

## Effects of Electroacupuncture on Inflammatory Response and Ferroptosis in Rat Models of Postoperative Cognitive Dysfunction: Postprint

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### Abstract

**Background** Postoperative cognitive dysfunction (POCD) is one of the important causes of increased postoperative morbidity and mortality in patients. Inflammatory response and ferroptosis are important mechanistic hypotheses for the occurrence of POCD, while the mechanism by which electroacupuncture improves learning and memory functions in POCD patients remains unclear. **Objective** To observe the effects of electroacupuncture on learning and memory, inflammatory cytokines, and hippocampal neuronal ferroptosis in aged rats with POCD, and to explore the mechanism by which electroacupuncture improves POCD. **Methods** From January 2022 to February 2023, 72 Sprague-Dawley (SD) rats aged 18-20 months were selected and divided into 3 groups according to the random number table method: control group (n=24), model group (n=24), and electroacupuncture group (n=24). Based on two observation time points at 3 and 7 days postoperatively, each group was further divided into 2 subgroups (control group 3-day postoperative subgroup, control group 7-day postoperative subgroup, model group 3-day postoperative subgroup, model group 7-day postoperative subgroup, electroacupuncture group 3-day postoperative subgroup, electroacupuncture group 7-day postoperative subgroup), with 12 rats in each subgroup. A POCD model was established using exploratory laparotomy, and electroacupuncture stimulation was administered at Baihui (GV20) and Neiguan (PC6) acupoints in the electroacupuncture group rats. The Morris water maze apparatus was used to detect behavioral performance in rats, enzyme-linked immunosorbent assay (ELISA) was used to detect the contents of interleukin (IL)-6, IL-10, and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) in serum and hippocampus, hippocampal tissue lipid peroxide (LPO) and Fe<sup>2+</sup> were measured, and Western blotting was used to detect the protein expression levels of acyl-CoA synthetase long-chain family member 4 (ACSL4), ferritin heavy chain 1 (FTH1), and lysophosphatidylcholine acyltransferase 3 (LPCAT3) in the hippocampus.

Transmission electron microscopy was used to observe the ultrastructure of neurons in the hippocampal region. Results At 3 days and 7 days postoperatively, there was no interaction between group and time on the escape latency of preoperative cognitive function training in rats ( $P_{\text{interaction}} > 0.05$ ), the main effect of training time on escape latency was significant ( $P_{\text{time}} < 0.05$ ), and the main effect of group on escape latency was not significant ( $P_{\text{group}} > 0.05$ ). The escape latency in the model group 3-day postoperative subgroup was higher than that in the control group 3-day postoperative subgroup and electroacupuncture group 3-day postoperative subgroup, the number of platform crossings and target quadrant dwell time were lower than those in the control group 3-day postoperative subgroup and electroacupuncture group 3-day postoperative subgroup, and the number of platform crossings in the electroacupuncture group 3-day postoperative subgroup was lower than that in the control group 3-day postoperative subgroup ( $P < 0.05$ ). The escape latency in the model group 7-day postoperative subgroup was higher than that in the control group 7-day postoperative subgroup and electroacupuncture group 7-day postoperative subgroup, the number of platform crossings was lower than that in the control group 7-day postoperative subgroup, and the target quadrant dwell time was lower than that in the control group 7-day postoperative subgroup and electroacupuncture group 7-day postoperative subgroup ( $P < 0.05$ ). Serum IL-6 and TNF- $\alpha$  in the model group 3-day postoperative subgroup were higher than those in the control group 3-day postoperative subgroup and electroacupuncture group 3-day postoperative subgroup, TNF- $\alpha$  in the electroacupuncture group 3-day postoperative subgroup was higher than that in the control group 3-day postoperative subgroup, and IL-10 was higher than that in the control group 3-day postoperative subgroup and model group 3-day postoperative subgroup ( $P < 0.05$ ). Serum IL-6 in the model group 7-day postoperative subgroup was higher than that in the control group 7-day postoperative subgroup, TNF- $\alpha$  was higher than that in the control group 7-day postoperative subgroup and electroacupuncture group 7-day postoperative subgroup, and IL-10 in the electroacupuncture group 7-day postoperative subgroup was higher than that in the control group 7-day postoperative subgroup and model group 7-day postoperative subgroup ( $P < 0.05$ ). Hippocampal IL-6 and TNF- $\alpha$  in the model group 3-day postoperative subgroup were higher than those in the control group 3-day postoperative subgroup and electroacupuncture group 3-day postoperative subgroup, IL-6 and TNF- $\alpha$  in the electroacupuncture group 3-day postoperative subgroup were higher than those in the control group 3-day postoperative subgroup, and IL-10 was higher than that in the control group 3-day postoperative subgroup and model group 3-day postoperative subgroup ( $P < 0.05$ ). Hippocampal IL-6 and TNF- $\alpha$  in the model group 7-day postoperative subgroup were higher than those in the control group 7-day postoperative subgroup and electroacupuncture group 7-day postoperative subgroup, and IL-10 in the electroacupuncture group 7-day postoperative subgroup was higher than that in the control group 7-day postoperative subgroup and model group 7-day postoperative subgroup ( $P < 0.05$ ). Fe<sup>2+</sup>, LPO, ACSL4, and LPCAT3 in the model group 3-day postoperative subgroup were higher than those in the control group 3-day postoperative subgroup and electroacupuncture

