32 Human Brain Stimulation with Transcranial Ultrasound

Potential Applications for Mental Health

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Interest in noninvasive brain stimulation for therapeutic effects on mental health has increased in recent years. The ability to directly modulate brain activity in targeted or diffuse regions noninvasively, that is, from outside the skull, has enormous potential for the treatment of psychiatric and neurological disorders. Brain stimulation also holds promise for the functional mapping of brain systems by coupling stimulation with subjective report and imaging techniques such as functional magnetic resonance imaging (fMRI). A potential advantage of therapeutic brain stimulation over pharmacological intervention is that targeted stimulation of brain circuits implicated in psychiatric disorders might minimize global effects on the brain and body, potentially minimizing or eliminating side effects.

Frequently used noninvasive brain stimulation methods, including transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS), have limitations (reviewed below), restricting their scientific and therapeutic applications. Ultrasound is a less frequently used brain stimulation technology, but may have advantages over other techniques. Ultrasound consists of mechanical vibrations above the threshold for human hearing (>20,000 Hz). Ultrasound can penetrate biological tissue, and echo off surfaces to give anatomical images, such as fetuses in the womb. Although primarily used for medical imaging, ultrasound can also modulate neural activity, both peripherally and in the brain. This chapter reviews the history of ultrasound neurostimulation and the first clinical trials in humans. Future directions in this emerging field are discussed.

INTRODUCTION: BRAIN STIMULATION

There are two broad approaches for human brain stimulation: invasive and noninvasive. Invasive procedures require surgical implantation of a device to a targeted area of the brain or central nervous system [e.g., deep brain stimulation (DBS)]. DBS electrodes deliver high-frequency electrical pulses to anatomically selected brain regions with millimeter precision to influence neuronal function and signaling. The ability to electrically stimulate the brain has provided significant benefit for neurological patients[1] and shows promise for psychiatric disorders[2] like depression.[3] Yet the invasiveness of surgical implantation of electrodes and microcontroller devices limits the application of DBS to only the most extreme of cases. Moreover, DBS cannot easily be used with imaging devices, such as fMRI, for brain mapping due to safety concerns and imaging artifact produced by stimulation devices.[4] Therefore, methods that allow noninvasive excitation or modulation of brain activity have been developed.

Among noninvasive methods, TMS and tDCS are the most commonly employed. Each takes advantage of different electromagnetic principles. TMS uses strong magnetic coils to focus induced currents in the brain via electromagnetic induction. One specific variety of TMS—repetitive TMS

(rTMS)—has been shown to excite (~5–20 Hz) or inhibit (~1 Hz) neural activity, and the effects of rTMS can last up to 30 min or more, before neural activity returns to baseline levels.[5] Applying low amplitude (~1 mA) direct current or alternating current to the brain (tDCS and tACS, respectively) can also induce reversible changes in neural activity. Like magnetic stimulation, tDCS and tACS can both excite or inhibit brain activity depending on the cathode/anode configuration of the stimulating electrodes on the scalp.[6]

Both methods are currently used as scientific tools and have shown promising, albeit limited, therapeutic application. Major limitations include the fact that the stimulation fields are relatively nonspecific (1 cm or more),[2] lacking the millimeter spatial resolution of DBS. Moreover, current approaches are incapable of targeting deep brain structures. TMS is relatively expensive, requires specialized facilities, and highly trained staff. Both methods can cause sensations on the skin, creating difficulties for blinding participants to treatment conditions. Finally, a potentially powerful use of noninvasive brain stimulation is the ability to functionally map brain areas by exciting or inhibiting targeted areas to observe the effects on behavior or during brain imaging (like fMRI). However, the ability of both electrical brain stimulation[7] as well as magnetic brain stimulation[8] for human brain mapping may be untenable due to technical difficulties. These issues have motivated researchers to seek alternative technologies for brain stimulation.

