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Increasing Adult Hippocampal Neurogenesis affects Neuronal Output Pattern from the Ventral Subiculum to the Hypothalamus

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Abstract:

Adult Hippocampal Neurogenesis, or AHN, has been discovered to alleviate anxiety and depressive behavior, thus leading to the presumption that the mechanisms underlying it might be somewhat linked to the regulatory circuitry of anxiety and depression. The subiculum is a region of the Hippocampus connecting the CA1 neurons and the Entorhinal cortex. It is the main output region of the Hippocampus. A great portion of the subiculum neurons form neuronal projections to the lateral hypothalamus, which is known to play a crucial role in the regulation of anxiety via hormone secretion in both rodents and humans. Thus, we hypothesize that Adult Hippocampal Neurogenesis regulates anxiety via altering the neural output from the subiculum to the lateral hypothalamus. In this research proposal we aim to prove that increasing AHN causes a direct impact on neuronal activity in the main output region of the Hippocampus--the subiculum region, under the effect of anxiety. This study uses the Fos-TRAP2 model to induce tdTomato expression in neurons with high recent activity while the mice was under the effect of corticosterone-induced anxiety. We compare the amount of TRAPed neurons in the subiculum region of the brain during anxiety, in mice with and without weeks of voluntary exercise (running), which leads to an increased neurogenesis. This provides insight into the effects of AHN on the subiculum-hypothalamus pathway, thus the mechanisms underlying its effect of anxiety alleviation.

Keywords: Adult Hippocampal Neurogenesis, Anxiety, Subiculum, Neural Activity, Hypothalamus, HPA axis, Fos-TRAP2.

1. Introduction

The Dente Gyrus is one of the only two regions proved to be sites for adult neurogenesis in rodents. In the recent decade, adult neurogenesis occurring in this region, also known as Adult Hippocampal Neurogenesis (AHN), has been a focus of interest and various studies done has implicated the existence of a relationship between AHN, anxiety, and depression. AHN has been shown to alleviate anxious behavior and depressive symptoms. Increasing AHN via knockout of the BAX gene promoted resilience in mice under chronic stress [4]. Another study demonstrated convincing evidence to prove the reversal effect of newborn neuron increase on depressive behavior induced by the UCMS model[1]. Furthermore, intake of common antidepressants, such as the serotonin selective reuptake inhibitor Fluoxetine, have also been found to increase neurogenesis in mice [3]. Moreover, it has been recently discovered and proved that voluntary running at a moderate intensity also boosts neurogenesis [2]. The subiculum is a crucial anatomical composition of the hippocampus, as it serves as its main output. Neurons in the subiculum extend axon projections to the entorhinal cortex and further regions of the brain. The role of the subiculum in anxiety regulation via its neuronal projections to the lateral hypothalamus has been long studied. The subiculum is the region in which connects the hippocampus to the hypothalamus, the first component of the HPA axis in terms of neural circuitry. It has been implicated that, lesions in the ventral subiculum region lead to an increased baseline level of Corticotropin Releasing Hormone (CRH) mRNA in the parvocellular paraventricular nucleus of the hypothalamus, suggesting a regulatory relationship between the two regions [5]. Thus, in this study, we aim to validate that one of the pathways through which AHN affects anxiety is the subiculum-hypothalamus pathway. The Fos-TRAP2 system is used to mark neurons activated upon CORT-induced anxiety with tdTomato fluorescence, and used to compare the amount of TRAPed neurons in mice with and without receiving increased neurogenesis via 4 continuous weeks of voluntary running. The focus of the study is on the neuronal activity in the subiculum region considering its crucial role of connecting the hippocampus, site of adult neurogenesis, and the Hypothalamus, site of intermediate anxiety regulation, and we wish to reveal the connections between the two in terms of their functions in anxiety regulation.

2. Materials, methods and Predicted Results

2.1 Animal

We use the offspring of transgenic FosTRAP2 and Ai14D mice, with equal numbers of male and female, all 11 weeks old, purchased from Jackson Laboratory. The mice were kept in standard conditions (12 hour light-dark cycle, temperature controlled at 25±1°C, group housed and with ad libitum access to food and water) for 11 weeks since birth before the experiment begins [7].

2.2 Fos-TRAP2 system

The $Fos^{2A\text{-}iCreER}$ knock-in allele (also called $Fos^{2A\text{-}iCreERT2}$ or «TRAP2») was designed to have expression of a tamoxifen-inducible, improved Cre recombinase ($iCreER^{T2}$) from the *Fos* promoter/enhancer elements - without disrupting endogenous *Fos* expression. The donating investigator reports this allele preserves endogenous *Fos*, including the highly conserved first intron and the 3' untranslated region critical for mRNA destabilization [8] .

2.3 Running wheel

In the first 4 weeks of experiment, group 1 and group 2 mice are group housed in cages with access to running wheels. Voluntary running increases adult neurogenesis in this group of mice, while the other half is housed in standard conditions without running wheel access. All mice live under regular 12 hour light-dark cycle with ad libitum access to food and water[2].

2.4 Saline injection and environment habituation

In order to prevent additional stimulus, all the mice are individually housed in dark cages and receives saline injection continuously for 5 days after the first 4 weeks. This makes the mice habitualized to the pain from the injection and the environment in which they will be injected with tamoxifen. To a certain degree, this can prevent activation of neurons out of our interest after tamoxifen injection[7].