ture group 3-day postoperative subgroup, and those in the electroacupuncture group 3-day postoperative subgroup were higher than those in the control group 3-day postoperative subgroup; FTH1 in the model group 3-day postoperative subgroup was lower than that in the control group 3-day postoperative subgroup and electroacupuncture group 3-day postoperative subgroup, and that in the electroacupuncture group 3-day postoperative subgroup was lower than that in the control group 3-day postoperative subgroup ( $P<0.05$ ). Fe2+, LPO, ACSL4, and LPCAT3 in the model group 7-day postoperative subgroup were higher than those in the control group 7-day postoperative subgroup and electroacupuncture group 7-day postoperative subgroup, and FTH1 was lower than that in the control group 7-day postoperative subgroup and electroacupuncture group 7-day postoperative subgroup ( $P<0.05$ ). In the model group 3- and 7-day postoperative subgroups, the nuclear double membrane structure was clear in the hippocampal tissue field of view, the perinuclear space showed no obvious widening, the morphology was irregular with an uneven surface; intranuclear chromatin was condensed and marginated; a small number of mitochondrial membranes were ruptured in the cytoplasm with disappearance of membrane structure; some endoplasmic reticulum was markedly dilated; and some myelin sheath breakage and disordered arrangement were observed; these findings were significantly improved in the electroacupuncture group 3- and 7-day postoperative subgroups compared with the model group. Conclusion Imbalance of inflammatory cytokines and neuronal ferroptosis may be important pathogenic mechanisms underlying POCD occurrence; electroacupuncture can improve the learning and memory abilities of aged rats with POCD, and its mechanism of exerting neuroprotective effects may be related to its regulation of systemic and central inflammatory cytokine levels as well as the neuronal cell ferroptosis pathway.

## Full Text

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## Abstract

### Background

Postoperative cognitive dysfunction (POCD) represents a significant contributor to increased postoperative morbidity and mortality. Inflammatory responses and ferroptosis constitute principal hypotheses regarding POCD pathogenesis, yet the mechanisms through which electroacupuncture improves learning and memory function in POCD patients remain unclear.

### Objective

This study investigated the effects of electroacupuncture on learning and memory, inflammatory cytokines, and hippocampal neuronal ferroptosis in aged rats with POCD to elucidate its therapeutic mechanisms.