Ultrasound can be transmitted through the skull to remotely influence brain activity, known as transcranial ultrasound (TUS). Depending on the focusing beam of the ultrasound, millimeter precision and deep brain structures can be targeted. Clinical ultrasound imaging machines, widely available in hospitals and clinics, can also be used but deliver less focused beams of ultrasound energy. Further, several decades of medical and therapeutic applications has demonstrated the safe output levels of ultrasound on biological tissue. Thus, researchers have begun to investigate the use of TUS in humans for scientific and neurotherapeutic purposes. A brief history of ultrasound for brain stimulation is outlined below, followed by descriptions of the first human studies and a discussion of future directions.

HISTORY

Discovery and Development of Safety Protocols

Ultrasound (US) consists of cyclic mechanical vibrations (acoustic pressure) in a frequency range higher than the upper limit of human hearing (>20,000 Hz or >20 kHz). At high intensity, US can cause tissue heating, but at lower levels, US is safe for human tissue. US was used for therapeutic purposes (e.g., physiotherapy) before being developed as a tool for medical imaging.[9] Wood and Loomis showed in 1927[10] that high intensity US could produce lasting changes on biological tissue. Shortly thereafter, Harvey and Loomis[11] showed that US could stimulate excitable tissue, including the heart and other muscles. These seminal studies launched investigations into the safety of US for therapeutic applications. In the 1930s and 1940s, researchers realized the potential to use US for medical imaging of brain tumors.[12] Since then, diagnostic ultrasound has developed into an indispensable tool with a wide range of medical applications.

Therapeutic and imaging ultrasound can be generally divided into high and low intensity.[9] High intensity ultrasound can destroy biological tissue by heating or cavitation (the creation of small, gas or vapor filled cavities that may explode[13]). For example, high intensity focused US is used therapeutically for lithotripsy (using shock waves to break apart kidney stones). In contrast, low intensity ultrasound, where exposure is chosen to have minimal lasting effects on biological tissue, has been used for diagnostics and therapeutic applications on the body safely for more than 70 years.[9] Virtually every part of the body, including the brain, has been safely imaged with low intensity ultrasound in humans.[14]

The US Food and Drug Administration regulates the acoustic output levels for ultrasound.[15] Most therapeutic ultrasound is continuously delivered below 1 MHz, whereas ultrasound for diagnostic imaging is pulsed (i.e., cycles of ultrasound separated by brief periods of rest) at frequencies between 1 MHz and 15 MHz. The monitoring and quantification of acoustic output levels allows the ultrasound operator to estimate the temperature changes resulting from ultrasound propagating through tissue or bone [thermal index (TI)] as well as the mechanical pressure induced by ultrasound waves [mechanical index (MI)]. Ultrasound exposures can be defined in terms of acoustic pressure or intensity. Pressure in an acoustic field varies spatially, and temporal variation is introduced by pulsing the ultrasound; thus, there are different ways to quantify the intensity output.[9] When calculating intensity, the spatial peak or spatial average of the pressure in the acoustic field can be included, as well as whether the time during the pulse (pulse average) or the total stimulation time including the on/off period of the pulse (temporal average) is included.[9] The current FDA limits for diagnostic ultrasound are limited to an MI of 1.9 and spatial peak, temporal average (I_{SPTA}) of 720 mW/cm^2 (I_{SPTA} will be used when discussing intensity below). At or below these output levels, US applied to biological tissue, including the brain, has been shown to leave no lasting bioeffects.[9,14,16,17]

Early Neuromodulation Studies

A seminal study by Harvey in 1928[18] showed that US could excite nerve and muscle tissue in frogs and turtles. Later, Fry[19] conducted a series of studies showing that high intensity US could lesion brain tissue. Interestingly, this was used to treat Parkinson's disease with some success, but was ultimately abandoned because craniotomy was necessary to deliver the ultrasound to deep brain structures.[20] Fry also found that aiming the ultrasound towards the lateral geniculate nucleus in cats resulted in the suppression of electrical potentials recorded over visual cortex.[20] US modulated the activity of nerve fibers differentially depending on the intensity parameters and size/type of fibers in animals.[21]

Gavrilov and colleagues[22] showed that focused US delivered to nerves in the hand could cause sensory perception in humans. Excitatory and/or inhibitory effects were reported in animal spinal cord[23] and cortex,[24] and in human cranial nerves.[25] There are numerous other early studies investigating US for neural stimulation;[26] for a more extensive review, including a discussion of the interesting work from scientists in the USSR in the 1970s who attempted to introduce auditory information to deaf patients via auditory nerve stimulation. Altogether, neuroscientific evidence from humans and nonhuman animals suggest that US can be used to modulate (stimulate or inhibit) neural activity.

