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REVIEW

A review of low-intensity focused ultrasound pulsation

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With the recent approval by the Food and Drug Administration (FDA) of Deep Brain Stimulation (DBS) for Parkinson's Disease, dystonia and obsessive compulsive disorder (OCD), vagus nerve stimulation (VNS) for epilepsy and depression, and repetitive transcranial magnetic stimulation (rTMS) for the treatment of depression, neuromodulation has become increasingly relevant to clinical research. However, these techniques have significant drawbacks (eg, lack of special specificity and depth for the rTMS, and invasiveness and cumbersome maintenance for DBS). This article reviews the background, rationale, and pilot studies to date, using a new brain stimulation method—low-intensity focused ultrasound pulsation (LIFUP). The ability of ultrasound to be focused noninvasively through the skull anywhere within the brain, together with concurrent imaging (ie, functional magnetic resonance imaging [fMRI]) techniques, may create a role for research and clinical use of LIFUP. This technique is still in preclinical testing and needs to be assessed thoroughly before being advanced to clinical trials. In this study, we review over 50 years of research data on the use of focused ultrasound (FUS) in neuronal tissue and live brain, and propose novel applications of this noninvasive neuromodulation method.

Keywords ultrasound; neuromodulation; neuroimaging; brain; treatment

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As early as 1955, Fry predicted that focused ultrasound (US) would have a major impact on neurology, not only for surgical treatment of chronic pain and Parkinson's disease,[1] but also for investigating structure and function of brain circuitry. Since then, he and others have used high-intensity focused ultrasound (HIFU) to irreversibly ablate tissue in localized brain areas for movement disorders and chronic pain[2] without observable damage to intervening tissue[3] or vasculature.[4] Recently, transcranial magnetic resonance guided HIFU (tcMRgHIFU) was used to localize and ablate tissue for relief of idiopathic chronic pain.[5] This line of research shows that HIFU can focally ablate neuronal tissue.

In contrast to HIFU, the effects of low-intensity focused ultrasound pulsations (LIFUP) on neurons are reversible, and make possible both neuronal excitation and inhibition. Considering the volume of work on HIFU, LIFUP application to neuroscience remains surprisingly unexplored, despite the fact that early work LIFUP and HIFU occurred in parallel.[6] Coupled with functional neuroimaging, LIFUP could be used as a steerable neurostimulation device to deliver focused US pulses for both reversible excitation and suppression of neuronal activity (Figure 1). Given the potential impact for LIFUP in both clinical and scientific brain mapping and functional connectivity, we seek to provide a comprehensive review of the current state of LIFUP neuromodulation.

Current field of neuromodulation

Neuromodulation technology offers an advantage over pharmacology treatment because its influence on neuronal circuits is more direct and focal. These features make it appealing to neuroscientists and clinicians, as it can be used to diagnose malfunctioning circuits and modify the biologic mechanisms underlying neurologic and psychiatric disorders. The development of modern high-resolution neuroimaging techniques makes the application of neuromodulation treatment even more appealing, as some of these imaging techniques (eg, fMRI, diffusion tensor imaging) might be used to visualize specific neuronal circuits, identify pathologic changes, and target them for treatment via neuromodulation. Neuroimaging can also monitor the effect of stimulation both during and after the neuromodulation procedures.

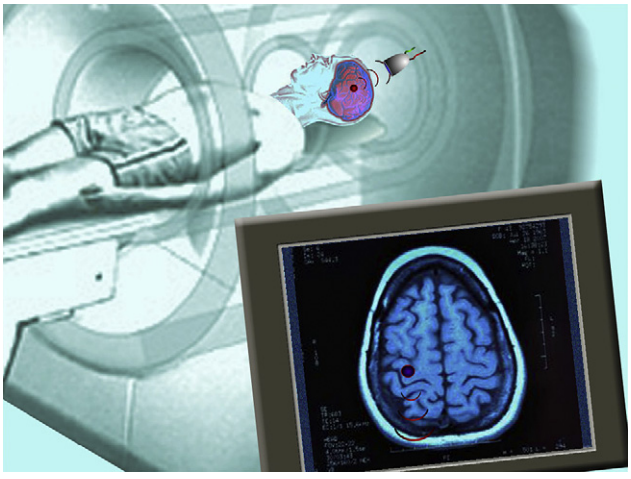

Figure 1 The proposed LIFUP used simultaneously within rtfMRI imaging. Low intensity focused ultrasound pulsation will be send to a specific point within the brain, targeted by MRI and the responses from the focus and other points in the brain functionally connected to the focus will monitored by rtfMRI.