### Methods

From January 2022 to February 2023, 72 healthy female Sprague-Dawley rats aged 18–20 months were randomly divided into three groups: control (n=24), model (n=24), and electroacupuncture (n=24). Each group was further subdivided into postoperative day 3 and day 7 observation subgroups (n=12 each). A POCD model was established via exploratory laparotomy under propofol anesthesia. Electroacupuncture stimulation was applied to Baihui (GV20) and bilateral Neiguan (PC6) acupoints in the electroacupuncture group. Cognitive performance was assessed using the Morris water maze. Enzyme-linked immunosorbent assay quantified interleukin (IL)-6, IL-10, and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) levels in serum and hippocampus. Hippocampal lipid peroxides (LPO) and Fe<sup>2+</sup> content were measured, while Western blotting determined protein expression of acyl-CoA synthetase long-chain family member 4 (ACSL4), ferritin heavy chain 1 (FTH1), and lysophosphatidylcholine acyltransferase 3 (LPCAT3). Ultrastructural changes in hippocampal neurons were examined via transmission electron microscopy.

### Results

No significant interaction between group and time was observed for escape latency during preoperative cognitive training at either postoperative day 3 or 7 (Pinteraction>0.05). Training time showed significant main effects (Ptime<0.05), while group effects were non-significant (Pgroup>0.05). At postoperative day 3, the model subgroup exhibited prolonged escape latency,

reduced platform crossing frequency, and decreased target quadrant dwell time compared to both control and electroacupuncture subgroups ( $P < 0.05$ ). Electroacupuncture subgroup showed intermediate performance. Similar patterns persisted at day 7. Serum and hippocampal IL-6 and TNF- $\alpha$  levels were significantly elevated in model subgroups at both time points, while IL-10 increased only in electroacupuncture subgroups ( $P < 0.05$ ). Hippocampal Fe<sup>2+</sup>, LPO, ACSL4, and LPCAT3 were markedly higher in model subgroups, whereas FTH1 was lower ( $P < 0.05$ ). Electroacupuncture intervention significantly attenuated these ferroptotic changes. Transmission electron microscopy revealed that model subgroups exhibited disrupted mitochondrial membranes, condensed chromatin, and disorganized myelin sheaths, while electroacupuncture subgroups showed substantially improved ultrastructure.

### Conclusion

Imbalanced inflammatory cytokines and hippocampal neuronal ferroptosis represent critical pathogenic mechanisms in POCD. Electroacupuncture at Baihui and Neiguan acupoints ameliorates learning and memory deficits in aged POCD rats, likely through modulation of systemic and central inflammatory responses and suppression of neuronal ferroptosis pathways.

### Keywords

Cognitive dysfunction; Postoperative cognitive complications; Electroacupuncture; Baihui; Neiguan; Inflammation; Ferroptosis

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Postoperative cognitive dysfunction (POCD) constitutes a common neurological complication following surgery. Epidemiological data indicate that POCD occurs in up to 56% of patients over 60 years undergoing non-cardiac surgery, with incidence increasing with age [1-2]. POCD severely impairs postoperative rehabilitation and quality of life while increasing economic burden, disease morbidity, and mortality [3]. Current international and domestic multidisciplinary consensus guidelines emphasize perioperative prevention as the primary strategy for reducing POCD incidence [4-5]. Pharmacological interventions for POCD carry inherent adverse effects and may increase surgical risks when combined with anesthetic agents. Electroacupuncture has demonstrated efficacy in improving cognitive function in POCD patients with fewer adverse effects and lower costs [6], though its specific mechanisms remain undefined.

Ferroptosis, a newly identified iron-dependent form of programmed cell death, participates in POCD-related neuroinjury and triggers inflammatory responses [7]. Inflammation represents a crucial pathological factor in POCD development, as anesthesia and surgery induce massive expression of pro- and anti-inflammatory cytokines that exacerbate neuronal death [8]. Whether electroacupuncture alleviates POCD by inhibiting ferroptosis and restoring cytokine balance remains unreported. Therefore, this study employed an aged rat POCD model to investigate the effects of electroacupuncture at Baihui and Neiguan acupoints on postoperative learning and memory, pro- and

anti-inflammatory cytokines, and ferroptosis.

