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Non-thermal focused ultrasound induced reversible reduction of essential tremor in a rat model

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ABSTRACT

Background: Essential tremor (ET) is one of the most common movement disorders of adults, characterized by postural and kinetic tremor. With drug treatment only partially efficient, new treatments are being developed.
Objectives: The goal of this study was to demonstrate the feasibility of non-thermal focused-ultrasound (FUS) to induce tremor-suppression in an ET rat model.
Methods: Harmaline-induced tremor rats were treated with FUS along the inferior olivary (IO) system. EMG was recorded continuously during treatment in order to quantify FUS-induced tremor suppression. T2-weighted MRI was performed immediately following treatment and periodically thereafter.
Results: FUS treatment at an intensity of 27.2 W/cm^2 (Isppa) induced significant reduction of tremor in 12 out of 13 ET rats. Tremor frequency was reduced from 6.2 ± 2.8 to 2 ± 1 Hz, $p < 0.0003$. In 6 of the 12 responding rats, tremor was completely suppressed. Response duration was 70 ± 61s, on average.
FUS induced motor response, depicted as movement of the tail and/or the limbs synchronized with the FUS sonication, was also demonstrated both in ET rats and in naïve rats when treated in the medulla oblongata region.
Conclusions: These results demonstrate the feasibly for obtaining significant tremor reduction or tremor suppression induced by non-thermal, non-invasive, reversible focused-ultrasound.

1. Introduction

Essential tremor (ET) is one of the most common movement disorders of adults, characterized by postural and kinetic tremor [1,2]. The most recognized feature of ET is a kinetic tremor (tremor occurring during voluntary movements) of the arms, hands or fingers [3,4], however it can also involve the head, vocal cords or other body parts during voluntary movements such as eating and writing. The tremor is usually apparent in both arms, although as a rule it is slightly asymmetric, with the tremor being of greater amplitude in one arm than in the other [5]. The frequency of this tremor varies widely (range 4–12 Hz) and has been shown to relate to several parameters such as age [6].

Harmaline is a tremorigenic β-carbolines that upon administration to experimental animals, induces an acute postural and kinetic tremor of axial and trunci musculature. This drug-induced action tremor has been proposed as a model of ET. Investigations have shown that Harmaline induces rhythmic burst-firing activity in the medial and dorsal accessory inferior olivary nuclei (ION) that

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is transmitted via climbing fibers to Purkinje cells and to the deep cerebellar nuclei, and then to brainstem and spinal cord motor neurons [7].

Recently, there is growing awareness to ultrasound as a potential therapeutic modality for ET. Since the introduction of focused ultrasound (FUS) in recent years, better understanding of its interactions with biological structures and the mechanism of action of neuromodulation is being gained [8]. Major advances in the design of the ultrasound applicators [9] and development of imaging modalities has opened a broad spectrum of potential clinical applications for FUS. In July 2016, FUS thalamotomy was approved by the Food and Drug Administration (FDA) for unilateral treatment of ET. The main advantage of FUS thalamotomy is that no open surgery of the skull is required. The most important disadvantage is its irreversible nature. Therefore, if side effects occur, they are permanent. However, transient FUS induces neuromodulation may allow testing to a certain extent, of the suppression of tremor while evaluating unwanted side effects by real time reversible inhibition of the target region.The neuromodulatory potential of FUS was suggested by the pioneering work of Fry and colleagues in 1958 [10], which demonstrated that FUS administered to the lateral geniculate nuclei of the thalamus reversibly inhibited the visual pathway in cats. Recent studies have demonstrated the excitatory and suppressive neuromodulatory properties of FUS not only in the central nervous system [11,12], but also in the peripheral nervous system [13]. To achieve the desired modulation, a wide range of ultrasound pulse schemes and frequencies have been explored [14–16]. The neuromodulatory effects of FUS have been demonstrated via electrophysiological recordings such as electroencephalographic (EEG) [11,15,17] and electromyographic (EMG) [14,18], and using imaging techniques such as functional MRI [19,20] and PET-CT [21]. FUS-induced functional modulation was also shown by direct measurement of extracellular levels of neurotransmitters and metabolic changes and by direct recording of microelectrodes [22].

To the best of our knowledge, the potential of neuromodulatory non-thermal FUS for tremor suppression has not been explored yet. The goal of this study was to study the neuromodulatory effects of non-thermal FUS and to demonstrate the feasibility of non-invasive, non-thermal, FUS-based reversible suppression of tremor in Harmaline treated Rats.

2. Materials and methods

Thirteen lightly anesthetized rats with visible Harmaline-induced tremor were treated with FUS along the inferior olivary (IO) system. EMG was recorded continuously during treatment in order to quantify FUS-induced tremor suppression. In order to assess possible brain toxicity, the rats were scanned by T1- and T2-weighted MRI immediately following treatment and periodically thereafter.

2.1. Magnetic Resonance (MR) guided FUS system (MRgFUS)

Rats were treated using a A hemispheric transducer intended for brain treatments (ExAblate 4000, 230 kHz; Insightec Ltd, Haifa, Israel). The transducer is a phased array with 1024 elements that are located on flat tiles placed on a hemisphere with a radius of 150 mm. Each tile contains nine elements, sized 10.7 × 10.7 mm. The phase and amplitude of each element are individually controlled, with all elements transmitting at a frequency of 230 kHz. Out of the 1024 elements 916 elements were active during the treatments. The system was embedded within a clinical 1.5 T MRI scanner (Signa HDxt, GE Healthcare, Chicago, Illinois, USA) with a custon integrated 2-channel head coil (InSightec, Tirat Carmel, Israel). Rats were scanned with conventional T2-weighted MRI prior to treatment for localization of the focus.

2.2. Animals

The study was performed using 21 Sprague-Dawley, male rats weighing 250–350 gr at the day of the treatment. Thirteen rats were in the treatment group (ET rats) and 8 were used as controls: 5 naïve rats that were treated by FUS without Harmaline administration and 3 ET rats that underwent a sham procedure. The study was approved by Sheba Medical Center's institutional ethics committee for animal experiments.

