

*Original Research*

# Electroacupuncture Improves Depression-Like Behavior by Regulating the Abundance of Lactobacillus and Staphylococci in Mice

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## Abstract

**Background:** Growing evidence suggests that gut microbiota can affect depression-like behavior, and electroacupuncture (EA) can regulate the composition and abundance of gut microbiota. At the same time, not a lot of research has been done on how EA affects gut microbiota to depression-like behavior. The objective of this study was to study the associated mechanisms by which EA exerts antidepressant effects by modulating gut microbiota. **Methods:** Twenty-four C57BL/6 male mice were randomly divided into three groups, one group (n = 8) was the normal control group (NC). And the other two groups was chronic unpredictable mild stress for modeling + electroacupuncture group (CUMS + EA) (n = 8) and chronic unpredictable mild stress for modeling group (CUMS) (n = 8). Both CUMS and EA groups were subjected to 28 days of CUMS, but only the EA group received an additional 14 days of EA procedure. Behavior tests were used to determine the antidepressant effect of EA. Sequencing of the 16S ribosomal RNA (rRNA) gene was applied to examine alterations in the intestinal microbiome between groups. **Results:** The findings were compared to those of the NC group, the sucrose preference rate and the total distance of Open Field Test (OFT) in CUMS group decreased, the abundance of Lactobacillus decreased, while the abundance of staphylococci increased. After the intervention of EA, the sucrose preference index and the total distance of OFT increased, the abundance of Lactobacillus increased, while the abundance of staphylococci decreased. **Conclusions:** These findings indicated EA may play an antidepressant effect by adjusting the abundance of Lactobacillus and staphylococci.

**Keywords:** electroacupuncture; depression gut microbiota; Lactobacillus; staphylococci

## 1. Introduction

Depression is a common psychiatric disorder characterized by heterogeneous symptoms: persistent low mood, loss of interest, low self-esteem and energy, weight changes, insomnia or drowsiness, and impairment of cognitive functions such as attention and memory [1]. Persistent depression seriously affects people's daily work and life, and severe depression can even lead to suicide, threatening the stability and development of society [2]. Therefore, it is important to explore the pathogenesis and treatment of depression. Researchers have increased focus on the clinical efficacy and brain-gut axis theory of electroacupuncture (EA) in the treatment of depression [3].

Combined with the advantages of acupuncture and electrical stimulation, EA is used in the treatment of stroke, insomnia, depression, and other neurological diseases [4]. The acupoints Baihui and Yintang (GV20 and GV29) are widely used to treat depression [5]. Combining EA (GV20 and GV29) with the treatment of selective 5-hydroxytryptamine (5-HT) reuptake inhibitors (SSRIs) has been shown to have early and durable control of symptoms in patients with depression with good efficacy [5,6]. The antidepressant mechanism of EA is not clear, and the brain-gut axis theory may explain the antidepressant effect of EA.

The brain-gut axis is known as a bidirectional pathway between the enteric nervous system and the central nervous system. In recent years, the brain-gut axis has been identified as an important mechanism affecting depression [7]. Clinical experiments have proved significant differences in the composition of the gut flora between patients with depression and healthy controls, while transplantation of the "depressed microbiota" from MDD patients into germ-free (GF) mice resulted in a similar depression-like effect compared to transplantation of the "healthy microbiota" from healthy control individuals. It indicates that gut flora is closely related to depression [8]. Animal experiments indicate that chronic prebiotic fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS) treatment exhibited both antidepressant and anxiolytic effects [9]. However, exactly how the brain communicates with the gut and how changes in the type and abundance of intestinal flora affect depression remains controversial. The microbiota and the brain communicate with each other through various pathways, including the immune system, metabolism of tryptophan, the vagus nerve, and the enteric nervous system, involving microbial metabolites such as short-chain fatty acids, branched-chain amino acids, and peptidoglycans [10]. The abundance and structure of intestinal mi-



Microorganisms in different diseases change differently. Studies have proved that EA can regulate the abundance of Delta-proteobacteria and Epsilon-proteobacteria to balance the structure of intestinal microorganisms and improve the learning and memory abilities in senescence-accelerated mouse prone 8 (SAMP8) mice [11]; EA alleviates behavioral defects in rotarod performance tests and pole tests and partially rescues dopaminergic neurons lost in the Parkinson's disease model in mice. This may be due to the decrease of intestinal microbial Erysipelotrichaceae by EA [12]. However, few researchers have studied the changes in intestinal microbial abundance and structure of EA antidepressants. In our study, we intend to detect the effect of electrospray on depressed intestinal flora using the 16s rRNA method.

## 2. Materials and Methods

### 2.1 Animals

South Medical University's Experimental Animal Center, Guangzhou, China, supplied the animals (Certificate No. SCXK [Guangdong] 2021-0041). In the course of the research, twenty-eight male 4-week-old C57BL/6 mice (20 g) were housed in a controlled environment ( $24 \pm 2$  °C, 50–60% humidity, 12:12 hours light/dark cycle). During this period, good indoor ventilation was ensured. After three days of quarantine, all mice were kept in the new environment for at least one week before use.

