Trans-Spinal Focused Ultrasound Stimulation Selectively Modulates Descending Motor Pathway

Evgenii Kim, Jeungeun Kum, and Hyungmin Kim

***Abstract*— Compared to current non-invasive methods utilizing magnetic and electrical means, focused ultrasound provides greater spatial resolution and penetration depth. Despite the broad application of ultrasound stimulation, there is a lack of studies dedicated to the investigation of acoustic neuromodulation on the spinal cord. This study aims to apply focused ultrasound on the spinal cord to modulate the descending pathways in a non-invasive fashion. The application of trans-spinal focused ultrasound (tsFUS) was examined on the motor deficit mouse model. tsFUS was achieved using a single-element focused ultrasound transducer operating at 3 MHz. The sonication was performed on anesthetized 6 week-old mice targeting T12 and L3 vertebrae. The effect was analyzed by comparing electromyography responses from the hindlimb induced by electrical stimulation of the motor cortex. Further, the mouse model with the Harmaline-induced essential tremor (ET) was selected to investigate the potential clinical application of tsFUS. The safety was verified by histological assessment. Sonication at the T12 area inhibited motor response, while sonication over the L3 region provided signal enhancement. Sonication of T12 of the ET mouse also showed the ability of ultrasound to suppress tremors. Meanwhile, the histological examination did not show any abnormalities with the highest applied acoustic pressure. In this work, a non-invasive motor signal modulation was achieved using tsFUS. Moreover, the results showed the ability of focused ultrasound to manage tremors in a safe manner. This study provides a stepping stone for the trans-spinal application of focused ultrasound to motor-related disorders.**

***Index Terms*— Descending pathway, neuromodulation, spinal cord, trans-spinal focused ultrasound.**

I. Introduction

NUMEROUS neurological movement disorders are associated with the malfunction of the corticospinal tract, the pathway responsible for movement control [1]–[3]. For example, stroke, spinal cord injury (SCI), and Parkinson's disease can lead to muscle hypo-/hyper-tonia, tremor, and paralysis. The stimulation of the central nervous system has been demonstrated as an alternative therapy to decrease motor dysfunction [4], [5], especially when pharmacological treatment fails.

The epidural electrical spinal cord stimulation developed to manage pain has been applied to restore locomotion during tremors [6]–[8] and after a SCI paralysis [9]–[11]. The electrode implantation offers a high spatial precision but requires surgical intervention. The non-invasive electric and magnetic trans-spinal stimulations were proposed to overcome the downside for some applications [12], but still suffered from low targeting precision. On the other hand, focused ultrasound has already shown the ability to modulate neural circuits with high spatial selectivity in a non-invasive fashion [13]–[15]. The application of focused ultrasound neurostimulation has already been greatly extended for brain research [16], peripheral nerve stimulation [17], and sub-organ modulation [18] but not spinal cord stimulation. Therefore, in this work, we examined the effect of non-invasive tsFUS neuromodulation on healthy and motor deficit mouse models.

Firstly, we investigated the influence of tsFUS on descending pathways on healthy mice by stimulating the motor cortex via electricity and recording muscle activity of the hind limb. The sonication effect was evaluated by varying acoustic pressure as well as aiming at different spinal areas. An additional experiment was performed to observe the influence of spinal cord sonication on the ascending tract.

Both transient suppression and facilitation of motor signals were achieved through trans-spinal sonication. While the degree of neuromodulation was correlated to acoustic pressure, the inhibition/facilitation was determined by the sonicated area. In contrast, the same sonication protocol did

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not significantly affect the signals from limbs to the cortex. To demonstrate the potential in clinical application, the non-invasive motor signal modulation was examined on the essential tremor (ET) model induced by the Harmaline injection. The implementation of tsFUS showed the reduction of the motor deficit, providing a stepping stone for the trans-spinal application of focused ultrasound to motor-related disorders.

II. Methods

A. General

All experiments were performed with 6-week old male ICR mice in accordance with the guidelines of the Care and Use of Laboratory Animals. A total number of mice in this study was 50 (n = 20 descending, n = 10 ascending, n = 10 tremors, n = 5 histology, n = 5 supplementary). Animals were anesthetized via intraperitoneal injection of ketamine-xylazine cocktail (80:10 mg/kg) followed by shaving the skin above the selected segment of the spinal cord. During the experiment, an additional dose of ketamine (30 mg/kg) was administered when necessary. The eyes were treated with eye lubricant to keep the cornea moist, and body temperature was maintained at 37 °C using a feedback-regulated heating pad monitored by a rectal probe.

