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Restoring brain health: Electroacupuncture at GB20 and LR3 for migraine mitigation through mitochondrial restoration

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Abstract:

BACKGROUND: Electroacupuncture (EA) is a promising alternative therapy for migraine, with mitochondrial dysfunction hypothesized as a pivotal mechanism in migraine pathophysiology. This research endeavors to investigate the therapeutic potential of EA in addressing migraines and shed light on the associated mechanisms linked to mitochondrial anomalies.

MATERIALS AND METHODS: Migraine in rats was induced by 10 mg/kg nitroglycerin, followed by 2/15 Hz EA treatment at GB20 and LR3. Nociceptive behavior was recorded via a camera and analyzed using EthoVision XT 12.0 software. The hind-paw withdrawal threshold was assessed using the von Frey test. We assessed the levels of calcitonin gene-related peptide (CGRP), nitric oxide (NO), and endothelin (ET) – key parameters in migraine pathophysiology using immunohistochemistry and enzyme-linked immunosorbent assay. Mitochondrial morphology in brain tissues was observed through transmission electron microscopy. Reactive oxygen species (ROS) level in mitochondria was measured by flow cytometry. The levels of PINK1 and Parkin were assessed using Western blot analysis.

RESULTS: EA at GB20 and LR3 decreased nociceptive behaviors (resting and grooming) and increased exploratory and locomotor behaviors in migraine rats. The hind-paw withdrawal threshold in migraine rats was significantly elevated following EA treatment. Post-EA treatment, levels of CGRP and NO decreased, while ET level increased, suggesting an alteration in pain and vascular physiology. Notably, EA treatment mitigated the mitochondrial damage and reduced ROS level in the brain tissues of migraine rats. EA treatment upregulated the expression of PINK1 and Parkin in migraine rats.

CONCLUSION: EA at GB20 and LR3 may treat migraine by alleviating PINK1/Parkin-mediated mitochondrial dysfunction.

Keywords:

Electroacupuncture, GB20, LR3, migraine, mitochondrial dysfunction, PINK1/Parkin pathway

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Introduction

Migraine, a recurrent and episodic neurological disorder, is typically characterized by severe headaches, sensory disturbances, and motor anomalies.[1,2] It stands as a significant contributor to partial disability among individuals aged 15–49 years globally, with an estimated prevalence of 15%.[3,4] The multifaceted nature of migraines results in unilateral head pain and numerous neurological symptoms.[5] Recent research has explored several contributing factors to migraines, including vasodilation, neurotransmitter abnormalities, inflammatory responses, genetic factors, and environmental triggers.[6] Some studies posit a connection between migraine episodes

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and abnormal dilation of cerebral blood vessels, which increases neurotransmitter and inflammatory mediator release, thereby instigating headaches.[6,7] The existing armamentarium of migraine treatments predominantly comprises mild analgesics such as acetaminophen, nonsteroidal anti-inflammatory drugs, and triptans for severe headaches.[8] Despite their utility, these agents often exhibit limited efficacy, low adherence, and adverse side effects such as fatigue, dizziness, chest discomfort, somnolence, and nausea.[8] The dearth of effective clinical treatments to prevent and delay migraine progression underscores the need for comprehensive investigation into alternative therapies.

Acupuncture, a time-tested practice within traditional Chinese medicine, has gained traction as a potent and safe alternative for migraine treatment.[9] Electroacupuncture (EA), an evolved form of traditional acupuncture, incorporates pulsed electrical currents transmitted through acupuncture needles for healing benefits.[10] EA has shown promising results for neuropathic and persistent pain management, as well as analgesia, making it a viable option for migraine treatment.[11-14] The GB20 (Fengchi) acupoint, situated at the intersection of the sternocleidomastoid and trapezius muscles, is proximal to the region impacted by migraines. Prophylactic acupuncture at GB20 can alleviate migraine attacks and mechanical cephalic cutaneous hypersensitivity by modulating neuronal discharge from the trigeminocervical complex.[15] Earlier research indicated that EA application on GB20 could reduce hyperalgesia and inflammation in a rat migraine model.[16,17] The LR3 (Taichong) acupoint, situated on the foot, is another focal node for EA treatment, which can instigate extra-segmental neuromodulation to aid in migraine management.[18,19] Stimulating LR3 can facilitate the resolution of inflammatory mediators and contribute to pain relief in migraines.[20] While EA at the GB20 and LR3 acupoints shows promise in migraine treatment, the precise mechanisms underlying its efficacy remain largely elusive.

