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Ultrasonic modulation of neural circuit activity

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Ultrasound (US) is recognized for its use in medical imaging as a diagnostic tool. As an acoustic energy source, US has become increasingly appreciated over the past decade for its ability to non-invasively modulate cellular activity including neuronal activity. Data obtained from a host of experimental models has shown that low-intensity US can reversibly modulate the physiological activity of neurons in peripheral nerves, spinal cord, and intact brain circuits. Experimental evidence indicates that acoustic pressures exerted by US act, in part, on mechanosensitive ion channels to modulate activity. While the precise mechanisms of action enabling US to both stimulate and suppress neuronal activity remain to be clarified, there are several advantages conferred by the physics of US that make it an appealing option for neuromodulation. For example, it can be focused with millimeter spatial resolutions through skull bone to deep-brain regions. By increasing our engineering capability to leverage such physical advantages while growing our understanding of how US affects neuronal function, the development of a new generation of non-invasive neurotechnology can be developed using ultrasonic methods.

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Introduction

There have been numerous advances in our ability to modulate neural circuit activity and brain function over the past several decades. In addition to chemical or genetic approaches, device-based methods include non-invasive ones like transcranial magnetic stimulation (TMS) and invasive approaches like deep-brain stimulation (DBS). Some optical methods like optogenetics require genetic modifications [1], whereas others use high-energy, infrared light pulses to achieve spatially precise thermo-mechanical neurostimulation [2]. Regardless of the methods implemented, our ability to non-invasively and precisely modulate neural circuits remains ultimately limited by physics and biology. For example, the anisotropy of brain tissues makes it difficult to accurately focus TMS, and the need for genetic alteration is a hurdle that hinders use of optogenetics in humans. In efforts to overcome some of the physical limitations posed by electromagnetic radiation for neuromodulation, investigations into the use of ultrasound (US) have begun to demonstrate new possibilities for interfacing with neurobiology using mechanical forces.

Ultrasound is a pressure wave that has a frequency higher than the range of human hearing (>20 kHz). Besides it use in diagnostic medical imaging, US can be used to produce various thermal or mechanical effects on biological tissues [3,4]. High-intensity focused ultrasound (HIFU) is used to cause significant tissue heating for therapeutic ablations, whereas low-intensity US produces mechanical effects on tissues that do not cause heating or damage. Because of the way US interacts with matter including biological tissues, it provides neuroscience with some rather unique capabilities. For example, US can be transmitted across the skull and precisely focused into deep-brain regions of humans 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 into the effects of US on neural activity date back nearly a century. Over the past decade in particular there has been growing evidence that US is a viable tool for non-invasively modulating neural activity and brain function [7•].

It was first shown that high-intensity US could alter neuronal activity in frog and turtle neuromuscular systems [8]. A few decades later it was first reported that US could produce effects on brain activity when it was demonstrated that HIFU transmitted to the lateral geniculate nucleus of cats reversibly suppresses *in vivo* visual cortex activity [9]. Because heating tissues with HIFU poses inherent risks for creating irreversible damage, more recent investigations have focused on the use of low-intensity US for non-invasively modulating neuronal activity. Studies in this area were propelled forward by observations made in brain slices that low-intensity US can directly stimulate action potentials and synaptic transmission through mechanisms involving the non-thermal activation of ion channels (Figure 1) [10]. In the remainder of this article, we describe additional evidence that low-intensity US can modulate neural activity

through mechanical (non-thermal) mechanisms at high spatial resolutions in a variety of experimental models and organisms. We further describe how these observations have collectively contributed to the emergence of new tools for neuroscience and open possibilities for developing unique diagnostics, therapies, and human-computer interfaces using ultrasonic neuromodulation (UNMOD) technology [7•].

Basic characteristics of ultrasound

Several factors influence the interaction of US with biological tissues. For in depth discussions of these principles, we refer the reader to some other sources covering the basic biophysics of US (reviews see Refs. [3,4]). Some critical factors affecting outcomes include the acoustic frequency and intensity profiles of US waveforms used to affect biological activity. First, the spatial resolution that can be achieved with US is a function of both the acoustic frequency and transducer aperture used. It is possible to focus US fields in the brain using acoustic lenses or with phased array methods that can correct for aberrations or distortions caused by tissue interference [5]. In soft tissues like brain, muscle, and fat that have acoustic properties similar to water [4], the diffraction-limited spatial resolutions for 1.0 MHz and 100 MHz US beams in the far-field are 0.75 mm and 7.5 μm respectively. While the spatial resolution of US increases with frequency, power loss due to absorption and scattering of US by tissues also becomes more significant as frequency increases. In other words, higher frequencies of US can provide fine spatial resolutions, but are less capable of being transmitted through tissues. For the transcranial modulation of human brain circuits, <0.7 MHz US has been deemed particularly useful [11••,12,13•] and up to 5 MHz has been used to modulate the intact brain circuits of mice with thinner skulls. When US transmission through a skull is not required, frequencies up to 43 MHz have been used to modulate neuronal activity [14].

