Exploring the Antidepressant Effect and its Molecular Mechanisms of Pattern Separation Training on Newborn Neurons

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Abstract: Newborn neurons in the dentate gyrus (DG) region of the hippocampus play a crucial role in the ability to perform pattern separation, and impairments in this ability have been associated with depression. Therefore, prior research has focused on designing interventions to enhance the number and activity of new neurons as a potential antidepressant approach. However, these interventions primarily emphasize physical exercise, with few studies exploring cognitive training. This proposal outlines a series of pattern separation training exercises in mice to investigate whether such training can exert antidepressant effects by improving the number, activity, or synaptic plasticity of newborn neurons. Additionally, it aims to determine the optimal timing for the training and the specific mechanisms underlying the antidepressant effects. The expected outcome is that the proposed pattern separation training will enhance the number, activity, and synaptic plasticity of newborn neurons, leading to a prophylactic antidepressant effect. This proposal contributes to the development of cognitive training programs, including pattern separation training, and provides support for the positive effects of similar cognitive interventions on newborn neurons.

1. Introduction

1.1. Depression

Major Depressive Disorder (MDD) has emerged as a global concern, affecting approximately 3% of the global population in 2020[1]. Depression is recognized as a leading cause of mental disabilities worldwide[2], and research on this condition has been a primary focus for the past two decades. However, the pathogenic mechanisms underlying depression are highly complex, presenting challenges for the development of effective treatment strategies. Impaired episodic memory is one of the factors contributing to the onset of depression.

1.2. Episodic Memory Function

Episodic memory function is a critical component of the major neurocognitive memory systems[3]. It refers to the ability to recall events within a spatial and temporal context[4]. The relationship between episodic memory impairment and depression is bidirectional: on one hand, impaired episodic memory can manifest as a symptom of depression, as cognitive features of depression often include deficits in episodic memory[5]. On the other hand, impaired episodic memory may contribute to the development of mental disorders such as depression and anxiety. Individuals suffering from depression frequently interpret ambiguous stimuli as threatening or negative, for instance, perceiving neutral facial expressions as signs of hostility.

Episodic memory capacity involves multiple processes, including pattern separation and pattern completion. Pattern separation refers to the ability to differentiate between similar experiences[6], and is mainly completed by neurons in the hippocampal DG region while pattern completion is predominantly carried out by neurons in the CA3 region of the hippocampus. Consequently, targeted pattern separation training may enhance episodic memory function.

1.3. Adult hippocampal Neurogenesis

Over the past two decades, recent studies have demonstrated that neurogenesis occurs throughout much of human life. In the human brain, the most extensively studied regions of neurogenesis are the subventricular zone (SVZ) and hippocampal DG region. Even though adult born born neurons (ABNs) are relatively few in number compared to existing mature neurons, they play significant roles in various functions. For instance, neurogenesis in hippocampal DG region, which is termed as adult hippocampal neurogenesis (AHN), is involved in processes such as memory encoding and pattern separation (see Figure 1).

The impairment of AHN can lead to disruptions in normal brain function and subsequently result in psychological or neurological disorders. For instance, the ability to discriminate between very similar situations relies on the activity of ABNs in the hippocampal DG region; therefore, damage to AHN can adversely affect

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pattern separation. Additionally, episodic memory function may be compromised, which is one of the contributing factors to depression. Conversely, enhancing the number and activity of ABNs in the DG may also promote an antidepressant effect by improving pattern separation abilities.

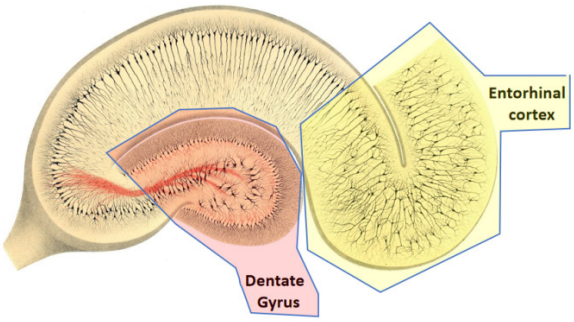

Figure 1. Partial regions of the hippocampus[7]. The original image was digitally adjusted and modified for clarity. The hippocampal DG region and the entorhinal cortex are visible.