Mitochondrial dysfunction is posited as a potential mechanism implicated in migraine pathophysiology.[3] Magnetic resonance spectroscopy indicates diminished mitochondrial phosphorylation capability and energy metabolism in the brains of migraine sufferers during interictal periods.[21] Enhanced brain mitochondrial function could potentially mitigate migraine severity.[22] The PTEN-induced putative protein kinase 1 (PINK1)/Parkin pathway regulates mitochondrial function. In compromised cells, PINK1 attracts Parkin to the mitochondria, safeguarding against excessive reactive oxygen species (ROS) production.[23] EA is reported to mitigate neuronal injury by inhibiting PINK1/Parkin-mediated mitochondrial dysfunction in cerebral ischemia-reperfusion.[24]

Building upon the existing evidence, we hypothesize that EA treats migraines by modulating PINK1/Parkin-mediated mitochondrial dysfunction. This study aims to validate this hypothesis and delve deeper into the mechanisms underlying the application of EA at the GB20 and LR3 points. We utilized a nitroglycerin (NTG)-induced rat migraine model to investigate the therapeutic efficacy of EA and to clarify its underlying mechanisms.

Materials and Methods

Animals

In this research, animal trials received approval from the Institutional Animal Care and Use Committee at Xiamen University (XMULAC20220034-19). Sprague Dawley rats (male, $n = 24$), pathogen-free and weighing 200 ± 10 g, were sourced from GemPharmatech Co., Ltd., China. Before experimentation, each rat underwent a week-long acclimatization in a laboratory setting with regulated temperature (21°C ± 2°C) and humidity (40%–70%), operating on a 12 h light/dark rotation. Throughout this adaptation phase, the rats were provided with unrestricted access to food and water.

Group assignment and treatment

All rats were randomly allocated into four distinct groups, with six rats in each: control, model, model with nonacupuncture (NA) point EA, and model with EA groups. A rat migraine model was developed by giving intraperitoneal injections of 10 mg/kg NTG on the 1st, 3rd, and 5th days. For the model + EA group, EA was applied daily to the GB20 and LR3 points for 5 days. GB20 acupoint, positioned at the junction of the sternocleidomastoid and trapezius muscles, is near the affected area in migraine. LR3 acupoint is located on the foot about two finger widths above the place where the skin of your big toe and the next toe join. Specifically, two stainless steel acupuncture needles (0.25 mm in diameter and 25 mm in length) were penetrated 2–3 mm deep into the GB20 and LR3 acupoints. Subsequently, the handles of these needles were linked to an electrical stimulator. This apparatus was activated daily for 15 min, using a frequency of 2/15 Hz (pulse width of 0.5 ms) and an intensity ranging between 0.5 and 1.0 mA. This frequency was chosen based on its well-documented effectiveness in pain management through promoting the release of various endogenous opioids and neurotransmitters. The treatment duration of 20 min, which aligns with standard practice in EA research, optimizes the balance between therapeutic efficacy and animal comfort. For the model + NA group, EA was administered at a nonacupoint location (roughly 10 mm above the iliac

crest) using identical procedures in the model + EA group. The nonacupoint location, situated approximately 10 mm above the iliac crest, does not correspond to any traditional acupuncture point.[25] Rats in the control and model groups were merely restrained in fixtures for 15 min without undergoing acupuncture. After a 5-day treatment period, rats were sedated using an intraperitoneal injection of 0.3% pentobarbital sodium (0.1 ml/10 g) and then humanely euthanized via cervical dislocation. Peripheral blood and brain tissues were harvested for subsequent experimentation.

Spontaneous behavioral analysis

Following 5 days of NTG administration, rats were allowed to acclimate in separate transparent acrylic observation cages for 20 min. Rats in both the model + NA and model + EA groups underwent EA treatment in a different cage and were then individually monitored for a duration of 1 h. The spontaneous behavior of rats was recorded by a camera and analyzed by EthoVision XT 12.0 software (Noldus IT, Wageningen, Netherlands). Four main spontaneous behaviors were recorded, as follows:

1. Exploratory behavior: Walking, hanging, nurturing, and sniffing
2. Locomotor behavior: Turning, stretching, walking, jumping, and walking slowly
3. Resting behavior: Head rested on flexed forepaws for sleeping
4. Grooming behavior: Using forepaws to groom face and body.

