ADVANCING TRANSCRANIAL FOCUSED ULTRASOUND FOR NONINVASIVE NEUROMODULATION OF HUMAN CORTEX

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Advancing Transcranial Focused Ultrasound for Noninvasive Neuromodulation of Human Cortex

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Abstract

Ultrasound waves are mechanical undulations above the threshold for human hearing, and have been used widely in both the human body and brain for diagnostic and therapeutic purposes. Ultrasound can be controlled using specially designed transducers into a focus of a few millimeters in diameter. Low intensity ultrasound, such as used in imaging applications, appears to be safe in adults. It is also known that ultrasound waves can penetrate through the skull and be focused within the brain for ablation purposes, employing the heat generation properties of high intensity focused ultrasound. High intensity focused ultrasound is thus used to irreversibly ablate brain tissue in localized areas without observable damage to intermediate tissue and vasculature. Ablation with high intensity focused ultrasound guided by magnetic resonance imaging is used for abolishing brain tumors, and experimentally for pain.

Low intensity ultrasound can be utilized beyond imaging in neuroscience and neurology by focusing the ultrasound beam to investigate the structure and function of discrete brain circuits. In contrast to high intensity focused ultrasound, the effects of low intensity focused ultrasound on neurons are reversible. Considering the volume of work on high intensity focused ultrasound, low intensity focused ultrasound remains decidedly underdeveloped. Given the great potential for impact of low intensity focused ultrasound
in both clinical and scientific neuromodulation applications, we sought to advance the use of low intensity focused ultrasound for noninvasive, transcranial neuromodulation of human cortex.

This dissertation contains novel research on the use of low intensity transcranial focused ultrasound for noninvasive neuromodulation of human cortex. The importance of mechanical forces in the nervous system is highlighted throughout to expand beyond the stigma that nervous function is governed chiefly by electrical and chemical means. Methods of transcranial focused ultrasound are applied to significantly modulate human cortical function, shown using electroencephalographic recordings and behavioral investigations of sensory discrimination performance. This dissertation also describes computational models used to investigate the insertion behavior of ultrasound across various tissues in the context of transcranial neuromodulation, as ultrasound’s application for neuromodulation is relatively new and crudely understood. These investigations are critical for the refinement of device design and the overall advancement of ultrasound methods for noninvasive neuromodulation.
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1 Introduction

Advancing ultrasonic neuromodulation
1.1 Introduction

Neuromodulation technology offers benefits over pharmacological treatment because its influence on neuronal circuits is more direct and focal. These features make it appealing to clinicians and researchers, as it can be used to diagnose malfunctioning neural circuits and modify the biologic mechanisms underlying pathologic conditions. Neuromodulation is used currently to treat many disorders. Surgical, invasive neuromodulation techniques on the United States market or nearing the market include deep brain stimulation, vagus nerve stimulation, implanted electrocortical stimulation, and epidural cortical stimulation. A number of minimally or noninvasive neuromodulation technologies exist as well, such as repetitive transcranial magnetic stimulation, transcranial direct current stimulation, cranial electrotherapy stimulation, and trigeminal nerve stimulation.

The modulation and monitoring of the nervous system is pertinent to the treatment of neurologic and psychiatric diseases, as well as to the scientific investigation of the neural mechanisms of cognitive, sensory, and motor functions. Therefore, improved approaches to the transcranial modulation of human brain circuit activity are sought to support global brain mapping efforts, as well as to advance diagnostics and therapies. Conventionally, interfacing with the nervous system has been conducted using electrical and chemical means, such as micro-dialysis and deep-brain stimulation. Recently, the development of devices utilizing mechanical energy to interact with the nervous system has received considerable attention. While numerous mechanical events have been observed and associated with neuronal activity, it has not been until very recently that technology has started to be adapted to capitalize on these mechanical events to allow the observation, and even modulation, of nervous tissue. The recent burst in development of
these methods and devices represent early technological advances to exploit the coupling between neuronal function and mechanical forces that still require further improvement and study before achieving wide spread adoption and seamless implementation. The overarching goal of this work was to progress the efforts of mechanical neuromodulation by investigating and refining the use of transcranial focused ultrasound for focally modulating cortical function in humans. The research presented here advances this goal in several steps, as follows.

1.2 Document organization

In this chapter, the overall content of the dissertation is discussed. Other than the first and last chapters of the dissertation, each chapter highlights work that has either been published or submitted with the aim to increase the consideration of mechanical forces as a means to interface with the nervous system, and to promote work advancing the use of low intensity transcranial focused ultrasound for the noninvasive neuromodulation of human cortex. Additionally, some background information is provided in Chapter 1 on a few selected concepts to help provide an understanding of the discussions to follow.

Chapter 2 is a topical review focused on the discussion of the role of mechanical forces in the nervous system. The physiological functions of nervous systems are primarily regarded as being regulated by electrical and chemical driving forces, and it is not widely understood how other forces, such as mechanical ones, impart actions upon neural function. This chapter highlights some contexts where it has been shown how mechanical forces and thermodynamics also govern neuronal excitability, signaling, and development.
Chapter 3 discusses work probing the influence of transcranial focused ultrasound targeted to the human primary somatosensory cortex. Despite observations in different animal models, it had remained untested whether ultrasound can focally modulate the activity of intact human brain circuits. These observations help establish that focused ultrasound can be used to focally modulate sensory evoked brain activity and cortical function in humans.

Chapter 4 expands on the observations of the previous chapter to investigate the feasibility and effects of focused ultrasound on phase dynamics. Both phase and phase rate has been previously identified to be modulated by other conventional non-invasive neuromodulation methods. These investigations support that transcranial focused ultrasound modulates electroencephalographic oscillatory dynamics similar to existing technologies.

Chapter 5 explores the sensitivity of the transcranially focused ultrasound beam to variations in tissue layers, their geometry, and their material properties. The intracranial manifestation of effects by ultrasound depends on its insertion behavior across the various layers of tissue. Using computational modeling, the sensitivity of the transcranially focused ultrasound in the context of neuromodulation was investigated. An understanding of the insertion behavior in the context of neuromodulation is key to the continued advancement of ultrasound stimulation methods.

Chapter 6 considers the results in the previous chapters to suggest future lines of academic enquiry and possible technical improvements. This chapter also attempts to foresee future developments in the field.
1.3 Background

Some brief background information is provided on a few selected concepts to help provide context and appreciation of the discussions to follow.

1.3.1 Current non-invasive neuromodulation methods

Noninvasive neuromodulation is of great appeal in the clinical and scientific realm as it allows one to probe neural activity with minimal preparation and manipulation of subjects, which is especially appealing in humans. Fairly established methods for noninvasive neuromodulation include transcranial magnetic stimulation (TMS), transcranial alternating current stimulation (tACS), and transcranial direct current stimulation (tDCS). In tACS and tDCS, large electrodes are applied to the scalp and connected to a power source to induce low amplitude currents in the head. Evidence suggests tACS and tDCS currents do not explicitly elicit action potentials in the underlying nervous tissue, but rather modify the transmembrane potential, influencing their excitability and modulating their firing in response to their inputs [1]. In TMS, a large brief current is pulsed through handheld coil windings to create a brief, intense magnetic field that passes through air and tissue unimpeded to generate intracranial currents via electromagnetic induction. Evidence suggests that these currents can be of sufficient magnitude to elicit action potentials, and that TMS can be used to modulate cortical excitability as well [1].

However, with the gain in advantage of being noninvasive, these electrical and electromagnetic induction methods of transcranial neuromodulation suffer from a limitation in spatial resolution. This can be conceptualized as being due to the devices of
intervention being located far from the target tissue; in contrast to an electrode implanted directly next to the target tissue in the brain. These electrically based transcranial methods generate electric fields having centimeter length scales in the human brain (Figure 1.1) and thus provide a crude resolution that is poorly suited to focally modulate discrete human brain circuits.

1.3.2 Ultrasound

Ultrasound waves are mechanical undulations at frequencies above the upper limit of human hearing, approximately 20 kHz and greater. Ultrasound has been used widely in the human body for both diagnostic (e.g. Doppler imaging and medical sonography) and therapeutic (e.g. thrombolysis and tissue ablation) purposes. Because ultrasound energy is mechanical rather than electromagnetic, its simultaneous use with electromagnetically based diagnostic systems (e.g. magnetic resonance imaging and electroencephalography) requires minimal accommodation and is relatively straightforward.

1.3.3 Primary somatosensory cortex

The primary somatosensory cortex (S1) is located in the lateral postcentral gyrus of the parietal lobe of the human brain. It is the main sensory receptive area in the cortex for the sense of touch, and similar to other sensory areas, is associated with a map of sensory space called the sensory homunculus. Lesions affecting the primary somatosensory cortex produce characteristic symptoms including deficits in the sense of vibration, proprioception, and fine touch.
1.3.4 Electroencephalography

Electroencephalography (EEG) is the recording by an array of electrodes on the scalp of the brain’s electrical activity, generated by the cumulative ionic current flows of neural elements within the brain. Some of the activity recorded by scalp electrodes is generated by the action potentials of cortical neurons, but most are generated by excitatory postsynaptic potentials [2]. While the fine details of EEG generation are not fully understood, EEG is a widespread clinical diagnostic and research tool. This can be attributed to the availability of EEG systems, its noninvasiveness, and the advent of computers and digitization leading to quantitative methods that allow for objective measures and automation.

1.3.4.1 Event related potentials

Derivatives of electroencephalography (EEG) include the analysis of event related potentials, which involves averaging the EEG activity time locked to the presentation of a stimulus (i.e. the potentials evoked by the stimulus event). The stimulus can be of any number of forms including visual, somatosensory, or auditory. Somatosensory evoked potentials (i.e. event related potentials from a somatosensory stimulus) recorded from the scalp are a useful noninvasive means of observing the processing of physical stimuli by inspection of the recorded cortical activity. The event related potential (ERP) and has been extensively studied and used in cognitive neuroscience and psychophysiological research.

Two models addressing the generation of ERPs include the evoked model and the phase reset model. The evoked model (Figure 1.2B) proposes that the evoked response
generating the ERP is additive and completely independent of ongoing background EEG activity [3]. The phase reset model (Figure 1.2C) proposes a resetting of ongoing brain oscillations as the neural basis of the ERP [3]. The debate between the evoked and oscillatory models of ERP generation continues in literature, as the exact roles of EEG amplitude and frequency, two independent wave parameters, on the generation of ERPs remains largely conjectured.

1.3.4.2 Neural oscillations

Oscillatory neural activity overall has become recognized as a basic signal in cognitive function, and the entrainment of neuronal populations to these oscillating frequencies of the brain is implicated as participation in complex brain functions [4, 5]. Phase has been associated with various functions, including the timing of communication between functionally related neural populations, the exchange of information between global and local neuronal networks, and the timing of neuronal processing in response to stimuli [6]. Thus, complex brain functions are likely manifested in the superposition of several oscillating neuronal populations, however the meaning behind a specific value of instantaneous phase within a given frequency band and its implication on cognitive function remains obscure.

Further complicating the matter, phase coupling between populations are more complex than a direct 1:1 relationship in instantaneous phase values. For example, the difference in phase between regions may preserve a constant value to maintain a high coherence in phase between regions [6]. Even more complicated is phase synchronization across frequencies, including m:n phase synchronization, where populations oscillating at
different frequencies exhibit a more complex synchronization to exchange information independent of absolute instantaneous phase angles [6]. Phase rate may represent a parameter beyond instantaneous phase more accessible for inspection of complex cognitive functions, possibly as the facilitation of signal transmission between populations with similar or resonant oscillatory phase characteristics. The variation of phase rate and its relation to phase and the EEG signal is illustrated in Figure 1.3. Note that sudden shifts in phase lead to large jumps in phase rate (Figure 1.3 left column), and that phase rate modulation leads to a dampening, or acceleration, of changes in the phase of the EEG signal (illustrated with a frequency modulated signal in the right column of Figure 1.3A).

