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Research article

Electroacupuncture ameliorates gastrointestinal dysfunction by modulating DMV cholinergic efferent signals to drive the vagus nerve in *p*-MCAO rats

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ABSTRACT

Background: The use of proton pump inhibitors in the acute phase of cerebral infarction may lead to adverse long-term outcomes, this study aims to explore the potential of electroacupuncture (EA) in replacing omeprazole in exerting post-stroke gastrointestinal protection.
Methods: A permanent middle cerebral artery infarction model was established using the modified Longa thread occlusion technique. Gastrointestinal motility, gastrointestinal mucosal damage, cerebral infarct volume, and alterations in choline acetyltransferase (ChAT)-positive neurons within the dorsal motor nucleus of the vagus nerve (DMV) were assessed after 7 days of EA at Zusanli (ST36) or omeprazole intervention. To evaluate the role of the vagal nerve in mitigating post-stroke gastrointestinal dysfunction, we employed subdiaphragmatic vagotomy and the ChAT-specific inhibitor α-NETA. Additionally, we utilized methyllycaconitine (MLA), a selective inhibitor of the α7-type nicotinic acetylcholine receptor (α7nAChR), and PNU282987, an agonist, to identify the target of EA.
Results: EA restored ChAT neurons lost in the DMV, activated the vagus nerve and conferred cerebroprotection while ameliorating gastrointestinal mucosal injury and gastrointestinal motility disorders. In addition, following the administration of the α7nAChR antagonist, the attenuation of gastric mucosal injury and inflammatory factors induced by EA was hindered, although gastrointestinal motility still exhibited improvement.
Conclusion: EA at ST36 promotes the restoration of cholinergic signaling in the DMV of stroke-afflicted rats, and its excitation of the vagal nerve inhibits gastrointestinal inflammation after stroke via α7nAChR, while improvement in gastrointestinal motility could be mediated by other acetylcholine receptors.

1. Introduction

Patients with ischemic stroke often experience various complications, including pulmonary infections, stress ulcers, and swallowing issues [1–3]. Gastrointestinal complications are among the most common causes of poor prognosis in patients [4]. The use of

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antiplatelet medications in the acute phase of ischemic stroke can increase the risk of gastrointestinal bleeding [5], necessitating the careful use of gastric mucosal protective drugs. However, a multicenter retrospective study involving 4542 patients with acute ischemic stroke has revealed that 73.4 % of the patients who received proton pump inhibitors (PPIs) for either prevention or treatment had poor long-term outcomes [6].

Most studies on post-stroke gastrointestinal dysfunction have primarily focused on the stress-induced disruption of the gastrointestinal barrier [7]. Stress-induced sympathetic excitation is often accompanied by vagal inhibition, and changes in the parasympathetic vagal system after a stroke are often assessed for cardiopulmonary function using techniques such as tilt table testing, Valsalva maneuver, and heart rate variability [8]. However, there has been limited research on its role in post-stroke gastrointestinal dysfunction.

The parasympathetic system is primarily governed by the vagus nerve. Two key centers within the dorsal medulla of the brainstem, the nucleus tractus solitarius (NTS) and the dorsal motor nucleus of the vagus nerve (DMV), play central roles in parasympathetic signal transmission. Activation of cholinergic neurons expressing choline acetyltransferase (ChAT) serves as an indicator of vagal excitation [9]. Studies on the DMV in experimental mice have revealed that functional gastrointestinal issues often manifest in the early stages of Parkinson's disease and are accompanied by the loss of ChAT-positive neurons [10]. Additionally, gastric ulcers occurring postoperatively or due to severe systemic trauma have been linked to dysfunction in the brainstem vagal centers, particularly in the NTS region [11–13].

Inflammatory signaling is closely associated with the cholinergic anti-inflammatory pathway (CAP), which is regulated by the parasympathetic nervous system [14] and involves the activation of DMV cholinergic neurons that release acetylcholine (ACh) into the bloodstream via vagal efferent fibers. ACh then binds to α7-type nicotinic acetylcholine receptors (α7nAChR) present in immune cells,