### 2.2 Group

After baseline screening (excluding the mice with less than 20% decrease in sucrose preference after the CUMS), 28 mice were randomly divided into the normal control group ( $n = 8$ ), and CUMS model group ( $n = 20$ ). The normal control group did not have any intervention. The model group underwent CUMS model establishment for 28 days. The successful establishment of our CUMS model was determined by significant differences in depressive-like behaviors acquired on an experimental day 28 when compared with those of the normal control group. After confirming the modeling was successful, they were randomly divided from the CUMS model group into CUMS model group ( $n = 8$ ) and the EA intervention group ( $n = 8$ ). EA intervention group underwent EA intervention for 14 days after 28 days of CUMS modeling. CUMS group also grasped fixed but did not do EA intervention.

### 2.3 CUMS Procedure

According to the method of Zhang [5], model group animals were housed individually and repeatedly exposed to the following CUMS conditions: (1) water deprivation for 24 hours; (2) food deprivation for 24 hours; (3) immobilization for 12 hours; (4) horizontal shaking for 5 minutes; (5) strobe for 12 hours; (6) day and night reversal. One stressor was applied in random order each day, and the whole stress process lasted 28 days.

### 2.4 Interventions

CUMS modeling was followed by a 14-day intervention. EA was performed on mice in the EA group at GV20 (the center of the parietal bone) and GV29 (the middle of both brow bones) [3]. The mice were lightly immobilized in a clear acrylic mouse holder (number of version: SMT-HC-GDS, Smart (Guangzhou) Biotechnology Co Ltd, Guangzhou, Guangdong, China) and disposable acupuncture needles (lot number: 210802A, 0.22 mm  $\times$  5 mm, SUZHOU ACUPUNCTURE & MOXIBUSTION APPLIANCE CO LTE, Suzhou, Jiangsu, China) were inserted horizontally into the two points at a depth of 2–3 mm. After insertion, the needles were connected to the electrodes and a sparse wave electrical stimulation (current of 1 mA, frequency of 2 Hz) was performed, when the mice's heads trembled slightly. The entire EA treatment was administered for 30 minutes at a time, once a day. During the EA treatment, mice in CUMS groups were also lightly immobilized in the clear acrylic mouse holder in the same way as those in EA.

### 2.5 Behavioral Testing Method

Upon completion of 14 days of intervention (at day 42), the Sucrose Preference Test (SPT) and Open Field Test (OFT) were administered.

#### 2.5.1 Open Field Test (OFT)

The OFT was used to assess spatial exploration behavior in a quiet room in a black square arena (40  $\times$  40  $\times$  40 cm) with dim lighting. In the arena, mice were placed individually and allowed to explore independently for five minutes. Above the arena, a video camera recorded the movements of the mice. An analysis of the total distance traveled during the 5 minutes was conducted using a video computer tracking system (SMART 3.0; Panlab SL, Barcelona, Spain). The arena was cleaned with ethanol after each test.

#### 2.5.2 Sucrose Preference Test (SPT) and Weighing

Before SPT, mice were trained to acclimate to sucrose solution (1% w/v). Two bottles of sucrose solution were placed in each cage for 24 hours, and then 1 bottle of sucrose solution was replaced with pure water for 24 hours. After acclimation, mice were deprived of water and food for 12 hours. During the 24 h SPT, mice were housed in individual cages. For 24 hours, each mouse had access to two bottles, one containing (1% w/v) sucrose solution and the other pure water. Two bottles were switched places at 12 hours to prevent place preference, and each bottle was weighed before and after the test. The sucrose preference rate was calculated according to the formula of water consumption/(sugar water consumption + water consumption)  $\times$  100%. Weight measurement at the end of the intervention.

## 2.6 16s rRNA Sequencing

### 2.6.1 Fecal Sample Collection

After 28 days of CUMS modeling and 14 days of EA intervention, six mice in each group were randomly selected and their feces were collected and placed in sterile conical tubes and immediately frozen at 80 °C. Fecal samples were thawed on ice. Mobio lysis buffer was added to these fecal samples and further vortexed to mix. The fecal suspension was centrifuged and the supernatant was placed in a MobioGarnet bead tube containing Mobio buffer.

### 2.6.2 DNA Extraction and PCR Amplification

Microbial community genomic DNA was extracted from CUMS model group samples Using the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) as directed by the manufacturer. 1% agarose gel DNA extract was tested for concentration, purity, and DNA concentration with NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, NC, USA). With the use of an Agilent GeneAmp® 9700 PCR thermocycler (ABI, CA, USA), the hypervariable region V3-V4 of the bacterial 16S rRNA gene was amplified with primer pairs 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTA AT-3'). Following are the steps taken to amplify the 16S rRNA gene by PCR: Three minutes of denaturation at 95 °C, followed by 27 cycles of denaturing at 95 °C for 30 seconds, annealing at 55 °C for 30 seconds, and extending at 72 °C for 45 seconds. After that, a single extension at 72 °C for 10 minutes is followed by a cool down at 4 °C. The PCR mixtures contain 2.5 mM dNTPs 2 µL, 5 × TransStart FastPfu buffer 4 µL, template DNA 10 ngf, 0.8 mL of reverse primer (5 µM), 0.4 µL of TransStart FastPfu DNA polymerase, and finally ddH<sub>2</sub>O up to 20 µL. Triplicate PCR reactions were performed. Based on the manufacturer's instructions, AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) was used to extract PCR product from 2% agarose gel and to purify it and quantitate it using Quantus™ Fluorometer (Promega, Madison, WI, USA).

