The Efficacy of Pattern Separation Training in Antidepressant Treatment and the Exploration of Its Drug-Target Alternatives

Jia Liu *

Department of Marine Science, Sun Yat-Sen University, Zhuhai 519000, China

Abstract: Current treatments for MDD focus on pharmacological treatments, psychotherapeutic interventions, and somatic treatments. The core goal of this study is to develop an innovative non-pharmacological treatment method, which produces antidepressant effects similar to those of antidepressant drugs, mainly through pattern separation training in cognitive training, which has the advantages of being non-invasive, having fewer side-effects, and reducing relapse. At the same time, we use the transcriptome to detect changes in molecular mechanisms brought about by pattern separation training, find differentially expressed genes, and probe deeply into the molecular mechanisms behind them in order to find potential drug targets. Through this approach, we hope to provide more diverse treatment options for patients with depression, while reducing dependence on drug therapy.

1. Introduction

1.1. MDD and current treatments

The most prevalent and dangerous mental disorder worldwide is Major Depressive Disorder (MDD), commonly referred to as depression[1]. It is currently the third biggest cause of disease burden and is expected to rise to the top by 2030, according to the World Health Organization[2]. It is one of the most deadly and incapacitating diseases in the world. Major Depressive Disorder includes, Persistent low mood, loss of interest, sleep disturbances, poor concentration, ruminative thinking, cognitive rigidity, recurrent suicidal thoughts, perseverative or inflexible behavior, cognitive rigidity[3].

Current treatments for MDD primarily involve a combination of psychotherapy, medication, and somatic therapies[4]. The doctor will give different treatment options according to the degree of depression in the patient (Figure 1). (1) In terms of psychological treatment, the most common ones are Cognitive Behavioral Therapy (CBT) and Interpersonal Therapy (IPT). CBT assists patients in recognizing and altering harmful thought habits. IPT can lessen depressed symptoms by assisting patients in creating stronger social support networks and interactions. (2) On medication, Selective Serotonin Reuptake Inhibitors (SSRIs) and other antidepressants are commonly used to regulate neurotransmitter levels. When it comes to specifically targeted medications, NMDA receptor antagonists and 5-HT receptor antagonists are the most often used new pharmaceuticals[1]. (3) The most well-known somatic treatment technique for treating resistant depression is ECT. Recent years have seen the proposal of many techniques, including transcranial direct current stimulation (tDCS) and repetitive transcranial magnetic stimulation (rTMS)[5].

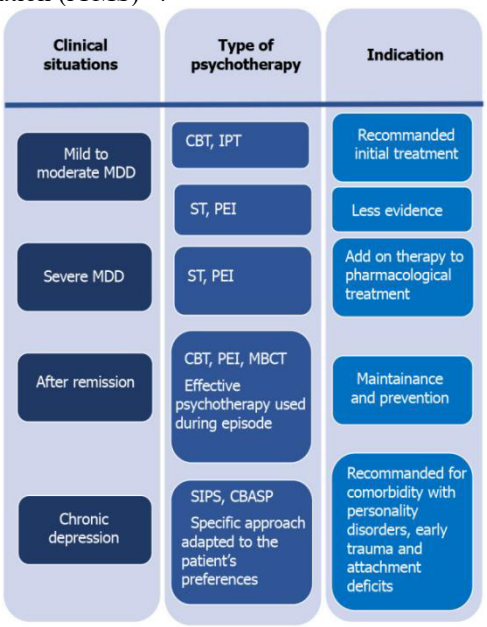

Figure 1. Overview of psychotherapy in various depression clinical settings[4]

1.2. The concept of pattern separation and its role in emotion regulation

The ability to discern between similar but distinct experiences or stimuli is known as pattern separation, and it is a fundamental cognitive process that is mainly mediated by the hippocampus in the brain, specifically within the dentate gyrus. The main goal of pattern separation is to reduce interference between similar

* liuj398@mail2.sysu.edu.cn

memories by allowing the storage of unique representations for each experience, which improves memory and learning and allows people to recall distinct events without confusion[6].

An aspect of episodic memory is pattern separation. Pattern separation issues have been linked in studies to the onset and severity of depression. The hippocampus, which is essential for pattern separation, frequently shows structural and functional abnormalities in depressed people. For example, stress-induced reduction of adult hippocampal neurogenesis may hinder pattern separation during encoding and cause memory loss[7]. These changes might make it harder to tell apart unpleasant experiences from one another, which could result in a more widespread and negative cognitive bias.

