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Altering adult Hippocampal Neurogenesis in the dentate gyrus through varying glucocorticoid receptor concentrations

Minheng Cui

YKPAO School High School Campus, China, Shanghai

jonney_minheng@hotmail.com

Abstract. Adult Hippocampal Neurogenesis, short for AHN, is an essential yet foundational process of producing new nerve cells in the dentate gyrus of the hippocampus. It is a fact that increasing AHN will enhance people's ability to learn, memorize, regulate mood, etc. Therefore, scientists strive to pursue the factors which influence AHN. Until now, research had proved that GR activity is associated with the degree of AHN. Additionally, a high GR activity will potentially reduce the level of ANH and an optimal level of GR activity will create the optimized level of AHN. Consequently, how a low level of GR activity will possibly influence AHN will be examined in this paper. For Experiment 1, the GR antagonist reduces the GR activity in 8-year-old male mice: C57BL/6 and BrdU highlights the newly generated neurons in the dentate gyrus. As an ideal result compared to the control group, low GR activity will lead to a decrease in the AHN. For Experiment 2, 3 minor experiments will be constructed to test the GR activity and AHN of the mice in 3 different conditions (low, controllable, and uncontrollable stress). And Nanobit Assay that is separately attached to the GR and the ligands will present fluorescent when combined. Additionally, the method of CRISPR-CAS9 is used to knock down either GRα or GRβ when the other one is measured, and BrdU is also used to light up the neurons to measure the degree of AHN. The ideal result will present a number greater than 1 for the ratio between GRα/GRβ and AHN decreases for the low-stress group and uncontrollable-stress group. Oppositely, the ratio will be smaller than 1 for the controllable stress group, meaning AHN increases. Conclusively, the output will demonstrate a reversed "U" shape with the x-axis being the level of GR activity and the y-axis presenting the level of AHN. Despite the experiments that have proceeded on mice, the significance of them is people will have certain references to the influences of AHN to prevent those conditions or stress which will negatively affect AHN.

Keywords: Glucocorticoid Receptors, Adult Hippocampal Neurogenesis, Cortisol

1. Introduction (Big & Small Pictures)

Previous research has proved that GR is released as a stress response of the body and it will inhibit AHN. Specifically, GR can be classified into two types: GRα and GRβ (types of nuclear receptors) are α and β isoforms(two or more than two similar functional proteins with different amino acid sequences) of Glucocorticoid Receptors which affect Adult Hippocampal Neurogenesis heavily. Specifically, GRα inhibits the scale of AHN and GRβ hinders the function of GRβ, so if more GRβ is present than GRα, the level of AHN will certainly increase [1].

Elaborating more base on that, a prediction will be altering the concentration of GR will influence the degree of AHN. Additionally, previous experiments had tested that AHN is maximized at the optimal

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level of GR activity with controllable stress and AHN is minimized at the high level of GR activity with uncontrollable stress [2].

Consequently, this paper seeks to test how will the level of AHN be affected by the low level of GR activity (low stress). Therefore, a meaningful hypothesis is that a low level of GR activity will present a low level of AHN, and the relationship between GR activity (x-axis) and AHN (y-axis) will emerge a parabola concaving downwards.

2. Method

1) **CRISPR-CAS9 Genome Editing Technology:** Allows researchers to amend DNA sequences of living organisms. By breaking this technology down, "CRISPR" refers to a DNA sequence containing special sequences, and "CAS9" chops off certain DNA. It will be employed in the experiments to eliminate either GRα or GRβ while the other one is being examined, so each of them could be separately counted and the ratio between them could be calculated. By designing a complementary RNA sequence to GRα or GRβ the CAS-9 enzyme could be synthesized with it to locate the position of the targeted DNA which contains the GR [1]. When it's found, the CAS9 enzyme automatically produces the CRISPR system to bind with the DNA and cut it off which ultimately knocks out the gene [3].

2) **GR antagonist:** Since the following experiments want to confirm how the level of AHN will alter when the GR activity is low expressed, the GR antagonist will be a suitable choice. The GR antagonist first performs a competition with the cortisol for binding to a GR. When it's successfully coalesced, it allows a molecular change to perform certain interactions with DNA sequences to modulate gene expression [4]. Since then, it will entail the signaling pathways to block cellular reactions so less protein and gene transcription will be created. Overall, the GR antagonist could alter the cellular response produced by the GR combining with glucocorticoid hormones or arouse a negative feedback system to decrease the amount of glucocorticoid production to decrease GR activity [5].

3) **Nanobit Fluorescent Assay:** This technology introduces a change in fluorescence when GR and GC are combined to form a conformational change. It contains two complementary fragments of Nanobit and the GR mergers with a large bit with a size of 18 kDa. Additionally, the cortisol is cloned with a smaller Nanobit with a size of 1.3 kDa. Thus, when the receptor and cortisol bind with each other, the Nanobit will trigger to get closer and finally present fluorescent after a certain amount of time. The time factors also vary the extent of luminescence [6].