2.3. Animals preparation

Harmaline, which was freshly dissolved in saline at the morning of the experiment, was injected IP at a dose of 20 mg/kg. Once Tremor was clearly visible (within minutes post injection), the rats were deeply anesthetized (600 μL of 0.75 mL/kg ketamine and 1.3 mL/kg xylazine, intra muscular). Once anesthetized, the tremor stopped and their heads were shaved in order to prevent air bubbles during FUS treatment. The anesthetized rats were strapped onto a holder in the supine position. The holder was designed to ensure that the rats heads will not move during treatment despite the tremor and that the shaved skull will be exposed to as many elements as possible (Fig. 1).

The holder was placed in the FUS/MR system so that the rats' skull was placed in the center of the transducer and fully submerged in degassed water (lower than 1 ppm).

Once the rat was placed in the brain system, T2-weighted MRI was performed for depicting the brain anatomy, and the locations of the sonications were planned using the images. While the animals remained strapped to the holder, the MR table was detached from the scanner and was pulled out to a position where the rat was easily visible and the electrical noise from the MRI scanner was not affecting the EMG readings. Once the tremor was observed again, upon awakening, the EMG electrodes (MP150 system with EMG100cMRI with EL452 12 mm unipolar needle electrodes, BIOPAC systems Inc, Goleta, California, USA) were placed. Two recording electrodes were placed in the muscles which showed the most intense tremor in the limbs or tail (The biceps femoris or the sacrocaudalis ventralis medialis). The ground electrode was placed under the chest skin.

Gain was set to 500. a band-pass filter from 10- 500 Hz was applied.

Next, differential EMG was recorded continuously, consisting of a baseline reading of the pre-treatment tremor/muscle activity, and reading during and after treatment. The treatment was performed only after verification that the tremor was clearly recorded by the EMG.

2.4. FUS treatment

ET rats were treated in the region of the olivo-cerebellar system (Fig. 2) at 27.2 W/cm2 (Isppa). Treatment duration was 52s, consisting of 100 mSec on and 2900 mSec off. The pulse duration was chosen since it was found previously to effectively induce neurostimulation [23].

EMG was measured up to 5 min after the treatment or in case of tremor suppression, until after the tremor recovered. If no change in tremor was visible, the same treatment was repeated with a 1 mm shift in the focus location. If there was no change in tremor after 3 such treatments – the rat was considered non-responding to treatment.

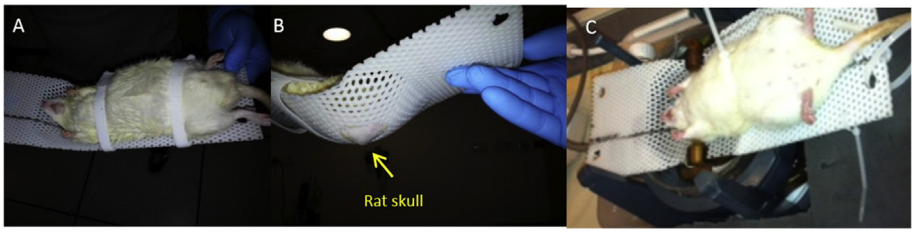

Fig. 1. Rat positioning in the transducer: (A) Rat is laying supine on the holder. (B) The skull is exposed outside of the holder (C) The holder is located in the FUS/MR system and the skull is fully submerged in degassed water. (D) location of sonication.

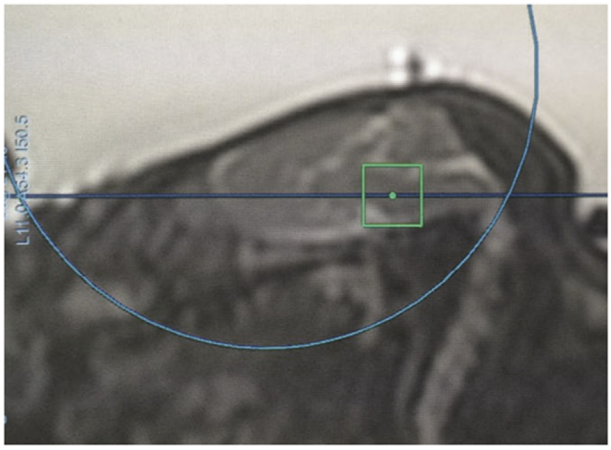

Fig. 2. MR image used for sonication localization. The dot represents treatment location.

Six control rats underwent the same treatment without receiving Harmaline, and 3 control rats received only Harmaline and underwent a sham protocol identical to the treated rats.

2.5. MRI follow-up

All rats were scanned by MRI 3 days and 1 month post FUS treatment. The rats were scanned under full anesthesia, using a clinical GE 1.5 T MRI system (Optima MR450w, GE Healthcare, Chicago, Illinois, USA) with a clinical phased array knee coil. The scan consisted of T2-weighted MRI (in plain 0.23 mm × 0.23 mm and 1 mm slice thickness) for assessing possible permanent brain tissue damage and Gradient Eco (GE) for assessing hemorrhages (pixel size 1 × 1 mm).

2.6. Neurological assessment

Routine weekly assessments of the well-being and neurological condition of the rats were conducted based on a score sheet for evaluating the severity of symptoms for traumatic brain injury and subarachnoid hemorrhage [24]. The parameters examined was weight loss, physical appearance (grooming, shiny eyes, clean fur), breathing, changes in behaviour (reduced mobility, abnormal movements/gait, apathy, lack of social contact), reaction to handling and basic neurological evaluation (limb flexion, decreased resistance to lateral push and seizures). Neurological damage was determined as a change in at least one of the parameters evaluated.

2.7. EMG data analysis

The EMG signal of ET rats typically consisted of multiple peaks, representing both Harmaline-induced tremor and background noise (including rat motion).

The aim of the analysis was to separate the tremor peaks from the background noise and then count the number of peaks per second (tremor frequency) as a function of time, in order to quantify the extent and duration of tremor suppression. A dedicated algorithm was developed using the Matlab software package (Version R2010a; MathWorks, Natick, Massachusetts, USA). Results of the different steps of the algorithm in a 4s period of representing data are shown in Fig. 3.

