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Impact of Adult Hippocampal Neurogenesis on Neuronal Output Patterns from the Subiculum to the Lateral Hippocampus During Anxiety

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Abstract:

Adult hippocampal neurogenesis (AHN) has an effect on mood regulations. Promoting AHN could recover stressed-induced symptoms such as depression and anxiety. In this study, we used voluntary physical exercise, fosTRAP2, functional magnetic resonance imaging (FMRI) and positron emission tomography (PET) technology to evaluate the effect of AHN on mice during unpredictable chronic mild stress (UCMS). Additionally, we inject fluoxetine to increase AHN.

Keywords: Adult Hippocampal Neurogenesis, Depression, Hippocampus, Subiculum.

1. Introduction

Adult hippocampal neurogenesis (AHN) is a process when new neurons make the senate gyrus (DG) then they turn into mature neurons and combine with the existing neural circuitry, which is related with pattern recognition, cognitive flexibility, emotion accommodation and resilience to stressful conditions.[1]

The hippocampus, which is located in the inner region of the temporal lobe, is an important brain region associated with the cognitive processes such as memory. Studies in animal models have provided many information for the role of adult hippocampal neurogenesis by learning and memorize space and context.

Major depression (MD) is a mood disorder that causes a feeling of lost of interests and sadness. It affects millions of people worldwide and an estimated 3.8% of the population experiences depression, including 5% of adults (about 4% among men and 5% among women). Approximately, there were about 280 million people experiences depression. Moreover, it is also a main contributor to the global burden of healthcare and the economy.[2]

Anxiety is a phycological disorder that occurs when a person faces stressful or dangerous conditions.[3] The difference between MD and anxiety is mainly about the symptoms. Anxiety involves worrying and fear whereas MD involves a feeling of sadness.[4]

In my previous research shows that increasing AHN alleviates depressive-like behaviors [5]; it also promotes recovery of depressive-like symptoms such as eating less and aches and pains but also in protective parts [6]. In the clinical treatment of major depression (MD), the effects of antidepressants are also meditated by increased neurogenesis [7]. Furthermore, inside our brains, the subiculum of hippocampus plays a key role in stress response. The ventral subiculum is principally responsible for ventral hippocampal actions on the HPA stress response, which involves in the regulation of depression [8].

In this study, we use fosTRAP2, which is use to capture neural activation patterns during anxiety and depression; FMRI, which is a tool use for measuring and mapping brain activities; PET, which is a polymer use to manufacture items for a low cost, technologies to display the activity of stress-related neurons in the ventral subiculum in the brain, assessing the effect of increasing AHN in the stress response.

2. Previous methodology

2.1 Animals

Mice at the age of 11 weeks and is genetically modified—transgenic fosTRAP2 (CreER2) and uses Ai4D 26sortm (CAG-tdtomato).

2.2 Corticosteroids

Inject corticosteroids to the mice with a needle after 1 hour since tamoxifen injection. Suspended in saline with 1% Tween 80 (polyoxyethylene sorbitan monoleate) and

0.1% DMSO (dimethylsulphoxide)

2.3 Tamoxifen

Inject tamoxifen 1 hour before corticosteroid injection to the 2 of each group. Use 4-hydroxy-tamoxifen (4-OHT) intraperitoneal at a dose of 50mg/kg. 4 ATM (Sigma H6278) was dissolved in an aqueous solution with 10% DMSO and Tween-80 in saline. All cages were changed the next day to avoid re-uptakr of 4-hydroxy-tamoxifen.[9]

2.4 Fluoxetine intake

The selective serotonin re-uptake inhibitor (SSRI) Fluoxetine, has been known to suppress anxiety and depressive-like behavior and increase neurogenesis in mice. 3 out of 12 mice received chronic fluoxetine intake for 10mg/kg/day in drinking water for 4 weeks.

2.5 Voluntary physical exercise (running)

The mice will be housed individually in cages with access to running wheels and ad libitum access to food and water. [10]

2.6 fosTRAP2:

The mice are divided into 4 groups, 2 of which receive increase in AHN by voluntary physical exercise—running and fluoxetine intake before corticosteroids (CORT) injection respectively. For the rest of the two groups, both groups receive no AHN increase. However, one with CORT injection, one with no stress.

All four groups will undergo a four weeks six days and 12 hours experiment. For the first four weeks, groups will be access to either food and drink or fluoxetine injection. Then for 5 days, the mice will be placed in a dark cage and habitualized the pain of injection during saline injections. On the next day, the first three groups will receive tamoxifen and corticosteroid injections and remains standard conditions for the fourth group. After 12 hours of placing each mice in individual dark cages without disturbance, the mice will be sacrificed.

2.7 FMRI

Materials used: strong magnet to have a clearer view and resolution of the mice brain. Use anesthesia if needed; isoflurane (anesthesia) concentration between 4-5%+0.8-1L/min; electronic equipment for body temperature and blood pressure, visual monitoring for oxygenation.

