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Adult Hippocampal Neurogenesis Affect Place Cells and Affects the Spatial Memory Ability of Mice

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Abstract. Background: The fact that AHN can improve spatial memory is already very clear. However, place cells directly affect spatial memory, but they are located in CA1 region. AHN happens in DG, which is not directly connect with CA1.So,the the impact mechanism of AHN on place cells and spatial memory is still unclear. Method: I use the injection of tamoxifen and the extracellular recording to observe place cells in CA1 region. Running training is used to stimulate the AHN. Clozapine N-oxide (CNO) Injection is used to silence old place cells. Morris water maze is used to test spatial memory ability. The difference memory ability before and after CNO injection and AHN can reflect the impact of AHN on place cells and spatial memory ability. Expected Result: AHN mice can still have spatial memory even after CNO injection, because AHN create some new place cells or activate some old ones. However, no-AHN mice will completely get lost even after training. Conclusion: If the results is similar to our expectation, it can be proved that AHN can improve spatial memory by increasing the number or the activity of place cells.

Keywords: AHN, place cells, Morris water maze, spatial memory, running training, extracellular recording.

1. Introduction

Previous study[1]has shown that adult hippocampal neurogenesis (AHN) can significantly improve the cognitive performance of mice.In maze test,mice spent less time to find direction after AHN had been stimulated(especially in Morris water maze).

There also a study[2]provide a result that when mice accumulate spatial memory, a special type of neuron named place cells exhibits activity, which can be demonstrated as fluorescent development image of CA1 region in hippocampus.

So there comes a problem: AHN happens in dentate gyrus(DG),place cells located in CA1 region, there is a long distance between them. It have been proved that[3]the new neurons created by AHN can compete with the old neurons and finally replace them to improve memory ability, but this replacement only happens in CA3 region, which is directly linked with DG. It is still unclear whether AHN can creates new place cells or improve their activity.

The effect of AHN on place cells is really an interesting topic. I design an special experiment to clearly identify the impact. In our experiment, mice are divided into two groups. We use Morris water maze with different pictures on the container wall to test the memory ability of mice, and at the same time, we use extracellular multi-celluar ‘field’ recording to observe the CA1 region. Running is used to

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stimulate AHN, Tamoxifen[4] are used to open neuron pathway. We use Clozapine N-oxide(CNO) injection[5] to inhibit the function of the old place cell, and we aim to figure out if AHN will create more new place cells or replace the old ones.

My hypothesis is, AHN in DG can effectively activate some of the place cells in CA1 region, even can create some new place cells, so that AHN is verified to enhance spatial memory.

Due to limited research conditions, I am currently unable to conduct a complete scientific experiment to verify my hypothesis. I will provide an estimate of the results and make corresponding discussions in the following text.

2. Literature Review

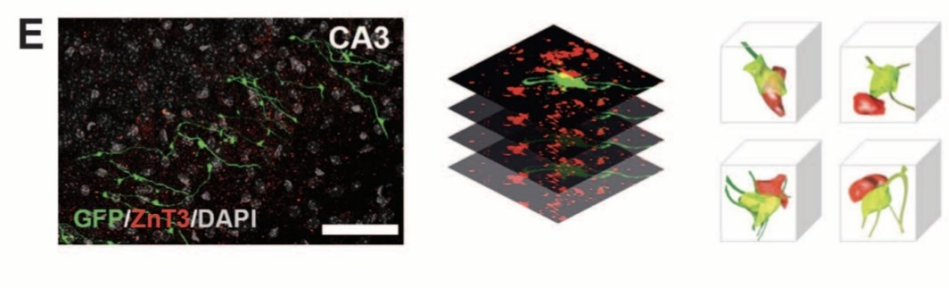


Figure 1. New neurons replace old neurons(under microscope)[3]

In the study of Katherine G. Akers et al[3],they proved that after AHN happens in DG, the newlyborn neurons have higher activity, they compete with the old neurons located in CA3 region(some of them are long term potentiation, LTP)and replace them. The process of the replacement can be observed through microscope, which is shown as figure1.