1.4 References


1.5 Figures

**Figure 1.1** Comparison of electric field distributions induced by transcranial direct current stimulation (left) and transcranial magnetic stimulation (right). Color map corresponds to the independently normalized electric field strength in the FEM models of whole human brain. Note that the red colored peaks span multiple gyral crowns across a few centimeters, thus spanning multiple cortical regions and circuits.
Figure 1.2 Conceptual illustration of the generation of event related potentials by the evoked and phase reset models. (A) Averaging of unrelated trials that are not time locked to a stimulus results in a flat line. (B) The evoked model proposes that in each single trial a constant evoked response is added onto the background EEG signal. Averaging of the trials reflects the isolated evoked response. (C) The phase reset model proposes that the event related potential is generated by phase resetting of the oscillatory background EEG signal, without any additive component. Adapted from [3].
Figure 1.3 Illustration of changes in phase rate due to a shift in phase (left column) and variation of phase rate (right column) for theoretical data (A) and example EEG data (B). A sudden change in phase of the EEG signal leads to discontinuities in the calculation of phase and corresponding spikes in the phase rate (left column). A modulation of phase rate leads to smooth evolutions of the phase and phase rate parameters (right column).
2 Background

Role of mechanical forces in the nervous system

Work presented in this chapter has been published as:


2.1 Abstract

The fundamentals of neuronal membrane excitability are globally described using the Hodgkin-Huxley (HH) model. The HH model however does not account for a number of biophysical phenomena associated with action potentials or propagating nerve impulses. Physical mechanisms underlying these processes, such as reversible heat transfer and axonal swelling, have been compartmentalized and separately investigated to reveal neuronal activity is not solely influenced by electrical or biochemical factors. Instead, mechanical forces and thermodynamics also govern neuronal excitability and signaling. To advance our understanding of neuronal function and dysfunction, compartmentalized analyses of electrical, chemical, and mechanical processes need to reevaluated and integrated into more comprehensive theories. The present perspective is intended to provide a broad overview of biophysical forces that can influence neural function, but which have been traditionally underappreciated in neuroscience. Further, several examples where mechanical forces have been shown to exert their actions on nervous system development, signaling, and plasticity are highlighted to underscore their importance in sculpting neural function. By considering the collective actions of biophysical forces influencing neuronal activity, our working models can be expanded and new paradigms can be applied to the investigation and characterization of brain function and dysfunction.

2.2 Introduction

Examining the importance of mechanical forces on neuronal activity began gaining attention several decades ago [1] and continues to motivate questions in the modern study
of brain function [2]. Outside of neuroscience, there exist well-established models describing the interplay between mechanical and electro-chemical signaling in tissues [3-7]. In fact the importance of mechanical cues on physiology has been thoroughly characterized in several organ systems. For instance mechanical forces have been broadly implicated in regulating heart function, and stretch-activated channels (SACs) are known to play a role in cardiac pacing [8-12]. The activity of SACs and mechanosensitive channels (MSC) generate ordered signals influencing the colon [13], bladder [14], and muscles [15]. In sensory neuroscience it is well established that MSCs are involved in signal transduction processes, such as those exemplified by the role of transient receptor potential (TRP) channels and hair cells in hearing [16] or free nerve endings and similar TRP channels or mechanoreceptors in touch [17].

Currently, the physiological functions of nervous systems are primarily regarded as being regulated by electrical and chemical driving forces. For instance, we have an intimate portrait of how electrical signaling along axonal fibers is converted to chemical signaling at the synapse between neurons. It is not well understood however how other forces, such as mechanical ones, impart actions upon neural function although there are numerous cellular and molecular players known to sense and transduce mechanical signals in neurons, glial cells and brain circuits [2]. These components include a viscoelastic plasma membrane, stretch-sensitive ion channels, cytoskeletal proteins, such as actin and microtubules that can generate and sense intracellular force, as well as extracellular matrix proteins that provide adhering and coupling forces between cells to name a few (Figure 2.1).
Several mechanical events have indeed been experimentally observed in nervous system preparations and are thought to play important roles in regulating neuronal activity. Only recently however have technological advances in the tools used to study mechanobiology enabled expanded investigations into the actions biophysical forces exert on neural circuits [2]. In the present article, we provide an overview of biophysical formulations and mechanisms thought to influence nervous system activity on a variety of levels spanning development, signaling, plasticity, and injury (Figure 2.2). In addition to these basic formulations, we describe models and technology for mechanically manipulating and monitoring biophysical forces in the nervous system (Figure 2.2). To advance our understanding of how nervous systems operate it is important to develop comprehensive models where electrical, chemical, and mechanical energies are not compartmentalized from one another, but rather cooperate in a synergistic manner to regulate neuronal excitability and signaling. By starting to consider the interplay between electrical, chemical, and mechanical energy, new paradigms for understanding and studying the biophysics of neural systems will advance our comprehension of brain function.

2.3 Electro-mechanical coupling and deformation forces
The Hodgkin-Huxley (HH) model is a bioelectric description of neuronal excitability based on conductance of ion-selective channels and a membrane capacitor, and is the currently accepted model for describing the action potential [18]. However, there are a number of observations related to the action potential that are not electrical or electro-chemical in nature. Several studies have shown the geometric dimensions of nerve fibers
change in phase with action potential propagation, exerting forces normal to the membrane surface [1, 19-23]. Additionally, there is a reversible change in heat generation during action potential propagation, where heat released during the first phase of the action potential is compensated by heat uptake in the second phase [23-27]. The HH model however is based on irreversible processes and does not include thermodynamic variables required to sufficiently explain all the physically observed features of a nerve impulse. Despite this shortcoming, the equivalent RC circuit formalism of the HH model [18] has, no doubt, acquired global support through a bewildering number of independent observations over the past 60 years. While separate models accounting for the other non-electrical behaviors observed during the action potential have been proposed, there is not a broadly accepted model unifying electrical, chemical, and mechanical descriptions of the neuronal action potential. Regardless, the coupling of mechanical and electrical energy has undergone considerable research and development (for example, piezoelectricity) and its consideration as applied to the nervous system is briefly highlighted below.

2.3.1 The flexoelectric effect

The flexoelectric effect is a liquid crystal analogue to the piezoelectric effect in solid crystals. Flexoelectricity refers specifically to the curvature dependent polarization of the membrane [28]. As opposed to area stretching, thickness compression, and shear deformation in solid crystals, the flexoelectric effect includes the deformation of membrane curvature. This effect is manifested in liquid crystalline membrane structures, as a curvature of membrane surface leads to a splay of lipids and proteins. The molecules
would otherwise be oriented parallel to each other in the normal flat state of the local membrane. Similar to piezoelectricity of solids, flexoelectricity is also manifested as a direct and a converse effect, featuring electric field induced curvature. The flexoelectric effect provides a basic mechanoelectric mechanism enabling nanometer-thick biomembranes to exchange responsiveness between electrical and mechanical stimuli. Consideration that cellular membranes possess mechanoelectric properties has raised concerns regarding the possible origin of inductance in early circuit models of the neuronal membrane and giant squid axons [29].

Experimentally, the generation of alternating currents by membranes subjected to oscillating gradients of hydrostatic pressure was observed in the early 1970’s [30]. This observed vibratory response was assumed to be due to changes in membrane area and thus capacitance, though a detailed explanation of the mechanisms with regard to transmembrane potential was not offered at the time. The oscillations of membrane curvature in these experiments can be credited as a displacement current due to the oscillating reversal of flexoelectric polarization of the curved membrane [28]. According to the Helmholtz equation, an electric potential difference appears across a polarized surface. For a membrane curvature that oscillates in time, and assuming spherical curvature for simplicity, the flexopolarization leads to a transmembrane AC voltage difference with first harmonic amplitude described by Equation 2.1:

$$U_{\omega} = \frac{f}{\varepsilon_0} 2c_m$$  (2.1)
where $f$ is the flexoelectric coefficient, measured in coulombs, $\varepsilon_0$ is the absolute dielectric permittivity of free space, and $c_m$ is the maximal curvature. Similarly, a displacement current due to oscillating flexopolarization can also be calculated by considering a membrane capacitance described by \textbf{Equation 2.2}:

$$C_0 = \frac{\varepsilon_0 S_0}{d} \quad (2.2)$$

where $S_0$ is the flat membrane area and $d$ is the capacitive thickness of the membrane. The first harmonic amplitude of the membrane flexoelectric current can then be described by \textbf{Equation 2.3}:

$$I_\omega = f \frac{C_0}{\varepsilon_0} 2c_m \omega \quad (2.3)$$

where $\omega$ is the angular frequency of oscillations. Thus, the current through or potential across the membrane can be determined from the associated flexoelectric coefficient of the membrane and the radius of curvature. Flexoelectricity (current generation from bending) and converse flexoelectricity have been demonstrated in lipid bilayers and cell membranes [28, 31]. Flexoelectricity provides a linear relationship between membrane curvature and transmembrane voltage and is likely involved in mechanosensitivity and mechanotransduction in biological systems [31]. The direct and converse flexoelectric effects have been used to describe the transformation of mechanical into electrical energy by stereocilia and the electromotility of outer hair cell membranes for hearing [28, 31].
Many membrane functions involve the manipulation of membrane curvature (for example, exocytosis, endocytosis, and cell migration) and the prospects that flexoelectricity is intricately involved in these processes thereby relating membrane mechanics and electrodynamics is likely, but requires further inspection.

### 2.3.2 Voltage-induced changes in membrane tension

Mechanical equilibrium in membranes requires that the cellular radius depend on surface tension in order to maintain a constant pressure across the membrane, as related by the Young-Laplace equation:

\[
\Delta P = \gamma \left( \frac{1}{R_1} + \frac{1}{R_2} \right)
\]  

(2.4)

where \( \Delta P \) is the pressure difference across the membrane, \( \gamma \) is the surface tension, and \( R_1 \) and \( R_2 \) are the principal radii of curvature. Considering now electric field mediated effects on the membrane, the relation between surface tension and an applied electrostatic potential is given by the Young-Lippmann equation:

\[
\gamma = \gamma^0 - \frac{CV^2}{2}
\]  

(2.5)

where \( \gamma \) is the total chemical and electrical surface tension, \( \gamma^0 \) is the surface tension at zero electric field, \( C \) is the capacitance of the interface, and \( V \) is the surface potential at the interface. Electrowetting on dielectrics is one application concerned with surface
tension as related to an applied voltage [32-34]. In electrowetting, a thin insulating layer (analogous to the cell membrane) is used to separate conductive liquid (extracellular environment) from metallic electrodes with an applied voltage (voltage regulated intracellular environment) to avoid electrolysis. Modulation of the applied voltage of the metallic electrode is used to change the interfacial surface tension and contact angle of the conductive liquid with the insulating layer for applications such as electronic displays, adjustable lenses, and microfluidic transport of droplets [33]. Extend this construct however such that the insulating layer is deformable (neuronal membrane) and there is a modulation of surface tension of liquids on both sides of the insulating layer (intracellular and extracellular environments), and the premise of combining and extending **Equation 2.4** and **Equation 2.5** as a model for modulation of the tension of the deformable membrane becomes more readily apparent.

Such a model would require a solution to the surface potential at the interfaces based on the distribution of ions in the electric double layer, which is obtainable from the Poisson-Boltzmann equation. Assuming both positive and negative ions of valence 1 are present, the surface potential \( V \) near a surface with charge density \( q \) is given by **Equation 2.6** [35]:

\[
V = \frac{2k_B T}{e_0 \sinh^{-1} \left( \frac{q}{2\sqrt{2nk_B T \epsilon_w \epsilon_0}} \right)}
\]  

(2.6)

where \( k_B \) is Boltzmann’s constant, \( T \) is the absolute temperature, \( e_0 \) electronic charge, \( \epsilon_0 \) the permittivity of free space, \( \epsilon_w \) the relative permittivity of water, and \( n \) ionic strength of the solution. Differences in tension between the intracellular and extracellular interfaces
will create changes in membrane curvature, referred to earlier as converse flexoelectricity. Thus, modulation of membrane tension by transmembrane voltage in a neuron will cause movement of the membrane with magnitude and polarity governed by the neuronal membrane stiffness and surface potentials at the membrane interfaces in order to maintain pressure across the membrane. Such an effect has been observed in real-time using atomic force microscopy (AFM) and voltage clamped HEK293 cells [35]. In these studies Zhang et al. (2001) observed that depolarization caused an outward movement of the membrane, with amplitude proportional to voltage. By applying Equation 2.5 and Equation 2.6 to both interfaces of the lipid bilayer, a mathematical model able to predict the membrane tension over a range of surface potentials was developed [36]. The sum of the two interface tensions yields the total tension in the membrane ($\gamma$) as described by Equation 2.7:

$$
\frac{(\gamma-\gamma^0)e_0}{\sqrt{(2k_BT)^2\varepsilon_w\varepsilon_0}} = \sqrt{n_{ex}} \left[ \sinh^{-1} \left( \frac{\sigma_{ex} - C_m V}{2\sqrt{2n_{ex}k_BT\varepsilon_w\varepsilon_0}} \right) \right]^2 + \ldots
$$

$$
\sqrt{n_{in}} \left[ \sinh^{-1} \left( \frac{\sigma_{in} + C_m V}{2\sqrt{2n_{in}k_BT\varepsilon_w\varepsilon_0}} \right) \right]^2
$$

where $k_B$ is Boltzmann’s constant, $T$ is the absolute temperature, $\varepsilon_0$ the permittivity of free space, $\varepsilon_w$ the relative permittivity of water, $e_0$ electronic charge, $n$ ionic strength of the solution, $\sigma$ structural charge density at the interface, $C_m$ specific capacitance of the membrane, $V$ voltage, $\gamma^0$ the voltage independent portion of membrane tension, and the subscripts $ex$ and $in$ refer to the external and internal membrane interfaces respectively. Thus, the tension in the membrane is related to the voltage and ionic charges across the
membrane. Consequently, the change in voltage with a nerve impulse is associated with a change in membrane tension, which will result in an alteration of cell radius to keep pressure constant across the membrane. This offers a mechanism and quantitative description for the observed change in the diameter of nerve fibers during the action potential, as opposed to alternative hypothesized mechanisms such as cell swelling due to water transport [20].

