ISSN 2959-409X

Effects of Increased Adult Hippocampal Neurogenesis on Neuronal Output from the Subiculum to the Lateral Hippocampus under Anxiety

Lienyi Tang

The high school affiliated to the Yunnan Normal University, Kunming, 650106, China, ynsdfz2010@126.com

Abstract:

Numerous studies assessed the important involvement of adult hippocampal neurogenesis (AHN) in the regulation of stress. Although the precise processes underlying the promotion of AHN on the recovery of anxiety-induced symptoms have been thoroughly examined, they are still unclear.We observed the subiculum region and its neuronal output to the lateral hippocampal formation, which may serve as a conduit between AHN and the recovery from depression.In order to cultivate AHN with exercise and fluoxetine injection independently, we employed a mouse model. We then used FosTRAP2, PET, and fMRI to measure the subiculum's neural activity during anxiety.When considered collectively, our findings imply that raising AHN in anxious mice modifies the way some subiculum neurons in the lateral hippocampal region output.

Keywords: Major depression, Adult hippocampal neurogenesis, subiculum, lateral hippocampus

1. Background

1.1 Major depression

Major depression (MD) is an extremely crippling condition that affects between 100 and 200 million people globally on a 3% to 5% prevalence basis. Despite not being a homogenous illness, MD presents with basic symptoms such anhedonia and a tense mood, which are frequently linked to food and sleep difficulties, mental health issues, and psychomotor changes.

The majority of antidepressants used now are active compounds that alter monoamine systems; among traditional medications are selective serotonin reuptake inhibitors (SSRIs), like fluoxetine. The fact that these medications take time to become effective suggests that long-term downstream alterations as well as direct modulation of monoamines may contribute to their antidepressant effects.[1,2]

1.2 Adult hippocampal neurogenesis(AHN)

The hippocampal region is crucial for mood and thought processes. The exceptional ability of this brain region to generate new neurons in the dentate gyrus, where they mature into mature neurons and integrate functionally into the existing neural circuitry, is one of its most intriguing characteristics.[3] It's interesting to note that AHN is connected to mood and stress management in addition to cognitive processes like memory and learning.[4]Additionally, it is possible that raising AHN could lessen the negative effects of stress on mood and cognition because AHN buffers stress responses [5].In summary, this research lends credence to the notion that adult-born neurons may play a major role in the regulation of MD, even though the exact processes are yet unknown.

Accordingly, AHN's effects on stress-induced deficits were the subject of numerous studies, which mostly examined AHN's impacts on recovery but also touched on its protective role—a topic that has gained a lot of attention in the medical community and AHN-related research.

2. Literature Review

2.1 Increased AHN alleviates depression[1]

Increased AHN is sufficient to decrease depressive-like behaviors, according to earlier research on the neurotransmitter.[6]Furthermore, some results indicate that raising AHN both preventatively and in the recovery of depressive-like symptoms.[1] Increased neurogenesis is another way that antidepressants work in the therapeutic treatment of MD. Interestingly, the increase in adult hippocampal neurogenesis (AHN) would be causative rather than contemporaneous because its reduction or suppression limits

some of the benefits of antidepressants. This research work focuses on the intricate mechanism of AHN's depression-alleviation impact, which is currently under investigation.

2.2 Subiculum of the hippocampus is a key part of the stress response

A dorso-ventral segregation of function exists within the subiculum, as evidenced by studies of synaptic plasticity in the hippocampal-subicular pathway and anatomical data on the subiculum's dorso-ventral organization in relation to the hypothalamic-pituitary-adrenal (HPA) axis: the dorsal component appears primarily concerned with the processing of information about space, movement, and memory, while the ventral component appears to play a major regulatory role in the inhibition of the HPA axis.[7]
Lesion and stimulation studies also indicate that the hippocampus, acting via output neurons of the ventral subiculum, acts to attenuate stress-induced glucocorticoid release, suggesting that the ventral subiculum proper is principally responsible for ventral hippocampal actions on the HPA stress response[5,8],which is involved in the regulation of depression.
Therefore, we used the FosTRAP2 technique, an active neural labeling technique, fMRI and PET technology, which are two medical imaging techniques, assessing the effect of AHN increase in the subicular neural activity.