### 2.6.3 Illumina MiSeq Sequencing

The purified amplicons were pooled in equimolar concentrations and sequenced on an Illumina MiSeq PE300 platform/NovaSeq PE250 platform according to the standard protocols by Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China).

### 2.6.4 Processing of Sequencing Data

Fastp version 0.20.0 was used to demultiplex 16S rRNA gene sequence reads and FLASH version 1.2.7 to merge them with the following criteria: (i) the 300 bp reads were truncated at any site receiving an average quality score of <20 over a 50 bp sliding window, and the truncated reads shorter than 50 bp were discarded, reads containing ambiguous characters were also discarded; (ii) only overlap-

ping sequences longer than 10 bp were assembled according to their overlapped sequence. The maximum mismatch miceio of the overlap region is 0.2. Reads that could not be assembled were discarded; (iii) samples were distinguished according to the barcode and primers, and the sequence direction was adjusted, exact barcode matching, 2 nucleotide mismatch in primer matching [13,14].

A 97% similarity cutoff was used to cluster Opimiceional taxonomic units (OTUs), and chimeric sequences were identified and removed by using UPARSE version 7.1 [15,16]. The taxonomy of each OTU representative sequence was analyzed by RDP Classifier version 2.2 against the 16S rRNA database (silva138/16s\_bacteria) using a confidence threshold of 0.7 [17].

## 2.7 Statistical Analysis

All statistical analyses were performed using SPSS 22.0 (IBM Corp., Chicago, IL, USA) and GraphPad Prism 9 software (GraphPad, San Diego, CA, USA). All data were presented as mean ± SD both complied with normal distribution. Differences between two groups were assessed by independent samples *t*-test, and differences between groups were assessed by multivariate ANOVA followed by LSD post hoc tests. Statistical significance was given by *p* < 0.05, or LDA scores >2.

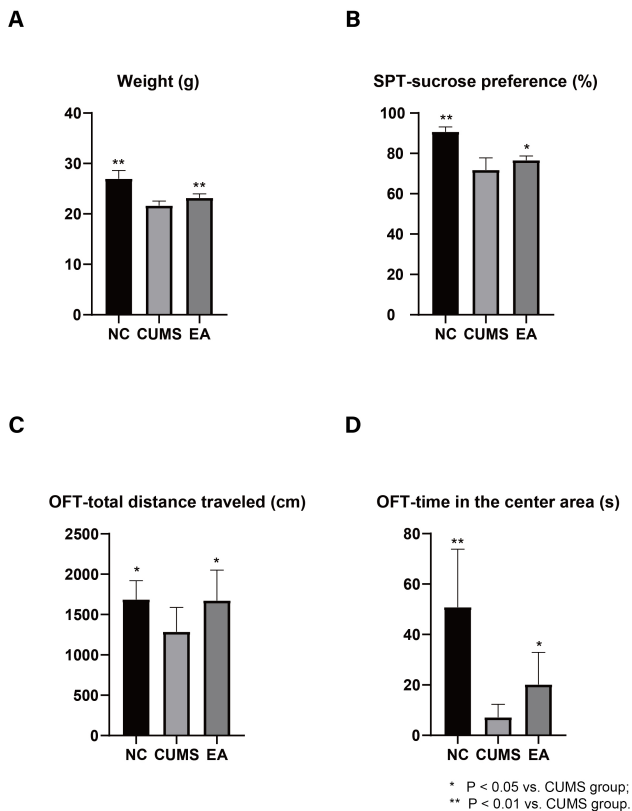
## 3. Results

### 3.1 CUMS-Induced Depression-Like Behaviors Were Reduced by EA

After 28 days of CUMS modeling. Firstly, excluding the modeling failure, In the remaining 18 mice, approximately 80% of them successfully modeled CUMS-induced depression based on SPT, weight, and OFT evaluations (**Supplementary Fig. 1**). They were then divided into CUMS and EA groups with 8 mice in each group. As the intervention progressed, CUMS models remained stable, with significantly different behavioral results from the control group. The EA group was accepted 14 days of EA intervention. Neither the model establishment nor the intervention period resulted in any mouse deaths. Furthermore, the EA group showed positive results compared with the CUMS group, indicating that the treatment of EA improved mice's depression-like behavior (weight, NC: 26.9700 ± 1.66, CUMS: 21.64 ± 0.91, EA: 23.18 ± 0.82, *p* = 0.0001; SPT, NC: 0.91 ± 0.024, *p* = 0.0001, CUMS: 0.72 ± 0.059, EA: 0.76 ± 0.021, OFT-total distance traveled, NC: 1686.63 ± 234.04, CUMS: 1285.69 ± 303.86, EA: 1674.47 ± 375.81, *p* = 0.027; OFT-time in the center area, NC: 50.83 ± 22.98, CUMS: 7.20 ± 5.11, EA: 20.19 ± 12.74, *p* = 0.0004) (Fig. 1).