1.4. Previous Studies

An increasing number of studies have demonstrated a connection between depression and impaired neurogenesis. Episodic memory function, including pattern separation, acts as a critical link between these two phenomena. Impaired pattern separation arises from a diminished capacity to discriminate information at the level of basic sensory processing, resulting in stimulus confusion. This confusion may further contribute to reduced mental flexibility and is closely associated with the activation of automatic thoughts, as described in cognitive behavioral theory, in patients with depression[4]. It is well-established that impaired neurogenesis can lead to deficits in pattern separation; therefore, targeted training designed to enhance neurogenesis activity may yield antidepressant effects.

Previous studies have demonstrated that running exercises in mice enhance neurogenesis and spatial pattern separation[8]. Furthermore, there is increasing evidence that physical exercise, dietary restriction, and environmental enrichment are associated with cognitive improvements, likely resulting from enhanced pattern separation and increased hippocampal neurogenesis in adults[9]. While most training in these studies has concentrated on physical exercises, cognitive training remains a promising avenue for improving neurogenesis and pattern separation. Research involving women with HIV has indicated that six weeks of mental and physical (MAP) training, combined with meditation and aerobic exercise, resulted in significant, lasting improvements in rumination and depressive symptoms, as well as enhanced discrimination of similar patterns of information[10].

Compared to humans, designing cognitive training for pattern separation in mice is both valuable and challenging. This proposal draws on cognitive-behavioral tests utilized in previous studies and adapts them for pattern separation training. The aim is to investigate whether this training can provide a novel approach to antidepressant treatment by enhancing the number, activity, or synaptic plasticity of ABNs.

1.5. Outline

The core purpose of this proposal is to investigate whether pattern separation training can promote AHN or improve the activity and synaptic plasticity of ABNs to exert antidepressant effects. Therefore, the main idea of the experiment is to give mice equal durations of pattern separation training and stressful situations, followed by behavioral tests to assess the level of depression in the mice after exposure to stress. However, the impact of pattern separation training may yield two potential outcomes: prophylactic and prognostic. Therefore, two parallel groups were established based on whether the pattern separation training occurred before or after the stress exposure. Additionally, to monitor the number of excitatory ABNs in response to challenging situations during behavioral tests in real time, it is essential to observe these neurons continuously. This proposal employs retrovirally transfected gene editing technology to introduce the GCaMP gene into the ABNs located in the DG region of the mice. Subsequently, excitatory ABNs will be visualized using a miniature microscope. To assess ABN activity and synaptic plasticity, the experiment will measure the levels of brain-derived neurotrophic factor (BDNF) and the expression of NMDA receptor (NMDAR) mRNA.

To summarize, the experiment is divided into four sections:

1) Detection of Viral Transfection Efficiency: To determine whether the retroviral transfection efficiency is sufficiently high to proceed with the subsequent experiments.

2) Efficacy Test of Pattern Separation Training: To evaluate whether pattern separation training has an antidepressant effect in mice.

3) Exploration of Prophylactic or Prognostic Effects: To investigate the optimal sequence of pattern separation training and stress exposure.

4) Exploration of Molecular Mechanisms: To investigate whether the quantity, activity, or synaptic plasticity of ABNs is altered.

2. Methods

2.1. Timeline

11-week-old C57BL/6 mice are selected for the experiment. 1 week after virus transfection, flow cytometry (FCM) is employed to assess the virus transfection rate. The experiment continues for a total of 9 weeks following the tamoxifen injection (see Figure 2). Subsequently, the mice are euthanized, and samples are collected.

The 9-week experiment is divided into three sessions, each lasting three weeks. In the first stage, no experimental interventions are imposed, as the ABNs are in the process of establishing synapses. In the second and third phases, pattern separation training or unpredictable

chronic mild stress (UCMS) is administered for three weeks, with the order of application varying according to the different groups. Behavioral tests specific to the degree of depression are conducted at the end of the 9-week experiment, and fluorescence imaging are observed using a miniature microscope.

After 9 weeks of experiment, the mice were euthanized, and the hippocampal DG region was isolated from the brains to determine the content of BDNF and NMDAR mRNA.

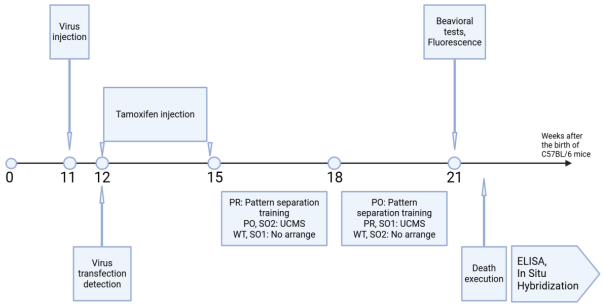

Figure 2. The timeline of all experiments was created using BioRender.com. The time cue is the duration from birth to death, with each test's time node and duration clearly indicated.

