Neuromodulation by Focused Ultrasound

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Abstract

Ultrasound (US) is most widely recognized in medicine for its use in imaging as a diagnostic tool. Over the past decade, US has become increasingly appreciated for its ability to non-invasively modulate cellular activity including neuronal activity. Data from the past ten years show that low-intensity US can reversibly modulate the physiological activity of neurons in peripheral nerves, spinal cord, and intact brain circuits. Empirical evidence and modeling data indicate acoustic pressure profiles exerted by US act to alter the gating dynamics of mechanosensitive ion channels, including voltage-gated ones to modulate activity. The exact mechanisms of action enabling US to both stimulate and suppress neuronal activity are perplexing and challenge several fundamental issues in modern neuroscience related to the role of electro-mechanical coupling in endogenous nervous system function. Neuromodulation by focused ultrasound (NeuroFUS) offers several advantages over existing noninvasive neuromodulation methods. These stem from the physics of US and its ability to be transmitted and focused across bone and other tissues, deep into the body or brain, to millimeter and submillimeter targets with high accuracy and precision. By increasing general awareness of, engineering capabilities for, and medical research into NeuroFUS, science and medicine can begin to leverage such physical advantages to develop new treatment approaches to many pervasive neurologic and psychiatric disorders.

Introduction & Overview of Modern Medical Ultrasound

Ultrasound (US) is a mechanical wave or sound pressure wave with a frequency higher than about 20 kHz or the upper frequency limits of human hearing. US represents, by far, the most widely used and arguably the safest medical imaging modality in the world [1-3]. Just over a decade ago, medical US was conducted using large, expensive machines costing tens of thousands of dollars available almost exclusively in clinical settings. With recent advances in ultrasound transducer materials, digital signal processing, and medical device engineering, some of the most advanced medical US imaging systems now cost less than a personal computer and can fit in the pocket of a physician's lab coat. Changes in medical ultrasound are rapidly elevating standards of care in medicine. At the forefront, is a surge in the use of point-of-care ultrasound (POCUS) imaging devices in routine physical examinations, to image cardiovascular activity, to more easily enable image-guided nerve blocks or anesthesia, by first responders

in emergency medical situations, and at the bedside of millions of patients around the world [1]. These advances are so important in fact that the University of California at Irvine School of Medicine just gifted each one of their incoming first-year medical students with a POCUS imaging device [4]; as if it were as indispensable to the modern physician as a stethoscope of years past.

Besides its use in diagnostic medical imaging, US can be used to produce thermal and nonthermal or mechanical effects on biological tissues for therapeutic applications [2,3] (**Figure 1**). High-intensity focused ultrasound (HIFU; $> 200 \text{ W/cm}^2$) is used to cause significant tissue heating by exposing the target tissue to several seconds of continuous wave US for therapeutic ablations. Low-intensity US ($< 50 \text{ W/cm}^2$) on the other hand is typically delivered in a pulsed wave mode of very brief bursts of energy to produce mechanical bioeffects on tissues and that do not cause heating or damage. Due to the manner, in which US interacts with physical matter including biological tissues, it provides neuroscience research,

as well as neurosurgery, neurology, and neuropsychiatry with unique capabilities and opportunities. Most notably, US can be transmitted across the human cranium and precisely focused into deep-brain regions with millimeter spatial resolution for achieving therapeutic HIFU ablations as later discussed [5,6]. Although recent breakthroughs in engineering and medicine have enabled such feats, studies exploring the effects of US on neural activity

date back nearly a century. Over the past decade and of particular topic in this overview article, there has been growing evidence that US is a viable tool for non-invasively modulating neural activity and brain function [7,8] (**Figure 2**). The major goal of this medRxiv critical review is to educate the medical research community on the past decade of advances made in the study and application of neuromodulation by focused ultrasound (NeuroFUS).

Portability & Flexibility

Ultrasound is experiencing growth due to expanded point-of-care applications enabled by chip-based, wireless imaging platforms; and the development of advanced real-time ultrasound imaging modalities.

POCUS:
Portable,
Handheld

Non-invasiveness

Phased-array and beamforming methods have enabled transcranial, MR-guided, high-intensity focused ultrasound (MRgHIFU) for incisionless functional neurosurgery via thermal ablation of thalamus.

NeuroFUS:
Nonthermal,
No exogenous
agents required

Nonthermal Applications

Ultrasound-mediated drug delivery is being enabled by the synthesis of acoustically-sensitive carriers and nanoparticles. Sonogenetics is using gene targeting methods incorporating mechanosensitive channels into neural circuits.

Figure 1. Recent advances in medical ultrasound. The figure highlights several advances in medical ultrasonic over the past decade. The size, weight, and power requirements (SWaP) of devices has been reduced significantly resulting in the emergence of point-of-care ultrasound (POCUS) imaging. Transcranial HIFU can be used to perform stereotactic ablations of brain circuits for disease treatments. Advances have also been made in ultrasound-mediated drug delivery and non-invasive neuromodulation as illustrated.

History of Neuromodulation by Focused Ultrasound

Prior to the late 2000's, several bodies of evidence had emerged that HIFU was capable of reversibly modulating nerve and brain activity by producing thermal effects. In some related pioneering

studies, Fry and colleagues demonstrated in a series of investigations that HIFU could treat several neurologic conditions by focally and functionally ablating brain circuits [9-13]. These observations were shelved by practicing medicine at the time primarily because accurately focusing ultrasound through skull bone was difficult without a major

Figure 2. Neuromodulation by focused ultrasound of brain and nerve. The figure shows modulation of human S1 brain circuits by low-intensity, transcranial, pulsed, focused ultrasound in the *upper-left*. Panel adapted from reference [14]. In the *upper-right* data showing modulation of peripheral nerves and receptors in the hand by pulsed ultrasound. Panel adapted from reference [15]. The mechanisms of action underlying NeuroFUS are due to the mechanical sensitivity of the nervous systems and their cellular components as portrayed in the lower left. Panel adapted from reference [16]. Thermal and cellular safety data show NeuroFUS is safe and does not cause macroscopic tissue heating or damage when used properly. Image compliments of IST, LLC.

