Preventive and Therapeutic Effects of Low-Intensity Ultrasound Stimulation on Migraine in Rats

Leiqiang Yao, Rong Chen, Hui Ji, Xingran Wang, Xiangjian Zhang, and Yi Yuan

***Abstract*— This study sought to systematically evaluate the prophylactic and therapeutic effects of low-intensity transcranial ultrasound stimulation on migraine in rats. We used video recordings to assess the head scratching behavior and laser speckle contrast imaging to record the changes in cerebral blood flow velocity of freely moving rats in a healthy group, migraine group, migraine group with ultrasound prevention, and migraine group with ultrasound therapy. Results demonstrated that (1) head scratching during migraine attacks in rats was accompanied by an decrease in cerebral blood flow; (2) both ultrasound prevention and therapy significantly reduced the number of head scratches but did not reduce the cerebral blood flow velocity; and (3) the number of head scratches in the ultrasound stimulation groups was not affected by the auditory effect. These results reveal that low-intensity ultrasound has the potential to be used clinically in the prevention and therapeutic treatment of migraine.**

***Index Terms*— Transcranial ultrasound stimulation, migraine model, laser speckle contrast imaging, cerebral blood flow, freely moving rat.**

I. Introduction

MIGRAINE is a recurrent, chronic, and disabling neurological disease. Patients can present with reversible neurological and systemic symptoms lasting for 4–72 hours [1], [2], [3]. Most migraine cases are moderate or severe and can occur in any part of the head. They are often accompanied by non-headache symptoms such as nausea and/or vomiting [4], [5], [6]. Clinical drug treatment is the main treatment for migraine. Commonly used drugs include, but are not limited to, beta-blockers, anti-seizure drugs, anti-depressants, calcium antagonists, non-steroidal anti-inflammatory drugs, and angiotensin inhibitors [7], [8]. The ultimate goal is to end the pain if it continues to progress, reduce recurrence following treatment, and alleviate associated symptoms to minimize adverse reactions and economic burden. However, in recent years, many studies have shown that drug resistance and addiction caused by the long-term and improper use of drugs are very common, thereby complicating a patient's condition [9], [10], [11]. Due to these, many patients with migraine have to terminate treatment and experience frequent occurrences of migraine.

Nitroglycerin can induce migraine and is often used in experiment to prepare animal models of migraine. Nitroglycerin induces hypersensitivity of trigeminal nerve fibers by causing meningeal vasodilation, formation of neurogenic inflammation, and activation of neuronal functions in the hypothalamus, brainstem, and spinal cord segments [12], [13]. Previous studies have shown that nitroglycerin is first converted into nitric oxide (NO) in the body. NO is a key factor in migraine and vascular headache, and is closely related to the occurrence of cerebral symptoms [14], [15], [16], [17]. NO acts on the corresponding receptors to promote the synthesis of cyclic guanosine monophosphate, inhibit the influx of cellular Ca^{2+}, reduce the activity of K^{+} channels in the cell membrane, and relax the vascular smooth muscle. NO can directly lead to endothelial-derived vasodilation, resulting in sterile inflammation. Furthermore, NO can produce neurogenic vasodilation by acting on nerve fibers around blood vessels. This molecule is also involved in the generation and transmission of noxious stimuli, has neurotoxic effects, and can stimulate the trigeminal nerve-vascular reflex. The above phenomena may explain the pathophysiological mechanism of nitroglycerin-induced migraine. Tassorelli *et al.* reported that nitroglycerin induces migraine in animals, and its migraine behavior (head scratching, head shaking, cage climbing) and pathological and biochemical changes are similar to those in human migraine attacks [18]. Other studies also found

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that the migraine rat model by nitroglycerin replicates the neurogenic inflammation and hyperalgesia of migraine, and its behavioral manifestations are similar to those of human migraine [19], [20].

Migraine is considered as a neurovascular disease in which the coupling between the neuronal function and blood vessels plays a key role. Activated and sensitized intracranial perivascular nociceptors transmit nociceptive signals through parasympathetic nerves throughout the cerebral blood vessels. Headache is a major source of migraine; under-triggering, dysfunctional sympathetic nerves can lead to excessive vasomotor fluctuations, causing migraine attacks [21], [22], [23]. Previous studies have shown that potential biomarkers associated with migraine mainly include calcitonin gene-related peptide, serotonin 1F, glutamate, β-endorphin, pituitary adenylate cyclase, nitric oxide synthesis enzymes, etc. [24], [25], [26], [27], [28], [29], [30], [31]. In addition, cerebral hemodynamics in different stages of migraine attacks show changes in cerebral blood flow and perfusion, vascular caliber, as well as cortical and subcortical functions. Therefore, cerebrovascular reactivity may be a marker of migraine severity. A previous study found that the cerebral blood flow velocities of nitroglycerin-induced migraine were significantly decreased [30].

