Received: 3 June 2022 | Revised: 27 July 2022 | Accepted: 17 August 2022

DOI: 10.1002/ar.25065

SPECIAL ISSUE ARTICLE

The Anatomical Record WILEY

Electroacupuncture modulates gut microbiota in mice: A potential target in postoperative cognitive dysfunction

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Funding information
Ningbo public welfare science and technology, Grant/Award Number: 202002N3141; Ningbo University, Grant/Award Number: 201804; Zhejiang science and technology plan of traditional Chinese Medicine, Grant/Award Number: 2020ZB236

Abstract

The detailed mechanism of inflammation in postoperative cognitive dysfunction (POCD) is unclear. This study aimed to determine whether electroacupuncture (EA) ameliorates POCD by modulating gut microbial dysbiosis. Compared to the control group, mice in the EA group were treated at the acupoints Zusanli (ST36), Quchi (L111), Baihui (GV20), and Dazhui (GV14) 1 week before appendectomy. Novel object recognition and the Morris water maze tests were used to assess learning and spatial reference memory deficits, whereas hippocampus samples and stool samples were collected for central inflammatory tests and 16S-rRNA sequencing of intestinal flora, respectively. In amyloid precursor protein/presenilin 1 (APP/PS1) mice, EA enhanced spatial memory and learning deficits. The fecal microbial community was altered in APP/PS1 mice in the absence of EA following surgery. Among them, *Coprococcus* and *Bacteroidetes* were more abundant in the EA groups than in the control groups; however, *Actinobacteriota*, *Helicobacteraceae*, and *Escherichia/shigella* constitute the minor bacterial colonization in the EA groups. Furthermore, we found a significant negative correlation between *Firmicutes* and escape latency (Pearson correlation coefficient − 0.551, $p < 0.01$) and positive correlation between *Proteobacteria* and escape latency (Pearson correlation coefficient 0.462, $p < 0.05$). Electron microscopy revealed signs of blood–brain barrier (BBB) impairments and immunofluorescence images showed glial cells activated in the hippocampus of APP/PS mice without EA, and serum diamine oxidase levels were increased in these mice; whereas EA treatment significantly relieved the above pathological changes. Our findings implied that EA decreases hippocampal inflammation of APP/PS1 by upregulating benificial gut microbiota, reducing BBB and intestinal barrier dysfunction, thus alleviates postoperative cognitive dysfunction. This may provide a novel target in POCD management.

KEYWORDS

blood–brain barrier, electroacupuncture, gut microbiota, postoperative cognitive dysfunction

Abstract

术后认知功能障碍(POCD)中枢炎症的详细机制尚不清楚。本研究旨在确定电针刺激（EA）是否通过调节肠道微生物菌群失调来改善 POCD。本实验将接受手术的淀粉样前体蛋白/早老素 1（APP/PS1)和同窝野生小鼠各20只，随机分为

非电针组(WT-C和APP/PS1-C)和电针组(WT-E和APP/PS1-E)。与非电针组相比，电针组小鼠在阑尾切除术前一周分别在足三里(ST36)、曲池(L111)、百会(GV20)和大椎(GV14)穴位进行电针。新物体识别和莫里斯水迷宫测试用于评估小鼠学习和空间参考记忆缺陷，而海马样本和粪便样本分别用于中枢炎症因子测试和 16S-rRNA 测序评估肠道微生物菌群。结果发现:在APP/PS1小鼠中，电针组改善了空间记忆和学习缺陷。非电针组APP/PS1 小鼠术后的粪便微生物群落发生了改变。电针组的粪球菌和拟杆菌属非电针组明显，而放线菌、螺杆菌科和大肠杆菌/志贺氏菌表则显著低于非电针组。此外，我们发现厚壁菌与逃潜伏期之间存在显著相关(相关系 - 0.551, $p < 0.01$)，变形杆菌与逃潜伏期相关(相关系0.462, $p < 0.05$)。电镜显示非电针组APP/PS1小鼠血脑屏障 (BBB)明显 受损，免疫荧光图像显示其海马神经胶质细胞被激活，血清二胺氧化酶水平升高；而电针治疗显著缓解了上述病理改变。综上所述，电针通过上调有益的肠道微生物群、减轻血脑屏障和肠道屏障损伤来降低海马炎症反应， 从而改善APP/PS1 小鼠术后认知功能障碍。因此针灸有望为 POCD 提供一种新的防治方法。

1 | INTRODUCTION

In the old adult population, postoperative cognitive dysfunction (POCD) is particularly becoming the major cause of disability and mortality, affecting up to 54% in the first few weeks following surgery (Alam et al., 2018; Eriksson et al., 2019; Feinkohl et al., 2017). Currently, the incidence of POCD within 3 months following discharge is 12.9% (Nemeth et al., 2017). Elderly patients with POCD have a greater risk of developing dementia or cognitive trajectory deterioration (Needham et al., 2017).

As the detailed pathological mechanism of POCD remains unknown, recent evidence suggests that expansion of peripheral pro-inflammatory cytokines initiates damage-associated molecular patterns (DMAPs), allowing inflammation to develop in the central nervous system (Safavynia & Goldstein, 2018).

Recently, dysbiosis of the gut microbiota has been identified as a risk factor for mental illnesses by triggering brain inflammatory response (Bourassa et al., 2016; Shen et al., 2017). Wu et al. demonstrated that *Enterobacter* infection accelerated the course of Alzheimer's disease by inducing tumor necrosis factor (TNF)/c-jun N-terminal kinase-mediated neurodegeneration (Wu et al., 2017). Furthermore, Lian et al. reported that POCD is associated with adverse changes in gut microbiota and fecal metabolites (Lian et al., 2021). Lately, Luo et al. observed numerous pro- and anti-inflammatory gut microbiota that can help prevent high-fat diet-induced obesity and metabolic inflammation (Luo et al., 2022). Previously, the gut microbiota has been reported to possess a strong influence on postoperative cognitive impairment and may be a viable therapeutic target (Jiang et al., 2020; Luo et al., 2021) but there is no clear evidence regarding the involvement of gut microbiota in cerebral inflammation.

Electroacupuncture (EA) is a non-pharmacological technique used to treat many neuropsychiatric illnesses. EA improves cognitive function through many processes, such as reducing neuronal death (Guo et al., 2015), promoting neurogenesis (Li et al., 2014), driving distinct neuropeptide Y (NPY)-expressing sympathetic pathways (Liu et al., 2020; Liu, Wang, et al., 2021), and enhancing endogenous Nrf2-mediated antioxidative mechanism (Liu et al., 2022). Most importantly, these pathways enhance the overall anti-inflammatory response.

Previous studies have shown that EA could improve chronic colitis by modulating gut microbiota (Wang et al., 2020). Furthermore, research on the animal mechanism of EA-induced improvement in spatial learning and memory revealed the regulatory effects of combining acupoints, Baihui (GV20) and Zusanli (ST36) (He et al., 2021) but these studies did not explore how EA-induced gut microbiota modulation could influence neuroinflammation. Hence, in this study, we investigated the role of EA in alleviating neuroinflammation and improving behavioral defects by modulating gut microbiota in POCD mice (overall design figure see Figure 1).