2.3.3 Opto-electric and electro-mechanical coupling

Our early understanding of the phenomena of electrical coupling with the mechanical modification of the neuronal membrane has already begun to yield innovative methods and technologies for interfacing to the nervous system. The modulation of refractive index or thickness of the cell due to transmembrane potential dependent deformations has allowed label free imaging of the membrane potential without the need of organic dyes or optogenetic probes which themselves likely alter membrane dynamics [37]. By measuring milliradian scale phase shifts in the transmitted light, changes in the membrane potential of individual mammalian cells have been detected using low coherence interferometric microscopy without the use of exogenous labels [37]. Using this technique, it was also demonstrated that propagation of electrical stimuli in gap junction-coupled cells could be monitored using wide-field imaging. This technique offers the advantages of simple sample preparation, low phototoxicity, and no need for photobleaching. Previous successes in label-free imaging of electrical activity has been possible in invertebrate nerves and neurons, as mammalian cells are smaller, optically transparent, and scatter light significantly less [37]. While such approaches still require
further refinement to enable a resolving power capable of imaging single action potentials, these methods have been able to experimentally confirm that the source of light phase shifts are due to potential-mediated changes in membrane tension, as opposed to swelling due to water transport or electrostriction of the cell membrane [37].

Regarding probing the mechanical response of mammalian cells to electrical excitation, atomic force microscopy (AFM) is the most commonly used tool for quantifying cellular deformation despite its invasiveness. Recently, piezoelectric nanoribbons have been developed for electro-mechanical biosensing and have demonstrated that cells deflect by 1 nm when 120 mV is applied to the membrane [36]. Furthermore, these nanoribbons support the model of voltage induced membrane tension discussed earlier, and support previous investigations of cellular electro-mechanics using AFM. Nanoribbons are made using microfabrication techniques, and so can be scaled more readily than AFM probes. Additionally, advances in microfabrication techniques could allow the manufacture of thinner nanoribbons to enhance their sensitivity, and facilitate the electro-mechanical observation of smaller neural structures, such as axons, dendrites, and dendritic spines. It will be interesting to see how further innovative methods and technologies develop to advance our understanding of the electrical coupling with the mechanical modification of the neuronal membrane.

2.4 Mechanically-sensitive ion channels

Mechanical forces acting on cell membranes or through cytoskeletal filaments can be transformed into consequences on membrane bound and cytoskeletal-tethered protein activity (Figure 2.3). Many ion channels exhibit spring-like structures, rendering their
gating kinetics sensitive to mechanical forces. Further, the effects of pressure, tension, stretch, and stress on cell membranes are known to be capable of activating and inactivating a broad range of MSCs. From bacteria to primates, nearly all animal cells express MSCs. Further, many classic voltage-gated channels known to be involved in regulating neuronal excitability have been shown to exhibit mechanosensitive changes in gating dynamics [2].

To better understand the factors influencing ion channel activity, consider the simple case of a two state (open and closed) channel using Boltzmann statistics, where open channel probability ($P_o$) can be described by \textbf{Equation 2.8} as:

$$P_o = \frac{1}{1 + e^{\frac{\Delta G}{k_B T}}} \quad (2.8)$$

where $k_B$ is the Boltzmann constant, $T$ is the absolute temperature, and $\Delta G$ is an intrinsic energy difference between open and closed states ($G_{open} - G_{closed}$). The $\Delta G$ dictates the likelihood of the channel occupying each state. The change in free energy can also be expressed as the sum of changes due to chemical ($\Delta G_{chem}$), electrical ($\Delta G_{elec}$), and mechanical ($\Delta G_{mech}$) contributions, and channels can be responsive to a combination of these factors (polymodal). Various analytical models quantifying changes in free energy due to these different mechanisms have been detailed in literature [38-44].

Regarding mechanical stimulation, if a force $f$ is exerted on the channel and the gating domain moves a distance $b$, then work is done, and the change in free energy is described by \textbf{Equation 2.9}:
\[ \Delta G_{\text{mech force}} = -fb + \Delta u \]  

(2.9)

where \( \Delta u \) is the intrinsic energy difference between states in the absence of applied force. A larger movement of the gate swing \((b)\) requires less force \((f)\) to obtain the same \(\Delta G\). Note that this expression is equivalent to the gating of voltage-dependent channels as described by Equation 2.10:

\[ \Delta G_{\text{elec force}} = f_{\text{elec}} \times \text{distance} + \Delta u \]

\[ = -E q \times b + \Delta u \]  

(2.10)

\[ \approx V_m \left( \frac{q b}{m} \right) + \Delta u \]

where \( E \) is the electric field strength, \( q \) the net charge on the gating region, and \( \Delta u \) is the intrinsic energy difference between states in the absence of an electric field. The electric field strength is \( E = V_m / m \) where \( V_m \) is the transmembrane potential and \( m \) is the distance over which the field drops, typically approximated as the membrane thickness. Due to difficulties in determining \( b \) and \( m \), the term \( q b / m \) is utilized, and referred to as the equivalent gating charge. The equivalent gating charge is typically 4-6 electron charges per subunit for voltage-gated channels, such that an energy difference of 1 \( k_b T \) is produced by a membrane potential of \( \sim 5 \text{ mV} \) [38].

A similar expression for the free energy holds for channels influenced by tension in the membrane. Tension is the energy excess per unit area resulting from any type of stress. Most work on mechanically gated channels uses patch-recording electrodes to
apply suction to a patch of membrane to generate tension. A lateral stretch of a membrane generates an expanded area, producing work, lowering the free energy difference in states, and can be expressed by Equation 2.11 as:

$$\Delta G_{\text{mech tension}} = -\gamma \Delta a + \Delta u$$

(2.11)

where $\gamma$ is lateral tension, $\Delta a$ is the change of the in-plane area of the channel after opening, and $\Delta u$ is the intrinsic energy difference between states in the absence of tension. Other forms of stress relevant to biological membranes include shear stress and bending stress, which can also contribute to changes in free energy. However, as the area elasticity modulus is much larger than the shear and bending moduli, the contributions to free energy from lateral tension will typically dominate.

The mechanosensitivity of voltage-dependent channels has been investigated for a multitude of ion channels. Mammalian cells express several families of polymodal-gated ion channels, including transient receptor potential (TRP) and potassium two-pore domain (K$_{2P}$) channels, which have been shown to be activated by mechanical stimuli including membrane stretch and hydrostatic pressure (for reviews see [45-48]). The TRP channel heteromers TRPC1/C3 and TRPC1/P2 are of particular interest as they have been shown to form calcium-permeable MSCs in mammalian cells including neurons (for reviews see [47-49]). The polymodal K$_{2P}$.1 channel TREK-1 is mechanosensitive and is active at rest while mediating potassium leak currents to regulate the resting membrane potential and excitability of neurons [45, 50]. Neurons are also known to express a variety of pressure-sensitive channels, which both exhibit polymodal gating mechanisms.
As briefly mentioned above, several of the voltage-gated ion channels expressed in neurons (for example, the voltage-gated sodium channels Nav1.2, Nav1.5, and the voltage-gated potassium channel Kv1.1) possess mechanosensitive properties that render their gating kinetics sensitive to transient changes in lipid bilayer tension [38, 46]. In one particular investigation, voltage-gated potassium (Kv) channels were shown to exhibit sensitivity to small physiologically relevant mechanical perturbations of the cell membrane, producing shifts in the channel activation curve and an increase in the maximum open probability [51]. A theoretical model was developed that could reproduce the shift in gating kinetics, and the model held only by having the tension act predominantly on the pore opening transition of the channel to favor the open conformation, and not if membrane tension acted mainly on the channel’s voltage sensor conformational changes [51]. This mechanically-induced shift in activation kinetics could allow Kv channels, and likely other voltage-dependent ion channels related in function and structure such as voltage gated sodium and calcium channels, to play a role in mechanosensation and contribute to the variability of cellular responses to mechanical forces.

The heart is of particular interest for investigating the role of mechanosensitivity in voltage-gated channels, as the heart could represent a more readily accessible model of mechano-electric feedback than the nervous system. If the varying mechanical environment of the myocardium and vasculature are indeed involved in the control of cardiac rhythmicity by the routine deformations of the bilayer membrane structure, then the heart makes for a well-characterized model to study the contributions of stretch-activated currents due to mechano-electric feedback. The heart could also serve as a
possible model for investigating the role of mechanically abnormal bilayers (such as in diseased heart tissue) to electrical pathologies of the heart [8]. One early attempt to model the effects of MSCs on heart function include the incorporation of stretch-activated currents into an existing guinea pig ventricular cell model as a linear current introduced by Equation 2.12 [9]:

\[ I_{SAC} = \frac{|V - V_{rev}| \gamma \rho A}{1 + Ke^{-\alpha(L-L_0)}} \]  

where the stretch activated current \( I_{SAC} \) is determined by the membrane potential \( V \), the channel’s reversal potential \( V_{rev} \), channel conductance \( \gamma \), channel density \( \rho \), cell area \( A \), an equilibrium constant \( K \) controlling the amount of current at \( L_0 \), sarcomere length \( L \), and a sensitivity parameter \( \alpha \). By using sarcomere length as an analog of membrane tension, simulated ventricular action potentials successfully captured a number of experimentally observed behaviors. In this manner, more macroscopic characterizations of the cell and tissue can be considered to investigate their effect on stretch-activated currents, and effectively MSCs as well. By examining the stretch-activated currents in heart cells as a whole, in addition to the investigation of the mechanical sensitivity of individual ion channels, further insights on the influence of mechanical forces on the activity of ion channels can be obtained. We should strive to apply these observations and models to nervous systems since it is unimaginable how they might escape factors regulating the influence of mechano-electric coupling on channel activity and cellular excitability.
2.5 Phospholipid membranes

The properties of the phospholipid bilayer membrane determine in part the behavior of various dynamic and relaxation processes of neuronal function. Processes influenced include the propagation and attenuation of mechanical waves, the decay of thermal shape fluctuations, and the translational and rotational diffusion of membrane components [52]. When subjected to lateral stretching or compression, bilayer membranes behave as a viscoelastic material with anisotropy, and this can serve as a mechanism to modulate the state of the membrane, and hence all associated neuronal membrane processes such as channel activity. Thermodynamic investigations of lipid phase transitions have shown that lipid density pulses (sound or mechanical waves) can be adiabatically propagated through lipid monolayers, lipid bilayers, and neuronal membranes to influence fluidity and membrane excitability [53-55] (Figure 2.4). Interestingly, recent evidence indicates such sound wave propagation in pure lipid membranes can produce depolarizing potentials ranging from 1 to 50 mV with negligible heat generation [53], linking mechanical waves in neuronal membranes to changes in transmembrane potentials.

How the properties and changes in density of phospholipid bilayers influence the propagation of mechanical waves and neuronal processes, such as action potential initiation and propagation, is not precisely known. Anesthetics though, make for an interesting case to examine the influence of phospholipid bilayer state on neuronal function. It is known that anesthetics affect various functions of the neurons, including membrane permeability, hemolysis, and the function of ion channels and proteins. The Meyer-Overton rule for anesthetics relates that the critical dose is linearly proportional to the membrane solubility of the anesthetic molecules in the neuronal membrane,
independent of the chemical ligand actions of the molecule [56]. This rules out specific binding effects based on protein models for the wide variety of anesthetics that follow the Meyer-Overton rule. For example, voltage-gated sodium and potassium channels are slightly inhibited by halogenated alkanes and ethers, but not by xenon and nitrous oxide, despite all these anesthetics following the Meyer-Overton rule [56]. It is known however that anesthetics have a pronounced effect on the physical properties of lipid bilayers, such as their lipid melting point phase transitions. This change in physical properties of the membrane can be related to the alteration of neuronal function by anesthetics, providing a mechanism for the alterations in function dependent on their solubility in the membrane and independent of their chemical nature. Such changes in the properties of the neuron’s membrane would then influence such mechanisms as flexoelectricity, voltage induced membrane tension, the forces in cytoskeletal and extracellular matrices, ion channels, and all other mechanically-sensitive processes coupled to the membrane (Figure 2.5).