Low-intensity transcranial ultrasound stimulation (TUS) generally uses low-intensity ultrasound waves through the entire skull to modulate neural function [32], [33]. It has the advantage of being non-invasive and having a high spatial resolution and high stimulation depth [34], [35]. Researchers have leveraged ultrasound to conduct non-invasive high-precision stimulation in healthy rodents/non-human primates/humans. At the macroscopic level, previous studies found that TUS can induce motor responses in mouse/rat limbs, whiskers, and tail sites [36], [37], [38]. At the mesoscopic level, previous studies have demonstrated that TUS can modulate neural information encoding, such as the spike rate and amplitude, power spectrum, and low-frequency or high-frequency phase amplitude coupling intensity of local field potential, in the cortex/hippocampus/thalamus of mice [39], [40], [41], [42], [43]. It can also alter cerebral hemodynamics and cerebral blood oxygen metabolism, such as increasing cerebral blood flow (CBF) speed and enhancing the intensity of neurovascular coupling between electrophysiology and brain blood oxygen [44], [45], [46], [47]. At the microscopic level, researchers have demonstrated that TUS can modulate protein expression in brain tissue, such as by increasing the levels of brain-derived neurotrophic factor, glial cell-derived neurotrophic factor, and vascular endothelial growth factor and by reducing the levels of acetylcholine and Aβ [48], [49], [50]. In summary, TUS can modulate the functional activity and metabolism of healthy brain tissues.

In addition, researchers have found that TUS can treat neuropsychiatric diseases, including brain injury [51], ischemic stroke [52], Parkinson's disease [53], and epilepsy [54], [55]. In our previous study, we found that low-intensity TUS can inhibit cortical spreading depression by modulating neural activity and hemodynamics [56]. Cortical spreading depression is a pathophysiological process of cortical activity inhibition, which is closely related to migraine. However, whether ultrasound stimulation can prevent and protect migraines remains unclear.

Studies have shown that neural activity elicited by ultrasound stimulation may be confounded by activation of auditory pathways in certain animals [57], [58]. Ultrasound application causes mechanical waves to stimulate the inner ear structures of the cochlea. Activation of the cochlea leads to excitation of auditory pathways, including the contralateral auditory cortex. Cross-modal projections from these auditory areas modulate neural activity throughout the cortex, including neurons within the ultrasound focal zone and observed auditory cortex responses to ultrasound stimulation to induce motor responses. Studies have also shown that ultrasound stimulation can indeed produce auditory system activity in normal hearing mice based on auditory brainstem responses [59]. In addition, the researchers found a direct correlation between the duration of the ultrasound pulse and the duration of the muscle electromyography (EMG) response. This supports the hypothesis that the motor response evoked by ultrasound stimulation is not occurring via the peripheral auditory system [59]. However, whether the auditory pathways impact ultrasound interventions for migraine remains unclear.

To answer the question of whether ultrasound stimulation can prevent and protect against migraines, we first developed a wearable laser speckle imaging system and a behavioral system to jointly assess the attack of nitroglycerin-induced migraine in rats by simultaneously monitoring the changes in CBF and the number of scratches in freely moving rats in real time. Next, we used low-intensity ultrasound to prevent migraine and treat the rat migraine model, and analyzed the number of head scratching and blood flow changes across different groups (healthy group, migraine group, migraine group with ultrasound prevention, and migraine group with ultrasound therapy). Finally, chemically deafened rats were used as a control to assess the effect of auditory effects on ultrasound protection and therapy.

II. Methods

A. Animal Surgery and Anesthesia

Fifty-six Sprague-Dawley rats were used in these experiments (all male; body weight, 200 $\pm$ 20 g; Beijing Vital River Laboratory Animal Technology Co., Ltd., Beijing, China). All procedures were conducted in accordance with the guidelines of the Animal Ethics and Administrative Council of Yanshan University. The rats were housed in standard cages under a 12-hour light/dark cycle and had *ad libitum* access to food and water. The model of migraine was established by subcutaneous injection of nitroglycerin (10 mg/kg, 1 ml, 5 mg, No. H11020289, Beijing Yimin Pharmaceutical Co., Ltd.) into the posterior neck of rats.

B. Experimental Groups

Eight groups were used in our experiments. In Group 1, seven rats were used as the control group (control group). In Group 2, seven rats were subcutaneously injected with nitroglycerin to induce migraine (migraine group). In Group 3, seven rats underwent TUS for 15 min, followed by