While the acoustic frequency of US used determines the spatial resolution, the acoustic intensity and exposure times are major factors in determining the dominate bioeffects (thermal or mechanical) on cells and tissues. Heating tissues or ablating diseased brain circuits for therapeutic purposes is generally performed using continuous wave HIFU at intensities greater than 200 W/cm^2. In contrast to HIFU, low-intensity US (0.5–100 W/cm^2) delivered in a pulsed mode for brief periods of time are less likely to produce tissue heating, but can still be focused through the skull and other tissues to produce prominent mechanical bioeffects on cells [3,11••]. The peak acoustic intensity recommended for diagnostic US imaging is 190 W/cm^2, which is higher than what has been shown capable of non-thermally modulating neural activity over the past decade [7•].

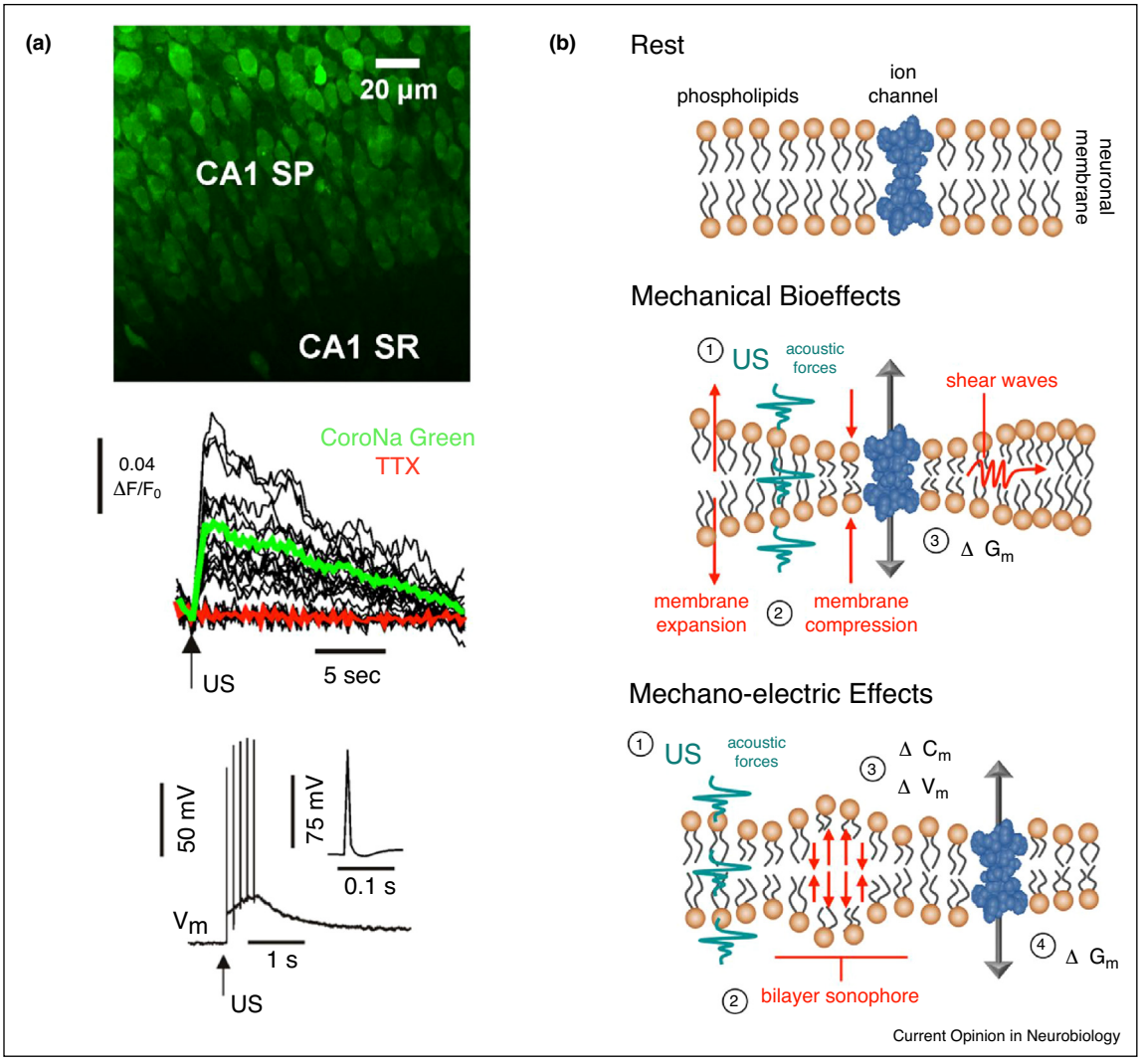
Ultrasonic modulation of central nervous system activity

