ISSN 2959-409X

Using fluorescence, PET, and fMRI to detect the alteration of patterns and activation of specific neurons in the subiculum in response to hippocampus neurogenesis

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Abstract:

In this paper, the questions started with “What will happen to the brain if AHN(adult hippocampus neurogenesis) increases” and then going further on the question became “How would certain neurons in the hypothalamus and subiculum change if AHN increases”. Therefore, this paper will address this question and propose three experiments and a conclusion. For the experiments, PET and fMRI are used to scan the brain and see the levels of glucose and therefore the activation of neurons. Furthermore, FosTRAP2 with fluoresce allows a better in-depth look at the parts where neurons were active, or in this case, activated due to stress. The three experiments should show more activity/ fluorescence in the hypothalamus and subiculum in groups with neurogenesis compared to the control groups with CORT injection. In conclusion, if the results are in accordance with the hypothesis, this allows a better insight into the mechanism and link between anxiety and AHN. This will also mean a correlation with the subiculum, HPA, and AHN.

Keywords: Depression, anxiety, neurogenesis, hypothalamus, subiculum, brain glucose activity.

1. Introduction

Major depressive disorder (MDD) is increasing in the population and MDD pathogenesis has been proven to have negative effects on the person including loss of interest, depressive mood, change of appetite, and even loss of pleasure in rewarding actions. In detail, stress can cause MDD which can lead to more molecular effects. In the brain depression decreases the volume of the hippocampus, increases inflammation, oxygen restriction, changes the connections between parts of the brain and changes the chemical levels in the brain and body, and can impair AHN.[1]

When the brain gets stressed the hypothalamus releases corticotropin-release-hormone (CRH) in the hypothalamic-pituitary axis (HPA) and then adrenocorticotropic hormone (ACTH) which at the same time affects the adrenal glands which then increases levels of cortisol which has a negative feedback to the brain [2]. It also has a negative effect on the glucocorticoid receptor binding which suppresses pro-inflammatory immune response.

AHN is a process by which new neurons are continuously generated in the dentate gyrus (DG) approximately 700 newborn granule cells, where they develop into mature neurons and functionally integrate into the existing neural circuitry, which is relevant for pattern discrimination, cognitive flexibility, emotional processing and resilience to stressful situations [3] [4] .

The mechanism for AHN has been studied and newborn neurons are born in the ventral dentate gyrus, in the ventral hippocampus, which is more specialized in emotions and reward modulation circuitry [5].

Increasing AHN alters the patterns and activation of specific neurons in the subiculum to the lateral hippocampus under anxiety-depression conditions. As the subiculum is related to the regulation of depression, this research shows a further approach in which part of the ventral subiculum displays activity under anxiety-depression conditions.

1.1 Literature review

The ventral subiculum proper is principally responsible for ventral hippocampal actions in the HPA stress response, which involves the regulation of depression. When the brain gets stressed the parvocellular neurons of the paraventricular nucleus of the hypothalamus secrete corticotropin-release hormone (CRH) in the hypothalamic-pituitary axis (HPA). This then stimulates the synthesis of it stimulates to synthesize adrenocorticotropic hormone (ACTH) which at the same time affects the adrenal gland which then increases levels of glucocorticoids (GCs)

found mainly in the amygdala. The prefrontal cortex and the hippocampus GCs have negative feedback to the brain. It also has a negative effect on glucocorticoid receptor binding which suppresses pro-inflammatory immune response. Long-term administration of GCs such as corticosterone induces depressive-like behaviors in experimental animals, suggesting that stress-related hormones play an important role in depression [6].

In recent studies, it has been shown that increasing adult hippocampus neurogenesis, and newborn neurons in the hippocampus have been proven to promote resilience toward depression [7]. Boosting neurogenesis before any symptoms of depression is also sufficient to alleviate depression and anxiety-like behavior [8], recovery of depressive-like symptoms but also in protective part, and the effects of antidepressants are also mediated by increased neurogenesis. Other studies conclude that AHN could be denied by depression and anxiety [9] but can be accelerated by antidepressants which can also restore the hippocampus volume back to normal. However, hippocampus volume cannot change if not treated with antidepressants. In addition, neurons within the DG are critical for hippocampal negative control of the HPA axis and support a direct role for adult neurogenesis in depressive illness. DG can modulate reward circuitry and emotional behavior through projections to the nucleus accumbens, prefrontal cortex, amygdala, and stress responses by regulating the HPA axis [10]. However, there is no investigation in depth of how neurons in the subiculum act when there are more neurons in the hippocampus.