As a result, individuals may have difficulty forming unique memories, which may exacerbate depressive symptoms such as lack of pleasure and cognitive inflexibility[8].

1.3. Purpose and hypothesis of the study

The current study only looked at the impaired ability to separate patterns caused by depression, where individuals have difficulty distinguishing similar negative events. Pattern separation is a part of episodic memory. This study attempts to develop a pattern separation training mouse model. Through pattern separation training, we can improve the cognitive level and adult hippocampal neurogenesis of mice, and achieve antidepressant effects similar to those of antidepressants.Then, the differentially expressed genes induced by pattern training and their related pathways were detected by omics to explore potential cognitive antidepressant drug targets and the molecular mechanism of pattern separation training.

2. Literature Review

2.1. Most relevant studies

Biased processing of personal memories, especially emotional memories, is a central feature of mood disorders, anxiety disorders, and stress-related disorders. Studies have shown that Autobiographical Episodic Memory Training (AET) can improve MDD[9]. AET includes memory specificity training, concreteness training, competitive memory training, etc. However, the effect of different training schemes varies greatly[10]. Cognitive Training (CT) is a type of training designed to improve an individual's cognitive abilities, which may include memory, attention, executive function, processing speed, and multitasking[11]. Between AET and CT, pattern separation training is training that improves the accuracy and reliability of an individual's memory, and helps an individual better identify and distinguish between different emotional memories. The following are two of the most relevant articles on the effects of different training on depressive mood.

The "everyday memory" test was used in Annet Glas's study[12] to examine the effects of trial spacing on memory and neuronal ensemble activity in mice. Sixty minutes between trials increased memory retrieval but decreased memory encoding. Trial spacing improved the similarity of population activity patterns in the dorsomedial Prefrontal Cortex (dmPFC) during encoding and retrieval trials, according to in vivo calcium imaging. The size of the entire neuronal ensemble and the subpopulations devoted to particular task-related behaviors and events, however, remained unaffected by trial spacing. These results imply that spaced learning, maybe through enhancing synaptic connection within the ensemble, encourages the reactivation of prefrontal neuronal ensembles processing episodic-like memories.

The combined benefits of physical and cognitive training on adult hippocampal neurogenesis (AHN) in mice were examined in F. Ávila-Gámiz's study[13-14]. After exercising on a treadmill for ten days, mice were trained to use their working memory in the Morris water maze. Comparing the combined training to each therapy alone, the results demonstrated a considerable improvement in the survival of newly-generated hippocampus neurons. According to this, exercise primes the hippocampus for increased neuroplasticity, which cognitive difficulties can then build upon to provide improved AHN and possibly other cognitive benefits.

Both papers explore the positive effects of cognitive training on the brain and cognition, with a focus on AHN as a key mechanism. This suggests that cognitive stimulation can promote the survival of newborn neurons, especially during the critical survival period of 1-3 weeks after cell division. Both papers highlight the important role of AHN in hippocamp-related processes such as learning, memory, and emotion regulation, and indicate that enhancing AHN can improve cognitive function or restore neurological damage.

2.2. Innovation and Contributions to Ongoing Conversations in the Field

Although there are some training models for AET and CT, pattern separation training model has not been proposed. At present, the mechanism of AET solving emotional disorders is not clear, and this study aims to explore a pattern training model, which will effectively reduce the symptoms of depression and anxiety. In addition, the specific molecular mechanism of antidepressant was explored by multi-omics methods of transcriptome and metabolome, which laid a theoretical foundation for the development of new antidepressants.

3. Methods

3.1. Vector Construction and Transfection

Using the P1 phage Cre/loxP recombination system (Figure 2), we generated eight-week-old C57BL/6 J male mice with hippocampal DG newborn neuron-specific labeling. The animals were housed in ventilated, temperature- and humidity-controlled rooms with a 12-hour light/dark cycle, with three to four mice per cage. Nesting material, water, and food were provided ad libitum in standard laboratory cages. Subsequently, the mice were injected with

tamoxifen for four consecutive weeks at a daily dose of 15 mg/kg via intraperitoneal injection.