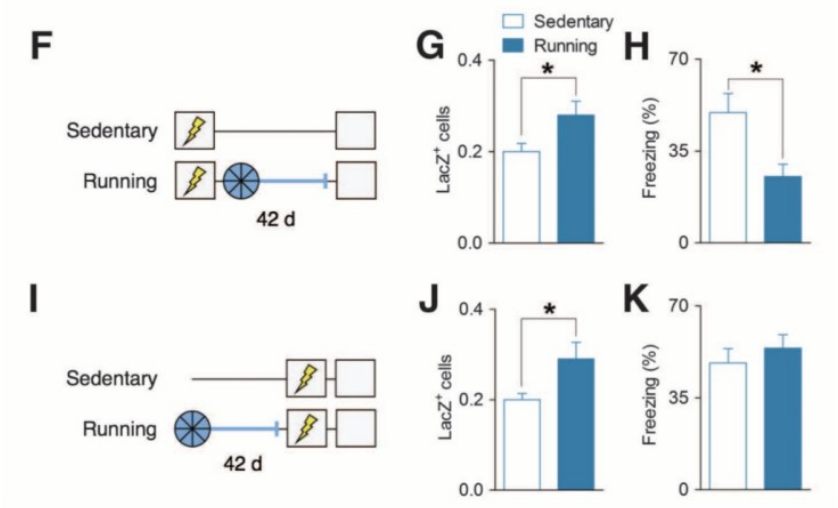


Figure 2. AHN can improve memory ability and clean up old memory[3]

This replacement improve the memory ability of mice, as figure 2(F,G,H),they doing running training on one group of mice after fear conditioning. The running mice(who have AHN)has obviously better memory performance than those who did not running.

But if the used LTP is replaced, some of the old memory will be cleaned up. Shown as figure 2(I,J,K),after the previous experiment left the memory, running training has been done immediately, which can stimulate AHN. The result demonstrated that AHN results in the decline of old memories.

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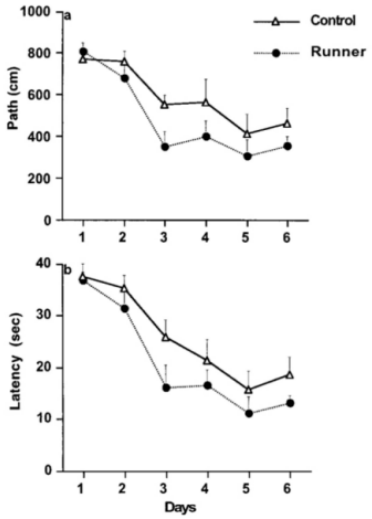


Figure 3. Running can improve spatial memory ability[1]

In the study of Henriette Van Praag et al[1],they also did running training to stimulate AHN, and let the runners and the control group do Morris water maze together. It is clear that the runners who has AHN can find the platform more quickly(figure3),which means AHN mice have better spatial memory.