Von Frey test

The von Frey test was used to assess the sensitivity to mechanical stimuli in our rat migraine model. We chose to measure this in the hind paws of the rats due to the common occurrence of extracephalic allodynia, or sensitivity to pain outside of the head, in migraine sufferers. This test provides an indirect reflection of the state of migraine by measuring changes in overall body pain sensitivity. Von Frey filaments (1.4, 2.0, 4.0, 6.0, 8.0, 10.0, 15.0, and 26.0 g; North Coast Medical, Inc., USA) were successively applied to stimulate the central part of the hind paws of rats. Each stimulation lasted for 2 s and was repeated 5 times with a 5-s interval. Paw withdrawal was considered a positive reaction. The mechanical threshold of rats was determined by the "up-and-down" method.[26]

Immunohistochemistry

Immunohistochemical analysis was conducted on harvested brain tissues, particularly emphasizing the trigeminal nucleus caudalis region. The tissues were first fixed in 4% formaldehyde for a span of 24 h, then dehydrated through a progressive ethanol sequence, and finally encapsulated in paraffin. Thin sections (4–7 μm) were then generated from these embedded samples. Following the deparaffinization and rehydration process, the sections underwent antigen retrieval and subsequent blocking of endogenous peroxidase activity. Thereafter, each section was treated with 50 μl normal goat serum for a 15-min duration to block nonspecific binding sites before being incubated with 50 μl anti-calcitonin gene-related peptide (CGRP) antibody (5 μg/ml; #ab47027, Abcam, UK) overnight at 4°C. The following day, these sections were meticulously washed three times with phosphate-buffered saline, with each wash lasting for 5 min. Next, these sections were incubated with 50 μl goat anti-rabbit immunoglobulin G (IgG) H and L (HRP) (1:2000; #ab205718, Abcam, UK) for a duration of 15 min. The target signal was made visible using diaminobenzidine staining for 10 min, followed by a 3-min counterstain with hematoxylin. Post dehydration, the sections were mounted and then examined using a light microscope (Olympus, Japan).

Enzyme-linked immunosorbent assay

Given its significant role in migraine pathophysiology, CGRP levels were assessed using enzyme-linked immunosorbent assay (ELISA). Elevated during migraine attacks, CGRP, a potent vasodilator and neuromodulator, contributes to pain transmission and modulation.[27] Serum concentrations of CGRP (CGRP; Rat CGRP ELISA kit, Mibio, China), nitric oxide (NO; NO assay kit, Nanjing Jiancheng, China), and endothelin (ET; ET1 ELISA kit, Mibio, China) were measured by ELISA according to manufacturers' protocols.

Transmission electron microscopy

Transmission electron microscopy (TEM) was employed in this study to observe the ultrastructural changes in brain mitochondria, as it provides high-resolution images at the nanometer scale. Brain samples, roughly 1 mm^3 in size, were preserved in 2.5% glutaraldehyde for a duration of 48 h and subsequently in 1% osmium tetroxide for 2 h at a temperature of 4°C. Tissues were then dehydrated and embedded for cutting into 70 nm sections. After staining with uranyl acetate and lead citrate, the sections were examined using a JEM-1400Flash Electron Microscope (JEOL, Germany).

Measurement of mitochondrial reactive oxygen species level

The mitochondrial ROS level in brain tissues was detected by flow cytometry. Mitochondria were isolated from brain tissues by supercentrifugation. Mitochondria, once resuspended, were treated with a 10 mM H2DCFDA probe (sourced from Invitrogen, CA, USA) and incubated at 37°C for 15 min in darkness. Subsequently, ROS levels were assessed using flow cytometry, employing an excitation wavelength of 488 nm and an emission wavelength of 525 nm. Higher