MODERN NEUROMODULATION STUDIES

Recent studies have shown direct effects on the firing rates of single neurons in slice preparations or populations of neurons with brain imaging. Tyler and colleagues[27] reported that focused low intensity US stimulated single action potentials and synaptic transmission in rodent hippocampal slice cultures and *ex vivo* brains. Further, they found that the spatial resolution was on the order of 2 mm. Another study showed that focused TUS (<180 mW/cm^2) was sufficient to elicit motor potentials in intact mouse motor cortex, and caused region-specific muscle contractions after motor cortex stimulation (e.g., tail, forepaw, and whisker movements; see supplemental Movie S2 in[28]). Importantly, remote stimulation affected hippocampal activity, suggesting TUS is capable of targeting deep subcortical structures.[27]

Min and colleagues[29] showed that low intensity US pulsed at a rate of 100 Hz (130 mW/cm^2) could suppress epileptic activity in an animal model. Yoo and others[30] recently demonstrated region specific increases (1.6 W/cm^2) or decreases (160 mW/cm^2) of brain activity in anesthetized rabbits by coupling focused ultrasound with fMRI. Tsui and colleagues[31] found that shorter durations of US pulses led to excitation and longer durations to inhibition of action potentials in peripheral nerves, suggesting that US pulse duration is important for stimulatory or inhibitory effects of US. Finally, Min and colleagues[29] recently showed that focused US stimulation of the rat thalamus increased neurotransmitter (dopamine and serotonin) in the frontal cortex. The ability to modulate region specific brain activity along with the modulation of neurotransmission demonstrates that TUS can be used as a brain mapping technique.[29] Indeed, given that US energy is mechanical rather than electromagnetic, several researchers have noted that US is well suited to complement brain imaging techniques, such as fMRI, for functional brain mapping without causing significant artifact.[32,33]

Searching for Mechanisms

The mechanisms by which ultrasound modulates neural activity are poorly understood. Tyler and others[34] proposed that ultrasound excites neural activity by mechanical stretching of membrane lipid bilayers, membrane proteins (integrins), and extracellular proteins that causes membrane depolarization. Indeed, voltage-gated ion channels on neurons and neurotransmitter receptors possess mechanosensitive properties that make them susceptible to mechanical forces. Tyler and colleagues[27] showed that voltage-gated sodium and calcium channels were activated by focused US. Moreover, US can reversibly induce increases in calcium uptake in fibroblasts[35] and modulate potassium influx and efflux in rat thymocytes.[36]

Ultrasound may inhibit neural activity by disrupting synaptic signaling possibly via thermal effects (i.e., heating)[37]; however, inhibitory effects have also been found with low intensity US that results in almost no thermal effects (160 mW/cm^2), leading others to propose that higher pulse repetition frequency may lead to inhibitory effects ultrasonic effects on neural tissue.[30,31]

Another view is that US affects neural activity through resonant vibrations in microtubules, major components of the cytoskeleton. Microtubules grow, organize and regulate neurons and synapses, and have been implicated in mood, memory, and conscious awareness.[38] Composed of the brain's most prevalent protein, microtubules have resonance frequencies in the MHz range,[39–41] making them susceptible to US vibrations affecting neural activity and mental states.