4) **Bromodeoxyuridine:** Short for BrdU, will be used to show the surge or proliferation of new neurons to conclude the changes in the level of AHN. In the following experiments, BrdU will be injected into the mice intraperitoneally or eaten together with its diet. Furthered more, a microtome will be used to dissect its brain. Then, DNA denaturing is needed for exposing the BrdU and it could be achieved by putting the mice's slice of brain into a 95 degrees Celsius water bath. Afterward, anti-BrdU antibodies will merge with BrdU to create illumination. Finally, observing microscopely the brain slice can examine the level of AHN [7].

3. Student Design Experiments

3.1. Equipment Needed

1) Microscope: observing the brain slice.
2) Water bath: denaturing DNA.
3) Microtome: slicing the mice's brains.
4) Mice: C57BL/6J, short for B6, 8-week-old male mice.
5) Three Cages (Crude, enriched and normal): for mice in the low, controllable and uncontrollable stress group.
6) Mice Exercising Equipment: provide exercise for the controllable stress group.
7) Noise Machine: provides inescapable shock for the uncontrollable stress group.
8) Petri Dish: for storing the slices of mice's brains.
9) Mice food & Water: for incorporating BrdU or for simply maintaining alive.

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3.2. Experiment 1: When low GR activity is expressed, how would the level of AHN alter?

Procedure:

1) Prepare the materials in the list except the crude cages, enriched cages and noise machine.
2) Inject GR antagonist into the body of the mice, provide the mice with food and incorporate BrdU into the diet.
3) Slice the mice's brain using a microtome and place it under the microscope to observe the presence of nerve cells.
4) Record the number of newly generated neurons in the dentate gyrus and compare the changes with normal C57BL/6J mice not subjected to GR antagonist, serving as the negative control.

3.3. Experiment 2: Constructing 3 minor experiments, investigate the mechanism of GR activity and how it affects AHN.

Procedure for Experiment 2:

1) Prepare all the materials in the equipment list.
2) Inject GR antagonist into the body of the mice and put it inside the crude cage/mundane cage and provide chronic noise as inescapable shock/luxurious cage and provide accompanies, enriched variety of food and exercising equipment.
3) Inject the Nanobit Assay into the mouse.
4) Repeat and construct the identical steps 3 to step 4 of Experiment 1.
5) Record the number of newly generated neurons and compare the changes with the C57BL/6J mice that were not subjected to stress treatment, serving as the negative control.

3.4. Results

3.4.1. Experiment 1

Table 1. Low level of GR activity contributing the level of AHN

	Level of GR Activity	**Level of AHN**
Low GR Activity	Decrease	Increase
		Decrease
		No change

When decreasing the GR activity, the level of AHN will present 3 possible results and a decrease in AHN will be expected.

3.4.2. Experiment 2

Minor Experiment 1:

Table 2. Low level of stress (poor environment and sedentary lifestyle) contributing to AHN

	GRα/GRβ	**Adult Neurogenesis**		
Low Stress	>1	Increase	No change	Decrease
	1	Increase	No change	Decrease
	<1	Increase	No change	Decrease

When adjusting the level of stress to low, the ratio between GRα/GRβ will display 3 possible results and each will match 3 results of the level of AHN. A ratio larger than 1 and a decrease in the level of AHN will be expected.

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Minor Experiment 2:

Table 3. Controllable stress (enriched environment, physical activity, learning) contributing to AHN

	GRa/GRb	Adult Neurogenesis		
Controllable Stress	>1	Increase	No change	Decrease
	1	Increase	No change	Decrease
	<1	Increase	No change	Decrease

When adjusting the level of stress to controllable, the ratio between GRα/GRβ will display 3 possible results and each will match 3 results of the level of AHN. A ratio smaller than 1 and an increase in the level of AHN will be expected.

Minor Experiment 3:

Table 4. Uncontrollable stress (chronic stress, inescapable shock, social defeat) contributing to AHN

	GRa/GRb	Adult Neurogenesis		
Uncontrollable & High Stress	>1	Increase	No change	Decrease
	1	Increase	No change	Decrease
	<1	Increase	No change	Decrease

When adjusting the level of stress to uncontrollable, the ratio between GRα/GRβ will display 3 possible results and each will match 3 results of the level of AHN. A ratio larger than 1 and a decrease in the level of AHN will be expected.

4. Discussion

The experiments and the ideal results demonstrated that the activation of different concentrations of GR will influence AHN by acting like a parabola concaving downwards. The possible mechanism that will explain that will be when the mice are under low stress or uncontrollable stress, the possibility of the production of GRα will increase. So as more GRα could combine with GRα, it will inhibit AHN [1, 8]. However, in controllable stress conditions, the possibility of producing GRβ will gradually increase. When more GRβ combines with GRα, it will prevent GRα from inhibiting AHN, therefore increasing the level of AHN (Note: the scenario of two GRβ will possibly happen, but their activity sometimes is too low for the presence of Nanobit fluorescent so it will not be accounted in the experiment).

Elaborating more, there are also other interpretations of this phenomenon. Controllable stress could influence positively on releasing cortisol since it plays an essential role in regulating immune responses and influencing the secretion of brain-derived neurotrophic factor (BDNF). BDNF is well known to assist the growth and survival of newborn neurons in the dentate gyrus and increase the level of AHN [9]. The other profound mechanism of why does different stress level associate with AHN could be investigated in the future. Conclusively, different stress can contribute to different activation of GR levels and the differential activation of GR will trigger the AHN separately [2].

