Involvement of perineuronal nets in anti-depressant effects of electroacupuncture in chronic-stress-induced depression in rats

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Research Article

Keywords: electroacupuncture, depression, PNNs, Baihui, Yintang

Posted Date: September 28th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2100411/v1

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Abstract

Acupuncture help alleviate depression-like behaviors, but the neural mechanisms behind such antidepressive impacts are still unknown. Abnormalities in the perineuronal net (PNN) have been documented in multiple psychiatric disorders. The modulation and neural mechanism of PNNs in the antidepressant process of electroacupuncture (EA) at Baihui (GV20) and Yintang (GV29) points were investigated in this work. A rat depression model was induced by chronic unpredicted mild stress (CUMS). Acupuncture was performed on model rats in the EA group at GV20 and GV29 acupoints every other day for 30 min each time. The fluoxetine (FLX) group of model rats were gavaged with 10 mg/kg fluoxetine each day. Immunohistochemistry and western blot assays were used to evaluate the density and components of PNNs, the protein expression levels of the main synthase of GABA, GAD67, and of the synaptic proteins GLuA1, and PSD95 in the pre-limbic (PrL) and sub-limbic (IL) of mPFC. We found that four weeks of CUMS could decrease the levels of PNN component proteins aggrecan and brevican and GAD67. Electroacupuncture exhibited significant anti-depressive effects on depressive rats by altering the levels of PNNs. Specifically, aggrecan and brevican are involved in the anti-depression mechanism of electroacupuncture. After electroacupuncture treatment, the decreased expression of GAD67, GLuA1 and PSD95 in the mPFC induced by CUMS for four weeks was also reversed. This indicates that the mechanism of acupuncture's antidepressant effect may be based on reversing the stress-induced decline in PNN expression, the functional impairment of GABA neurons, and the regulation of excitatory synaptic expression.

Introduction

Depression is a highly prevalent and highly recurrent chronic mental illness that demonstrates marked disability and suicide rates, and it affects both mental and physical health [1, 2]. However, the pathogenesis and pathological features remain to be thoroughly uncovered. The onset of depression is related to many factors, such as neuroplasticity, heredity, inflammation and cytokines, neurotransmitter abnormalities, and neuroendocrine abnormalities [3]. Neuroplasticity is the basis of many physiological activities of the nervous system. Perineuronal nets (PNNs) are special structures inside the extracellular matrix (ECM) in the central nervous system, and they are closely associated with the synaptic plasticity of the central nervous system [4, 5]. Current research results indicate that in the pathogenesis of mental disorders such as schizophrenia and bipolar disorder, PNNs in the medial prefrontal cortex (mPFC) are core participants in regulating neuroplasticity [6], suggesting that abnormal PNN levels may be involved in depression pathogenesis. The structural and functional changes of PNNs in the pre-limbic (PrL) and sub-limbic (IL) of mPFC are not completely clear. Previous results suggested that PNNs in the PrL of vulnerable rats, which are prone to develop depression-like behaviors, exhibit low expression of component proteins and low densities [7].

Depression has yet to see the development of a radical cure or effective treatment; and most of the existing clinical antidepressants cannot completely relieve patients' depressive symptoms, restore normal function, or have certain side effects [8]. In recent years, acupuncture treatment of depression has
gradually been widely promoted, playing an irreplaceable role in the treatment of depression due to advantages such as less pain, unobvious side effects, simple operation, and a definite curative effect [9, 10]. The guidelines for the treatment of depression issued by the American Medical Association in 2016 included acupuncture as a non-drug therapy [11]. Experimental studies have also shown that electroacupuncture can improve depression-like behaviors in rats after CUMS [12]. The data of a large number of existing experiments and clinical literature studies on acupuncture treatment of depression have found that the Baihui (GV20) and Yintang (GV29) points are the most widely used in clinical treatment of depression and have good therapeutic effects [13, 14]. However, the roles of PNNs in electroacupuncture treatment of depression remain not clarified.

In the present study, we investigated the role of PNNs in the PrL and IL of the mPFC in the mechanisms of the anti-depressive effect of electroacupuncture, so as to provide more evidence for depression treatment with acupuncture.