2 | MATERIALS AND METHODS

2.1 | Animals and experimental procedure

Male amyloid precursor protein/presenilin 1 (APP/PS1) mice and its littermate wild-type (WT) mice aged male

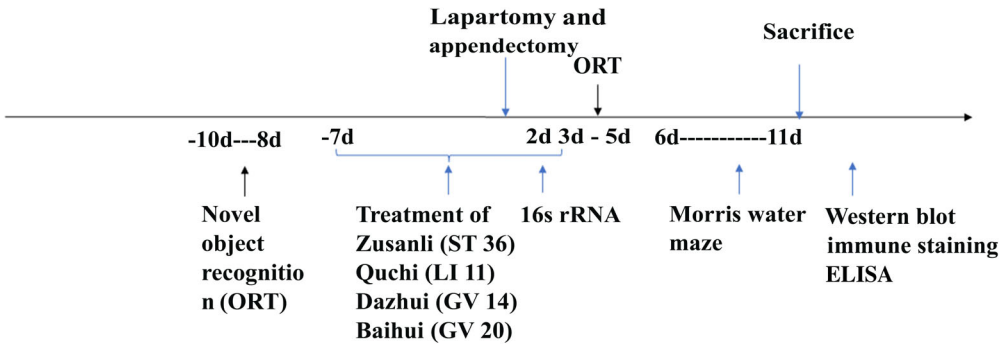

FIGURE 1 Experiment design. The mice received a novel object recognition test after acclimatization, then EA was performed daily from preoperative day 7 until postoperative day 3, except on the day of surgery. Aged mice were anesthetized in a chamber prefilled with 1.5% isoflurane, and underwent 20 min abdominal exploration. Novel object recognition and Morris water maze analysis were used to evaluate the effects of EA on cognition. On postoperative day 11, animals were sacrificed for biochemical study. EA, electroacupuncture

(5-month-old; weight, 30–40 g) were used for all experiments. Animals were purchased from the Jiangsu Ai Lingfei Biotechnology Co., Nanjing, China and bred under standardized housing conditions with food and water fed ad libitum and kept at 23 ± 2°C with a 12-hr light/dark cycle. This study was approved by the Animal Ethics Committee of the Affiliated Hospital of Medical School of Ningbo University (approval number NBU20210003) and followed the National Institutes of Health's "Guide for the Care and Use of Laboratory Animals."

Following a 1-week acclimatization period, APP/PS1 ($n = 20$) and WT ($n = 20$) mice were randomly divided into POCD control groups (WT-C and APP/PS1-C), and POCD + EA treated groups (WT-E and APP/PS1-E). POCD model was generated by appendectomy as previously described (Xu et al., 2019). Briefly, mice were subjected to appendectomy under 3% sevoflurane, and the surgical procedure was performed under sterile conditions for approximately 15 min during which the animal body temperature was maintained at 36.3–37.2°C using heat pads. The SpO2 was measured to ensure that there was no hypoxia (SpO2 < 90%) throughout the procedure, whereas potential motor and memory deficiencies were assessed using novel object recognition and the Morris water maze (MWM) tests.

2.2 | Electroacupuncture treatment

EA was performed daily for seven consecutive days before surgery and continued additional 2 days after surgery. EA treatment was performed on four acupoints, Zusanli (ST36) which is located on the tibialis anterior muscle, Quchi (L111) located at the outer end of the elbow stripes, Baihui (GV20) located on the scalp, and Dazhui (GV14) located at the seventh cervical spinous process (Liu, Yin, et al., 2021). Acupuncture needles were inserted at a depth of 2–3 mm, and the EA instrument (China Huato 3800, A-M Systems) provided continuous-wave stimulation at a frequency of 15 Hz for 30 min to the connected acupoints in the EA-treated mice.

2.3 | Novel objective recognition test

The object recognition tests were carried out before and after surgery in a square chamber made of white non-porous plastic (approximately 40 cm × 40 cm × 40 cm) (Lueptow, 2017). The tests comprised 1-day adaptation and 1 day of training followed by another day of testing (see the design figure). On the ninth day before surgery and the fourth day after surgery, the mice spent 5 min exploring two of the same objects (a red wooden block with a length of each side = 5 cm). On the eighth day before surgery and the fifth day following surgery, a new object with a different form (a blue wooden cylinder with a height of 7 cm) replaced one of the objects, and the mice were provided another 5 min to explore the area. The mice's exploration learning time was calculated as sniffing or touching the objects with their nose and/or forepaws at a distance of less than 2 cm. Sitting on the objects was not counted as exploratory behavior. The exploratory behavior was monitored by a video camera, and object recognition times were manually scored by a blinded investigator. The cognitive function was measured using a novel object recognition ratio (also known as recognition index), which was calculated as the exploration time of the novel object compared with the total exploration time. The field was decontaminated with 25% ethanol solution to avoid scent clues.

2.4 | The Morris water maze test

The MWM test was performed as previously described (Zhou et al., 2020). Briefly, MWM was performed in a

circular pool (120 cm in diameter and 50 cm in depth) filled with water as deep as 30 cm at 22 ± 3°C. A platform (10 cm in diameter) was positioned at 1 cm above the surface of the water for the first day and below the water surface for the rest 5 days. On the first day, each mouse was allowed to swim freely to familiarize itself with the environment and water temperature in the circular pool, from the second day to the fourth day, each animal was trained to locate the hidden platform with four trials per day at an interval of 30 min. If the mice did not find the platform within 90 s, they would guide to the target platform and stay for 15 s. On the fifth day, the platform was removed, and the trained mice were allowed to probe the target quadrant where the removed platform had been located for 90 s. The number of platform crossings and swimming time in each quadrant of the pool were recorded. All trials were monitored by a video camera linked to a video tracking system (Duoyi, shanghai) and assessed with ANY-maze software (Stoelting, UK). After each test, the water was drained, and the pool was cleaned with 25% ethanol to minimize olfactory cues between each test.

2.5 | Enzyme-linked immunosorbent assay

Hippocampal tissues were collected and homogenized using/ an ultrasonic tissue disruptor. The quantities of interleukin-6 (IL-6), IL-1, and TNF-α in the homogenized samples were determined using ELISA kits (Cat# PI326 for IL-6, Cat# P5898 for IL-1, Cat# PT512 for TNF-α, Beyotime, China) per the manufacturer's instruction. D-Lactic acid, diamine oxidase (DAO), and endotoxin lipopolysaccharide (LPS) levels in mice plasma were also measured using commercially available ELISA kits (Cat#E-EL-M0412c, Elabscience, China, and Cat# CSB-E13066m, Cusabio, China).