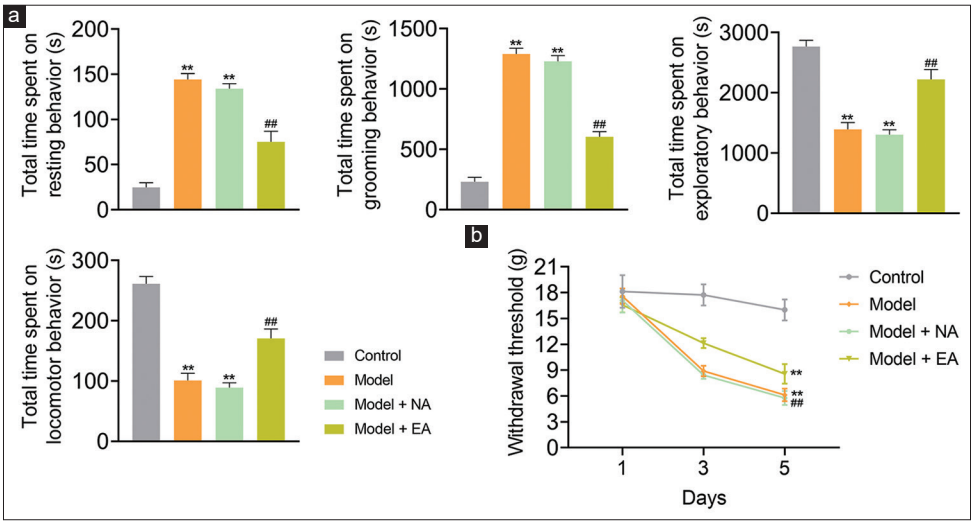

Figure 1: Effects of electroacupuncture (EA) treatment on migraine-like nociceptive behaviors and hind-paw allodynia. (a) Spontaneous behavior analysis for rats, including resting, grooming, exploratory, and locomotor behaviors. (b) Hind-paw withdrawal threshold of rats. A rat model of migraine was established by intraperitoneal injection with 10 mg/kg nitroglycerin and then treated by EA. $^{**}P < 0.01$ versus control; $^{\#\#}P < 0.01$ versus model + nonacupuncture point EA

ROS level indicates the occurrence of oxidative stress that can lead to mitochondrial dysfunction, thereby resulting in the development of migraine symptoms.

Western blotting

Brain tissue proteins were extracted utilizing the RIPA lysis buffer (Beyotime, China), supplemented with the protease inhibitor PMSF (Beyotime). The protein concentration was determined using the bicinchoninic acid technique. A quantity of 25 μg of protein was separated on a 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) via electrophoresis at 90 V. It was then transferred onto a polyvinylidene fluoride (PVDF) membrane at 65 V for a duration of 2 h. This PVDF membrane was subsequently blocked using 5% nonfat milk for 1 h and later incubated with the primary antibodies overnight at a temperature of 4°C. Primary antibodies were PINK1 antibody (1:1000; #DF7742, Affinity, CA, USA), Parkin antibody (1:500; #AF0235, Affinity), and anti-GAPDH antibody (1:1000; #ab8245, Abcam, UK). Following three washes with 1× TBST, each lasting 10 min, the membrane was exposed to goat anti-rabbit IgG H and L (HRP) (1:2000; #ab205718, Abcam) for 1 h, ensuring no light exposure. Excess secondary antibody was then rinsed away with 1× TBST. The protein bands were subsequently detected using an ECL chemiluminescence kit (Servicebio, China).

Statistical analysis

Data were processed and analyzed using GraphPad Prism software (version 7.0, GraphPad Software, San Diego, CA, USA) and are presented as mean ± standard deviation. For comparisons involving multiple groups, one-way ANOVA was employed, and for pairwise comparisons, Tukey's test was used. $P < 0.05$ was considered to indicate a significant difference.