1.2 Hypothesis

Increasing AHN in mice alters the patterns and activation of specific neurons in the subiculum to the lateral hippocampus under anxiety-depression conditions.

2. Material and Methods

2.1 Material

· For the FosTRAP2 experiment use genetically modified mice, transgenic FosTRAP2 (Jackson Laboratory; Fos2mt.1(iCreERT2)Luo/J; strain #(030323)), and Ai14D(Jackson Laboratory; B6l;129S6-Gt(ROSA)26sortm14(CAG-tdtomato)Hze/J; Strain #007908)for the fluorescence, mice lines were crossed in-house to generate FosTRAP2-Ai14 mice (hereafter referred to as FosTRAP2)

· Tamoxifen: 4-hydroxy-tamoxifen (4-OHT) intraperitoneal at a dose of 50mg/kg. 4TM (Sigma H6278) dissolved in an aqueous solution containing 10% DMSO and 10% Tween-80 in saline

· Fluoxetine: The selective serotonin reuptake inhibitor (SSRI) Fluoxetine is efficient in suppressing anxiety and depressive behavior and increasing neurogenesis in mice. The second group of each experiment will receive chronic fluoxetine intake (10mg/kg/day, in drinking water) for 4 weeks

· Corticosterone: Suspended in saline with 1% Tween 80 (polysorbate 80, polyoxymethylene sorbitan monooleate) and 0.1% DMSO (dimethylsulphoxide). Use a dose of 50mg/kg.

· Isoflurane: This is a non-flammable volatile anesthetic but carries a strong, pungent odor that makes it difficult to use for inhalational induction of general anesthesia and leads to an overall decrease in minute ventilation. Use a concentration between 4-5%+0.8-1L/min, or 0.3 - 0.5 mL/hr, only if needed as it can also bring unexpected results and can promote neuronal apoptosis.

For the FosTRAP2 experiment use the cell detection feature in NeuroInfo to identify FosTRAP2+cells and count them. Then use the DAPI channel as a method to register it in Mouse Brain Atlas. Have a microscope that can be set at 10x and can take images from different wavelengths to capture DAPI and tdTomato. Also, use GraphPad Prism and R (version 4.0.2) for figure design and statistical analyses.

Histology:

Use DAPI protocol. Equilibrate the sample briefly with phosphate-buffered saline (PBS). Dilute the DAPI stock solution to 300 nM in PBS. Add approximately 300 µL of this 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 for better contrast.

2.2 Methodology and timeline

To see the neurons of the brain or the activity, PET, fMRI, and FosTRAP2 with fluorescence are going to be carried out. PET and fMRI can be used to have a view of the activation of neurons in the subiculum as they can scan the glucose level in the brain, neurons need glucose to work and be active. FosTRAP2 with fluorescing can allow a clearer view of the neurons as they are fluorescing and can be seen clearly on the microscope. PET and fMRI are needed as in contrast with FosTRAP2, PET and fMRI scans keep the mice alive. Use PET and fMRI to have more reliable data as both should give the same results. PET and fMRI have been proven to be effective in mice mapping [11].

For each experiment there would be 4 groups, one group will increase AHN with physical activity, the second group will also increase AHN with fluoxetine, and the third and fourth group will not have any type of AHN

increase. The first, second, and third groups will also receive an injection of CORT during the experiment. This will give anxiety-like effects. Use CORT to avoid sensory stressors, as this will make other parts of the brain activate/fluoresce. The fourth group remains in control.