Neuromodulation, in various guises, is used currently to treat many disorders. Several types of surgical, invasive neuromodulation techniques are on the United States market or nearing the market: vagus nerve stimulation (VNS)[7-9]; deep brain stimulation (DBS)[10-15]; implanted electrocortical stimulation (IES) and epidural cortical stimulation (ECS).[16] Several minimally or noninvasive neuromodulating technologies exist as well, including repetitive TMS (rTMS)[17-21]; cranial electrotherapy stimulation (CES),[22] transcranial direct current stimulation (tDCS),[16,23] and trigeminal nerve stimulation (TNS).[24]

Advantages of LIFUP for neuromodulation and brain stimulation

The currently available neuromodulating and brain stimulating techniques have a variety of important limitations. Table 1 summarizes the advantages and disadvantages of the two most frequently used techniques, and their comparison to LIFUP. For example, DBS requires relatively complex neurosurgery,[25] and neurostimulators periodically require battery replacement. VNS is also invasive, and its spatial resolution is poor.[9] Although CES and tDCS are considered noninvasive, they have poor spatial resolution, and their value in brain mapping is doubtful.[23] DBS coupled with fMRI has been problematic for safety reasons: although its spatial resolution is excellent, the location of stimulation cannot be changed easily and the simultaneous use with fMRI is cumbersome [26,27] and potentially hazardous.[28-30] Similar to CES and tDCS, the spatial resolution of noninvasive rTMS is poor. The typical focus is several centimeters in diameter, and the method cannot target deeper structures without stimulating more proximal tissue. In addition, the brain penetration is minimal, unless using a deep (H-coil), which has an even larger focus than traditional coils.[31] Furthermore, the use of simultaneous rTMS/fMRI for brainmapping entails technologic problems that are not resolved easily.[32-34] Nonetheless, some progress in this area of research has been achieved,[35,36] and neuronavigation using prestimulatory fMRI has been used in psychiatric and neurologic disorders.[37,38] A noninvasive brain stimulation technique that would overcome these difficulties would be extremely beneficial for further development of the field.

Table 1 Comparison of LIFUP with other common neuromodulation treatment modalities

	Stimulation modality		
Biophysics	Deep brain stimulation (DBS)	Transcranial magnetic stimulation (TMS)	Focused ultrasonic pulsation (LIFUP)
Energy delivery	Electrical	Magnetic	Mechanical (most likely)
Invasiveness	Invasive	Noninvasive	Noninvasive
Stimulation source	Voltage/current source + electrical conducting probe	Alternating magnetic field	Low intensity pulsating ultrasound
Stimulation configuration	Implantable electrodes	Magnetic coils	US transducer
Biophysical principle	Direct conduction	Faraday induction	Ion channel alternation
Spatial resolution	Fractions of mm	~3-5 cm	2-5 mm
Depth of penetration	Unlimited	~1-1.5 cm unless H coil is used	10-15 cm or more
Duration of the neuromodulation effect after the stimulation is stopped	~5 s	~5 s	Possibly ~10-40 mins
Use with fMRI for brain mapping (simultaneously)	Possible but difficult	Possible but difficult	Could be used simultaneously
Use with fMRI to guide the treatment and evaluate the effect	Used for implantation	Has been used	Could be used

In 2002, while conducting neuroimaging experiments in psychiatric patients, we proposed that LIFUP could be used for neuromodulating purposes (Bystritsky, 2002, USPTO 7,283,861).[39] US waves are mechanical undulations that are above the threshold for human hearing (approximately 20 kHz). US has been used widely in the body and brain for diagnostic purposes,[40,41] as well as therapeutic purposes, such as thrombolysis.[41-43] Low-intensity US, as used in Doppler imaging, appears to be safe in adults,[44] but this has been recently questioned in fetal evaluations.[45] US can be directed using specially designed transducers to a focus of only a few millimeters in diameter.[46] For some time, it also has been known that US waves can penetrate the skull and be focused within the brain for ablation purposes, using the thermal properties of HIFU.[47-49] Because US energy is mechanical rather than electromagnetic, its simultaneous use with fMRI for high-spatial resolution brain mapping is relatively straightforward. When HIFU is used therapeutically, determining significant biologic effects on the brain and precise spatial resolution of effected loci are vital for fMRI image guidance and feedback responses. Ablation with HIFU, guided by fMRI, has been used experimentally for pain,[5] and for eradicating brain tumors.[50]