3. Research Approaches

3.1 Fluoxetine intake

Fluoxetine, a kind of selective serotonin reuptake inhibitor (SSRI), has been known to suppress anxiety and depressive behaviour and increase neurogenesis. It was used to increase the neurogenesis of the experimental mice(3 out of 12 sub groups in the Control-experiment Plan)

3.2 Voluntarily physical exercise [running]

Previous studies showed that mice allowed to run on a wheel had increased AHN compared to sedentary mice. Additionally, another study found that regular aerobic exercise was associated with increased gray matter volume in the hippocampus, which may be related to AHN.[9]
Thus, we adopted the running method to promote an increase in mice's neurogenesis(3 out of 12 sub groups in the Control-experiment Plan), and compared with the fluoxetine-treated groups. The mice would be housed individually in cages with access to running wheels and add libitum access to food and water.

3.3 CORT injection

Glucocorticoids are primary stress hormones used to give mice stress. However it is also involved in the decrease of AHN, so we inject tamoxifen 1hr before the CORT injection to reverse the effect of corticosteroids on AHN.[10]

3.4 FosTRAP2 system[11,12]

We used Fos-TRAP2 mouse technology to capture brain-wide neural activation patterns elicited during the initial stress exposure, especially in the subiculum region.

3.5 fMRI & PET

Both are medical imaging techniques, which are used to scan and read mice's stress-response images during their stressed state without killing them.

3.6 Histology and statistical analysis

Statistical Analysis Software Used: Graphpad Prism and R (version 4.0.2) were used for figure design and statistical analyses.
Imaging parameters: a miscroscope at a 10x can take images from different wavelengths able to capture DAPI and tdTomato
Cell counting and Registration: Used cell detection feature in NeuroInfo to identify FosTRAP2+cells.And used DAPI channel as a method to register results in Mouse Brain Atlas.

4. Methodology

4.1 Control-experiment plan

Table 1

FosTRAP2	G1	Physical activity+CORT injection
	G2	Fluoxetine Injection+CORT injection
	G3	no AHN increase+ CORT injection
	G4	noAHN increase+ no stress
fMRI	G1	Physical activity+CORT injection
	G2	Fluoxetine Injection+CORT injection
	G3	no AHN increase+ CORT injection
	G4	noAHN increase+ no stress

PET	G1	Physical activity+CORT injection
	G2	Fluoxetine Injection+CORT injection
	G3	no AHN increase+ CORT injection
	G4	noAHN increase+ no stress

G1-G4 corresponds to Group 1-Group 4
The mice are divided into 4 groups, 2 of which receive increased AHN before undergoing CORT injection; We used 2 different methods to increase neurogenesis-voluntary physical excercise and fluoxetine injection. Each group is divided into 3 sub groups, one subgroup will go under the FosTRAP2 fluoresence, another subgroup will have the fMRI procedure and the last third will receive the PET procedure.

4.2 Animals

Use male mice at the age of 11 weeks.
Genetically modified mice. Transgenic FOS_TRAP2 [CreER2]
And Ai14D 26sortm[CAG-tdtomato]

4.3 Tamoxifen:

Inject tamoxifen 1hr before the injection of corticoroid to half of each group. Use 4-hydroxy-tamoxifen [4-OHT] intraperitoneal at a dose of 50mg/kg. 4TM [Sigma H6278] was dissolved in an aqueous solution containing 10% DMSO and 10% Tween-80 in saline. All cages were changed the following day to avoid the re-uptake of 4-hydroxy-tamoxifen.

4.4 Corticoesteroids:

Inject corticoesteroids to the mice with a needle after 1hr has passed since the injection of tamoxifen. Suspend the mice in saline with 1% Tween 80 [polysorbate 80, polyoxyethylene sorbitan monooleate] and 0.1% DMSO [dimethylsulphoxide]

4.5 FosTRAP2 experiment groups

During the first 4 weeks, the first group is housed in cages with access to running wheels and add libitum access to food and water. The second group receives regular fluoxetine injections. And the third and fourth groups are housed in standard conditions with ad libitum access to food and water. After that, the mice will be placed in a dark cage and habitualized to the pain of injections via regular saline injections for 5 days. At the next day, the first, second and third group undergo an injection of tamoxifen and afterward receive afterwards an injection of corticoesteroid.
The fourth group remains under standard conditions(unstressed), and also receives tamoxifen injections. After 12 hours of housing in individual cages in darkness without disturbance stimulus, the mice will be sacrificed.