subcutaneous injection of nitroglycerin [TUS (Pre) group], and the ultrasound wave entered the brain tissue directly above the skull. In Group 4, seven rats were subcutaneously injected with nitroglycerin, followed by TUS for 15 min [TUS (The) group], and ultrasound waves entered the brain tissue from the side of the skull. In Group 5, seven rats used for preparing deafness models underwent TUS for 15 min, followed by subcutaneous injection of nitroglycerin [TUS (Pre)-Sham group-1]. In Group 6, seven rats used for preparing deafness models were subcutaneously injected with nitroglycerin, followed by TUS for 15 min [TUS (The)-Sham group-1]. Ultrasound waves were applied close to the ear but did not pass through the brain tissue in groups 5 and 6. In Group 7, five rats used for preparing deafness models underwent TUS for 15 min, followed by subcutaneous injection of nitroglycerin [TUS (Pre)-Sham group-2]. The stimulation mode of group 7 was the same as that of group 3. In Group 8, five rats used for preparing deafness models were subcutaneously injected with nitroglycerin, followed by TUS for 15 min [TUS (The)-Sham group-2]. The stimulation mode of group 8 was the same as that of group 4. Two rats died during the operation to open the skull window. Two deafened rats model were used for laser speckle contrast imaging.

C. Deafened Rat Model

The deafened rat model was prepared as previously described [57]. First an injection of kanamycin (1 g kg-1 subcutaneously, 030201211, 100 mg/ml-1; North China Pharmaceutical Group Corporation Ltd, Shijiazhuang, China) was given. Thirty minutes later, furosemide (200 mg/kg^{-1} intraperitoneally, 041101181, 10 mg ml^{-1}; Shanxi Zhaoyi Biology Ltd, Yuncheng, China) and saline (1.5 ml subcutaneously) were given. This cocktail was expected to produce partial deafening within 30 min after furosemide and saline administration.

D. Experimental Procedures

In these experiments, rats were anesthetized with isoflurane (5.0% initial and 2.0% for maintenance), and the oxygen level was set to a delivery rate of 0.5 L/min. The anesthetized rats were fixed in a stereotaxic frame (ST-5ND-C, Stoelting Co., USA) with ear bars and a clamping device. A midline incision was made over the scalp, and the exposed tissues were cleaned with a scalpel to expose the surface of the skull. A 4-mm × 4-mm square-shaped skull section was removed to expose the brain tissue, and a 4-mm × 4-mm glass plate was affixed to the opening of the window. Next, the skull adapter was affixed to the skull. The body temperature of the rats was maintained at 37°C using a closed-loop animal temperature controller (69002, RWD., Shenzhen, China). Prior to attaching the imaging tube, the rats were anesthetized using 2% isoflurane. The cranial window was cleaned using lens cleaning tissue and ethanol.

E. Ultrasound Experimental Setup and Parameters

The ultrasound system was similar to that used in our previous study [56]. The fundamental frequency, stimulation duration, pulsed repetition frequency, and duty cycle were 500 kHz, 400 ms, 1 kHz, and 50%, respectively (Figure 1(E)). The peak-to-peak pressure of ultrasound was 0.5 MPa and the corresponding spatial peak and pulse-average intensity (I_{sppa}) values were 8.3 W/cm^2. The ultrasound transducer (V323-SU, Olympus, USA) was connected to the rat skull using a conical coupling cone filled with ultrasound coupling gel. A calibrated needle-type hydrophone (HNR500; Onda, USA), moved by a 3D electric translation platform, was used to measure the ultrasound field distribution under the skull. As shown in Figure 1 (F) and (G), the full width of the ultrasound spot at half maximum (FWHM) was ~6.6 mm. In this study, the whole rat brain was stimulated by ultrasound and there were no specific brain targets.

F. Video Recording System

A camera (BASLER acA1280-60 gm, USA) was used for video recording, and video images were acquired by a computer. Manual counting was performed to quantify the number of head scratches within 2 hours.

G. Laser Speckle Contrast Imaging (LSCI) System for Freely Moving Rats

A custom imaging system was configured to perform LSCI in freely moving rats [60], [61]. A miniature rolling-shutter CMOS camera (XiMu, Ximea Inc., Golden, CO, Germany) was used to record raw images. The skull adapter was fixed to the skull using dental cement. A tube was used to connect the CMOS camera and skull adapter and fix the light source. When we performed ultrasound stimulation, the imaging tube was removed from the skull adapter. When recorded, the imaging tube was reinstalled on the skull adapter. The tube was screwed onto the skull adapter plate, and the image stream from the camera was used to assess the location of the best focus by carefully rotating the imaging tube about the threads on the skull adapter. Once the location of the best focusing of the brain was found, the imaging tube was secured to the skull adapter using two setting screws. A diode laser (HL6322G, 638 nm, 15 mW, Hitachi, Japan) was used as the light source. The laser speckle images were acquired at 20 frames per second, and the exposure time was 8 ms.

H. LSCI Image Reconstruction

The speckle contrast value (K) is calculated as the ratio of the standard deviation (σs) to the mean intensity of each binning window <I> [62], [63]. The raw images recorded by the camera were converted to speckle contrast images using Eq. (1).

$$K = \frac{\sigma_S}{\langle I \rangle} = \left\{ \frac{\tau_C}{2T} \left[1 - \exp\left(-\frac{2T}{\tau_C} \right) \right] \right\}^{0.5} \quad (1)$$

where T is the exposure time of the camera and τ_c is the correlation time, which is assumed to be inversely proportional to the velocity of the scattering particles. In our study, a temporal laser speckle contrast analysis algorithm was used to calculate the contrast value from speckle images.