2.6 | Immunofluorescence staining

For immunofluorescence assay, mice were severely sedated with intraperitoneal pentobarbital sodium (50 mg/kg) and slowly perfused with 40 ml of saline, followed by perfusion of 40 ml of 4% paraformaldehyde (PFA) as previously described (Tao et al., 2016). Coronal brain tissues were

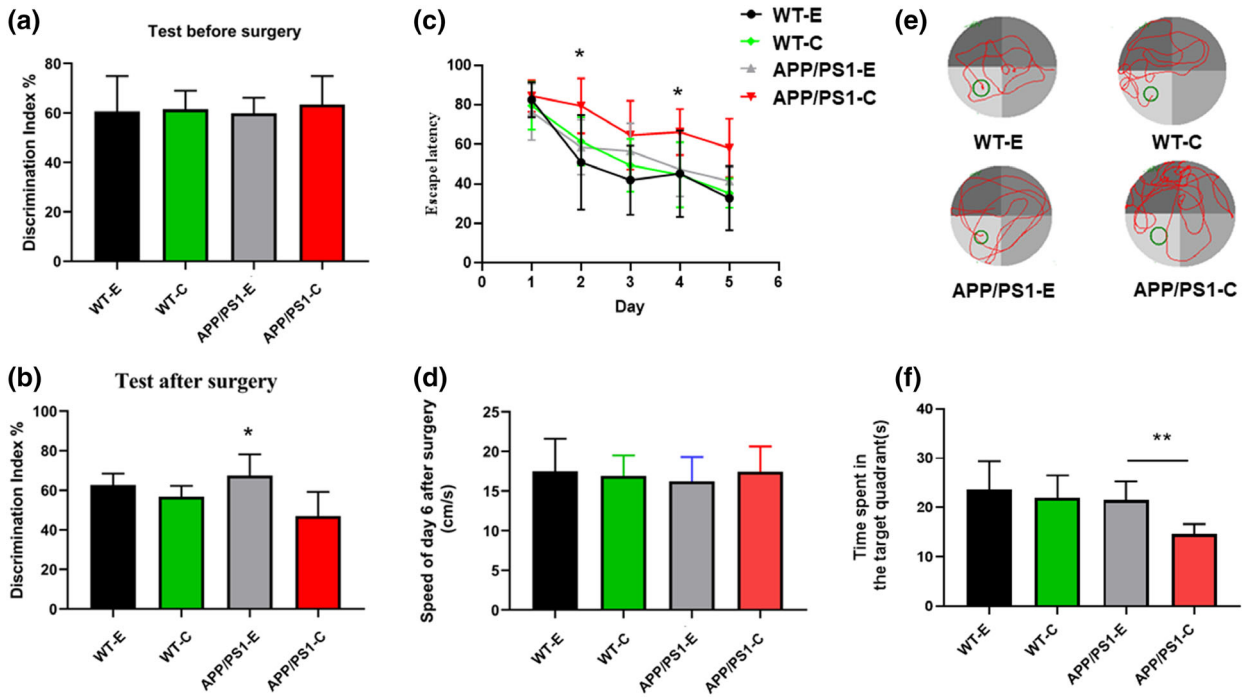

FIGURE 2 EA reduces the defects in recognition caused by abdominal surgery under general anesthesia in 5-month-old amyloid precursor protein/presenilin 1 (APP/PS1) mice. Following 1 day of open field adaption, novel object recognition tests were performed on 8-9 days before surgery and 3–5 days following surgery. (a) During the test session of new object recognition before EA intervention, all groups displayed comparable overall exploration time. (b) During a training session on new object recognition 5 days following surgery, the APP surgery + EA (APP/PS1-E) group exhibited a higher recognition index than the amyloid precursor protein surgery (APP/PS.C) group (one-way ANOVA $F_{[3,16]} = 5.046$, $p < .05$). (c) EA pretreatment mitigates surgery-induced spatial memory impairments in APP/PS1 mice, on days 7 and 9 following surgery, and prolonged escape latency was observed in the APP/AP1-C group whereas no significant difference was detected among the WT groups (two-way ANOVA $F_{[9,104]} = 1.995$, $p < .05$). (d) The speed of four groups during the training and probe session. (e) Probe trials revealed how much time was spent in each quadrant. (f) The time spent by each group in the target quadrant, thereby, measuring memory retention capabilities (one-way ANOVA $F_{[3,29]} = 9.756$, $p < .001$). Values are represented by mean ± SEM, *$p < .05$, **$p < .01$, versus APP/AP1-C. EA, electroacupuncture; WT, wild type

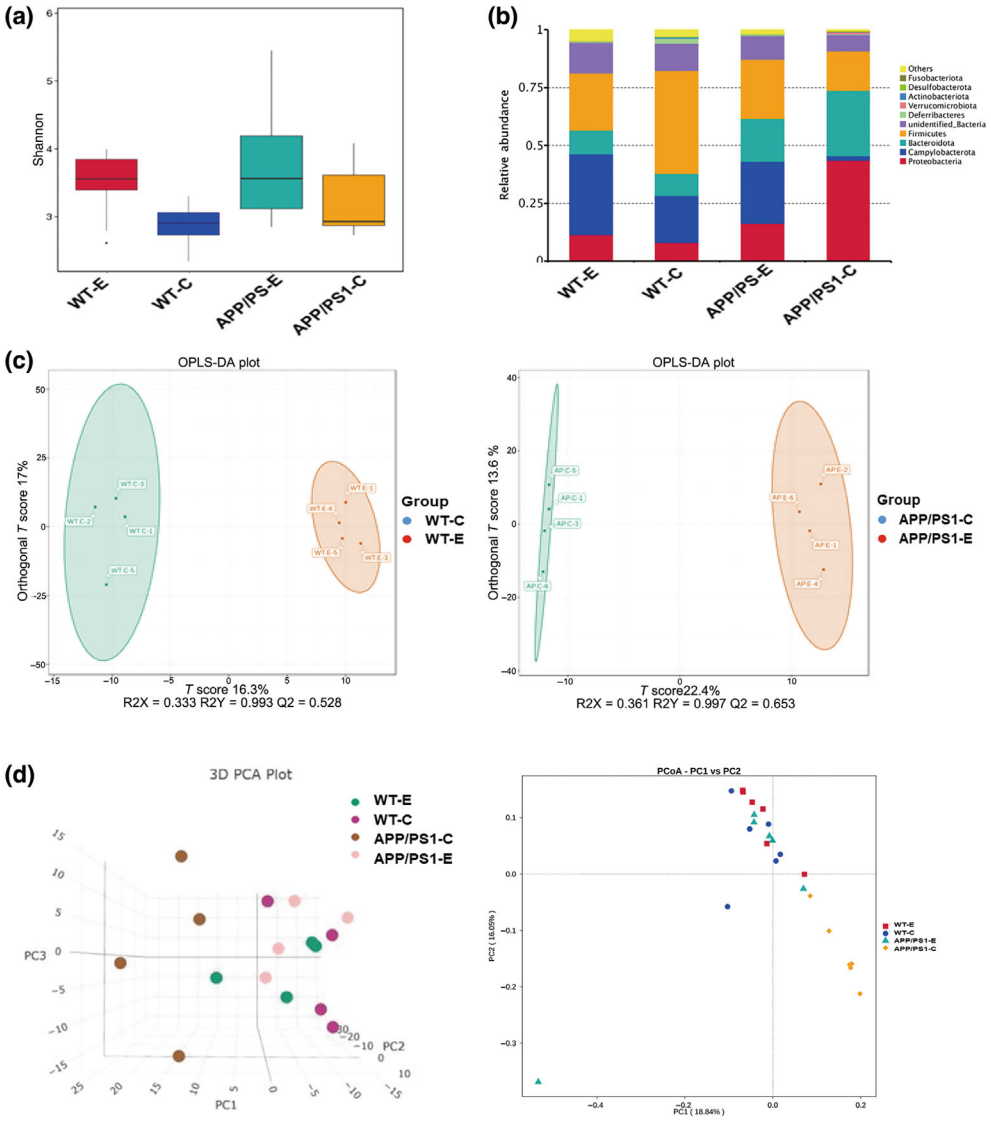

FIGURE 3 Effect of EA on the differential profiles of gut microbiota among WT-E (wild-type surgery + EA), WT, APP/PS1-E (amyloid precursor protein [APP] surgery + EA), and APP/AP1-C (APP surgery) groups. (a) The Shannon index of gut microbiota in four groups, $F_{[3,20]} = 2.568$, $p < .01$, (b) using the orthogonal partial least squares discriminant analysis (OPLS-da) model, analyzing the metabolome data, and drawing the score map of each group, we received R2Y = 0.997, Q2 = 0.504 for the distinction of posion between WT-E and WT-C (WT surgery) groups and R2Y = 0.997, Q2 = 0.653 for the distinction of posion between APP/PS1-E and APP/AP1-C groups. (c) and (d) represent 2D and 3D analyses of within-group metabolome differences, respectively. EA, electroacupuncture