3. Materials and Methods

3.1. Experiment Timeline

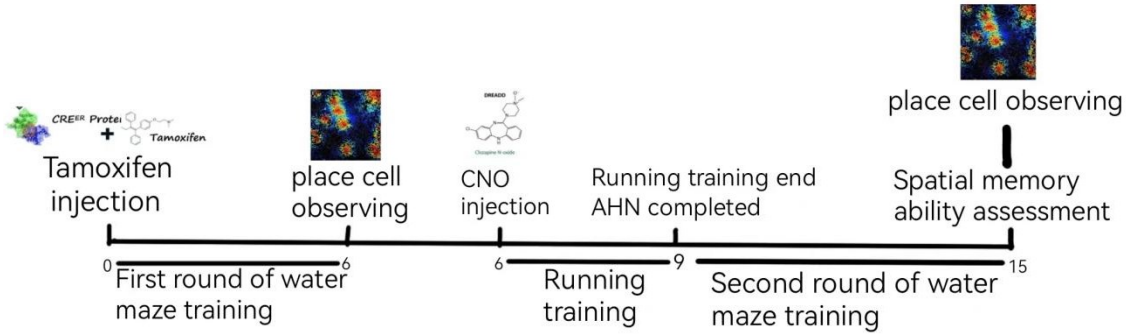


Figure 4. Experiment timeline

The entire experiment must follow a strict order. Before any training begins, Tamoxifen has been injected in mice brain. Next is the first round of water maze training, which takes about six days. There are four times of training each day. Statistical data will be recorded during this time, and extracellular recording(CA1 region only)is used to observe place cells of each mice. Then comes one day for mice to rest. CNO injection(CA1 region only)will be done before running training. After that is 3 day's running training(20 minute each round, 2 rounds each day, for experimental group only)to sufficiently stimulate AHN. After AHN is completed, there is second round of maze training, which also takes six days. When all training have been done, final spatial memory ability assessment and the second extracellular recording(CA1 region only)will take place.

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3.2. Experimental and control group

Two groups of adult female mice,3 months old[1].Each group include 10 individuality.One is experimental group,the other is control.They both in good physical condition.The swimming and running ability show no significant difference.

3.3. Morris water maze

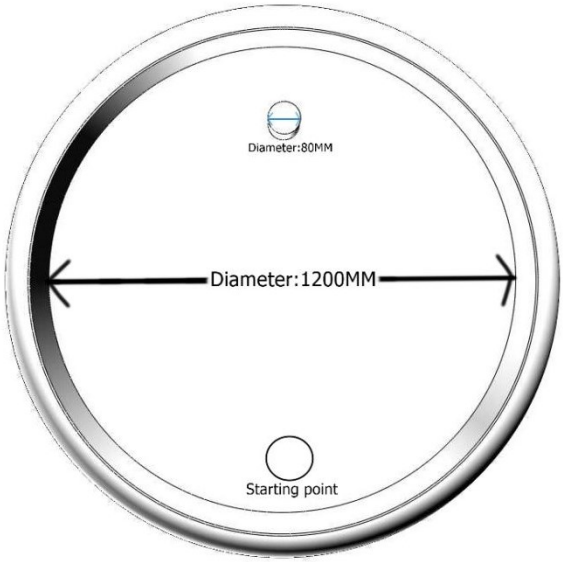


Figure 5. Morris water maze vertical view

The Morris Water Maze features a circular water container with a diameter of 1200mm and a depth of 400mm(deep enough so it is impossible for mice to escape from the container).The diameter of platform is 80mm,which is big enough for rat to stand on. It is located 100mm away from the edge of the container. Starting point and the platform are connected by a line of the same diameter, 50mm away from the other side of edge. The water depth and platform height are the same, both being 80mm(impossible for mice to step on the bottom).

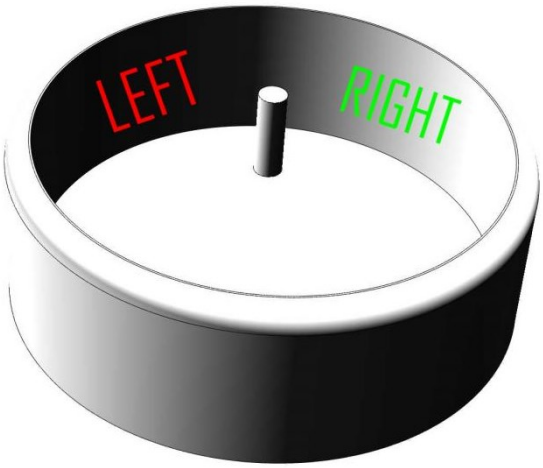


Figure 6. Layout of Morris water maze(3D view)

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Driven by fear, mice will try their best to find the platform. There are two big character: one red 'LEFT' on the left side of platform, one green 'RIGHT' on the right side of platform, mice can clearly observe them, and these characters are easy to remember. In first round of training, repeat training multiple times until every mouse can directly find the platform. In second round of training, according to our expectation CNO-injected mice without running will get lost, while the mice in running group who have AHN can find the platform.