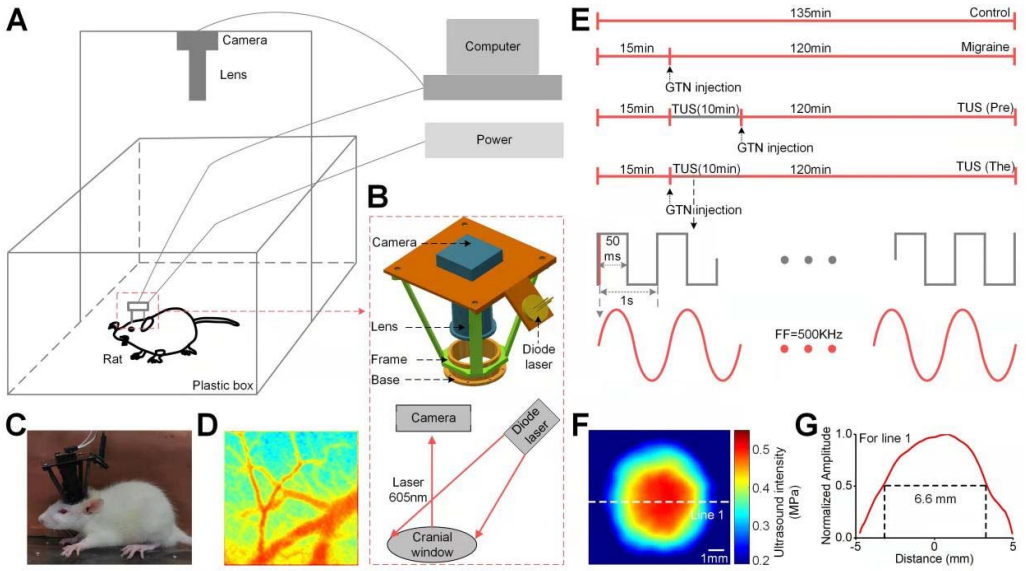

Fig. 1. Experimental setup and protocol. (A) Schematic of the design of behavior monitoring and laser speckle contrast imaging. (B) Design of the miniature head-mounted laser speckle imager for a free-moving rat; this design includes a camera, lens, frame diode laser, and base. (C) A photograph of the imager mounted onto the head of a free-moving rat during the experiment. (D) A typical laser speckle contrast image from a freely moving rat. (E) Time sequence of different groups [control group, migraine group, transcranial ultrasound stimulation (TUS) (Pre) group and TUS (The) group]; ultrasound parameters: stimulation duration (SD) = 15 min, fundamental frequency (FF) = 500 kHz, pulsed reception frequency = 1 Hz, and duty cycle = 5%; (F) 2D distribution of the ultrasound field; and (G) reconstruction profile along the white line in the ultrasound spot and the full width at half maximum (FWHM) is ~6.6 mm.

I. Statistical Analysis

The Kruskal-Wallis test was performed to test for statistical significance. Differences were considered significant at p-values of <0.05. Statistical analyses and data processing procedures were performed using MATLAB (MathWorks, Natick, Massachusetts, USA).

III. Results

A. Behavior and CBF Response in Normal and Migraine Rats

We first analyzed the number of head scratches in the control (video 1) and migraine groups (video 2). As shown in Figure 2(A), we found that, compared with the control group, the number of head scratches in the migraine rats increased significantly (control group: 2.3 ± 0.5, migraine group: 42.5 ± 3.5, mean $\pm$ SEM, N = 7 for each group, $^{***}p < 0.001$, Kruskal-Wallis test). Next, we assessed the change in CBF velocity within 120 min in the control and migraine groups. Laser speckle contrast images of the cortex from a typical rat in the control group are shown in Figure 2(B). There was no significant change in CBF velocity from −5 min to 120 min. Figure 2(C) represents the mean change in the CBF velocity of seven rats. We noticed that the CBF velocity was approximately stationary from −5 min to 120 min. Figure 2(D) shows the laser speckle contrast images of the cortex from a typical rat in the migraine group. The average blood flow of the seven rats changed from −5 min to 120 min, as shown in Figure 2(E). Results demonstrated that the CBF velocity of rats gradually decreased and then gradually returned to levels before the injection of nitroglycerin over time. Finally, we quantitatively analyzed the mean changes in CBF between