B. Evoking Descending Signal and Recording

The trimmed mouse scalp was disinfected with 0.1% Betadine in water, followed by 70% ethanol. Following an incision along the midline of the skull, the fascia and fat overlying the skull surface were scraped away. A small hole with a diameter of 0.5 mm was carefully drilled in the skull over the areas of the brain which control the hindlimb, 0.5 mm posterior and 1.5 mm lateral to the bregma. Microelectrodes (bipolar stainless steel electrode, 30G, EL451 Biopac Systems Inc., USA) were inserted into the brain through the hole at a depth of ∼1 mm until the limb movement was identified. The stimulation was achieved by applying biphasic trains of negative current stimulation: 0.2 ms pulse, 200 Hz, 100 ms train, 0.7-1.2 mA (Fig. 1A). The currents applied to the stimulation area were 150% of the minimum current necessary to observe a hindlimb twitch.

The muscle activity was recorded through stainless steel wires (45 AWG, California Fine Wire, USA) inserted into lateral gastrocnemius, gluteus medius, and neck muscles, as recording, reference, and ground electrodes, respectively. The signal was obtained at a sampling rate of 100 kHz with an active notch filter at 60 Hz, and a band passed filter between 10 Hz and 1 kHz (ADInstruments, Sydney, Australia).

The experiment protocol consisted of 10 min of acquisition time with three equally divided time segments: baseline, tsFUS, and recovery. Each section included 100 motor signal stimulations with 2 s intervals. Animals (n = 20) were randomly divided into two groups depending on the sonication target. Each animal in the first group received three consecutive sonication sessions to demonstrate the neudomodulatory effect on acoustic pressure delivered to the T12 area. The animals from other group experienced two consecutive sessions to show neuromodulatory effects of sonicating the L3 area under different sound pressure. The pressure value for each session was fixed with an interval of 5 min between sessions. The order of pressure levels for each animal was selected randomly.

C. Trans-Spinal Focused Ultrasound Neuromodulation

In this study, the in-house focused ultrasound transducer was designed as described in the previous work [19]. The transducer was made based on 3 MHz PZT-4 ceramics with a diameter of 6 mm. The ultrasound beam was spherically focused by a plano-concave 3D printed acoustic lens with a 10 mm radius of curvature. The acoustic pressure profiles (Fig. 1C, D) were obtained in free water space by needle-type hydrophone (HNR-0500, 0.5 mm probe, ONDA Corp., USA). Hydrophones with 0.5 mm in diameter is wildly used in the measurement of high-frequency ultrasound [13]. However due to the close similarity of the transducer focal size and the hydrophone surface, minor underestimation of the measurement can be expected.

The trans-spinal sonication was delivered 1 s before electrical stimulation, as a pulse train lasting for 200 ms with 0.5 ms of tone-burst duration and pulse repetition frequency (PRF) of 1 kHz, with an interstimulus interval of 2 s (Fig. 1B). Three acoustic pressures (2.2, 1.4, and 0.8 MPa in peak negative pressure) were administered on T12 spinal cord location, and two pressures (2.2 and 1.4 MPa) for L3 vertebra. T13 vertebra was used as a reference point as it is the natural top of the mouse spine. The approximate positions of the T12 and L3 vertebrae were -2.5 and +7 mm from the reference point, respectively. By drawing crosslines on the skin using a marker, the transducer was aligned and placed directly on top of the shaved skin with acoustic gel. The gel helped to minimize acoustic impedance mismatch and keep the small transducer in place during the whole experiment. During the ascending modulation session, T12 and L3 were administered with a sound pressure of 2.2 MPa. The ET suppression was examined with 1.4 MPa. The detailed sonication parameters are listed in Table I.

D. Acute Essential Tremor Model

The ET was induced by the Harmaline IP injection (30mg/kg freshly dissolved in saline). Once the tremor was clearly visible, the mouse was anesthetized with the ketamine-xylazine mixture allowing us to place the ultrasound transducer on T12, the suppression location of the spinal cord, and insert hindlimb recording and reference EMG electrodes. After the injection, the tremor was diminished due to deep anesthesia but returned once the anesthesia stabilized to a mild level. The tremor motion was evaluated by a recorded EMG signal. The baseline tremor activity was recorded for 1 min, followed by tsFUS for 1 min and another minute of rest. The ultrasound protocol was in line with the previous experiment.