Materials And Methods

Animals

The experiments were approved by the Ethics Committee of Beijing University of Chinese Medicine under the ethics review number of BUCM-4-2020080301-3004. SPF healthy male Sprague Dawley (SD) rats with body weights between 220–240 g were obtained from Beijing Vital River Laboratory Animal Technology Company Limited [animal license: SCXK (Jing) 2016-0011] and housed in the animal laboratory of Beijing University of Chinese Medicine (a clean environment) at a room temperature of 22 ± 2°C, a relative humidity of 50–60%, and a light/dark cycle of 12 h/12 h at 8:00 a.m. and 20:00 p.m.. The rats can eat standard food and drink water ad libitum. Each animal cage housed five rats. Before the experiments, all rats were subjected to 1-week environment acclimation.

Depression Induction by CUMS

Following the method of Willner and Hennessy, a depression model was induced [15, 16]. All model rats were administered 10 mild but unpredictable stressors for 3 or 4 weeks, and two stressors were administered per day. The stressors included food deprivation, limited access to water, crowding, tilted cages (45°), continuous light, soiled bedding (each lasting for 24 h), tail clipping for 1 min (1 cm from the tail tip), swimming in cold water (5°C) for 5 min, limited activities for 2 h, and playing white noise (100 decibels) for 4 hours. The same stress was not applied continuously to prevent the animal from adapting.

Experimental Intervention

To explore the effect of CUMS on PNN component proteins levels, rats in the CUMS 3 group or CUMS 4 group were exposed to CUMS stress for 3 or 4 consecutive weeks (Fig. 2A). The rats in the control group did not receive any stress and were provided enough food and water. To explore the effect of EA on depressive behaviors and PNN levels, rats in the CUMS group were exposed to CUMS stress for 4 consecutive weeks. The EA group received electroacupuncture treatment every other day from day 8 to
day 28, and electroacupuncture was given 1 h before the CUMS exposure. Each electroacupuncture session lasted 30 min[17]. The Baihui (GV20) and Yintang (GV29) acupoints that correspond to human anatomical regions were determined according to “Experimental Acupuncture Science” [18], and sterilized disposable stainless acupuncture needles (0.3 mm × 10 mm) were inserted into the two acupoints at a depth of 2–3 mm. The EA was applied with a frequency of 2 Hz and a current intensity of 0.6 mA, which was strong enough to induce microvibration on the rat head. The FLX group, which served as the positive comparator for anti-depressant effect, received fluoxetine (10 mg/kg; Innochem Technology, Beijing, China; catalog no. F0750) via intraperitoneal injection 1 h before stress exposure, and the injection frequency was once a day from day 8 to day 28[19]. The rats in the control group did not receive any stress and were provided enough food and water. (Fig. 3A).

**Behavioral Observation**

**Sucrose Preference Test (SPT)[20]**

SPT was conducted to assess anhedonia in rats housed in individual cages after CUMS. The test was divided into two stages. In the first stage of adaptation, rats were habituated to two bottles of 1% sucrose solution for 24 h on the first day. On the second day, each cage received one bottle of 1% sucrose and another bottle of pure water; after 12 h, the sucrose solution and pure water bottles switched positions. In the second stage of testing (3rd day since the beginning of SPT), rats were housed in individual cages with access to two bottles ad libitum for 24 h, one containing 1% sucrose and the other pure water. Sucrose preference was defined as the consumption ratio of sucrose solution in total liquid.

**Forced Swim Test (FST)[21]**

FST was performed in a φ20 cm×50 cm transparent glass cylinder. Water at 25℃ was added to a depth of 30 cm. On the first experiment day, rats were trained for 15 min of swimming adaptation, and on the second day (24 h later), rats were tested for 5 min of forced swimming; video recordings were made to analyze the floating immobility time. During the test, the surrounding environment was kept quiet, and an opaque baffle was placed between each two swimming containers so that the rats could not observe the situation of the other rats.

**Novelty-Suppressed Feeding Test (NSFT) [20]**

NSFT was conducted to assess anxiety-like behaviors in rats. Briefly, rats were fasted for 24 h before being placed in an open field (75 cm × 75 cm × 45 cm) corner, and a tiny amount of food was introduced in the field center. The latency time was recorded as the latency interval the mice experienced to approach food and start eating, and each test lasted 10 min. The surrounding environment was kept quiet during the test, and food consumption within 5 min after test initiation was recorded as a measurement for hunger. After each test, the cage was thoroughly cleaned with 75% ethanol.