After the injection, hippocampal DG region cells were collected, and the transfection efficiency was detected using a flow cytometer. By selecting appropriate lasers and filters, different fluorescent markers could be distinguished. During data acquisition, gating was set to exclude non-cellular particles and debris. Finally, by analyzing the signal intensity of RFP and G-Camp fluorescent markers, the transfection efficiency was calculated by dividing the number of yellow fluorescent cells by the number of red fluorescent cells.

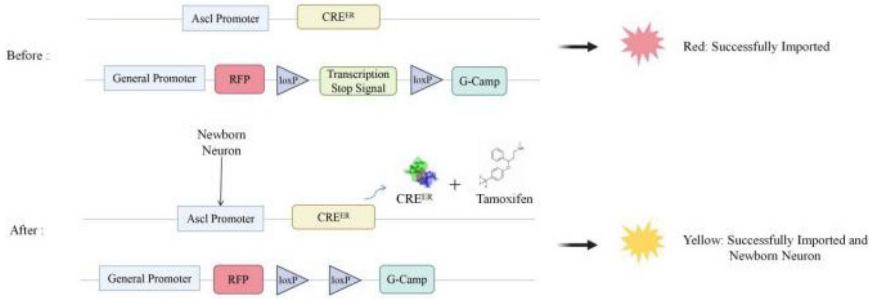

Figure 2. Cre/loxP recombination system

3.2. Pattern Separation Training and Unpredictable Chronic Mild Stress (uCMS)

3.2.1 Pattern Separation Training

The mice are trained in pattern separation in an order from the easiest to the most difficult over a period of four weeks. There are three types of training, each lasting six days, with testing conducted on the seventh day. The scenarios for the three types of training are as follows:

(1) Object Recognition Experiment: Train the mice to recognize different objects and test their ability to distinguish between similar objects. A familiar object is kept constant. If the mouse spends significantly more time exploring the new object compared to the familiar one and ultimately chooses the familiar object, it is given an appropriate amount of food pellet as a reward. The training lasts for 10-15 minutes and is conducted twice daily.

(2) 8-Arm Maze Training: The mice learn how to find the rewards placed at the ends of similar arms. During the training, data such as the time taken to find the reward and the number of entries into each arm are recorded. Each training lasts for 10-15 minutes and is conducted twice daily.

(3) Water Maze Training: The mice are trained to find a hidden platform using different spatial cues to distinguish between two similar situations. The training lasts for 10-15 minutes and is conducted twice daily.

3.2.2 uCMS

Over a period of 28 days, animals are randomly subjected to 7 types of stimuli (Table 1), including: warm water swimming (37°C, 5 minutes), cold water swimming (4°C, 5 minutes), water deprivation for 24 hours, food deprivation for 24 hours, tail clamping (1 minute at 1cm from the base of the tail), foot shock (30V voltage, each lasting 5 seconds), horizontal shaking (160Hz, 5 minutes). One type of stimulus is administered each day, with each stimulus being used three times, and the same stimulus cannot occur on consecutive days, preventing the animals from anticipating the occurrence of a particular stimulus. The control group of rats receives no stimulation, while the treatment group is given fluoxetine at 2mg/kg body weight, administered once daily by gavage. This treatment lasts for 28 days, during which behavioral tests and a sugar water consumption test are conducted.

Table 1. Timeline of daily stressors for the unpredictable chronic mild stress procedure

Weeks	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
1th	A cold swim	Water deprivation	Tail clamping	Foot shock	A warm swim	Food deprivation	A warm swim
2th	Water deprivation	Horizontal shaking	A warm swim	Food deprivation	A cold swim	Tail clamping	Foot shock
3th	Tail clamping	A cold swim	Foot shock	A warm swim	Water deprivation	Food deprivation	Horizontal shaking
4th	Foot shock	Water deprivation	Tail clamping	Food deprivation	Horizontal shaking	A cold swim	Horizontal shaking

3.3. Behavior Test

Behavioral tests were conducted on four groups of mice to assess the levels of depression and anxiety. The Forced Swim Test and Tail Suspension Test were used to evaluate depressive-like conditions, while the Open Field Test and Elevated Plus Maze Test were used to assess anxiety-like conditions. The following are the specific experiments:

(1) Forced Swim Test (FST): The mice were made to swim by submerging them in 30 cm of pure water at a temperature of (25±1)°C in a cylindrical container. A video system was used to capture the amount of time that the subject was immobile for the four minutes that followed the six-minute forced swim phase.