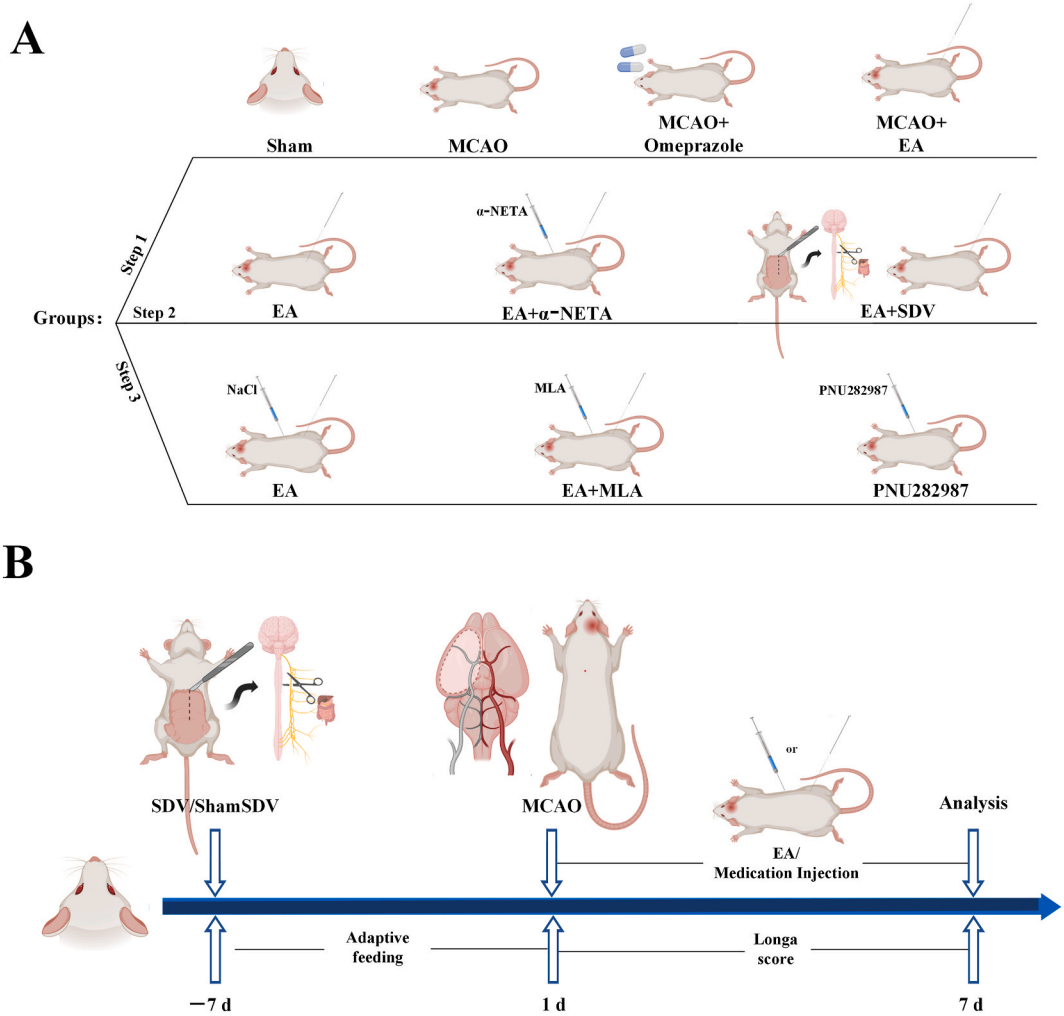

Fig. 1. Experimental design overview. (A) Groups and experimental steps. (B) Flowchart of the study interventions. SDV, Sub-diaphragmatic vagotomy; MCAO, Permanent middle cerebral artery occlusion; EA, Electroacupuncture; α-NETA, Non-competitive ChAT inhibitor; MLA, Methyllycaconitine citrate (a selective α7nAChR antagonist); PNU282987, α7nAChR agonist.

such as macrophages and T cells [15], thereby promoting the release of anti-inflammatory cytokines such as interleukin (IL)-10 and inhibiting the release of pro-inflammatory cytokines such as IL-6 and tumor necrosis factor-alpha (TNF-α) [16]. Subsequently, ACh is enzymatically degraded into acetate and choline by acetylcholinesterase (AChE), thereby terminating neural signaling and its associated anti-inflammatory effects [17]. Both animal studies and long-term clinical evidence have suggested the effectiveness of AChE inhibitors and α7nAChR agonists in improving neurological deficits and cognitive deficits after stroke [18–20].

Electroacupuncture (EA), an innovative acupuncture therapy that combines traditional acupuncture with electrical stimulation, is widely used to treat stroke and its associated complications. Emerging evidence supports the idea that EA, specifically when applied at ST36 acupoints, activates cholinergic neurons within the DMV, leading to enhanced vagal anti-inflammatory responses and improved gastrointestinal motility [21,22].

In this study, we hypothesized that stress-induced sympathetic hyperexcitability can inhibit vagal function and cause the loss of DMV cholinergic signaling, which in turn induces post-stroke gastrointestinal mucosal damage and dysmotility. In contrast, EA at ST36 can restore stroke-induced gastrointestinal dysfunction by upregulating the number of ChAT-positive neurons in the DMV. To test the above hypothesis, we evaluated cerebral infarction volume, DMV cholinergic signaling, gastric emptying rate, intestinal propulsion rate, expression of ChAT and α7nAChR protein, serum inflammatory factors TNF-α and IL-6, as well as the gastrointestinal motility-related hormones gastrin (MTL) and vasoactive intestinal peptide (VIP), which are released in a vagus nerve-dependent manner.

2. Methods

2.1. Animal and ethics statement

We purchased 120 male Sprague Dawley rats, each weighing between 250 and 280 g, from Guangdong Weitonglihua Laboratory Animal Technology Co., Ltd (animal certificate: SCKK 2022-0063), which were then housed in cages (n = 4 mice/cage), maintained at a temperature of 22 °C, with a relative humidity of 55 %. They had unrestricted access to both water and food and were maintained under a 12-h light and 12-h dark cycle.

2.2. Animal grouping

In total 120 rats were used in this study. After a 7-day acclimatization period, the 120 rats were randomly allocated to 10 groups using a random number table method, with each group comprising 12 rats.

The experiment was divided into three distinct parts, as illustrated in Fig. 1A. The first part included four groups as follows: a sham surgery group (Sham group), a middle cerebral artery occlusion (MCAO) group, a model group treated with Omeprazole (MCAO + Omeprazole group), and a model group treated with EA (MCAO + EA group).

The second part of the experiment included three groups: an EA treatment group (EA group); an EA treatment group, with vagotomy before stroke (EA + SDV group); and an EA treatment group, with α-NETA injection before (EA + α-NETA group).

The third and final part of the experiment included three groups: an EA treatment group, with NaCl injection before (EA + NaCl group); an EA treatment group, with α7nAChR inhibitor MLA injection before (EA + MLA group), and an α7nAChR agonist PNU282987 treatment group (PNU282987 group).

2.3. Model preparation

A permanent MCAO (*p*-MCAO) model was established under isoflurane anesthesia utilizing the modified Longa thread occlusion method [20]. The rats were anesthetized and placed in a supine position on an operating Table An incision is made on the left side of the neck along the midline to expose the common carotid artery (CCA). The internal carotid artery (ICA) and external carotid artery (ECA) were carefully isolated along the CCA. The CCA and ECA were sequentially ligated, and the ICA was clamped using a vascular clip. An incision was made in the ECA, and a silicon-coated threaded bolt was inserted through this incision and gently advanced into the ICA. Following release of the vascular clip, the threaded bolt was further advanced until slight resistance was encountered, after which it was ligated and sutured together with the ICA. To maintain the body temperature of rats, they were positioned on 37 °C warming pads throughout the procedure. Subsequently, 2 h after regaining consciousness, the neurological function of rats was assessed using the Longa score. Rats with scores ranging from 1 to 3 were included in the study, whereas those with scores of 0 or 4 were excluded. Subsequently, the Longa scores of the rats were evaluated daily to ensure exclusion of false-positive models.

In the Sham group of the first part, all procedures were performed identically as described above, except that suturing was performed without inserting the thread bolt.

2.4. EA intervention

All the procedures were performed according to the methods described by Na-Na Yang [22]. To prevent restraint-induced stress, all treatments were conducted under anesthesia and gentle immobilization. Two acupuncture needles (diameter: 0.3 mm, were sterilized and inserted into the bilateral ST36 acupoints at a depth of approximately 3 mm. The ST36 point is situated 2 mm lateral to the anterior tibial tuberosity and 3 mm below the knee joint. Subsequently, an EA device (SDZ-II, Hwato) was connected using stimulation parameters consisting of a 10-Hz continuous wave and a current intensity of 1 mA.

EA treatment was performed once daily for 20 min over 7 consecutive days (Fig. 1B).

2.5. Sub-diaphragmatic vagotomy (SDV)

SDV was performed 7 days before establishing the *p*-MCAO model (Fig. 1B). Following the method outlined by Houston et al. [23], a longitudinal incision was made in the upper abdomen of the rats. The esophagus and stomach were exposed under an operating microscope, and the ventral and dorsal branches of the vagus nerve attached to the esophagus were subsequently separated and excised for a length of 1 cm each, followed by suturing and adaptive feeding for 7 days.

In the second part of the experiment, the EA group and the EA + α-NETA group underwent the same surgical procedures as described above, with the exception of vagus nerve resection, which was not performed in these groups.

2.6. Medication injection

Omeprazole was administered intraperitoneally at a dose of 20 mg/kg (MB1692, Meilunbio), in line with the dosages commonly used in animal experiments [24,25].