## Materials and Methods

### Study Period and Animals

This study was conducted from January 2022 to February 2023. Seventy-two healthy female SPF-grade Sprague-Dawley rats aged 18–20 months (body weight 350–450 g) were provided by the Animal Experiment Center of Gansu University of Chinese Medicine [license: SCXK(Gan)2020-0001]. Rats were housed under standard conditions (22–24°C, 40–70% humidity, 12-hour light/dark cycle) with ad libitum access to food and water. The study protocol was approved by the Experimental Animal Ethics Committee of Gansu University of Chinese Medicine (2021-200).

### Experimental Grouping

After one week of acclimatization, rats were randomly divided into three groups: control (n=24), model (n=24), and electroacupuncture (n=24). Each group was further subdivided into postoperative day 3 and day 7 observation subgroups (n=12 each) based on the two postoperative time points.

### POCD Model Establishment

Following previously described methods [9–10], we established the POCD model via exploratory laparotomy. The control group received equivalent volume of 0.9% saline intraperitoneally and underwent binding fixation, while other groups received 150 mg/kg propofol anesthesia. After loss of righting reflex and absence of tail pinch response, abdominal hair was shaved and the surgical field disinfected with iodophor. A 3 cm midline incision was made approximately 1 cm below the xiphoid process. The abdominal muscles and peritoneum were incised to expose viscera. Systematic exploration of liver, spleen, stomach, intestines, and kidneys was performed three times (3 minutes each, 7-minute intervals). During intervals, approximately 10 cm of small intestine was exteriorized, covered with saline-soaked gauze, and gently massaged for 3 minutes before replacement. Total exploration time was approximately 30 minutes. The incision was closed in layers with 4-0 surgical sutures and covered with sterile dressing. Successful modeling was confirmed by significant differences in Morris water maze performance compared to controls.

### Electroacupuncture Intervention

Electroacupuncture group rats were fitted with custom-made jackets and immobilized with bandages. Baihui (GV20) and bilateral Neiguan (PC6) acupoints were selected according to “Standardized Nomenclature and Location of Commonly Used Acupoints in Experimental Animals Part 2: Rats” [11]. Disposable sterile acupuncture needles (0.25 mm × 25 mm) were inserted obliquely 2 mm at Baihui and perpendicularly 1 mm at Neiguan. Electroacupuncture was delivered using an electronic acupuncture apparatus (Suzhou Medical Supplies Factory) at 20/100 Hz sparse-dense wave frequency and 1 mA intensity for 20–30 minutes. Treatment commenced 2 days before modeling and continued once

daily for 5 consecutive days. Control and model group rats underwent identical immobilization without needling.

### **Exclusions**

Four rats were excluded during the experiment: one control rat with visual impairment during cognitive training, and three rats that died postoperatively (one model day 7, two electroacupuncture day 3 and day 7). A total of 68 rats were included in the final analysis.

### **Morris Water Maze Testing**

Preoperative cognitive training was conducted using the place navigation test. Rats were placed in the water facing the pool wall, and escape latency was recorded. Rats finding the platform within 120 seconds remained for 10 seconds; those failing within 120 seconds (latency recorded as 120 s) were guided to the platform for 15 seconds. Training lasted 5 days with 4 trials daily from fixed quadrants, with 15–20 minute inter-trial intervals. Postoperative testing was performed on days 3 and 7 using identical procedures. Spatial probe trials were conducted after platform removal, recording swimming paths and platform crossing frequency within 120 seconds.

### **Sample Collection**

After behavioral testing, rats were anesthetized with 3% pentobarbital sodium (30 mg/kg intraperitoneally). Upon loss of righting and tail pinch reflexes, the abdominal cavity was opened to expose the abdominal aorta for blood collection. Blood was allowed to clot at room temperature for 30 minutes, then centrifuged at 3,000 rpm for 15 minutes (radius 8 cm). Serum was aliquoted into cryovials. Brains were rapidly harvested on ice, and hippocampi were dissected.