Early in the history of medical ultrasonics research, it was shown HIFU can reversibly suppress sensory-driven or electrically-evoked activity by transiently heating the brain or spinal cord [9,15]. Several other studies have since shown that HIFU can modulate evoked activity in the CNS (for reviews see Refs. [7•,16]). Although thermal neuromodulation by HIFU provides immense clinical opportunities, the margins of safety are too narrow for widespread use in neuroscience. To address these thermal limitations, the influence of low-intensity US on brain circuits began to draw consideration. Subsequently, it was shown that low-intensity US can directly stimulate action potentials and synaptic transmission in hippocampal slices [10]. Further, evidence showed these effects were partially mediated by the activation of voltage-gated sodium and calcium channels [10]. These observations inspired numerous studies over the past decade that have explored the effects of low-intensity US on CNS activity [7•].

Following the *in vitro* findings that low-intensity US could modulate CNS circuits, Tufail and colleagues (2010, 2011) described methods using low-intensity transcranial US for conducting *in vivo* stimulation of mouse motor cortex and hippocampus [17], as well as for rapidly attenuating seizure activity in mice [18]. Subsequent studies designed to quantitatively evaluate the optimal parameters for effectively modulating intact rodent brain activity led to an expansion of research in the field [19,20]. Several others have since shown that the mechanical (non-thermal) bioeffects of low-intensity US (<100 W/cm^2) can safely modulate the activity of intact cortical, thalamic, and hippocampal circuits in mice and rats [19–22], rabbits [23], sheep [24], and pigs [25••]. Other studies have continued to provide insights into the acute effects of US on brain activity across a range of parameters while accumulating various safety observations. The acute modulation of CNS activity by low-intensity US was shown safe in numerous animal models, which helped pave the way for conducting more recent human research studies.

Since it had become clear from clinical HIFU studies that US can be transmitted and focused across the skull into discrete brain regions, investigators began to further study whether low-intensity transcranial focused ultrasound could be used for non-invasive neuromodulation in non-human primates and humans. 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 [26]. More recently this work has been expanded to show that transcranial LIFU can stimulate individual cortical neurons in awake behaving macaques [27]. In humans, Legon and colleagues (2014) first showed that