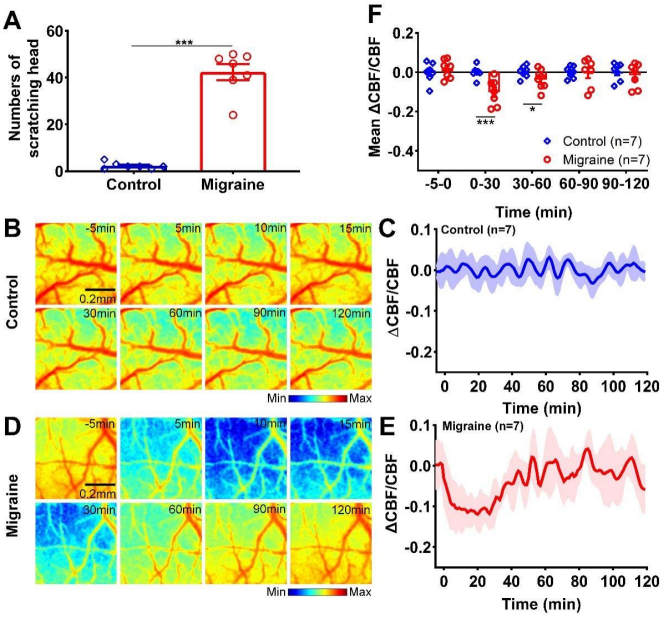

Fig. 2. Behavior and cerebral blood flow (CBF) in the control and migraine groups. (A) The number of head scratches in the control and migraine groups. (B) The raw CBF images of a typical rat in the control group from −5 min to 120 min. (C) The change in CBF of seven rats in the control group from −5 min to 120 min (n = 7). (D) The raw CBF images of a typical rat in the migraine group from −5 min to 120 min. (E) The change in CBF of seven rats in the migraine group from −5 min to 120 min (n = 7). (F) The mean change in CBF of all rats in the control and migraine groups at −5-0 min, 0-30 min, 30-60 min, 60-90 min, and 90-120 min (mean $\pm$ SEM, n = 7 for each group, $^{*}p < 0.05$, $^{***}p < 0.001$, Kruskal-Wallis test).

the control group and migraine group at −5-0 min, 0-30 min, 30-60 min, 60-90 min, and 90-120 min. We found that,

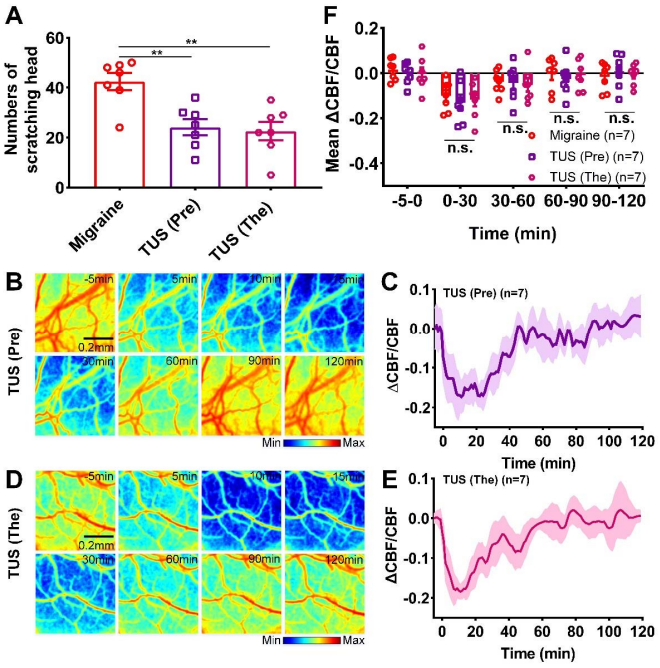

Fig. 3. Behavior and cerebral blood flow (CBF) in the transcranial ultrasound stimulation (TUS) (Pre) and TUS (The) groups. (A) The number of head scratches in the migraine, TUS (Pre), and TUS (The) groups. (B) The raw CBF images of a typical rat in the TUS (Pre) group from −5 min to 120 min. (C) The change in CBF of seven rats in the TUS (Pre) group from −5 min to 120 min (n = 7). (D) The raw CBF images of a typical rat in the TUS (The) group from −5 min to 120 min. (E) The change in CBF of seven rats in the TUS (The) group from −5 min to 120 min (n = 7). (F) The mean change in CBF of all rats in the migraine, TUS (Pre), and TUS (The) groups at −5-0 min, 0-30 min, 30-60 min, 60-90 min, and 90-120 min (mean ± SEM, n = 7 for each group, $^{**}p < 0.01$, Kruskal-Wallis test). n.s.: no significance.

in the migraine group, the mean ΔCBF/CBF at 0-30 min and 30-60 min was much lower than that before the injection of nitroglycerin (−5-0 min). We also noticed that, compared with the control group, the mean ΔCBF/CBF was also significantly reduced at 0-60 min in the migraine group (Mean ± SEM, n = 7 for each group, $^{*}p < 0.05$, $^{***}p < 0.001$, Kruskal-Wallis test). The above results indicate that when rats are injected with nitroglycerin, the number of head scratches will increase significantly, and the average CBF velocity at 0-60 min will decrease significantly. We used this result as the basis to further study the effects of ultrasound on the prevention and therapy of migraine.