The ethical issue is a major concern in experiments that requires dissections of mice's brain slices. It's not just significant for the welfare of animals and also for the honesty and credibility of the experiments. It's indispensable to prioritize the welfare of the mice in experiments to use quick and humane methods for slicing in order to minimize their distress and pain. Therefore, it's crucial to finish a transparent report to justify the experiment to the Institution of Animal Care and Use Committee to gain approval for the experiment. Experimenters should always maintain reverence for life and lofty thoughts when dealing with animals, and be grateful for their outstanding contributions to the field of science [7].

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5. Conclusion

In Experiment 1, there could be three possible results for the level of AHN (a decrease/increase compared to the control group or no significant change compared to the control group). According to the hypothesis stated before of the reversed "U" shape diagram, the ideal and prospective result will be a decrease in the level of AHN compared to control group.

Experiment 2 is divided into 3 minor experiments. In minor experiments 1 & 3 with low stress and uncontrollable stress, in the ideal results, the ratio between GRα and GRβ demonstrates > 1, indicating that more GRα is present so the production of new neurons is hindered meaning that the level of AHN has decreased. Furthermore, the controllable stress group illustrates an opposite ideal result of the ratio between GRα and GRβ performs to be < 1, so as more GRβ is present, it inhibits the function of GRα [1]. Consequently, the level of AHN increases because the GRα is being hindered by GRβ.

Additionally, knowing how different kinds of stress or GR activity are will be distinctly important. Knowing that controllable and a suitable amount of stress associated with education, communication, and exercise promotes AHN. Oppositely, acknowledging negative stress for people for example intensive shock and loneliness could contribute to harming AHN. Therefore, utilizing positive stress could develop the cognitive function of humans and increase overall being, people will have the tendency or alert to incorporate physical activity and education to promote brain health [10].

For future expectations and experiments, the other profound mechanism of why does different stress level associate with AHN and how will cortisol levels influence the activation of GR activity could be further investigated.

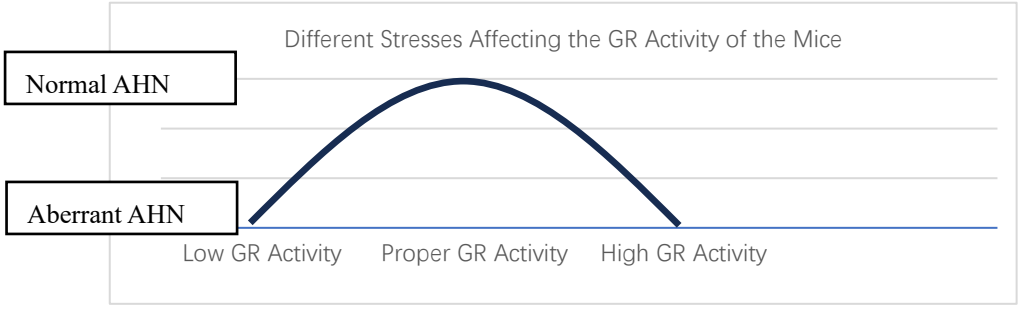

Figure 1. The relationship between GR Activity and the level of AHN

The relationship of GR activity and AHN will display as a parabola concaving downwards. An optimum level of AHN will present when at Proper GR Activity and an aberrant level of AHN will present at Low/High Level of AHN.

6. Limitations

For Nanobit Fluorescent Assay, there are several factors that will influence the reaction of this technique so it requires the experimenter's high sensitivity and exquisite control of the factors. For example, the fluorescence will become brightest around 30 minutes and if the time is not enough, the bright light will not have enough time to appear [6]. If the time is too long, then the fluorescence will gradually dim. Additionally, it will also be affected by the pH level and temperature. A slightly neutral alkali pH level in the range of 7.0-8.0 will be preferred for this reaction to happen. Also, the Nanobit Assay reaction will result in the fastest at around 37 degrees Celsius instead of room temperature.

An issue that Bromodeoxyuridine perceived is that it will not immaculately highlight all the proliferated cells. As the BrdU is coalesced into the DNA in the Synthesizing Phase when DNA is replicated so it only labels cells that are actively dividing. Consequently, it will not highlight all units of the proliferating cells because it only the other period of cell division won't incorporate will BrdU [7].

Although CRISPR-CAS9 is an unprecedented technology, it still presents the drawback of off-target effects. In detail, the guide RNA should be fully complementary to the DNA that's intended to be modulated so the CAS9 enzyme could be successfully guided to the location. However, the CAS9 sometimes won't be bothered by the mismatches of the DNA and RNA [3]. Moreover, because of the

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appearance of homologous or repetitive sequences which are similar to the target site, this also increases the possibility of a mismatch that leads to fallacious editing by CAS9. Therefore, as the false DNA or GR could be knocked out, it potentially leads to misinterpretations of the experiment results as the concentration of the GR could be inaccurately examined.

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