First, the median value of the signal was subtracted from the signal data so that the median signal value was set to zero (Fig. 3A). Then, a threshold for separating the noise was calculated using a moving median filter (red lines). This enabled the threshold to adapt in cases in which signal became noisier during the measurement. Next, the signal was divided by the threshold value so that within the noise area, the signal values ranged between 0-1. After this division, the signal to the power of 4 was calculated: this way the noise was almost eliminated and the peaks were amplified (Fig. 3B). Finally, convolution of the resulting noise-reduced data with a time kernel corresponding to 100 Hz allowed us to merge adjacent peaks in order to ensure that in cases that a single tremor produced a few peaks, they would be counted once (Fig. 3C).

The effects of the FUS treatment on the tremor were studied by calculating, for each animal, the tremor frequency before, during and following the FUS treatment. Therefore, the average tremor frequencies before, during and after the FUS treatments were calculated and compared using paired *t*-test. Significance level was set to 0.05. Duration of tremor suppression was also calculated from EMGs. Definition of tremor suppression was reduction in tremor frequency lasting at least 15 s.

3. Results

All 16 baseline EMG signals of the ET rats recorded pre-treatment demonstrated distinct tremor. The average frequency of this tremor was 6.2 ± 2.8 Hz. The baseline tremor frequency of the sham rats was 5.7 ± 2 Hz. Results are summarized in Table 1.

3.1. Tremor suppression

Response to FUS was defined as reduction in tremor frequency that lasted for at least 20s. There was large variability in the response to the 27.2 W/cm^2 FUS-treatments, with respect to both the decrease in tremor frequency and the duration of this suppression: 12 of the 13 treated animals exhibited significant decrease in tremor frequency during or after the FUS-treatment. Complete tremor suppression was measured in 6 of the responding rats. The Average tremor frequency of the responding rats was significantly lower than the tremor frequency before treatment (2 ± 1 Hz vs 6 ± 1 Hz, $p < 0.0003$). No significant difference was found between the tremor frequency of the sham rats prior and after the sham protocol (5.7 ± 2 Hz and 5.4 ± 2.3 Hz for before and after sham treatment respectively. Paired test $p < 0.3$).

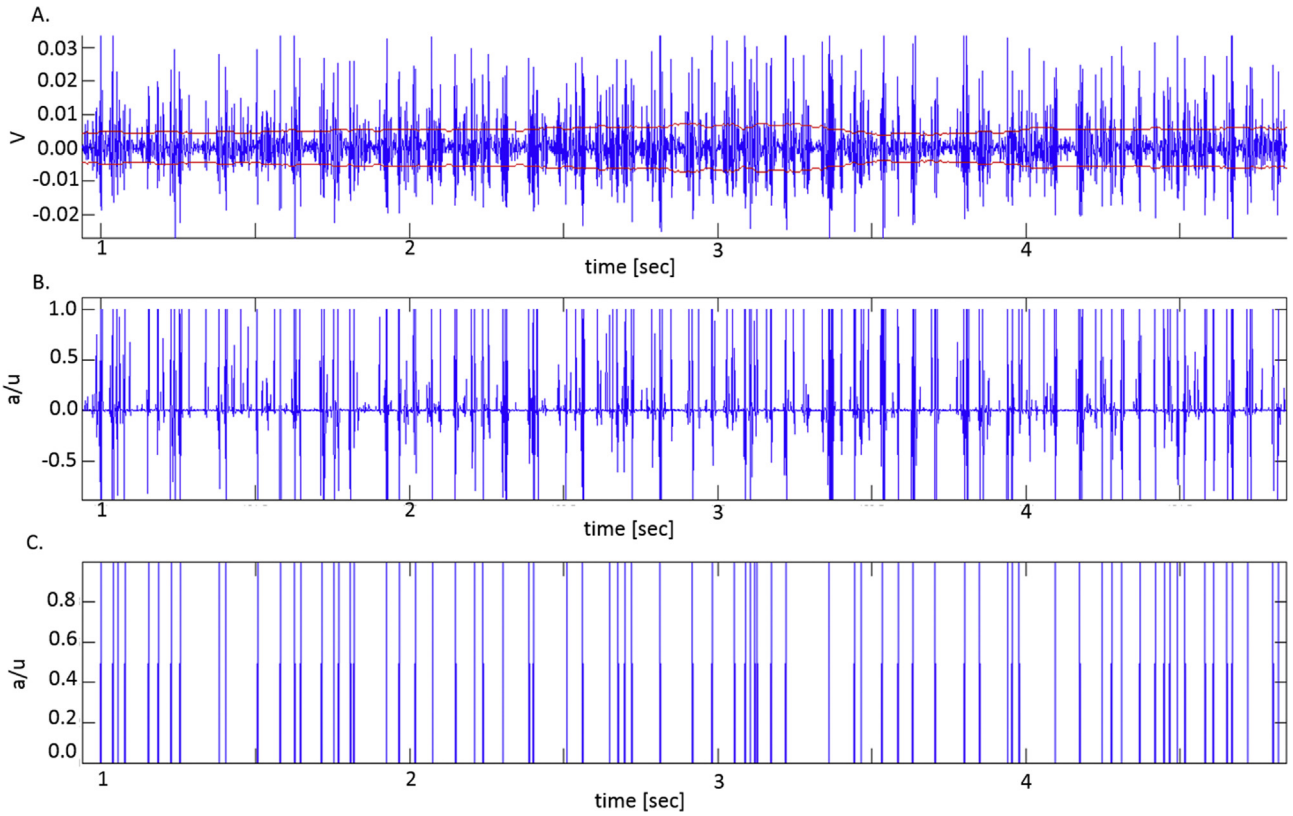

Fig. 3. EMG analysis: (A) Raw EMG data and moving median filter (red lines). (B) EMG signal after noise- reduction (C) Number of peaks counted. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Frequency of tremor prior to treatment, during response time, duration of response and time of response from the start of the sonications.