### 3.2 Bioinformatic Analysis

A total of 872,581 single-end reads were obtained by sequencing; the DADA2 plug-in in the QIIME2 process was used for noise reduction of the sequenced reads,



**Fig. 1. Electroacupuncture has an antidepressant effect.** Comparison of weight (A), SPT (B), OFT-total distance traveled (C) and OFT-time in the center area (D) among 3 groups (mean  $\pm$  SEM).

and the noise reduction steps included filtering noise and correcting sequence errors, removing chimeras and single sequences, and de-duplicating sequences to obtain high-resolution ASVs for subsequent analysis; a total of 4,85,522 sequences were generated from 18 samples after DADA2 noise reduction, with 22,615–33,287 sequences per sample, and 4239 ASVs were obtained.

### 3.3 Gut Microbial Species Abundance Analysis Indicates Adequate Sample Size

The variation of total species and core species with sample size was described using PAN/Core species analysis. In microbial diversity studies, the species abundance and the number of core species in the environment are widely used to determine whether the sequencing sample size is sufficient. The results of PAN/Core species analysis showed (Fig. 2A) that the number of species under the genus classification stabilized and the curve leveled off as the sample size increased. The number of species of gut microorganisms no longer increased with the increase in sample size, indicating that the sample size was sufficient.

### 3.4 EA Restores the Intestinal Flora Composition Induced by CUMS

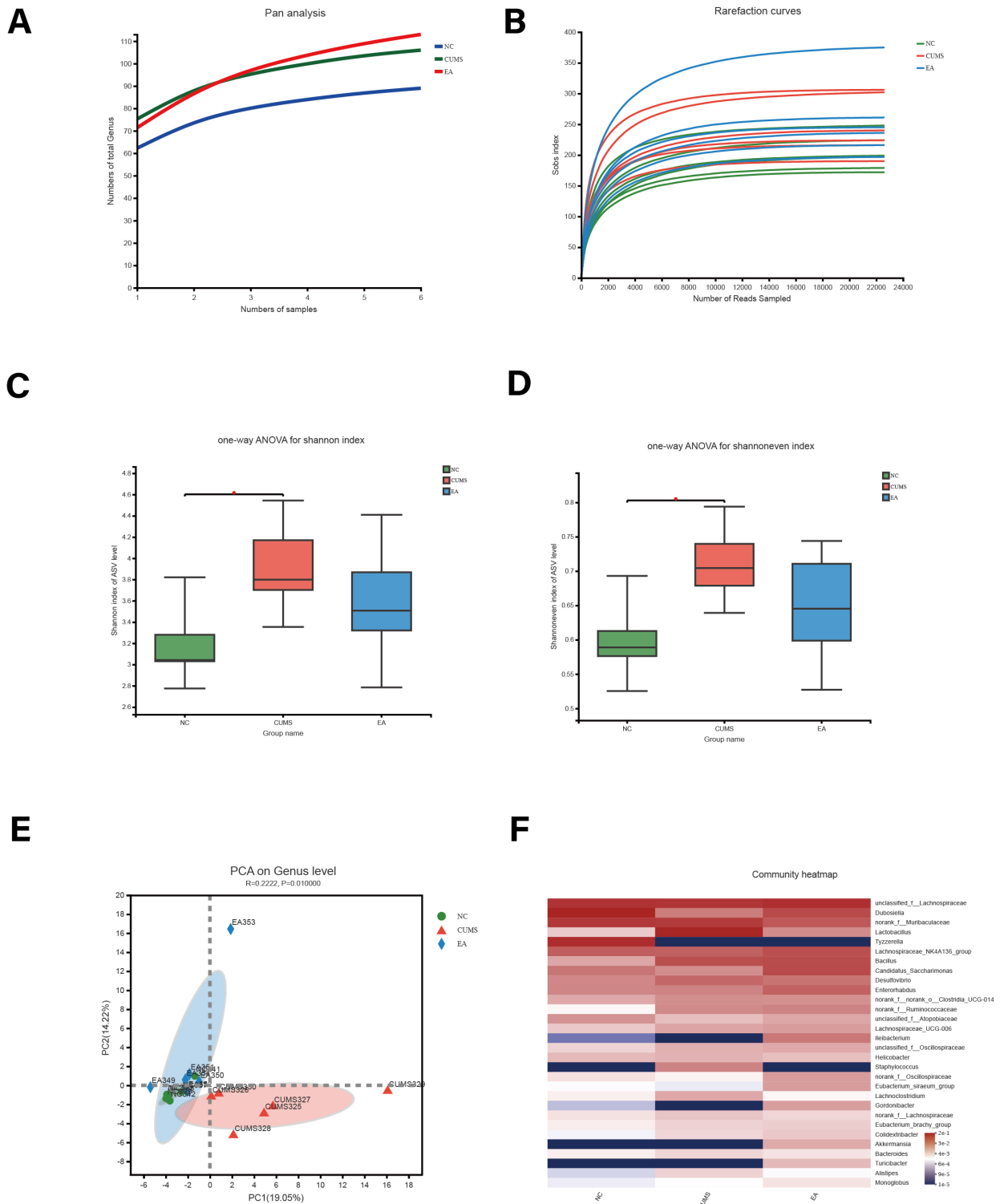
Alpha community diversity analysis and beta community diversity analysis are commonly used indicators to analyze species diversity. The results of the Alpha diversity index to test the differences between groups proved that the Shannon index and shannoneven index (ASV level) were lower in the NC groups (Fig. 2C,D) than in the CUMS group ( $p = 0.036$ ) ( $p = 0.022$ ). There was also a difference in the Simpson, Chao and Ace index in the NC group compared to the CUMS and EA groups, but it was not statistically significant (Supplementary Fig. 2). The results of the beta-diversity result to test the differences between groups proved that the PCA analysis compared the differences in microbial communities between the NC, CUMS, and EA groups (Fig. 2E). The PCA analysis of results showed significant differences between the EA and CUMS groups ( $p = 0.01$ ,  $R = 0.2222$ ). The above results indicated that the composition of intestinal flora was different between NC, CUMS, and EA groups, and the main composition of the intestinal flora of mice after CUMS intervention was more different from that of the NC group, while the main composition of the intestinal flora of mice after receiving EA intervention was more similar to that of NC group (Fig. 2E).