craniectomy. Some decades later, Hynynen and colleagues made key breakthroughs by describing methods for precisely focusing ultrasound through human skull bone using ultrasound transducers operating as phased arrays [17-19]. Focusing methods and approaches have been continuously refined in medical ultrasonics over the past decades and holographic methods are beginning to emerge as discussed below. Today, the use of transcranial, magnetic resonance imaging-guided HIFU (MRgHIFU) for incisionless neurosurgery is becoming increasingly adopted for treating pervasive neurological and neuropsychiatric diseases. For example, transcranial stereotactic ablations of brain circuits in the thalamus by HIFU is now an FDA-cleared treatment for essential tremor and

Parkinson's disease [5,6,17,20]. Other clinical indications including MRgHIFU ablation-mediated treatment of pain, epilepsy, and obsessive compulsive are expected to be cleared next since several investigations have shown damage promise for each. While HIFU produces thermal effects through waveforms delivered to target tissues in a continuous-wave mode, as mentioned above low-intensity ultrasound delivered in a pulsed-wave mode can produce mechanical or non-thermal effects on cellular activity.

Low-intensity, pulsed ultrasound was first shown capable of directly stimulating action potentials and synaptic transmission in brain slices [21]. Using optogenetic reporters of synaptic vesicle activity, calcium channel reporters, and whole-cell

electrophysiological recordings combined with pharmacology, it was shown low-intensity, pulsed ultrasound produced these effects by activation of voltage-gated sodium and calcium channels [21]. These initial studies have inspired numerous investigations over the past decade exploring the effects of low-intensity, pulsed ultrasound on central

and peripheral nervous system activity [7,8]. The remainder of the review discusses data and insights gained over the past ten years in this burgeoning new field of NeuroFUS.

Figure 3. Low-intensity focused ultrasound for functional deep-brain mapping. (A) MR-thermometry images of pig brains showing the focal heating of targeted thalamic nuclei by high-intensity focused ultrasound (HIFU; *top*) and a lack of heating produced by low-intensity focused ultrasound (LIFU; *bottom*). (B) Electrophysiological recordings of SSEPs evoked by trigeminal (*left*) and tibial (*right*) nerve stimulation during baseline (*black*) and when LIFU (*red*) was targeted to regions (*yellow*) near (*top*) or in the sub-nuclei of the pig thalamus (VPM *middle* and VPL *bottom*). Collectively, these data show LIFU can be used to functionally map different somatotopic regions of the pig thalamus without causing tissue heating (adapted from Ref. [35]).

NeuroFUS for Brain Applications

As mentioned above, it has long been known that US can produce a variety of thermal and nonthermal effects on cells and tissues depending on several factors including frequency, intensity, duty cycle, and exposure time. The acoustic frequency of US used for a particular application will define the spatial resolution. In soft tissues, like the brain, the diffraction-limited spatial resolution of 0.5 MHz US is about three millimeters while it is about 15 microns for 100 MHz US, which is more amenable to peripheral nerve targeting. However, power loss due to absorption and scattering of US by tissues becomes greater as the acoustic frequency increases. For instance, the optimal gain for transcranial transmission and brain absorption of US is between 0.2 and 0.65 MHz [5,22]. Higher US frequencies (for example, 2 – 10 MHz) are routinely used in transcranial imaging applications because only nominal acoustic intensities are required for imaging

applications. Thus, greater power loss at these higher US frequencies can be tolerated for imaging application. In other non-imaging applications of transcranial US, such as NeuroFUS that require higher acoustic intensities to be generated in brain tissues, lower US frequencies (< 0.7 MHz) should be used.

Soon following the initial observations that US can directly stimulate action potentials in brain slices by Tyler and colleagues (2008), methods using low-intensity transcranial US for conducting *in vivo* stimulation and modulation of neurocellular activity in the mouse motor cortex and hippocampus [23], as well as for rapidly attenuating seizure activity in mice [24] were described. Subsequent studies designed to quantitatively evaluate and confirm our basic observations led to an expansion of research in the field of NeuroFUS [25,26].

Others have also since shown that the mechanical (non-thermal) bioeffects of low-intensity, pulsed US (< 50 W/cm²) can safely modulate the activity of intact cortical, thalamic, and hippocampal

circuits in mice and rats [25-28], rabbits [29], sheep [30], monkeys [31-34], pigs [35], and humans [14,36-38] as discussed further below (**Table 1**). These studies have continued to provide valuable insights into the acute effects of US on brain activity across a range of parameters while collecting safety data and observations. The acute modulation of brain activity by low-intensity US has been shown safe in numerous animal models, which helped pave the way for conducting more recent human NeuroFUS research studies.

In non-human primates, it was first demonstrated that transcranial low-intensity focused ultrasound (LIFU) can evoke visuomotor behaviors when targeted to frontal eye field regions of cortex [31]. This work was soon thereafter expanded upon to show that transcranial LIFU can stimulate individual cortical neurons in awake behaving macaques [32]. Most recently, brain network plasticity was shown to be triggered by transcranial LIFU in a series of elegant, back-to-back fMRI studies in which the effects of FUS on cortical and deep-brain activity lasted up to several hours following treatment without producing damage or side effects [33,34]. An ability to produce plasticity or such lasting changes in brain circuit/network activity is a hallmark of all neurotherapeutics that act to alter activity for producing beneficial outcomes.

In humans, Legon and colleagues (2014) provided the first electrophysiological and behavioral evidence demonstrating that LIFU (0.5 MHz, < 50 W/cm²) can modulate human brain activity [14]. The authors demonstrated that a 0.5 MHz transcranial LIFU beam, having a lateral spatial resolution of about 5 mm and an axial resolution of about 18 mm, targeted to the somatosensory cortex at S1 can focally suppressed evoked EEG activity and produced a functional enhancement in somatosensory discrimination thresholds [14]. These fine spatial resolutions obliterate those provided by other noninvasive neuromodulation methods. While these studies point to a promising future, more studies are required to fully understand the safety and efficacy of focused US for acute applications in the brain, as well as to define the safety envelop for emerging chronic applications.