4.6 PET experiment groups

During the first 4 weeks, the treatment of the PET groups is consistent with the FosTRAP2 groups. Then, the mice will be placed in a dark cage and habitualized to the pain of injections via regular saline injections for 5 days. At the next day, the first, second and third group receives an injection of corticoesteroid. Fourth remains as a control. Scan the mice after injection and after 1 hour from last injection.
Materials:
18-F-FDG less than 10% of total blood volume between [1.6-3.2 mL] .
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:
Perform cross calibration of the clocks used and between PET scanner and dose calibrator. Control temperature between 26-34 ℃. Keep mice fasting for 8 to 12h and for 20h to decrease the blood glucose level.
Inject 18-F-FDG to mice before the reading. (Have the mice tied up to prevent any movement so the image doesn't get blury. Only use anesthesia and provoke surgical stresses if necessary .The main stressors associated with surgical procedures are physical.)

4.7 FMRI experiment groups

During the first 4 weeks, the treatment of the PET groups is consistent with the FosTRAP2 groups. The mice will be placed in a dark cage and habitualized to the pain of injections via regular saline injections for 5 days. At the next day, the first, second and third group receives an injection of corticosterone. Fourth group is the control. Scan the brain after injection and after 1 hour from the injection.
Materials:
Have a stronger magnet than usual to have a clearer view and resolution of the mice brain. 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 movement

so the image doesn't get blury. (only use anesthesia and provoque surgical stresses if necessary. The main stressors associted with surgical procedures are physical.)

5. Discussion

5.1 Expected results

Under the assumption that our hypothesis is correct, we should observe more fluorescent activity in the hypothalamus and subiculum in the groups with neurogenesis compared to the control groups with the CORT injection.

Furthermore, in order to validate our experimental methods, we should observe that the control groups without CORT injection have less TRAPed neurons in the subiculum and lateral hypothalamus.

According to *figure A*, the neurons activity is greater in the dorsal thalamus and hippocampus than other regions(like BLA-LA and S1BF)[13]

According to *figure B*, there is greater neural activity in the hippocampus region with physical activity or fluoxetine treatment.[13]

Thus, increasing AHN in mice under anxiety alters the output pattern of specific neurons in the subiculum to the lateral hippocampus.

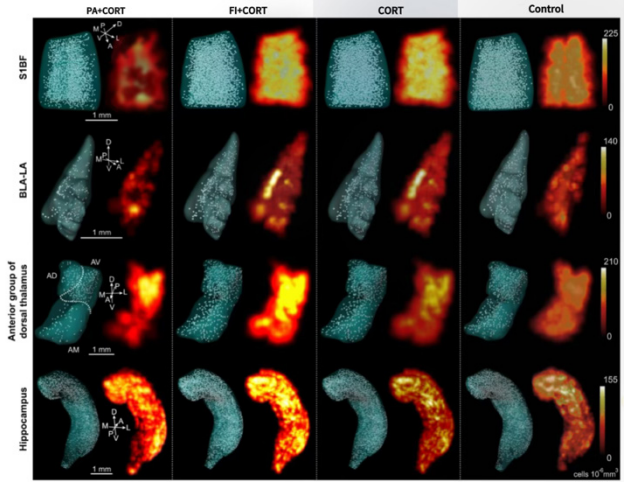

Figure A[stimulated]The image of the hippocampus,anterior group of dorsal thalamus,BLA-LA and SiBF in the FosTRAP2 groups.

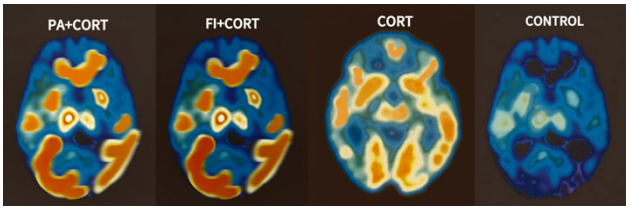

Figure B[stimulated] The image of the whole brain[sectional figures from the top] under the PET scanner.