3.4. Extracellular recording

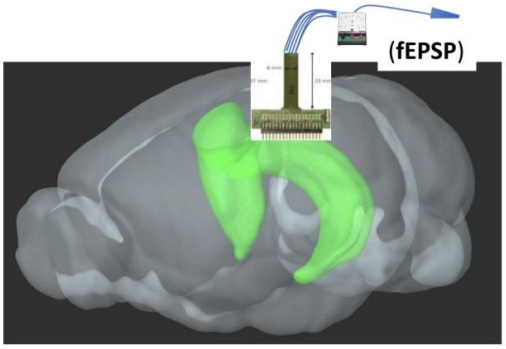


Figure 7. Extracellular recording of CA1 region[6]

During the both round of maze training and running training, recording electrode stay in the CA1 region of hipppocampus to record regions with high activity of place cells(through fEPSP),They can constitute a spatial map in mice brain. After CNO injection, there are no active place cells. After running training, there will be small number of place cell observed in particular mice.

3.5. Tamoxifen and Clozapine N-oxide (CNO) injection

Before all training begin,moderate and appropriate concentration of Tamoxifen has been injected in every mice in both two groups.The injection method of tamoxifen is based on the study of Jonathan Whitfield et al[7].First mix tamoxifen powder with corn oil,obtain corn oil suspension of tamoxifen,whose concentration is 10 mg/mL.[7]We use 1 mL syringe and a 21-gauge 5/8 needle(100μL to inject 1 mg) to inject intraperitoneally.This injection will stimulating the opening of neural pathways.

Before the running training, Clozapine N-oxide (CNO)will be injected. The method of CNO injection is from the study of Loris L Ferrari et al[8].I use CNO's physiological saline solution as an injection solution,0.5mL for each mice, concentration is 0.03mg/mL[8].Cerebral cortex injection is used to inject the medication directly into CA1 region. This injection will silence all the place cells in CA1 region.

3.6. Running training



Figure 8. ZL-015 mouse passive running wheel system[9]

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Running training aims to create a group of AHN mice, for the purpose of conducting comparative research with control group.

After first round of maze training, every mice in experimental group do running training. Each individual mouse runs on a passive running wheel system[9].Running training lasts for three days, twice a day, with each run lasting twenty minutes.

Previous study of Md Jahangir Alam et al[10] has proved that running can effectively stimulate adult hippocampal neurogenesis(AHN),so after this running training, we can obtain a group of AHN mice. Control group do not do any running so there is no AHN in their hippocampus.

3.7. Memory ability assessment and Statistical analysis

In the first round of maze training ,our requirement is that each mouse in both groups can quickly and accurately find the platform.

Table 1. Experimental Group Statistical Table

Average data for all mice Experimental group	Day 9 E	Day10 E	Day11 E	Day12 E	Day13 E	Day14 E	Day15 E
Path(cm)	Lost	850	800	670	660	655	650
Latency(sec)	Lost	34	25	23	22	21	21

Table 2. Control Group Statistical Table

Average data for all mice Control group	Day 9 c	Day10 c	Day11 c	Day12 c	Day13 c	Day14 c	Day15 c
Path(cm)	Lost	Lost	Lost	Lost	Lost	Lost	Lost
Latency(sec)	Lost	Lost	Lost	Lost	Lost	Lost	Lost

In the second round of maze test, two groups of mice training and testing individually. We use two indicators to testing the spatial memory ability of mice: the total distance they swam(path) and the time it took them to find the platform(latency).So we can have these statistical tabs.(Table1A and Table1B)

In the statistics, we will collect the path and latency of each mouse in each group, and take the average of the remaining data after removing the maximum and minimum values, then fill in the data into the table and prepare for the production of the statistical line chart.

If one mouse can not find the platform Within 40 seconds or their travel path within 1000 centimeters, they are judged as lost.