The first 4 weeks and 5 days will be the same for each experiment. During the first 4 weeks, the first group will be in cages with access to running wheels and water. While the second group will receive regular doses of injections of tamoxifen. The third and fourth groups are caged in standard conditions and all groups will have ad libitum access to food and water. In the next 5 days, all groups will be placed in a dark cage, immobilized, and with their ears covered to prevent any noise, to accustom them to the injection of regular saline injections and environment.

PET: After the 4 weeks and 5 days protocol, on the day after the first, second, and third groups the after day the first, second, and third groups received an injection of 18-F-FDG before CORT injection. In case the image is not clear and immobilizing the mice is not enough, use isoflurane as an anesthesia to immobilize the mice. Inject CORT while mice are immobilized with the lights off and ears covered to prevent any new stress in the mice. After injection of CORT, scan the brain and scan it again after 1 hour to compare the activity as CORT takes time to reach the brain of the mice. This improves the reliability of the data.

fMRI: After the 4 weeks and 5 days procedure, the following day the first, second, and third groups will receive the CORT injection while the fourth remains as control. In case the image gets blurry and is not clear, use the procedure mentioned before in PET with anesthesia. Inject CORT while mice are immobilized with the lights off and ears covered to prevent any new stress in the mice. After injection of CORT, scan the brain and scan it again after 1 hour compare the activity as CORT takes time to reach the brain of the mice. This allows us to have more reliable data.

FosTRAP2: After the 4 weeks and 5 days procedure, the following day the 4 groups will receive an injection of tamoxifen. Right after the injection of tamoxifen inject another injection of CORT to the first, second, and third groups. Maintain them in dark cages, immobilized, and ears covered for the next 12 hours in individual cages without any other stimulus. Then the mice will be sacrificed to see the fluorescence neurons.

3. Expected results

With the fluorescence experiment, more neurons should be seen under the microscope in the regions of the hypothalamus and subiculum when there is an increase in neurogenesis compared to the third and fourth groups. As with PET and fMRI scans, images should show more levels of glucose in the same regions where there were fluoresce as neurons consume energy for them to be active. Three experiments should express the same hypothesis, otherwise it is unclear why experiments would show when mice are alive or dead.

If the results end up having the same prediction as the hypothesis, this will have a high significance as it allows a better insight into the mechanism and link between anxiety and AHN, and more research could be done to reveal how the neural circuits are altered from the neurogenesis in a more cellular and molecular view.

4. Ethical issues

To put in balance the human and animal interests to decide whether animal experiments are morally justified or not [12] the 3Rs must be implemented (Replacement, Reduction, Refinement). Following the 3Rs, use 8 transgenic mice and the other 16 can be domestic mice to reduce costs. As this experiment does not put mice in lasting stress or harm it should be adequate and should not have any problem with ethical concerns.

5. Discussion

The result should show that the subiculum and hypothalamus have more activity, this finding can imply that other ways of making this region work, like electric shock or other medicaments. This can also imply that neurogenesis does not happen in the hypothalamus because it is more effective in the DG as more neurogenesis would result in more activity in the hypothalamus. The images of the PET and fMRI scans can be hard to see. Sound cannot be fully blocked so other parts of the brain can be active, regions related to sensory stimuli. Another limitation of the study is if corticosterone has the same effect on mice as on humans as they have different physiology, this also means that using anesthesia, or fluoxetine may have side effects. However, even with these limitations, the results should show that there is more activity in the hypothalamus and subiculum, as the sensory circuit is located in the cortex. For future research, scientists could get deaf mice from birth or use other drugs that are better studied in mice to prevent any side effects but still have a similar effect to anesthesia, or promote depressive behavior. Using IV drip as an alternative method to make the corticosterone inside the system can be consider as a method less stressful for the mice. Also rewards can be used after mice been tied up, so when the experiment happend mice would know that this action will lead to a reward and therefore it will not lead to stress.

In conclusion, increasing AHN in the brain has been shown to prevent depression and anxiety effects. Using fluoxetine as a method to increase AHN and using fMRI and PET to map the brain of the mice, alongside fluorescence mapping in dead mice allow to conclude that AHN may have the same effects in mice alive and post-death.

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