### 3.5 Community Heatmap

A hierarchically clustered heatmap was further constructed to evaluate the relationships among the samples at the genus level based on the top 30 most abundant microbial communities and the abundance was distinguished by the shade of color. The result showed that the 24 samples could be separated into three groups (Fig. 2F). The most abundant genera were *Dubosiella* (20.77%) in NC, *Lactobacillus* (17.42%) in CUMS, and *Lachnospiraceae* (17.07%) in EA, respectively. In comparison to CUMS group, the bacterial taxonomic groups of *Lactobacillus*, *Lachnospiraceae\_NK4A136\_group*, *Bacillus*, *Desulfovibrio*, *Candidatus\_Saccharimonas*, and *Staphylococcus*, were detected to have higher similarity in colony composition at the genus level in NC and EA group. The heatmap analyses (Fig. 2F) agree with the results of the PCA (Fig. 2E), with Both analyses showing a high degree of similarity between NC and EA mouse intestinal flora sample characteristics and microbial communities.

### 3.6 Community Composition Analysis

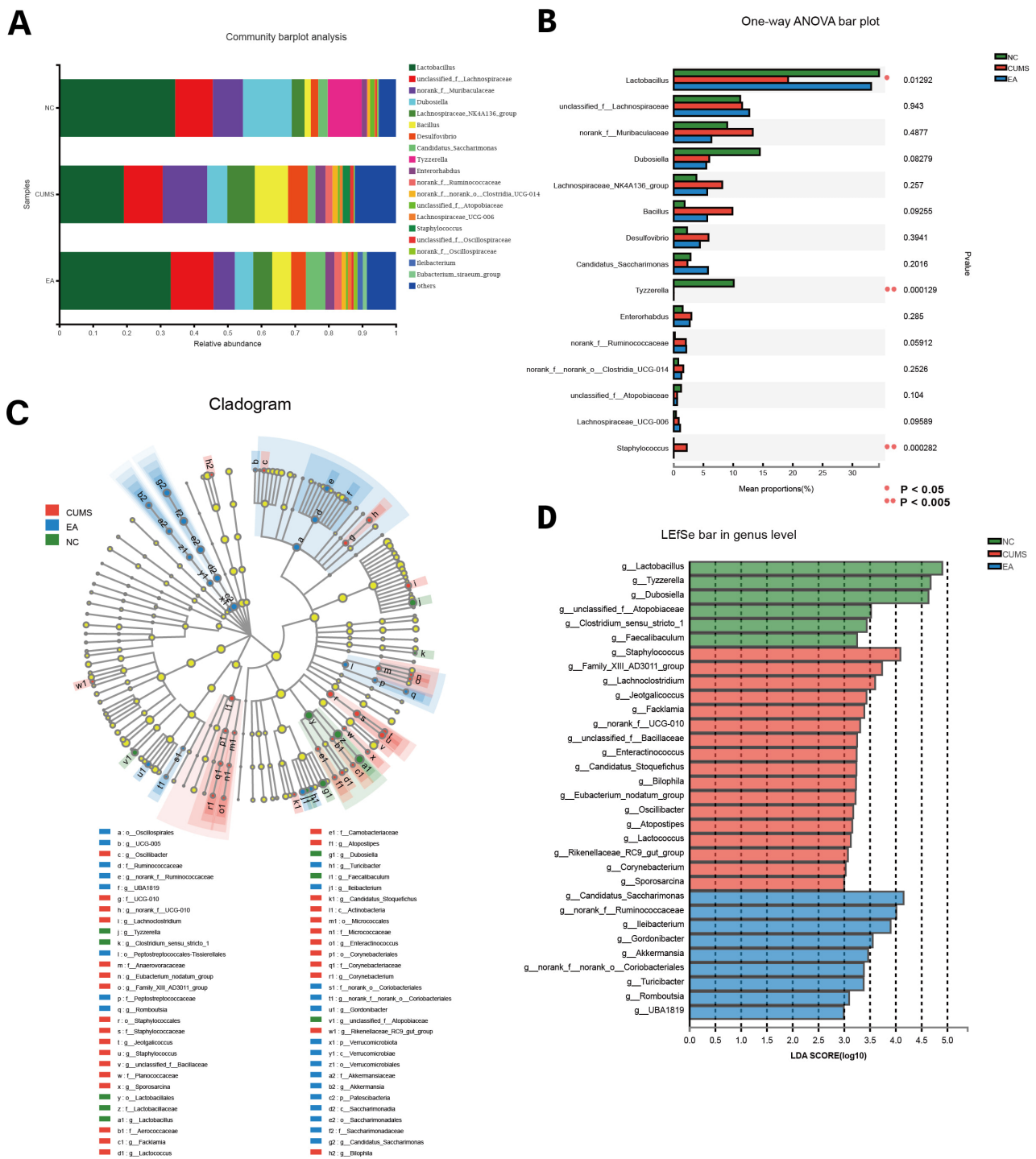
Analyzed at the genus level (Fig. 3A), the highest relative abundance in the three groups was found for *Lactobacillus*. The proportion of *Lactobacillus* in the NC group was 34.45%, 33.13% in the EA group, and 19 in the CUMS group. The relative abundance of *Lactobacillus* in the CUMS group was significantly lower than in the EA group, while other genera that showed more significant differences in the multi-group comparative analysis of the three groups, such as *Staphylococcus*, were not found in the NC and EA