Technological advances in the field have been made by developing and demonstrating LIFU targeting methods that account for individualized variations in anatomy. For example, one recent study developed realistic models using individualized

measures of skull density and brain anatomy, so LIFU beams could be accurately delivered to specific regions of somatosensory cortex. Using these methods, Lee and colleagues (2015) first showed that transcranial LIFU (0.25 MHz, 3 W/cm²) targeted to S1 of human volunteers can directly stimulate and evoke somatosensory potentials [36]. A particularly unique observation in these studies was that LIFU targeted to S1 could elicit different thermal/mechanical/pain sensations in the hands and fingers of volunteers in the absence of peripheral stimuli [36]. More recently the authors extended these by showing that transcranial LIFU (0.27 MHz, 16.6 W/cm²) targeted the primary visual cortex can stimulate visual sensations and evoke sensory potentials in different visual fields of humans as indicated by fMRI BOLD responses [37]. Efforts to replicate these findings are under way and will help determine optimal parameters for stimulating or suppressing activity in various brain regions of humans. With the continued safety observations and the further refinement of focusing methods aiming to decrease the costs of equipment and complexity of procedures required, transcranial LIFU can support several unique approaches to functional brain mapping as discussed further below.

Because US is compatible with EEG, MRI and other standard neurophysiological assessments, the use of focused US for high resolution, non-invasive brain mapping represents a potentially transformative opportunity. Dallapiazza and colleagues (2017) showed that pulsed LIFU (0.22 – 1.14 MHz, 25 – 30 W/cm²) can be transmitted to sub-nuclei of the pig thalamus to functionally modulate somatosensory evoked potentials induced by the stimulation of different peripheral nerves [35]. Data from MR-thermometry combined with these neurophysiological observations confirmed LIFU can focally (1.14 MHz focal volume = 1 x 1 x 3 mm) modulate deep-brain activity for functional circuit mapping without causing tissue heating [35] (**Figure 3**). These observations are a critical step towards realizing the full potential of using LIFU to clinically map and functionally validate brain targets prior to DBS and other neurosurgical interventions. Excitingly, such non-invasive deep-brain mapping methods for basic research applications is also becoming a realistic possibility since it was recently demonstrated that transcranial LIFU can modulate the thalamus of healthy humans [38].

Table 1. Sample of NeuroFUS Brain Studies.

Authors	Af (MHz)	ISPPA (W/cm2)	PRF	PL/PD (msec)	DC (%)	SD	Target	Species	Outcomes
Tyler et al. 2008	0.44, 0.67	2.9	0 - 0.1 kHz	20 - 80	0-75	0.25 - 15 sec	Hippocampal slices	mouse	Directly stimulated action potentials,
Tufail et al. 2010	0.25, 0.5	0.07 - 0.3	1.2 - 3 kHz	20 - 300	19 - 86	0.02 - 0.3 sec	Ctx and hippocampus	mouse	Stimulated hippocampal and cortical single- and multi-unit activity including sharp wave ripple activity.
Tufail et al. 2011	0.25, 0.5	0.07 - 0.3	1.2 - CW	0.02 - 300	10-100	0.02 - 3 sec	Ctx and hippocampus	mouse	Stimulation of cortex; termination of KA-induced seizure activity.
Yoo et al. 2011	0.69	3.3 - 12.6	0.01	2	5	0.5 - 8 sec	V1, M1	rabbit	Stimulation of fMRI BOLD signal
Deffieux et al 2013	0.32	13.5	-	100	100	0.1 sec	FEF	monkey	Transcranial; modulation of saccades by targeting FEF in NHPs
Legon et al 2014	0.5	16.4	1 kHz	0.36	36	0.5 sec	S1	human	Suppression of evoked potentials
Lee et al. 2014	0.25	1.4 - 15.5	500 Hz	1	50	50-150 msec	S1/M1	sheep	Stimulation of evoked potentials
Lee et al 2015	0.25	3	500 Hz	1	50	0.3 sec	S1	human	Stimulation of sensory cortex + sensations
Dallapiazza et al 2017	0.22, 0.65, 1.14	23	10 Hz	43.7	43.7	40 sec	Thalamus	pigs	No skull; no heating confirmed by Insightec HIFU 20 W/cm2 for 20 sec
Lee et al. 2016	0.27	35	500 Hz	1	50	0.3 sec	V1	human	Stimulation of VEP's and fMRI BOLD signals
Wattiez et al 2017	0.32	5.6	-	100	100	0.1 sec	FEF	monkey	Transcranial; modulation of saccades and single-unit recordings in FEFs
Lee et al 2017	0.21	35	500 Hz	1	50	0.5 sec	Ctx	human	Transcranial s1 at two cortical sites simultaneously
Legon et al. 2017	0.5	14.56	1 kHz	0.36	36	500 msec	Thalamus	human	Modulation of human thalamus and evoked potentials.
Lee et al. 2018	0.6	2.3-14.9	500 Hz	1	50	300 msec	Ctx	rat	Wearable transducers for ultrasonic neuromodulation in freely moving rats.
Legon et al 2018	0.5	11 to 17.12	1 kHz	0.36	36			human	Transcranial; safety analysis 120 subjects from 7 experiments
Ai et al. 2018	0.5	16.05	1 kHz	0.36	36	0.5 sec	M1	human	Transcranial; 7t fMRI BOLD signal modulation
Pang et al 2018	0.25	29.5	2 kHz	0.25	50	300 msec	Ctx	monkey	Stimulation of fMRI BOLD signal
Yang et al. 2018	0.25	29.5	2 kHz	0.25	50	300 msec	Ctx	monkey	Stimulation of fMRI BOLD signal
Zhou et al. 2018	3.8	1	1 kHz	-	50	1 sec	STN	mouse	Behavioral and cytochemical improvements in MPTP model of ET/PD
Sharabi et al. 2019	0.23	27.2	0.3 Hz	100	3	52 sec	Ctx	rat	Reduced tremor in MPTP model of PD/ET
Folloni et al. 2019	0.25	18 - 64	10 Hz	30	30	30 msec	ACC, Amygdala	monkey	Long lasting changes in network activity by fMRI BOLD imaging
Verhagen et al. 2019	0.25	24 - 31	10 Hz	30	30	30 msec	SMA, FPC	monkey	Long lasting changes in network activity by fMRI BOLD imaging

Table 2. Sample of Peripheral NeuroFUS Studies.