Rat	# of treatments	Baseline tremor (Hz)	Response tremor (Hz)	Duration of response (s)	Time to response (s)
1	2	2.3 ± 1.9	0.5 ± 0.8	25	52
2	3	5.9 ± 5.5	3.4 ± 2.3	52	67
3	3	5.5 ± 4.2	3.6 ± 2.2	30	44
4	3	3.9 ± 2.5	0.3 ± 0.7	130	86
5	1	7.6 ± 2.4	2.9 ± 1.6	15	47
6	1	10.5 ± 1.7	0.1 ± 0.5	33	60
7	1	3.2 ± 1.7	0.7 ± 1.5	34	50
8	3	7.2 ± 2.7	5.3 ± 2.5	26	52
9	3	3.3 ± 3.1	0.4 ± 0.8	110	58
10	2	11.7 ± 2.4	2.8 ± 2	40	40
11	1	5.7 ± 2.9	1.9 ± 1.7	80	97
12	1	5.9 ± 2.9	0.4 ± 0.7	215	41
13	3	7.5 ± 2.3	7.5 ± 2.2	0	NO RESPONSE
14	sham	3.5 ± 2.4	3.4 ± 2.6		
15	sham	5.9 ± 2.9	4.8 ± 3.4		
16	sham	7.6 ± 2.4	7.9 ± 2.8		

In 6 cases (3 in which the tremor was completely suppressed and 3 in which the tremor frequency was decreased) the suppression of the tremor occurred during the treatment, 20±7s on average from treatment initiation, and in the other 6 cases, the suppression occurred after the treatment has ended, 40±7s on average from the end of the treatment.

Representative examples of EMG recordings and signal analysis before, during and after FUS treatments in which there was either: (a) no response (b) a decrease in tremor frequency and (c) complete suppression of tremor, are shown in Fig. 4.

Duration of tremor suppression: in the 12 responding rats, response duration was 70 ± 61s, on average. The average response duration for rats showing complete suppression was significantly higher than the response duration for rats showing partial tremor suppression (104 ± 34 s vs 40 ± 9 s, $p < 0.04$).

3.2. FUS-induced motor response

Muscle contraction reflected by movement of the tail and/or the limbs, which was synchronized with the FUS sonication, was observed in 6 of the 13 treated rats (including the non-responsive rat). The movement, clearly observed every 3s during the FUS treatment, was observed in at least one of the 3 tested locations, not always in the location in which tremor suppression occurred. An example of an EMG of a rat showing tremor and motor response can be seen in Fig. 5. Additional 6 naïve rats (no Harmaline) were

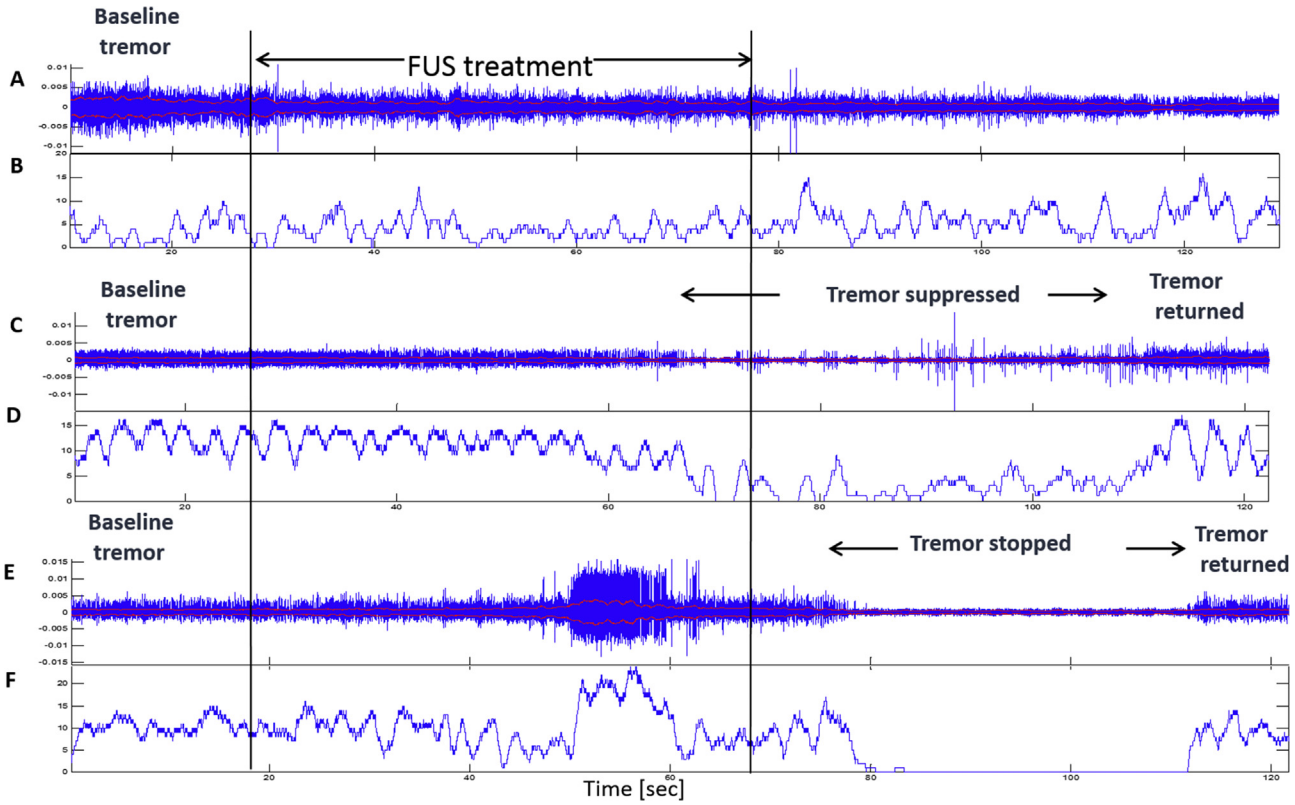

Fig. 4. Examples of EMG recordings and signal analysis before, during and after FUS treatments: (A + B) No response. (C + D) tremor frequency is reduced during the treatment. (E + F) Tremor stopped post treatment.