The ChAT inhibitor α-NETA (HY-138097, MedChemExpress) was dissolved in 10 % dimethylsulfoxide as per the instructions and then injected intraperitoneally at a dosage of 3 mg/kg prior to EA treatment [26].

Both the selective α7nAchR antagonist MLA (HY–N282987A, MedChemExpress) and the selective α7nAchR agonist PNU282987 (P6499, Sigma) were dissolved in sterile saline according to the manufacturer's instructions and subsequently administered intraperitoneally at dosages of 1 mg/kg and 10 mg/kg, respectively, prior to EA treatment [27].

The EA and EA + SDV groups in the second part and the EA + NaCl group in the third part received sterile saline injections.

2.7. Assessment of gastric emptying rate and intestinal propulsion rate

To prepare 150 g of black fluid food, the following ingredients were mixed with 125 mL of distilled water: 5 g carboxymethyl cellulose, 8 g milk powder, 4 g sugar, 4 g starch, and 8 g 5 % activated carbon powder. After their last treatment, the rats in each group were subjected to a 24-h fast without access to water. Subsequently, 4 mL of fluid food (equivalent to 4.4 g) was administered via oral gavage. After 30 min, the rats were euthanized using an overdose of isoflurane. The abdomen was opened to expose the stomach and the intestines. Medical sutures were used to ligate the cardia and pylorus, and the entire stomach was isolated and weighed. The stomach body was then incised along the greater curvature and the stomach contents were rinsed with saline and dried using filter paper. The net weight of each stomach sample was determined. The entire intestine was rapidly extracted and unfurled, and both the total length of the intestine and length from the pylorus to the end of the black-fluid food in the intestine were measured. The gastric emptying and intestinal propulsion rates were calculated as follows:

Gastric emptying rate = [1 − (full stomach weight − net stomach weight) / fluid weight] * 100%

Intestinal propulsion rate = (length of fluid propulsion / total length of intestine) *100%

2.8. 2,3,5-Triphenyltetrazolium chloride (TTC) staining

After euthanizing the rats, the brains were quickly extracted and stored at −20 °C. A 2 % TTC solution was prepared by dissolving 2 g of TTC powder in 100 mL of distilled water and then stored in a dark environment. The brains were sliced into six coronal sections from the frontal to the occipital lobes, promptly immersed in 2 % TTC solution at 37 °C, shielded from light, and incubated for 30 min. Subsequently, the slices were fixed in a 4 % paraformaldehyde solution for 12 h. Images were captured, and the percentage of the cerebral infarction area was quantified using the ImageJ software.

2.9. Enzyme-linked immunoassay (ELISA)

The serum levels of MTL (CSB-E08208R, Cusabio), VIP (ER1429, FineTest), TNF-α (ER1393, FineTest), and IL-6 (ER0042, FineTest) were determined using ELISA. Blood samples were obtained from the tail vein of each rat prior to euthanasia and were subsequently centrifuged at 12,000 rpm for 15 min at 4 °C. The resulting supernatant was stored at −80 °C until analysis. Each ELISA kit was used according to the manufacturer's instructions, and the optical density of each reaction was measured at 560 nm using an ELISA microplate reader (ELX450, BioTek).

2.10. Hematoxylin and eosin (HE) staining

After euthanizing the rats, the hearts were promptly perfused with 4 % paraformaldehyde. Gastric tissues were fixed in a 4 % paraformaldehyde solution for 12 h. Then, the sections were dehydrated in an ethanol gradient, embedded in paraffin, and cut into 4 μm sections. Next, the sections were subjected to xylene dewaxing, ethanol gradient hydration, hematoxylin and eosin staining after washing, ethanol gradient dehydration, xylene transparency, and sealing with neutral gum. Finally, the sections were observed under an optical microscope (TCS SP8 SR, Leica) at a 200 × magnification. The complete slice and detailed particulars can be found in Supplementary Material 1.

2.11. Western blot

A Western blot analysis was performed to assess the expression of ChAT protein in the cerebral cortex on the infarcted side and in gastric tissues. The total protein concentration in each group was determined using the BCA Protein Assay Kit (G2026-200T, Servicebio). Subsequently, 10 μg of each sample was loaded onto a 10 % SDS-PAGE gel for electrophoresis. Following electrophoresis, the proteins were transferred onto PVDF membranes (IPVH00010, Millipore) and blocked with 5 % skim milk for 1 h at room temperature. The membranes were then incubated overnight at 4 °C on a shaker with primary antibodies, including ChAT (1:1000, ab181023, Abcam), α7nAchR (1:1000, ab216485, Abcam), and β-actin (1:1000, GB15003, Servicebio). On the following day, the membranes were incubated with a fluorescent secondary antibody (1:10000, SA5-35571, Invitrogen) and visualized using an Odyssey imager.