B. Behavior and CBF Response in Normal and Migraine Rats With Ultrasound Prevention and Therapy

We adopted two ultrasound intervention modes for the migraine rats. One is to administer ultrasound stimulation for 15 min before the injection of nitroglycerin [TUS (Pre) group], and the other is to provide ultrasound stimulation following the injection of nitroglycerin [TUS (The) group]. First, we analyzed the number of head scratches in the rats under the two intervention modes. As represented in Figure 3(A), we noticed that the number of head scratches in migraine rats decreased significantly with ultrasound prevention (video 3) and therapy (video 4) (migraine group: 42.5 ± 3.5, TUS (Pre) group: 24.1 ± 3.2, (TUS (The) group): 22.5 ± 3.6, mean ± SEM, N = 7 for each group, $^{**}p < 0.01$, Kruskal-Wallis test). However, there was no significant difference between the ultrasound prevention and therapy groups. The above results reveal that both ultrasound prevention and therapy can reduce the number of migraine attacks in rats, and there is no difference between prevention and therapy groups. Next, we assessed the changes in CBF in the ultrasound prevention and therapy groups. As represented in Figure 3(C) and (E), the CBF velocity of the rats was significantly reduced and continued to gradually return to the level before the injection of nitroglycerin within 120 min following the injection of nitroglycerin in both ultrasound prevention and therapy groups. These results are similar to the changes in CBF in the migraine group. Finally, we quantitatively analyzed the mean ΔCBF/CBF in the migraine group and the TUS prevention and therapy groups. The average CBF velocity in different time periods in the ultrasound prevention and therapy groups was similar to that in the migraine group (n = 7 for each group, Kruskal-Wallis test). Results demonstrated that ultrasound stimulation could not alter CBF velocity in migraine rats. The above results show that both ultrasound prevention and therapy can reduce the number of migraine attacks in rats, but does not modulate CBF velocity.

C. Evaluation of Auditory Effect on TUS in Migraine Rats

Finally, we compared the number of head scratches in migraine rats in different groups, including the migraine group, TUS (Pre) group, TUS (The) group, TUS (Pre)-Sham group, and TUS (The)-Sham group. We tested the CBF before and after chemical deafening in two rats. As shown in Figure 4(A) and (B), we found that chemical deafening did not change the CBF of those rats. There was no significant difference between migraine and deaf migraine rats considering only the sound output (Figure 4 (C)). This indicates that the sound effect alone does not improve the number of head scratches in migraine rats. Furthermore, the number of head scratches in the TUS (Pre)-Sham-1 group (n=7) was close to that in the migraine group (n=7), and the number of head scratches in the TUS (Pre) (n=7) and TUS (Pre)-Sham-2 (n=5) groups were significantly lower than that in the TUS (Pre)-Sham-1 group (Mean ± SEM, $^{**}p < 0.01$, $^{***}p < 0.001$, Kruskal-Wallis test). As shown in Figure 4(D), the results of ultrasound therapy were similar to those observed with ultrasound prevention (Mean ± SEM, $^{**}p < 0.01$, $^{***}p < 0.001$, Kruskal-Wallis test). The above experiments demonstrate that the reduction in migraine attacks after ultrasound stimulation is not due to auditory effects.

IV. Discussion

In our study, we prepared a rat migraine model by subcutaneous injection of nitroglycerin and stimulated the brain tissue of migraine rats using low-intensity ultrasound. The head scratching behavior and CBF changes of the freely moving rats were monitored in real time using a video recording system and a wearable miniature LSCI system. We demonstrated that,