TRANSCRANIAL ULTRASOUND IN HUMANS

Skull Penetration

The evidence reviewed so far points to the possibility of using noninvasive US to modulate human brain activity. Ultrasound can be focused to penetrate targeted areas as small as 2 mm[27] and metamaterials for focusing US may increase spatial resolution even further (<1.0 mm). Focused TUS is also capable of stimulating deeper brain structures than other stimulation methods like TMS and tDCS.[33] Thus, it has been proposed that US could mitigate the need for invasive DBS implants for the treatment of refractory psychiatric disorders (e.g., depression, obsessive compulsive disorder), Parkinsonism, and other disorders that are currently treated via this method.[28] However, the skull reflects, refracts, absorbs, and diffracts the US field, thus presenting a major obstacle for transcranial US.

Transcranial Doppler ultrasound (TDU) is used frequently in clinical procedures to measure blood flow velocities in brain arteries,[42] and is capable of penetrating the skull through the trans-temporal window or other areas where the skull is thinnest (Figure 32.1) with low intensity US (~100 mW/cm^2).[43] Many TDU devices use the standard "b-mode" (found on many hospital grade ultrasound machines) in which a linear array of transducers emit ultrasound to scan a plane through the brain or body. As TDU must penetrate the skull and reflect back to the transducer to produce an image, this *prima facia* demonstrates that imaging US can penetrate the skull at intensities under the FDA limit. Mathematical modeling, along with experimental data, shows that the optimal gain for focused US transmission through the skull and for brain absorption occurs at frequencies below 0.70 MHz.[44,45] In fact,

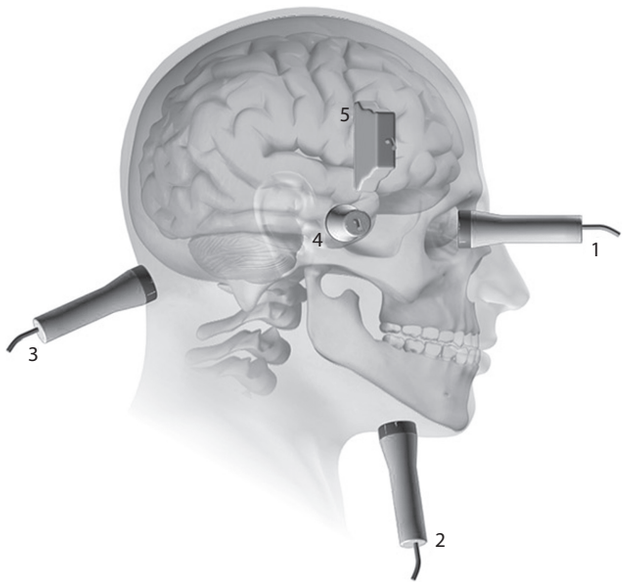

FIGURE 32.1 Typical locations used for transcranial ultrasound in medical imaging 1–4. The fifth location was the stimulation site in Hameroff et al.[56] Stimulation was on the opposite side to where the patient experienced chronic pain. (Adapted with permission from Hameroff S et al. *Brain Stimul* 2013;6(3):409–15.)

Hynynen and colleagues[46–48] have developed a technique for precise targeting of focused ultrasound from outside the skull [magnetic resonance guided focused ultrasound (MRgFUS)]. The feasibility of using this method for low intensity neuromodulation is not yet clear, yet the fact that ultrasound can penetrate the skull with such precision is encouraging. Indeed, research groups are currently working on designs for low intensity TUS arrays for the treatment of refractory psychiatric disorders.[49,50] Functional studies must be now performed that examine how US, at varying output parameters, affects intact brain circuits and behavior in humans.