Potential challenges

There are several questions that need to be answered before the use of LIFUPs in humans for diagnostic and therapeutic purposes can become a reality. First, does US have reversible effects on neuronal conductivity, and what is the nature of the effect? Second, can LIFUP produce effects on brain tissue that are visible via fMRI? Third, what parameters (ie, intensity, frequency, and duration of US bursts and the length of interpulse interval) should be used for either stimulation or inhibition of neuronal tissue? Fourth, is it possible for LIFUP to penetrate the skull similarly to HIFU? The answers to all of these questions have been addressed recently.

Early work on LIFUP

Although the first attempts to study the effect of US on neuronal tissue began nearly 80 years ago (Harvey et al, 1929),[51] systematic scientific exploration of this field did not start until the 1950s. At that time, several articles, in different languages, described the effect of US on neuronal tissues,[52-57] with the most relevant English language articles arising from Fry's laboratory.[3,4,6,58] The above studies demonstrated that US could induce reversible physiologic effects on nervous tissue, ranging from increased activity[56] to reversible suppression of visual evoked potentials.[4] Notably, Fry's studies documented both excitation and inhibition of neuronal tissue without concomitant histologic changes in the sonicated area.

Between 1960 and 1990, only a few papers were published on this topic. Much of the data came from the laboratory of Gavrilov in Moscow.[2,59-62] These reports showed that FUS, in both humans and animals, was capable of stimulating inner ear structures, as well as the auditory nerve directly. With respect to safety, these studies further documented reversible neuromodulation with US, without observable damage of neuronal tissue. Several other laboratories, also exploring the effects of US on neuronal tissue, found similar results.[63-66] All of the above studies found reversible enhancement, or depression, of neuronal activity in brain slices or in peripheral nerves of animals and humans, without histologic findings characteristic of thermal damage or cavitation.

The 1990s saw increased interest in the use of FUS for several practical applications: use of HIFU for ablation,[47,67] stroke thrombolysis,[68,69] and peripheral nerve blocking.[70-72] Interest increased with the discovery that FUS can be used also to open the blood brain barrier (BBB), and deliver drugs to the brain focally.[73,74] Disrupting the BBB with US could be done with or without use of a contrast agent that enchases the cavitations at lower power of ultrasonic application. Disruption of the BBB at lower powers was usually reversible, and accompanied only by minimal evidence for apoptosis and ischemia. Although disruption of the BBB opens another chapter in direct drug delivery to specific areas within the brain, a full review of this topic is beyond the scope of this manuscript.[75] Low-energy FUS has also been effective therapeutically for accelerating postfracture healing time in bone (for a review meta-analysis.[76] However, functional modulation of brain activity remains one of the most interesting possible applications of FUS.

Recent experimental literature

The last decade has seen increased research on the neuromodulating properties of LIFUP. With advancements in multiarray transcranial transmission of US and real time functional imaging for guidance, the possibility of LIFUP for human brain mapping is approaching rapidly. Tsui et al.[77] found US parameters that lead to modulation of action potentials in peripheral nerves. Shorter duration pulses of US seem to activate, whereas longer pulses seem to inhibit, the amplitude and velocity of action potentials.[77] A recent publication by a Arizona State University group not only confirmed reports of LIFUP induced neuromodulation in mouse hippocampal preparations, but also offered insight into possible mechanisms of this effect, such as influencing voltage gated sodium and calcium channels.[78] Later, a paper by the same group[79] described for the first time neuromodulation using US pulsation in vivo in the motor cortex of mice. Using LIFUP focused on motor cortex, they were able to evoke movements of the paws during transcranial stimulation. In this same report, when focusing LIFUP on the hippocampus, they were able to increase spike activity in CA1. In both cases, they found no evidence of BBB disruption or apoptosis.