Figure 1

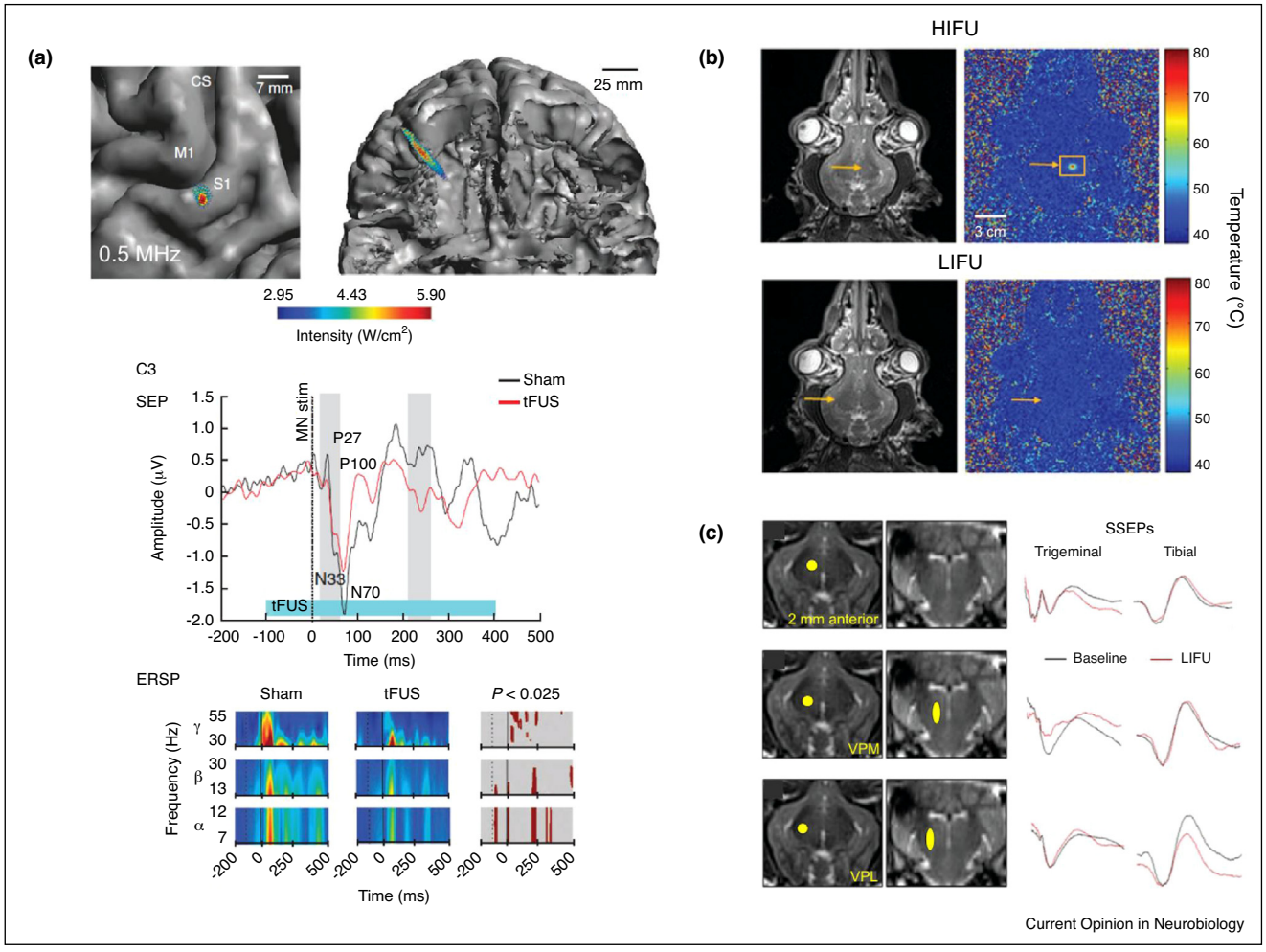


Non-thermal modulation of neural activity by low-intensity ultrasound. **(a)** Data showing that low-intensity US stimulates voltage-gated sodium channels and action potentials in hippocampal neurons and synapses located in CA1 *stratum pyramidale* (SP) and *stratum radiatum* SR; (adapted from Ref. [10]). **(b)** Illustrations showing a neuronal membrane and ion channel at rest and in response to low-intensity US. One model (*middle*) shows a case where the acoustic pressure of US (1) causes mechanical effects on membranes and channels (2) to change membrane conductance and channel activity (3). Another model (*bottom*) describes the acoustic pressure of US (1) causes the formation of a bilayer sonophore (2) that causes mechanically originated displacement currents (3) altering changes in membrane capacitance (C_m) and voltage (V_m). These actions subsequently alter neuronal membrane conductance (G_m) by affecting the voltage-mediated activity of ion channels (4).

LIFU (0.5 MHz, <50 W/cm^2) can modulate human brain activity (Figure 2) [11••]. 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 [11••]. 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