2.5 Tamoxifen

All mice were injected with tamoxifen 1 hour before CORT injection. We use 4-hydroxyl tamoxifen (4-OHT) at a dose of 50mg/kg body weight, dissolved in aqueous solution. The mice are then kept in a dark cage individually, in avoidance of sensual stimulus [7].

2.6 Corticosterone injection

1 hour after tamoxifen injection, we inject group1 and the first control group with corticosterone (CORT, 40mg/kg, suspended in saline with 1% Tween 80 (polysorbate 80, polyoxyethylene sorbitan monooleate) and 0.1% DMSO (dimethylsulphoxide)).(Yao Z et al. 2023) Group 2 and the second control group receives vehicle injection of the same volume. Then we house all mice individually in dark cages for 12 hours without food and water, before they are sacrificed for inspection[6].

2.7 Histology details

After 12 hours of stimulus avoidance, the mice are perfused transcranially in 4% PFA. The brains are dehydrated with 20% sucrose. Coronal sections of 20μM was collected with a cryostat and immediately mounted to a glass slide. Slides were washed with PBS and incubated with DAPI. 1:10000 for 10 min [7].

2.8 Imaging

Images were taken with a microscope at 10x objective and imaged at 546nm (FosTRAP cells) and 405nm (DAPI) and then the images are later processed for data collection [7].

2.9 Cell counting

We use the automated cell detection function of NeuroInfo (MBF Bioscience, Willson) to collect data from the images. The NeuroInfo 'Registration' feature was used on the DAPI channel to register individual sections to the Allen Mouse Brain Atlas [7].

2.1 0. Data processing

We use GraphPad Prism for figure deign, data processing and statistical analysis [7].

3. Predicted Results

3.1 Validate the effectiveness of the running wheel method

Through comparing the neural activity in the lateral hypothalamus from the first control group (without neurogenesis and stressed) and the second control group (without increased neurogenesis and unstressed), we validate our experimental method of corticosterone injetcion was effective in inducing stress. We compare the amount of active neurons in the lateral hypothalamus of mice in group 2 (increased neurogenesis and unstressed) with the second control group (no increase neurogenesis and unstressed) to examine the effectiveness of the running wheel method in increasing neurogenesis.

3.2 Increasing voluntary excercise alters neuronal activity in the subiculum region

We plan to compare the amount of active neurons marked with tdTomato in the group that receives increased adult neurogenesis before undergoing stress (running wheel group) and the group that doesn't receive neurogenesis before undergoing stress (the first control group), and

through statistical analysis we wish to discover a difference in the activity of neurons in the subiculum region of the brain. If a distinct difference is discovered then it is sufficient to suggest that adult neurogenesis affects the output behavior of subiculum neurons to the lateral hypothalamus, thus interferes with the neural circuitry of anxiety regulation.

3.3 A distinct population of subiculum neurons, the behavior of which under anxiety is specifically altered by AHN, is identified.

Through further analysis of the images from group 1 which were stressed after neurogenesis and group 2 which was unstressed after neurogenesis, we wish to identify a specific group of neurons, the alteration in behavior of which is directly influenced by hippocampal neurogenesis specifically under anxious conditions.

4. Discussion

Our study aims to indicate how AHN affect neural output activity in the subiculum region to the hypothalamus, which is a pathway known for anxiety regulation. Thus, through this study, we hope to provide more insight into the mechanism, in terms of neural circuitry, behind the regulatory effect of AHN on anxiety.

The neural circuitry connection between the hippocampus and the hypothalamus has been widely and deeply studied. Theories on their anatomical and functional connections regarding with anxiety regulation are well established in the field. The hypothalamus receives input from cells expressing glucocorticoid receptors in the subiculum region of the hippocampus and such input plays a negative feedback control mechanism on the HPA axis which most directly regulates anxiety via corticosterone secretion regulaion. This mechanism allows the hippocampus to monitor the concentration of circulating glucocorticoids in the blood and respond back to the HPA axis via the projections mentioned above. Considering that AHN has been implicated to alleviate anxious and depressive symptoms, our study aims to dive further into the circuitry structure that AHN affected that eventually caused anxiety alleviation. Compared to preexisting studies throughout the field, our study offers more insight into the details of its mechanism. One of the main focuses of previous studies focusing on AHN is its direct impact on alleviating anxiety and depressive symptoms, which is mainly asses with behavioral models; Studies found that increasing AHN before chronic stress promotes resilience in UCMS depression model [1]; Another study indicated that, increasing neurogenesis via BAX gene knockout alleviates anxiety and depression-like behavioral symptoms [4].

5. Conclusion

This study discovers the effect of AHN deirived via voluntary running on specific neuronal populations in the subiculum region projecting to the hypothalamic terminal of the HPA axis; It identifies the specific population, the behaviour of which under specifically anxious conditions is altered by adult hippocampal neurogenesis.

6. Future directions

Given that a homologous characteristic exists between anxiety regulation circuits of mice and humans, which in this case the HPA axis, and the similarities of both anatomical structure and neuronal mechanisms of the hippocampus, the findings of our study could help form the crucial groundwork that supports the clinical applications of AHN on anxiety alleviaion in humans.

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