sliced into sections of 30 μm thickness using a microtome cryostat (Leica). Following PBS washing for 15 min, brain sections were using QuickBlockTM (Beyotime, China) following manufacturer guidelines, and then incubated with primary antibodies against the glial fibrillary acidic protein (GFAP, Abcam, ab68428) and ionized calcium-binding adaptor molecule-1 (Iba1, Abcam, ab178846) overnight at 4°C. After probing with goat anti-rabbit Alexa Fluor 488 secondary antibody (Invitrogen; A11008), the fluorescence intensities were captured with a fluorescence microscope (Leica, Germany), and the images were analyzed using Image J software (version 1.52a; National Institutes of Health).

2.7 | Western blotting

Western blotting was performed according to the standard protocols. Hippocampal tissues were homogenized in lysis buffer (RIPA:phosphatase inhibitor:protease inhibitor = 97:1:2). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was used for protein separation, and then transferred to polyvinylidene fluoride membranes. Following non-specific blocking with skimmed milk, the membranes were incubated with antibodies against Occludin (Abcam, UK), GFAP (Abcam, UK), platelet-derived growth factor (PDGF) receptor β (Cell Signaling Technology, US), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH, Abcam, UK), and the protein expression were assessed relative to GAPDH, which considered as a loading control. All the tests were repeated three times and the specific bands were detected and quantified using Odyssey Infrared Imaging System (LI-COR).

2.8 | Electron microscopy

Mice hippocampal tissues were fixed in 2.5% glutaraldehyde for 1 hr before being treated with 1% osmium

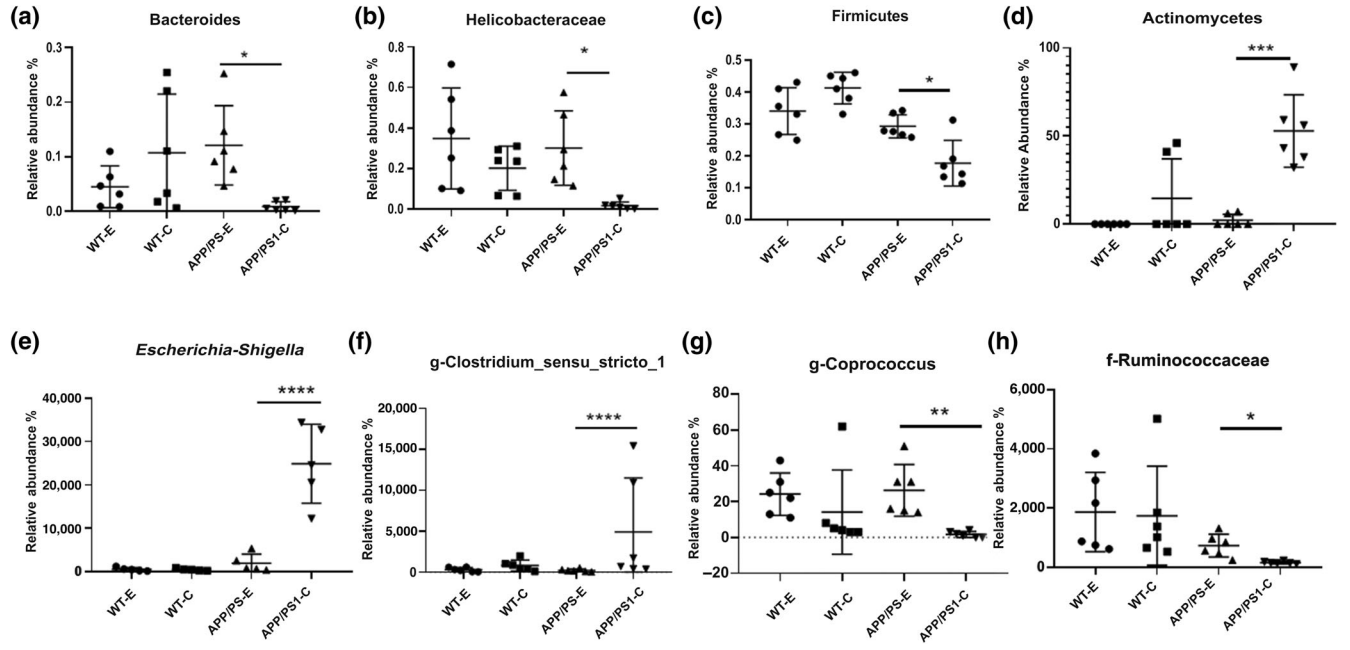

FIGURE 4 EA reduced the modification of intestinal flora caused by surgery. The top eight gut bacteria changed between WT and amyloid precursor protein/presenilin 1 (amyloid precursor protein [APP]/PS1) mice fecal samples at six phylogenetic levels (phylum, class, order, family, genus, and species). (a) Bacteroides ($H_{(3,22)} = 10.46$; $p = .015$); (b) Helicobacteraceae (Kruskal–Wallis non-parametric test, $H_{(3,22)} = 13.82$; $p = .003$); (c) Firmicutes (Kruskal–Wallis non-parametric test, $H_{(3,22)} = 8.315$; $p = .02$); (d) actinomycetes (Kruskal–Wallis non-parametric test, $H_{(3,22)} = 17.695$; $p = .02$); (e) *Escherichia-shigella* (Kruskal–Wallis non-parametric test, $H_{(3,22)} = 12.921$; $p = .0034$); (f) g-*Clostridium*-sensu-stricto-*1* (Kruskal–Wallis non-parametric test, $H_{(3,22)} = 7.953$; $p = .047$); (g) *Bifidobacterium* (Kruskal–Wallis non-parametric test, $H_{(3,22)} = 17.695$; $p = .001$); (h) f-Ruminococcaceaes (Kruskal–Wallis non-parametric test, $H_{(3,22)} = 13.012$; $p = .001$). EA, electroacupuncture; WT, wild type

tetroxide. Following staining with uranyl acetate and lead citrate, the tissue slides were examined under an electron microscope (Electron, Japan) and the alterations in the basal laminas, tight junctions, mitochondria, and endoplasmic reticulum surrounding the capillaries were analyzed by expert investigators who were ignorant to the treatment schedule.

2.9 | 16S rRNA of fecal samples

Fecal samples were collected on the second day after the operation. DNA was extracted using a Genomic DNA Kit (Qiagen, Germany) per the manufacturer's instructions. The primers 341F and 805R were used to amplify bacterial 16S rRNA gene sequences (V3–V4 regions) from the whole genome. On an Illumina NovaSeq platform, amplicon libraries were sequenced (Metware Technology, China). The raw paired readings from each sample were trimmed by removing the primer and barcode sequences. Fqtrim version 0.94 (https://ccb.jhu.edu/software/fqtrim/index.shtml) was used to filter raw tags to collect high-quality clean tags, and Vsearch (VSEARCH version 2.3.4, GitHub, https://github.com/torognes/vsearch) was used to filter chimeric sequences. Shannon deduced alpha diversity (diversity). QIIME2 was used to determine beta diversity. Principal coordinate analysis (PCoA) plots were created using UniFrac's unweighted and weighted distance metrics, and principal component analysis was used to evaluate gut microbiota composition before and after intervention at the phylum and genus levels.