(2) Tail Suspension Test (TST): The mice's bodies were free to float in midair without coming into contact with the

sides or bottom of the container thanks to a suspension system attached to their tails. The amount of time spent hanging upside down in immobility was recorded.

(3) Open Field Test (OFT): Using the number of grid crossings, time spent in the central region, number of grooming instances, and number of standing instances as observational indicators, this test assesses the exploratory and spontaneous behavior of experimental animals in a novel setting.

(4) Elevated Plus Maze Test (EPM): The animals were positioned with their backs to one of the open arms in the middle of the maze. A typical recording of an animal's behavior in the maze lasted between five and ten minutes. We counted and noted the number of admissions into the open and closed arms, as well as the amount of time spent in each arm.

3.4. New neurons were detected by fluorescence labeling

Bromodeoxyuridine (BrdU) was injected into the experimental animals, a thymidine analog that can be integrated into the DNA of dividing cells. The administration was carried out at specific doses and schedules to label proliferating cells within a defined time frame. After the administration of BrdU and the necessary time for neuron development, the animals were euthanized, and brain tissue was collected. The tissue was then cut into thin slices using a vibratome or microtome for subsequent immunohistochemical processing. The tissue sections were permeabilized to allow the antibodies to access the cellular antigens. The tissue sections were incubated with a primary antibody that specifically recognizes BrdU. This primary antibody is usually conjugated to a fluorophore or a molecule that can be used to bind a fluorophore-labeled secondary antibody in subsequent steps. To maintain the fluorescence and stop it from fading, the slices were mounted using a fluorescent mounting media after the unbound antibodies were removed. After that, the sections were studied using a fluorescent microscope that had the necessary filters. A camera with a high resolution was used to take the images. The quantity of fluorescently labeled cells, which indicate new neurons, in the hippocampal DG region was counted using image analysis software. Analysis of the data between the experimental groups was done statistically.

3.5. Multiple Omics Select Targets

This study primarily employs a combination of untargeted metabolomics and single-cell transcriptomics for the analysis of potential drug targets.

3.5.1 Metabolomics analysis

Collect fasting blood from each group of mice, and use a centrifuge to separate the plasma. Store it in a -80°C freezer to completely halt all enzymatic and chemical activities, thereby preventing the consumption or transformation of existing metabolites or the appearance of new compounds or decomposition products during the preparation process. Subsequently, grind the samples, separate them, and perform rapid filtration and aspiration to extract, purify, and concentrate various metabolites as completely as possible. Utilize an ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS) system. The mixture is separated by liquid chromatography, and the separated components are then introduced into the mass spectrometer for analysis and identification.

Finally, data processing is conducted. Start with data preprocessing, which includes extracting mass spectrometry information, aligning mass spectrometry peaks, removing isotope peaks, and performing quality control, calibration, and noise reduction to arrange the useful information in the mass spectra into a matrix. Then, conduct multivariate statistics and pattern recognition to identify and extract metabolic features and patterns from the data by comparing the degree of separation between groups, including Principal Component Analysis (PCA), Principal Coordinates Analysis (PCoA), and Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA). Next, compare the metabolite differences between groups using variables like fold changes, P-values, and Variable Importance in Projection (VIP) scores to identify chemicals that are differentially metabolized. Finally, use functional and pathway analysis to examine modifications in metabolic pathways by using databases such as KEGG and MBRole.

3.5.2 Single cell transcriptome analysis

Cell isolation, single-cell RNA extraction and purification, single-cell transcriptomic library building, high-throughput sequencing, and ensuing data processing and analysis are the main steps in the single-cell transcriptomic sequencing process. In particular, it starts with the separation of individual cells from the hippocampal tissue of mice through the use of methods like laser capture microdissection and microfluidics. These single cells' entire RNA is then isolated and purified. Reverse transcription is then performed on the purified RNA to create cDNA libraries at the single-cell level. These cDNA libraries are sequenced using high-throughput sequencing platforms. Finally, the raw sequencing data undergoes bioinformatics analysis, including quality control, alignment, quantification, and differential expression analysis, to uncover the gene expression characteristics and patterns at the single-cell level.

Based on the results of transcriptomic and metabolomic analyses, identify potential targets that may be involved in metabolic or signaling pathways related to depression, and further screen for drugs or compounds that may affect these targets. Finally, use Computer-Aided Drug Design (CADD) technology to predict the binding affinity of these drugs to targets.