2.2. Animal Models

The animal model used in this experiment was the commonly utilized C57BL/6 mice. All experiments should be conducted in accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals. Mice will be housed three per cage in a 14:10 hour light:dark cycle environment. Pattern separation training, UCMS and behavioral tests will be performed during the light phase.

These mice should be divided into five groups as follows. However, due to the effects of hormonal fluctuations on CRE protein levels, data from male and female mice should be analyzed separately to ensure the rigor and applicability of the results. Each set of experiments was conducted in triplicate. Mice in either group received identical conditions for viral transfection and Tamoxifen injection, and were ultimately subjected to behavioral testing.

1) Pre-prognostic mice (PR): The pattern separation training occurred prior to the administration of stress.

2) Post-prognostic mice (PO): Stress exposure occurred prior to the pattern separation training.

3) Stress Only Group 1 (SO1): The distinction from the PR group is that no training is performed.

4) Stress Only Group 2 (SO2): The distinction from the PO group is that no training is performedtraining.

5) Wild Type (WT): Neither pattern separation training nor stress was administered.

2.3. Transgene Construction

Since the aim was to visualize ABNs in the hippocampal DG region and distinguish them from other mature neurons, this research selected the GCaMP gene to construct the genetic circuit. GCaMP, a widely used tool in neuroscience research, consists of a fusion protein of calmodulin and green fluorescent protein (GFP). It enhances green fluorescence when binds to Ca^{2+}, thereby enabling live imaging of neuronal activity at the single-cell level[11] (see Figure 3).

In addition, this research constructs a gene circuit in all mice through viral transfection and employs retrovirus-mediated single-cell gene editing (see Figure 4). The constructed retroviral vector contains the red fluorescent protein (RFP) gene, the GCaMP gene, and the Cre^{ER}-loxP system. Following the injection of the virus into the DG region, the viral DNA integrates into the dividing neuronal progenitor cells, resulting in the transfected cells becoming Cre^{ER}-positive and expressing RFP (Cre+; red).

Ascl1, one of the proneural transcription factors (TFs) and a primary regulator of vertebrate neurogenesis, is essential for the complete process of neurogenesis[12]. Cre^{ER} was positioned downstream of Ascl1 to ensure that only ABNs would express Cre^{ER} following viral transfection.

Cre^{ER}-loxP enables precise gene editing. In this experiment, loxP is positioned downstream of the terminator, and GCaMP is followed, both regulated by the general promoter. Consequently, when Cre^{ER} is expressed, the transcriptional stop signal is removed and Cre^{ER}-mediated recombination occurs to allow for GCaMP expression.

In summary, the final observations under the fluorescence microscope indicate that the transfected neurons in the hippocampal DG region exhibit red fluorescence. Meanwhile, the ABNs in an excitatory state express green fluorescence, which, when mixed with the red fluorescence, results in a yellow fluorescence.

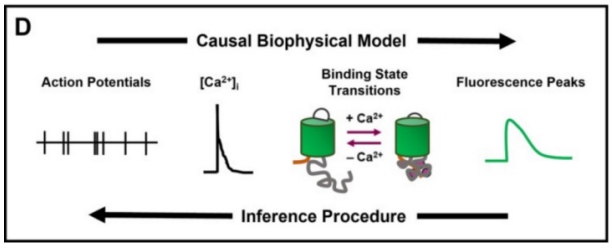

Figure 3. Schematic representation of the GCaMP structure and its function[11]. Neuronal excitation is accompanied by the influx of Ca^{2+} from the extracellular environment into the cell, which enables the cell to fluoresce under a fluorescence microscope.

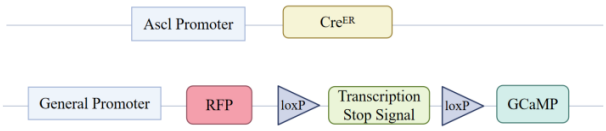

Figure 4. Gene circuits in the hippocampal DG region for successful viral transfection. The Cre^{ER} protein can be activated by tamoxifen, which subsequently leads to recombination. The transcriptional stop signal located between the two loxP sites is removed, allowing for the expression of the GCaMP gene.

2.4. Flow CytoMetry (FCM)

1 week after virus transfection, an equal number of mice from each group will be randomly selected and

euthanized. The hippocampal DG neurons will be isolated and analyzed using FCM.