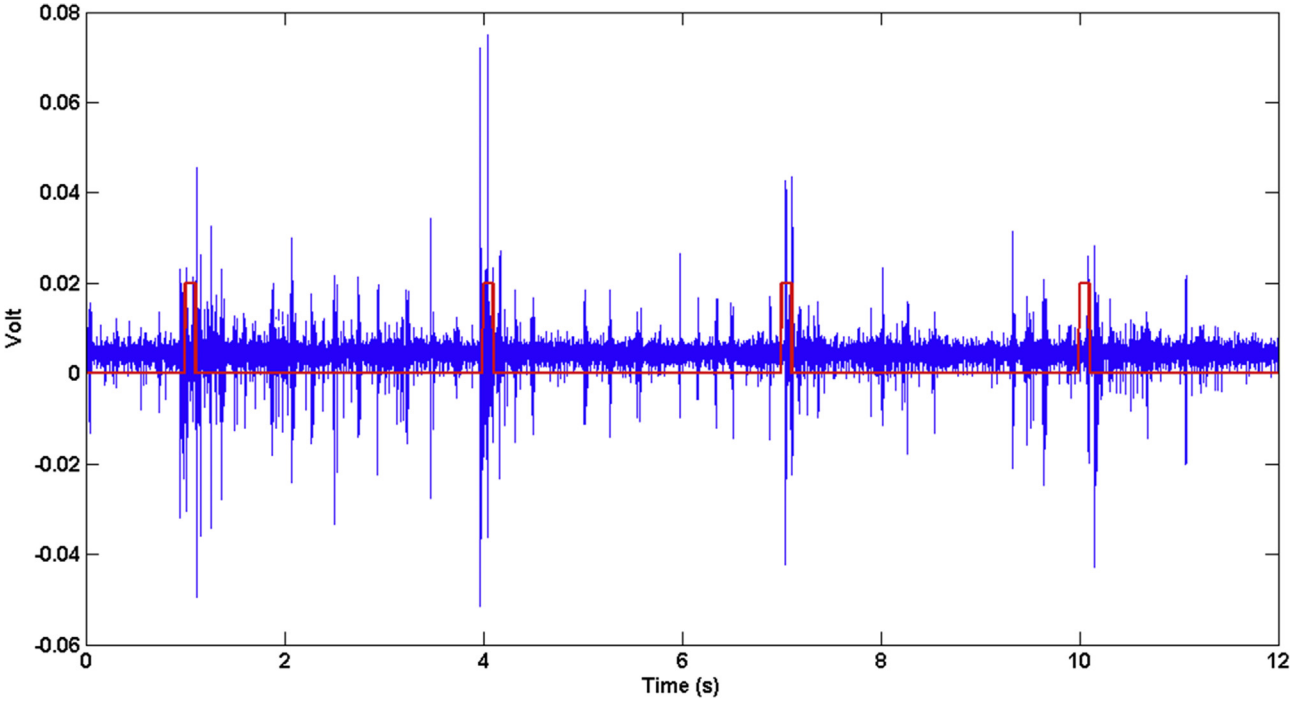

Fig. 5. Example of motor response (seen every 3 s) corresponding to treatment timing and the trigger from the FUS system corresponding to sonication timing.

treated with FUS using the same protocol at the same location. A trigger from the FUS system was connected to the EMG so the synchronization could be recorded. A motor response identical to the response observed in the ET rats was observed and recorded by EMG in all 6 rats (Fig. 6). The location of the motor response (tail/limbs) as well its duration varied between the rats. The average duration of the response was 316.9 ± 45.8 ms with latency of 73.2 ± 6.8 from the beginning of the sonication.

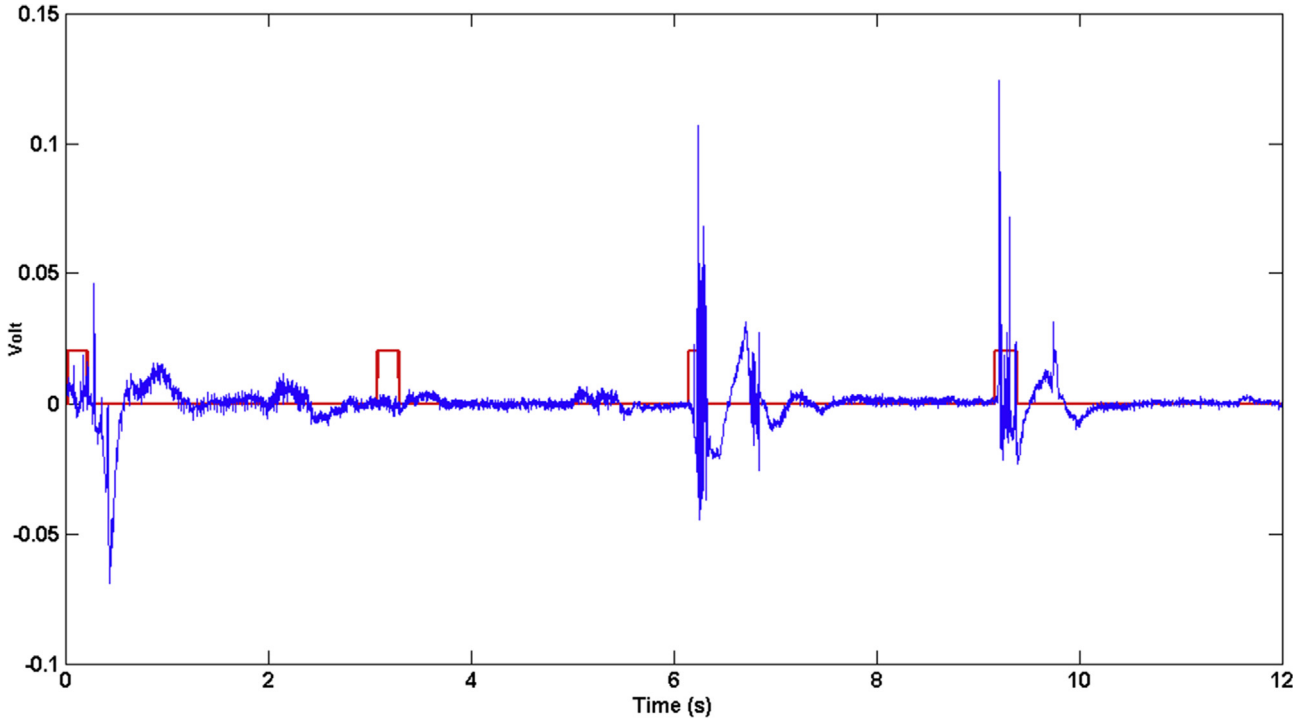

Fig. 6. Example of motor response (seen every 3 s) and the trigger from the FUS system corresponding to sonication timing of a naïve rat.