2.5.1 The soliton model of action potentials

An alternate representation of the action potential recently proposed is that of a self-propagating density pulse (soliton) in a cylindrical membrane. This model is better able to explain the phenomena of fluctuations in nerve fiber thickness and reversible heat change associated with the action potential, rather than solely the electro-chemical behavior captured by the HH model [18]. The soliton model of action potentials is based on the thermodynamics and phase behavior of the lipid components of the biological membrane, which are in a fluid state at physiological temperatures. It has been shown
that properties of the lipid membrane slightly above melting transition temperature are sufficient to allow the propagation of mechanical solitons in a cylindrical membrane [55]. The soliton model is derived from the wave equation for sound given by Equation 2.13:

\[
\frac{\partial^2 \Delta \rho}{\partial t^2} = \frac{c^2 \partial^2 \Delta \rho}{\partial x^2} 
\]

(2.13)

where \( \rho \) is the lateral density of the nerve membrane, \( \Delta \rho \) is the change in density compared to the resting membrane, \( t \) is time, and \( x \) is position. The propagation velocity \( c \) is typically considered constant when describing sound propagation in air, however, the speed of sound is a sensitive function of density and frequency close to the melting transition in membranes [57]. This nonlinearity of both the density and frequency dependence of sound velocity makes soliton propagation possible. The differential equation resulting from expansion of the wave equation is complex, but can be expressed as the following localized analytical solution [56] describing the shape of a propagating density excitation through Equation 2.14:

\[
\Delta \rho^A(x) = \frac{p}{q} \cdot \frac{1 - \left( \frac{v^2 - v_{min}^2}{c_0^2 - v_{min}^2} \right)}{1 + \left( 1 + 2 \sqrt{\frac{v^2 - v_{min}^2}{c_0^2 - v_{min}^2}} \cos h \left( \frac{c_0}{h} z \sqrt{1 - \frac{v^2}{c_0^2}} \right) \right)} 
\]

(2.14)

where \( \Delta \rho^A \) is the change in lateral membrane area density, \( z \) is the position along the axon, the parameters \( p \) and \( q \) describe the dependence of the sound velocity on density, \( v \)
is the propagation velocity, \( v_{\text{min}} \) is the minimum velocity allowed for soliton propagation, \( c_0 \) is the velocity of small amplitude sound, and \( h \) is a parameter describing the frequency dependence of the speed of sound (dispersion). Area density is a two dimensional property and is calculated as the mass per unit area. As required by the equation, and observed experimentally [55], the soliton profile has a maximum \( \left[ \partial (\Delta \rho^A) / \partial z = 0 \right] \) about which it is symmetric.

Of particular interest is the comparison of the energy carried by solitons with the electrostatic energy associated with the conventional modeling of pulse propagation in a nerve. If the empirically observed energy is greater than the electrostatic energy, then the conventional HH mechanism for pulse propagation is insufficient. This inequality has in fact been shown, as the electrical energy released and reabsorbed from a membrane capacitance is unable to account for more than half of observed temperature changes during an action potential [58]. The associated energy of a propagating soliton will have potential and kinetic energy contributions, which, using a Lagrangian formalism, the energy density is given by Equation 2.15 [55]:

\[
e_{\text{sol}} = \frac{c_0^2}{\rho_0^A} (\Delta \rho^A)^2 + \frac{p}{3\rho_0^A} (\Delta \rho^A)^3 + \frac{q}{6\rho_0^A} (\Delta \rho^A)^4
\] (2.15)

Assuming a maximum voltage change at the peak of the soliton, \( V_0 \), and that the capacitive energy of a membrane is due to compression and the accompanying voltage change, the capacitive energy density is given by Equation 2.16 [55]:
\[ e_{cap} = \frac{1}{2} C \cdot \left( \frac{V_0 \Delta \rho^A}{\Delta \rho_{max}^A} \right)^2 \] (2.16)

where \( \Delta \rho_{max}^A \) is the maximum amplitude of the change in lateral membrane area density and \( C \) is the capacitance of the membrane. Comparisons of the estimates of energy using these equations found that the electrostatic energy density was more than one order of magnitude smaller than the energy of the corresponding soliton [55].

Clearly, the HH model alone cannot capture a number of behaviors of the propagating nerve pulse, such as the reversible transfer of heat and mechanical changes that are captured in the soliton model of propagating activity. However, proteins do not function as active components or channels in the model of soliton propagation through the phospholipid bilayer, but rather tune the thermodynamics of the membrane. Whether initiation of the mechanical soliton is used for communication is therefore left unanswered, and the numerous investigations supporting the role of proteins in the electrical propagation of nervous signals are left neglected. Also, the collision of action potentials is known to block the propagation of nervous signals. The collision of solitons according to the above equations based on adiabatic and reversible physics though, allows pulses to pass through each other with minimal loss of energy [59]. The inclusion of proteins for electro-mechanical coupling may resolve these issues, however the combination of the soliton and HH models, which are based in separate mechanisms, is not straightforward.
2.5.2 Hypothesis of neuro-mechanical signaling

Beyond the role of anesthetics to change the mechanical properties of the lipid bilayer, and hence neuronal function, it has recently been proposed that propagating density pulses in the neuronal membrane may serve as the actual signal that modulates the function of membrane-bound enzymes, operating as an alternative mechanism for signaling and nerve pulse propagation [60-62]. In this hypothesis, the activity or conformational fluctuations of one protein initiates a local mechanical disturbance, which propagates along the lipid interface to another protein, transiently changing the thermodynamic state of the second protein’s environment and influencing its activity (Figure 2.5B). Communication of this form would not require energy since mechanical pulse propagation is adiabatic and enzymes work reversibly. Additionally, the dielectrical properties of the interface would lead to a propagating voltage pulse coupled to these density oscillations, as discussed via earlier electro-mechanical coupling mechanisms.

Dynamic studies of the biological lipid interface using thermodynamic concepts rather than the currently prevailing electrical theory were used to explore this hypothesis. By monitoring 2D pressure pulses in lipid monolayers, the degree of excitability of the interface was found to depend on its thermodynamic state. Close to the maximum of compressibility of the interface, the pulse signal becomes very weak, illustrating that the thermodynamic state of the interface influences propagation speed and strength [61, 63]. Furthermore, by investigating different interfaces at different temperatures, correlations of the optical and mechanical states of lipid monolayers was found to be a property of the thermodynamic state, and not due to the nature of the molecules investigated [62]. This coupling between fluorescent intensity and pressure pulses were clearly resolved when
the system was excited within or nearby the transition region of the membrane, as was the condition for self-propagating solitons discussed earlier.

To investigate the phenomena in biological systems, the temperature dependence of an excitable medium’s mechanical material properties were derived and also correlated with temperature-dependent relaxation of pulse propagation in blackworms, nerves, and gels [60]. It is typically assumed that temperature sensitivity of the duration of refractory period is determined by the timescales required by metabolic processes to reestablish resting ion gradients and channel kinetics. However, using solely the framework of thermodynamic theory and no assumptions about metabolic reactions or equilibration processes, the predictions of relaxation times based solely on thermodynamic theory compared well with experimental data. The velocity-temperature relationship for vessel pulsations followed a conserved pattern for pulses in excitable systems: linear increase of velocity with increasing temperature ultimately interrupted by a heat block. It was concluded that conservation of temperature dependence of pulse propagation velocity is likely a consequence of some well conserved physical mechanism, such as mechanical state, and not dependent on metabolic reactions [60]. Thus, oscillations in the thermodynamic state of the lipid interface is sufficient to propagate density pulses, and as required thermodynamically, any proteins or enzymes located in the path of such a density pulse will exhibit an altered kinetic behavior. These observations and conclusions form the basis of the proposed hypothesis of mechanical signaling along the neuronal membrane.
2.6 Mechanics in the brain

Mechanobiology is a rapidly growing field investigating the role of mechanical forces in cellular biology and physiology [3]. One common approach to mechanobiology involves the application of analysis to the cytoskeletal and extracellular matrices of a cell and determining its associated effects on cellular and molecular processes. One such approach is based on the concept of tensegrity architecture as a simple mechanical model of cell structure to relate cell shape, movement, and cytoskeletal mechanics, as well as the cellular response to mechanical forces [64]. Another approach examines interactions between microtubules and actin as basic phenomena behind many fundamental processes, classified as either regulatory or structural interactions [65]. Regulatory interactions encompass where microtubule and actin systems indirectly control each other through effects on signaling cascades, such as by the Rho family of small GTPases [65]. Structural interactions encompass where microtubules and actin are physically linked, such as by a static connection with a binding protein, or a dynamic interaction with a microtubule- or actin-based motor protein [65]. The application of mechanobiology to neuronal function represents an actively growing field of interest [2].

The structural interactions of neurons lend well to mechano-biological analysis of the forces in the structural matrices. Intracellular forces may be generated by the polymerization and depolymerization of cytoskeletal elements, such as actin filaments. Actin forms soft macromolecular networks of entangled and cross-linked fibers to establish part of the neuronal cytoskeleton. The polymerization and depolymerization of actin filaments and microtubules generates forces that are important to many neuronal processes, such as motility [66], to counteract plasma membrane tension and deformation.
changes during clathrin-mediated endocytosis [67], and to act as a molecular tension sensor regulating numerous aspects of intracellular homeostasis and function [68]. Actin is in fact one of the best-recognized cytoskeletal contributors to synaptic function. It has been well established that the cytomechanics of axonal growth cone navigation and branching are largely mediated by actin-generated forces and exemplify some of the most important path-finding events occurring in the development of the nervous system [69]. The actin motor proteins myosin are capable of generating forces sufficient to contract muscle tissue and are known to participate in molecular cargo shuffling during motility and growth [70]. Recent measurements of growth cone mechanical properties have shown growth cones have a low elastic modulus (\(E = 106 \pm 21 \text{ N/m}^2\)) and that considering its retrograde flow actin may generate internal stress in growth cones on the order of 30 pN/\(\mu\text{m}^2\) [71]. These results indicate growth cones are a soft and weak force generators rendering them sensitive to the mechanical properties of their environment [71]. Thus, the mechanical forces of structural elements, such as actin, and the mechanical environment have broad implications on neural function.

2.6.1 Mechanical forces and neural development

During development, the mechanical properties of nervous tissue are prone to alteration, and neurons encounter different mechanical cues depending on their location and developmental stage. Indeed, the elasticity and mechanical properties of the brain has been shown to change across different stages of development [72-74]. Thus, neurons and growth cones are likely to encounter environments with differing mechanical properties as they migrate in situ. Growth cones are highly motile structures that are the leading tips
of developing axons and dendrites, which generate the forces during neuronal growth thru polymerization of actin at their leading edge. In vitro, many neuronal cell types adapt their morphology to the stiffness of their substrate, and as such neuronal growth is likely a mechanical process influenced by the interaction with the mechanical environment in vivo as well. Even between neuronal types, dorsal root ganglion neuron growth cones have been shown to generate significantly greater traction forces compared to hippocampal neuron growth cones, as determined using traction force microscopy [75]. Moreover these neuronal types exhibited differential cytoskeletal adaption to substrate stiffness [75]. Such differences in cytoskeletal mechanics pose the possibility that different forces generated by actin may serve unique mechanical scripts for synapse formation, maturation, and operation in neurons and between their types. Perhaps a mechanical environment, such as the extracellular matrix (ECM) within a given anatomical area can change to optimize the growth dynamics of specific groups of invading axons across distinct stages of development [2]. Any such cellular mechanical matching for tuning patterned synapse formation is certainly a tantalizing concept.

Several additional observations seem to provide evidence for such mechanical tuning mechanisms of growth. Quantified with atomic force microscopy (AFM), different layers of the hippocampus have been shown to possess significantly different rigidities in the rodent brain (CA1 stratum pyramidale = 0.14 nN/µm², CA1 stratum radiatum = 0.20 nN/µm², CA3 stratum pyramidale = 0.23 nN/µm², and CA3 stratum radiatum = 0.31 nN/µm²; [76]). Observed on substrate rigidities ranging from 0.5 and 7.5 nN/µm², hippocampal axons increase their length faster on softer substrates [77]. Neurons from embryonic spinal cord develop a five-fold higher neurite branch density on soft substrates
(0.05 nN/µm$^2$) compared to more rigid ones (0.55 nN/µm$^2$) [78]. Neurons plated on top of various geometrically constrained micropatterns revealed neuronal polarization was sensitive to external constraints and that axon polarization was favored along straight lines, such as may be used by newborn neurons extending their axon along pre-existing straight structures [79]. Prolonged exposure to ultrasound disrupts neuronal migration in the embryonic cerebral cortex in mice [80], underscoring the effects of mechanical forces during neural development. Beyond growth cones and the mechanical environment, mechanical tension along neurites and axons affect network development as well. Applied tension determined axonal specification in undifferentiated minor processes of cultured hippocampal neurons, and could even induce a second axon in an already polarized neuron [81]. Furthermore, once a neurite has connected to its target, tension promotes its stabilization and at the same time causes retraction or elimination of collateral neurites [82]. Supported by these aforementioned primary observations, neuroscience should focus efforts on characterizing changes in growth cone traction force, elasticity, or viscosity across different anatomical and cellular regions, levels of activity, and stages of development.

The critical importance of proper force distributions during the development of the central nervous system can be underscored by the disorders resultant from abnormalities in cortical folding. The folding of the gyrencephalic cortex is a major mechanical event, and abnormalities in folding have been identified in neurological disorders including autism and schizophrenia [83, 84]. The two major theories currently considered to explain cortical folding are differential expansion [85] and a tension based theory [86]. Differential expansion proposes that the tangential expansion of cortical
regions is the driving force of cortical folding, propelled by the local proliferation of cells and changes in their sizes and connections [85]. The tension based theory hypothesizes that tension along axons in the white matter drives the folding of cortex [86]. However, micro-dissection arrays have shown that while axons are under significant tension, the patterns of tissue stress are not entirely consistent with the tension based hypothesis. Tension directed across developing gyri by axons was not found in developing ferret brains [87], and observed relaxation of tissue after cutting was suggested to be due to enhanced growth in the gray matter compared to white matter in adult mouse brain [88]. Overall, there is currently no theory that can explain all observations related to cortical folding and proof for either differential expansion or the tension hypothesis is still incomplete.