**Western Blot**
Following the behavioral tests, rats were decapitated in each group to obtain the brains and and and bilateral tissue punches (12-gauge) were collected in PrL and IL regions (Fig. 1A)[22–24]. The tissue was lysed with RIPA lysis buffer, homogenized with a mixture of protease inhibitors and a phosphatase inhibitor using an electrical disperser (15 s × 3, 5 s intervals), and then incubated on ice for 30 minutes. The homogenate was then centrifuged for 30 minutes at 10,000 × g. All of the preceding processes were carried out at 4°C. A BCA assay kit (Applygen Technology, P1511) was implemented to measure the protein concentrations in all samples. After that, RIPA lysis buffer was used to dilute the samples and obtain normalized protein concentrations.

The PrL and IL of rat mPFC were dissected before homogenization in a specific solution [1 protease inhibitor cocktail, 1 mM EDTA, 0.32 M sucrose, 20 mM HEPES (Applygen Technology), 1 mM sodium vanadate and 5 mM NaF] to filter crude synaptoneurosomes. The homogenate was centrifuged at 2,800 rpm at 4°C for 10 min. The pellet (nuclear fraction) was discarded before further centrifugation of the supernatant at 12,000 rpm for 10 min. Following that, the supernatant was discarded, and protein lysis buffer was used to resuspend and sonicate the pellet. Similarly, a BCA protein assay was implemented to measure the protein concentrations [25].

The samples were separated on SDS-PAGE for 1 h at 120 V in the resolving gel and 30 min at 80 V in the stacking gel. After that, proteins were transferred to PVDF transfer membranes by electrophoresis at 250 mA for 1–2.5 h. After that, the membranes were incubated at room temperature for 1 hour in blocking buffer (5% skim milk in TBST). TBST was used to wash the membranes, which were then incubated at room temperature with antibodies against Aggrecan, Brevican (1:1000, Abcam), Glutamate Receptor 1, GAD67, PSD95, GAPDH (1:2000, Abcam), and β-actin (1:2000, Sigma-Aldrich) in TBST plus 5% skim milk for 1 h. Subsequently, the cells were rinsed in TBST buffer for 15 min for 3 times and cultured with 100 µl horseradish peroxidase (HRP)-conjugated secondary antibodies (goat anti-mouse and anti-rabbit IgGs, Beijing Zhong Shan Jinqiao Company) at room temperature in blocking buffer at a dilution of 1:2000 and incubated for 1 h. Blots were then rinsed with TBST for 15 min for 3 times, and an EZ-ECL chemiluminescence detection kit was used to visualize immunostaining. Band intensities were quantified by the NIH ImageJ software, and those for aggrecan, brevican, and GAD67 were normalized to β-actin, while those for PSD95 and GluA1 were normalized to GADPH.

**Immunohistochemistry**

After behavioral testing, four rats per group were taken and fixed with 4% paraformaldehyde by slow perfusion. The brains were fixed with 4% paraformaldehyde for 24 h after removal. After dehydration with 30% sucrose (w/v) in 0.1 M phosphate buffer, the post-fixed brains were stored at −80°C until analysis. According to previous studies[7, 26], the brains were coronally sectioned apart into 20 µm thick sections along the rostrocaudal axis of the PrL and IL (within 4.2 mm to 2.52 mm from Bregma) (Fig. 1B). To avoid counting the same cell multiple times, we kept only one of every three brain sections in sequence, and 5 to 6 sections from each rat were randomly selected for staining, and the average cell numbers on both sides of the target brain region of all sections was taken as the number of immunoreactive cells in each rat.
All sectioned parts were immersed in blocking solution [3 percent donkey serum (Applygen Technology), 1 percent BSA (Amresco), and 0.3 percent (v/v) Triton X-100 (Sigma-Aldrich) in PBS] for 1 hour at 25°C. Primary rabbit anti-parvalbumin antibodies (Sigma-Aldrich) and biotin-conjugated lectin WFA were incubated overnight at 4°C on the sections (Sigma-Aldrich). All pieces were rinsed three times in PBS after incubation and then incubated with FITC-conjugated streptavidin (Sigma-Aldrich) and goat anti-rabbit Alexa Fluor 594. (Beijing Zhongshan Jinqiao Biotechnology). The data were observed using an Olympus VS120 fluorescent microscope. The NIH ImageJ software was used to count the number of PV⁺ cells, WFA⁺ PNNs, and their colocalizations in the mPFC.