3.3. Damage/toxicity

All the rats survived the treatment. Twelve hours post Harmaline administration tremor subsided and was no longer visible in both treated and untreated rats. None of the treated rats, nor the control rats, showed any signs of neurological damage or reduced well-being based on the score sheet.

The MR images of all scanned rats showed no abnormalities, or signs of treatment-induced toxicity (Fig. 7), although since resolution of the images was not high enough to establish whether small damage occurred.

4. Discussion

Non-thermal FUS is emerging as a promising approach for noninvasive modulation of brain activity and presents numerous potential applications in the treatment of neurologic and psychiatric diseases.

In the presented results we demonstrate the feasibility for non-invasive, non-thermal, FUS-based, reversible suppression of tremor in an ET model in rats. Twelve of the 13 treated animals exhibited significant decrease in tremor frequency induced by the FUS-treatment, out of which complete suppression of tremor was recorded in 6 rats. Tremor suppression occurred either during treatment, or after the treatment has ended, and lasted 70 ± 61s on average. None of the treated rats exhibited any signs of neurological damage and no damage was found in follow up MRIs. This is in accordance with other studies, conducted by us and others that found no damage following similar treatment parameters [20,21,25], although due to relatively low resolution of the images, additional histological analysis should be considered in the future to rule out small damage not depicted by the MRI.

Harmaline-induced tremor has been the most studied ET model in mice and rats. The Harmaline acts on the olive-cerebellar system resulting transient tremor [7]. Although it is still the most common ET model, there are some limitations to the model. First, this is an artificial, toxin-induced animal model of tremor and not the naturally occurring chronic human disease ET. Second, Although The traditional model of ET, proposed in the early 1970s, suggested that the ION was the prime generator of tremor, more recent studies found alterations in the components of the cortico-bulbo-cerebello-thalamo-cortical network as abnormal activation in the bilateral cerebellum, red nucleus, and thalamus in patients with ET [26–28]. Despite the limitations, we chose to use this model for the simplicity of it and the short duration of the tremor, reducing the suffering of the rats. Nevertheless, the effect of FUS on ET in human could differ from the rat model and other models should be considered in the future.

The Harmaline-induced tremor frequency in rats was previously reported to be in the range of 8–12 HZ [7], which is in accordance with most of the rats treated in this study. Thus some of the rats exhibited lower tremor frequency, resulting in a lower average tremor frequency (6.2 ± 2.8 Hz). A possible explanation may be the effect of anesthesia. Harmaline-induced tremor, similar to ET, is an action-induced tremor [29], and therefore, even though anesthesia was light at the time of the treatment, it still may have reduced spontaneous movements.

Hermaline was suggested to involve modulation of rhythm-generating ionic currents and facilitating rhythm generation in the ION [7,30], resulting in activation of glutamatergic climbing fibers and generalized tremor. Our hypothesis is that FUS may be suppressing this activation.

Our results and hypothesis are in accordance with the results of Min et al. [17]. They found that pulsed FUS sonications suppressed the number of epileptic signal bursts caused by chemical induced abnormally excessive or synchronous neural activity in rats. Further support for the suppressive effects of FUS was demonstrated by Yoo et al. [20], which showed suppression of visual evoked potentials in rabbits. Although tone burst pulses were used, with much shorter duration which might evoke a different mechanism of action.

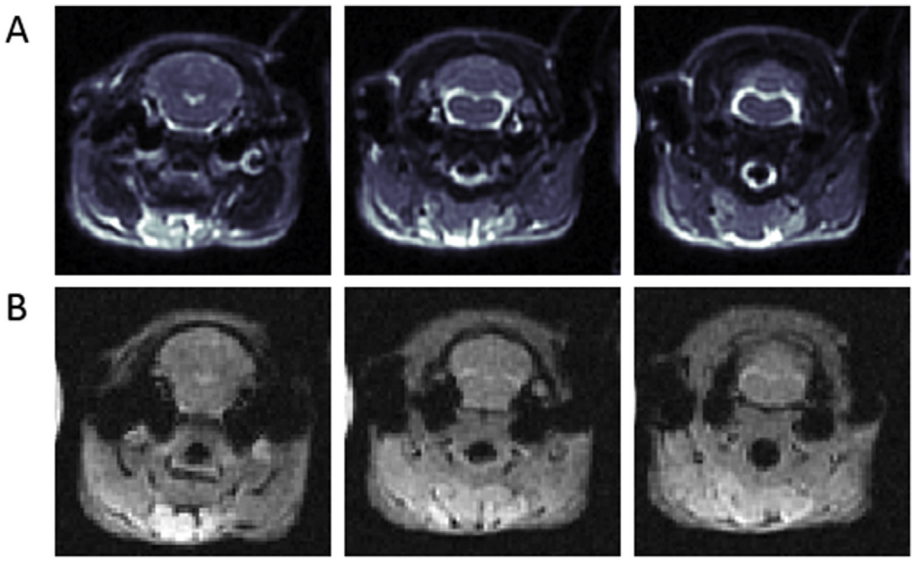

Fig. 7. MRI of ET rat, 30 days post FUS treatment. (A) T2-weighted MRI for assessment of damage (B) T1-weighted MRI with contrast agent for assessment of blood brain barrier disruption (C) Gradiant-eco MRI for assessment of bleeding. No signs of damage, blood brain barrier disruption or bleedings are observed.

Although continuous wave sonication was found to be associated with higher success rate in inducing neuromodulation than pulsed ultrasound, especially with intensities over 10 W/cm2 [14], there is increasing interest in pulsed-burst sonication as it may decrease the risk of temperature buildup in the tissue [11,15]. Here we chose a pulsed pattern with relatively long pulses (100 ms) but at a low duty cycle (3%). Kim et al. [31] found that changing the duty cycle from 5% to 8.3% yielded a significant change in response using the same pulse intensity - from suppression of the signal during treatment to elevated signal immediately post treatment. This finding may support our hypothesis that the treatment induces suppression of activation of glutamatergic climbing fibers.