4. Experiment Design

In this experiment, we aim to compare the antidepressant capabilities of four different groups of mice under various conditions. The entire process is shown in Figure 3. All four groups received four weeks of tamoxifen injections.

The first group underwent pattern separation training followed by unpredictable chronic mild stress. The second group experienced the opposite sequence of the first group. The third group only underwent unpredictable chronic mild stress. The fourth group only received pattern separation training.

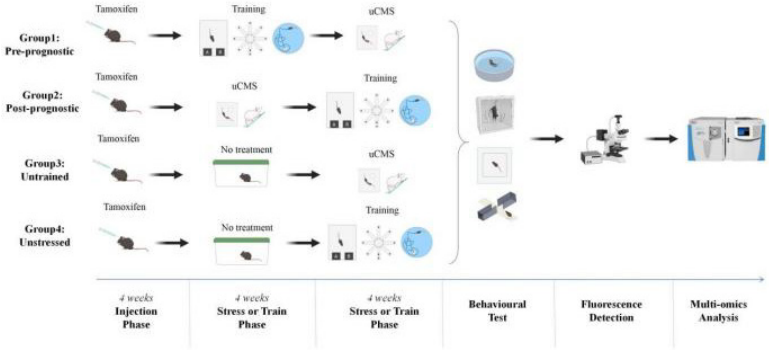

Figure 3. The experimental procedure used in this study

Subsequently, all groups underwent behavioral tests to detect the levels of depression and anxiety-like conditions. Then, immunofluorescence detection was used to quantitatively analyze the AHN results. Finally, a combination of full-spectrum metabolomics and single-cell transcriptomics was used to analyze potential antidepressant drug targets.

5. Expected Results

5.1. Behavior Test of Depression and Anxiety

Through the depression behavior tests, we predict that the US group will be the most active in the FST (Figure 4A) and TST (Figure 4B), followed by the PRP group, and then the POP and UT groups. Through the anxiety behavior tests, we predict that the US group will be the most active in the OFT (Figure 4C) and EMT (Figure 4D) , followed by the PRP group, and then the POP and UT groups.

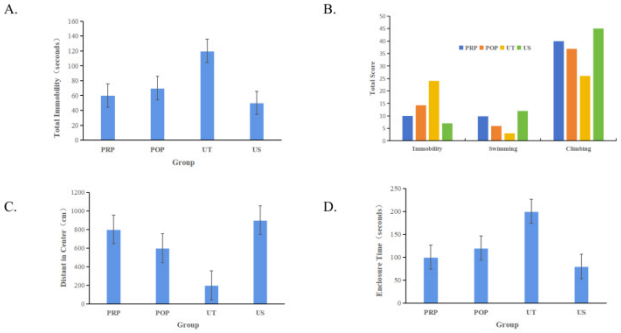

Figure 4. Prediction results of depression and anxiety-like behavioral tests in mice. PRP:Group1 Pre-prognostic; POP: Group2 Post-prognostic; UT: Group3 Untrained; US: Group 4 Unstressed. A. Forced swim test result; B. Tail suspension test result; C.Open field test result; D. Elevated plus maze test result

5.2. The activity and amount AHN

In this study, we predict that the US group will have the highest number of fluorescent signals, followed by the PRP group, then the POP group, and finally the UT group with the least fluorescent signals. The experimental results reveal a significant difference in fluorescence intensity among the different experimental groups.

5.3. Multiple Omics Result

This study was predicted by the combined analysis of metabolome and transcriptome. The counting of each cell type in the hippocampus in the four groups using UMAP yielded significantly more cell type 4 in the POP group than in the other groups. cell type 2 in the UT group was significantly lower than in the other groups (Fig. 5A). The volcano plot score analysis of differential gene expression yielded five genes with significantly higher expression and five genes with significantly lower expression (Fig. 5B), and these differentially expressed genes may be associated with antidepressant effects and inflammatory responses.