Authors	Af (MHz)	ISPPA {ISPTA} (W/cm ²); [Pressure] (Mpa)	PRF	PL (PD)	DC (%)	SD	Target	Species	Notes
Gavrilov et al. 1977	0.48	0.4 to 2.5		0.1 to 100 msec	100	0.1 sec	pacinian corpuscle	frog	Activation of receptors
Foster and Wiederhold. 1978	5	30		68 microsec	100		auditory nerve	cat	Activation
Tsirulnikov et al. 1988	2.5	{1 - 5}	0.125 - 8 kHz	50 - 100 microsec	50		acoustic nerve	human	Activation of fibers
Moore et al. 2000	1 - 3	{1}			50 - 100	480 sec	median nerve	human	Increased conduction velocity
Tsui et al. 2005	3.5	{1 - 3}	2		< 1		sciatic nerve	frog	Enhanced conduction, increased AP velocity
Legon et al. 2012	0.35	{11 - 55}	70 - 100 Hz	2 - 10 msec	10-20	0.5 - 1 sec	peripheral receptors/nerves	human	Activation of mechanical and thermal sensations
Wahab et al. 2012	0.825	[0.1 - 0.7]	100 Hz		0.1	15-75 sec	giant axon	earth worm	Inhibition of conduction; decreased AP velocity
Juan et al. 2014	1.1	18.7-93.4	20 - 1000 Hz			15 sec	vagus nerve	rat	Inhibition of conduction; decreased AP velocity
Yoo et al. 2014	0.65	{1 - 3}	50 Hz		10	1 sec	accupuncture pts	human	Activation
Casella et al. 2017	0.25	[0.9]	2 kHz	63 cycles		300 msec	posterior tibial	rat	Bladder contraction
Ilham et al. 2018	1.1	{0.91 28.2}	200 kHz		20 - 40	40 sec	sciatic nerve	mouse	Increased conduction velocity
Downs et al. 2018	3.57	[0.7 - 5.4]	1 kHz		15 - 100	1-10 msec	sciatic nerve	mouse	Stimulation of AP's
Daniels et al. 2018		2.3-4.6					auditory nerve	rat, pig	Suppression of AEPs
Lin et al. 2018	2.1	[0.1 - 1.0]		5 msec	1	5 msec	axon	crayfish	Stimulated AP trains or depolarization

NeuroFUS for Peripheral Nerves

In addition to the modulation brain activity, US has also been shown capable of differentially modulating the activity of peripheral nerves in a variety of *in vitro* and *in vivo* experimental models (for reviews see Refs. [8,39-41]. Studies spanning several decades have shown US can modulate peripheral nerve activity for time periods lasting from milliseconds to days through different mechanisms depending on the intensity, frequency, and exposure times implemented. Some of the pioneering studies demonstrating that focused US could modulate the sensory functions of the peripheral nervous system activity were been conducted by Gavrilov and colleagues showing different ultrasonic stimuli could impair or induce a variety of sensations in human psychophysical tests [42,43]. In other investigations, electrophysiological recordings from frog sciatic nerves [44], crab leg nerves [45], earthworm giant axons [46] and others [8,40] have shown that US can reversibly modulate neural activity by exerting non-thermal actions (**Table 2**). Several years ago it was reported that NeuroFUS can directly modulate the activity of prominent peripheral or cranial nerves, such as the vagus [47]. These findings have recently given way to a host of new studies evaluating the potential of peripheral NeuroFUS in treating various disorders or diseases as discussed in more detail below.

There are several considerations that need to be highlighted when discussing peripheral NeuroFUS. For example, somatosensory receptors naturally encoding mechanical stimuli are responsive to US. In fact, studies have shown low-intensity US delivered to the hands of humans can differentially activate peripheral nerve structures and produce EEG, as well as fMRI BOLD activity patterns like those obtained using more conventional somatosensory stimulation methods [15]. Thus, one must be able to distinguish direct effects on peripheral nerve fibers from those on different neuronal structures, such as somatosensory system receptors. These neurosensory receptors and many fine nerve endings encoding pain reside just below the epidermis. As described in numerous studies, different NeuroFUS waveforms can produce different sensations and effects on nerves.

Experiments aimed at investigating how low-intensity, pulsed US may be acting on peripheral nerves to modulate activity have also begun to shed light on potential mechanisms. A recent study demonstrating the modulation of crab leg nerves by US seem to indicate that cavitation may be a mechanism in the periphery [45]. These observations may not reflect natural conditions since it can be incredibly difficult to control cavitation *in vitro*. In fact, evidence from others indicates cavitation is not a predominant mechanism when stimulating mammalian peripheral nerves *in vivo* [48]. This elegant study recently demonstrated that focused US can robustly stimulate rat sciatic nerves in a manner similar to electrical stimulation [48]. Downs and colleagues (2018) further demonstrated that brief (0.8 - 10 msec) pulses of 3.5 MHz focused US stimulate peripheral nerve activity in a manner that indicates acoustic radiation force is a likely mechanism of action. As discussed below, other recent evidence also indicates the influence of radiation forces are likely involved mechanisms. While it may not be necessary to fully understand the mechanisms of action before implementing the basic methods, it will certainly advance our ability to use NeuroFUS as our understanding of the underlying mechanisms increase.

NeuroFUS Mechanisms of Action

Considering the physical properties of neurons and their circuits, there are several ways in which NeuroFUS may act to influence their electrical activity. Further complicating matters, the interactions of US with fluids including biological tissues is complex. One straight-forward possibility however is that mechanical forces exerted by the acoustic pressure of NeuroFUS waveforms act on mechano-sensitive ion channels to alter neuronal activity. Initial data in support of this hypothesis came from observations that US can stimulate brain activity through a non-thermal mechanism involving the activation of voltage-gated sodium channels and calcium transients as previously mentioned [21]. Numerous studies have confirmed that LIFU can modulate neuronal activity without causing significant tissue heating [8]. Due to the experimental approaches used in these studies to assay activity however, whether the effects of US involve the direct

mechanical modulation of ion channels has remained obscure until recently.