E. Evoking Ascending Signal and Recording

In a separate experiment, the tsFUS was applied for modulating the sensory signal. Following the verification of the

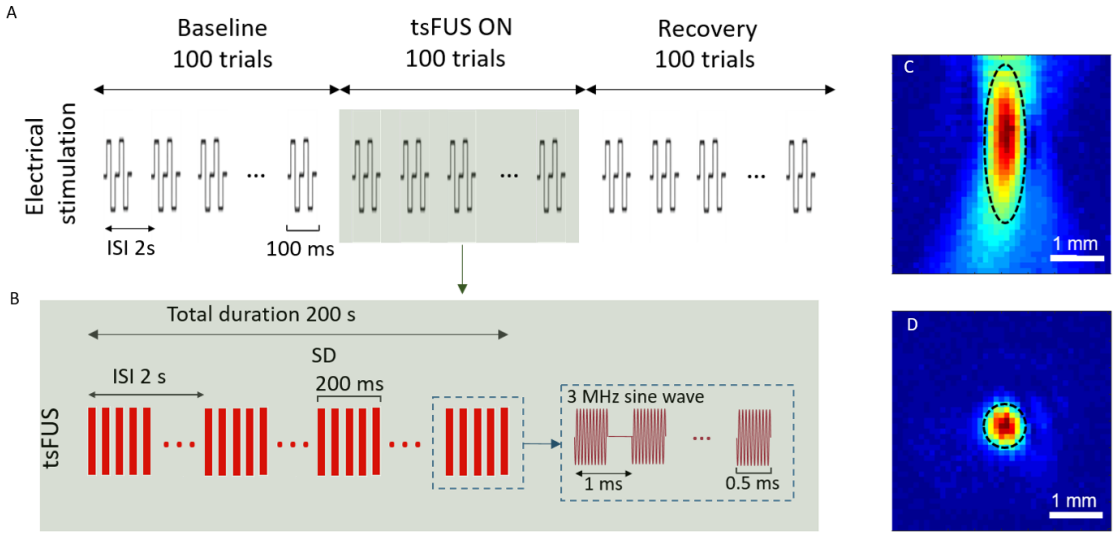

Fig. 1. (A) The protocol of electrical corticospinal stimulation. (B) Pulse parameters for the tsFUS representing one ultrasound pulse train. (C) Axial and (D) lateral acoustic pressure profiles in free water. The focal spot size of the transducer extends 3.2 mm longitudinally and the focal size is 0.6 mm in diameter, as determined by the FWHM of the acoustic intensity (black dashed contour).

TABLE I
DETAILED SONICATION PARAMETERS FOR EACH EXPERIMENTAL SESSION

Experiment	Spinal target	Pressure [MPa]	I_{SPPA} [W/cm^2]	I_{SPTA} [W/cm^2]	Mechanical index
Descending pathway modulation	T12	0.8	9.9	4.95	0.46
		1.4	28	14	0.8
		2.2	76	38	1.27
	L3	1.4	28	14	0.8
		2.2	76	38	1.27
Ascending pathway modulation	T12	2.2	76	38	1.27
	L3	2.2	76	38	1.27
Tremor suppression	T12	1.4	28	14	0.8
Safety	T12	2.2	76	38	1.27

correct sonication target by achieving a brief motor pathway modulation, the function of the electrodes was switched, maintaining the position of ultrasound transducer. The sensory electrical stimulation was delivered to the hindlimb using the same electrodes from the EMG recording, while the somatosensory evoked potential (SSEP) was recorded by a stainless steel screw which replaced the electrodes in the brain previously used for brain stimulation. An additional screw was implanted over cerebellum as a reference electrode. The ground electrode was kept untouched in the neck muscle. Similar to the previous experiment, each session (pre-tsFUS, tsFUS) consisted of 100 trials with 2-sec intervals. SSEP was evoked by single biphasic pulses (2-4 mA). The recording was performed with a 100 kHz sampling rate, 60 Hz notch filter, and band-pass filter of 0.3 to 40 Hz.