Results

Effects of electroacupuncture treatment on migraine-like nociceptive behaviors and hind-paw allodynia

Initially, we examined the impact of EA treatment on spontaneous behaviors resembling migraines and allodynia. A rat migraine model was created using an intraperitoneal injection of NTG. As shown in Figure 1a, model rats spent more time on resting and grooming behaviors than control rats within 60 min ($P < 0.01$). The EA treatment notably reduced the average resting and grooming activities observed in the model rats ($P < 0.01$). In contrast, model rats spent less time on exploratory and locomotor behaviors than control rats ($P < 0.01$). After EA treatment, time spent on exploratory and locomotor behaviors by model rats was significantly increased [$P < 0.01$; Figure 1a]. In addition, the nociceptive withdrawal threshold of rats at three time points (days 1, 3, and 5) is shown in Figure 1b. On the initial day of EA treatment, there were no significant disparities in the nociceptive thresholds of the hind-paw across the groups ($P > 0.05$). At subsequent time points (days 3 and 5), model rats showed a lower nociceptive withdrawal threshold than control rats ($P < 0.01$). EA treatment significantly attenuated the lowered hind-paw nociceptive threshold of model rats at days 3 and 5 [$P < 0.01$; Figure 1b].

Impact of electroacupuncture therapy on serum and brain tissue levels of calcitonin gene-related peptide, nitric oxide, and endothelin in rats with migraines

CGRP is a vasodilator neuropeptide, playing a critical role in the pathophysiology of migraine.[28] CGRP can

stimulate the synthesis and release of NO. Compared to control rats, CGRP expression in the brain tissues of model rats was increased, which was decreased by EA treatment to some extent [Figure 2a]. In serum, CGRP content was also elevated in model rats when compared with control rats, with the increased NO level ($P < 0.01$). EA treatment significantly reduced the serum levels of CGRP and NO in model rats [$P < 0.01$; Figure 2b]. Contrarily, the serum level of ET (a vasoconstrictor peptide) was decreased in model rats compared to control rats, which was rescued by EA treatment [$P < 0.01$; Figure 2c].

Electroacupuncture treatment alleviates PINK1/Parkin-mediated mitochondrial dysfunction in brain tissues of migraine rats

Mitochondrial dysfunction is a potential mechanism for the pathophysiology of migraine.[3] By TEM, we found that control rats had neatly arranged mitochondrial cristae in brain tissues, while model rats showed mitochondrial crest fracture and swelling. EA treatment ameliorated mitochondrial swelling and injury in model rats [Figure 3a]. The overproduction of ROS is a critical factor causing mitochondrial dysfunction.[29] As expected, the ROS level was elevated in brain tissues of model rats, which was reversed by EA treatment [$P < 0.01$; Figure 3b]. The PINK1/Parkin pathway is pivotal in modulating mitochondrial function. Through Western blot analysis, we observed a reduced expression of PINK1 and Parkin in the model rats. However, with EA intervention, their expression levels were significantly augmented [$P < 0.01$; Figure 3c].

Discussion

Our study provides evidence that EA treatment at the GB20 and LR3 points can ameliorate migraine-like spontaneous behaviors and allodynia. We suggest that the therapeutic efficacy of EA on migraines is associated with the modulation of mitochondrial dysfunction, regulated by the PINK1/Parkin pathway.

Electroacupuncture treatment alleviates cutaneous allodynia in migraine

In our research, we developed a rat migraine model through NTG induction, a technique commonly used to emulate acute or chronic migraine conditions.[30] Consistent with previous studies,[31,32] our migraine rat model displayed increased nociceptive behaviors (resting and grooming) and decreased exploratory and locomotor behaviors. These behaviors are reflective of the clinical symptoms experienced during migraine episodes. Notably, we found that the application of EA treatment resulted in a reversal of these behavioral changes in our rat model. Furthermore, cutaneous allodynia, characterized by skin hypersensitivity triggered by nonnoxious stimulation, often accompanies migraine episodes and can increase the risk of the condition becoming chronic.[33] Cutaneous allodynia manifests in the facial region on the affected side, progressively extending to territories innervated by the trigeminal nerve, thereby encompassing limbs and potentially the entire body.[34] EA at GB20 alleviates migraine pain and associated cutaneous allodynia by modulating the ascending pathway of the trigeminovascular