FCM is a high-throughput technique utilized at the cellular level to obtain information about individual cells by detecting cellular fluorescence signals. The cells successfully transfected with the virus express RFP, while only those transfected with the GCaMP gene exhibit yellow fluorescence. Consequently, FCM can assess transfection efficiency by measuring the ratio of yellow fluorescence to red fluorescence, thereby enabling further evaluation of the number and intensity of virus-infected cells. The flow cytometer comprises four fundamental components (see Figure 5): a fluid system for transporting cells, a focused light source for excitation energy, a filter and photodetector for collecting scattered light and light emitted from fluorescently labeled cells, and a computer system for data storage and analysis. As cells traverse the point where the laser intersects the fluid flow path, those transfected with the virus containing RFP are excited by the laser, resulting in the emission of light at higher wavelengths. This emitted light passes through a filter, is collected and amplified by a photomultiplier tube, and is subsequently converted into digital information for storage in a computer for further analysis or transformed into image form[13].

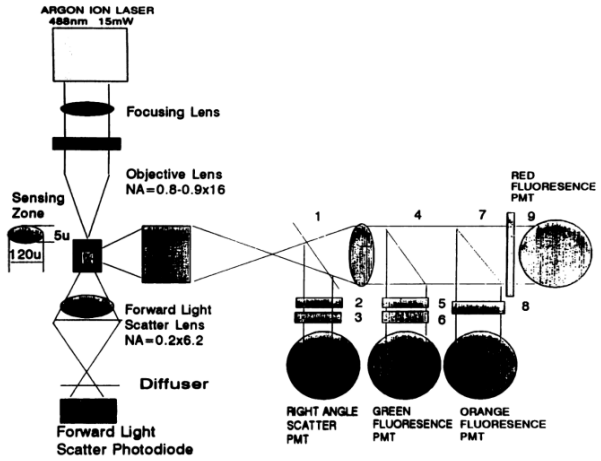

Figure 5. Schematic representation of a classical flow cytometry analyzer with an optical system[13]. The diagram illustrates the four fundamental components of the instrument. This experiment utilized a similar apparatus, the specifics of the components may vary.

2.5. Pattern Separation Training

Three weeks after the tamoxifen injection, the PR group receive three weeks of pattern separation training. The PO group will begin their training six weeks after the tamoxifen injection. The pattern separation training comprises three different tasks from easy to difficult. The simplest task is object recognition training, followed by eight-arm maze training and water maze training. Each of the three experiments lasts for 1 week.

Object recognition training is adapted from the novel object recognition test commonly used in research[14]. It takes place in a relatively open field, with two holes in the center, separated by a specific distance and measuring a few centimeters in diameter. Two different objects will be positioned above the holes, lightweight enough for the mice to push aside while adequately covering the holes. During the first phase of training, the mice will be allowed to freely explore the field for a designated period, during which they can push the objects aside to reveal the holes beneath. In the second phase, one of the objects will be replaced with a similar yet slightly different object, and a small food reward will be placed under this new object. If the mice can discern the difference, they will spend more time exploring and moving the altered object, ultimately discovering the reward. These two phases will be repeated several times. Experiments should be conducted when the mice are in a state of hunger.

The eight-arm maze training setup features one entry channel and seven exit channels, all radiating outward from a central point. Each channel terminates in a cup where a food reward can be placed. In each training session, only three of the passages contain a small food reward. Starved mice will be placed in the maze to search for food for a limited duration and will be removed immediately at the end of this time.

The water maze test employs the standard Morris water maze methodology[15]. Mice are tasked with locating a transparent circular platform situated on the surface of the water within an open pool. After the platform is consistently placed in a specific location for several trials, the time taken by the mice to find the platform gradually decreases. Subsequently, the position of the platform is altered, and the training is conducted once more.

2.6. Unpredictable Chronic Mild Stress (UCMS)

The PR and SO1 groups receive UCMS six weeks after the tamoxifen injection, while the PO and SO2 groups receive UCMS three weeks after the tamoxifen injection. UCMS, which consists of a variety of unpredictable chronic mild stressors, is used to induce long-term behavioral deficits and anhedonia in mice, which can be reversed[16]. The mice will be exposed to a stressful environment according to a pre-arranged schedule (see Table 1). Initially, the mice will be transferred to a clean room designated for UCMS manipulation and then placed in a stressful environment for at least 2h. The types of UCMS tests that can be selected are as follows:

1) Wet bedding: Pour approximately 500 ml of water into the mice's cage to moisten the bedding without creating standing water.