F. Statistical Analyses

The muscle response was evaluated with the area under the curve (AUC) value obtained from the EMG signal. All statistical analyzes were performed in the SPSS software (IBM Inc., USA). Two-way repeated measures MANOVA with further the multiple comparisons, Tukey's HSD was implemented to compare AUC values within motor signal suppression and separately during excitation experiments. The amplitude of SSEP responses was analyzed by the two-sample paired *t-test.* To identify a statistically significant difference in tremor modulation, the baseline AUC values, averaged from 0 to 60 s, were compared with AUC values from each time point by the two-sample paired *t-test* with the Bonferroni correction. The statistical power was above 0.85 for every test.

G. Safety Evaluation

The temperature change caused by sonication was measured using a micro needle-type thermocouple probe (29g, T-29X, Thermoworks Inc., USA) inserted into the spinal cord in proximity ultrasound focal point. The temperature was recorded for 2 min during 2.2 MPa pressure, identical to the previous experimental protocol. Moreover, hematoxylin and eosin (H&E) staining was performed to examine the potential spinal cord damage produced by tsFUS. The animals (n = 5) were sacrificed on the next day after spinal cord sonication. The intracardiac perfusion with buffered 4 % paraformaldehyde was performed before overnight postfixation. Stained

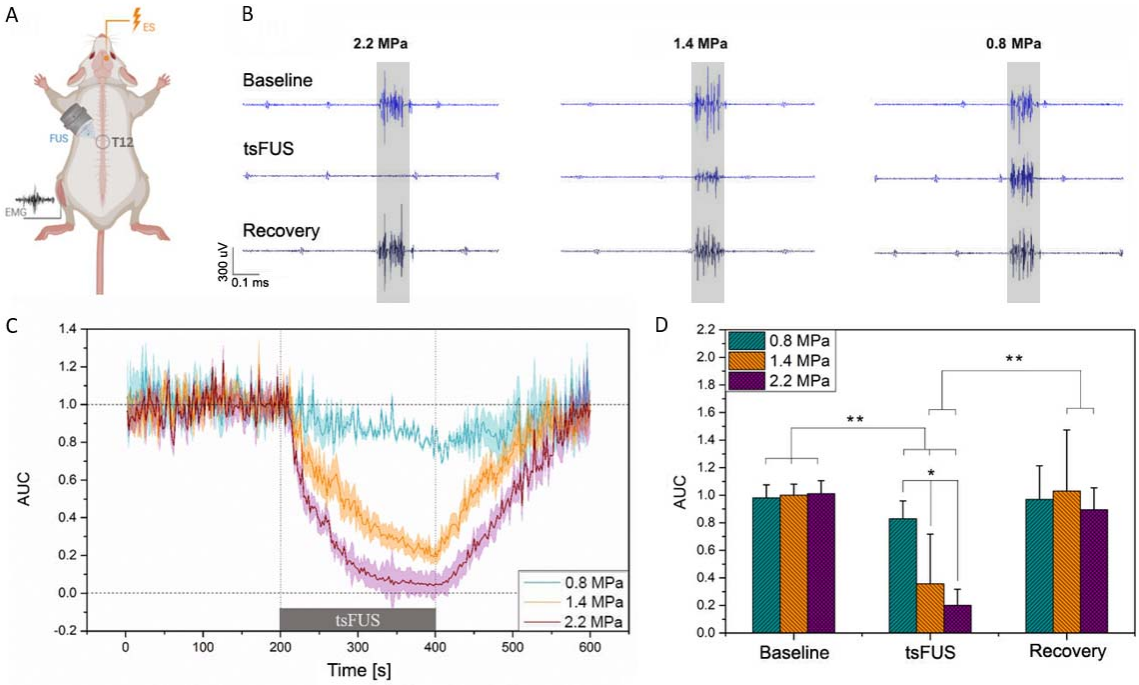

Fig. 2. (A) The schematic diagram of the inhibitory setup as implemented motor cortex stimulation and hindlimb EMG recording. (B) Representative EMG response at different experiment periods at 3 sonication conditions. The signals within the gray shaded area correspond to hindlimb movement, other pulses are artifacts related to heartbeat. (C) The grand-averaged AUC values with standard error are plotted over time. (D) AUC values at the end of each session. The error bars indicate the standard error for 10 mice. The statistically significant difference is indicated by * ($p<0.01$), and ** ($p<0.001$).

coronal sections of the sonicated segment were cryosectioned at 20 μm thick and compared to those from control, i.e., untreated mice.