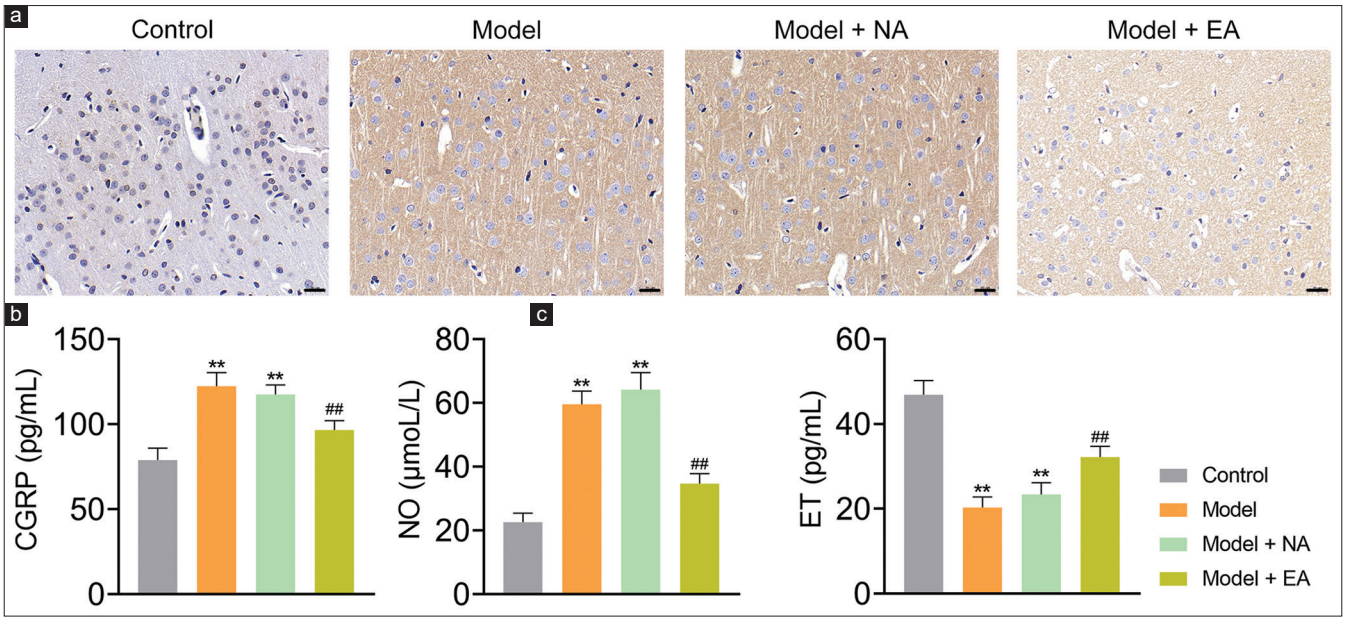

Figure 2: Effects of electroacupuncture (EA) treatment on calcitonin gene-related peptide (CGRP), nitric oxide (NO), and endothelin (ET) levels in serum and brain tissues of migraine rats. (a) The expression of CGRP in brain tissues of rats was measured by immunohistochemistry (scale bar = 20 μm). (b and c) The levels of CGRP, NO, and ET in the serum of rats were measured by enzyme-linked immunosorbent assay. A rat model of migraine was established by intraperitoneal injection with 10 mg/kg nitroglycerin and then treated by EA. $^{**}P < 0.01$ versus control; $^{\#\#}P < 0.01$ versus model + nonacupuncture point EA