### 2.6.2 Mechanical forces influence neuronal signaling and plasticity

Mechanical tension along axons contributes not only to neuronal network development, but also to regulation of neural function, and the evidence of the influence of mechanical forces on neural function is numerous. It has recently been shown that mechanical tension within axons plays an essential role in the accumulation of proteins at presynaptic terminals; biochemical signaling and recognition of synaptic partners is not sufficient [89]. Presynaptic vesicle clustering at neuromuscular synapses vanished upon severing the axon from the cell body and could be restored by applying tension to the severed end, and further stretching of intact axons could even increase vesicle clustering [89]. Furthermore, rest tensions of approximately 1 nN in axons were restored over approximately 15 minutes when perturbed mechanically, implicating mechanical tension
as a modulation signal of vesicle accumulation and synaptic plasticity [89]. Axonal tension modulates local and global vesicle dynamics [90], and increased axonal tension from the resting state may induce further actin polymerization and increased clustering via mechanical trapping or interactions between F-actin and vesicles [89].

Regarding the function of neurites in neural networks, actin in dendritic spines has been shown to regulate synapse formation and spine growth [91], activity-dependent spine motility [92-94], and plasticity [95-97]. In gelsolin knockout mice, reduced actin depolymerization has been shown to enhance NMDA-mediated and voltage-gated calcium activity in hippocampal neurons [98]. However, the contribution of mechanical force changes to any of the above observations is not clearly understood. The viscoelasticity of dendritic spines was found to be critical to their function through AFM elasticity mapping and dynamic indentation methods [99]. Through this mechanical characterization, the activity-dependent structural plasticity, metastability, and congestion in the cytoplasm of spines are all gauged by merely a few physically measurable parameters. The degree of which spines are able to remodel and retain stability is determined in large part by viscosity; where soft, malleable spines have properties likely associated with morphological plasticity for learning, and the properties of rigid, stable spines are likely associated with memory retention [99]. Perhaps the stabilization or destabilization of actomyosin networks produces direct mechanical consequences on synaptic activity by increasing or decreasing plasma membrane tension to coordinate the bending or compression of presynaptic compartments and dendritic spines. Given the dynamic nature of the actin cytoskeleton in the regulation of membrane tension and channel activity, the aforementioned idea seems natural for expanded investigations.
Additionally, the contribution of the various other elements composing the cytoskeletal and extracellular matrices besides actin as discussed here can analogously be investigated.

2.7 Mechanical interfacing to nervous systems

The modulation and monitoring of the nervous system is pertinent to the treatment of neurologic and psychiatric diseases, as well as the scientific investigation of the neural mechanisms of cognitive, sensory, and motor functions. Conventionally, interfacing with the nervous system has been conducted using electrical and chemical means, such as micro-dialysis and deep-brain stimulation. Recently, the development of devices utilizing mechanical energy to interact with the nervous system has received considerable attention. These devices include ultrasound for noninvasive imaging of brain function [100], noninvasive neural stimulation using ultrasound [101-103], and magnetic resonance elastography [104] or ultrasonic elastography [105, 106] for noninvasive palpitation and mechanical characterization of the brain.

Ultrasound imaging uses the amplitude of backscattered ultrasonic echoes to generate a tomographic image in real time. Due to the prevalent medical use of ultrasound as a diagnostic tool, the biological effects of ultrasound have been well studied and reviewed to ensure human patient safety [107]. Ultrasound provides good spatio-temporal resolution in comparison to functional magnetic resonance imaging, which has excellent depth penetration but lacks good spatial and temporal resolution. Good spatial and temporal resolution is critical to imaging complex transient events such as epileptic seizures, and functional ultrasound has recently been developed to allow high spatio-
temporal resolution imaging of whole brain vasculature dynamics in response to brain activation [100]. Using this technique, task evoked brain activation and the propagation of epileptiform seizures were observed through a cranial window in rats [100].

Besides its use for diagnostic imaging, ultrasound at low intensity is able to nondestructively excite nervous tissue [101-103]. The mechanisms behind ultrasound stimulation however are not well established. There are two classes of mechanisms primarily considered, thermal and mechanical. Ultrasound can heat tissue, analogous to transcranial high-intensity focused ultrasound ablation [108-110], and temperature sensitive ion channels can be activated through tissue heating. However, negligible temperature increases have been measured during pulsed ultrasound stimulation protocols [101, 111-113]. The premise behind the hypothesized mechanical mechanisms of ultrasound is that deformation of the cell membrane, or the proteins embedded therein, could affect ion channel kinetics and/or membrane capacitance to induce transmembrane currents to initiate action potential discharge [114, 115]. Recently, intramembrane cavitation has been proposed as a mechanism for the effects on nervous tissue by ultrasound [116, 117]. Using models of the cellular membrane, the mechanical energy from ultrasound would be absorbed and transformed by the membrane into expansions and contractions of the space between bilayer membrane leaflets [116]. Linking this model with electro-deformation, ultrasound led to action potential excitation via currents induced by membrane capacitance changes within the computational model [117]. This model is referred to as the bilayer sonophore, and offers explanations on the requirement for long ultrasonic stimulation pulses and other experimentally observed phenomena.
Magnetic resonance imaging is the most common imaging modality for investigating central neurological disorders as it is noninvasive and provides a number of contrast mechanisms. Magnetic resonance elastography determines the shear modulus of tissues in vivo through the application of mechanical shear waves and the use of a phase sensitive magnetic resonance imaging sequence to produce a map (elastogram) of the shear modulus of the tissue [118]. Additionally, ultrasound has also been used as the source of mechanical waves for producing elastograms [105, 106]. The elastogram is used for clinical diagnostics, as a change in cellular elasticity is associated with many diseases [119], due to the altering the microstructural environment of the central nervous system through neuroinflammation, neurodegeneration, and disruption of the glial matrix. This is analogous to the palpitation of tissue to identify lesions based on their differential stiffness to surrounding tissues such as breast tumors. Application of elastography to the brain may have useful applications for characterizing brain disease based on the mechanical properties of the tissue. The mechanical properties of the human brain have shown a high sensitivity to neurodegeneration in initial investigations of Alzheimer’s disease, multiple sclerosis, normal-pressure hydrocephalus, and cancer [118]. However, elastography of the human brain has yet to gain traction in clinical applications as mechanical properties are largely reported as global averages rather than local values. Recent work on brain elastography has been focused on generating high-resolution, reliable, and repeatable estimates of local mechanical properties in the human brain. The mechanical properties of the corpus callosum and corona radiate were recently measured in healthy individuals using high-resolution magnetic resonance elastography and atlas-based segmentation [120]. Both structures were found to be stiffer than the overall white
matter, and demonstrated the feasibility of quantifying the mechanical properties of specific structures in white matter architecture for the assessment of the localized effects of disease. The ability to reliably estimate local mechanical properties noninvasively represents a possible revolution in the clinical assessment of neurodegeneration of the human brain.

### 2.8 Conclusions

As discussed throughout this perspective, the HH model alone does not capture a number of biophysical phenomena associated with propagating nerve impulses or action potentials. With respect to physical forces, the significance of dynamic mechanical changes occurring in membranes during action potential propagation remains poorly understood [1, 2]. However, the mechanisms underlying these associated processes of the action potential have been investigated and modeled separately. Based on these observations we should consider the importance of electro-mechanical coupling on neuronal excitability. Mechanical forces acting on neuronal membranes or through cytoskeletal filaments can also be transformed into consequences on membrane bound and cytoskeletal tethered protein activity. Membrane bound protein activity is influenced by the properties of the phospholipid bilayer and how the properties and changes in density of phospholipid bilayers influence the propagation of mechanical waves and neuronal processes, such as action potential initiation and propagation, is not precisely known. It has recently been proposed that propagating density pulses in the membrane may also serve as the signal that modulates the function of membrane bound enzymes, operating as an alternative mechanism for signaling and nerve pulse propagation.
The recent development of methods and devices utilizing mechanical energy to interact with and observe the nervous system (such as ultrasound for neural stimulation, magnetic resonance elastography for noninvasive palpitation of the brain, and label free imaging of membrane potential) represent initial technological advances that capitalize on the coupling between neuronal function and mechanical forces. The extent to which cellular-mechanical dynamics influences neuronal activity, and effectually the interfacing to the nervous system using mechanical forces, remains largely unexplored. To advance neuroscience and our understanding of the complex nervous system, the compartmentalization of analyses and processes due to electrical, chemical, or mechanical energies in system characterization and manipulation needs to be stepped away from. While numerous mechanical events have been observed and associated with neuronal activity, it has not been until very recently that technology has started to be adapted to capitalize on these mechanical events to allow the observation, and even modulation, of nervous tissue. By starting to consider the interplay between electrical, chemical, thermal and mechanical energy, rather than separately compartmentalizing them, fresh insights into nervous system function and dysfunction will likely evolve. We are certainly curious to learn how integrated models spanning neuroscience, engineering, and physics drive our thinking about neural function 20 years from now.

2.9 References


2.10 Figures

**Figure 2.1** Cellular and molecular components that sense and transduce forces in neurons. (A) Various cellular, intracellular, and extracellular components that are affected by mechanical forces are illustrated including the plasma membrane, ion channels, actin cytoskeleton, microtubules, spectrins, cadherins, integrins, and extracellular matrix proteins. Each of the illustrated components is known to play significant roles in regulating neuronal function. (B) An expanded illustration of the plasma membrane highlights various micromechanical forces transduced and sense neurons including expansion, pushing, pulling, coupling, bending, and buckling forces (shown in orange).
Figure 2.2 Concept map highlighting the role of mechanical forces in the nervous system and its relation to a principles discussed in the article.
Figure 2.3  Mechanical sensitivity of embedded membrane proteins during neuronal events. (A) At rest in the axon membrane, a voltage-gated channel (VGC) is in a closed, low conductance conformation. (B) During an action potential in the axon, wave propagation along the neuronal membrane triggers transient voltage changes and lipid phase changes. Changes in the tension and density of the membrane may result in conformational changes of the VGC favoring increased conductance states. (C) At rest in the membrane of a dendritic spine, post-synaptic density (PSD) adapter proteins, a N-methyl-D-aspartate receptor (NMDA-R), and VGC, all associated with the membrane, are in inactive closed states. (D) During the actin-mediated contraction (“twitch”) of a dendritic spine, movement translated throughout the neuronal membrane may result in conformational changes of the membranous proteins leading to changes in gating kinetics.
Forces and transitions in the neuronal membrane during wave propagation. (A) At rest the intramembrane force of charges against each other (labeled $F$) minimally repels each other due to their small concentration and produces a slight tension (labeled $T$) in the phospholipid bilayer. The phospholipid bilayer is in a liquid phase and individual phospholipids are able to translate and swap with neighboring phospholipids. (B) An increase in the density of intramembrane charges, such as during membrane excitation, causes the intramembrane repelling force of charges to increase, expanding the bilayer membrane and increasing its tension. Under sufficient tension in the membrane the phospholipid bilayer will transition to the gel phase, a transition associated with the release of heat (labeled $H$). (C) During wave propagation along the neuronal membrane, such as during an action potential, there is an interplay between the transient voltage and phase changes, both affecting the membrane potential difference. Due to changes in the tension of the phospholipid bilayer, the area and thickness of the membrane, and hence capacitance, changes. This leads to a capacitive current due to the imbalance of charges across the membrane and is observed as a voltage pulse. The reversible heat observed during the action potential is proposed as a result of the production of heat during lipid transition from fluid to gel phase, which is reabsorbed as the membrane returns to the fluid phase.
The interaction of forces between ion channels and the neuronal membrane. (A) At rest, the ion channels are closed to ion transport and the forces between the phospholipid bilayer and channel protein are equalized. (B) When the channel opens to allow ion transport, phospholipids are displaced by the conformational change of the channel and this produces a mechanical wave that propagates through the viscous phospholipid bilayer membrane towards other channels to possibly influence their behavior. (C) Anesthetic compounds (illustrated in orange) that follow the Meyer-Overton rule will solubilize in the phospholipid bilayer and cause a redistribution of membrane forces that can shift channel conformational equilibrium towards the closed state, as channel opening would require a greater force to overcome that of the anesthetized membrane.
3 Stimulation of Human Motor Cortex

*Influence of ultrasonic stimulation on sensory discrimination and evoked potentials*

Work presented in this chapter has been published as:

3.1 Abstract

Improved methods of noninvasively modulating human brain function are needed. Here we probed the influence of transcranial focused ultrasound (tFUS) targeted to the human primary somatosensory cortex (S1) on sensory-evoked brain activity and sensory discrimination abilities. The lateral and axial spatial resolution of the tFUS beam implemented was 4.9 mm and 18 mm respectively. Electroencephalographic recordings revealed tFUS significantly attenuated the amplitudes of somatosensory evoked potentials (SEPs) elicited by median nerve (MN) stimulation. We also found tFUS significantly modulated the spectral content of sensory-evoked brain oscillations. The changes produced by tFUS on sensory-evoked brain activity were abolished when the acoustic beam was focused one centimeter anterior or posterior of S1. Behavioral investigations revealed tFUS targeted to S1 enhanced performance on sensory discrimination tasks without affecting task attention or response bias. Based on our observations we conclude tFUS can be used to focally modulate human cortical function.