2.10 | Statistical analysis

The data are expressed as the mean standard error of means (SEMs) and were statistically analyzed using Prism 8 (GraphPad Software Inc., CA). Kruskal−Wallis *H* tests were used for statistical analysis of gut microbial species. Pearson product–moment test was used for correlation analysis, and one-way or two-way analysis of variance (ANOVA) was used for statistical comparison between groups. Furthermore, changes in intestinal microbial alpha diversity were statistically analyzed using Bonferroni's multiple comparisons test, and post hoc analysis was done using Tukey's multiple comparisons test. $p < .05$ was considered statistically significant.

3 | RESULTS

3.1 | Electroacupuncture reversed appendectomy-induced cognition impairment in postoperative cognitive dysfunction mice

The novel object recognition test demonstrated that APP/PS1 mice had impaired learning ability, as there was no significant difference in the recognition index before surgery (Figure 2a), but the index was significantly altered ($p < .05$) after appendectomy (Figure 2b). Furthermore, during the postoperative period, on days 2 and 4 of the positioning cruise experiment (days 7 and 9 following surgery), the APP/PS1-E group had a significantly higher escape latency than the APP/AP1-C group ($p < .05$, Figure 2c) whereas the average speed during the MWM test exhibited no significant difference between the groups ($p > .05$, Figure 2d). During the test period, the APP/AP1-C group spent significantly less time in the target area than other groups ($p < .01$, Figure 2e,f), indicating that the APP/AP1-C group developed cognitive impairment after surgery.

3.2 | Electroacupuncture up-regulated anti-inflammatory flora and down-regulated pro-inflammatory flora

EA significantly increased the diversity of microbial species, as evidenced by the Shannon Index (Figure 3a). As presented in Figure 3b, the analysis of the phylogenetic species showed significant variations in the relative microbial enrichment between groups. According to the Orthogonal Partial Least Squares Discriminant Analysis model, the cations and anions were significantly different in the APP/PS1.C group compared with those in the APP/PS1.E group, indicating that the model was relatively stable. PCoA of the bacterial composition in three and two dimensions suggested that the bacterial community of the APP/PS1.C group was different from the other three groups (Figure 3c,d), which suggested that EA could facilitate vulnerable individuals to restore the proper intestinal flora distribution after surgery.

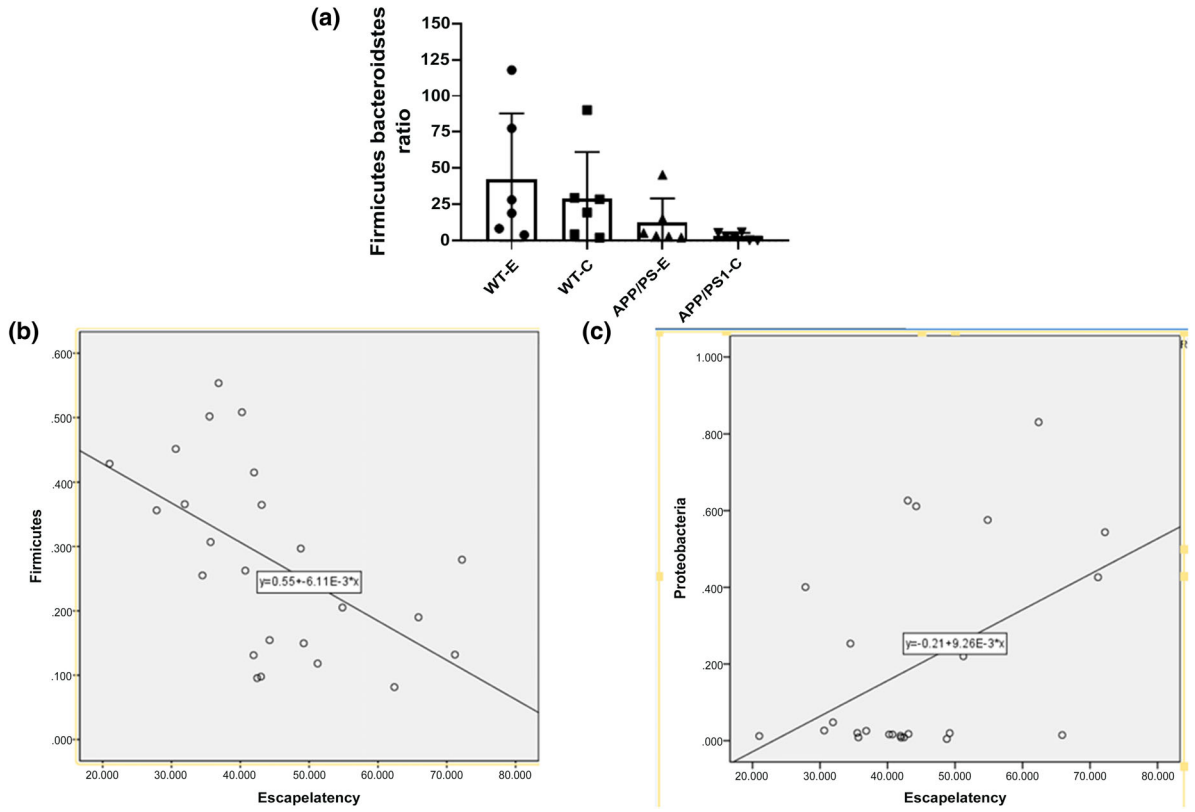

FIGURE 5 The common flora ratio and correlation between flora changes and behavior. The Firmicutes to Bacteroidetes ratio was measured using GraphPad prism 8, and there was no significance among the four groups ($F_{[3,20]} = 2.568$, $p = .1169$) (Figure 4a); the Pearson correlation analysis revealed that Firmicutes negatively correlated with cognitive impairment ($p = .005$, Figure 4b). Proteobacteria were positively correlated with cognitive impairment ($p = .023$, Figure 4c), and the correlation analysis form is presented (Figure 4d)