**Statistical Analysis**

GraphPad Prism 7.0 software was implemented to perform statistical tests on all data, which were expressed as the mean ± SEM. Firstly, we used a frequency distribution to see if the data for each group follows a normal distribution. Then, one-way analysis of variance (ANOVA) was used to determine differences between groups followed by Tukey's post hoc test if the F value indicated significance. *P* < 0.05 was considered statistically significant for the test.

**Results**

**Effect of CUMS on PNN component proteins expression in the mPFC of rats**

The behavioral observation results in our previous study revealed that[27], rather than three weeks of CUMS, four weeks of CUMS could induce depressive- and anxiety-like behaviors in rats, which is consistent with the findings in a prior study [28]. The expression of PNN components in the mPFC was investigated. In the PrL, significantly reduced expression of aggrecan was observed in CUMS 4 rats in comparison with control rats (*P* < 0.05; Figure 2B). In the IL, CUMS rats exhibited notably reduced expression of aggrecan and brevican relative to control rats (*P* < 0.01), while no significant difference in aggrecan, brevican expression was observed in the CUMS 3 group compared with the control group (*P* > 0.05; Figure 2C).

**Effect of EA on depressive behaviors, PNN, GAD67, GLuA1 and PSD95 expression in the mPFC of rats**

In the SPT, CUMS rats exhibited significantly lower sucrose preference than control rats (*P* < 0.01; Figure 3B), while rats in the EA and FLX groups showed notably increased sucrose preference in comparison to rats in the CUMS group (*P* < 0.05; Figure 3B). Briefly, SPT results suggested that both electroacupuncture and fluoxetine treatment improved the symptoms of anhedonia induced by CUMS in rats.

In the FST, CUMS rats presented with increased floating immobility time compared to control rats (*P* < 0.05; Figure 3C), while the floating immobility time of rats in the EA and FLX groups was significantly reduced in comparison with rats in the CUMS group (EA: *P* 0.05; FLX: *P* 0.01; Figure 3C). FST results indicated that both electroacupuncture and fluoxetine treatment alleviated the desperate behaviors of rats induced by CUMS modeling.
In the NSFT, the latency time of CUMS rats was significantly prolonged compared with that of control rats ($P < 0.05$; Figure 3D), whereas the EA and FLX groups had evidently shorter latency time than the CUMS group (EA: $P = 0.05$; FLX: $P = 0.01$; Figure 3D). Meanwhile, there was no significant difference among the four groups of rats in food consumption within 5 min ($P > 0.05$; Figure 3E). Collectively, these findings suggested that both electroacupuncture and fluoxetine significantly improved the anxiety-like behaviors of rats induced by CUMS modeling.

Then, the expression of PNN components and the expression of the main synthase of GABA, GAD67 in the mPFC was investigated. In the PrL, significantly reduced expression of aggrecan, brevican and GAD67 was observed in CUMS rats in comparison with control rats (aggrecan and GAD67: $P < 0.01$; brevican: $P < 0.05$). In contrast to the CUMS group, the EA group brought about an increased in the expression of aggrecan, brevican and GAD67 (aggrecan and brevican: $P < 0.01$; GAD67: $P < 0.05$). Similarly, the expression of aggrecan and GAD67 of rats in the FLX group was significantly higher than that in the CUMS group ($P < 0.05$) (Figure 3F). In the IL, CUMS rats exhibited notably reduced expression of aggrecan, brevican and GAD67 relative to control rats (aggrecan: $P < 0.01$; brevican and GAD67: $P < 0.05$), while a significant increase in aggrecan, brevican and GAD67 expression was observed in the EA group compared with the CUMS group (aggrecan: $P < 0.01$; brevican and GAD67: $P < 0.05$). In addition, rats in the FLX group had significantly higher expression of aggrecan than those in the CUMS group ($P < 0.05$) (Figure 3G).

Furthermore, we investigated the expression of synaptic proteins in the mPFC, including GLuA1 and PSD95. In the PrL, GLuA1 and PSD95 expression of rats in the CUMS group was significantly decreased in comparison with that in the control group ($P < 0.01$). The EA group ($P < 0.05$) and the FLX group ($P < 0.01$, $P < 0.05$) showed significantly higher GLuA1 and PSD95 expression than the CUMS group (Figure 3H). In the IL, a significant reduction in GLuA1 and PSD95 protein expression was observed in CUMS rats compared to control rats ($P < 0.01$). In contrast to the CUMS group, the EA group presented with elevated GLuA1 and PSD95 ($P < 0.01$, $P < 0.05$), and the FLX group had increased GLuA1 ($P < 0.01$) (Figure 3I).