Several studies on modulation of nerve conduction by FUS were recently reported from the Brigham and Women's Hospital, Harvard Medical School (see below). In a recent paper, Colucci et al.[80] investigated the safety thresholds for conductivity suppression using LIFUP in the sciatic nerve of bullfrogs. The authors determined that stimulation of the nerve with FUP could suppress conductivity reversibly for up to 45 minutes. Some of the effects were found to be thermally mediated, and some could not be explained by the thermal suppression. Specifically, nonthermal effects were present with low frequency sonication (690 kHz). Yoo et al.[81] investigated LIFUP for an in vivo real time functional MRI (rtfMRI) neuromodulation study in rabbits (n = 19). LIFUP caused observable changes in the blood oxygenation level dependent (BOLD) fMRI signal, but did not interfere with the recording. Both activation and suppression of the BOLD signal could be achieved by varying FUS pulse parameters. Visual cortex responses to a strobe light stimulation could be suppressed reversibly for up to 11 minutes (Figure 2) without causing BBB or tissue damage on postmortem histologic analysis.[81-83]

Since then, the Brigham and Women's Hospital group have confirmed BOLD signal suppression using EEG visual evoked potentials (VEP), and studied the suppression effect on epileptic seizures.[83,84] The rat pentylenetetrazol (PTZ) seizure model was used, wherein the rats were injected with PTZ and then underwent LIFUP stimulation to suppress the seizures. The results from a study of 30 animals reveal that low-intensity, pulsed FUS sonication suppressed the number of epileptic signal bursts observed in EEG recordings after the induction of acute epilepsy via intraperitoneal injection of PTZ. These finding suggest a potential role for LIFUP in the treatment of epilepsy, but this has not yet been tested in human experiments.

Possible cellular mechanisms of US induced neuromodulation

US introduces a mechanical pressure wave as it traverses through tissue.[85] Despite being the subject of study since the original work by Harvey et al.[51] the underlying origin of ultrasonic neuromodulation remains unclear. Given the nature of the US waveform, cavitation, thermogenic effects, and mechanical agitation represent the three possible cellular mechanisms by which US may exert its effects.[86] Therapeutic US can be defined as either low or high intensity, with the cellular responses varying greatly depending on the US parameters.[87]

At high intensities, the bioeffects of US are primarily thermal,[77] and these heating effects have been shown to

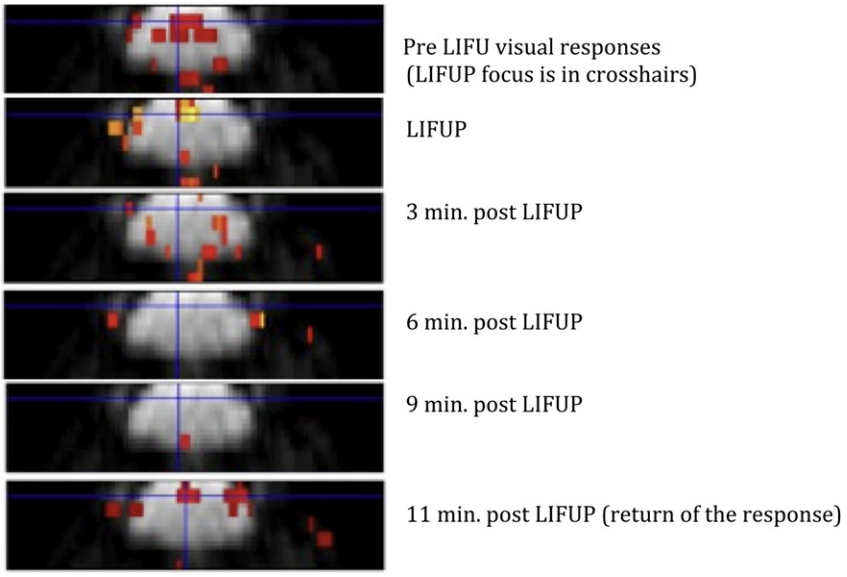

Figure 2 The sequence of rtfMRI recorded LIFUP suppression of the rabbit's visual cortex responses to strobe light stimulation (as described in[83]).

homogenize tissue, denature protein,[88] and cause DNA fragmentation.[89]

In contrast, the effects of LIFUP are thought to be nonthermogenic in nature. For example, Heckman et al.[90] and Gavrilov et al.[91] suggested that neuromodulation by low-intensity US delivered as short pulses could reduce the time-average power deposition to tissue. They proposed that this method might alter neuronal transmission and cause action potential changes by mechanical, rather than thermal, means. Bachtold et al.[92] documented that FUS pulses altered action potentials in hippocampal slices in rats.[91,92] Rinaldi et al.[66] reported that evoked potentials from the rat hippocampus can be attenuated by LIFUP.

