Exploring the Competitive Relationship of Pattern Separation Training and External Stress on Building Synapses with ABN and it's Molecular Mechanisms

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Abstract: Previous studies have shown that pattern separation training can play an antidepressant role, but the underlying mechanism is not clear. The ability of pattern separation is affected by newborn neurons, which also affect the treatment of depression. Thus, there is a structural hierarchical association between pattern separation ability and depression. That is, at the structural level of newborn neurons, pattern separation and depression may be able to interact. To investigate the mechanism by which pattern separation training plays an antidepressant effect, this paper tries to start with the factors influencing the pattern separation ability and the level of depression. In the paper, we choose pattern separation training and stress as the key influencing factors. We propose that the reason why pattern separation training can have antidepressant effect is partly because the competitive relationship between pattern separation training and external stress on building synapses.

1. Background

1.1. Previous study

Pattern separation ability is an ability of the brain to separate similar but different memories or experiences while processing information to prevent them from confusing each other.In pattern separation, CA3 receives signals from the EC through three pathways, while the input from mossy fibers from granule cells in the DG is a strong unidirectional input[1, 2]. There is substantial generation of granule cells in the course of hippocampal neurongenesis that may join pathways that separate processing patterns. It has been demonstrated that voluntary exercise contributes to enhanced pattern separation ability in mice and humans[3, 4], but if the neurongenesis isn't increased, the pattern separation ability will not be enhanced. This shows that the pattern separation ability in mice is closely related to neuronogenesis.

The hippocampus has been found to have reduced size and suppressed neurogenesis in depression[5-7], while severe depression showed a loss of pattern separation ability[8]. From this, we can infer that there seems to be a correlation in the hippocampal structure between pattern separation, and depression. Although experiments have been proved that pattern separation training can play a certain antidepressant effect, there have not been systematic experiments to prove the effect and elucidate its mechanism. Because they may be structural related, the factors that affect them could also be competitive at the structural level.

1.2. Introduction

We speculate that stress and pattern separation training may have a competitive relationship for building synapses with ABN in the DG. Take a step closer,pattern separation training leads to anti-depression through enhancing episodic memory function, competing with external stress to build synapses with ABN. This competitive relationship is reflected in Figure 1. And changing the order of pattern separation training and stress also changes the efficiency of synapse formed with ABN, thus affecting the antidepressant effects of pattern separation training. Therefore, we hope to observe the effect of pattern separation training and stress on the same batch of both newborn neurons to verify the above view.

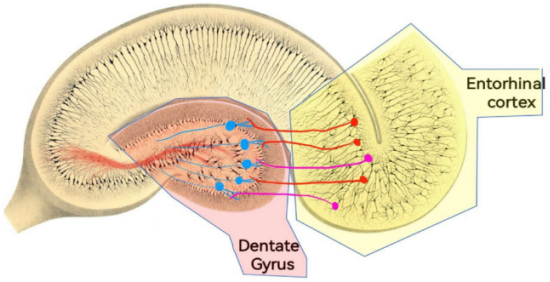

Fig.1 Pattern separation training leads to anti-depression through enhancing episodic memory function, by competing with external stress to build synapses with ABN.

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In this work, we injected a retroviral virus into the mouse hippocampus. The genetic design of the retroviruses is illustrated in Figure 2. After retroviral virus transfection of newborn neurons, we injected tamoxifen to enable the expression of G-camp in these neurons, thus allowing us to observe their activity in response to various stimuli.

We plan to divide the treated mice into three groups，and the heads of these mice will be embedded in transparent windows to allow for fluorescence observations. In this experiment, we opted to use pattern separation training (eight-arm maze) and stress (chronic unpredictable mild stress,CUMS) as influencing factors instead of drugs. This approach aims to align our study more closely with conventional way of influence,thereby making it more representative of general conditions.

Although the aforementioned experiments have partially explored the competitive relationship between pattern separation training and external stress on building synapses with ABN, the underlying molecular mechanisms have not been elucidated. Numerous existing studies indicate that NMDA receptors play a crucial rule in in synaptic plasticity[9-11]. Additionally, BDNF (Brain-Derived Neurotrophic Factor) is a key regulator of nervous system function, involved in both synapse formation and maturation[12-14]. So we designed experiments to detect the BDNF content and the RNA content of NMDA receptors, hoping to find the molecular mechanisms that may influence this competitive relationship. Synapses are further established through the rapid expression of essential assembly proteins and are functionally connected by the induction of synaptic plasticity. Synapses are eventually stabilized by eliminating weaker synapses through microglial phagocytosis.[15] We propose that microglia play a role in the competitive relationship of pattern separation training and stress on synapse building. We therefore also designed experiments that quantitatively measure the number and aggregation sites of microglia in the hippocampus, aiming to demonstrate the concept. By investigating these molecular mechanisms, we can enhance our understanding of this treatment. Furthermore, once we comprehend the involved molecular mechanisms, we can optimize treatment outcomes through the use of molecular drugs.