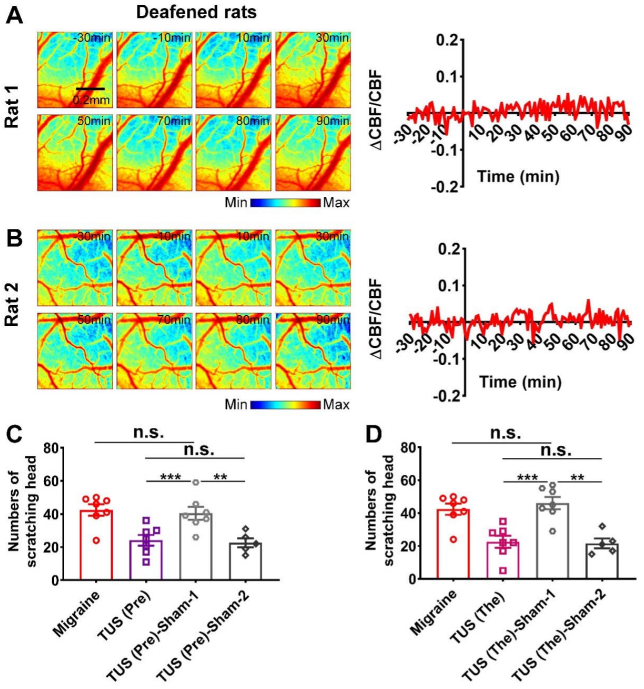

Fig. 4. Assessment of auditory effect on transcranial ultrasound stimulation (TUS) for migraine rats. (A) and(B), the raw cerebral blood flow (CBF) images of two deafness model rats from −30 min to 0 min (before deafness) and from 0 min to 90 min (after deafness), and the corresponding change in CBF. (A) rat 1, (B) rat 2. (C) The number of head scratches in the migraine, TUS (Pre), TUS (Pre)-Sham-1 and TUS (Pre)-Sham-2 groups. (D) The number of head scratches in the migraine, TUS (The), TUS (The)-Sham-1, and TUS (The)-Sham-2 groups (mean ± SEM, n = 7 for migraine, TUS (Pre), TUS (Pre)-Sham-1, TUS (The), and TUS (The)-Sham-1 groups; n = 5 for TUS (Pre)-Sham-2 and TUS (The)-Sham-2 groups; $^{**}p < 0.01$, $^{***}p < 0.001$, Kruskal-Wallis test).

compared with normal rats, the number of head scratches in migraine rats was significantly increased, and the CBF rate was significantly reduced 60 min after the injection of nitroglycerin. We found that both ultrasound prevention and therapy can significantly reduce the number of head scratches in migraine rats, but cannot alter the changes in CBF. To the best of our knowledge, this is the first time that ultrasound prevention and therapy have been found to inhibit migraine attacks.

Migraine is a vascular and nerve dysfunction disease caused by the interaction of vascular and neural mechanisms [64], [65]. Nitroglycerin can cause migraine in humans and animals [18], [30], [66], [67]. In a study with human subjects, researchers tested six healthy adults with an average age of 27.5 years (range 22-38 years). Nitroglycerin at a dose of 0.5 lg/kg/min was infused through the cubital vein for 20 minutes. During the nitroglycerin infusion, five out of six subjects experienced headache [30]. In an animal experiment, 10 mg/kg of nitroglycerin was intraperitoneally injected in Wistar rats, which resulted in a statistically detectable increase in head scratching time compared to controls [67]. In addition, nitroglycerin was injected intraperitoneally at a dose of 10 mg/kg to Sprague-Dawley rats. Two hours after the injection the rats showed a significant increase in the number of withdrawals and tremors compared to controls. The above studies demonstrate that a dose of 10 mg/kg is effective in a stable migraine model in rats. In our experiment, a nitroglycerin dose of 10 mg/kg was used to produce the migraine model of Sprague-Dawley rats, and the rats' behavior was evaluated. We also found that the number of head scratches increased significantly compared with the control group.

In this study, we first noticed that the number of head scratches in migraine rats was much higher than that in normal rats, which is consistent with previous results [18]. The onset of headaches leads to multiple head scratching movements. We also revealed that the CBF velocity of migraine rats was significantly lower than that of normal rats. This was caused by the drug nitroglycerin, which is used to induce migraine in rats. Nitroglycerin produces nitric oxide (NO) in the body, and NO diffuses to the middle meningeal artery, which not only has a strong effect on dilating cerebral blood vessels but also reduces the rate of CBF [68], [69].

Our experimental results also demonstrate that ultrasound prevention and therapy can inhibit migraine attacks but cannot alter CBF velocity. This may be closely related to the potential mechanism of ultrasound stimulation. Ultrasound as a mechanical wave can cause the mechanically sensitive ion channels of neuron cell membranes to open or close, depolarize or hyperpolarize neurons, thereby generating neuronal action potentials [70], [71], [72], [73]. In addition, ultrasound can open the TRPA1 channel in astrocytes, and the Ca^{2+} that enters through TRPA1 causes astrocytes to release glutamate through the Best1 channel. Finally, the released glutamate activates NMDA receptors in neighboring neurons, triggering action potential discharges [74]. Based on the above underlying mechanism of ultrasound stimulation, we speculate that ultrasound acts on neurons to change the network connection of the central nervous system and reduces headaches. However, ultrasound stimulation did not modulate the pharmacology of nitroglycerin. Therefore, it could not alter CBF velocity. In addition to blood vessels, migraine is closely related to neuronal activity. Importantly, functional neuron-type selectivity was recently associated with ultrasound stimulation[75]. Therefore, we can selectively excite or inhibit specific types of neurons by controlling ultrasound parameters, which could personalize treatment plans for migraine in the future.