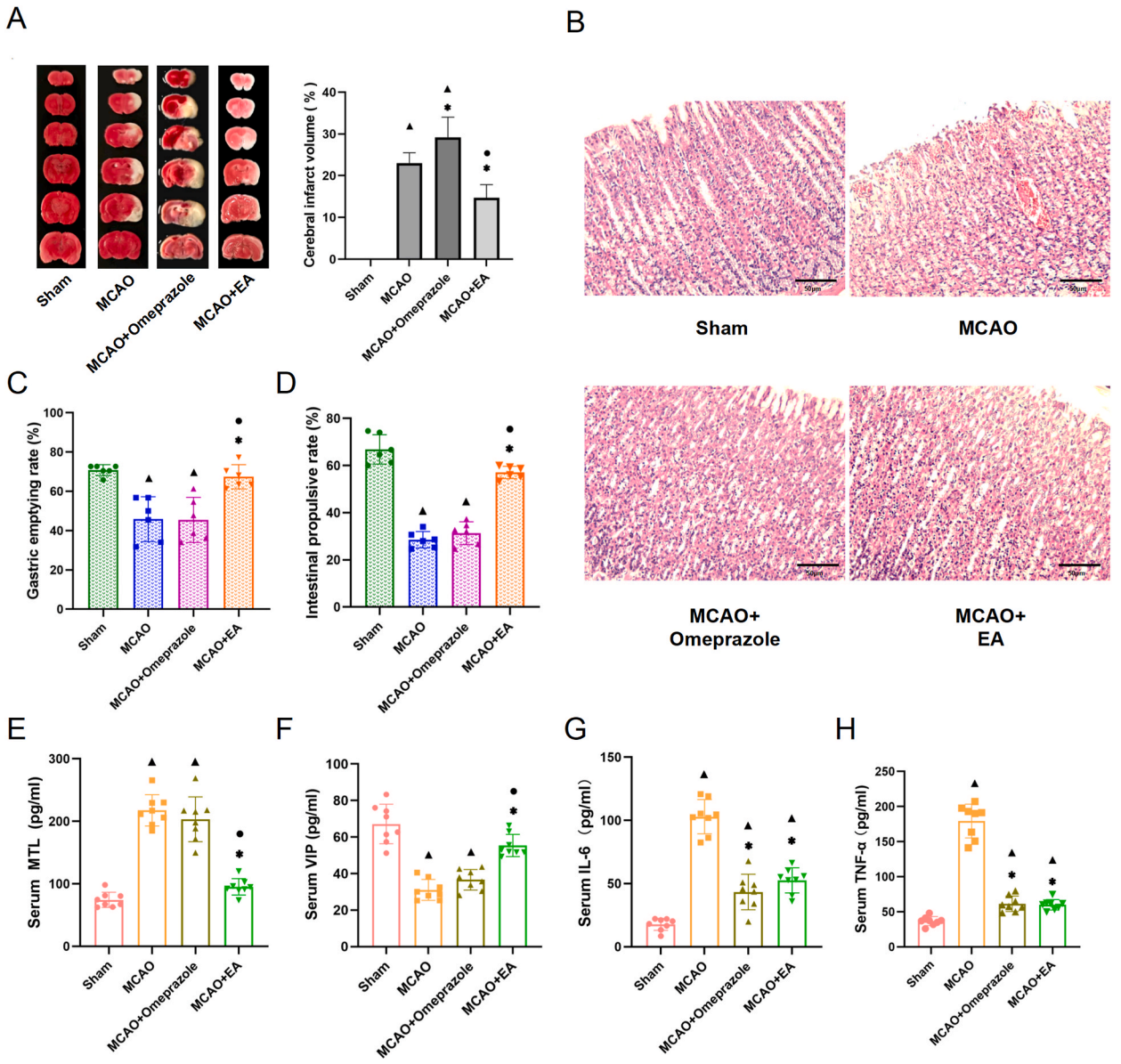

Fig. 2. Protective effects of EA on the gastrointestinal tract in MCAO rats. (A) Brain tissue staining using TTC (n = 6, F (3, 20) = 92.98, P < 0.0001). (B) Gastric mucosa histology assessed by HE staining (n = 6, 200 ×), the 40 × and 400 × fields of view are shown in Supplementary Material 1. (C) Gastric emptying rate results (n = 6, F (3, 20) = 15.93, P < 0.0001). (D) Intestinal propulsion rate findings (n = 6, F (3, 20) = 106.8, P < 0.0001). (E) Serum MTL protein expression levels (n = 8, F (3, 28) = 77.35, P < 0.0001). (F) Serum VIP protein expression levels (n = 8, F (3, 28) = 41.18, P < 0.0001). (G) Serum IL-6 protein expression levels (n = 8, F (3, 28) = 81.08, P < 0.0001). (H) Serum TNF-α protein expression levels (n = 8, F (3, 28) = 164.5, P < 0.0001). ▲P < 0.05, vs. Sham group; *P < 0.05, vs. MCAO group; ●P < 0.05, vs. MCAO + Omeprazole group.

2.12. Immunofluorescence

After sacrifice, the rats were rapidly perfused with 4 % paraformaldehyde, and the medulla oblongata was carefully extracted. The extracted tissues were embedded in optimal cutting temperature and 10-μm sections were obtained, after which they were sealed with 5 % bovine serum albumin at room temperature for 1 h. Subsequently, the sections were incubated with a ChAT primary antibody (1:500, ab181023, Abcam) overnight at 4 °C. The following day, Alexa Fluor 594 secondary antibody (1:500, A-11012, Invitrogen) was added and incubated at room temperature in the dark. DAPI (C1005, Beyotime) was used to label the cell nuclei. Finally, the DMV on the infarcted side was examined at 100 × magnification using a fluorescence microscope (TCS SP8 SR, Leica).

2.13. Statistical analysis

GraphPad Prism 8 and ImageJ software were used for image and data processing. The data are presented as means ± standard deviations. To assess the normal distribution of the data, the Kolmogorov–Smirnov test was utilized, and differences between groups