Yoo et al. [20] suggested that long exposure of neural tissue to FUS (on the order of a few seconds or minutes), divided into shorter pulses, can cause neurons to be less excitable. Decreased neurons excitability can be a possible explanation for the tremor suppression observed in our study. Alternatively, a different hypothesis can be alteration in extracellular transmitters level. Such alterations have been demonstrated by Min et al. [17] who found significantly increased extracellular concentrations of cortical Dopamin and serotonin and reduced levels of gamma-Aminobutyric acid (GABA) [22] after non-thermal FUS treatments in the thalamus. None of the hypotheses suggested here were tested and the accurate mechanism of action remains unclear, thus other studies needs to be conducted in order to determine the exact mechanisms involved in the tremor suppression.

In ~46% of the cases the treatment also induced motor response of the tail and limbs that was synchronized with the FUS sonications. This effect was also reproduced in the naïve rats experiments and has been previously reported [14,32]. The ultrasound focus in those studies was targeted to the motor cortex and not to the medulla oblongata as in the current study. King et al. [14] used similar pulse parameters (continuous single 20−480 ms pulses at up to 79.02 W/cm^2) targeted to the basal ganglia and the motor cortex of the mouse brain. The latencies of the motor response in their study were in accordance with our findings.

It is somewhat puzzling that the same pulse parameters yielded two different effects often in the same treatment: suppression of tremor on one hand and activation of motor response on the other. One possible explanation may be found in the neurotransmitters hypothesis. While some neurotransmitters, such as Dopamine and serotonin [33], were found elevated post treatment, others, such as GABA, were reduced [21]. Thus, the same pulse parameters yielded opposite effects in different brain systems which are both within the focus of the ultrasound in rats. Another possible explanation may be activation of inhibitory neurons in the IO system along with activation of excitatory neurons in the pyramidal system in the close vicinity of the IO. Again, additional studies should be conducted in order to determine the cause.

The use of relatively low US frequency is both an advantage and a limitation. Low frequency was found to induce larger affected area compared to high frequency [34], especially when sonicating small brain as rats. Since the head of the rat is relatively small compared to the focus size (3.8 mm × 3.8 mm × 8.6 mm) it is possible that eventhough the focus was aimed to the ION olivocerebellar fibers or other structures located in close proximity are effected > It is also possible that multiple areas are affected. For this reason we used the term region of the olivo-cerebellar system rather than the ION as our target. Nevertheless, our results showed high spatial resolution, often a 1 mm shift in focus resulted in reduced or no effect, evoking the need to shift the focus location. These results are in accordance with Yunan et al. [16] who found that when applying low frequency FUS to small brain, stimulation of very specific structures such as the oculomotor system or a single whisker was observed. The choice in low frequency also reduces attenuation in tissues and the skull and reduce heating. Excessive attenuation can lead to harmful heating effects [35], reduce the ability to reach deeper structures [18] and reduce success rate [18,36]. Constans et al. [35] concluded that thermal effects can be neglected in the rat brain when using similar treatment parameters.

Since Hermaline effects the ION, we chose to target the olive-cerebellar system. Although the focus was aimed to the ION it is possible that olivocerebellar fibers or other structures located in close proximity are effected since the head of the rat is relatively small compared to the focus size (3.8 × 8.6 mm).

During the time in which treated rats were monitored, there was no visible brain damage on follow-up T2-weighted MRIs, no BBB disruption was visible on T1-weighted MRI with Gadolinium-DOTA obtained 24 h post treatment and no hemorrhages were visible on GE. Despite the lack of histological analysis that might depict small

damage not visible by the MRI, this is also in accordance with other neuromodulatory studies conducted by as and others that found no damage to the treated brains either via MRI or histological analysis [20,22].

5. Conclusion

Essential tremor (ET) is one of the most common movement disorders of adults, where systemic treatments are only partially efficient, and therefore new treatment approaches are required. Our results demonstrate the feasibly and safety of applying non-invasive, non-thermal, reversible FUS for obtaining tremor suppression in ET rats. Although further research is needed in order to understand the mechanism of action underlining this effect, the results of the presented study provide evidence that non-thermal FUS holds promise as a noninvasive tool to obtain reversible tremor suppression. Such transient suppression may be applied for improved targeting (by screening specific brain regions) of ablative treatments and reduce side-effects for motion disorders such as ET and Parkinson's disease.