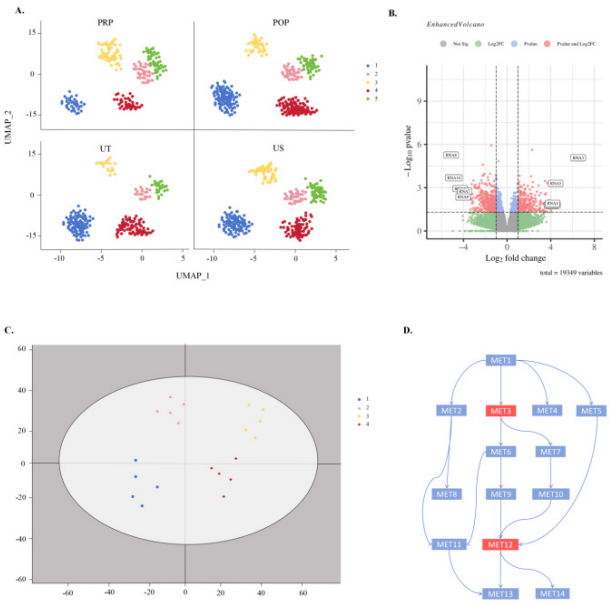

Figure 5. Prediction results of single cell transcriptome and full spectrum metabolome. PRP:Group1 Pre-prognostic; POP: Group2 Post-prognostic; UT: Group3 Untrained; US: Group 4 Unstressed. A. UMAP of 5 clusters split by samples; B. Volcano map of differentially expressed genes; C.OPLS score plots of four groups; D. Analysis of the pathway using all of the significant metabolites

OPLS analysis was performed using data from UPLC-MS (Figure 5C). A total of 14 differentially expressed metabolites between the four groups were identified, and

pathway analyses were performed using two of the key metabolites strongly associated with MDD (Figure 5D). These differentially expressed metabolites and genes were used as the basis for further screening of drugs sensitive to pattern training for antidepressant use.

6. Discusssion

Our predictive results suggest that, under conditions without external stress-induced depression, the US group performs the best in both behavioral tests and fluorescence detection, indicating that the number of new neurons produced in mice is the highest without stress stimulation, showing a significant increase in neural activity and the lowest level of depression.

In contrast, when depression is induced by stress, the PRP group performs better than the UT and POP groups, demonstrating significant neural activity in behavioral tests and fluorescence detection and a lower level of depression. This indicates that pattern separation training before stress is more effective in reducing depression than training after stress or no training at all. Therefore, for depressed mice, preliminary pattern training is the most effective. The UT group, compared to the other groups, exhibits the highest level of depression, thus proving the effectiveness of pattern separation training, which can help alleviate depressive symptoms. Expanding to human depression, effective pattern training can be conducted before the high incidence of depression in adolescence to reduce the risk of developing depression.

In addition to testing the effectiveness of pattern training, we also analysed 10 differentially expressed genes with 14 key metabolites that are critical for pattern separation through multi-omics, and conducted further excavation of metabolic pathways. The discovery of differentially expressed genes and metabolite markers may help researchers to understand the underlying stress and pathophysiological mechanisms associated with pattern-segregation deficits in depression, and provide a promising opportunity to generate new potential antidepressant targets.

This study also has some limitations. Specifically, the current model of pattern separation training is not yet fully developed, and it is unable to achieve the level of distinguishing various similar memories after only three training sessions; the evaluation indicators for pattern separation training are relatively single; and the performance of pattern separation training in humans has not been clearly understood, as it only been conducted on mice.

Future research directions can focus on utilizing potential drug targets identified through omics data analysis for drug development. This includes conducting in-depth research on identified biomarkers and molecular pathways to design and optimize small molecule compounds or biologics with therapeutic potential. At the same time, successful schema training models tested on mice can be translated and migrated to humans to assess training effects. Finally, new pattern separation training models can be developed and evaluated, which may include cognitive training programs, virtual reality technologies, or other innovative approaches to facilitate memory and emotional processing and enhance cognitive functioning and emotion regulation.

7. Conclusion

The aim of this study is to develop an innovative non-pharmacological treatment for Major Depressive Disorder treatment in the development of an innovative non-pharmacological treatment, namely pattern separation training, to achieve comparable efficacy with conventional antidepressant drugs. Meanwhile, the molecular mechanism of pattern separation training was explored through transcriptomics and metabolomics analyses, and potential drug targets were sought. Through the use of mouse models, behavioural tests, fluorescent labelling of newborn neurons, and histological analyses in order to explore the potential of pattern separation training as an alternative or complement to conventional antidepressant drugs. The expected findings suggest that pattern separation training can effectively alleviate depressive symptoms and promote de novo neuron generation in the hippocampal DG region. Multi-omics analysis revealed the molecular mechanism of pattern separation training and identified potential drug targets. This study provides new ideas and directions for the treatment of depression and drug development.

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