Mechanistic investigations have indeed shown that LIFU (10 MHz, < 10 W/cm²) can modulate the activity of voltage-gated sodium channels (NaV1.5) and two-pore-domain potassium channels (TREK-1, TREK-2, and TRAAK) in *xenopus* oocytes [49]. Although demonstrated under unique conditions containing exogenous microbubbles, sonogenetic methods of activating TRP-4 channels in *c. elegans* has been shown a viable of regulating neuronal activity [50]. More convincing empirical evidence has recently shown that LIFU acts in a mechanical manner to modulate the activity of ion channels and neuronal activity [51]. Kubanek and colleagues (2018) conducted an insightful study in which they knocked out thermosensitive ion channels in *c. elegans* and found this did not affect behavioral responses to LIFU. When the authors knocked out mechanosensitive ion channels however, LIFU responses were abolished. Further, altering LIFU parameters to accentuate acoustic radiation forces produced by US elicited more robust responses [51]. These observations provide additional support to the hypothesis that LIFU acts, in part, by exerting mechanical actions through radiation forces on native ion channels.

Other complex physical mechanisms may also be involved in NeuroFUS. For example, it has been hypothesized that mechano-electric effects underlie the influence of LIFU on neuronal activity. More specifically it is believed that LIFU can induce the formation of bilayer sonophores, which are small regions of phospholipid membrane that experience expansions and contractions [52]. These bilayer sonophores (microscopic membrane deformations) produced by US could theoretically generate capacitive displacement currents leading to charge build-up occurring over the course of tens of milliseconds [52,53]. Computational models incorporating these basic mechanisms have describes in some cases how US differentially affects neural activity depending on several factors including the types of ion channels expressed, the targeted neurons, and the duty cycle of the NeuroFUS waveform used [53]. Whether or not these models continue to hold up to empirically obtained physiological observations remains to be determined. The possibility that such mechanisms may remain to be uncovered however, does indeed indicate that we should not exclude non-damaging cavitation as a putative mechanism of action. Beyond direct effects on electrical activity, low-intensity US has been shown to modulate the activity

of neurotrophic factors that could produce secondary effects on neural activity and plasticity [23]. Therefore, more studies are needed to refine our working models of how low-intensity US affects neuronal function versus activity. Large cross-disciplinary efforts aimed at solving these issues are likely to reveal some completely novel information about how NeuroFUS and its mechanical forces act to regulate neuronal activity and plasticity.

Safety of NeuroFUS

Over the past decade, numerous studies cited throughout this review have examined the safety of NeuroFUS methods in addition to observing basic physiological effects. Many of the studies in the literature have examined the safety profile of NeuroFUS by analyzing thermal margins, histopathological assays, cytochemical assays for cell death, assays for blood-brain barrier disruption, imaging studies of structure and function, behavioral assays and surveys [54]. Overall, it has been found that when used under proper conditions, NeuroFUS can safely modulate brain and peripheral nervous system activity without causing macroscopic heating or tissue damage [54,55].

Legon and colleagues (2018) performed a comprehensive safety analysis on data they collected across seven different studies in human participants [55]. They collected safety data during experiments on 120 subjects across the seven experiments exposed to different NeuroFUS protocols. There were no severe adverse events reported in the study. In fact, no studies using NeuroFUS or low-intensity pulsed ultrasound for neuromodulation to date have reported a severe adverse event. During their studies, Legon and colleagues (2018) recorded specific safety data from 64 of the 120 volunteers. Of these about 10% (7 of the 64) reported mild to moderate adverse reactions including anxiety, neck pain, difficulty paying attention, muscle twitches, scalp tingling, headache, and sleepiness that subsided following treatment (**Figure 4A**). None of the participants reported any of the symptoms to be 'directly' related to, but rather 'possibly' or 'probably' due to the NeuroFUS protocols implemented (**Figure 4B**).

When calculated across all possible adverse reaction/event positive responses, Legon et al (2018) observed a 4.3% positive response rate (55 out of 1280 possible). Further 69% (38 of 55) of the positive responses were reported to be unrelated to, 18% unlikely due to, 7% possibly due to, 5% probably due to, and 0% definitely related to the NeuroFUS

protocols used. Finally, they reported a significant correlation of response rates with the intensity (spatial-peak, pulse average; I_{SPPA}) of US across the ranges (11.56 – 17.12 W/cm² in free water) used. The estimated intensity reaching the brain targets after skull attenuation is estimated to be about 3 to 4 fold lower producing intensity values lower than the peak and temporal average outputs maximums recommended for diagnostic imaging by the FDA (I_{SPPA} = 190 W/cm², I_{SPTA} = 720 mW/cm²). These data demonstrate that NeuroFUS can provide a safe means of noninvasive neuromodulation when used properly.

more detail elsewhere [14,36,37,55]. There are several important factors readers must consider before attempting NeuroFUS experiments.

Because the skull readily absorbs ultrasound, it is more prone to tissue heating than brain or nervous tissues. It is essential to ensure NeuroFUS waveforms avoid target tissue heating, but also the heating of any intervening tissues such as bone or skull. Tissue heating can be minimized by using brief durations of ultrasonic pulses, as well as implementing an inter-stimulus interval of a several to tens of seconds. This will help ensure that the buildup of heat from US absorption is not an issue. The number of sonication events in any one experiment on an individual should also be minimized.