### **Cytokine and Biochemical Assays**

Serum and hippocampal IL-6, IL-10, and TNF- $\alpha$  levels were quantified using ELISA kits (Shanghai Enzyme-linked Biotechnology) according to manufacturer protocols. Absorbance was measured at 450 nm using a microplate reader (Molecular Devices), and concentrations were calculated from standard curves. Hippocampal Fe<sup>2+</sup> content was determined using an iron assay kit (Abcam, ab83366) following centrifugation of homogenized tissue at 3,000 rpm for 20 minutes (4°C, radius 8 cm). LPO relative content was measured at 586 nm using a LPO assay kit (Nanjing Jiancheng Bioengineering Institute, A106-1-2).

### **Western Blot Analysis**

Hippocampal ACSL4, FTH1, and LPCAT3 protein expression were analyzed by Western blot. Hippocampal tissue (100 mg) was lysed and total protein extracted. Protein concentration was determined by BCA assay. Samples were separated on SDS-PAGE gels (80 V initially, then 120 V) and transferred to PVDF membranes. After blocking with 5% skim milk for 120–150 minutes, membranes were incubated overnight at 4°C with primary antibodies against ACSL4 (Immunoway, YT8070), FTH1 (Abcam, ab75972), LPCAT3 (Abcam, ab232958), and GAPDH (Immunoway, YM3029). Following TBST washes, membranes were incubated with secondary antibodies for 1.5 hours at room

temperature. Protein bands were visualized using ECL reagent and quantified using ImageJ software.

### **Transmission Electron Microscopy**

Hippocampal tissues were fixed overnight in 2.5% glutaraldehyde, dehydrated, embedded, and sectioned into ultrathin slices (60-80 nm). Sections were double-stained with uranyl acetate and lead citrate, washed, dried, and examined under a transmission electron microscope (Hitachi).

### **Statistical Analysis**

Data were analyzed using GraphPad Prism 8.0. Normally distributed data are presented as mean  $\pm$  standard deviation ( $\bar{x}\pm s$ ) and analyzed by one-way ANOVA with Bonferroni post-hoc test. Non-normally distributed data are expressed as median (P25, P75) and analyzed by Kruskal-Wallis H test with Dunn's test. Two-way ANOVA was used for preoperative training data.  $P<0.05$  was considered statistically significant.

## **Results**

### **Cognitive Function**

No significant group  $\times$  time interaction was observed for escape latency during preoperative training at either postoperative day 3 or 7 ( $P_{\text{interaction}}>0.05$ ). Training time showed significant main effects ( $P_{\text{time}}<0.05$ ), while group effects were non-significant ( $P_{\text{group}}>0.05$ ).

At postoperative day 3, significant differences were observed in escape latency, platform crossing frequency, and target quadrant dwell time ( $P<0.05$ ). The model subgroup exhibited prolonged escape latency, reduced platform crossings, and decreased target quadrant time compared to control and electroacupuncture subgroups. The electroacupuncture subgroup showed intermediate performance with fewer platform crossings than controls ( $P<0.05$ ). Swimming speed did not differ significantly among groups ( $P>0.05$ ).

Similar patterns were observed at postoperative day 7, with the model subgroup showing impaired performance compared to control and electroacupuncture subgroups ( $P<0.05$ ).

### **Inflammatory Cytokines**

At postoperative day 3, serum IL-6 and TNF- $\alpha$  were significantly higher in the model subgroup versus control and electroacupuncture subgroups ( $P<0.05$ ). Electroacupuncture subgroup TNF- $\alpha$  remained elevated compared to controls, while IL-10 was higher than both control and model subgroups ( $P<0.05$ ). At day 7, model subgroup serum IL-6 and TNF- $\alpha$  were elevated compared to controls, while electroacupuncture subgroup IL-10 was higher than both other groups ( $P<0.05$ ).