3.2 Introduction

Current non-invasive neuromodulation methods, such as transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS) confer low spatial resolutions. These methods typically produce electric fields having length scales on the order of several centimeters, which span anatomically and functionally distinct human brain circuits [1, 2]. As a result current transcranial approaches often modulate activity in the intended target, as well as in surrounding brain circuits [1, 3]. Therefore, improved approaches to the transcranial modulation of human brain circuit activity are sought after to support global brain mapping efforts, as well as to advance diagnostics and therapies in
neuroscience. In the present study we investigated the potential use of pulsed ultrasound (US) for focally modulating cortical function in humans.

Studies examining the direct effects of US on neuronal activity date back to 1929, when US was first shown to excite nerve fibers in isolated turtle and frog muscle preparations [4]. Evidence accumulated since then has shown US can directly modulate neuronal activity in peripheral nerves [5, 6], elicit action potentials in hippocampal slices [7, 8], and stimulate retina [9]. Further, US can noninvasively stimulate the hippocampus and motor cortex of intact mice [10, 11], modulate monosynaptic and polysynaptic spinal reflexes in cats [12], and disrupt seizure activity in cats [13], rats [14], and mice [15]. Additional evidence from animal models has demonstrated US can elicit fMRI blood oxygen level dependent (BOLD) contrast signals in the visual and motor cortices of rabbits [16], reversibly suppress the amplitudes of visual evoked potentials in both cats [17] and rabbits [16], and functionally modulate neuronal activity in the frontal eye fields of awake behaving monkeys [18]. At low-intensities for short exposure times tissue heating does not occur, so the mechanisms underlying the effects of US on neuronal activity are thought to partially stem from mechanical pressure effects of US on cellular membranes and ion channels [5, 10, 16, 19, 20]. These mechanical actions of US have not been reported to cause tissue damage when used to modulate neuronal activity [5, 9-11, 15, 16, 19].

Despite observations in different animal models, it has remained untested whether US can focally modulate the activity of intact human brain circuits. Therefore we aimed to determine if transcranial focused ultrasound (tFUS) is capable of functionally modulating brain activity in the human primary somatosensory cortex. Our findings
indicate tFUS can focally modulate sensory evoked brain activity and cortical function in humans. These observations may help advance the development of enhanced non-invasive neuromodulation strategies.

3.3 Methods

3.3.1 Quantitative acoustic field mapping

We measured the acoustic intensity profile of the ultrasonic waveform using a calibrated hydrophone (HNR-0500, Onda Corporation, Sunnyvale CA) whose signal was amplified by an AH-1100 preamplifier (Onda Corporation). The hydrophone, US transducer, and skull fragment were positioned within a 58 liter acrylic water tank. The hydrophone was mounted on a three-axis stage (LTS300, Thorlabs Inc, Newton NJ) using an assortment of optomechanical components (Edmund Optics Inc., Barrington, NJ and Thorlabs Inc., Newton, NJ). The US transducer and skull fragment were positioned similarly. Custom software written in LabVIEW (National Instruments, Austin, TX) was used to control the three-axis stage as well as the timing of transducer excitation and recording of the corresponding waveform as measured by the hydrophone. Acoustic field scans were performed at 400 µm (2 to 122 mm away from transducer in a 10.4 mm x 10.44 mm region) and 200 µm (2 to 72 mm away from transducer in a 5.6 mm x 5.6 mm region). For finding the final focal plane as well as the spatial peak location, the field map obtained from the earlier scans were used as locators for conducting 100 µm resolution scans. Scans around the axis (Z axis) were first performed to find the focal distance; next, a 12 mm x 12 mm scan was performed at this distance to obtain an XY acoustic power map at the focal plane. Scans were first performed without the skull in between the
transducer and hydrophone. Subsequently, to test the effects of a human skull on FUS fields, we inserted a 6 mm thick fragment of human cortical bone (rehydrated for 48 hours) in between the transducer and the hydrophone and repeated our scans using the same procedures, except that the starting distance to the transducer was increased to 10 mm to avoid colliding the skull and hydrophone.

3.3.2 Projection of tFUS fields into a realistic head model
A realistic head FEM model was created using SimNibs [21]. Briefly, GM, WM, CSF, skin and skull were segmented from the MR images and based on the binary tissue masks a 3D FEM model of the head was created containing approximately 1.7 million tetrahedral elements with increased resolution inside WM and GM. To estimate the acoustic field distribution in the brain during US stimulation the measured tFUS field was projected into the brain assuming that the face of the transducer was placed tangential to the scalp over CP3 as conducted in our EEG experiments described below. The density ($\rho$) of brain was specified as 1,030 kg/m$^3$ and the speed of sound ($c$) was 1550 m/sec [22]. Acoustic intensity in the mesh nodes was computed using a nearest-neighbor interpolation. It was assumed that the acoustic properties of GM, WM and CSF were similar enough such that effects due to impedance mismatch at the tissue interfaces were negligible.

3.3.3 tFUS waveform
Transcranial ultrasonic neuromodulation waveforms were generated using a two-channel, 2 MHz function generator (BK Precision Instruments) as previously described [15, 23].
Briefly, channel one was set to deliver US at a pulse repetition frequency (PRF) of 1.0 kHz and channel two was set to drive the transducer at a 0.5 MHz acoustic frequency (\(A_f\)) in a bursting mode with channel one serving as an external trigger for channel two. The pulse duration (PD) of the waveform was set to 0.36 ms by adjusting the number of cycles per pulse (c/p) on channel two to 180 while the stimulus duration (0.5 sec) was set by adjusting the number of pulses (np) on channel one to 500. The output of channel two was sent through a 40W linear RF amplifier (E&I 240L; Electronics & Innovation) before being sent to a custom designed focused ultrasound transducer (Blatek, Inc., State College, PA) having a center frequency (\(f_c\)) of 0.5 MHz, a diameter (d) of 30 mm, and a focal length (F) of 30 mm. The waveform employed for tFUS stimulation had the following parameters: \(A_f = 0.50 \text{ MHz}, \ PD = 360 \mu\text{sec}, \ PRF = 1.0 \text{ kHz}\) and \(np = 500\) to produce a stimulus duration of 0.5 sec yielding a peak rarefractional pressure (\(p_r\)) of 0.80 MPa, a mechanical index (MI) of 1.13, and a spatial-peak pulse-average intensity (\(I_{SSPA}\)) of 23.87 W/cm\(^2\) prior to transcutaneous and transcranial transmission. We have previously verified this waveform does not produce heating of the skin or skull bone. The transducer was coated with acoustic coupling gel and placed on the scalp at the 10-20 electrode location CP3 before being secured in place with athletic pre-wrap bandaging.

3.3.4 The effects of tFUS on sensory-evoked brain activity

3.3.4.1 Participants

The Institutional Review Board at Virginia Tech approved all experimental procedures. Ten volunteer study participants (5 Male, 5 Female, aged 18-47 with a mean age = 27.0 ±
9.5) provided written informed consent to participate in the study. None of the volunteers reported any neurological impairment and were all self-report right-hand dominant.

3.3.4.2 **Experimental Setup**

Participants were seated in a high-back desk chair with their right forearm fully supported in supination. During testing, subjects were required to sit passively while viewing a fixation cross on a screen. A total of 120 ultrasonic waveforms (see below) were delivered from the 10-20 EEG electrode site CP3 at an inter-stimulus interval (ISI) of 6 sec with a positive randomization of 4 sec. The tFUS treatment condition involved acoustically coupling the active face of the ultrasound transducer to the scalp at EEG site CP3 using ultrasound gel. The sham condition involved having the US transducer coupled to the head at CP3, but flipped upside down such that the inactive face of the transducer (symmetrical to the active face) made contact with the scalp but ultrasonic energy was not transmitted into the head. This approach was used to account for a chirping sound when the transducer was active. This chirping sound was identical for both the both sham and tFUS condition and no subjects reported any sensory or perceptual differences between the two conditions. The order of sham or tFUS treatment was randomized for each subject. Total collection time was approximately one hour.

3.3.4.3 **Electroencephalography**

Electroencephalography (EEG) data were acquired using a DC amplifier (BrainAmp MR Plus, Brain Products GmbH, Gilching, Germany) with four 10 mm gold over silver cup electrodes placed at electrodes sites C1, CP1, CP5, and P3 referenced to the left mastoid
and grounded to the left ulnar styloid process. Cup electrodes were filled with a conductive paste (Ten20 Conductive; Weaver and Company, Aurora, CO) and held in place with tape. The scalp was first prepared with a mild abrasive gel (Nuprep; Weaver and Company, Aurora, CO) and rubbing alcohol. Electrode impedances were verified (<5 kΩ) prior to recording. EEG data were on-line filtered (DC – 200 Hz) and digitized at 1000 Hz before being stored on a computer for subsequent off-line analysis. Somatosensory evoked potentials (SEPs) were elicited in response to right median nerve (MN) stimulation using a 0.2 ms square-wave pulse driven by SD-9 stimulator (Grass Technologies, Warwick, RI) delivered through a bar electrode (2 cm electrode spacing) affixed to the wrist. Intensity was adjusted to elicit a slight twitch of the thumb. Stimuli were delivered at an inter-stimulus interval of 6 seconds with a 4 sec positive randomization. In each treatment condition a total of 120 MN stimuli were delivered of which 100 random EEG responses to stimuli were used due to artifact rejection in analyses. Median nerve stimuli were time-locked to occur 100 ms after the onset of tFUS waveforms. The experimenters conducting experiments were not blinded to the experimental condition, but the researcher processing and analyzing the acquired EEG data was.

3.3.4.4 Statistical analysis of somatosensory evoked potentials

EEG data were preprocessed using EEGLAB v12.0.0.0b [24] and Matlab v7.10.0 (The Mathworks, Inc., Natick, MA). Data were band-pass filtered (2 – 90 Hz) and notch filtered (60 Hz). Data were epoched around median nerve stimulus (-200 to 500 ms) and baseline corrected (-200 to -100 ms). Data were inspected for artifact using automatic
rejection criteria absolute peak-to-peak amplitude of 75 µV and 60 µV/msec. Waveform peak amplitude and latency were identified and quantified using custom software written in LabVIEW (National Instruments, Austin TX, USA). All classically defined SEP components were assessed. This included the N20, P27, N33, P50, N70, P100, N140 and late potential (LP). The LP was defined as the positive (CP1 and C3) or negative (CP5 and P3) potential with a latency in the 200 ms range. A distinct inflection of the waveform was necessary for inclusion in statistical analyses. Statistical analyses were performed on mean peak-to-peak amplitudes for the N20/P27, N33/P27, P50/N33, N70/P50, P100/N70, N140/P100, and LP components of SEPs recorded during sham and tFUS treatment conditions (n = 10 subjects, 100 trials each for each condition). To statistically analyze these SEP components recorded from multiple electrodes and at different time regions of interest, we used nonparametric permutation statistics, which appropriately control for multiple comparisons problems encountered in analyses of complex EEG data sets [25]. Randomization tests were conducted as similarly described by Maris & Oostenveld (2007) where statistical P-values represent the proportion of 1,000 random partitions resulting in a test statistic larger than the t-value calculated by a conventional paired t-test (two-tailed, df = 9) on the data. A P < 0.025 was considered statistically significant. Values for SEP amplitudes are reported as mean ± SEM.

To assess the immediacy/longevity of the tFUS effect, amplitudes of the above stated SEP potentials were quantified from individual trials for each subject for both tFUS and sham conditions. Due to the inability to reliably detect SEP peaks from individual trials, amplitude was quantified from set time windows centered upon peak latency of each potential of interest (i.e N20 = 20 ms) with a time envelope approximated
to the full-width half maximum of the potential of interest rounded to the nearest millisecond quantified from the grand average (n = 10) trace recorded from electrode site C3. Thus, for example, the N20 amplitude for each trial was taken as the average from time points 18 to 22 ms. Data points from each time window were averaged to create a single value for each potential of interest for each trial. These data were averaged across each subject (n = 10) and are presented as mean +/- SEM for both tFUS and sham condition.