Cavitation, or the formation of gas bubbles that explode generating shockwaves, has been considered as a possible mechanism of neuromodulation.[93] However, early work by Wall and colleagues[94] also demonstrated that cavitation was not a major cause of US induced neuromodulation. In this and other papers, histologic analysis of tissue confirmed that low-energy stimulation of neuronal tissue did not produce cell damage characteristic of either cavitations or high-energy thermal damage. Earlier, Mihran et al.[95] demonstrated excitability and then reduction in action potential in frogs' sciatica nerve preparation by short (0.5 millisecond) bursts of FUS. This effect was also determined to be of nonthermal, mechanical nature.

For quite some time, it has been known that neuronal soma and fiber tracts, as compared with grey matter or blood vessels, are more susceptible to the effects of US.[1,96] This differential susceptibility is consistent with recent hypotheses that US induced neuromodulation occurs via mechanical stretch of the lipid bilayer.[78] This is particularly interesting since the gating kinetics of many voltage-gated ion channels are also responsive to a stretch component.[97] In addition, mechanosensitive channels respond to changes in the local fatty acid content in the lipid bilayer within their transmembrane domain. These ubiquitous channels typically transduce osmotic stress stimuli into ion fluxes and may be activated via LIFUP induced mechanical stress.[98]

The excitatory effects of LIFUP may also be modulated by mechanical and stretch activation of voltage-gated Na+ channels, which can in turn lead to depolarization and excitatory activity. Furthermore, TTX, a Na+ channel blocker, seemed to attenuate these effects.[78] Calcium channels, which also have a stretch component,[99] may also be altered by LIFUP, as evidenced by a reduction in calcium transients with the addition of cadmium in divalent cation form.[78] Inhibition of action potentials may also be stretch mediated. In preparations of rat sciatic and dog peroneal nerves, both increases and decreases in compound action potentials were apparent after application of mechanical stress.[100]

There is also considerable interest in the vasodilatory effects of ultrasound, which are thought to be mediated through nitric oxide release.[101,102] These effects could possibly contribute to increased activation of the tissue, as well as enhance the BOLD signal generated by LIFUP neuromodulation, and need to be further investigated.[103]

Safety issues

The safety of FUS has been assessed in multiple experimental papers (Table 2). In sharp contrast to HIFU, none of the studies using LIFUP suggest any problems with either histologic, BBB, or behavioral data. Although earlier

Table 2 Summary of ultrasound parameters and results from selected papers

Author	Year	Ultrasound parameters	Result	Safety considerations
Colucci et al.	2009	Organism: Frogs. Frequency: 0.661 and 0.1986 MHz. Duration: continuous for 30 s, or pulsed at 1 ms or 10 ms at 10 or 20 Hz Energy: 100-875 W/cm^2; (LIFU to HIFU)	Decrease in sciatic nerve action potentials.	Histology revealed little or no damage.
Foley et al.	2008, also in 2004 and 2007	Organism: Rats Frequency: 5.7 Mhz (high frequency) Duration = 5 s exposure Energy = I_{sptp} = 280-8200 W/cm^2 (HIFU)	Exposure dependent effects on compound muscle action potentials.	Lowest exposure showed no histological variation from controls. Intermediate exposure (2255 W/cm^2; 5 s) showed minor axonal disruption. Highest exposure (3310 and 7890 W/cm^2; 5 s), showed fewer axons, some hemorrhagic regions present, and necrosis.
Fry et al.	1958	Organism: Cats Frequency: Not specified Duration: 20-120 s exposure Energy: Not specified – (LIFU)	Stimulating in LGN leads to reversible suppression of visually evoked potentials in visual cortex	No hazardous effects mentioned
Gavrilov et al.	1977	Organism: Frogs Frequency: 0.48 MHz Duration: 1 ms or 100-1600 Hz for 20 ms. Energy: 0.01-2.5 W/cm^2 (LIFU)	Evoked response in midbrain auditory area of the frog. For a 1 ms pulse responses were detected with a minimum of 0.1 to 1.0 W/cm^2. Pulses of 100 ms only required 0.01-0.1 W/ cm^2.	No hazardous effects mentioned
Gavrilov et al.	1996	Organism: Humans Frequency: 0.48-3 Mhz Tactile paradigm - Duration: 1-100 ms stimulus Energy: 8-4500 W/cm^2; (LIFU to HIFU) Auditory paradigm: Duration: Amplitude modulation or pulse modulation (pulse width 0.05 ms-0.1 ms) at 125-8000 Hz	Tactile - Induction of tactile, pain, and temperature sensation in the skin Auditory - Stimulation of hair cells and auditory nerve fibers	No hazardous effects mentioned (though heating of several 10 s of degrees was mentioned in reference to other reports (eg, Gavrilov et al.1976 and 1980)
McDannold et al.	2005	Organism: Rabbits Frequency: 1.63 MHz Duration: Burst length of 100 ms at 1 Hz for 20 s Energy: Pressure amplitude 0.7 to 1.0 MPa (Note: Injection of ultrasound contrast agent) HIFU	Disruption of blood brain barrier	Mild inflammatory response, almost no apoptosis or ischemia