2) No bedding: All bedding material is removed from each cage.

3) Tilted cage: The cage is tilted at an angle of 45° and secured to prevent swaying as the mice move.

4) Light cycle disturbance: Mice are exposed to regular room light at night or have the lights switched off during the day.

5) Social stress: Mice are transferred from their own cage to the cage of neighboring mice that have been removed for 3h.

6) No bedding + water: Remove all bedding from each cage and add water to a specified depth. Dry the animals with a towel before placing them in a clean cage.

7) Restraint stress: Mice are placed in 50 mL plastic falcon tubes with openings on both ends to allow for breathing for 1h.

8) Predator smells: A filter paper soaked in 5 μl of 10% 2,4,5-trimethylthiazoline (a component of fox feces and the most commonly used synthetic reagent to induce innate fear in mice) is placed inside the cage.

9) No bedding + tilted cages: After the bedding is removed, each cage is tilted at an angle of approximately 45° and secured in place to prevent swaying due to mouse activity.

Table 1. An example diagram of the experimental arrangements at UCMS[17]. The tests conducted at UCMS were scheduled in a logical sequence over the course of 3 weeks.

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Week 1	Restraint stress	No bedding	Predator smells	Tilted cage	No bedding+ tilted cage	Predator smells	Light cycle disturbance
	Wet bedding	Tilted cage	No bedding+water	Social stress	Wet bedding		
Week 2	No bedding+water	Tilted cage	Predator smells	No bedding	Social stress	Social stress	Restraint stress
	Predator smells	Social stress	Wet bedding	No bedding+ tilted cage	Wet bedding		
Week 3	Tilted cage	Social stress	Predator smells	Social stress	No bedding	Restraint stress	Light cycle disturbance
	Wet bedding	No bedding+water	No bedding+ tilted cage	Wet bedding	No bedding+ tilted cage		

2.7. Behavioral Tests

After six weeks of pattern separation training or stress exposure, all mice undergo behavioral tests to assess the degree of depression. The mice are transferred to the testing arena 1h prior to the assessment to acclimate to the environment. All instruments used for each test are sterilized before each trial. Behavioral analyses are conducted by an experimenter who is blinded to the experimental conditions. All mice are euthanized following the final behavioral test[17]. The behavioral tests are divided into two parts: the tail suspension test (TST) and the forced swim test (FST).

For the TST, tape is affixed 2cm from the tip of the tail, and a cylindrical plastic tube is secured to the bottom of the tail. The mice are then suspended from a bar that is 30cm high. The test duration is between 5-10minutes, during which the experimenter should measure the duration of immobility in the mice[17].

In the FST test, mice are placed in a pool containing water that is 30cm deep and maintained at a temperature of 35°C. The pool has a diameter of 25-30cm and a height of 40-50cm. The test duration ranges from 5-10minutes[18]. The experimenter should record the behavioral performance and duration of the mice, including swimming time, struggling time, and floating time. A mathematical scoring model is employed to assess the degree of depression in the mice. Swimming, struggling, and floating behaviors are assigned low, medium, and high scores, respectively, and the total score is weighted to calculate the depression index.

2.8. Fluorescence

In order to observe the activity of ABNs in the hippocampal DG region in real time during behavioral tests, the mice are implanted with a gradient index lens (Grin Lens) on their heads. Fluorescence imaging of GCaMP-labeled active ABNs in the hippocampal DG region is conducted using miniature microscopy, and these images are recorded.

Micro-microscope technology represents a significant technological advancement in recent years. Weighing only 3g, this device allows for the observation of mice in their natural state while they engage in life activities, thereby minimizing any negative impact on the animals. It enables high-speed, real-time imaging at the sub-cellular level of approximately 0.4mm^2 of brain tissue. Data from over 200 neurons were captured simultaneously[19]. In this experiment, hippocampal neurons are recorded using a miniature microscope during behavioral tests. In the images, yellow fluorescence indicates ABNs in the hippocampal DG region while red fluorescence represents mature neurons in the same region or ABNs that have failed to undergo viral infection.

2.9. ELISA

BDNF is a widely recognized indicator of neuronal activity. To assess neuronal activity in the hippocampal DG region of mice, the enzyme-linked immunosorbent assay (ELISA) is employed to measure the content of BDNF in the hippocampal DG region following the euthanization of the mice.