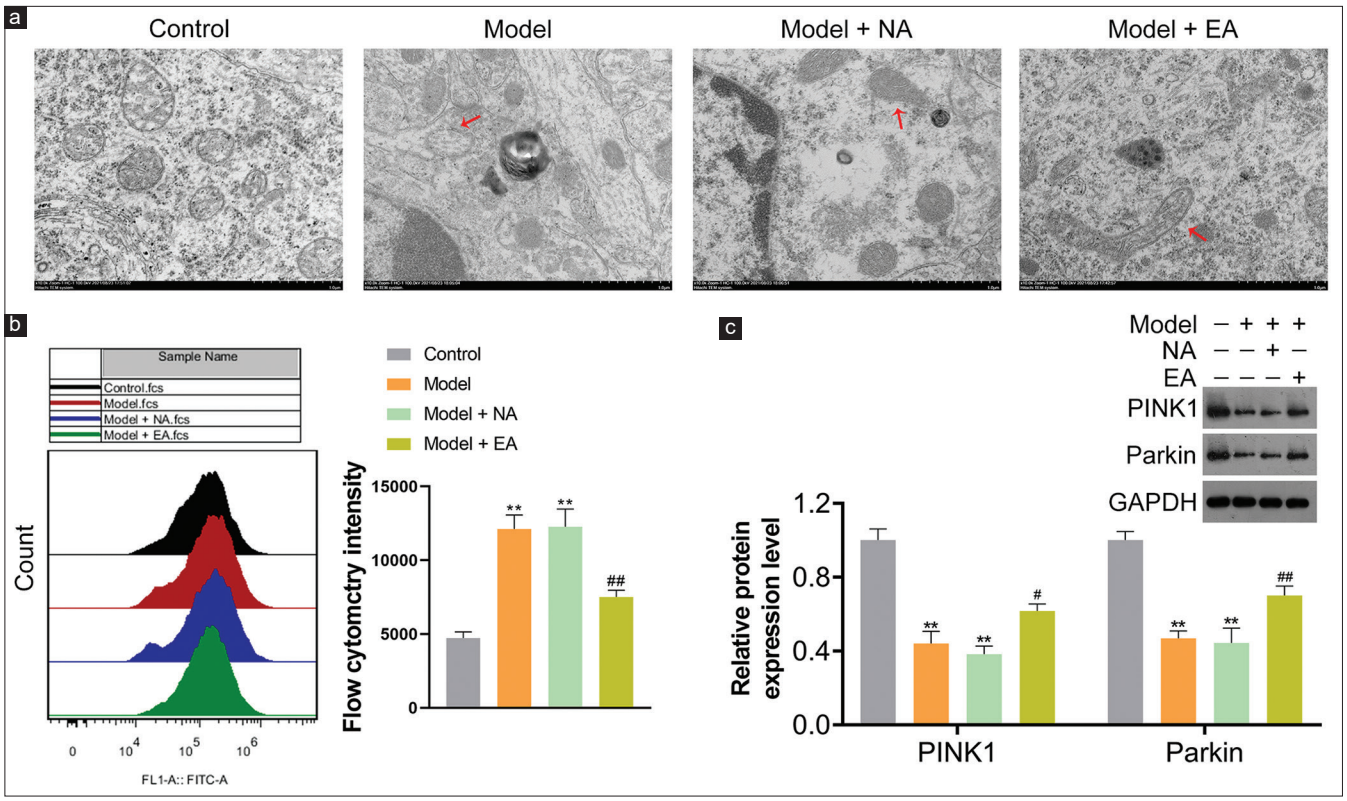

Figure 3: Electroacupuncture (EA) treatment alleviates PINK1/Parkin-mediated mitochondrial dysfunction in brain tissues of migraine rats. (a) Mitochondrial damage in brain tissues of rats was observed by transmission electron microscopy (scale bar = 1 μm). Red arrows indicate mitochondria. (b) Reactive oxygen species level in the mitochondria of rats was measured by flow cytometry. (c) The expression of PINK1 and Parkin was detected by Western blotting. A rat model of migraine was established by intraperitoneal injection with 10 mg/kg nitroglycerin and then treated by EA. $^{**}P < 0.01$ versus control; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ versus model + nonacupuncture point EA

system.[35] Consistently, our research revealed that applying EA to GB20 and LR3 markedly counteracted the migraine-caused decrease in the hind-paw nociceptive threshold. This underscores the alleviative effects of EA on cutaneous allodynia associated with migraine.

Electroacupuncture treatment recovers vasomotor function in migraine

In addition, we explored how EA impacts the concentrations of NO and CGRP, both of which are pivotal to migraine pathogenesis. Overproduction of NO leads to vasodilation. This, in turn, stimulates nociceptive nerve fibers and drives the release of vasoactive compounds, among them CGRP.[36] During migraine episodes, increased levels of CGRP have been documented, playing a role in the progression to chronic migraines.[30] In addition, ET, an endogenous vasoconstrictor, affects vasomotor function in migraine.[37] Our findings suggest that EA therapy markedly decreased the concentrations of NO and CGRP and concurrently elevated the ET levels in rats experiencing migraines. This suggests that EA treatment could regulate vasomotor function, providing a therapeutic effect for migraines.

