 combinations four times a day. During this process, the mice will learn this pattern. The initial two days are dedicated to the A-1 combination, followed by an identical two-day period for the B-2 combination.

The second stage is the flexibility test. The mice that have been exposed to the A-1 and B-2 combinations over the course of four days have demonstrated proficiency in learning the requisite patterns. The A-1 and B-2 combinations will be alternated. The mice will undergo a total of six times , with the first, third, and fifth tests utilizing the A-1 combination, and the remaining tests employing the B-2 combination. It was anticipated that the mice would demonstrate an ability to learn the previously presented pattern. The cognitive score will be higher for the mice that are able to find the correct path in the shortest time and at the highest level.

The final stage of the experiment is the inhibition test. The combination was altered. The context and task will be evaluated in a covert manner. The A-2 and B-1 combinations will be subjected to testing. The condition that must be met in order to identify the optimal route has been derived from the data collected during the initial five-day period. The mice must abandon their established pattern and redirect their attention to locating the reward. This would

serve to assess the capacity of the mice to alter their established behavioral pattern in order to pursue a goal-directed one.

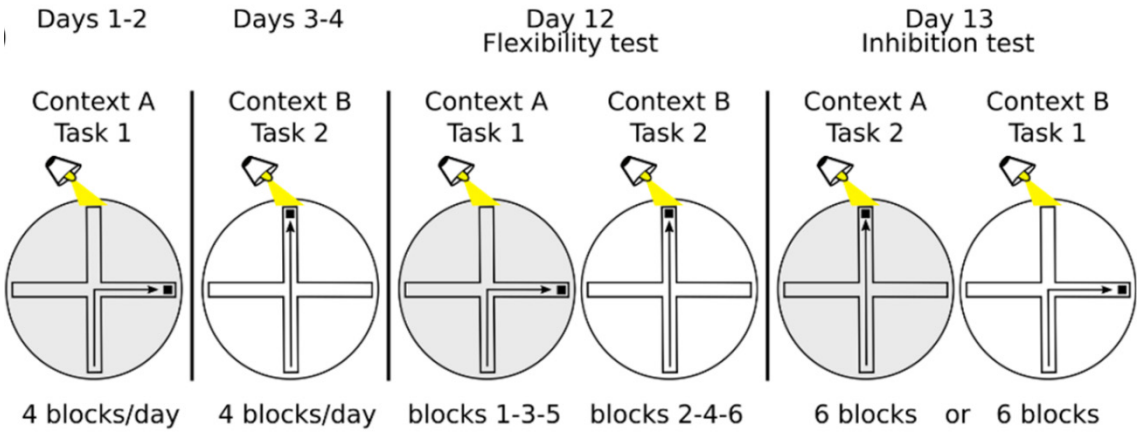

Fig.4 By Barbara et al., 2021 image in the paper Increasing Adult Hippocampal Neurogenesis Promotes Resilience in a Mouse Model of Depression.

2.1 1. Limitations

The experiment is, in theory, viable. Nevertheless, the result may not be an accurate reflection of the outcome. The mice were derived from embryos. It is conceivable that a period of 24 weeks may prove insufficient to establish a stable neural network. Furthermore, should the preliminary tests indicate that the neurons with synapses must be modified to prevent apoptosis, it will be necessary to remove all the hippocampus neurons' target genes. The apoptosis would not manifest in the brain. Consequently, the brain would be overpopulated with neurons, which could potentially compromise the mice's health. Additionally, the CRE-LoxP system exhibits some degree of leakage, which could result in unintended outcomes in non-target tissues. This could introduce inaccuracies in the experimental outcomes. To ensure the precise deletion of the gene, the LoxP sequences must be meticulously selected. Therefore, the experiment must be conducted multiple times and rigorously tested to minimize any potential adverse effects.

3. Discussion

3.1 Expected Results

It is anticipated that the results will indicate that the UCMS groups have a higher prevalence of depressive symptoms compared to the NS group. Conversely, the Tamoxifen group is expected to demonstrate a lower incidence of depressive symptoms compared to the Vehicle group. The UCMS Tamoxifen group is expected to exhibit the highest prevalence of depressive symptoms, while the UCMS Vehicle group is expected to have the second highest prevalence. The NS Tamoxifen and NS Vehicle groups are expected to have similar prevalence of depressive symptoms, but both are expected to be lower than the UCMS groups.

3.2 Contribution

The previous experiments haven't discussed how Hippocampal synaptic plasticity affects the resilience to depression. This research provides a new perspective of depression and anxiety. The water maze test will also shedding light on how synaptic plasticity affects cognitive functions.

3.3 Conclusions

The experiment would reflect the connection between the missing hippocampal synaptic plasticity and the MD. The experiment will evident that plasticity of synaptic is vital for both preventing depression and resilience.

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