**Effects of EA on the number of PNNs and the percentage of PNN$^+$ PV$^+$/PV$^+$ neurons in the mPFC of rats**

Lectin WFA specifically binds to the chondroitin sulfate chain, which is commonly used to detect PNNs using immunofluorescence[29]. After the behavioral tests, we determined the number of WFA-labeled PNNs around cells and the percentage of PNN$^+$ PV$^+$ neurons in PV$^+$ neurons. In the PrL, CUMS rats displayed reduced number of WFA-labeled PNNs and decreased percentage of PNN$^+$ PV$^+$ neurons in PV$^+$ neurons compared to control rats ($P < 0.01$; Figure 4B), while the EA group and FLX group showed the opposite trend to the CUMS group, as indicated by the increased number of WFA-labeled PNNs and elevated percentage of PNN$^+$ PV$^+$ neurons in PV$^+$ neurons ($P < 0.05$, $P < 0.01$; Figure 4C).

In the IL, the number of WFA-labeled PNNs and the percentage of PNN$^+$ PV$^+$ neurons in PV$^+$ neurons were notably decreased in CUMS rats relative to control rats ($P < 0.01$, $P < 0.05$; Figure 5). The WFA-labeled PNNs in the EA group was significantly higher than that in the CUMS group ($P < 0.05$; Figure 5B),
and the number of WFA-labeled PNNs and the percentage of PNN+ PV+ neurons in PV+ neurons were also significantly increased in the FLX group compared with the CUMS group ($P < 0.01$, $P < 0.05$; Figure 5).

**Discussion**

In this study, we found a decrease in the number of PNNs and percentage of PNN+ PV+ cells among PV+ cells in the PrL and IL of the mPFC in four weeks of CUMS-exposed rats. CUMS also caused decreased levels of PNNs components aggrecan, brevican, GAD67, GLuA1, and PSD95 in the PrL and IL of the mPFC. Electroacupuncture and fluoxetine reversed the depression-like behavior that was induced by CUMS and the decrease in the number of PNNs and percentage of PNN+ PV+ cells among PV+ cells and normalized the levels of aggrecan, brevican, GAD67, GLuA1, and PSD95 in the PrL and IL of the mPFC. In summary, these results suggest that PNNs in the mPFC are important contributors of depression etiology and the antidepressant-like effects of EA.

EA has been widely applied to treat depression for decades, usually using acupuncture points on the head [30, 31]. Traditional Chinese medicine considers two acupoints, Baihui (GV20) and Yintang (GV29), to govern the meridian and correlate directly with the brain via multiple collaterals and channels [28, 32]. Acupuncture on Baihui and Yintang can clear the mind, lift the spirits, regulate the Du meridian, and calm the mind to promote good mood and peaceful sleep [33, 34]. And research shows that electroacupuncture treatment every other day for three weeks can improve depression symptoms[17]. Therefore, we chose these two acupoints for stimulation with EA instruments in this study. On the other hand, fluoxetine (FLX) is a selective serotonin reuptake inhibitor (SSRI) used widely for depression treatment, and also can alleviate depressive behaviors in animal studies[35, 36]. We found that electroacupuncture and fluoxetine could reverse the depression- and anxiety-like behaviors induced by CUMS. We also found no significant difference between the EA and FLX groups, suggesting that electrotherapy had similar effects to fluoxetine.

The mPFC is involved in the pathogenesis of various mental illnesses, and its volume reduction is one of the most documented abnormalities in major depression [37, 38]. The mPFC is the main area responsible for executive function in the cortex. The dysfunction of the mPFC is related to the cognitive and emotional defects caused by stress, and the neuronal atrophy and synaptic structural and functional changes of the mPFC are closely associated to depression pathogenesis [39, 40]. In rodents, it was observed that mPFC-injured rats were more susceptible to stress than normal rats and developed depression- and anxiety-like behaviors, which increased the activation of brain regions involved in neuroendocrine and autonomic response [41]. PNNs mainly surround γ-aminobutyric acid (GABA) interneurons with PV-positive neurons [42]. Van De Werd found that the expression level of parvalbumin (PV) in the mPFC was not significantly different between the PrL and IL [43], but the distribution difference of PNNs in these two sub-regions of the brain has not been studied and compared.