3.3.4.5 Statistical analysis of spectral content

We conducted spectral analysis using Matlab v7.10.0 (The Mathworks, Inc., Natick, MA). Spectral decomposition measures average dynamic changes in amplitude of a broadband EEG frequency spectrum as a function of time relative to an experimental event [26]. Spectral content was calculated using a short-time Fourier transform with a window size of 50 ms with 25 ms overlap. Each segment was windowed with a Hamming window. The color of each pixel in the generated spectral image then indicates the power (dB) at a given frequency and latency. Here, spectral decomposition was performed upon the raw on-line DC – 200Hz filtered data. For reasons cited above, statistical tests on the spectra between tFUS and sham conditions were conducted using nonparametric permutation statistics with a temporal cluster threshold of 13.4 ms and a $P < 0.025$ controlling for multiple comparisons as described by Maris & Oostenveld (2007). Data is presented parsed into the following frequency bands: $\alpha$-band (7 – 12 Hz), $\beta$-band (13 – 30 Hz), and $\gamma$-band (30 – 55 Hz).
3.3.5 The spatial specificity of tFUS on sensory-evoked brain activity

3.3.5.1 Participants

Eight volunteer participants (6 Male, 2 Female, aged 22-57 with a mean age = 28.8 ± 11.6) provided written informed consent to participate in the study. None of the volunteers reported any neurological impairment and were all self-report right-hand dominant.

3.3.5.2 Experimental Setup

The set-up and approaches were identical to those described above. However, tFUS was projected from transducers placed at sites 1 cm anterior and 1 cm posterior of CP3 during MN stimulation trials. Both anterior and posterior sites were collected in the same session. Placement of the transducer (anterior versus posterior) as well as treatment condition (tFUS versus Sham) was pseudo-randomly assigned between subjects such that either anterior or posterior placement was collected first in half of the subjects.

3.3.6 The influence of tFUS on two-point discrimination behavior

3.3.6.1 Participants

Twelve volunteer participants (5 Male, 7 Female, Mean age 30.4 ± 10.4 years; age range 23-57) all self-report right hand dominant and free from neurological impairment or peripheral neuropathy.
3.3.6.2 **Experimental Setup**

Subjects were seated in a desk chair with their right arm resting upon a tabletop with the pad of their index finger resting over a 1.3 cm hole through which stimuli were delivered. A total of nine pin separation distances (pin diameter = 200 µm) were used; 0, 0.7, 1.0, 1.3, 1.6, 1.9, 2.2, 2.5, and 2.8 mm. Each pin distance was randomly applied at a constant force of 1N to the fingertip 10 times during tFUS or sham treatment. After each stimulus, participants were required to report verbally whether they felt one or two pins. Prior to formal testing participants were familiarized with the sensations produced by pins separated by 0 (one pin), 1.6 and 2.8 mm and informed after each stimulus to the fingertip whether the stimulus was one or two pins. Practice sessions of 10 trials of each pin distance (0, 1.6 and 2.8) were conducted. Formal testing began once participants achieved 80% (8/10) correct responses in response to stimulation using 0 mm and 2.8 mm pin distances. Participants were not aware of how many pin distances were used or the ratio of single to double pins during formal testing. Participants were not allowed to look at their fingers, but allowed to have their eyes open or closed. It was not possible for the participant to see the pins as they were occluded from view under a table.

A custom-made motorized device was built to apply the pin to the fingertip that was controlled by custom-made software (LabVIEW, National Instruments, Austin, TX). This allowed for precisely controlled force (1N) and duration (250 ms) of the pins to the fingertip. The software also timed the onset of tFUS (500 ms duration) to occur 100 ms prior to the pins application to the fingertip. Participants underwent the sensory discrimination testing during both tFUS and sham treatment conditions in the same testing session. The order of sham or tFUS treatment was counterbalanced across
subjects. The tFUS methods and parameters were identical to those reported earlier. Total collection time was approximately 1 hour.

3.3.6.3 Statistical analysis

Signal detection theory was used to assess two-point discrimination thresholds as previously described [27]. In this case, a two-response (one pin or two pin) design was used. Signal detection theory provides for the analysis of the two stages of information processing: 1) signal processing from sensory evidence and 2) the decision whether the signal is present or not. Signal detection theory thus provides measures of participants’ true sensitivity (d’) and their bias for responding a certain way (c). To assess sensitivity, each participants’ percent correct at each pin distance was calculated. In instances where accuracy was 1 or 0, proportions were adjusted by 1/(2N) and 1/(1-2N) respectively where N is the number of trials per condition. Data were Z-score transformed and analyzed using two-response classifications and cumulative d’ values as previously described [27]. The detection threshold was chosen as d’ = 1. Thus, this is the smallest pin distance that can be determined by a d’ of 1. d’ data was fitted using a third degree polynomial. To assess threshold differences between tFUS and sham stimulation, the pin distance where each participant first achieved a d’ prime >1 was recorded for both tFUS and sham and subjected to a Wilcoxon rank sum test for statistical significance. To determine if responder bias contributes to the perceptual results the criterion value c was calculated using the data from the one pin and two pin trials using the formula c = 0.5*[z(H)+z(FA)] [27], where z is the inverse of the normal cumulative distribution function, H is hit rate and FA is the false alarm rate. H was defined as responding one pin
when one pin was present plus responding two pins when two pins where present. FA was defined as responding two pins when one pin was present. A criterion value of 0 reflects no bias for responding. Negative values indicate a tendency to report a stimulus when there is none (FA) and positive values the opposite. In this case a positive value reflects a tendency towards saying two pins when there was only one and a negative value a tendency towards saying one pin when there were two. The parameter c was calculated for each subject for both the tFUS and sham session and subjected to a Wilcoxon rank sum test for statistical significance. In addition, the percent correct (hits/total trials) for the one-pin trials were quantified for both tFUS and sham conditions and statistical significance was assessed using a Wilcoxon sign-rank test.

3.3.7 The influence of tFUS on frequency discrimination behavior

3.3.7.1 Participants

Twelve subjects (5 Male, 7 Female, Mean age 31.8 ± 11.8 years; age range 20-57) provided written informed consent to participate in the study. None of the volunteers reported any neurological impairment or neuropathic condition and all were self-report right hand dominant.

3.3.7.2 Experimental setup

The physical setup was similar to above. Subjects’ right index finger rested upon a 1.3 cm diameter opening in which air-puff stimuli were delivered. Air puff stimuli were generated using a Picospritzer III (Parker Instruments, Cleveland, OH) with a constant pressure of 14.5 PSI delivered through a 1.88 mm diameter aperture that contacted the
volar surface of the index finger. This translated to a force of 0.3 N applied to the fingertip. A two alternative forced choice method was employed where the first stimulus was always a constant frequency of 100 Hz and the second stimulus was either the same frequency or higher. Participants were required to orally respond after cessation of the second stimulus “higher” if they thought the second stimulus was higher in frequency or “same” if they thought it was the same. The experimenter recorded the response on a computer. Ten frequencies were used from 100 Hz to 150 Hz in 5 Hz steps. The stimulus duration was 500 ms and the inter-stimulus interval was 500 ms. A total of 120 pairs of stimuli were delivered at an average inter-trial interval (ITI) of 6 sec. A total of 40 catch trials (100 Hz – 100 Hz) were randomly delivered throughout the test protocol. The ITI was not constant due to varying response times of the participants. The timing of stimuli was controlled by a custom-made program in written in LabVIEW (National Instruments, Austin, TX). The program also controlled the timing of tFUS such that it was delivered at the onset of the second air puff stimulus. Ultrasound waveform parameters are identical to those reported earlier. Prior to formal testing participants were familiarized with the air puff stimuli at 100, 125, and 150 Hz. Practice sessions were conducted until participants achieved an 80% success rate on the maximally separated 100 - 150 Hz pair. Participants were not aware of how many frequency differences were used or the ratio of same to different frequencies. Participants were not allowed to look at their fingers, but allowed to have their eyes open or closed. Participants were provided with headphones that played white noise to block any auditory cues from the air puffer apparatus.
3.3.7.3 **Statistical analysis**

Signal detection theory was used to assess frequency discrimination thresholds similar as reported for two-point discrimination. Briefly, each volunteer’s percent correct at each frequency difference was calculated. In instances where accuracy was 1 or 0, proportions were adjusted by 1/(2N) and 1/(1-2N) respectively where N is the number of trials per condition. Data were Z-score transformed and analyzed using two-response classification and cumulative d’. Detection threshold was chosen as d’ = 1. Data were fitted using a third degree polynomial. The frequency difference where each participant first achieved a cumulative d’ > 1 was recorded for both tFUS and sham and subjected to a Wilcoxon rank sum test for statistical significance. In addition, the percent correct (hits/total trials) for the same frequency trials were quantified for both tFUS and sham conditions and statistical significance was assessed using a Wilcoxon sign-rank test.

3.4 **Results**

3.4.1 **Acoustic beam properties of tFUS**

The optimal acoustic frequencies for the transcranial transmission and brain absorption of US are known to be < 0.65 MHz [28, 29]. We used 0.5 MHz US based on prior observations that it can modulate mammalian brain activity [10, 11]. First we quantified acoustic pressure fields emitted from a single-element focused ultrasound (FUS) transducer having a center frequency of 0.5 MHz, a diameter of 30 mm, and a focal length of 30 mm. Using a calibrated hydrophone mounted on a motorized three-axis stage, we recorded acoustic pressure fields transmitted from the FUS transducer into the free space of an acoustic test tank, as well as through hydrated fragments of human
cranial (Figure 3.1). Our measurements revealed that when FUS was transmitted through the skull that the spatial-peak pulse-average intensity ($I_{\text{SPPA}}$) dropped by approximately four-fold (1/4.05) corresponding to a -6.07 dB insertion loss with our skull sample (Figure 3.1a–b). We found this loss varied slightly across acoustic powers (free space powers and pressures ranging from $I_{\text{SPPA}} = 0.12$ W/cm$^2$ and 0.12 MPa peak-to-peak pressure to $I_{\text{SPPA}} = 50$ W/cm$^2$ and 2.5 MPa peak-to-peak pressure respectively) from a 3.7 to 4.1 fold drop in intensity when transmitting 0.5 MHz FUS through human cranial bone.

We characterized the three-dimensional shape of FUS acoustic fields in free space and following transcranial transmission (Table 3.1). Transmitting FUS through human cranial bone caused an approximately 10% loss in lateral and vertical spatial resolution of the acoustic beam estimated by the intensity full width at half maximum (FWHM; Figure 3.1c). The lateral (X) and vertical (Y) dimensions of FUS beam cross-sections measured at the intensity FWHM were 4.33 and 4.48 mm in the free space condition and 4.56 and 4.89 mm following transcranial transmission (Figure 3.1c). We also characterized the acoustic field in the axial direction along the Z-axis perpendicular to the transducer face and skull from the spatial-peak intensity maximum to 50% and 20% maximum of intensity (Table 3.1). The FUS intensity half width of the half maximum (HWHM) was 20.4 mm in the free space condition and 18.0 mm following transcranial transmission (Figure 3.1c). Under these conditions, transmission of 0.5 MHz FUS through the skull led to a reduced pressure depth-of-field and an approximately 12% increase in the axial resolution. This natural focusing may be best described where nonlinear effects cause a cone of FUS to rotate back towards the skull insertion point creating a more compact
pressure ellipsoid-shaped acoustic field (Figure 3.1c). Thus, the skull is not an obstacle for transcranial focusing of US and may actually exert an acoustic lensing effect to enhance spatial resolution under certain conditions.

3.4.2 Targeting tFUS to the primary sensory cortex

We targeted left S1 by transmitting tFUS beams into cortex from a transducer positioned perpendicular to the scalp at EEG electrode site CP3 (Figure 3.2a). We visualized tFUS beam locations in the brain using realistic models of human heads generated using a finite element method (FEM). Briefly, the grey matter (GM), white matter (WM), cerebrospinal fluid, skin and skull were segmented from MR images and based on the binary tissue masks a 3D FEM model of the head was created containing approximately 1.7 million tetrahedral elements. When targeting S1, the tFUS beam displayed a first prominent maximum of acoustic field strength in the brain at the top of the gyral crown in the postcentral gyrus (Figure 3.3a). The tFUS field produced a second maximum of field strength in the posterior wall of the central sulcus at a depth of approximately 2 cm (Figure 3.3b–c). This bimodal acoustic intensity distribution was due to the acoustic wave behavior arising from transcranial transmission as observed during quantitative field mapping of tFUS beams (Figure 3.1b–c). We observed the acoustic intensity field dropped to approximately 50% of its maximum in brain regions 2 mm anterior or posterior of the beam center (Figure 3.3c).
3.4.3 tFUS modulates sensory-evoked brain activity

The Virginia Tech Institutional Review Board approved all procedures. In a within-subjects design, we studied the influence of tFUS on short-latency and late-onset evoked brain activity by examining the peak-to-peak amplitudes of somatosensory evoked potentials (SEPs) and the spectral content of evoked EEG activity elicited by right median nerve (MN) stimulation. We targeted 0.5 MHz tFUS beams to the crown and posterior wall of the left central sulcus (S1) in human participants (N = 10) by placing the FUS transducer at the 10-20 EEG electrode site CP3 (Figure 3.2a). EEG activity was recorded from four electrodes surrounding CP3 placed at C3, CP1, CP5, and P3. The tFUS stimulus consisted of individual pulses having a pulse duration of 360 µsec repeated at 1 kHz for 500 ms. Transmission of the tFUS stimulus began 100 ms prior to MN stimulation (Figure 3.2b–c). The sham condition was identical to the tFUS treatment except the geometrically symmetrical inactive face (versus the active face) of the transducer contacted the scalp to control for a chirping sound produced by the transducer during its excitation. Volunteers did not report any thermal or mechanical sensations due to tFUS transmission through the scalp. Similarly, there were no reports of perceptual differences between the sham and tFUS conditions.