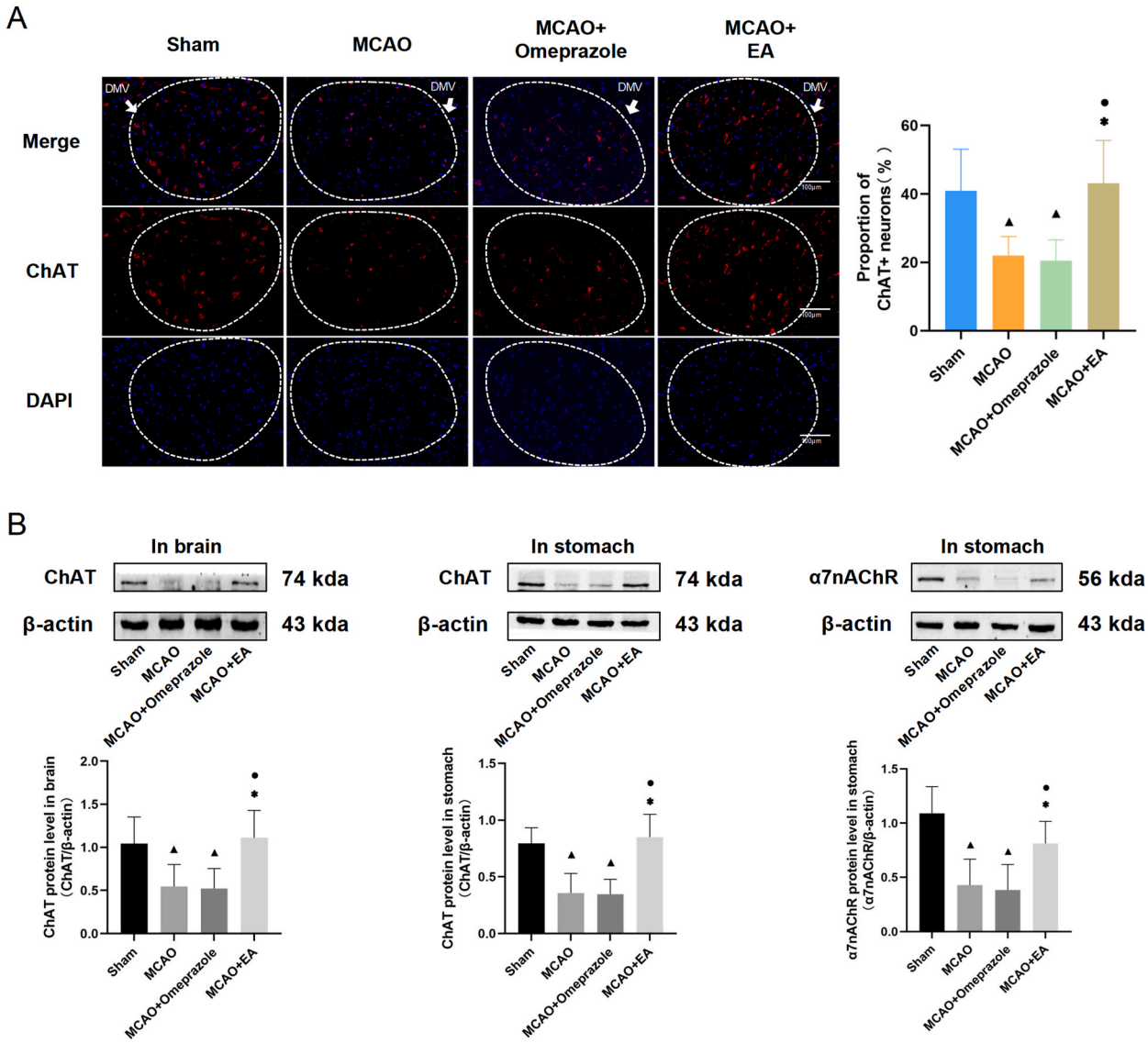

Fig. 3. Impact of EA on vagal efferent signals in MCAO rats. (A) Changes in the number of DMV ChAT-positive neurons (n = 6, F (3, 20) = 9.358, P = 0.0005). (B) ChAT protein expression levels in the cerebral cortex on the infarct side (n = 6, F (3, 20) = 7.508, P = 0.0015) and in gastric tissues (n = 6, F (3, 20) = 16.67, P < 0.0001), along with α7nAChR protein expression levels in the gastric tissues (n = 6, F (3, 20) = 12.56, P < 0.0001). The complete Western blot images are in the Supplementary Material 2. ▲P < 0.05, vs. Sham group; *P < 0.05, vs. MCAO group; ●P < 0.05, vs. MCAO + Omeprazole group.

were analyzed using a one-way analysis of variance (ANOVA), followed by post hoc analysis using the Tukey test. In cases where data demonstrated significantly unequal variances, the Welch's ANOVA test was used. Statistical significance was defined as $P < 0.05$.

3. Results

3.1. EA improved gastrointestinal motility and reduced the level of inflammation in MCAO rats

Using *p*-MCAO rats as a model, we investigated the effects of EA at ST36 on post-stroke gastrointestinal motility and inflammation (Fig. 2). Compared to the Sham group, the MCAO group displayed white infarct lesions in the brain sections (Fig. 2A). Furthermore, significant reductions were observed in the gastric emptying rate, intestinal propulsion rate, and serum VIP levels (Fig. 2C, D, and F; $P < 0.05$), as well as in elevated serum MTL levels (Fig. 2E; $P < 0.05$). Histological examination using HE staining revealed various degrees of gastric mucosal swelling, congestion, disorganized glandular arrangement, noticeable epithelial detachment, and