Rinaldi et al.	1991	Organism: Rats Frequency: 0.75 MHz (center frequency) Duration: 6 μs pulse at 150 kHz for 2-15 min Energy: I_{spta} = 80 W/cm^2 HIFU	Significant reduction in extracellular field potentials in hippocampal slices, with varying degrees of recovery.	Average temperature changes less than 1°C
Sheikov et al.	2004	Organism: Rabbits Frequency: 1.5 MHz and 1.63 MHz Duration: Burst length of 100 ms at 1 Hz for 20 s Energy: 0.55 or 3 W/cm^2 Note: Injection of ultrasound contrast agent that increases cavitation facilitating the tissue disruption at LIFU energy.	Peak temperature change measured during 3 W was 2°C, below level for thermal damage. BBB was opened effectively with 0.55 W, while largely preserving the tissue ultrastructure.	After 0.55 W/cm^2, category 1-2 of tissue damage, and no signs of cell death. Moderate to severe damage to vasculature from 3 W/cm^2.
Tsui et al.	2005	Organism: Frogs Frequency: 3.5 MHz (center frequency) Duration: 5 min Energy: 1-3 W/cm^2 LIFU but continuous stimulation –not pulsation	Increase in peak-to-peak amplitude of compound action potentials, and increase in nerve conduction velocity.	Temperature increase by 3°C for lowest energy, and 10°C for highest energy.
Tufail et al.	2010	Organism: Mice Motor paradigm- Frequency: 0.25-0.5 MHz Duration: 80-225 cycles per pulse for 0.16-0.57 ms, repeated at 1.2-3.0 kHz Energy: I_{sppa} = 0.075-0.229 W/cm^2 (I_{spta} = 0.021-0.163 W/cm^2) Hippocampal paradigm- Frequency: 0.25-0.35 MHz Duration: 40 cycles/pulse at 2 kHz for 650 pulses; or 50 cycles/pulse at 1.5 for 500 pulses every 2 s for 30 min Energy: I_{spta} = 0.036-0.084 W/cm^2	Motor paradigm: Triggered local field potentials, and increased cortical spikes in motor cortex. Evoked muscle contraction. Hippocampal paradigm: Triggered LFP in CA1 and increased spike frequency	Effects seen in absence of increase in brain temperature (<0.01°C). No damage to BBB. Did not increase apoptosis in neurons or glia. No effect on density of synapses, or number of docked vesicles. No effects on motor behavior. Never observed any neurologic abnormalities (in over 80 mice).
Tyler et al.	2008	Organism: Mice Frequency: 0.44-0.66 Mhz Duration: tone burst duration = 22.7 ms, cycles/tone burst = 10, pulse repetition frequency = 0-100 Hz, Number of tone bursts = 250 Energy: I_{sppa} = 2.9 W/cm^2 (I_{spta} = 0.023 W/cm^2)	In hippocampal slice cultures (CA1): activated voltage gated sodium channels, voltage dependent calcium transients, synaptic vesicle exocytosis, and synaptic transmission	Chronic stimulation (36-48 h) did not alter fine structure of neuronal membranes.