Safety Considerations

The major biohazard to consider when directing US into the brain is tissue heating and cavitation. At high intensities, US can cause thermal effects and damage due to explosions of microbubbles. Indeed, high intensity focused US (usually > 1000 W/cm^2) is used for tissue ablation in many areas of the body[51] including noninvasive cancer treatments,[52] whereas US intensities below 500 mW/cm^2 can produce biological effects without damage.[9,16] Recall that the FDA regulates the output level of diagnostic and imaging US (I_{SPTA} 720 mW/cm^2). These guidelines are based on decades of animal and human research showing that acoustic energy at or below these levels are safe for human adults, including the brain.[14] Histological analysis of animal brains that underwent US stimulation at intensities well below the FDA limits (e.g., ~50 mW/cm^2–250 mW/cm^2)[27] show no detectable signs of biological damage. In fact, US effects at intensities higher than the FDA limits also show no histological indicators of damage to the animal brain (e.g., ~3 W/cm^2).[30] It should be noted, however, that US may have detrimental effects on developing brains: neuronal migration is affected when rat fetus is stimulated with US for 30 min or more for at least two exposures.[53–55] Nonetheless, results from adult animal brains, and the fact that US is used routinely in medical procedures with no appreciable detrimental effects, suggests that low intensity US is safe and reversible for neuromodulation in adults.

Given the potentially wide ranging therapeutic application of TUS for the treatment of brain based mental disease, and the fact that low intensity US does not appear to cause biological damage in adults, the first human studies are warranted. The long-term effects of repeated low intensity US in humans have not been examined. Until the proper studies are conducted, these first TUS studies should take precaution to use only intensities that are known to be safe in the brain and to use the smallest exposure duration possible for effects.

Human Transcranial Ultrasound

The first published study attempting to use TUS to manipulate brain activity in humans was a double-blind, placebo-controlled experiment testing whether low intensity TUS (at 8 MHz) could alter self-reported pain and mood in an older population of chronic pain patients.[56] An FDA approved medical-grade imaging US device (General Electric LOGIQ e; GE Healthcare, Little Chalfont, UK) that emitted pulsed US in a scanning sequence (described above) was used. This device was chosen because it is used routinely for diagnostic imaging and the intensity output is well below the FDA guidelines. Thus, it presented little risk to the patients in regard to long-term effects. Participants received a single 15-second dose of TUS delivered to the temporal window opposite to the side of reported pain (Figure 32.1). Hameroff and colleagues collected self-reported pain (numerical rating scale for pain) and mood data (Visual Analogue Mood Scales) in 34 patients. Although self-reported pain did not significantly decrease, patients in the TUS group reported an improvement in mood compared to the placebo group at 10 min and at 40 min after TUS stimulation. As ultrasound images from each patient were collected, it could be verified that ultrasound had indeed penetrated the skull sufficiently to image brain tissue (Figure 32.2). These are the first TUS results in humans and, although preliminary, suggest that TUS can improve mood.

A follow-up report by Sanguinetti and coworkers[57] replicated these mood-altering effects in a healthy population of undergraduate students at the University of Arizona. TUS at lower frequencies should have a more robust effect on neural activity than TUS at higher frequencies insofar as lower frequency TUS is better at penetrating the skull.[44,45] Thus, Sanguinetti and colleagues examined self-reported mood following stimulation with either 2 MHz or 8 MHz TUS. Whereas Hameroff et al.[56] stimulated the contralateral side of the frontal cortex to where patients experienced pain, Sanguinetti et al.[57] applied stimulation to participants' right temporal window (Figure 32.3). Self-reported affect increased (i.e., became

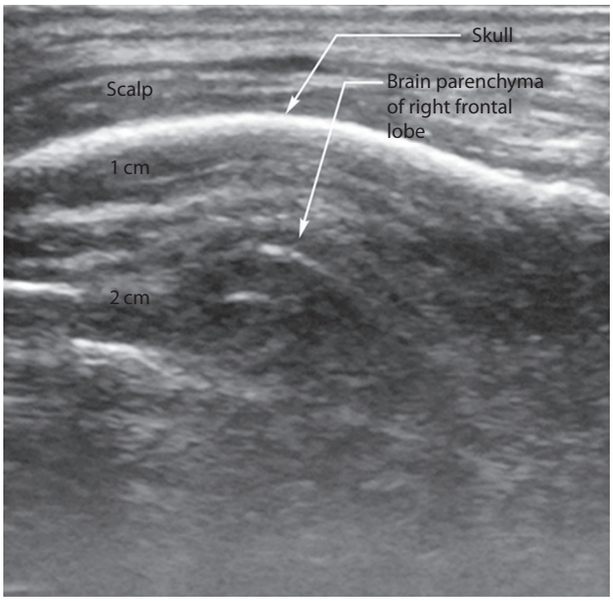

FIGURE 32.2 Example of image taken from a chronic pain patient. Longitudinal gray scale images taken from a session of transcranial ultrasound. Scalp, skull, and brain tissue are visible in the image. (Adapted with permission from Hameroff S et al. *Brain Stimul* 2013;6(3):409–15.)