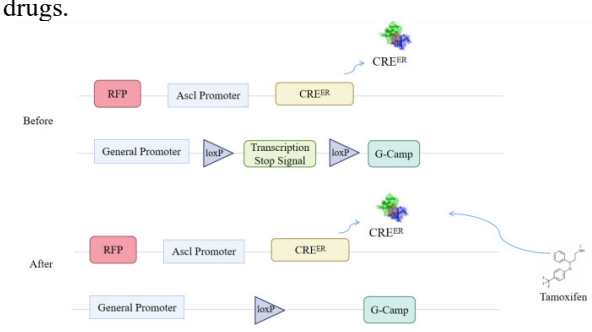


Fig.2 Genetic design of the recombinant virus.

Given that the above experiments build on short-term effects, we also set up long-term experiments. Experimental treatment of the three groups of mice was repeated once before injection of virus and tamoxifen. The additional mechanism exploration experiment is designed similarly to the short-term experiment.

2. Methodology

2.1. Retroviral Virus

Retroviruses were used to transfect hippocampal neurons, resulting in the exclusive expression of the CRE enzyme exclusively in newborn neurons（Fig.2). The CRE enzyme was activated following tamoxifen injection, which induced the expression of G-camp in these newborn neurons. Twenty-one days post-retrovirus, we collected samples from the mice and removed their hippocampus for homogenization. This homogenate was subsequently utilized to asses the viral transfection efficiency using flow cytometry (FCM).

2.2. Experimental and Control Mice

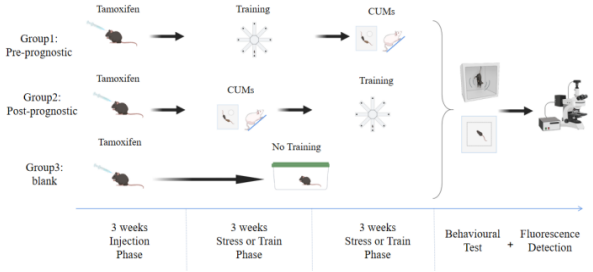
In the experiment, we used C57BL/6 mice as the experimental samples and divided the mice into three groups (Fig.3a): (1)Group 1: first pattern separation training and then stress (2)Group 2: First stress and then pattern separation training (3)Group 3: without any training and stress.

These three groups of mice were injected with a recombinant retrovirus into the hippocampus and received tamoxifen 21 days later. Experiments were conducted only three weeks after the tamoxifen injection.

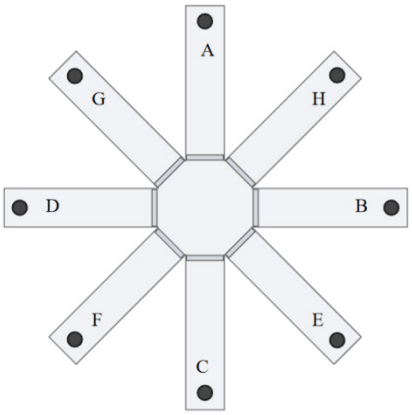
2.3. Experimental treatment

In the pattern-separation training, we utilized the eight-arm maze as the training modality. Mice were allowed 15 minutes to familiarize themselves with the maze before formal training, during which no food rewards were provided. Four arms of the eight-arm maze (A, B, C, D) were designated as reward arms, while the remaining four arms (E, F, G, H) were not rewarded (Fig.3b). In each experiment, two reward arms were randomly selected to receive food rewards at their ends. Mice were placed in the center of the maze and given 2 minutes to freely explore and select the arms. The reward arms for the food placement were changed every 5 rounds to enhance the pattern separation ability of the mice.

In our study on stress induction, we employed the Chronic Unpredictable Mild Stress (CUMs) model to investigate the effects of long-term stress on mice.



(a)Timeline of the three groups' experiments.



(b)Eight-arm maze design for pattern-separation training.
Fig.3 Experimental design of the three groups of mice.

2.4. Two-photon Microscopy

A small section of the mouse skull was carefully excised with a scalpel to expose the surface of the brain. A transparent window was then placed over the area where the skull was removed, ensuring that there was no compression or stimulation of the brain tissue. Following the experimental treatment, we assessed the mice's pattern separation ability using the eight-arm maze, and evaluated their levels of depression and anxiety through the Tail Suspension Test (TST) and the Open Field Test (OFT). Additionally, we recorded the activity of neurons and the number of synapses using Two-photon microscopy during the tests.