Figure 2

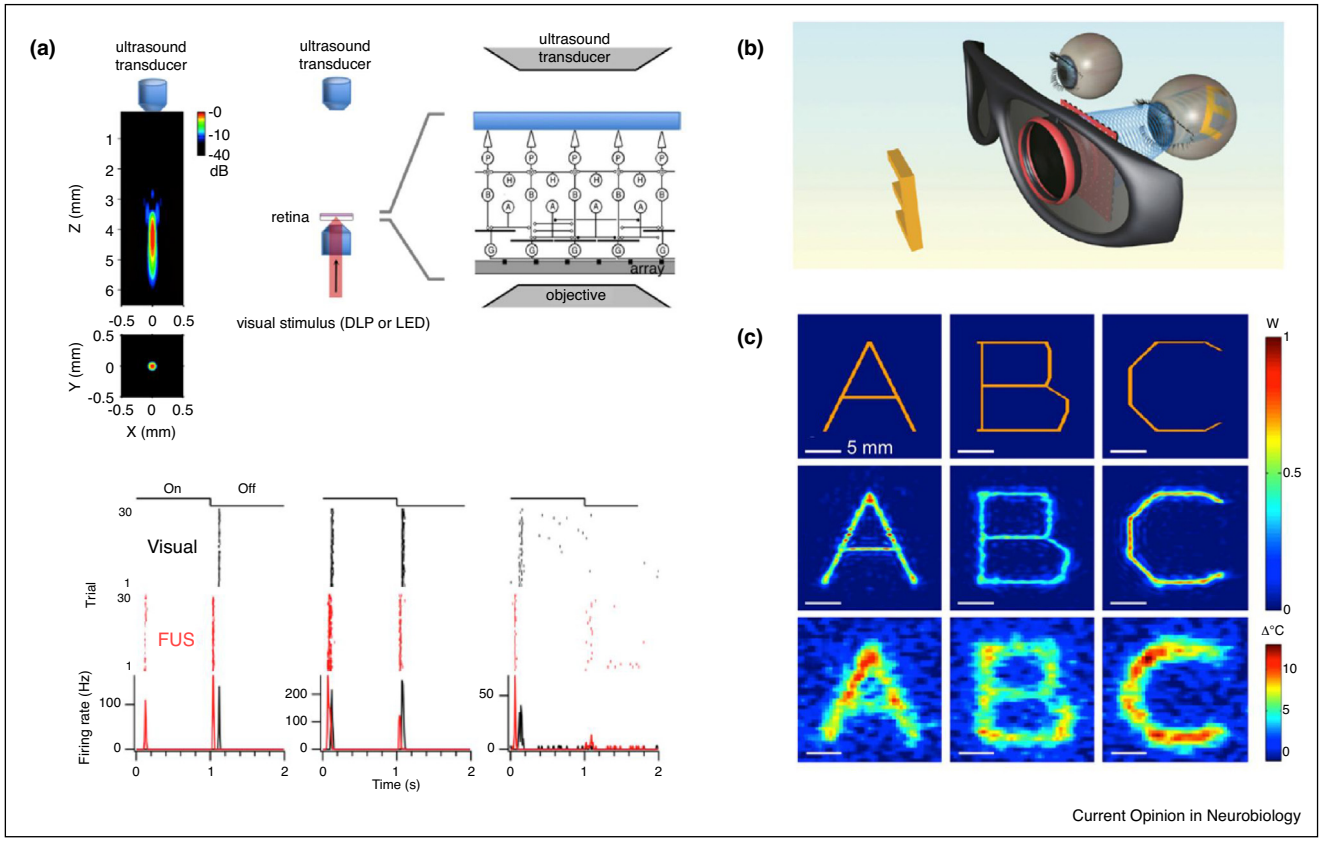


Low-intensity focused ultrasound for brain mapping. **(a)** Illustration showing a 0.5 MHz transcranial FUS beam targeted to the human primary somatosensory cortex S1 (*top*). The EEG data (*bottom*) show that FUS caused the suppression of somatosensory evoked brain activity (adapted from Ref. [11••]). **(b)** 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*). **(c)** 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 subnuclei 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. [25••]).

transcranial LIFU (0.25 MHz, 3 W/cm^2) targeted to S1 of human volunteers can directly stimulate and evoke somatosensory potentials [12]. 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 [12]. More recently the authors extended these by showing that transcranial LIFU (0.27 MHz, 16.6 W/cm^2) 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 [13•]. 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/cm^2) can be transmitted to subnuclei of the pig thalamus to functionally modulate

Figure 3



Retinal modulation using focused ultrasound and ultrasonic holography. **(a)** Data showing a beam plot (*top*) of an acoustic field used to stimulate a salamander retina at 90 μm spatial resolutions using 43 MHz FUS. Data showing retinal responses (*bottom*) stimulated by light (*black*) and FUS (*red*) for 'off' (*left*), 'on-off' (*middle*), and 'on' (*right*) ganglion cells (adapted from Ref. [14]). **(b)** The illustration of an acoustic retinal prosthetic (*top*) shows a concept for projecting ultrasonic holograms onto the retina for achieving multi-focal stimulation. **(c)** The images illustrate the product generated by an algorithm designed to produce multi-focal images (*top*) and the resultant ultrasound field intensities (*middle*) sufficient to stimulate retinal neurons as evidenced by the excess of power present indicated by the generation of thermal patterns in phantoms *bottom*; (adapted from Ref. [29]).

somatosensory evoked potentials induced by the stimulation of different peripheral nerves [25••]. Data from MR-thermometry combined with these neurophysiological observations confirmed LIFU can focally (1.14 MHz focal volume = 1 mm × 1 mm × 3 mm) modulate deep-brain activity for functional circuit mapping without causing tissue heating (Figure 2) [25••]. 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 [28••].