(*continued on next page*)

Table 2 (*continued*)

Author	Year	Ultrasound parameters	Result	Safety considerations
Velling and Shklyaruk	1988	Organism: Cats and Rabbits Frequency: Not specified Duration: 0.1-100 ms pulse width, 1-20Hz Energy: 1 $\mu W/cm^2 - 1400 W/cm^2$ LIFU to HIFU	Short pulse action ($<$ 1 ms) with intensities 1 m $W/cm^2 - 1400 W/cm^2$ had no effect on bioelectric activity. Extending durations $> =$ 1 s showed intensity and stimulating frequency dependent increases or decreases in cortical excitability. Cortical activation at 1-100 mW/cm^2, and suppression at 1-100 W/cm^2	Intensities of $>$1000 W/cm^2 caused damage.
Min, Bystritsky et al.	2011	Organism: Rats (with induction of acute epilepsy by PTZ) Frequency: 690 Khz Parameter: Bursts of 0.5 ms at 100 Hz Duration: 3 min, twice, with a 10 min gap between. Energy: $I_{sppa} = 2.6 W/cm^2$ $I_{spta} = 130 mW/cm^2$ LIFU	The occurrence of epileptic EEG bursts from epilepsy-induced rats significantly decreased after sonication when it was compared to the presonication epileptic state	No damage found from the control group (i.e., the non-epileptic animals that underwent sonication). No TUNEL positive activity.
Yoo, Bystritsky et al.	2011	Organism: Rabbits Frequency: 690 Khz Motor paradigm- Duration: Bursts of 50 ms at 10 Hz (for 1-2 s for success Energy: $I_{sppa} = 12.6 W/cm^2$ ($I_{spta} = 6.3$ W/cm2) Suppression paradigm- Duration: Bursts of 0.5 ms at 100Hz (for $<$8 s) Energy: $I_{sppa} = 3.3$ and 6.4 W/cm^2 ($I_{spta} = 160$ and 320 mW/cm^2)	Motor paradigm: motor cortex activation, and motor activity detected (only $I_{sppa} = 1.6$ W/cm^2 was required to elicit cortical activation). Suppression paradigm: magnitude of p30 VEP component was reduced	27 s continuous sonication of $I_{sppa} = 23$ W/cm^2 ; $I_{spta} = 1.15$ W/cm^2) produced a slight (~0.7 °C) temperature rise from the sonicated area. Shorter sonications did not change temperature. No evidence of apoptosis or ischemia.

The spatial peak-pulse average intensity (I_{sppa}) is the maximum intensity in the beam averaged over the pulse duration (for pulses of nonconstant amplitude). The spatial peak-temporal average intensity (I_{spta}) is the maximum intensity in the beam averaged over the pulse repetition period. I_{spta} is the best measure of the amount of heat delivered to a tissue by ultrasound. In diagnostic imaging I_{spta} is usually below 100 mW/cm^2, although higher for Doppler imaging. The spatial peak-temporal peak intensity(I_{sptp}) is the maximum intensity when the pulse is on.

publications did not consistently report on, or even look for evidence of damage, several recent studies looked thoroughly for evidence of damage caused by LIFUP and found none.[78,81,84,104] Even with chronic stimulation of LIFUP for 48 hours, no alterations were seen in the fine structure of neuronal membranes.[78]

The low frequency and low energy of LIFUP falls well below the threshold to induce damage. Even at high frequency (5.7 Mhz) and high energy (280 W/cm^2), tissue may not be damaged at all,[71,72] and the threshold for damage may be even higher.[65]

As a further illustration of the safety of LIFUP, several studies injected a contrast agent with the intention of disrupting the BBB and generally used higher intensity continuous FUS and did not report any damage outside of the application focus.[75,105] Even with BBB disruption, at low energies there was minimal evidence of apoptosis or ischemia, with a mild inflammatory response within the focus.

As a whole, US-induced tissue damage appears to be caused by heating, yet an increase in temperature is not required to exert effects on neural activity.[79] Thus, LIFUP most likely can modulate neural activity without injuring tissue. Also, although ablation studies using HIFU damage the neuronal tissue in the focus as intended, none of them reports the cellular damage outside of the focus where the intensity of US still exceeds that of LIFUP. Over the past 50 years numerous studies using US administration to the brain suggest that overall the LIFUP method is safe and may be introduced carefully into human use.

Barriers to progression to human trials

Our review of past literature and recent experiments in several laboratories around the country confirms that neuromodulation of central neuronal circuits using LIFUP is possible, and most likely safe. Our experiments demonstrated that this technology could be used simultaneously with rtfMRI and navigated by MRI. Most of the scientific literature agree that low-intensity FUS does not damage tissue unless excess thermal effects are present.