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4. Expected Results

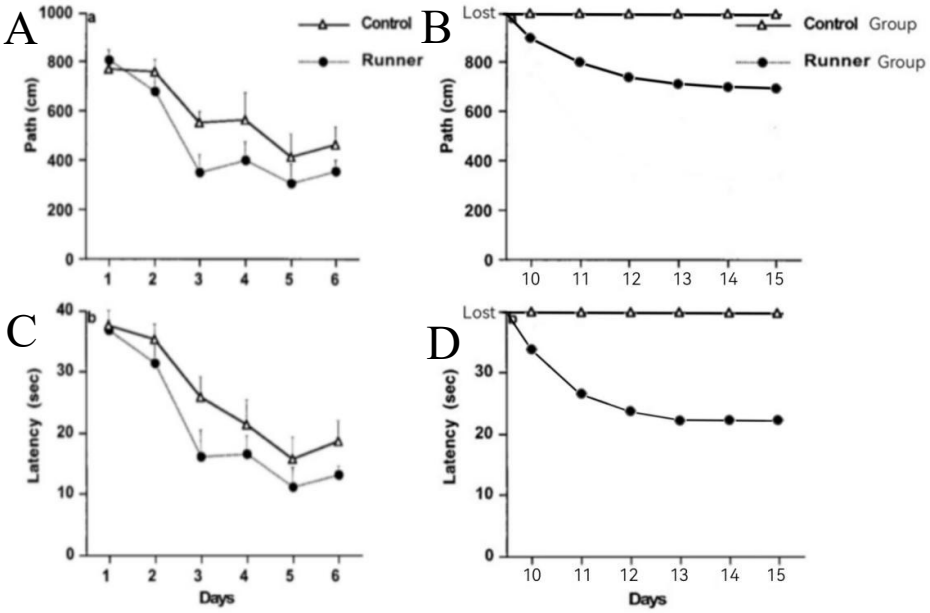


Figure 9. Expected results shown as line charts

My expected results shown as figure9(B,D),based on the statistical results in table1.I expect that at the second round of maze training, mice of both two groups will get lost, because their spatial memory ability has been dramatically weaken by the injection of CNO. Control group will get lost in every rounds of maze training, but experimental group who has AHN can find the platform in the end(although its spatial memory ability is weaker than before, shown as the comparison between figure9A/C and figure9B/D).

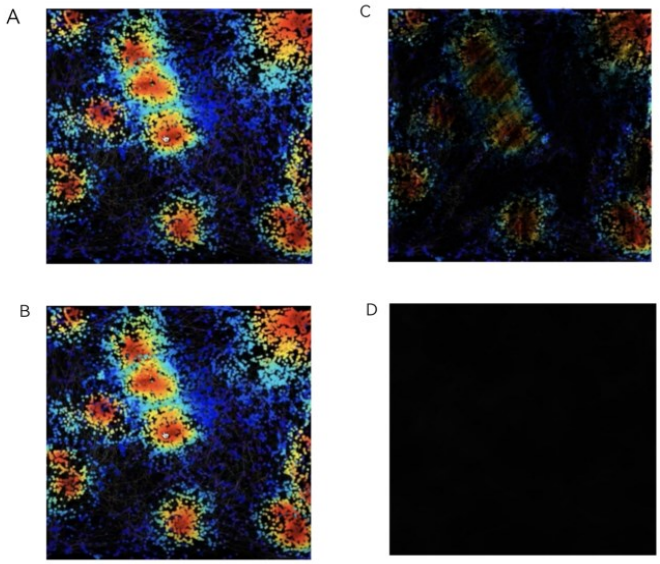


Figure 10. Spatial map in CA1 region[6]

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During first round of maze training, the CA1 region shows the same in all participants(figure 10A and figure 10B)[6].In the second round of maze training, we expect that the place cells of mice of control group who have no AHN is completely inactive and invisible, so the spatial map in CA1 region is dark(figure 10D).However, according to our expectation, AHN mice can activate new place cells or activate some new ones although the old ones have been silenced. So, in their CA1 region, we can observe an spatial map, but due to limited AHN capabilities, place cells activity levels will be lower than before CNO injection, resulting in a darker color tone displayed on the spatial map in CA1 region at this time(figure 10C).Accordingly, there is no spatial map can be observed in CA1 region of mice who is in the control group.