Other important technical advances highlight how the physics of US provide unique capabilities for interfacing with central nervous system circuits. As mentioned previously, <0.7 MHz US is used for transcranial applications, but higher frequencies can be readily used when skull transmission is not required. The highest spatial resolution achieved for ultrasonic neurostimulation to date is 90 μm, which was demonstrated using 43 MHz US to stimulate single neurons in salamander retina [14]. Interestingly, LIFU stimulated responses in retinal neurons faster than light stimulation because endogenous phototransduction cascades are bypassed when acoustic pressure was used as a stimulus (Figure 3) [14]. In advanced embodiments using LIFU for retinal stimulation, efforts to develop acoustic retinal prosthetics that project ultrasonic holograms onto the retina for neurostimulation have begun (Figure 3) [29]. Engineering an acoustic retinal prosthetic presents several technical and intellectual challenges ahead. However, proving out such an application would clearly validate the utility of ultrasound neuromodulation for advanced neural interfaces.

Modulation of peripheral nerve activity by ultrasound

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. [7•,16,30,31]). 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 most heroic studies investigating the influence of focused US on peripheral activity have been conducted by Gavrilov and colleagues [32,33]. In other investigations, electrophysiological recordings from frog sciatic nerves [34], crab leg nerves [35], earthworm giant axons [36] and others [7•,31] have shown that US can reversibly modulate neural activity by exerting non-thermal actions.

There are several considerations that need to be highlighted when discussing peripheral modulation by US. 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 similar to those obtained using more conventional somatosensory stimulation methods [37]. Thus, one must be able to distinguish direct effects on peripheral nerve fibers from those on somatosensory system receptors when transmitting US through the skin. Clear demonstrations that peripheral LIFU (1.1 MHz, 14–93 W/cm^2) can modulate the rat cervical vagus nerve has opened several therapeutic possibilities for exploration [38]. 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 [39]. Other studies directly measuring nerve responsivity to US indicate peripheral UNMOD will have clinical applications in neuromodulation and bioelectronic medicine [40]. Other potential clinical applications may support neuro-rehabilitation therapies or have implications for advanced prosthetics because low-intensity US has been shown to enhance nerve regeneration following nerve injury [41] and nerve grafts in rats [42]. Increased research into the utility and application of UNMOD for modulating the activity and plasticity of the peripheral nerves will continue to reveal expanded options where US can have significant impacts on medicine and neurotechnology beyond imaging.