In a previous study, researchers used low-intensity pulsed transcranial ultrasound stimulation before modeling to minimize or eliminate the risk of stroke. The results of the study showed that rats had smaller ischemic areas and smaller infarct volumes than controls during stroke induction and 24 h and 48 h after stroke. These results suggest that the application of TUS prior to photothrombosis may provide neuroprotection by increasing the brain's tolerance to subsequently induced focal ischemic injury. Moreover, the authors speculated that this neuroprotective pretreatment with ultrasound leads to cerebral metabolic inhibition and promotes ischemic tolerance [76]. Stimulation of a mouse/rat stroke model with TUS reduces apoptosis by promoting microglial polarization and modulating interleukin (IL)-10 signaling in the ischemic brain, apoptosis and induction of brain-derived neurotrophic factor (BDNF)

to promote neurorehabilitation after cerebral ischemia. The above results show that both ultrasound protection and ultrasound therapy can have a protective effect on the nerves of stroke [52], [77], [78], [79]. In this study, we found that both ultrasound prevention and therapy significantly reduced the number of head scratches. We speculate that the prevention and treatment with ultrasound may regulate migraine-related proteins and downstream effects, and thus play a protective effect on nerves. In future research, we will conduct in-depth studies to reveal the underlying mechanism for this effect.

This study was limited by the experimental conditions in the laboratory. For this reason, we only evaluated behavior and hemodynamics as biomarkers of the therapeutic and protective effects of ultrasound stimulation on migraine. Therefore, others potential biomarkers related to migraine were not evaluated, and how these change under ultrasound stimulation remains unknown. In the future, we will perform biochemical analysis to verify the modulatory effect of ultrasound stimulation on other biomarkers. Blood flow velocity in migraine rats after injection of nitroglycerin was significantly lower than that before injection, and gradually returned to baseline over time. We speculate that as nitroglycerin is metabolized, blood flow velocity is gradually restored, and the symptoms of migraine are gradually improved. Further research will be performed to establish the relationship between CBF velocity and migraine symptoms.

In this study, the ultrasound parameters were FF: 500 kHz, SD:15 min, PRF: 1 Hz, DC: 5%, I_{sppa}: 8.3 W/cm^2. We found that ultrasound prevention and therapy did not reduce the CBF velocity in migraine rats. In previous research, there were significant changes of blood flow velocity following ultrasound stimulation, and it returned to baseline levels after 10 s of ultrasound stimulation [44], [45], [46], [47] under different ultrasound parameters (Yoo *et al.* [44], FF: 690 kHz, I_{sppa}: (3.3, 6.4, 9.5, and 12.6 W/cm^2), PRFs: (10, 20, 100, and 1 kHz), SDs (0.5, 1, 1.5, and 2 s); Kim *et al.* [45], FF: 425 kHz, PRFs (375, 750, 1.5 kHz), N cycles: (80, 40, and 20), I_{sppa}: 1.84W/cm^2; Yuan *et al.* [46], FF: 500 kHz, I_{sppa}:1.1 W/cm^2, SD: 400 ms; Yuan *et al.* [47], FF: 500 kHz, I_{sppa}:10.1W/cm^2, PRF:1kHz, SD: 400ms). We hypothesized that blood flow velocity returned to the baseline level due to the long delay after stimulation. Therefore, the change in the blood flow velocity was not detected. In the future, an in-depth study will be performed to investigate why blood flow velocity was unchanged.

Ultrasound parameters, including ultrasound frequency, ultrasound intensity, pulsed repetition frequency, and duty cycle, play a key role in ultrasound modulation of neural activity, cerebral hemodynamics, and intervention in neuropsychiatric diseases [80], [81], [82]. Our study demonstrated that low-intensity TUS can play a preventive and therapeutic role in migraine in rats. However, the relationship between the modulation effect and ultrasound parameters remains unclear. In future work, we will assess the intervention effect of different parameters of ultrasound on migraine to obtain the most effective parameters.

To probe the thermal effects induced by ultrasound in the tissue, the potential temperature increase due to ultrasound parameters was estimated by the equation $\Delta T = \frac{2\alpha I t}{\rho C}$ [83], where α is the absorption coefficient and equals 0.032 cm^{-1} at the ultrasound frequency of 500kHz, I is the ultrasound intensity, t is the stimulation duration of ultrasound, ρ is the density of brain tissue, C is the specific heat capacity of brain tissue, and the product ρC is equal to 3.811 J cm^3 C^{-1}. In our study, the maximum ultrasound intensity (I_{sppa}) was 8.3 W/cm^2, and the maximum stimulation duration was 0.05 s. Therefore, the maximum temperature enhancement induced by TUS was $\sim 8.2 \times 10^{-3}$°C, which is far below the temperature threshold predicted to induce tangible thermal bioeffects.

V. Conclusion

Ultrasound prevention and therapy can significantly reduce the number of head scratches but without reducing the CBF velocity. Results are not affected by the auditory effect. These results demonstrate that low-intensity ultrasound stimulation has the potential to be used clinically for the prevention and therapy of migraine.

Conflict of Interest Statement

The author(s) declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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