5.2 Limits of our experimental designs

Since the factors causing the changes in the output pattern of the subiculum region are multiple and AHN's influence on the whole brain is elusive, we still have a lack of reliable evidences for our assumptions.

Additionally, optimization for the detailed operations in our experiments is required. For instance, tamoxifen injections can induce the knockout of the Baxgene, which can cause AHN to rise. Thus, that may affect the anticipated conditions that no AHN increase in the third and fourth groups.

Besides, how to accurately obtain the information of the activity of the neurons in the subiculum and lateral hippocampus should be refined.

5.3 Further direction

We could prove the effect of AHN on anxiety in the subiculum and hypothalamus region and could take this experiment further with improvements. The effect and mechanism of the HPA axis should be deeply studied, especially for the connection with the subiculum. And the experiment at the molecular level to obtain more detailed results is promising.

References

[1] Planchez, B. et al. [2021] Increasing adult hippocampal neurogenesis promotes resilience in a mouse model of depression, Cells. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8143348/ [Accessed: 05 August 2024].

[2] Wang, G. et al. [2021] Activation of 6–8-week-old new mature adult-born dentate granule cells contributes to anxiety-like behavior, Neurobiology of Stress. Available at: https://www.sciencedirect.com/science/article/pii/S2352289521000667 [Accessed: 05 August 2024].

[3] Anacker, C. and Hen, R. [2017] Adult hippocampal neurogenesis and cognitive flexibility - linking memory and mood, Nature News. Available at: https://www.nature.com/articles/nrn.2017.45 [Accessed: 05 August 2024].

[4] Carli, M. et al. [2021] Is adult hippocampal neurogenesis really relevant for the treatment of psychiatric disorders?, Current neuropharmacology. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8977631/ [Accessed: 05 August

2024].

[5] Herman, J. p et al. [2006] Role of the ventral subiculum in stress integration, Behavioural Brain Research. Available at: https://www.sciencedirect.com/science/article/abs/pii/S0166432806003287 [Accessed: 05 August 2024].

[6] Hill, A.S.; Sahay, A.; Hen, R. Increasing Adult Hippocampal Neurogenesis is Sufficient to Reduce Anxiety and Depression-Like Behaviors. Neuropsychopharmacology 2015, 40, 2368–2378. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1571536/ [Accessed: 05 August 2024].

[7] O'Mara, S. [2005] The Subiculum: What it does, what it might do, and what neuroanatomy has yet to tell us, Journal of anatomy. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1571536/ [Accessed: 05 August 2024].

[8] Herman, J. p et al. [1995] Contribution of the ventral subiculum to inhibitory regulation of the hypothalamo-pituitary-adrenocortical axis, Journal of neuroendocrinology. Available at: https://pubmed.ncbi.nlm.nih.gov/7550295/ [Accessed: 05 August 2024].

[9] Hill, A.S., Sahay, A. and Hen, R. [2015] Increasing adult hippocampal neurogenesis is sufficient to reduce anxiety and depression-like behaviors, Nature News. Available at: https://www.nature.com/articles/npp201585 [Accessed: 05 August 2024].

[10] Koutlas, I. et al. [2022] Characterizing and traping a social stress-activated neuronal ensemble in the ventral tegmental area, Frontiers. Available at: https://www.frontiersin.org/journals/behavioral-neuroscience/articles/10.3389/fnbeh.2022.936087/full [Accessed: 05 August 2024].

[11] Murra, D. et al. [2023] Patterns of neural activation during an initial social stress encounter are predictive of future susceptibility or resilience: A fostrap2 study, bioRxiv. Available at: https://www.biorxiv.org/content/10.1101/2023.03.10.532130v1 [Accessed: 05 August 2024].

[12] Tunc-Ozcan, E. et al. [2019] Activating newborn neurons suppresses depression and anxiety-like behaviors, Nature News. Available at: https://www.nature.com/articles/s41467-019-11641-8 [Accessed: 05 August 2024].

[13] D, R. et al. [2024] Innovative brain-wide mapping reveals a single memory is stored across many connected brain regions, SciTechDaily. Available at: https://scitechdaily.com/innovative-brain-wide-mapping-reveals-a-single-memory-is-stored-across-many-connected-brain-regions/ [Accessed: 05 August 2024].