ELISA is a widely utilized technique for detecting specific proteins in a sample, based on the fundamental principle that antigens bind specifically to antibodies. In this experiment, an antibody that specifically binds to BDNF is selected. Subsequently, a second antibody, labeled with a fluorescent marker, is used to bind to the BDNF antibody. Finally, the color intensity of the substrate reaction is measured using optical density to quantify the BDNF content in the sample.

2.10. In Situ Hybridization

In order to assess synaptic plasticity in the neurons of the hippocampal DG region of mice, the mRNA expression levels of NMDAR is measured using in situ hybridization following the euthanization of the mice.

Firstly, RNA probes that are specifically complementary to the NMDAR mRNA sequence are designed and labeled with either fluorescence or a radioisotope. These probes are then added to the samples to hybridize with the NMDAR mRNA sequence. Finally, fluorescence microscopy or autoradiography is employed to detect the hybridization signal between the NMDAR mRNA and the probes. The expression levels of NMDAR in the neurons of the hippocampal DG region will be quantified.

3. Expected Results

Mice that underwent pattern separation training exhibited superior performance on behavioral tests following stress exposure compared to those that did not receive such training. This group have higher number of ABNs, heightened activity, and enhanced synaptic plasticity. The sequence of stress application after pattern separation training proved to be better than the reverse order, yielding a more pronounced antidepressant effect, which means the prophylactic benefits of pattern separation training is better. In conclusion, the pattern separation training employed in this study may induce antidepressant effect through enhancing the number, activity, and synaptic plasticity of ABNs in mice.

3.1. Transfection Efficiency

The results of FCM in all groups of mice indicate that the virus transfection rate is sufficiently high, providing a foundation for the subsequent experiments.

3.2. Behavioral Tests Result

The results of the TST and FST tests indicate that the SO1 and SO2 groups of mice, which did not undergo pattern separation training but were conducted stress, exhibited the highest levels of depression. These levels were significantly greater than those observed in WT mice that either did not receive training or were not conducted stress, confirming that UCMS indeed induces depression in mice. The PR group, which underwent pattern separation training, displayed lower levels of depression compared to SO1, while the PO group that also received pattern separation training showed reduced depression compared to SO2. This suggests that pattern separation training is effective in alleviating depression in mice following stress. Furthermore, the degree of depression in the PR group is lower than that in the PO group, indicating that the antidepressant effects of pattern separation training are more pronounced in a prophylactic context rather than prognosis context (see Figure 6).

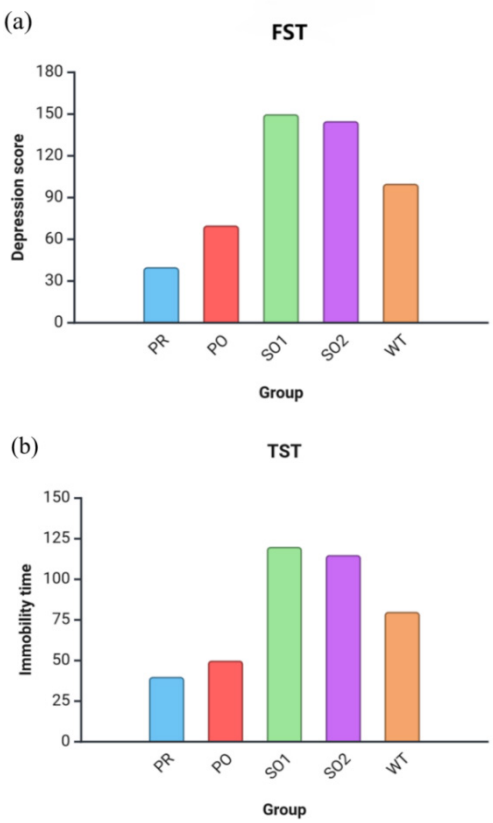

Figure 6. The expected results of the behavioral tests are illustrated in the image created with BioRender.com. (a) FST results indicate that PR and PO exhibit less depression compared to SO1 and SO2, with PR showing less depression than PO. (b) TST results demonstrate that PR and PO have less immobility time than SO1 and SO2, and PR has less immobility time than PO.

3.3. Florescence Result

The number of ABNs in the SO1 and SO2 groups should be lower than that in the WT group, as pressure delivery interferes with neurogenesis. The PR group exhibits a greater number of ABNs than the SO1 group, and the PO group has more ABNs than the SO2 group, indicating that pattern separation training enhances the production of ABNs. Furthermore, the number of ABNs in the PR group exceeds that in the PO group, suggesting that the prophylactic effect of pattern separation training is more pronounced. These findings align with behavioral studies, indicating that the increase in ABNs may be one of the mechanisms through which pattern separation training exerts a prophylactic antidepressant effect.