2.5. Molecular Mechanism

After conducting the aforementioned experiments, hippocampal tissue samples from the three groups of mice were collected. The tissue was placed in a mortar containing frozen saline and homogenized using an ultrasonic crusher or grinder. The resulting homogenate was then centrifuged, and the supernatant was collected. The supernatant from the hippocampal samples was then added to the sample wells of the ELISA plates. A capture antibody was introduced and incubated overnight at 4°C. The ELISA plates were subsequently washed with a washing buffer to remove any unbound antibodies. Next, assay antibodies were added and incubated at the appropriate temperature. Unbound detection antibodies were washed away. A substrate solution (e.g., TMB) was added to initiate a color reaction with the enzyme. The substrate reaction was terminated with a stop solution. The optical density values of each well were measured using an ELISA microplate reader at the appropriate wavelength, and the concentration of BDNF in the samples was calculated based on the standard curve.

Frozen sections were prepared from mouse hippocampus tissues after experiments. The tissue sections were treated with proteinase K to enhance probe permeability. Blocking the nonspecific binding site with blocking solution (eg., PBS containing normal serum). The bridized with were added to the slices and usually incubated overnight at the appropriate temperature. Wash away the unbound probes with the appropriate washing solution. Fluorescent signals were observed and recorded using a fluorescence microscope. The obtained images were quantitatively analyzed to assess the expression level of the NMDA receptor mRNA.

Frozen sections of the hippocampus were utilized to quantitatively analyze the number of microglia and aggregation sites. Immunofluorescence staining was conducted using antibodies against microglial markers, such as Iba1 and CD68. Labeling was performed with appropriate fluorescent secondary antibodies to visualize microglia under a fluorescence microscope. The acquired images were processed using image analysis software (e.g., ImageJ or Fiji). The collected images were compared to the fluorescence of active neurons in the same mice.

2.6. Longer-term Experiments

The pattern separation training and CUMs in mice were considered as a set of basic manipulations. For long-term study, we designed them to carry out basic manipulations first, then inject virus and tamoxifen, and finally carry out basic manipulations again. All the mechanistic experiments were repeated once on mice with long-term experiments except for different experimental treatments of mice.

3. Expected results

The virus transfection efficiency was more than 99% after 21 days, indicating a higher transfection efficiency of recombinant retroviruses and demonstrating that newborn neurons in mice can express the target genes.

In the eight-arm maze test, group 1 mice activated more and more widely distributed neurons than group 2，and the number of synapses involved in group 1 mice was significantly higher. In the TST and OFT tests, group 1 mice activated fewer and less distributed neurons than group 2 , and the number of synapses involved in group 1 mice was significantly lower. The predicted experimental results will validate that pattern separation training, as a pretreatment treatment can effectively improve its influence, that is, more new neurons can participate in pattern separation-related pathways, while the new neurons involved in depression and anxiety-related pathways are reduced.

In the molecular mechanism experiment, the BDNF content in group 1 mice was slightly higher than that in group 2, indicating that pattern separation training as a pretreatment treatment showed a limited increase in building synapses with ABN compared with prognostic treatment.

Group 1 mice had more NMDA receptor RNA from group 2, indicating that pattern-separation training as a pretreatment treatment could increase the number of NMDA receptors in neurons compared to prognostic treatment.

In the image contrast, both groups of mice, in the hippocampus, a certain number of microglia were clustered around the synapses of active neurons. Suggesting that microglia participate in pattern separation training and stress competition for synapse formation.

In the long-term mice, the situation described above was slightly improved. This indicates that long-term pattern separation training can achieve better antidepressant effects, and the reason why this phenomenon exists may be due to the positive regulation of the above mechanisms.

4. Discussion

In this paper, to explore the competitive relationship between pattern separation training and stress on building synapses with ABN, we controlled the newborn neurons expressing G-camp receptors to observe the number and distribution of activated neurons. Due to the time required for pattern separation training and stress, we could not observe the newborn neurons that were generated during the 6-week course. That is, we investigate the competitive relationship between pattern separation training and stress on pre-existing newborn neurons. We speculate that the competitive relationship between pattern separation training and stress for the newborn neurons generated during the course of the training and stress is also what we guessed in our experiment.

We propose that microglia play a role in the competitive relationship of pattern separation training and stress on synapse building. However, the mechanism leading to microglia involvement in this process has not been explored. We propose that pattern-separation training and stress may influence the release of molecules in neurons that regulate microglial localization and shear the synapses.

5. Conclusion

In this paper, we propose that the reason why pattern separation training can have antidepressant effect is partly because the competitive relationship of pattern separation training and external stress on building synapses. Pattern separation training may enhance the chance of forming synapses with newborn neurons by increasing BDNF content and NMDA expression in newborn neurons.At the same time, stress and pattern separation training may affect synapse formation by changing the neuronal release of molecules that affect microglial aggregation and shear synapses. At the same time, changing the order of pattern separation training and stress may affect the content of BDNF and NMDA expression, and then affect the antidepressant effect of pattern separation training. In future antidepressant studies, we may be able to reinforce the competitiveness of pattern separation training through molecular mechanisms in the expectation of achieving better treatment expectations.

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