**Fig. 2.** After electroacupuncture treatment, EA mice had a community diversity and composition similar to control mice. Pan/Core (A), species analysis (B), Rarefaction curve (C,D), Alpha diversity index inter-group difference test (E), PCA analysis (F), Community Heatmap at the genus level.

groups and 2.20% in the CUMS group. Ruminococcaceae was 0.20% in the NC group, 2.11% in the EA group, and 2.11% in the CUMS group. The proportion of Tyzzerella was 8.99% in the NC group, 6.33% in the EA group, and 13.23% in the CUMS group.

The results of the comparative analysis of multiple groups (Fig. 3B) showed that. *Lactobacillus* had the highest abundance levels and the differences between groups were statistically significant ( $p = 0.013$ ). Also, there was a statistically significant difference between groups for



**Fig. 3.** At the genus level, CUMS mice had multiple species that differed significantly from controls and EA groups. (A) Bar diagram of colony composition. (B) Multi-group comparison histogram. (C) LefSe multi-level species hierarchy tree diagram. (D) LDA Discriminant Bar Chart.

Staphylococcus and Tyzzerella (The abundance of Staphylococcus in the EA groups and Tyzzerella in the CUMS and EA groups were much shorter than other groups and could not be shown in the histogram). And norank\_f\_Ruminococcaceae was seen to have a more pronounced trend, but the difference between groups was not statistically significant ( $p = 0.059$ ).

### 3.7 Linear Discriminant Analysis of Effect Size (LEfSe)

LEfSe can be used to find the specific characteristics that best explain the differences between groups in both groups and the extent to which these characteristics influence the differences between groups. LDA discriminant histograms counted the microbial groups that played a sig-

nificant role in many groups. The LDA discriminant analysis shows that species with LDA >2 have a greater effect on intergroup variation (Fig. 3D). The LDA of the three species with significant differences in multiple comparisons at the genus level were *Lactobacillus* LDA = 4.90, *Staphylococcus* LDA = 4.03, *Ruminococcaceae* LDA = 3.98, and *Tyzzereella* LDA = 4.73.

## 4. Discussion

### 4.1 Establishment of Animal Model

CUMS had been chosen as the animal model in this study. And certain kinds of behavioral testing were involved to assess the success of modeling and intervention effect. Anhedonia and decreased locomotor activity are two common depressive behaviors shown in the CUMS model, which is widely used as an animal model for depression. Depression-related anorexia nervosa is characterized by a lack of enjoyment of food in humans and animals. The reduction in food intake may then lead to weight loss.

Sucrose preference tests are classic tests for detecting depression in rodents, and a reduced preference for sucrose is a key indicator of depression. In addition, OFT measures motor activity, and a decrease in motor activity indicates anxiety-like behaviors associated with depression [18]. Therefore, we used SPT, weighing, and OFT to evaluate depressive behaviors in CUMS mice in the present study.

After modeling, CUMS mice exhibited significant depression-like symptoms such as reduced preference for sugar water decreased spontaneous motor function and weight loss. These results are consistent with the findings in previous reports and support the success of the modeling [19].

### 4.2 Acupoint Selection

EA was chosen as the treatment in this study. EA is a commonly recommended treatment for depression [20]. Additionally, the 2014 “Evidence-based Guidelines of Clinical Practice with Acupuncture and Moxibustion: Depression” also recommended EA treatment and auricular acupuncture as treatments for depression [21]. And the behavioral tests described above were used to assess the effect of the intervention. Researchers have reported that EA at GV20 and GV29 acupoints alleviate depression-related symptoms in chronic unpredictable mild stress-induced rats [22].