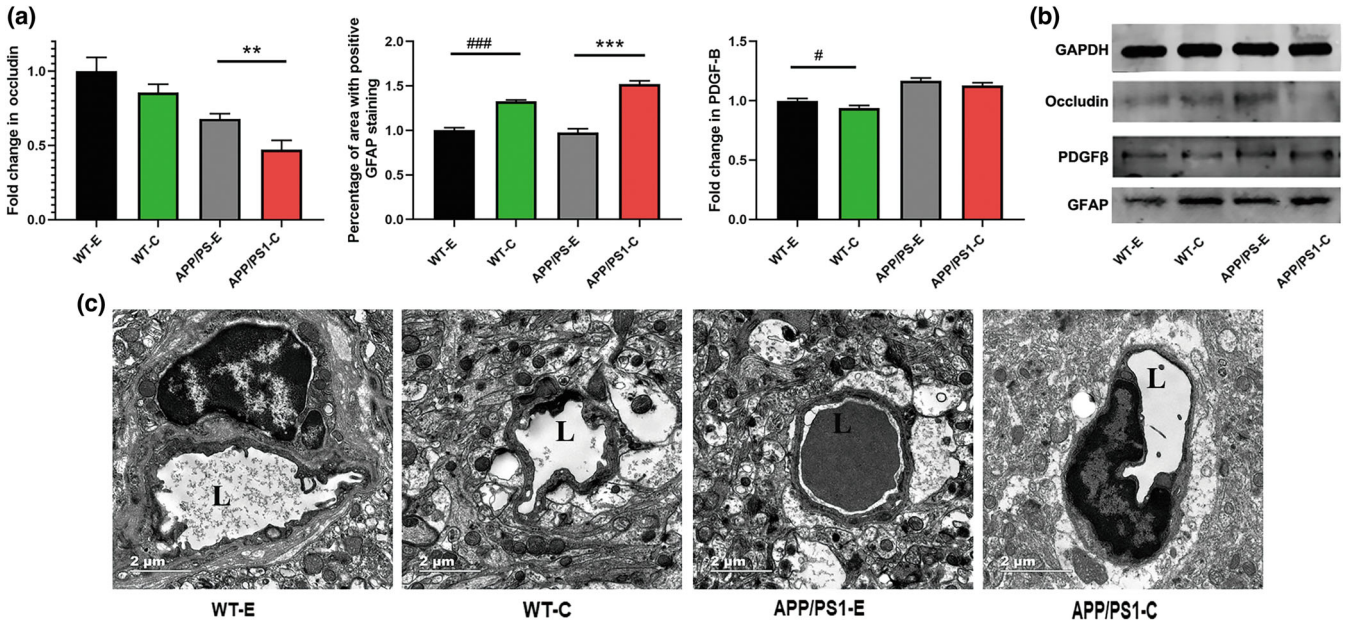

FIGURE 6 EA intervention attenuated the BBB disruption induced by surgery (a, b). There was an increase in the expression of tight junction protein Occludin in the APP/PS1-E (amyloid precursor protein [APP] surgery + EA) group compared with the APP/AP1-C (APP surgery) group ($p < .01$), and EA intervention decreased the expression of the GFAP following surgery in both WT and APP/PS1 mice. There was no difference in PDGF-β expression in the hippocampus of APP/presenilin 1 (PS1) mice; however, PDGF-β expression increased in the WT-E (wild-type surgery + EA) group than in the WT-C (wild-type surgery) group. Transmission electron microscopy revealed that the ultrastructure of the basal laminas of mice in the WT groups was continuous and integrated; however, the mice in APP/PS1 groups had BBB impairments on day 2 following surgery. In the APP/PS1 group, endfeet swelling of astrocytes (indicated by *) and scattered mitochondrial hypertrophy (indicated by #) were observed. The arrow represents the basal layer where the BBB was damaged. Results are presented as mean ± SEM ($n = 4$). Two-way ANOVA with post hoc Bonferroni test, $^{**}p < .01$, $^{***}p < .001$ versus APP/PS1-E; ### $p < .001$ versus WT-C. BBB, brain–blood-barrier; EA, electroacupuncture; GFAP, glial fibrillary acidic protein; WT, wild type

We performed 16S rRNA gene sequencing to analyze the gut microbial composition in both the WT and APP/PS1 mice, in which the relative abundances of *Bacteroides*, *Firmicutes*, *Coprococcus*, and *Ruminococcaceae* were increased in the APP/PS1-E group compared to APP/PS1-C mice (Figure 4a,c,g,h), whereas those of *Helibacteraceae*, *Actinomycetes*, *Clostridium* sensu stricto *1*, and *Escherichia/Shigella* were decreased following EA treatment in the APP/PS1 mice (Figure 4b,d,e,f). Meanwhile, analysis of microbial composition showed the insignificant difference between WT-E and WT-C groups. These findings fortify our hypothesis that EA intervention could alter the gut microbiota following POCD establishment.

No significant increase in the ratio of firmicutes to Bacteroidetes was observed in this study (Figure 5a), as demonstrated in Figure 5b,c, Pearson's correlation analysis showed significant ($p = .005$) negative correlations between *Firmicutes* relative enrichment and cognitive impairment, while *Proteobacteria* showed significant ($p = .023$) positive correlation with cognitive impairment. These findings indicated the relationship between gut microbiota and behavioral changes.

3.3 | Electroacupuncture mitigated the blood–brain barrier disruption and reduced gut permeability induced by surgery

The blood–brain barrier (BBB) integrity damage in the APP/PS1 groups was more obvious than that in the WT groups after surgery, and EA could alleviate the impairment of the BBB in APP/PS1-C group, as inflated foot edema in astrocytes and basal cell damage in APP/PS1-C were observed from transmission electron microscopy (Figure 6c). We further examined changes in the constituent cells that make up the BBB, as shown in Figure 6a,b that the expression of Occludin in the APP/AP1-C group was significantly lower than that in the APP/PS1-E group ($p < .05$), suggesting that EA can improve the destruction of the tight junctions of endothelial cells after surgery; meanwhile, the expression of GFAP in group APP/PS1-C was significantly higher than that in APP/PS1-E group ($p < .001$), suggesting that EA can reduce the activation of astrocytes after surgery. Furthermore, EA treatment increased the expression of PDGF in WT mice after surgery ($p < .05$), but the