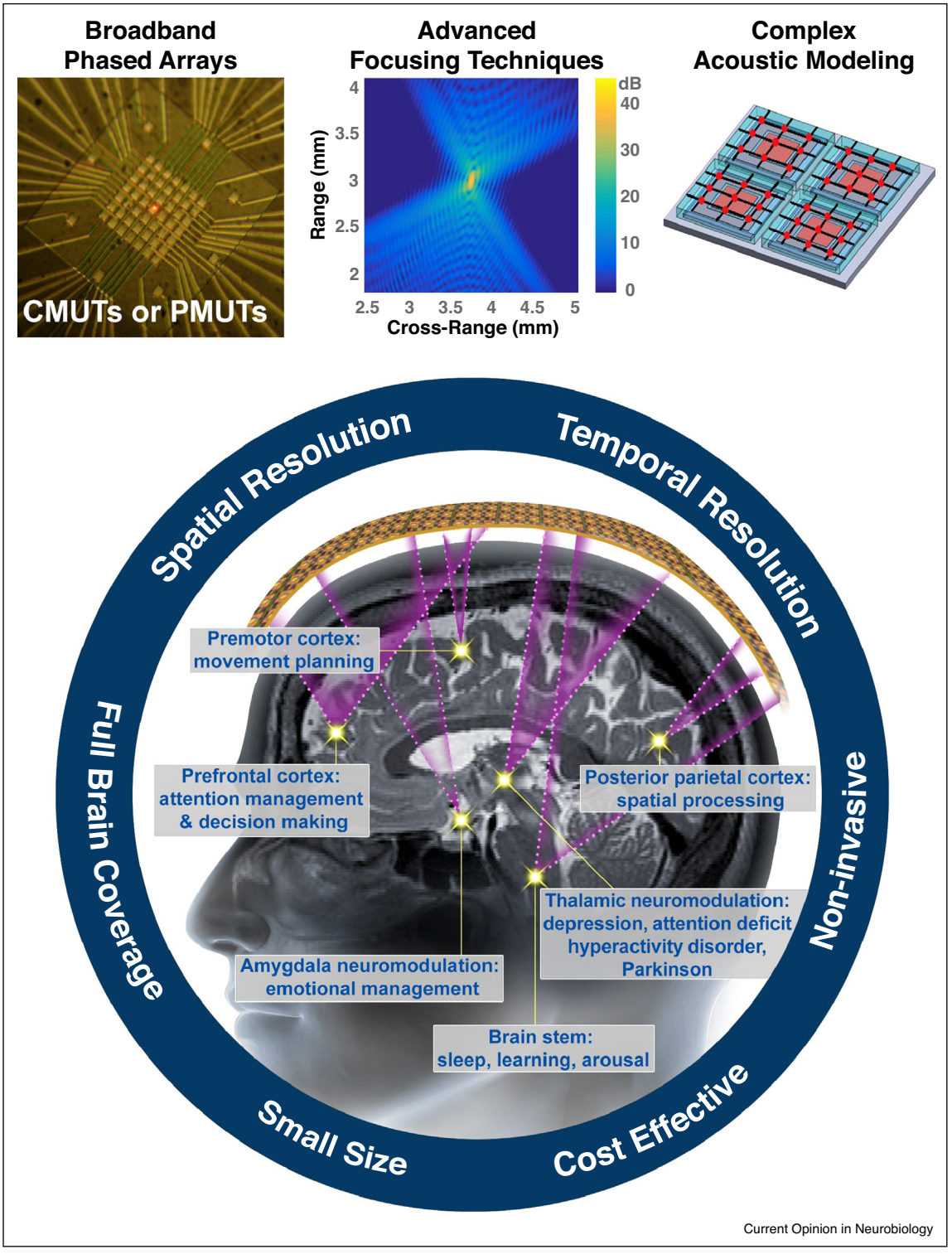
Some recent studies have begun to more carefully examine how US may be acting on peripheral nerves to modulate activity. A recent study demonstrating the modulation of crab leg nerves by US seem to indicate that cavitation may be a mechanism in the periphery [35]. These observations may not reflect natural 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* [43]. This elegant study recently demonstrated that focused US can robustly stimulate rat sciatic nerves in a manner similar to electrical stimulation [43]. Matthew and colleagues (2018) further demonstrated that brief (0.8–10 ms) 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 UNMOD once we uncover how it works.

Mechanisms of action

Considering the physical properties of neurons and their circuits, there are several ways in which US 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 US act on mechano-sensitive ion channels to alter neuronal activity (Figure 1). 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 [10]. Numerous studies have confirmed that LIFU can modulate neuronal activity without causing significant tissue heating [7•]. 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/cm^2) 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 [44]. Although demonstrated under unique conditions containing exogenous microbubbles, sonogenetic methods of activating TRP-4 channels in *Caenorhabditis elegans* has been shown a viable of regulating neuronal activity [45]. More convincing empirical evidence has recently shown that LIFU acts in a mechanical manner to modulate the activity of ion channels and neuronal activity [46]. 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.

Figure 4



Forward-looking engineering concepts for brain applications of neuromodulation by focused ultrasound. A multisite non-invasive neuromodulation system using focused ultrasound is illustrated. The concept draws from knowledge across different engineering disciplines of transducer design (*top left*), focusing techniques (*top middle*), and modeling capabilities (*top right*) shown as a whole-head phased array for multisite transcranial human neuromodulation using FUS. (*bottom*) A lightweight, monolithic, wearable CMUT array is able to stimulate or modulate one or more sparsely distributed regions in a simultaneous or sequential manner at high spatial and temporal resolutions. As illustrated multiple brain regions can be targeted using beam forming and focusing methods. These areas include deep-brain regions like the amygdala, thalamus, and brain stem,

Further, altering LIFU parameters to accentuate acoustic radiation forces produced by US elicited more robust responses [46]. 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 UNMOD. 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 [47]. 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 (Figure 1) [47,48]. 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 UNMOD waveform used [48]. 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 [17]. 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 mechanical forces act to regulate neuronal activity and plasticity.