5. Discussion

If the results is similar to our expectation,we can arrival at two possible conclusions ,one is AHN can activate some old unworkable place cells;the other is AHN can create some new workable place cells.

If the first conclusion holds true, it indicates that no new place cells have been produced. So, everything back to the signal which can be the stimulator.

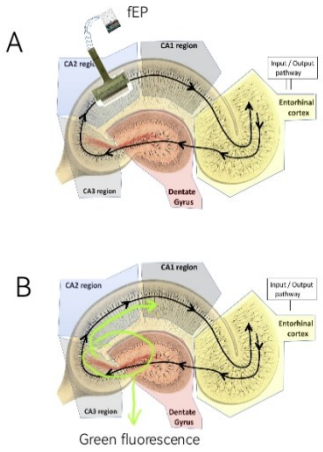


Figure 11. Detection method for potential AHN induced transmission of signals to the CA1 region[11]

It is clear that when AHN create new neurons which replace the old ones in CA3 region, there might be a signal transmit in hippocampal circuit. This signal will move into CA1 region and cause some changes. To confirm what the signal is, in the future maybe we can do some measurement on the entire hippocampal circuit, and find the area where the voltage change happens the most dramatically, then use centrifugation to separate the cellular components in the area, extract chemical substances that can be used as neurotransmitters, and determine what substance transmits the signal(figure 11A).Optogenetics is also an alternative solutions, we can use fluorescence to mark the new neurons in CA3 region, and observe the transfer of fluorescence. This method can also identify potential ways of signal transmission(figure 11B).

If the second conclusion holds true, this means there are some special cells located in CA1 or CA2 region, similar to neural stem cell in DG, who can divide into new neurons. Our experiment is not thoughtful, the CNO injection maybe destruct the entire CA1 region, it maybe inhibit the activity of potential neural stem cells.

What is also worth mentioning is that there was a result from Vegard H Brun et al[12] suggest that Rats with an isolated CA1 area showed normal acquisition of an associative hippocampal-dependent spatial recognition task. Spatial recall was impaired. These results suggest that the hippocampus contains two functionally separable memory circuits: The direct entorhinal-CA1 system is sufficient for recollection-based recognition memory, but recall depends on intact CA3-CA1 connectivity. So, this

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maybe lead to the third conclusion: AHN can directly linked with CA1 instead of transmitting any signals through hippocampal circuit.

In our experiment ,the conclusion that AHN can effect place cells has been proved. However, there is still much debate about its mechanism of action.

6. Conclusion

This study is based on three existing results: AHN enhances spatial memory ability, spatial memory ability is directly affected by place cells and AHN can repair damaged spatial memory[13]. Starting from the contradiction that DG is not directly connected to CA1, an experiment was designed to test whether AHN affects place cells. Due to limited conditions, the detailed experimental steps cannot be completed. But through speculation, I expect AHN to have an impact on positional cells, whether by creating new neurons in the CA1 region or enhancing the activity of old place cells. Correspondingly, the spacial memory of mice will be enhanced.

This study further tested the positive effect of adult hippocampal neurogenesis on memory and proposed new ideas for decoding spatial memory forms in the hippocampus

In the future study, gene editing[14] can be used as a means to knock out certain necessary proteins in order to inhibit place cells' activity, which can avoid the associated damage of CNO drug injection to the CA1 region. The connection between AHN and other brain regions can also be further studied, such as the hypothalamus and its associated emotional responses[15].

In summary, my study provides potential directions for future AHN and spatial memory research, and can enhance people's understanding and cognition of The operational mechanism of brain memory.

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