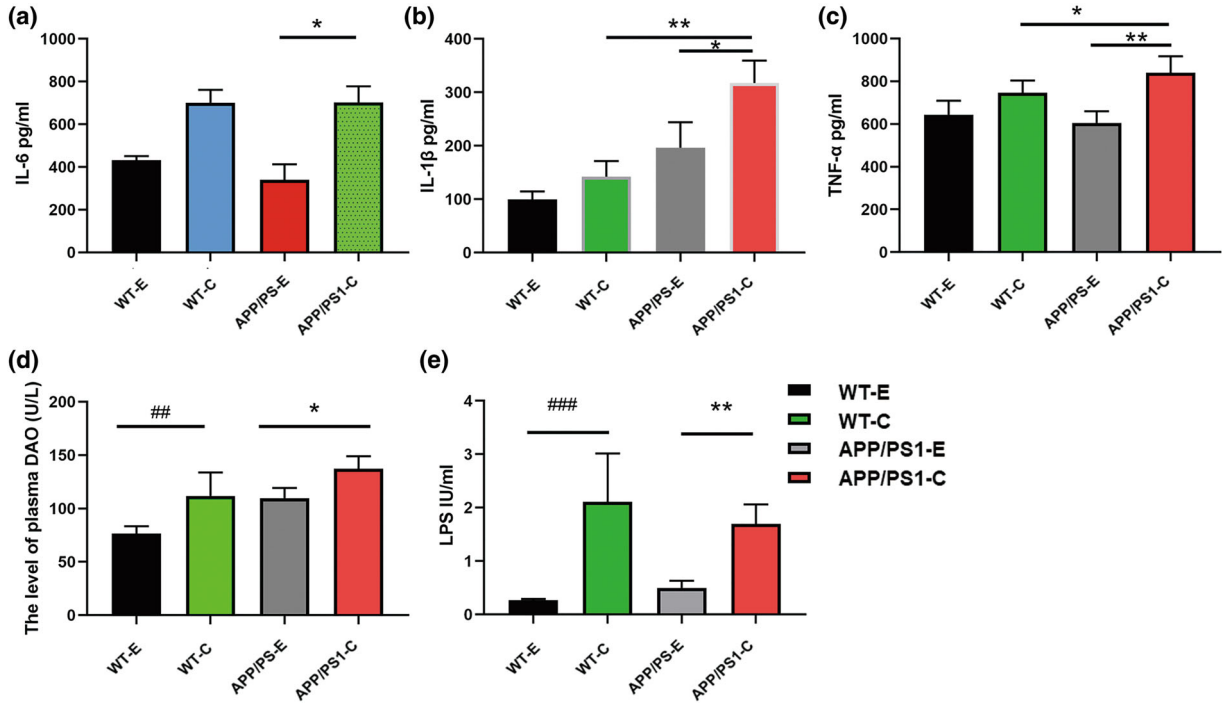

FIGURE 7 In amyloid precursor protein/presenilin 1 (APP/PS1) mice, EA reduces the expression of tumor necrosis factor TNF-α, interleukin IL-1, and IL-6 in the hippocampus caused by abdominal surgery under general anesthesia. On day 12 following surgery, mice were slaughtered. The expressions of (a) TNF-α, (b) IL-1, and (c) IL-6 in hippocampus extracts of mice were measured using ELISA. Data are represented as mean ± standard deviations ($n = 4$–6); ##$p < .01$ versus WT-C (wild-type surgery), *$p < .05$ and **$p < .01$ versus APP/AP1-C (APP surgery), and $p < .01$ versus APP/PS1-E (APP surgery + EA) (one-way ANOVA and Tukey's test). (f) EA attenuates the increase of the DAO and LPS expression in both APP/PS1 and WT mice. ##$p < .01$ versus WT-C, ###$p < .001$ versus WT-C *$p < .05$ and **$p < .01$ versus APP/AP1-C (APP surgery) (one-way ANOVA and Tukey's test). EA, electroacupuncture; ELISA, enzyme-linked immunosorbent assay

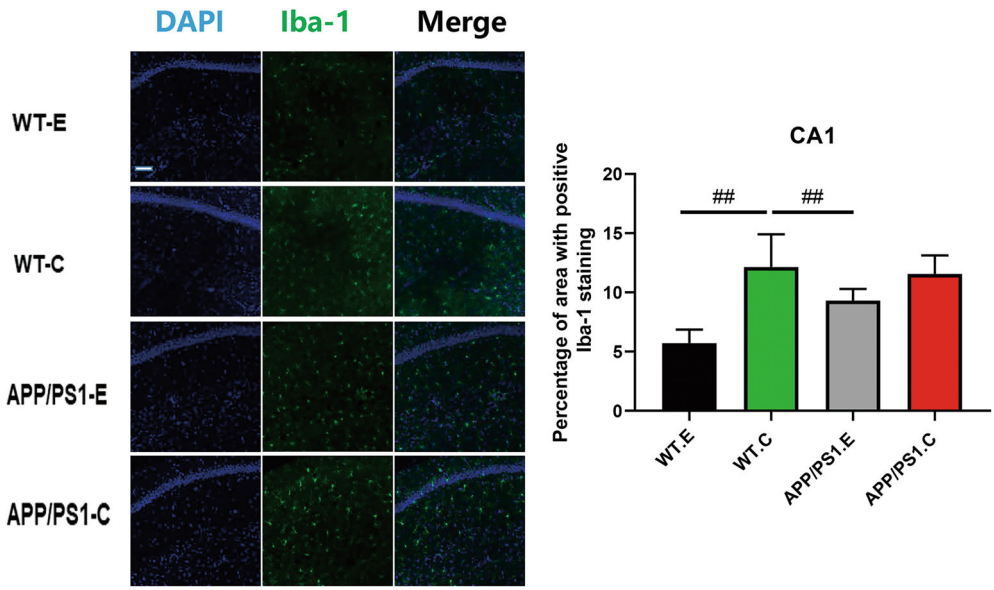

FIGURE 8 Immunofluorescence staining and quantification of ionized calcium-binding adaptor molecule (Iba1)-1 to identify glia in the CA1 region of the hippocampus. Scale bar, 100 μm. ##$p < .01$ versus WT-C

difference was not significant in APP/PS1 mice postoperatively ($p > .05$), suggesting that EA has little effect on improving postoperative pericyte injury.

In terms of intestinal barrier integrity, EA significantly reduced the levels of DAO and LPS in the serum of WT and APP/PS1 mice, suggesting that EA could

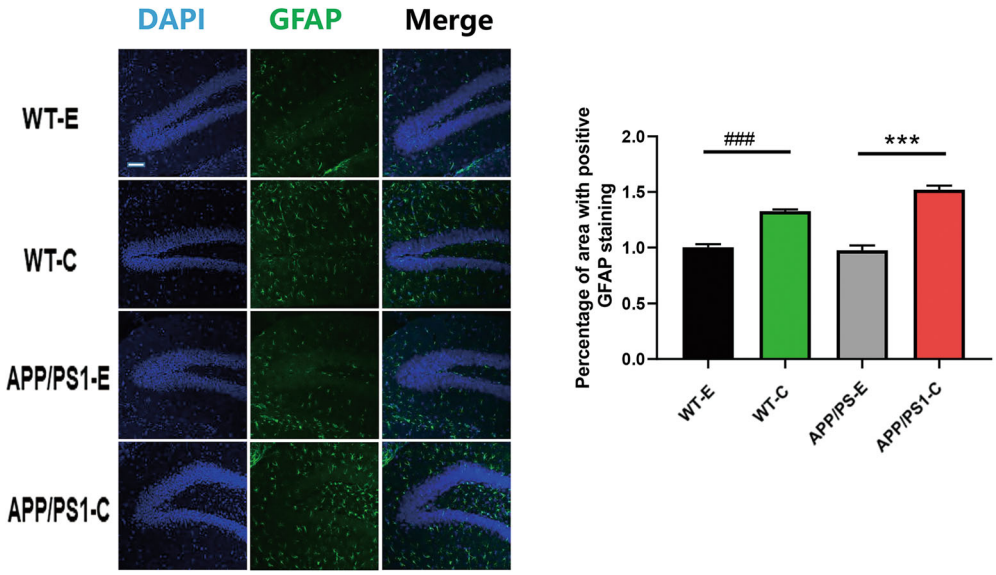

FIGURE 9 Immunofluorescence staining and quantification of GFAP to identify astrocytes in the hippocampus. Scale bar, 100 μm. ###$p < .001$ versus WT-C, ***$p < .001$ versus APP/PS1-C. GFAP, glial fibrillary acidic protein

protect the decreased intestinal barrier permeability after surgery ($p < .01$, Figure 7d,e).

These findings suggest that EA alters the integrity of key physiological barriers (gut and BBB) after surgery.

3.4 | Electroacupuncture decreased neuroinflammation in the hippocampus

Activated glia could lead to neuroinflammation by releasing proinflammatory factors. The percentage of area with GFAP+ cells was significantly reduced in the WT-E group compared with WT-C ($p < .01$, Figures 8). Compared with those without EA intervention groups, the percentage of area with Iba-1+ cells was significantly decreased in the EA-treated groups ($p < .01$, Figure 9). Furthermore, there was a substantial reduction in levels of or expression of TNF-α, IL-1, and IL-6 in the APP/PS1-E group's hippocampus ($p < .05$ for both, Figure 7a–c). These results suggested EA decreased neuroinflammation in the hippocampus.