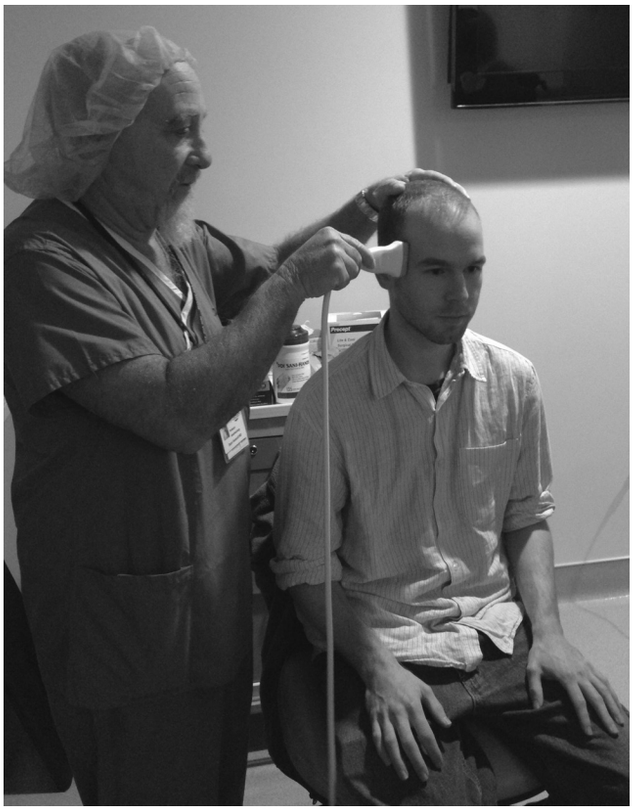

FIGURE 32.3 Example of stimulation to right frontal cortex with the GE LOGIQ e 12R transducer probe (GE Healthcare, Little Chalfont, UK). (Photo courtesy of J. Sanguinetti.)

more positive) following right frontal-temporal TUS stimulation at 2 MHz, but 8 MHz stimulation had no effect on mood relative to baseline. Interestingly, improvements in mood also appeared to be dose dependent: There was a greater improvement on mood following 30 s of stimulation than 15 s of stimulation. The effects on mood in these studies lasted more than 30 min, and it remains to be seen whether multiple stimulation sessions would lead to longer effects on mood. Taken together, these first two preliminary human studies suggest that TUS can affect mental states through frontal cortex stimulation. However, these experiments in humans do not demonstrate direct effects of ultrasound on the brain, therefore future studies in humans should be examined whether TUS affects brain activity directly with imaging methods (e.g., fMRI).

FUTURE DIRECTIONS

Much remains to be understood about the neurobiological mechanisms of TUS before it can be routinely used therapeutically in mental health fields. However, this should not prevent researchers from investigating the clinical applicability of TUS. Most of the noninvasive brain stimulation methods—TMS and tDCS, for example—were developed for scientific and clinical applications before extensive research on animal models to understand the biological mechanism were conducted.[2] This is due in part to the noninvasive nature of these techniques but also because the necessary work was done in animals to show these methods do not pose significant bioeffects and that the potential benefit outweighed the risks. There is a growing literature on the bioeffects of US at low and high intensities,[9,14,16,17] and low intensity US has a proven safety record in over 50 years of medical diagnostic applications. Thus given the extant evidence that TUS can stimulate brain activity in animals, and the pilot work in humans suggesting promise for elevating mood, researchers should cautiously proceed to investigate the applicability of TUS for mental health conditions. Although research to date suggests that mood can be increased acutely following TUS, whether repeated delivery of TUS can lead to sustained mood change in psychiatric disorders remains an important and unexamined question.