Technical challenges and future solutions

The identification and consolidation of optimal parameter sets for UNMOD will likely be a challenge for some time to come. There is always appeal however to ensure safety, optimize efficacy, limit defocusing, increase spatial and temporal resolution, as well as provide an ability to target anywhere in the brain with one or multiple foci. Already methods of conducting MR-guided transcranial LIFU neuromodulation for brain mapping using optical neuro-navigation systems amenable to basic neuroscience laboratory environments have been described [12,13•,28••]. Compared to HIFU ablative procedures that rely on MR-thermometry for guidance and targeting, LIFU faces the added challenge that new non-thermal feedback mechanisms will need to be explored in order to leverage adaptive focusing techniques, such as time reversal [49,50] or US-guided focused US treatments [51]. Alternate focusing methods can rely on advanced computational methods that develop predictive models based on acoustic simulations and neurophysiological observations. These tools will no doubt accelerate the development of best practices for using focused ultrasound (FUS) and may point to new phased array designs tailored to different treatment areas (both near brain surface as well as deep-brain stimulation) and excitation waveforms that are unique for every individual to account for the complexities of each individual skull.

Beyond correcting aberrations in the focusing, efforts have also begun to improve the spatial resolutions of FUS for neuromodulation beyond the diffraction limit. Advances in acoustic metamaterials and acoustic hyperlenses have enabled several applications in subdiffraction acoustics over the past decade [52]. Whether such advances in acoustic metamaterials or advanced beamforming methods can enable superresolution UNMOD has not been explored, but could enable unprecedented spatial control of neural activity. One particularly interesting method of improving UNMOD spatial resolution used two different US waveforms transmitting different carrier frequencies into the brain simultaneously to generate an interference pattern at the beat frequency. Mehic and colleagues (2014) showed that a local beat frequency of 0.5 MHz generated by 2.0 and 1.5 MHz transmitted across rodent skulls offered improved spatial resolutions compared to using 0.5 MHz alone for UNMOD and brain mapping [53]. Mixing frequencies in such a manner to create interference patterns has several practical applications that may be useful for enhancing spatial resolutions, but can also serve as a basis for developing adaptive focusing methods. Perhaps mixing high frequency electrical currents or magnetic fields with US can produce unique effects on neural activity that are useful.

Higher temporal resolution will rely on the ability to use larger bandwidths than typical piezoelectrics with the added benefit of reducing focal spot sidelobes. Newer transducers, such as capacitive or piezoelectric micromachined ultrasonic transducers (CMUTS or PMUTs), can help meet these demands while being able to be fabricated in many different array shapes and sizes [54,55]. These microfabricated arrays could conceivably be used

(Figure 4 Legend Continued) which are collectively implicated in emotion, arousal, sleep, depression, and attention. Superficial brain circuits that can be targeted include the prefrontal cortex, motor cortex, and posterior parietal cortex, which are required, respectively, for decision-making, movement planning, and spatial processing. Several different neurological conditions can be diagnosed and treated with FUS for functional neuromodulation as the field progresses and produces efficacy studies. Methods to accelerate cognitive and motor-skills learning can also be assayed with multi-site, high-resolution, FUS neuromodulation. (MR image adapted from Ref. [58]).

in nested structures to be able to focus in holographic patterns or multiple treatment areas simultaneously. New efforts towards engineering turn-key FUS hardware such as CMUTs and PMUTs and associated software systems designed specifically for neuromodulation will encourage greater adoption in neuroscience because it will undoubtedly reduce the time, costs, and technical barriers to entry. Once broad research and development exploring the utility of UNMOD and FUS can occur, it is expected that neuroscientists will identify unanticipated ways of using acoustics to study brain function (Figure 4). For example controlled drug delivery for neuromodulation using FUS combined with various types of pressure-sensitive and temperature-sensitive nanocarriers has been described [56], which may open new avenues for further enhancing spatiotemporal control over brain function [57].

Conclusions

The use of acoustics in neuroscience and implementation of FUS for neuromodulation has expanded over the past several years. Data from numerous organisms and experimental models agree that the mechanical forces of US can reversibly stimulate or suppress brain circuits through direct, non-thermal mechanisms of action (Figure 1). It will take time and efforts by multiple groups working collaboratively to continue to establish safety thresholds balanced with effective modulation protocols. Likewise, gaining a thorough understanding of the mechanisms of action will require additional multidisciplinary investigations conducted across a variety or experimental preparations and conditions. This is par for course given neuroscience grapples daily with similar issues using more familiar and mature electrical or magnetic stimulation methods. Despite future challenges, the foundation has been laid and it is anticipated that the UNMOD field will continue to proliferate. Methods and devices that incorporate US for neural interfaces indeed represent a powerful set of next generation, non-invasive tools for neuroscience, medicine, and neurotechnology.

Conflicts of interest statement

WJT is the inventor and co-inventor on issued and pending patents related to methods, systems, and devices for electrical and ultrasonic neuromodulation.

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- •• of outstanding interest

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