3.4. BDNF Content and Fluorescence Intensity of In Situ Hybridization

The BDNF content and NMDAR expression of SO1 and SO2 should be less than that of WT, because stress interferes with ABNs activity and synaptic plasticity. PR has more BDNF content and NMDAR expression than SO1, and PO has more BDNF content and NMDAR expression than SO2, indicating that pattern separation training improved ABNs activity and synaptic plasticity.

The results of PR are better than those of PO, indicating that the prophylactic antidepressant effect of pattern separation training is better. These results are consistent with the results of behavioral experiments, suggesting that one of the mechanisms by which pattern separation training has the antidepressant effect is to improve ABNs activity and synaptic plasticity (see Figure 7).

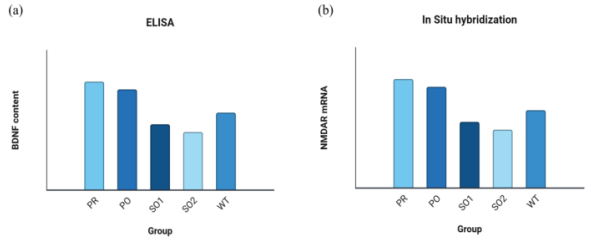

Figure 7. The expected results of the ELISA and in situ hybridization are illustrated in the image created with BioRender.com. (a) The ELISA results indicate that PR and PO exhibit higher BDNF content compared to SO1 and SO2, with PR showing greater levels than PO. (b) The in situ hybridization results demonstrate that PR and PO have elevated NMDAR expression relative to SO1 and SO2, with PR again showing higher levels than PO.

4. Discussion

Under the assumption that the fundamental operation of the experiment is sound, it is possible for the experiment to yield results beyond the expected results, which may lead to alternative conclusions. In addition to the expected results, the most probable outcomes are as follows: The prognostic effect of pattern separation training is superior to that of prophylactic measures; pattern separation training exhibits either a prophylactic or prognostic antidepressant effect; and pattern separation training positively influences one or more of the following: the number, activity, and synaptic plasticity of ABNs.

If the prognostic effect of pattern separation training is better, the level of depression in the PR group will be higher than that in the PO group. Additionally, the fluorescence, BDNF content, and NMDAR expression in the PO group will be superior to those in the PR group.

If pattern-separation training has only a prophylactic or prognostic effect, at least one of the differences between the PR and SO1 groups, or between the PO and SO2 groups, will consistently be non-significant in each test.

If the pattern separation training positively affects only one or two of the factors, the number, activity, and synaptic plasticity of ABNs, then the differences in fluorescence, BDNF content, and NMDAR expression between the groups with and without pattern separation training will be significant in only one or two tests.

It is also worth noting that if the results reveal no significant differences in the levels of depression among the PR, PO, SO1, and SO2 groups, this suggests that the pattern separation training employed in this experiment does not exert an antidepressant effect at the behavioral level. However, this does not imply that other forms of pattern separation training or cognitive training cannot yield antidepressant effects. In the future, alternative pattern separation training protocols can be developed for new experiments, and the design of cognitive training interventions can also be investigated.

Regarding the rigor of the experiment, the results of the FST and the TST of the five groups of mice should be similar. Additionally, the ranking of the depression levels among the five groups in both experiments, as well as the significance of the differences between the groups, should be consistent.

The method of real-time observation of the hippocampal DG region in mice using a head-mounted micro-microscope may have certain limitations, including a restricted field of view and insufficient resolution, which could impact the experimental results. Additionally, the assessment of the number of yellow fluorescently labeled neurons in the images may be subject to subjective bias during interpretation. Furthermore, another limitation of the current experiment is that BDNF content and NMDAR expression are measured in all neurons in the hippocampal DG region, rather than being restricted to ABNs. Pattern-separation training may also enhance the activity and synaptic plasticity of mature neurons, potentially contributing significantly to the antidepressant effects of this training. Therefore, future experiments should focus on the differential effects of pattern separation training on mature neurons compared to ABNs.

5. Conclusion

Based on the expected results, the pattern separation training proposed in this proposal may have a prophylactic effect by increasing the number, activity, and synaptic plasticity of ABNs in mice.