According to the current relevant research, ECM level changes in the brain are related to the occurrence of depression [44]. As a special component of the ECM, PNNs are widely present in the central nervous
system and important participants of the signal transmission between neurons and glial cells. The functions of PNNs are abnormal in many neuropsychiatric diseases, such as epilepsy, schizophrenia, and bipolar disorder, and have become the focus of depression research in recent years [45, 46]. These behavioral abnormalities caused by psychiatric diseases may be improved when the changes in the number of PNNs are reversed [47–49]. Generally speaking, PNNs include chondroitin sulfate proteoglycans (CSPGs), hyaluronan, tenascin-R, and link proteins [50]. Among them, CSPGs are mainly composed of aggrecan, brevican, versican, and neurocan [51, 52]. Human autopsy studies have shown that in schizophrenia and bipolar disorder, the neurons expressed by aggrecan and PV are significantly abnormal [53]. Studies have shown that chronic stress in young age or adulthood can change PNN density in the mPFC brain area and affect the plasticity and structure of inhibitory neurons, especially PV-expressing interneurons [54, 55]. Besides, long-term antidepressant therapeutic agents, such as venlafaxine and fluoxetine, could also change the PV and PNN densities in adult cerebral cortex and hippocampus [56, 57]. Some showed that FLX can decrease PNNs formation during critical period which is the time of onset and close of PNNs formation [58]. There are also studies which found that PNNs are required for the antidepressant effect of ketamine, which is a rapid antidepressant drug [59, 60]. In this experiment, we found that electroacupuncture and fluoxetine treatments could reverse the drop in the number of PNNs and the percentage of PNN$^+$/PV$^+$/PV$^+$ the mPFC after CUMS. Additionally, electroacupuncture treatment could reverse the decrease in the protein expression of PNNs components aggrecan and brevican in the mPFC caused by stress. Previous studies indicated that fluoxetine administration would make the inhibitory neurons in the adult cerebral cortex undergo structural changes, which probably occurs by alternating the plasticity-related molecules in neurons or adjacent ECM, and these molecules present in interneurons are crucial for the development of plasticity in juvenile brains [61]. The results in EA and FLX groups in our study were similar, indicating that both treatments had a similar outcome.

The PNN is a complex of extracellular matrix molecules that mostly surrounds GABAergic neurons in various brain regions, participates in synaptic plasticity, and protects and buffers against external oxidative stress [62, 63]. Reduced expression of GABA synthetase has been observed in the brain tissue of suicidal patients in previous studies; the main GABA synthetase, glutamic acid decarboxylase 67 (GAD67), is specifically expressed in GABAergic neurons [64]. Decreased expression levels of GABA and GAD67 can be observed in the brains of depression patients and animals under long-term stress [38]. The decrease in PNN levels may make GABA neurons more sensitive to stress and other stimuli, resulting in abnormal neuronal function. Reduced levels of PNNs and disruption of GABAergic circuits have also been found in psychiatric disorders such as schizophrenia [65]. Our experimental results show that the reduction of PNNs in rats with depression induced by CUMS may lead to the functional damage of GABAergic neurons. Electroacupuncture at Baihui and Yintang acupoints in the depression rats increased the expression level of PNNs, which could protect GABA neurons from external stress stimulation.

During brain development, PNNs appear when the critical period for developmental plasticity ends and affect the onset and closure of that period. Recent work has revealed the crucial role of PNNs in
controlling CNS plasticity [63, 66]. Abnormalities in the structure and composition of PNNs may lead to changes in synaptic plasticity and neuronal function [67]. Destroying PNNs or degrading ECM can reduce the glutamatergic input of GABAergic neurons and reduce the excitability of neurons [68, 69]. GluA1 is a subunit of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor, which exists in the postsynaptic membrane of excitatory synapses and participates in the regulation of synaptic plasticity. Its abnormal expression is a common mechanism of mental illnesses such as depression, schizophrenia, and chronic drug addiction [70]. PSD95 is the main scaffold protein located in the excitatory postsynaptic synapse density [71] and an important participant of synaptic plasticity, glutamatergic transmission, and dendritic spine morphogenesis during neural development [72]. The protein expression levels of GluA1 and PSD95 in the PrL and IL were increased after electroacupuncture treatment. Thus, electroacupuncture at Baihui and Yintang can exert an antidepressant effect by affecting the expression level of excitatory synaptic proteins on neurons.