Hippocampal cytokine patterns mirrored serum findings, with model subgroups showing elevated IL-6 and TNF- $\alpha$  at both time points, and electroacupuncture subgroups demonstrating increased IL-10 compared to controls and models

( $P < 0.05$ ).

### **Ferroptosis Markers**

Postoperative day 3 hippocampal  $\text{Fe}^{2+}$ , LPO, ACSL4, and LPCAT3 were significantly higher in model and electroacupuncture subgroups versus controls, with model values exceeding electroacupuncture ( $P < 0.05$ ). Conversely, FTH1 was lower in model and electroacupuncture subgroups, with model values lowest ( $P < 0.05$ ). Similar significant differences were observed at day 7. Representative Western blot bands are shown in [Figure 1: see original paper] and [Figure 2: see original paper].

### **Ultrastructural Changes**

Control subgroups exhibited regular nuclear morphology with clear double nuclear membranes, normal perinuclear space, and uniform chromatin distribution. Mitochondria appeared intact with abundant endoplasmic reticulum [Figure 3: see original paper]A,D. Model subgroups showed irregular nuclear morphology with condensed marginal chromatin, disrupted mitochondrial membranes, expanded endoplasmic reticulum, and disorganized myelin sheaths [Figure 3: see original paper]B,E. Electroacupuncture subgroups demonstrated substantial improvement, with more regular nuclear morphology, intact mitochondrial membranes, and reduced endoplasmic reticulum expansion [Figure 3: see original paper]C,F.

### **Discussion**

POCD manifests clinically as postoperative delirium, anxiety, personality changes, and impaired memory and cognition. In traditional Chinese medicine, POCD falls under categories of “dementia,” “forgetfulness,” or “stupor,” with pathology rooted in brain dysfunction related to heart, liver, spleen, and kidney systems. The primary pathogenesis involves marrow depletion and brain decline with loss of mental agility, arising from kidney essence insufficiency, qi-blood deficiency, or emotional injury leading to marrow sea depletion and brain malnourishment, or from blood stasis and pathogenic obstruction of brain collaterals [12].

Ferroptosis is a novel iron-dependent cell death mechanism characterized by lipid peroxidation and reactive oxygen species (ROS) accumulation, resulting from disrupted iron metabolism, amino acid antioxidant system imbalance, and lipid peroxidation [13]. Iron ions play a critical role, as metabolic dysregulation causes excessive intracellular  $\text{Fe}^{2+}$  accumulation, which generates ROS via Fenton reactions, inducing oxidative stress damage. FTH1 is an iron carrier protein that maintains intracellular iron homeostasis and possesses ferroxidase activity to prevent iron-mediated ROS production [13]. Studies demonstrate that anesthesia/surgery significantly increases cellular/brain iron content while decreasing FTH1 expression, reducing hippocampal neuronal viability and impairing cognitive function [14-15].

Lipid peroxidation accumulation, another ferroptosis hallmark, requires activa-

tion of key enzymes including ACSL4 and LPCAT3 [16]. ACSL4, a crucial polyunsaturated fatty acid metabolism enzyme, determines cellular sensitivity to ferroptosis. Human neuroblastoma cells exposed to sevoflurane show elevated  $\text{Fe}^{2+}$  and ACSL4 levels, while ACSL4 silencing suppresses sevoflurane-induced ferroptosis [17]. Our findings revealed significantly increased hippocampal  $\text{Fe}^{2+}$ , ACSL4, and LPO levels postoperatively with decreased FTH1, which electroacupuncture reversed in parallel with cognitive improvement.