In contrast to previous studies, this proposal aims to investigate whether the designed pattern separation training has an antidepressant effect through its positive influence on ABNs. Additionally, it seeks to provide a new direction for the design of cognitive training aimed at preventing depression. In the future, this research will continue to explore a more effective pattern separation training protocol and the mechanisms underlying the impact of pattern separation training on newborn neurons.

References

1. COVID-19 Mental Disorders Collaborators. Global prevalence and burden of depressive and anxiety disorders in 204 countries and territories in 2020 due to the COVID-19 pandemic. Lancet. 2021 Nov 6;398(10312):1700-1712.
2. WHO (World Health Organ.). 2021. Depression. Fact Sheet, WHO, Geneva. http://www.who.int/news-room/fact-sheets/detail/depression
3. Tulving E. Episodic memory: from mind to brain. Annu Rev Psychol. 2002;53:1-25.
4. Gandy K, Kim S, Sharp C, Dindo L, Maletic-Savatic M, Calarge C. Pattern Separation: A Potential

Marker of Impaired Hippocampal Adult Neurogenesis in Major Depressive Disorder. Front Neurosci. 2017 Oct 26;11:571.

5. James TA, Weiss-Cowie S, Hopton Z, Verhaeghen P, Dotson VM, Duarte A. Depression and episodic memory across the adult lifespan: A meta-analytic review. Psychol Bull. 2021 Nov;147(11):1184-1214.
6. Ngo CT, Michelmann S, Olson IR, Newcombe NS. Pattern separation and pattern completion: Behaviorally separable processes? Mem Cognit. 2021 Jan;49(1):193-205.
7. David Warmflash, MD， "The Founding of Neuroscience" Visionlearning Vol. SCIRE-2 (8), 2016. The Founding of Neuroscience | Scientists and Research | Visionlearning
8. Creer DJ, Romberg C, Saksida LM, van Praag H, Bussey TJ. Running enhances spatial pattern separation in mice. Proc Natl Acad Sci U S A. 2010 Feb 2;107(5):2367-72.
9. Brianne A Kent, Charlotte A Oomen, Pedro Bekinschtein, Timothy J Bussey, Lisa M Saksida, Cognitive enhancing effects of voluntary exercise, caloric restriction and environmental enrichment: a role for adult hippocampal neurogenesis and pattern separation?, Current Opinion in Behavioral Sciences, Volume 4, 2015, Pages 179-185, ISSN 2352-1546.
10. Millon, E.M., Lehrer, P.M. & Shors, T.J. Meditation and Aerobic Exercise Enhance Mental Health Outcomes and Pattern Separation Learning Without Changing Heart Rate Variability in Women with HIV. Appl Psychophysiol Biofeedback 47, 27–42 (2022).
11. Federico Iseppon, John E. Linley, John N. Wood, Calcium imaging for analgesic drug discovery, Neurobiology of Pain, Volume 11, 2022, 100083, ISSN 2452-073X.
12. Vasconcelos FF, Castro DS. Transcriptional control of vertebrate neurogenesis by the proneural factor Ascl1. Front Cell Neurosci. 2014 Dec 2;8:412.
13. McSharry JJ. 1994. Uses of flow cytometry in virology. Clin Microbiol Rev 7.
14. Lueptow LM. Novel Object Recognition Test for the Investigation of Learning and Memory in Mice. J Vis Exp. 2017 Aug 30;(126):55718.
15. Alfredo Meneses, Chapter 3 - 5-HT Pathways, Receptors and Transporter: Memory Functions and Dysfunctions, Editor(s): Alfredo Meneses, The Role of 5-HT Systems on Memory and Dysfunctional Memory, Academic Press, 2014, Pages 7-16.
16. Burstein O, Doron R. The Unpredictable Chronic Mild Stress Protocol for Inducing Anhedonia in Mice. J Vis Exp. 2018 Oct 24;(140):58184.
17. Tunc-Ozcan, E., Peng, CY., Zhu, Y. et al. Activating newborn neurons suppresses depression and anxiety-like behaviors. Nat Commun 10, 3768 (2019).
18. Antonio Armario, The forced swim test: Historical, conceptual and methodological considerations and its relationship with individual behavioral traits, Neuroscience & Biobehavioral Reviews, Volume 128, 2021, Pages 74-86, ISSN 0149-7634.
19. Zeng, YF., Yang, KX., Cui, Y. et al. Conjunctive encoding of exploratory intentions and spatial information in the hippocampus. Nat Commun 15, 3221 (2024).