In summary, the present study shows that the mechanism of electroacupuncture's anti-depression effect may work through reversing the stress-induced decline in PNN expression, the functional impairment of GABA neurons, and the regulation of excitatory synaptic expression. The results of this study provide a new theoretical basis for the study of the antidepressant mechanism of acupuncture.

**Declarations**

**Data Availability Statement**

The original contributions presented in the study are included in the article; further inquiries can be directed to the corresponding author.

**Ethics Statement**

The animal study was reviewed and approved by the Biomedical Ethics Committee for Animal Use and Protection of Beijing University of Chinese Medicine and carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

**Conflicts of Interest**

The authors declare that they have no competing interests.

**Funding Statement**

This study was supported by the National Natural Science Foundation of China (No. 81803857).

**References**


42. Pantazopoulos H et al (2015) Aggrecan and chondroitin-6-sulfate abnormalities in schizophrenia and bipolar disorder: a postmortem study on the amygdala. Transl Psychiatry 5:e496
57. Ohira K et al (2013) Chronic fluoxetine treatment reduces parvalbumin expression and perineuronal nets in gamma-aminobutyric acidergic interneurons of the frontal cortex in adult mice. Mol Brain 6:43
60. Yu Z et al (2022) Neurocan regulates vulnerability to stress and the anti-depressant effect of ketamine in adolescent rats. Mol Psychiatry,

Figures

Figure 1

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Figure 2

Effect of CUMS on PNN expression, GAD67 in the mPFC of rats (A) Experimental timeline. (B) Western blots and fold change quantification of PNNs components and GAD67 levels in the PrL. (C) Western blots and fold change quantification of PNNs components and GAD67 levels in the IL. The data are expressed as mean ± SEM. *$P < 0.05$, **$P < 0.01$ vs. the CUMS group. CUMS 3: rats were exposed to CUMS for 3 weeks; CUMS 4: rats were exposed to CUMS for 4 weeks.
Figure 3

Behavioral observations (n=10) and Effect of EA and FLX on PNN expression, GAD67, GLuA1 and PSD95 in the mPFC of rats (n=6) (A) Experimental timeline. (B) Sucrose preference in SPT. (C) Immobility time in FST. (D) Latency to feed and (E) food intake in the home cage in NSFT. (F) Western blots and fold change quantification of PNNs components and GAD67 levels in the PrL. (G) Western blots and fold change quantification of PNNs components and GAD67 levels in the IL. (H) Western blots and fold change quantification of PNNs components and GAD67 levels in the PrL.
quantification of GLuA1 and PSD95 levels in the PrL. (I) Western blots and fold change quantification of GLuA1 and PSD95 levels in the IL. The data are expressed as mean ± SEM. *P < 0.05, **P < 0.01 vs. the Control group; #P < 0.05, ##P < 0.01 vs. the CUMS group. EA: electroacupuncture; FLX: fluoxetine.

Figure 4
Effects of EA on the number of PNNs and the percentage of PNN⁺ PV⁺/PV⁺ neurons in the PrL of rats (n=4). (A) Immunofluorescence staining images of PNNs and quantification of PNNs⁺ and PNN⁺ PV⁺/PV⁺ neurons in the PrL. (B) Number of WFA-labeled PNNs in the PrL. (C) Percentage of PV⁺ neurons surrounding PNNs (%) in the PrL. The data are expressed as mean ± SEM. *P < 0.05, **P < 0.01 vs. the control group; #P < 0.05, ##P < 0.01 vs. the CUMS group. EA: electroacupuncture; FLX: fluoxetine.
Effects of EA on the number of PNNs and the percentage of PNN⁺ PV⁺/PV⁺ neurons in IL of rats (n=4). (A) Immunofluorescence staining images of PNNs and quantification of PNN⁺ and PNN⁺ PV⁺/PV⁺ neurons in the IL. (B) Number of WFA-labeled PNNs in the IL. (C) Percentage of PV⁺ neurons surrounding PNNs (%) in the IL. The data are expressed as mean ± SEM. *P < 0.05, **P < 0.01 vs. the control group; #P < 0.05, ##P < 0.01 vs. the CUMS group. EA: electroacupuncture; FLX: fluoxetine.