This finding is supported by our findings, which show that EA enhances sucrose uptake in SPT and reduces immobility times in OFT, allowing the reversal of the typical manifestations of depression [23]. Notably, EA treatment resulted in a structure and abundance of gut microbes in CUMS mice that more closely resembled controls, which is consistent with other studies showing that gut flora functions through the brain-gut axis to influence depression [9]. In conclusion, as a result of these findings, EA has demon-

strated its effectiveness in treating depression-like behaviors caused by CUMS.

### 4.3 Gut Flora Analysis

Intestinal flora in the feces of mice was analyzed in this study. The species of intestinal flora analyzed with statistically significant differences in the three sets of multiple comparisons at the genus level were *Lactobacillus* and *Staphylococcus*, and both species had LDA >2 (considered statistically significant). Therefore, we focused on the possible mechanisms by which changes in the abundance of *Lactobacillus* and *Staphylococcus* improve depression-like behavior in mice. In addition to this, there are *Ruminococcaceae* and *Tyzzereella* with LDA scores also >2. It has been suggested that they might also be the flora associated with depression-like behavior, so we discussed them as well.

This study found that NC and EA groups had higher relative *Lactobacillus* abundances than CUMS groups. Recent studies have shown that *Lactobacillus* has some depression-relieving effects. *Lactobacillus Plantarum* DP189 reduced hippocampal neuronal apoptosis in mice, attenuated depression-like behavior, and prevented hippocampal nerve damage by modulating immune function. DP189 reduced serum IL-1 $\beta$  and TNF- $\alpha$ , decreased mitogen-activated protein kinase 7 and c-jun amino-terminal kinase 2 levels in the hippocampus, and down-regulated the immune content of the pro-apoptotic protein Bax. These results suggest that DP189 therapy may prevent and/or attenuate cortisol-induced depression-like behavior and hippocampal neurological damage [24].

Meanwhile, it has been shown that *Lactobacillus* cases may improve the protein expression of the monoamines dopamine, norepinephrine, and 5-hydroxytryptamine, brain-derived neurotrophic factor (BDNF) and its tyrosine kinase receptor B (TrkB) receptor, N-methyl-D-aspartate receptor 1, through the BDNF-TrkB pathway, thereby improving depressive behavior in mice [19]. Long-term application of *Lactobacillus rhamnosus* (JB-1) induced region-dependent alterations in GAB1b mRNA in the brain and decreased the expression of GABA receptors in the hippocampus, amygdala, and blue spot, thus exerting an antidepressant effect, as well as reducing cortisol and improving cortisol-induced anxious-depressive behavior. These two effects are mediated by the vagus nerve [19].

In the present experiment, after chronic unpredictable mild stress (CUMS) stimulation, the number of lactobacilli in mice decreased, and after EA treatment, the number of lactobacilli increased close to normal levels and was statistically different ( $p = 0.013$ ), which is the same as the experimental results of other researchers. Therefore, we propose a hypothesis that CUMS stimulation can reduce the abundance of *Lactobacillus* and EA can exert antidepressant effects by upregulating the abundance of *Lactobacillus*. The specific mechanism of *Lactobacillus* for depression is still being explored and may be a potential target for depression treatment.

As compared to the other two groups, the CUMS group had a higher relative abundance of *Staphylococcus*. *Staphylococcus* is a gram-positive bacterium associated with infection and toxicity. *Staphylococcus aureus* produces staphylococcal enterotoxins and glutamine, which stimulate the vagus nerve and send signals to the brain, causing vomiting and nausea behavior [25], suggesting that staphylococci may damage the nervous system. Some studies have demonstrated the antimicrobial activity of antidepressants selective 5-hydroxytryptamine reuptake inhibitors (SSRIs), including sertraline, paroxetine, and fluoxetine, against *Staphylococcus* and reduced the abundance of *Staphylococcus* [11].

This is consistent with the results of this experiment, no *Staphylococcus* was detected in the NC group, and the abundance of *Staphylococcus* increased after CUMS modeling. The abundance of *Staphylococcus* decreased after EA treatment, with a statistically significant difference compared to the CUMS group ( $p = 0.0032$ ). This suggests a potential target of *Staphylococcus* for the treatment of depression and shows that EA may have some anti-inflammatory effects.

Ruminococcaceae were more abundant in CUMS and EA groups than in NC groups ( $p = 0.059$ ). Ruminococcaceae is a pathogenic bacterium [26]. Several studies have shown that *Ruminococcus* can reduce the antidepressant effect of antidepressant drugs and that antidepressant drugs can reduce the abundance of *Ruminococcus* [27]. Researchers have found that patients with autism spectrum disorders have higher levels of *C. tumefaciens* in their bodies [28]. These results imply that rumen bacteria may be a deleterious factor in depression.

In terms of the mechanism of action of rumen bacteria on depression, several studies have shown that rumen cocci can secrete tryptophan decarboxylase and catalyze the formation of the neurotransmitter tryptamine. Tryptophan induces the release of 5-hydroxytryptamine, which regulates intestinal motility. Since tryptophan decarboxylase secreted by *C. rumen* consumes tryptophan in the intestine causing a decrease in plasma tryptophan levels, leading to a decrease in 5-hydroxytryptamine levels in the brain, this may be a direct mechanism by which *C. rumen* affects anxiety-depressive behavior [29].