4 | DISCUSSION

In this study, we demonstrated that EA intervention, using a combination of four different acupoints, improved the cognitive dysfunction caused by abdominal surgery. We found that changes in gut flora composition were accompanied by alterations in the permeability of important barriers such as the BBB and intestinal barrier, allowing inflammatory mediators to enter the bloodstream, thus exacerbating hippocampal inflammation.

Since the clinical treatment does not typically use one single acupoint (Liu, Yin, et al., 2021), the synergistic complementary effects of varied acupoint compatibility may be beneficial for cognitive enhancement, and our model employed multiple EA points referring from early studies.

With the advancement of brain-gut research, many recent study has implicated the gut microbiota in a variety of illnesses, including autism, anxiety, and neurodegenerative diseases such as Parkinson's and Alzheimer's disease (Cryan et al., 2019). More and more researchers in the field of POCD have discovered that alterations in postoperative gut flora are directly associated with cognitive impairments (Han et al., 2020; Lian et al., 2021; Zhan et al., 2019). Although different research showed diverse results on bacterial changes, we attributed this occurrence to variations in anesthetic and surgical methods, as well as the kind and age of mice (Jiang et al., 2019). In our study, *Helibacteraceae*, *Actinomycetes*, *Clostridium* sensu stricto 1, and *Escherichia/Shigella* were decreased following EA treatment in the APP/PS1 mice. *Actinomycetales* are generally gram-positive and anaerobic bacteria with mycelia in a filamentous and branching growth pattern. It was discovered to be abundant in the model of cognitive dysfunction induced by chronic neuropathic pain (Hua et al., 2021), and our study confirmed the abundance of this bacteria could be modulated by EA. Also, *Escherichia/Shigella* is a well-known bacteria that has been linked to proinflammatory status since it can produce proinflammatory cytokines via an NLRP3-dependent mechanism (De la Fuente et al., 2014), Lian et al. found the abundance of *Escherichia/Shigella* enriched after anesthesia/surgery

compared to the baseline controls (Lian et al., 2021), and our study showed that *Escherichia/Shigella* could be a promising target and EA is effective in reducing the expression of this bacteria.

On the other hand, *Bacteroides*, *Firmicutes*, *Coprococcus*, and Ruminococcacea were downregulated microbiota induced by EA in our study. No significant increase in the ratio of *firmicutes* to *Bacteroidetes* was observed in this study (Figure 4a), which is an indicator of the health status of intestinal flora with fewer complications in the human study (Yoshida et al., 2022). We included fewer samples than expected, which may explain the lack of statistical difference. However, EA intervention significantly increased the abundance of *Coprococcus* and *Ruminococcaceae* following surgery in our study, which are two common bacteria producing short-chain fatty acids (SCFAs), such as acetic acid, propionic acid, and *i*-butyric acid. These SCFAs have the potential to positively modulate peripheral and central pathologic processes (Lee et al., 2020). Furthermore, as a product of bacterial imbalance, LPS increased BBB permeability and induced the release of pro-inflammatory cytokines including TNF-α, which exacerbates the central inflammatory response. In this study, we observed that serum LPS and DAO levels were higher in the APP/AP1-C and WT-C groups than in groups that received EA intervention, indicating gut barrier impairment (Du et al., 2019). Interestingly, our study detected the preserved integrity of both the intestinal barrier and BBB in APP/PS1-E mice, which are consistent with the findings of Wang et al. (2020) and Zhao et al. (2022). Tight junction protein levels and electron microscopy results revealed that the BBB was destroyed in the absence of EA and the concentration of inflammatory factors was increased in the hippocampal tissue. This suggests that infections and inflammatory mediators could enter the bloodstream via a "leaky gut" and eventually the brain. This putative underlying mechanism for the association between gut microbiota changes and central nervous system dysfunction is being progressively investigated (Alhasson et al., 2017).

Nevertheless, whether there was a sequence of changes in these two barriers is unknown. Although we measured an increase in LPS levels in the blood, more research into the amplification or resolution of central inflammation caused by different degrees of immune response induced by endotoxin and the interaction between the two important barriers is needed.

DAMPs produced by surgical trauma, in contrast, are felt by vagal afferents that terminate on the nucleus tractus solitarius (Safavynia & Goldstein, 2018) whereas EA has been reported to reduce the expression of HMGB1 (a key player of DAMPs) in a pain model of the mouse (Hsiao & Lin, 2022). Liu et al. recently documented a neuroanatomical basis for EA in driving specific autonomic pathways, indicating that EA has a potent effect at the beginning of the initiation of peripheral aseptic inflammation by surgery. This phenomenon was explained in our study through the lens of intestinal flora regulation, which helps to explain why EA pretreatment reduced perioperative inflammation.

This study had some limitations since animal models might not directly mimic the clinical pathogenesis, requiring detailed mechanistic studies. Moreover, in this study, we did not test the neurobehavioral effects of specific bacteria on mouse intestinal fecal bacteria transplantation, and the post-surgical SCFAs levels in the brain have not been calculated, which could help improve our understanding of the contributed role of SCFAs in the EA intervention. These concerns will be addressed shortly with suitable studies.

5 | CONCLUSIONS

In summary, EA ameliorated inflammatory cascade and improved POCD by modulating gut microbiota following surgery. Moreover, EA pretreatment reversed BBB and intestinal barrier dysfunction and decreased hippocampal inflammation. This may provide a novel potential target in POCD management.

AUTHOR CONTRIBUTIONS

Binbin Zhu: Conceptualization (equal); data curation (equal); formal analysis (equal); funding acquisition (equal); investigation (equal); methodology (equal); project administration (equal); software (equal); writing – original draft (equal); writing – review and editing (equal). **yanling Zhou:** Data curation (supporting); formal analysis (supporting); investigation (supporting). **Weijian Zhou:** Data curation (supporting); formal analysis (supporting); investigation (supporting); software (supporting). **Chunqu Chen:** Data curation (supporting); methodology (supporting); software (supporting). **jianhua Wang:** Conceptualization (equal); funding acquisition (supporting); supervision (supporting); validation (lead); visualization (supporting); writing – original draft (supporting); writing – review and editing (supporting). **shujun Xu:** Formal analysis (supporting); resources (supporting); supervision (supporting); writing – original draft (supporting). **qinwen Wang:** Conceptualization (supporting); investigation (supporting); resources (supporting); software (supporting); supervision (supporting); validation (supporting); visualization (supporting); writing – review and editing (supporting).

ACKNOWLEDGMENTS

Miss Lu Jiani and Miss Wu Can assisted in conducting the research; Dr. Gui Yu and Prof. Xu Zhipeng provided administrative assistance; Prof. Cui Wei donated the electroacupuncture apparatus used in the study.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest in this work.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available upon reasonable request from the corresponding author.

ETHICS APPROVAL

This study was approved by the Animal Ethics Committee of the Affiliated Hospital of the Medical School of Ningbo University (approval number NBU20210003) and the experimental procedures were conducted in accordance with the National Institutes of Health "Guide for the Care and Use of Laboratory Animals."

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How to cite this article: Zhu, B., Zhou, Y., Zhou, W., Chen, C., Wang, J., Xu, S., & Wang, Q. (2023). Electroacupuncture modulates gut microbiota in mice: A potential target in postoperative cognitive dysfunction. *The Anatomical Record*, *306*(12), 3131–3143. https://doi.org/10.1002/ar.25065