The only adverse events reported in the literature to date, microhemorrhages in sheep, used 0.25 MHz US at 6.6 W/cm² (I_{SPPA}) delivered 600 times once every second [30]. Whether or not this microhemorrhage was due directly to the treatment is not known. It is important to note that the mechanical index (a dimensionless number) that describes the mechanical effects on tissue increases and the likelihood of streaming or cavitation effects increases as the frequency decreases. Thus, using a higher acoustic frequency makes it less likely that cavitation or streaming damage may occur. In unpublished safety data collected in pigs (**Figure 2**) prior to pursuing our initial human studies, we found that 0.5 MHz NeuroFUS waveforms delivered at ten times the intensity used in our subsequent human experiments (with all other parameters equal) failed to exhibit any histopathological markers of cell death, edema, or microhemorrhage from data scored by an, independent, blinded, and experienced veterinary pathologist. Nonetheless, the microhemorrhage data from Lee and colleagues (2016) stresses the need for caution when designing NeuroFUS experiments.

For general safety, it is prudent to maximize the inter-stimulus interval, minimize the number and duration of exposures (i.e. As Low As Reasonably Achievable; ALARA), use brief pulse durations (0.02 to 100 msec) at moderate to high pulse repetition frequencies (10 Hz – 2 kHz) with NeuroFUS waveforms low duty cycles (< 50%) having an I_{SPPA} < 30 W/cm². These guidelines should be considered as a starting point as there are numerous studies in the literature for gaining more specific insights into the safe use of NeuroFUS.

Many reports employing NeuroFUS for transcranial brain applications have successfully used 70-100 sonications at 1 – 30 W/cm² lasting 0.5 sec separated by a 7-12 second inter-stimulus interval in

Figure 4. Acute safety outcomes from human NeuroFUS studies. (A) Histograms showing incidence of adverse reactions/ events rated as absent, mild, moderate, or intense severity in 64 subjects. (B) Histograms showing the subjective attribution of adverse reactions/ events as related to NeuroFUS being unrelated, unlikely, possible, probable, and definite. Figure panels adapted from Ref. [55].

Although the evidence to date has demonstrated convincingly that NeuroFUS is safe in acute use situations, appropriate safety precautions should always be taken when modulating brain or neural function with FUS since the full spectrum of safe and effective parameters are still being identified, optimized, and refined. We caution the readers to realize strict exposure limits, standard operating procedures, and technical guidelines were imposed in the human studies conducted to date as discussed in

humans and animals without producing any damage or reports of adverse events. In fact, more than 250 humans have been subjects in NeuroFUS experiments to date with no severe adverse events and only minor or moderate adverse reactions reported at < 10% rates [55], which are equivalent to those observed in TMS and transcranial electrical stimulation studies. Future studies should continue to make investigating the safety of NeuroFUS a priority. There are a number of unresolved questions related to the chronic safety of NeuroFUS and many studies will need to work toward defining the true safety envelope for

NeuroFUS. Chronic safety experiments should be conducted in several animal models prior to humans. For acute use, there is now sufficient data and evidence in the literature after the past decade of investigation on NeuroFUS however to support it as a low-risk or minimal-risk procedure when best practices are employed by properly trained individuals. Ongoing studies will need to continuously focus on designing measures for gaining further insights into the safety outcomes of acutely and chronic NeuroFUS.

Figure 5. NeuroFUS applications in the peripheral nervous system. (A) A functional prototype of a handheld, wireless 5 MHz POCUS imaging device is shown engineered with a single-element, broadband, 0.5 MHz focused ultrasound transducer for simultaneous or inter-leaved imaging and neuromodulation of peripheral nerve targets. In the particular application shown, POCUS imaging is used to localize the median nerve before delivering a pulsed, broadband NeuroFUS waveform to the median nerve. Such embodiments may be useful to treat inflamed or irritated nerves in neuropathies, carpal tunnel syndrome, radiculopathies, and other dysfunctions. (B) Averaged traces are shown from microneurography recordings of the median nerve before (blue) and after (red) NeuroFUS delivered with a different broadband 0.5 MHz focused ultrasound transducer showing suppression of evoked potential amplitudes. Panels (A) and (B) compliments of IST, LLC. (C) Illustration showing the principles of mid-air or touchless haptics for generating spatially and temporally discrete patterns on the hand. In this embodiment, NeuroFUS waveforms are transmitted from a phased array of 40 – 70 kHz ultrasound transducers to the body in a manner that affects the activity of neuronal structures including somatosensory receptors for communications, entertainment, and gaming applications.

Potential for NeuroFUS in Medicine

There is a critical need for new neuromodulation-based therapies and diagnostics in medicine. Numerous noninvasive and invasive neuromodulation methods have been used in the investigational or clinical treatment of almost every neurologic and psychiatric disorder imaginable. For numerous practical and technical reasons beyond the scope of this review, there have been many failures at demonstrating that neuromodulation treatment approaches can provide clinically significant benefits in psychiatry. In fact, electroconvulsive shock therapy remains one of the most effective neuromodulation-based approaches to treating debilitating psychiatric disorders like treatment-resistant depression (TRD). While transcranial magnetic stimulation (TMS) has been shown capable of treating TRD, the outcomes can certainly be improved upon. Outside of movement disorders and some pain syndromes, deep-brain stimulation of various brain targets has been used with mixed results depending on the disorder being treated.

Enhanced Brain Circuit Targeting by NeuroFUS

First it is essential to realize that for neuromodulation approaches to be effective treatments of neurologic and psychiatric disorders, brain regions and circuits should be targeted using functional signatures rather than anatomical landmarks alone. For example, targeting functionally localized prefrontal brain circuits using subject-specific realistic simulations of the electric field distributions generated by TMS pulses would likely improve clinical outcomes when TMS is used for treatment of TRD. The current methods of targeting TMS in clinical populations is poor to say the least. This is because subject-specific gyral curvatures and tissue specific anisotropies cause the electric field produced by TMS pulses to be uniquely shaped and distributed throughout cortex in a manner that cannot be easily or accurately predicted without knowing specific anatomical geometries beforehand [56]. This fundamental short coming of TMS highlights one potential advantage of NeuroFUS for medicine in that it can enable more accurate and precise targeting of brain structures.