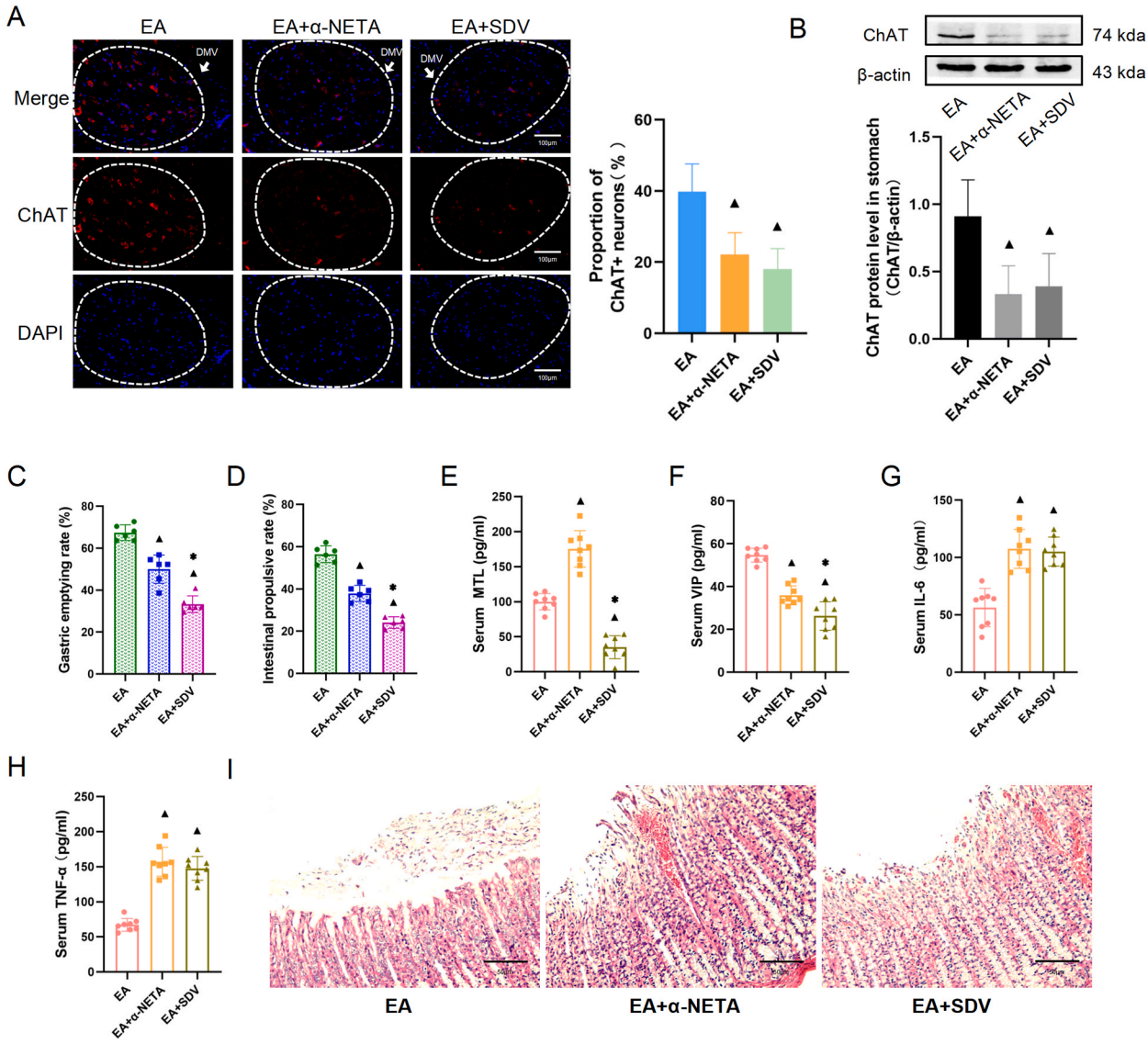

Fig. 4. Protective effect of EA nullified by ChAT inhibitors and vagotomy. (A) Changes in the number of DMV ChAT-positive neurons ($n = 6$, $F(2, 15) = 18.08$, $P = 0.0001$). (B) ChAT protein expression levels in gastric tissue ($n = 6$, $F(2, 15) = 10.28$, $P = 0.0015$). The complete Western blot images are in the Supplementary Material 2. (C) Gastric emptying rate results ($n = 6$, $F(2, 15) = 69.68$, $P < 0.0001$). (D) Intestinal propulsion rate findings ($n = 6$, $F(2, 15) = 126.0$, $P < 0.0001$). (E) Serum MTL protein expression levels ($n = 8$, $F(2, 21) = 109.2$, $P < 0.0001$). (F) Serum VIP protein expression levels ($n = 8$, $F(2, 21) = 67.43$, $P < 0.0001$). (G) Serum IL-6 protein expression levels ($n = 8$, $F(2, 21) = 27.61$, $P < 0.0001$). (H) Serum TNF-α protein expression levels ($n = 8$, $F(2, 21) = 73.50$, $P < 0.0001$). (I) Gastric mucosa histology assessed by HE staining ($n = 6$), the 40 × and 400 × fields of view are shown in Supplementary Material 1. ▲$P < 0.05$, vs. Sham group; *$P < 0.05$, vs. MCAO group.

infiltration of inflammatory cells in the MCAO group compared to the Sham group (Fig. 2B). Additionally, serum levels of IL-6 and TNF-α were significantly increased (Fig. 2G and H; $P < 0.05$). Compared to the MCAO group, the MCAO + Omeprazole group exhibited an increased cerebral infarct volume (Fig. 2A; $P < 0.05$), reduced gastric mucosal damage (Fig. 2B), and significantly lower serum levels of inflammatory factors IL-6 and TNF-α (Fig. 2G and H; $P < 0.05$). However, no significant differences in the gastric emptying rate, intestinal propulsion rate, or serum MTL and VIP levels were observed between groups. Conversely, compared to the MCAO group, the MCAO + EA group displayed reduced cerebral infarct volume, significantly increased gastric emptying rate and intestinal propulsion rate (Fig. 2A, B, $P < 0.05$), improved gastric mucosal damage (Fig. 2B), elevated serum VIP levels, and reduced serum MTL, IL-6, and TNF-α levels (Fig. 2C, D, F, and G, $P < 0.05$). These findings suggest that although omeprazole effectively reduces gastric

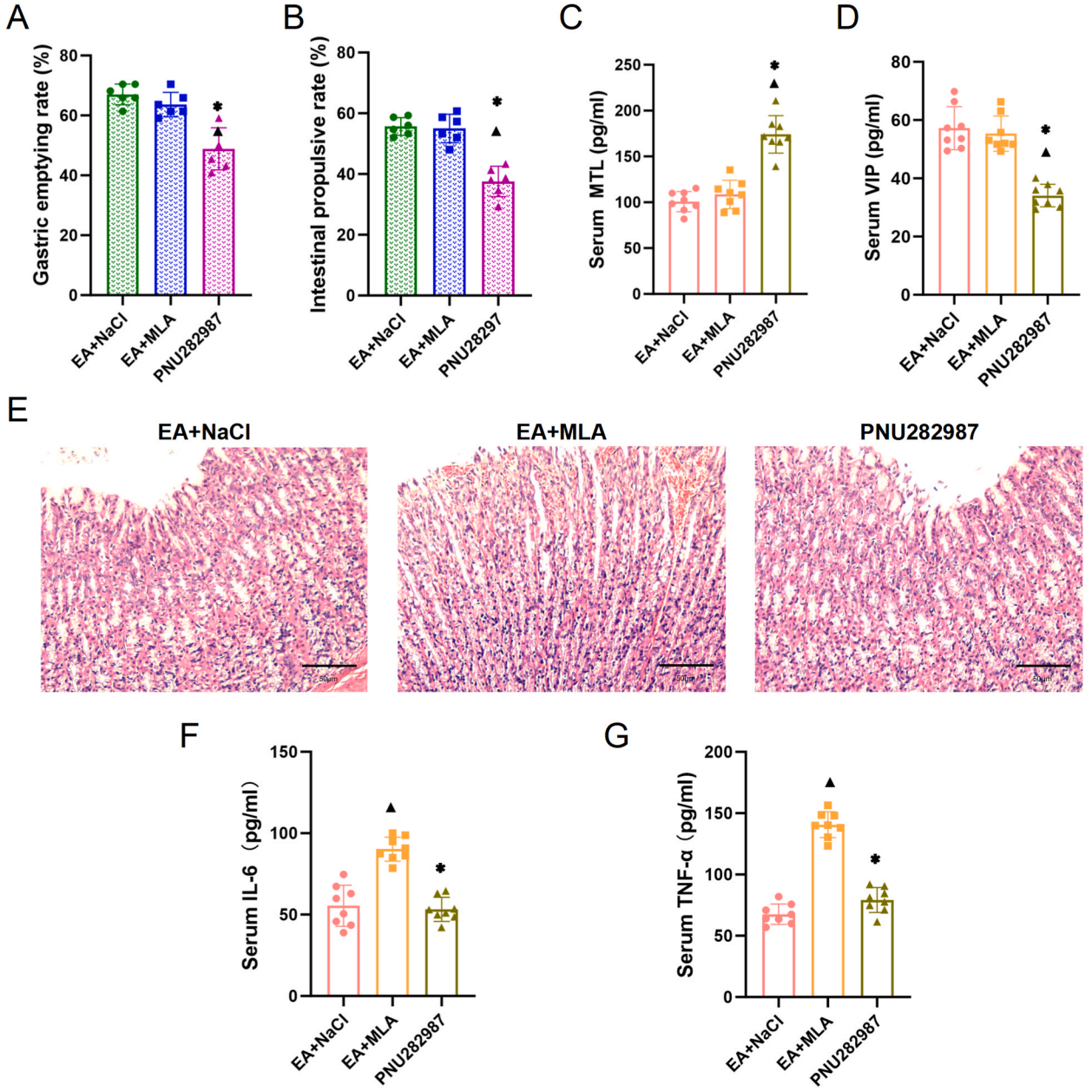

Fig. 5. α7nAchR mediates EA's effect on post-stroke gastrointestinal mucosal damage but not gastrointestinal motility. (A) Gastric emptying rate ($n = 6$, $F(2, 15) = 21.70$, $P < 0.0001$). (B) Intestinal propulsion rate findings ($n = 6$, $F(2, 15) = 33.99$, $P < 0.0001$). (C) Serum MTL protein expression levels ($n = 8$, $F(2, 21) = 49.86$, $P < 0.0001$). (D) Serum VIP protein expression levels ($n = 8$, $F(2, 21) = 67.43$, $P < 0.0001$). (E) Gastric mucosal histology assessed by HE staining ($n = 6$), the 40 × and 400 × fields of view are shown in Supplementary Material 1. (F) Serum IL-6 protein expression levels ($n = 8$, $F(2, 21) = 38.38$, $P < 0.0001$). (G) Serum TNF-α protein expression levels ($n = 8$, $F(2, 21) = 129.9$, $P < 0.0001$). $P < 0.05$, vs. Sham group; *$P < 0.05$, vs. MCAO group.

mucosal injury post-stroke, it also exacerbates cerebral infarction and may not positively affect gastrointestinal motility. By contrast, EA demonstrated a positive impact on improving cerebral infarction and its related complications.