III. Results

A. Transient Modulation of Descending Pathway

The influence of focused ultrasound on the spinal cord was initially investigated by sonication at the T12 vertebra during motor signal stimulation, Fig. 2A. The gradual inhibition of muscle response was observed during tsFUS. The change of AUC values from the EMG signal over time was illustrated in Fig. 2C. Both partial and complete inhibition were achieved by managing the acoustic pressure. Representative EMG responses at the different periods at various sonication conditions were presented in Fig. 2B. The statistical comparison of the last AUC values from baseline, tsFUS, and recovery showed the significant difference between tsFUS and baseline, tsFUS and recovery, and no difference between baseline and recovery, as shown in Fig. 2D. Moreover, a significant difference in the level of suppression was observed at the end of tsFUS phase depending on radiating pressure.

The sonication of the L3 vertebra was also tested with the expectation to facilitate the motor signal, Fig. 3A. The focused ultrasound with 2.2 MPa pressure on the lumbar area presented the facilitatory effect on EMG activity. The change of AUC values in time is shown in Fig. 3C, where the representative tsFUS-amplified muscle responses were shown in Fig. 3B.

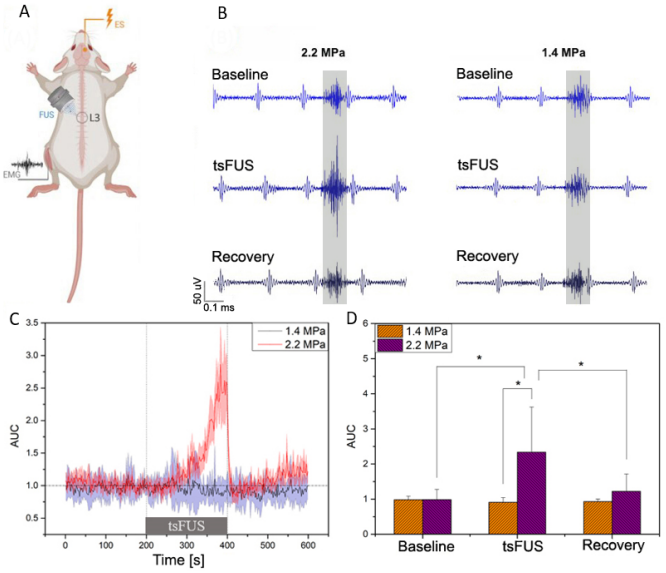

Fig. 3. (A) The schematic diagram of the enhancement setup. (B) Representative EMG response at different experiment periods at 2 sonication conditions. The signals within the gray shaded area correspond to hindlimb movement, other pulses are artifacts related to heartbeat. (C) The grand-averaged AUC values with standard error are plotted over time. The grey region represents the sonication duration. (D) AUC values at the end of each session. The error bars indicate the standard error for 10 mice. The statistically significant difference is indicated by * ($p<0.01$).

Similar to the suppressive effect with T12 stimulation, the facilitatory effect produced by tsFUS was temporal, and the EMG signal returned to the baseline value after sonication.