The physics and mechanisms of action underlying NeuroFUS are not as affected by gyral

curvature (shape), tissue orientation, or tissue anisotropy as the physics and mechanisms underlying TMS (electromagnetic induction). Simply put, NeuroFUS waveforms are not as greatly influenced by small differences in tissue shape or orientation as electrical fields which makes targeting a bit more straight-forward. This particular property of NeuroFUS may prove an advantage by minimizing variability in outcomes arising from tissue/energy interactions. Perhaps the most obvious and biggest advantage of NeuroFUS over TMS and other noninvasive electrical-based neuromodulation methods is that US can be transmitted across the skull and focused to almost any location including deep-brain targets. This advantage immediately opens the potential for functionally mapping deep-brain targets using NeuroFUS as a tool for clinical interventions and diagnostics (**Figure 3**).

NeuroFUS as Support or an Alternative to DBS

Deep-brain stimulation for psychiatric disorders has proven to be a difficult therapeutic platform to advance. This difficulty was most recently displayed when two different randomized clinical trials failed to demonstrate efficacy of DBS for the treatment of TRD [57], [58]. These trials targeted the ventral capsule/ventral striatum [57] and the ventral anterior limb of the internal capsule [58] with DBS electrodes to treat TRD. Other clinical trials and studies using DBS targeted to different brain regions including the nucleus accumbens, subgenual cingulate cortex, lateral habenula, inferior thalamic nucleus, and medial forebrain bundles for the treatment of TRD have also been wrought with similar shortcomings or lack appropriate controls to make reliable inferences [59]. One of the major problems facing DBS therapies in psychiatry is that emotion and mood tend to be more diffusely localized in the brain making target identification/localization difficult. Therefore, it has been proposed that DBS electrodes should be targeted to brain circuits that have been localized using functional neuroimaging approaches rather than anatomically localized [60,61].

The issues raised above highlight the critical need for a noninvasive neuromodulation method capable of reaching deep-brain targets with a high spatial resolution. As discussed above in this review, NeuroFUS can be focused to deep-brain regions.

Furthermore, the spatial resolution is about the same size as the spatial extent of electric fields generated by standard DBS electrodes. NeuroFUS methods are compatible with MRI and have been used to focally stimulate and modulate human BOLD responses at both 3T and 7T [37,62]. Besides directly treating affective or mental health disorders, the most logical application of NeuroFUS for psychiatry and neurosurgery is its use to interrogate and modulate potential DBS targets during functional neuroimaging experiments combined with measures of behavioral outcomes and neurophysiological assessments. In such embodiments NeuroFUS could enable exhaustive pre-surgical mapping and surgical planning studies in order to identify the best targets for treating a particular psychiatric or neurologic disorder in a highly personalized manner. Whether or not such approaches will help improve the clinical outcomes of DBS-based psychiatric therapies or not needs to be thoroughly investigated given it can be a game changer for neuromodulation-based medicine.

It has been well-established that DBS is effective for treating movement disorders, such as essential tremor and Parkinson's disease. Other forms of surgically-implanted cortical stimulators have been shown effective for treating epilepsy, pain, and other neurologic disorders. Interestingly, one of the early demonstrations that NeuroFUS could modulate intact brain circuits was provided in studies showing low-intensity, transcranial ultrasound can rapidly terminate kainic acid-induced, electrographic seizure activity [24]. These data suggest that NeuroFUS may be a powerful tool in treating *status epilepticus* in neurocritical care situations, as well as treating other forms of epilepsy. Wearable microelectromechanical systems (MEMS) fabricated as piezoelectric micromachined ultrasonic transducers (pMUTS) have begun to show promise for treating movement disorders, such as essential tremor by conducting NeuroFUS of brain and peripheral nerve targets in mice and rats. These transducers are energy efficient and engineered in manner that allows them to be body-mounted or worn for successfully treating models of neurologic and psychiatric disorders in animals and pave the way for similar embodiments in human patients [63-66].

NeuroFUS in Bioelectronic Medicine Applications

Studies have shown that low-intensity ultrasound (1.1 MHz, 14 – 93 W/cm²) can modulate the rat cervical vagus nerve and has opened several therapeutic possibilities given the widely recognized potential of vagal nerve modulation in treating inflammatory disorders, auto-immune disorders, neurologic, and psychiatric [47]. In other circuits, modulation of the tibial nerve activity by low-intensity US has been shown to affect rat micturition reflexes paving the way towards the development of ultrasonic devices for controlling bladder function [67]. Other studies directly measuring nerve responsivity to US indicate peripheral NeuroFUS will have clinical applications in neuromodulation and bioelectronic medicine [68]. Other potential clinical applications may support neuro-rehabilitation therapies and physical medicine or have implications for advanced prosthetics because low-intensity US has been shown to enhance nerve regeneration following nerve injury [69] and nerve grafts in rats [70].

Most recently in two independent studies led by General Electric Research and Medtronic using low-intensity NeuroFUS methods, it was shown that pulsed ultrasound targeted to nerve fibers innervating the spleen and liver can alter neurocellular and neuronal structure activity to produce significant therapeutic benefits in animal models of auto-immune disorders such as rheumatoid arthritis, as well as metabolic disorder like diabetes and obesity [71-74]. These data further bolster the position that NeuroFUS can be used as an alternative to bioelectronic medicine methods where surface (transcutaneous) or implanted electrical peripheral nerve stimulators have been used to provide therapeutic benefits.

State-of-the-art NeuroFUS Engineering and Development

Recently there have been several significant innovations in the fields of physical acoustics, materials engineering, and ultrasonics that can be integrated with basic NeuroFUS approaches to advance state-of-the-art neuromodulation and brain mapping tools.

NeuroFUS and Imaging Methods

One of the most logical manners by which these advances can have a near-term impact on brain mapping is through combining recently developed

Improving NeuroFUS Spatial Resolutions

Improving upon the spatial resolution of NeuroFUS is another area where advances are continuously being made despite the limits of physics. As mentioned previously, the diffraction limited spatial resolution of NeuroFUS is a function of the acoustic frequency or wavelength of a particular frequency in a tissue. For transcranial NeuroFUS applications where US has had to be transmitted across intact human skull bone, the acoustic frequencies have ranged from 0.7 to 0.3 MHz yielding theoretical spatial resolutions of about 2 to 7 millimeters respectively. Quantitative measurements of HIFU-induced thalamic lesions in humans [6,79] and functional localization of cortical NeuroFUS in humans have shown the actual spatial resolution of tFUS to be about 4 to 10 millimeters [14,36,37]. It was recently shown that when using mice as experimental models, higher US frequencies could be transmitted across their thin skulls with power sufficient to stimulate brain circuits at functional spatial resolution of about 0.3 millimeters for 5 MHz tFUS [80].