Declaration of interests

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References

[1] Zesiewicz TA, Chari A, Jahan I, Miller AM, Sullivan KL. Overview of essential tremor. Neuropsychiatric Dis Treat 2010;6:401–8.
[2] Louis ED. Essential tremor then and now: how views of the most common tremor diathesis have changed over time. Park Relat Disord Jan 2018;46(1):S70–4.
[3] Puschmann A, Wszolek ZK. Diagnosis and treatment of common forms of tremor. Semin Neurol 2011;31(1):65–77.
[4] Thenganatt MA, Louis ED. Distinguishing essential tremor from Parkinson's disease: bedside tests and laboratory evaluations. Expert Rev Neurother 2012;12(6):687–96.
[5] Benito-Leon J, Louis ED. Essential tremor: emerging views of a common disorder. Nat Clin Pract Neurol 2006;2(12):666–78.
[6] Brennan KC, Jurewicz EC, Ford B, Pullman SL, Louis ED. Is essential tremor predominantly a kinetic or a postural tremor? A clinical and electrophysiological study. Mov Disord Off J Mov Disord Soc 2002;17(2):313–6.
[7] Handforth A. Linking essential tremor to the cerebellum—animal model evidence. Cerebellum 2016;15(3):285–98.
[8] Plaksin M, Kimmel E, Shoham S. Cell-type-selective effects of intramembrane cavitation as a unifying theoretical framework for ultrasonic neuromodulation. eNeuro 2016;3(3).
[9] Aubry JF, Tanter M. MR-guided transcranial focused ultrasound. Adv Exp Med Biol 2016;880:97–111.
[10] Fry FJ, Ades HW, Fry WJ. Production of reversible changes in the central nervous system by ultrasound. Science 1958;127(3289):83–4.
[11] Kim H, Park MY, Lee SD, Lee W, Chiu A, Yoo SS. Suppression of EEG visual-evoked potentials in rats through neuromodulatory focused ultrasound. Neuroreport 2015;26(4):211–5.
[12] Bystritsky A, Korb A, Stern J, Cohen M. Safety and feasibility of focused ultrasound neuromodulation in temporal lobe epilepsy. J Therapeut Ultrasound 2015;3(1):1.
[13] Juan EJ, González R, Albors G, Ward MP, Irazoqui P. Vagus nerve modulation using focused pulsed ultrasound: potential applications and preliminary observations in a rat. Int J Imag Syst Technol 2014;24(1):67–71.
[14] King RL, Brown JR, Newsome WT, Pauly KB. Effective parameters for ultrasound-induced in vivo neurostimulation. Ultrasound Med Biol 2013;39(2):312–31.
[15] Lee W, Lee SD, Park MY, Foley L, Purcell-Estabrook E, Kim H, et al. Image-guided focused ultrasound-mediated regional brain stimulation in sheep. Ultrasound Med Biol 2016;42(2):459–70.
[16] Younan Y, Deffieux T, Larrat B, Fink M, Tanter M, Aubry JF. Influence of the pressure field distribution in transcranial ultrasonic neurostimulation. Med Phys 2013;40(8), 082902.
[17] Min BK, Bystritsky A, Jung KI, Fischer K, Zhang Y, Maeng LS, et al. Focused ultrasound-mediated suppression of chemically-induced acute epileptic EEG activity. BMC Neurosci 2011;12:23.
[18] Ye PP, Brown JR, Pauly KB. Frequency dependence of ultrasound neurostimulation in the mouse brain. Ultrasound Med Biol 2016;42(7):1512–30.
[19] Kim H, Chiu A, Lee SD, Fischer K, Yoo SS. Focused ultrasound-mediated non-invasive brain stimulation: examination of sonication parameters. Brain Stimul 2014;7(5):748–56.
[20] Yoo SS, Bystritsky A, Lee JH, Zhang Y, Fischer K, Min BK, et al. Focused ultrasound modulates region-specific brain activity. Neuroimage 2011;56(3):1267–75.
[21] Kim H, Park MA, Wang S, Chiu A, Fischer K, Yoo SS. PETCT imaging evidence of FUS-mediated (18)F-FDG uptake changes in rat brain. Med Phys 2013;40(3), 033501.
[22] Yang PS, Kim H, Lee W, Bohlke M, Park S, Maher TJ, et al. Transcranial focused ultrasound to the thalamus is associated with reduced extracellular GABA levels in rats. Neuropsychobiology 2012;65(3):153–60.
[23] Deffieux T, Younan Y, Wattiez N, Tanter M, Pouget P, Aubry JF. Low-intensity focused ultrasound modulates monkey visuomotor behavior. Curr Biol: CB 2013;23(23):2430–3.
[24] Pinkernell S, Becker K, Lindauer U. Severity assessment and scoring for neurosurgical models in rodents. Lab Anim 2016;50(6):442–52.
[25] Daniels D, Sharabi S, Last D, Guez D, Salomon S, Zivli Z, et al. Focused ultrasound-induced suppression of auditory evoked potentials in vivo. Ultrasound Med Biol 2018;44(5):1022–30.
[26] Louis ED. Essential tremor then and now: how views of the most common tremor diathesis have changed over time. Park Relat Disord 2018;46(Suppl 1):S70–4.
[27] Wills AJ, Jenkins IH, Thompson PD, Findley LJ, Brooks DJ. Red nuclear and cerebellar but no olivary activation associated with essential tremor: a positron emission tomographic study. Ann Neurol 1994;36(4):636–42.
[28] Wills AJ, Jenkins IH, Thompson PD, Findley LJ, Brooks DJ. A positron emission tomography study of cerebral activation associated with essential and writing tremor. Arch Neurol 1995;52(3):299–305.
[29] Park YG, Choi JH, Lee C, Kim S, Kim Y, Chang KY, et al. Heterogeneity of tremor mechanisms assessed by tremor-related cortical potential in mice. Mol Brain 2015;8:3.
[30] Miwa H. Rodent models of tremor. In: Grimaldi G, Manto M, editors. Mechanisms and emerging therapies in tremor disorders. New York, NY: Springer New York; 2013. p. 37–51.
[31] Kim H, Park MY, Lee SD, Lee W, Chiu A, Yoo S-S. Suppression of EEG visual-evoked potentials in rats via neuromodulatory focused ultrasound. Neuroreport 2015;26(4):211–5.
[32] Kamimura HA, Wang S, Chen H, Wang Q, Aurup C, Acosta C, et al. Focused ultrasound neuromodulation of cortical and subcortical brain structures using 1.9 MHz. Med Phys 2016;43(10):5730.
[33] Min B-K, Yang PS, Bohlke M, Park S, Vago DR, Maher TJ, et al. Focused ultrasound modulates the level of cortical neurotransmitters: potential as a new functional brain mapping technique. Int J Imag Syst Technol 2011;21(2):232–40.
[34] Constans C, Deffieux T, Pouget P, Tanter M, Aubry JF. A 200-1380-kHz quadrifrequency focused ultrasound transducer for neurostimulation in rodents and primates: transcranial in vitro calibration and numerical study of the influence of skull cavity. IEEE Trans Ultrason Ferroelectrics Freq Contr 2017;64(4):717–24.
[35] Constans C, Mateo P, Tanter M, Aubry JF. Potential impact of thermal effects during ultrasonic neurostimulation: retrospective numerical estimation of temperature elevation in seven rodent setups. Phys Med Biol 2018;63(2), 025003.
[36] Tufail Y, Matyushov A, Baldwin N, Tauchmann ML, Georges J, Yoshihiro A, et al. Transcranial pulsed ultrasound stimulates intact brain circuits. Neuron 2010;66(5):681–94.