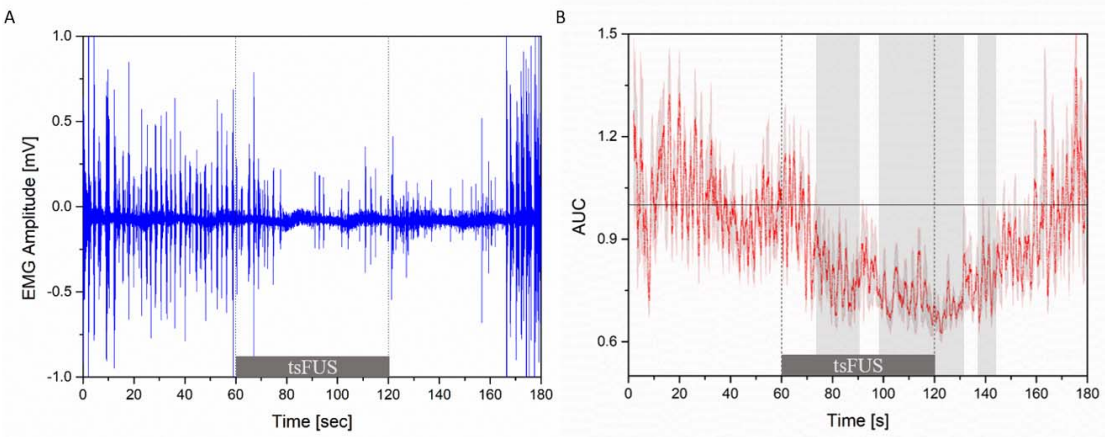

Fig. 4. (A) ET suppression from a representative mouse. (B) The grand-averaged AUC values over all mice are shown over the time with standard error. The shaded regions indicate the time when results have a statistically significant difference with baseline.

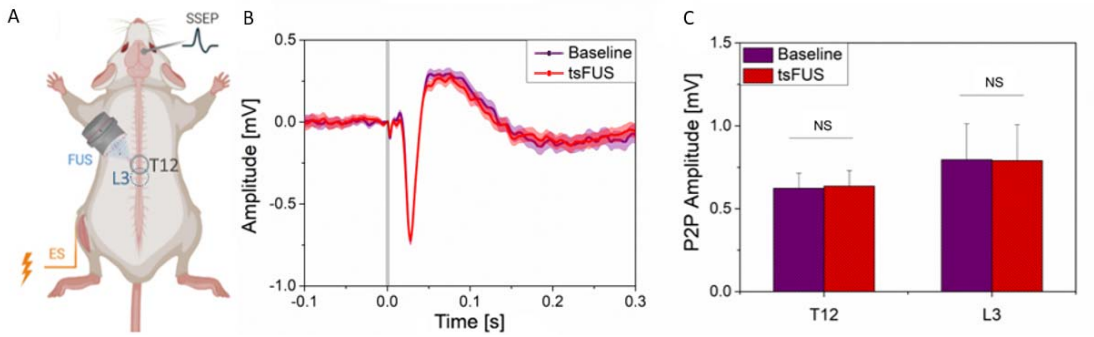

Fig. 5. (A) The sensory stimulation setup during tsFUS. (B) Averaged SSEP signals with standard error are shown during stimulation and non-stimulation from a representative animal. The black line represents the single ES stimulation. (C) The averaged peak-to-peak amplitude from 5 mice each for different sonication targets.

However, while the tsFUS with 2.2 MPa pressure induced a significant effect compared to baseline, there was no significant difference applying 1.4 MPa, as shown in Fig. 3D.

B. Tremor Suppression

The inhibitory application of tsFUS was examined on a drug-induced ET mouse model. The sonication over the T12 area showed the suppression of tremors of the hindlimb. The representative tremor inhibition was plotted as EMG from the selected mouse in Fig. 4A. The averaged AUC values from all mice (n = 10) were presented in Fig. 4B. The statistically significant suppression appeared 13 s after the sonication onset and last more than 23 s after sonication offset.

C. Ascending Consequences of Spinal Sonication

The interference to the sensory path was also evaluated during spinal cord stimulation as in Fig. 5A. The high pressure, which significantly modulated the descending tract, had no visible effect on the ascending path. The averaged SSEPs from the representative mouse are shown in Fig. 5B. The comparison of the peak-to-peak values from all animals did not show a statistically significant difference regardless of sonication target (Fig. 5C).

D. Safety of Ultrasound Stimulation

The ultrasound with the highest negative pressure applied in this study still maintained the mechanical index of 1.27, below 1.9 as mandated by the U.S. FDA to avoid cavitation-related tissue damage for general diagnostic ultrasound system including human neonatal transcranial use. The highest I_{SPPA} of 76 W/cm^2 in this study was also within the approved range, below 190 W/cm^2 [20]. Temperature elevation after two mins of sonication was 3.5, 1.6, 0.6 degrees for 2.2, 1.4, and 0.8 MPa, respectively. The histological assessment did not show any sign of hemorrhage or neuronal damage (Supplementary Fig. S1).

IV. Discussion

Focused ultrasound neuromodulation has attracted large interest as it would offer a non-invasive approach to stimulate deep structures with high precision. The data presented here demonstrates the ability of ultrasound to modulate motor signal pathways by sonicating the spinal cord without any surgical intervention. We found that both inhibitory and facilitatory effects could be induced by radiating at different spinal areas. The level of sonication influence on the motor signal was dependent on acoustic pressure. In any sonication protocol, the effects were transient and returned back to baseline within a few minutes.