In the present experiment, the number of Ruminococcaceae in mice increased after chronic unpredictable mild stress (CUMS) stimulation and was statistically different ( $p = 0.03$ ), which is consistent with the experimental results of other researchers. However, intriguingly, the number of rumen cocci did not change after EA treatment. EA treatment did not seem to affect the abundance of this flora, the mechanism of which is unclear.

The relative abundance of *Tyzzarella* in the NC group was significantly higher than other two groups in this study. Recent studies on *Tyzzarella* have focused on the relationship between *Tyzzarella* and cardiovascular disease and

obesity. Some studies have shown that *Tyzzarella* is associated with Obese [30]. Some studies have shown that *Tyzzarella* is associated with dietary patterns and has an elevated abundance in the intestinal flora of mice on a high-fat diet [31,32]. The relative abundance and bodyweight of *Tyzzarella* were elevated in high-fat diet mice in these studies. This is consistent with our experiments, where *Tyzzarella* abundance was higher in the NC group compared to the EA group ( $p = 0.001975$ ), while the CUMS group did not persist to *Tyzzarella*.

We speculate that this is related to the fact that the CUMS modeling intervention may affect the mice's food intake. However, some studies of postpartum depressive disorder have shown that a correlation was observed between *Tyzzarella* levels and the severity of depressive symptoms [33]. But in our experiments, a higher abundance of *Tyzzarella* was found in the NC group than in the other group. Even after the EA intervention, *Tyzzarella* abundance levels did not change significantly. This raises the hypothesis that *Tyzzarella* may affect mouse behavior, but that the altered abundance of *Tyzzarella* is not affected by CUMS intervention and EA treatment, but is more related to sex hormones and dietary patterns.

This study showed that the flora with statistically different alterations in the three groups of multiple comparisons was *Lactobacillus* and *Staphylococcus*. The possible mechanisms linking these two bacteria to depression-like behavior are also discussed. *Lactobacillus*, which has been shown in earlier studies to be possibly related to the pathogenesis of depression, was shown to increase in abundance and improve behavior in mice treated with EA in this experiment. *Lactobacillus* may improve depression-like behavior by inhibiting inflammatory substances, decreasing hippocampal neuronal apoptosis, and altering BDNF protein expression by the BDNF-TrkB pathway.

Patients with sepsis are known to have an ecological dysbiosis of the gut microbiota due to systemic inflammation. In turn, changes in the intestinal flora can exacerbate the symptoms of sepsis [34]. In addition, it has been shown that electroacupuncture improves the signs and symptoms of ulcerative colitis and restores the balance of the intestinal flora by a mechanism that may be immunomodulation, especially T-cell regulation [35]. Considering our findings, we hypothesize that immunomodulation may be one of the most important parts of the mechanism of the effects of EA on adjusting the abundance of the microbes.

In contrast to *Lactobacillus*, *Staphylococcus aureus* may lead to depressive-like behavior by producing staphylococcal enterotoxins that damage the nervous system, and EA treatment resulted in decreased abundance of *S. aureus* and improved behavior in mice.

In addition to this, some bacteria with possible roles are discussed. For example, *Staphylococcus tumefaciens* may be secreting tryptophan decarboxylase, which depletes plasma tryptophan causing a decrease in brain 5-



hydroxytryptamine levels and leading to depression-like behavior. Typhimurium was found to be associated with obesity and dietary patterns, suggesting that Typhimurium may influence host behavior [36]. These results should be translated into the human population to confirm preliminary animal data. Accordingly, further investigations and future research may be warranted.

#### 4.4 Limitations

Our study has some limitations. First, we have not yet explored the brain interaction intestinal mechanism, the next step to improve the metabolome and brain neural and other studies. Second, the research conclusions for mental disorders should be cautiously applied to human beings due to racial differences and mouse model limitations. Further research will be conducted to overcome these shortcomings.

### 5. Conclusions

These results suggested that the antidepressant effect of EA is related to the altered structure and abundance of the intestinal flora, particularly related to *Lactobacillus* and *Staphylococcus*.

#### Author Contributions

XQ, ZL—investigation, writing - original draft; ZZhang—conceptualization, data curation, writing - review & editing; SH, FW, ZF and JZ—investigation; XC—validation; ZZheng, SQ—formal analysis; YH—conceptualization, resources, funding acquisition; ZZhang—methodology, resources, funding acquisition.

#### Ethics Approval and Consent to Participate

The experiments were conducted in accordance with the NIH Guidelines for the Care and Use of Animals and were approved by the Animal Experimentation Ethics Committee of Southern Medical University (L0219007).

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#### Conflict of Interest

The authors declare no conflict of interest.

#### Supplementary Material

Authors of accepted manuscripts may provide related supplemental data to be posted online along with the pub-

lished manuscript. All Supplemental Data information (except videos) should be combined into a single Word/PDF file. Before submission, carefully review all files; if you wish to make changes to supplementary material during any stage of the process, please make sure to provide an updated file. The journal is not responsible for any errors contained in data supplements.

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