3.2. Cholinergic signals lost in the DMV of MCAO rats were restored by EA

To elucidate the alterations in vagal function following stroke and the effects of EA, we assessed the changes in cholinergic signaling within the DMV (Fig. 3A). Immunofluorescence analysis revealed that compared with the Sham group, the number of ChAT-positive neurons within the DMV was significantly reduced in rats in the MCAO group ($P < 0.05$). Conversely, the number of ChAT-positive neurons in the MCAO + EA group was significantly increaseed ($P < 0.05$). However, the differences between the MCAO + Omeprazole and MCAO groups were not statistically significant ($P > 0.05$). Next, we measured ChAT protein content in the cerebral cortex on the infarct side (Fig. 3B). Compared to the Sham group, the MCAO group demonstrated significantly reduced ChAT content in the cerebral cortex ($P < 0.05$), and the MCAO + EA group exhibited significantly higher ChAT content than the MCAO group ($P < 0.05$). However, no significant difference in the ChAT content was observed between the MCAO + Omeprazole and MCAO groups ($P > 0.05$). We also examined the ChAT and α7nAChR content in the rats' gastric tissues, which are innervated by the gastric branch of the vagus nerve (Fig. 3B), and the results revealed similar trends in gastric tissues as in brain tissues ($P < 0.05$). This consistent ChAT expression pattern in both the central and peripheral tissues indicates vagal function inhibition. Furthermore, EA may enhance vagal function by restoring cholinergic signaling within the DMV and acting on the gastric receptor α7nAChR to ameliorate post-stroke gastrointestinal dysfunction.

3.3. ChAT inhibitors and vagotomy both eliminate the protective effect of EA

Cholinergic signaling, mediated by the vagal nerve, plays a pivotal role in the regulation of gastrointestinal motility and inflammatory responses. To investigate the necessity of ChAT and the vagus nerve in managing gastrointestinal dysfunction post-stroke, α-NETA was intraperitoneally administered, and a subphrenic vagotomy was conducted prior to EA treatment (Fig. 4). Both the EA + α-NETA and EA + SDV groups exhibited significantly reduced cholinergic signals within the DMV and ChAT protein expression in the medulla oblongata compared to the EA group (Fig. 4A and B, $P < 0.05$). Subsequently, we re-evaluated the levels of gastrointestinal motility and inflammation in MCAO rats. Both the EA + α-NETA and EA + SDV groups demonstrated a reversal of the improvements observed in the EA group concerning gastric emptying rate, intestinal propulsion rate, VIP, IL-6, and TNF-α (Fig. 4C, D, F–I, $P < 0.05$). Serum MTL levels were significantly lower in the EA + SDV group than those in the EA + α-NETA group (Fig. 4E, $P < 0.05$) and even lower than those in the EA group (Fig. 4E, $P < 0.05$). We hypothesized that the release of MTL may rely on vagal efferent fibers and that denervation of the gastrointestinal tract following SDV results in a loss of MTL release.

3.4. α7nAChR mediated the improvement of post-stroke gastrointestinal inflammation by EA but did not affect gastrointestinal motility

Lastly, we evaluated the role of α7nAChR in ameliorating gastrointestinal dysfunction post-stroke through EA (Fig. 5). Our findings revealed contrasting effects on gastrointestinal motor function and gastric mucosal injury following the administration of the selective α7nAChR antagonist MLA and the agonist PNU282987. Compared to the EA + NaCl group, the EA + MLA group did not exhibit restoration of gastric mucosal damage or serum inflammation (Fig. 5E–G, $P < 0.05$). However, the gastric emptying rate, intestinal propulsion rate, VIP, and MTL returned to levels comparable to those in the EA + NaCl group (Fig. 5A–D, $P > 0.05$). Similarly, when compared with the EA + NaCl group, the differences in gastric mucosal damage and serum inflammation levels were not statistically significant in the PNU282987 group (Fig. 5E–G, $P > 0.05$). However, the improvements in the gastric emptying rate, intestinal propulsion rate, and serum gastrointestinal motility hormone levels were not significant (Fig. 5A–D, $P < 0.05$). These results suggest that α7nAchR may be a target for EA in reducing the level of gastrointestinal mucosal damage and inflammation after stroke, but it may not play a significant role in improving gastrointestinal motility through EA.

4. Discussion

Acupuncture has a longstanding history in China for the treatment of stroke and its associated complications. Modern research has demonstrated that transcutaneous electrical stimulation can effectively improve inflammatory pain, muscle spasms, and post-stroke limb dysfunction [28–30]. Consequently, a novel form of acupuncture, EA, was developed by combining traditional acupuncture with electrical stimulation. The vagus nerve is key target for the therapeutic effects of acupuncture and EA [21,31]. Numerous experiments have demonstrated that both direct stimulation of the vagus nerve [32,33] and EA stimulation [34] can reduce local and systemic inflammation by activating the CAP. The anti-inflammatory mechanism underlying EA's activation of the vagus nerve is associated with the intensity of the electrical current used for stimulation [21]. For example, current stimulation of 0.5 mA at the ST36 acupoint induces the expression of c-Fos in vagal efferent neurons in the DMV, resulting in a vagus nerve-dependent anti-inflammatory effect. By contrast, 3 mA of current did not produce the same effect. Recent studies have revealed that the frequency of EA stimulation influences the vagus nerve [35]. Specifically, EA at 2 Hz has a stronger excitatory effect on neurons in the NTS than at 100 Hz EA, which is less effective. In line with prior research, our experimental results indicated that EA administered at 2 Hz with an intensity of 0.5 mA significantly increased the number of ChAT-positive neurons in the DMV, leading to improved vagal function and enhanced gastrointestinal motility.