Many prior studies on ultrasound neuromodulation have shown that inhibitory and excitatory outcome is dependent on sonication parameters [21]–[23]; the short duty cycle with low PRF inhibits the brain network, and higher DC with faster PRF offers an excitatory result. Our work also demonstrates that the same acoustic protocol can produce both inhibitory and excitatory effects by changing the sonication area. The selected protocol produced the boost of neural excitability by sonicating the lumbar spine, and signal inhibition by sonicating the thoracic area. It is still unclear whether the suppression outcome is due to motor neuron inhibition or due to stimulation of inhibitory neurons. The study with optogenetics stimulation of the lower thoracic spine [24] may support the role of inhibitory neuron activation in our study, as it demonstrated a similar inhibition result on the descending pathway with no effect on the ascending tract. On the other hand, the L3 vertebra enclosing the S4-Co2 spinal segments has few neurons involved in hindlimb control but includes the lumbar nerves and axons linked with the limb. Previous research has already shown that the sonication on the axon increased the excitability of the motor neuron [25], [26], suggesting the facilitation mechanism in our study. Moreover, different neural compartments (soma, dendrite, and axon) respond differently on mechanical stimulation [27] may explain the observation that 1.4 MPa significantly affects suppression but not facilitation. The observed change in muscle activity over time (sharper increase and decrease of AUC values during facilitating sessions) also supports this claim. The observations remind the importance of considering a neuron type and its structural orientation within the sonication area. There may be different effects by sonicating white vs. gray matter region.

The experiment with ascending tract showed that the sensory information remained intact while having a significant change of motor signal induced by tsFUS. This could provide a great promise for many neurological movement disorders where any interruption on sensation is not desired. The preclinical application of tsFUS was demonstrated on a motor deficit mouse model mimicking ET [28]. The pulsed sonication of the T12 vertebra significantly suppresses the tremor. Considering the tremor signal is weaker in amplitude than voluntary activity, there is a promise to adjust ultrasound protocol to filter tremor maintaining voluntary movement. Moreover, recent studies suggested that ultrasound may offer lasting effects [29]–[31], which is crucial for future clinical applications.

The effect of high-pressure tsFUS alone was examined by concurrent recording of EMG activity and SSEP in a separate set of animals ($n = 5$). The tsFUS without electrical stimulation showed no response from the brain nor muscle (Supplementary Fig. S2), emphasizing that ultrasound did not produce involuntary muscle activity or any detectable tactile sensation. The temperature elevation was below the threshold of damaging the neuronal cell [32]. Moreover, the absence of hemorrhage and neural death from histological data supports the safety of the method. However, it has to be noted the potential of thermal risk due to cumulative thermal dose [33].

In this study, a single-element ultrasonic transducer with a relatively high frequency of 3 MHz offered a sub-millimeter focal size. Although the measurement of acoustic field distribution in the spinal canal faced the technical limitation due to the small size of the spine, previous works with microbubbles showed no significant degradation of sound propagation during blood-spinal cord barrier opening on small [34], [35] and middle-size animals [36]. A detailed acoustic simulation will be considered in future work [37].

Considering the use of tsFUS for clinical needs, delivering the required acoustic foci size through a human spinous process and lamina may be challenging. The solution to overcome this obstacle was suggested by adopting a multi-array transducer [38]. In addition, the image guiding technique, such as magnetic resonance imaging, also offers additional control of sonication targeting [39].

The underlying mechanism of tsFUS remains unclear, especially the role of the thermal aspect. Various studies showed that ultrasound neuromodulation does not require a tissue temperature change [40], [41], but some work demonstrated the evidence of dominant thermal nature in acoustic stimulation [42], [43]. Moreover, a recent ultrasound research showed the significant influence of temperature elevation on the mechanical effect [44]. Direct comparison between tsFUS with thermal-based stimulation techniques such as photothermal [45] or magnetorthermal [46] may clarify some of the questions.

V. Conclusion

In summary, tsFUS has been shown to be able to induce both inhibitory and facilitatory effects based on the sonication spot. The ultrasound interfered with motor but not sensory signals, meaning the effect was pathway-specific. The stimulation was applied for ET suppression, opening a new window for focused ultrasound in spinal cord stimulation to treat movement disorders.

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