Mitochondrial structural and functional alterations represent classic ferroptosis features, including mitochondrial shrinkage, increased membrane density, reduced or absent cristae, and outer membrane rupture [18]. Transmission electron microscopy confirmed that anesthesia/surgical trauma induced iron metabolism disruption, lipid metabolism abnormalities, and mitochondrial structural changes in aged rat hippocampal neurons, triggering ferroptosis and cognitive dysfunction. Electroacupuncture mitigated these ultrastructural pathologies.

Anesthesia/surgery also modulates inflammatory cytokine expression. Postoperative surgical site inflammation increases cytokine levels, activating brain-resident microglia and promoting neuroinflammation progression [19]. IL-6, a critical immune signaling molecule, regulates neuronal and synaptic functions including synaptic transmission and plasticity—essential cellular mechanisms for learning and memory [20]. Under physiological conditions, central nervous system IL-6 expression remains low, but markedly elevates in CNS diseases associated with cognitive and behavioral changes [21]. Pro-inflammatory  $\text{TNF-}\alpha$  also plays important roles in immunity, inflammation, and regulation of cell proliferation, differentiation, and apoptosis [22-23].  $\text{TNF-}\alpha$  induction stimulates production of multiple inflammatory cytokines including IL-1, IL-6, IL-8, colony-stimulating factors, interferons, and  $\text{TGF-}\beta$  [24]. IL-10, a cytokine synthesis inhibitory factor, regulates cell growth and differentiation while participating in inflammatory and immune responses. IL-10 negatively regulates expression of pro-inflammatory cytokines including IL-1, IL-2, IL-6, IL-8, IL-12,  $\text{TNF-}\alpha$ , and  $\text{IFN-}\gamma$  [24].

The POCD neuroinflammation hypothesis posits that peripheral inflammatory changes may affect the central nervous system, triggering CNS inflammatory responses. Our study demonstrated significantly elevated serum and hippocampal IL-6, IL-10, and  $\text{TNF-}\alpha$  levels postoperatively, with imbalance between pro- and anti-inflammatory cytokines representing another important pathological mechanism in POCD development.

Electroacupuncture combines traditional needling with electrical stimulation to enhance therapeutic intensity and efficacy [25]. Our previous systematic reviews confirmed that electroacupuncture effectively alleviates postoperative cognitive impairment in elderly patients and those undergoing gastrointestinal tumor resection [6,26]. This study selected Baihui and Neiguan acupoints, applying 20/100 Hz sparse-dense wave stimulation for 5 consecutive perioperative days, which effectively reduced postoperative cognitive deficits. Baihui and Neiguan

are the most frequently used acupoints in clinical practice for POCD prevention. Baihui, a governing vessel acupoint, functions to dispel wind-cold, warm meridians, ascend yang, calm wind, and clear orifices, serving as a critical point for restoring consciousness. Neiguan, a luo-connecting point of the pericardium meridian and one of the eight confluent points, harmonizes yin-yang, regulates qi, unblocks vessels, nourishes heart, clears brain, and restores consciousness [27].

In conclusion, neuronal ferroptosis and inflammatory cytokine imbalance represent important pathogenic mechanisms in POCD. Electroacupuncture at Baihui and Neiguan acupoints ameliorates POCD in aged rats by modulating neuronal ferroptosis pathways and inflammatory cytokine levels. These findings provide novel insights and approaches for electroacupuncture in POCD management, though further validation and in-depth mechanistic studies are warranted.

**Author Contributions:** QIN Xiaoyu and WANG Chunai conceived and designed the study. QIN Xiaoyu implemented the study, collected and analyzed data, and drafted the manuscript. QIN Xiaoyu, ZHANG Binsen, LU Xiaoting, ZHANG Xiaojia, and LIU Hongxin performed animal experiments and data collection. QIN Xiaoyu, ZHANG Binsen, and ZHANG Xiaojia conducted experimental assays. WANG Chunai supervised quality control and takes overall responsibility for the manuscript.

**Conflict of Interest:** The authors declare no conflicts of interest.

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*Note: Figure translations are in progress. See original paper for figures.*

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