Experimental middle cerebral artery infarction typically leads to damage to the cerebral cortex, although substantial evidence

indicates that damage can also extend to distant sites, including the hippocampus and hypothalamus [36,37], and to peripheral organs, such as the lungs, heart, stomach and kidneys [38–41]. Autonomic dysfunction plays a critical role in the development of peripheral organ damage following stroke [9]. Acute stress resulting from a stroke leads to heightened sympathetic nervous system activity, which is often accompanied by vagal inhibition. The activity of cholinergic neurons in the DMV directly influences the efferent activity of the vagus nerve. Although neuronal projections between DMV cholinergic neurons and higher brain centers have been the subject of limited research, existing evidence suggests significant downstream projections from various brain areas to the NTS and DMV. These brain regions include the paraventricular nucleus of the hypothalamus, the central nucleus of the amygdala, and the bed nucleus of the stria terminalis [9,42,43], and they contain neurons like oxytocin and orexin neurons, among others, which may exert indirect effects on DMV cholinergic neurons [44–46]. To the best of our knowledge, our study is the first to demonstrate that the development of gastrointestinal dysfunction after stroke is closely related to the loss of cholinergic signaling in the DMV, resulting in vagal depression. In addition, EA reversed the loss of cholinergic neurons in the DMV of MCAO rats, attenuated gastric mucosal injury and serum inflammation, and restored stroke-induced gastrointestinal motor inhibition. These findings suggest that EA si a promising treatment for gastrointestinal dysfunction after stroke.

The vagus nerve plays a pivotal role in gastrointestinal innervation through two distinct pathways: the excitatory cholinergic pathway and the inhibitory nonadrenergic noncholinergic (NANC) pathway [47]. In the cholinergic pathway, gastric tone is heightened by the release of ACh, which binds to its receptors, whereas the NANC pathway primarily reduces gastric tone by releasing VIP and other neurotransmitters. In our experiments, we observed a significant downregulation of ChAT, the rate-limiting enzyme responsible for ACh synthesis, after stroke. Conversely, ChAT expression was upregulated after EA treatment. VIP, which functions as both a neurotransmitter and a brain–gut peptide that induces smooth muscle relaxation, demonstrated a tendency to decrease after stroke [48], as confirmed by our experiments. This neurotransmitter imbalance suggests that the effects of the cholinergic and NANC pathways on gastric motility cannot be simply categorized as either an increase or decrease in gastric tone. Normal gastric motility depends on the balance between tense contractions and the receptive relaxation of the stomach. Excessive contraction or relaxation of the stomach suppresses the gastric motility. The inhibition of gastric motility after stroke may result from a combination of factors, including the inhibition of cholinergic, NANC, and other potential pathways. Therefore, we examined another brain–gut peptide, MTL, which is often used in conjunction with VIP to evaluate gastric motor function. The MTL is known to exhibit vagal-dependent release, possess neuronal projections from the PVN to the DMV, and induce strong contractions in the gastrointestinal tract [49]. Our results suggest that MTL and VIP exhibited opposite trends after stroke, indicating that the rats experienced strong gastric spasms. In addition, EA effectively alleviates the inhibition of gastric motility in MCAO rats with strong contractions, and evidence suggests that MTL release involves the activation of cholinergic pathways [50]. However, our experimental results revealed that EA downregulated serum MTL levels in MCAO rats and that MTL secretion was almost absent after vagotomy, which could be related to stomach denervation.

PPIs, H2 receptor antagonists (H2RA) and mucoprotective agents are often used to manage post-stroke heartburn symptoms or prevent gastroduodenal injury. However, the long-term use of PPIs and H2RA has been associated with unfavorable outcomes [6,51]. Recent studies have demonstrated that the clinically used PPIs, including omeprazole, lansoprazole, and rabeprazole, act as ligands for ChAT. High concentrations of these PPIs nearly completely inhibited ChAT activity, whereas low concentrations of omeprazole exhibited inhibition levels similar to those of the potent ChAT inhibitor, α-NETA [52]. Although H2RAs do not share these properties, they have been confirmed to antagonize the increase in ACh levels induced by H3 receptor antagonists [53]. Therefore, low-risk approaches to protect the gastric mucosa after stroke need to be identified urgently. Our experiments verified that EA significantly increased the expression of ChAT in both central and peripheral tissues in MCAO rats, reduced gastrointestinal mucosal damage, and enhanced gastrointestinal motility, while the administration of α-NETA and vagotomy reversed the improvements in gastrointestinal function achieved through EA.

The a7nAchR is recognized as a key target in CAP because its activation effectively mitigates both central and peripheral inflammatory damage [20,54]. Studies have demonstrated that EA can alleviate decreased intestinal motility and intestinal inflammation resulting from postoperative intestinal obstruction through the α7nAchR-mediated JAK2/STAT3 signaling pathway [22]. However, our experiments, following the administration of the α7nAchR antagonist MLA, revealed that EA still improved serum levels of MTL, VIP, gastric emptying rate, and intestinal propulsion rate in MCAO rats, although it did not alleviate gastric mucosal injury and inflammation levels. Conversely, when the α7nAchR agonist PNU282987 was administered, the extent of gastric mucosal injury and serum inflammation levels decreased in MCAO rats, whereas gastrointestinal motility did not improve. These results suggest that α7nAchR is a target of EA for improving gastric mucosal injury and inflammation after stroke, whereas the enhancement of gastrointestinal motility by EA may involve other ACh receptors or non-cholinergic pathways.

In conclusion, our study provides a new idea that the application of PPIs in the acute phase of cerebral infarction may exacerbate cerebral infarction despite its partial gastric mucosal protection, whereas EA can be used as a low-cost and low side-effect treatment alternative to PPIs for the protection of gastrointestinal function after stroke. However, our study has some limitations. Middle cerebral artery infarction primarily affected the brain regions supplied by the middle cerebral artery and did not directly affect the brainstem region supplied by the vertebral artery. Therefore, further experimental investigations are necessary to elucidate how ischemia in regions such as the cortex or hippocampus influences the activity of cholinergic neurons in the DMV of the brainstem. In future experiments, we plan to use retrograde neural tracing techniques to retrogradely label DMV cholinergic neurons through the vagus nerve, which could allow us to explore the projections of DMV cholinergic neurons at various levels, including the brainstem, hypothalamus, hippocampus, and cortex.

5. Conclusion

MCAO rats exhibited reduced DMV cholinergic signaling and suppressed vagal function after 7 days of ischemia, which was accompanied with suppressed gastrointestinal motility and increased inflammation levels. Although omeprazole reduced gastric mucosal injury and inflammation after stroke, it aggravated cerebral infarction. In addition, EA reduced gastric mucosal injury and serum inflammation levels, and improved gastrointestinal motility by enhancing vagal function to restore DMV cholinergic efferent signaling. a7nAchR was also identified to play an important role in EA by improving the level of gastric mucosal damage and inflammation instead of improving gastrointestinal motility after stroke.

Institutional review board statement

This study was reviewed and approved by the Institutional Animal Care and Use Committee of the Guangxi Medical University, with the approval number: 202205012.

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Data availability statement

No, data will be made available on request.

CRediT authorship contribution statement

Ziyan Jin: Writing – original draft, Methodology, Conceptualization. **Zihong Shen:** Writing – review & editing, Data curation. **Siyang Yan:** Methodology, Formal analysis. **Guolei Chen:** Methodology, Conceptualization. **Yalong Yin:** Software, Methodology. **You Zhang:** Methodology. **Xingui Wu:** Supervision, Resources, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e29426.

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