Methods: put the mice in the machine and stimulate the brain with stressor. Have the mice tied up to prevent any movements so the image don't get blurry (Use anesthesia and provoke surgical stresses if necessary. The main stressors associated with surgical procedures are physical).[11]

2.8 PET

Materials used: 18-F-FDG less than 10% of total blood volume between 1.6-3.2mL; isoflurane concentration between 4-5%+0.8-1L/min; electronic equipment for body temperature, blood pressure, visual monitoring for oxygenation.

Methods: perform cross calibration of the clocks used and between PET scanner and dose calibrator. Control temperature between 26-34 degree celsius. Keep mice fasting for 8-12 hours and for 20 hours to decrease blood glucose level.

Inject 18-F-FDG to mice before the reading. Tied up the mice to make sure the image don't get blurry. Only use anesthesia and provoke surgical stresses if necessary. The main stressors associated with surgical procedures are physical.[12] Lastly, scan the mice after injection and after 1 hour from last injection.

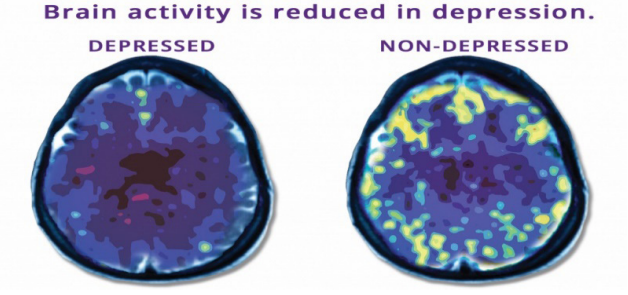

Figure 1: PET scanning of the brain when depressed and not depressed. There's an increase in blue color and decrease in yellow color show that there is a decrease in brain activity. [13]

2.9 Histology

Equilibrate the sample briefly with phosphate-buffered saline (PBS); Dilute the DAPI stock solution to 300 nM in PBS; Add approximately 300µL of dilute DAPI staining solution to the coverslip preparation, making certain that the cells are completely covered; Incubate for 1-5 minutes; Rinse the sample several times in PBS; View the sample using a fluorescence microscope with appropriate filters.

2.1 0. Statistical analysis software use

Graphed Prism and R (version 4.0.2) were used for figure design and statistical analysis.

2.1 1. Imaging parameters

Microscopy at a 10x objective lens is capable of imaging at multiple wavelengths, including those for DAPI and tdTomato fluorescence at a 10x to capture DAPI and tdTomato.

2.1 2. Cell counting and registration

Use cell detection feature in NeuroInfo to identify fosTRAP2+ cells. Use DAPI channel as a method to registered it in Mouse Brain Atlas.

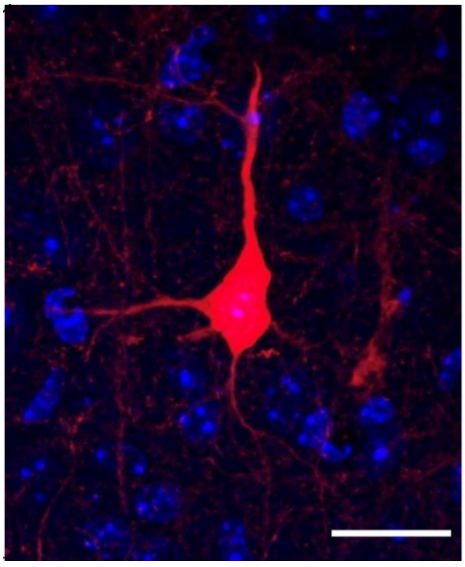

Figure 2: Neurons marked by fosTRAP2 technology. Red:fosTRAP, blue:DAPI [14]

3. Results from previous studies

The experiments proved that increasing AHN in mice alters the output pattern of specific neurons populations in the subiculum to the lateral hippocampus under anxiety conditions. The fluorescence neurons and activity in hypothalamus and subiculum should be observed in groups with neurogenesis compared to the control groups with CORT injection. Furthermore, control groups without CORT injection could also be observed to have less TRAPed neurons in the subiculum and lateral hypothalamus in order to verify the validity of the methods. Furthermore, the results indicate that it is possible to alleviate depression by increasing AHN in which could reduce the population of the neurons in the subiculum and lateral hippocampus and this method could be used in human.

4. Discussion

In the experimental methods, fosTRAP2, FMRI and PET technology were used to prove whether increasing AHN in mice alters the output pattern of specific neurons populations in the subiculum to the lateral hippocampus under anxiety conditions, which is also the hypothesis of this experiment.

In the future, we could make deeper research on the effect of AHN on anxiety and depression in hypothalamus and subiculum in order to take this experiment further and we can also research how to apply the methods from laboratory to clinical medicine. Moreover, the experimental methods can be improved by conducting experiments at the molecular level to obtain a more specific and detailed results and data.

5. Conclusion

Based on the results and discussion presented above, it is concluded that increasing AHN in mice alters the output pattern of specific neuron populations in the subiculum to the lateral hippocampus under anxiety condition.

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