Some methods have been developed to improve upon NeuroFUS spatial resolutions. A particularly interesting method implemented a beat frequency of 0.5 MHz by transmitting modulated higher, carrier frequencies such as 2.0 and 1.5 MHz US across rodent skulls to stimulate cortical activity [81]. This is an interesting approach to optimizing the spatial targeting of NeuroFUS, which demonstrated feasibility in animals and warrants further investigation in humans to understand the limits for using mixed combinations of high carrier frequencies in various applications that require US transmission across the skull. Advances in acoustic metamaterials and acoustic hyperlenses have enabled super-resolution acoustic imaging over the past decade by producing sub-diffraction US [82,83]. Whether such advances in acoustic metamaterials [82], hyperlenses [83], sound bullets [84], or propagation invariant acoustic field needle beams [85] can enable super-resolution NeuroFUS is not yet known, but most certainly worth exploring since early indications are they can be useful for enabling unprecedented spatial control of both superficial and deep-brain circuit activity, simultaneously in a manner that is noninvasive (**Figure 6A,B**).

Holographic Interfaces for NeuroFUS

One of the most interesting technical developments in acoustics recently has been the emergence and demonstrations of acoustic holography or holographic US [86]. This also brings us to perhaps one of the most fascinating embodiments of NeuroFUS, which involves the delivery of holographic US through an acoustic retinal prosthetic device capable of generating multi-focal, patterned neurostimulation of retinal circuits to convey fine spatial visual information [87,88] (**Figure 6C**). Similarly, generating acoustic holograms for transcranial NeuroFUS may enable the projection of structured US into brain circuits for the multi-focal, patterned neuromodulation of brain circuit activity.

Imagine a situation where one may wish to noninvasively and precisely replicate the flow of somatosensory information throughout the brain. This would require that sparsely distributed regions of both deep and superficial brain circuits (for example, regions of the thalamus, somatosensory cortex, prefrontal cortex, hippocampus, and amygdala) be synchronously and sequentially stimulated and modulated in a precisely timed manner. In other words, NeuroFUS would need to produce effects on circuits in many different brain regions at exactly or nearly the exact same time. Such an embodiment of NeuroFUS seems conceptually possible by projecting dynamically structured acoustic fields or ultrasonic holograms into the brain. In fact, recent studies have shown that arbitrary ultrasonic fields can be focused into the brain as holographic projections for NeuroFUS [89]. Similar approaches have also been described for peripheral NeuroFUS in embodiments of touchless or mid-air haptics (**Figure 5C**).

Touchless haptics, mid-air haptics, or ultrahaptics are different terms that use air-coupled US transducers to transmit sound pressure waves at peripheral neuronal structures and receptors to generate spatially or temporally patterned sensations (**Figure 5C**). Whether air-coupled or body-coupled, the conversion of the mechanical displacement energy (sound waves) on neuronal structures and receptors by NeuroFUS results in the processing of natural sensations as indicated by psychophysical testing, EEG, and fMRI BOLD imaging studies [15]. Mid-air haptic devices have numerous applications in medicine but have primarily been explored in human communications and entertainment applications thus

far. They have been studied as human-computer interfaces [90], incorporated large displays [91], touch display surfaces [92], to enhance movie watching experiences [93], and in multi-sensory art museum exhibits [94]. These applications provide initial feasibility that mid-air or touchless haptics using NeuroFUS can be used to transmit, convey, or provide medical information to physicians, patients, caregivers, and families in clinical applications. Further engineering is required and is underway by many groups around the world to make mid-air haptics systems scalable and adoptable. The generation of new classes of transducers or the improvement of existing technologies is sure to help.

Microfabricated NeuroFUS Transducers and Other Applications

Transducers, such as capacitive or piezoelectric micromachined ultrasonic transducers (CMUTs or PMUTs), can be fabricated in many different array shapes and sizes [95,96]. These microfabricated arrays can be used in nested structures to be able to focus in holographic patterns or multiple treatment areas simultaneously as mentioned. These transducers controlled by integrated circuits or application specific integrated circuits (ASIC) offer lightweight and power efficient methods for delivering NeuroFUS waveforms to neuronal structures or neurocellular sites in the brain or peripheral nervous system in a manner that allows them to be body-mounted or worn [63-66].

Other types of piezo-electric polymers or materials including CMUTs and PMUTs can be incorporated into head-mounted devices for brain-computer interfaces or to regulate or modulate human computer interactions. These transducers also enable incorporation into wrist watches, headphones, mobile devices like phones, smart phones, tablets, and laptops for various applications of NeuroFUS including for haptic interfaces or communication platforms as described to treat, diagnose, or manage medical disorders and diseases.

Conclusions

NeuroFUS represents a relatively new way of achieving focal neuromodulation that has evolved greatly over the past decade. The observations made in the field thus far have demonstrated that low-intensity US can reversibly stimulate and modulate intact brain circuits through nonthermal mechanisms of action. More work is required to unravel the optimal NeuroFUS parameters for modulating and stimulating brain or peripheral nerve activity. Likewise, understanding the mechanisms of action will require additional multidisciplinary investigations conducted across a variety of experimental preparations and conditions. Continuing to identify the safe parameters for NeuroFUS applications is also imperative. Despite the efforts that remain ahead, the foundation has been laid and it is anticipated NeuroFUS will continue to grow and mature as an indispensable tool for neuroscience research and medicine.

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Conflicts of Interest Disclosure

WJT is the inventor and co-inventor of issued patents and patents pending in the USA and internationally (Japan, Israel, Netherlands, France, Spain, Italy, U.K., Germany, India, Canada, Hong Kong, China, and others) covering ultrasonic and bioelectronic neuromodulation methods, systems, and devices. WJT is an equity holding co-founder of IST, LLC.