When conducting TUS on humans, the most important safety consideration is the acoustic output levels of the transducer. As mentioned above, the FDA limits the acoustic output to a MI of 1.9 and the spatial peak, temporal average (I_{SPTA}) of 720 mW/cm^2. Researchers should do the necessary background reading to understand what these outputs mean and they should only use equipment in which these outputs can be clearly read out or controlled with high certainty. Hameroff et al.[56] and Sanguinetti et al.[57] chose to use FDA approved medical imaging devices in which the acoustic output cannot be set to dangerous levels. These devices are prevalent in hospital and clinics, giving interested researchers and clinicians easy access for future TUS studies. However, these systems emit US in scanning beams of several centimeters (depending on the selected probe). Furthermore, many of these devices emit US at frequencies greater than1 Mhz.

If researchers desire greater spatial precision, and wish to emit US at lower frequencies that will penetrate the skull better, then devices will need to be designed specifically for human TUS. These devices should be designed in such a way that they can only emit low intensity US. Such devices are being developed, for example, by a pioneering team at Virginia Polytechnic Institute and State University.[23,55]

There appears to be an almost unlimited parameter space for researchers to explore TUS in humans. The optimal frequency for TUS transmission and brain absorption is ~0.5 MHz,[44,45] narrowing the parameter space considerably. But many unknowns remain. For example, much of the US brain stimulation results in animals have been on motor areas with focused US (with millimeter precision). It is unknown whether focused US or unfocused, scanning beam (like that emitted from medical imaging devices) is best for therapeutic effects. Although the animal research has primarily investigated US, researchers and clinicians who have access to medical grade imaging US devices might find that scanning beams are sufficient for many purposes.[56] For example, depression has been linked to alterations in lateralized frontal activity,[58] and it might be sufficient to stimulate the frontal cortex with unfocused US AS global effects might be more effective than localized effects.[57] For functional brain mapping, however, focused TUS will be necessary to linking changes in electrical or hemodynamic activity following TUS stimulation with behavioral changes.

Different properties of the US waveforms might affect how effective TUS works as a brain stimulation method.[28] The length of the stimulation pulse (pulse duration), how often the pulse is repeated (pulse repetition frequency), overall intensity (peak and temporal average intensities) might alter the effect of TUS on neural tissue. Lower pulse durations have been shown to stimulate brain activity (<50), whereas longer pulse durations (>100) have been found to inhibit.[30,31] However, it is not known which pulse durations give the strongest inhibitory/excitatory effects in human brain tissue and whether different cortical areas respond similarly to the same TUS parameters. The overall exposure duration will also affect stimulation efficacy as well. Given that Hameroff et al. and Sanguinetti et al. found effects of frontal cortex TUS stimulation on mood at 15 s and 30 s, respectively, it is recommended to start with the lowest exposure duration possible where an effect can be seen. The effects in these studies lasted more than 30 min. However, it is unclear whether multiple stimulation sessions would lead to longer effects. In addition to replications studies, and examining the impact of TUS on a variety of emotional disorders, future studies should also examine the parameter-space of TUS waveforms to increase therapeutic effects.

SUMMARY

Over 50 years of animal research suggests that US can stimulate or inhibit neural activity. Low intensity US is safe for use in adult humans and can be effectively transmitted through the human skull and absorbed by the brain. TUS has several advantages over established noninvasive magnetic and electrical neurostimulation methods. It can be focused with millimeter precision or unfocused to stimulate larger brain areas. TUS can easily be coupled with imaging techniques, such as fMRI, for brain mapping studies, and it is capable of targeting and stimulating subcortical brain structures. Two initial reports support the use of TUS for elevating mood in humans and future work will investigate mood-elevating properties of TUS with psychiatric patients (e.g., depression). Looking ahead, future studies will need to examine the TUS parameters most amenable for neurostimulation.

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