Electroacupuncture treatment ameliorates mitochondrial dysfunction in migraine

We further explore the potential mechanism of EA treatment for migraines. Previous studies indicate that mitochondrial dysfunction contributes to migraine progression.[38] Specifically, compromised mitochondrial metabolism is associated with migraine pathogenesis, as it lowers the threshold for initiating migraine attacks. Nattagh-Eshtivani *et al.* highlighted that mitochondrial dysfunction stands as a paramount factor causing migraines, primarily by resulting in an inadequacy in oxygen metabolism and disrupting mitochondrial energy processes.[39] In our study, we observed significant mitochondrial damage in the brain tissues of our migraine rat model, accompanied by elevated ROS levels. However, with the application of EA treatment, both mitochondrial damage and ROS levels were significantly reduced. These results indicate that EA can ameliorate mitochondrial dysfunction in migraine.

Electroacupuncture treatment mitigates mitochondrial dysfunction by activating the PINK1/Parkin pathway in migraine

The PINK1/Parkin signaling pathway is crucial for the modulation of mitochondrial functionality.[40] PINK1 functions as a sensor for detecting mitochondrial damage, while Parkin acts as an E3 ubiquitin ligase that targets damaged mitochondria for degradation.[41] PINK1 transfers Parkin to impaired mitochondria, leading to

the selective elimination of dysfunctional or surplus mitochondria through autophagy, thus preserving energy metabolism.[40] In specific subsets of migraine patients, impaired mitophagy and mitochondrial dysfunction have been observed.[42] Malfunctioning PINK1 and Parkin can lead to compromised mitophagy, causing an accumulation of damaged mitochondria. This buildup, coupled with heightened oxidative stress, plays a role in the onset and advancement of migraines. Targeting mitophagy pathways and restoring mitochondrial quality control hold promise as novel therapeutic strategies for treating migraines. Modulating PINK1/Parkin signaling, promoting proper mitophagy flux, or enhancing mitochondrial function could offer promising appropriate for intervention. Notably, in the context of cerebral ischemia-reperfusion, EA has been reported to ameliorate neuronal injury by reducing PINK1/Parkin-mediated mitochondrial dysfunction.[24] Furthermore, EA has shown the ability to regulate PINK1/Parkin-mediated mitophagy, thereby mitigating neurological deficits in intracerebral hemorrhage.[43] Therefore, it is plausible to speculate that EA may alleviate PINK1/Parkin-mediated mitochondrial dysfunction as a treatment for migraines. Our findings revealed a reduced expression of PINK1 and Parkin in migraine-affected rats, which was notably rectified following EA intervention. This finding suggests that EA mitigates mitochondrial dysfunction by activating the PINK1/Parkin pathway in migraine.

Despite yielding significant insights, our study has limitations. First, our results from the NTG-induced rat model of migraine might not fully translate to humans due to the complexity of migraines. Second, the specific regulatory mechanism for EA treating migraine through the PINK1/Parkin pathway is still incomplete, and further investigation will be performed using activators and inhibitors of the PINK1/Parkin pathway. Finally, the efficacy of acupuncture, including EA, can vary depending on treatment details. Thus, the exploration of various conditions and acupuncture points in future studies is advisable.

Conclusion

EA at GB20 and LR3 reduced nociceptive behaviors and increased the hind-paw withdrawal threshold of migraine rats. The therapeutic effect of EA on migraine was achieved by alleviating PINK1/Parkin-mediated mitochondrial dysfunction. Current research efforts are directed toward delving deeper into the mechanisms by which EA modulates mitochondrial dysfunction via the PINK1/Parkin pathway.

Author contributions

JL, LF, and BL substantially contributed to the conception and the design of the study, and manuscript drafting and critical revisions of the intellectual content. JL, LF, LW, and ZF was responsible for the acquisition, analysis, and interpretation of the data. JL, and BL confirm the authenticity of all the raw data. BL approved the final manuscript to be published. All authors have read and approved the final manuscript. The manuscript has been read and approved by all the authors, that the requirements for authorship as stated earlier in this document have been met, and that each author believes that the manuscript represents honest work.

Ethics Committee approval

Animal experiments in this study were approved by the Institutional Animal Care and Use Committee of Xiamen University (XMULAC20220034-19, dated on September 15, 2022) in accordance with the Helsinki Declaration of 1975.

Data availability statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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