In our recent study, we were able to measure temperature in the focus of LIFUP during stimulation.[83] We did not find any temperature elevation, even when using prolonged stimulation. We also identified both excitatory and inhibitory sonication parameters, which we successfully used in vivo in rabbits and rats.[83,106]

In addition to our in vivo research, Tyler and his group at the Arizona State University demonstrated activation in vivo in mice.[78,79] This group also elucidated possible mechanisms of the LIFUP effect on neuronal tissue (see the section on cellular mechanisms of LIFUP).[78,104]

Thus, many of the issues we discussed in the "potential challenges" have been studied. However, much more work needs to be done. Unfortunately, some of this work, such as precise focusing and navigation of LIFUP through the human skull, and identification of the effective and safe human parameters of this technique, can be done only in humans. We believe that it is time to carefully precede to the first human use trials.

The arguments for human trials are the following:

1. All of the more than 30 publications described in this review using LIFUP in different experimental setups (brains, peripheral nerves, and neuronal tissues) demonstrated biologic effects without damaging the tissues when subthermal stimulation was used.
2. Recent experiments at BWH and Arizona State University demonstrated safety and biologic effects (i.e., motor activation and seizure suppression) in several different types of animals including (frogs, mice, rats, and rabbits).
3. Focused US has been used in humans in the United States and in Switzerland[5] for ablation, which is destructive to the tissue in the focus. Outside the focus, the energy of the ablative ultrasound still exceeds the energy level at the focus of LIFUP device. However, no tissue damage was found in any other location but US focus.
4. Doppler US, which has been used extensively on the brains of adults and children, is similar to the energy used in the LIFUP method.
5. NOTE: "US is used in surgical guidance for dental applications in energies that exceed LIFUP." For example, US in nasopharyngeal surgery navigation, or for blood clot dissolution, has been safely used in humans (see the safety issues section of this Review above.)

Future experiments

We believe that future experiments will need to focus on several aspects of LIFUP such as pulse parameters for delivery through human skull. This problem has been solved in many ways in the application of HIFU for surgical ablation.[107] However, it is still unclear that low-intensity US pulsations would be able to penetrate into deep areas of the brain and be precisely navigated through an intact human skull, though there appears to be no a priori theoretical limitation. Some of this work could be done in phantom simulations and human skulls, but the final test will need to be done in humans.

The effects of LIFUP on larger brains have not been reported; pig or monkey experiments are needed to document the safety of LIFUP in larger volume brains. Those experiments are indeed on the way in several university laboratories. For example, we have recently stimulated the hypothalamic area of a minipig using LIFUP transcranially. The stimulation was delivered through the lower plate of the skull, which is similar in thickness to a human skull. In five experiments, stimulating the hypothalamic area consistently

increased both blood pressure and heart rate demonstrating an effect similar to that usually evoked by DBS in the same region.[108]

Given that the focusing of US in complex structures such as the human head is difficult to optimize, imaging will likely remain an important component of the practical application of LIFUP. A variety of methods have been put forward to guide ablative, HIFU therapy, such as MR thermometry [5,109,110] and more recently acoustic radiation force imaging (ARFI).[111,112] However, higher sensitivity could be required to visualize the effects of lower intensity sonication. For better navigation, and monitoring of thermal and BOLD effects, it is necessary to optimize the parameters to be used in the fMRI environment. Similarly, a more systematic, and broader, evaluation is needed of the duration of optimal treatment in different neuronal circuits, and structures, as well as how many treatments are needed to modify the circuits for a prolonged period.

Future application: brainmapping and therapeutic potential

Focused US, combined with rtfMRI, could potentially be used for brain mapping paradigms that help identify and diagnose functional disorders of the brain that currently lack clear neuronal underpinnings. For example, bipolar mania, OCD, depression, autism, and others could benefit from these studies. Treatment of neurologic disorders such as chronic pain, obesity, and Parkinson's might be possible via LIFUP induced neuroinhibition, as it may reach deep brain areas noninvasively. Therapeutic areas where invasive DBS has shown some promise – including pain, obesity, epilpesy, OCD, and other mental disorders, Parkinson's and other movement disorders – all may be treatable with LIFUP. Therapy with LIFUP may find a niche between medication treatments (which are still most convenient) and invasive strategies (i.e., ablation and DBS) that should be reserved for the most severe conditions that require permanent disruption or attenuation of neuronal circuitry. The unique properties of the LIFUP, which include noninvasiveness, small focus, and real time feedback from fMRI imaging, could provide us with better understanding of brain function and better targeted treatment of mental and neurologic disorders.

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