The SEP produced by MN stimulation during EEG recordings has been well studied and its components are named according to their negative (N) or positive (P) polarities and latencies (in ms) as N20, P27, N33, P50, N70, P100 and N140. The N20 component of the MN-evoked SEP has been shown to represent sensory input from the dorsal column-medial lemniscal pathway by thalamocortical fibers originating in the ventroposterolateral nucleus of the thalamus and terminating in area 3b (anterior bank of
the post-central gyrus facing the central sulcus) of S1 [30]. Subsequent slow-onset late potentials (LP) with a latency of about 200 ms or later are thought to reflect the ensuing serial processing of somatosensory information from S1 Area 3b to Areas 1 and 2, as well as to higher-level somatosensory processing areas including posterior parietal cortex (Areas 5 and 7) and secondary somatosensory cortex serving different roles in the encoding of stimulus representations [31, 32].

We found C3 most reliably captured both short-latency and late-onset brain activity evoked by MN stimulation. Compared to sham, we found tFUS elicited a significant reduction in the amplitude of the short-latency N20/P27 SEP complex recorded at C3 (Figure 3.4 and Table 3.2) and CP1 (sham = 1.22 ± 0.14 µV, tFUS = 0.73 ± 0.15 µV; \(P = 0.014\); Figure 3.4). We also observed tFUS produced a significant reduction in the amplitude of the short-latency N33/P27 SEP complex recorded at C3 (Figure 3.4 and Table 3.2). The reduction in amplitudes of these short-latency SEP components remained stable across the duration of experiments indicating there were no cumulative effects of tFUS on brain activity as studied (Figure 3.5). As illustrated in Figure 3.4, tFUS also produced significant effects on the amplitudes of the N70/P50 complex recorded at CP5 (sham = −3.30 ± 0.65 µV, tFUS = −2.85 ± 0.46 µV; \(P = 0.017\)) and P3 (sham = −0.66 ± 0.15 µV, tFUS = −1.42 ± 0.23 µV; \(P = 0.010\)). Lastly, the LP recorded from C3 was significantly attenuated by tFUS (Figure 3.4 and Table 3.2). In summary, we found tFUS targeted to S1 modulated the amplitudes of both short-latency and late-onset SEP complexes.

Spectral decomposition of EEG provides additional valuable information of ongoing oscillatory dynamics that is regarded to reflect cortical excitability and
information processing in the human brain [33, 34]. As such, spectral decomposition was performed on the time epoch of -200 to 500 ms around MN stimulation to further evaluate the effects of tFUS on sensory-evoked brain activity compared to sham treatment. Compared to sham, we found tFUS significantly \((P < 0.025)\) decreased the power of baseline alpha-band (7–12 Hz) and beta-band (13–30 Hz) activity recorded from EEG sites C3 and P3 in the 100 ms following the onset of tFUS transmission prior to MN stimulation (Figure 3.4). We also found tFUS produced a significant \((P < 0.025)\) attenuation in the power of short-latency evoked gamma-band (30–55 Hz) activity occurring within 70 ms of MN stimulation (Figure 3.4). Also evident at varying degrees across the EEG channels recorded, tFUS significantly modulated the power of late-onset alpha-, beta-, and gamma-band activity occurring about 200 ms or later following MN stimulation (Figure 3.4).

3.4.4 tFUS modulates sensory detection thresholds

We next examined the behavioral effects of tFUS delivered to S1 on sensory detection thresholds using two-point and frequency discrimination tasks. Briefly, participants \((N = 12)\) were required to decide whether they experienced one or two stimuli in response to one or two spaced pins (0.3 mm separation distances ranging from 0 to 2.8 mm) applied for 250 ms at a constant force to the pad of their right index finger during sham and tFUS treatments. The presentation of sensory stimuli began 100 ms after the onset of tFUS or sham treatment. In a separate experiment on a different day, 12 subjects (10 of the subjects completed both tasks) were required to decide if the frequency of the second of two discrete air puffs (500 ms duration each, 500 ms inter-stimulus interval) applied to
their right index finger was higher than the frequency of the first air puff. The frequency of the first air puff stimulus remained constant at 100 Hz while the frequency of the second stimulus varied randomly between 100 and 150 Hz in 5 Hz increments. Sham and tFUS conditions were counterbalanced across subjects in each experiment.

Data obtained from the two-point and frequency discrimination tasks were analyzed using signal detection theory [35]. During two-point discrimination catch trials, the percent correct response (sham = 85 ± 5\% versus tFUS = 84 ± 5\%) was not different during tFUS and sham treatment ($Z = 0.751, P = 0.453$; Figure 3.6a). These values indicate that participants’ attention was directed to the task and did not differ between tFUS and sham treatments. A Wilcoxon signed-rank test revealed no difference in criterion values between the tFUS condition and the sham condition ($Z = -0.756, P = 0.450$; Figure 3.6a). These data indicate tFUS did not affect response bias or influence participants to respond a certain way. We examined discrimination thresholds using the cumulative $d’$ where $d’ > 1$ was considered the discrimination threshold (Figure 3.7). A Wilcoxon signed-rank test revealed volunteers exhibited significant improvements in their ability to distinguish pins at closer distances during tFUS treatments compared to sham ($Z = 2.196, P = 0.028$; Figure 3.7a).

A Wilcoxon signed-rank test revealed subjects were also significantly better at discriminating small frequency differences between successive air puff stimuli during tFUS trials compared to sham ($Z = 2.102, P = 0.040$; Figure 3.7b). The percent correct response during air puff frequency discrimination catch trials (sham = 80 ± 6\% versus tFUS = 83 ± 5\%) did not differ between tFUS and sham conditions ($Z = 0.253, P = 0.800$; Figure 3.6b) indicating participant attention did not differ across treatments.
Likewise, tFUS did not alter participants’ response bias compared to sham as indicated by a Wilcoxon signed-rank test on the criterion values obtained during frequency discrimination testing ($Z = -0.203, P = 0.840$; Figure 3.6b). Collectively these data show tFUS enhanced the sensory discrimination abilities of participants as assessed by two-point and frequency discrimination tasks, without affecting response bias or task attention.

3.4.5 tFUS modulation of brain activity is spatially restricted

We next studied the focal specificity of tFUS by analyzing SEP complex amplitudes and the spectral content of EEG activity elicited by MN stimulation within volunteers ($N = 8$) when transducers were placed 1 cm anterior and 1 cm posterior to CP3 location in a counter balanced manner. Here we focused on examining the influence of tFUS on EEG activity recorded from electrode C3 since it best captured the early sensory components of SEPs as described above. With respect to targeting, we observed moving the FUS transducer 1 cm anterior of CP3 generated an acoustic beam in brain regions located across the central sulcus in the precentral gyrus. Similarly, displacing the transducer 1 cm posterior of CP3 resulted in the acoustic beam being focused in brain regions posterior to the crown of the postcentral gyrus (Figure 3.8a). Importantly, this translation of acoustic beams along the anterior-posterior axis enabled the targeting of non-overlapping and spatially discrete brain regions by tFUS. While tFUS targeted to the crown and posterior wall of the central sulcus (S1) produced a significant decrease in the amplitude of both short-latency (N20/P27 and P27/N33) and late-onset (LP) SEP complexes (Figure 3.4 and Table 3.2), moving the acoustic beam 1 cm anterior or posterior from this site...
abolished these effects. Specifically, there were no significant differences between the amplitudes of any SEP complexes recorded during tFUS and sham treatments when transducers were positioned 1 cm anterior or posterior of CP3 (Figure 3.8b and Tables 3.3–3.4). Spectral decomposition further confirmed this observation since moving the transducer either to the anterior or posterior position yielded strikingly similar spectral profiles across tFUS and sham treatments (Figure 3.8b). These similar spectral patterns are in contrast to our observations that tFUS targeted to S1 produced significant effects on the power of specific brain wave activity patterns. For example, when the acoustic beam was targeted to S1, we observed that tFUS significantly decreased the power of short-latency gamma-band activity occurring within 70 ms of MN stimulation (Figure 3.4). When the acoustic field was focused 1 cm anterior or posterior of the post-central gyrus however, tFUS failed to produce a significant effect on short-latency evoked gamma activity (Figure 3.8b). Curiously, when the acoustic beam was targeted to the precentral gyrus (1 cm anterior of CP3) tFUS significantly increased the power of late-onset gamma-band activity occurring around 300 ms following MN stimulation (Figure 3.8b). These results indicate tFUS differentially affected short-latency evoked gamma activity, as well as late-onset induced gamma activity as a function of the anatomical region targeted by the acoustic beam. Considering these anatomical and neurophysiological observations, we are led to conclude tFUS can modulate human cortical function while conferring a high spatial resolution in modular areas of cortex separated by about 1 cm or less. This spatial resolution of tFUS is better than those conferred by conventionally applied TMS or tDCS.
3.5 Discussion

Using a single element focused transducer we showed 0.5 MHz ultrasound can be focused through human skull to produce acoustic beam profiles having a lateral spatial resolution of approximately 4.9 mm and an axial spatial resolution of about 18.0 mm from the focal distance (Figure 3.1). Our electrophysiological observations demonstrate tFUS beams targeted to S1 can focally modulate short-latency and late-onset evoked cortical activity elicited in humans by somatosensory (median nerve) stimulation (Figures 3.4–3.5 and Table 3.2). Additional behavioral investigations revealed tFUS targeted to S1 enhanced the somatosensory discrimination abilities of volunteers (Figure 3.7). Collectively these observations demonstrate the utility of tFUS in the non-invasive modulation of human cortical function.

3.5.1 Targeting the spatiotemporal effects of tFUS

In the present study we implemented a sham condition, which controlled for sounds made by the transducer when it was active as described above. In fact, subjects reported the chirping sounds during sham and tFUS treatments were indistinguishable from one another. Further, they did not report any sensations specific to FUS transmission through their skin or skull. In contrast however, we have previously shown distinct US waveforms applied to the skin of the periphery can induce tactile and thermal sensations and differentially trigger brain activity patterns in sensory circuits [23]. Thus, it is important to distinguish several features of the tFUS waveform used in the present study with ultrasonic waveforms we have previously used to stimulate the somatosensory periphery [23].
The pulse duration (360 µsec) of the tFUS waveform utilized in the present study was too short and the pulse repetition frequency (1 kHz) too high to activate somatosensory receptors and fibers [23]. Using 64-channel EEG recordings, fMRI, and subjective reports we have previously shown US waveforms must be tuned for the activation of specific somatosensory receptors or fibers located in human skin [23]. For example, in our previous studies we showed low pulse repetition frequencies (10 - 70 Hz) and long pulse durations (7 - 10 ms) elicit vibratory or buzzing sensations transduced by skin receptors or mechanosensory fibers [23]. We also showed that thermal sensations can be elicited when delivering continuous wave US (100% duty cycle) to the skin for at least one second [23]. It is therefore critical to recognize that US waveforms having different spatial peak and temporal average energy profiles can exert unique effects on a variety of cellular populations and neuronal structures as described throughout the literature [11, 15, 36, 37]. The US waveform used in the present study was chosen partially for its inability to produce mechanical or thermal sensory effects on the skin or scalp.

In the present study we transmitted tFUS beams to the crown of the post-central gyrus (S1) and posterior wall of the central sulcus (Figure 3.3). The projection of the acoustic beam path is consistent with our physiological observations that tFUS significantly affected the amplitudes of short-latency SEP complexes (Figures 3.4–3.5 and Table 3.2). This claim is supported by the fact that the short-latency SEP components in monkeys and humans are generated in S1 Areas 3b and 1 on the posterior wall of the central sulcus and crown of the post-central gyrus [30, 38]. When targeted to brain regions 1 cm posterior or 1 cm anterior of the post-central gyrus, the effects of
tFUS on evoked brain activity elicited by MN stimulation were abolished (Figure 3.4 versus Figure 3.8b). In contrast, we have found that moving TMS coils in 1 cm or greater increments from a motor hotspot may not be sufficient to produce a significant change in the amplitudes of motor evoked potentials [2]. Our observations show the influence of tFUS on brain activity can be restricted to discrete modules of cortex located within 1 cm of each other. It is not yet known however whether US exerts its effects primarily on dendrites, axons, or the cell bodies of neurons. It will be important for future studies to examine these potential cellular sites of action since tFUS may exert specific effects on anatomically distinct regions of the neuropil.

The temporal dynamics of US-induced changes in brain activity have been shown to have somewhat delayed onset kinetics when compared to those observed with other stimulation modalities, such as electrical stimulation [10]. In the present study, we found tFUS produced an effect on baseline alpha- and beta-band activity within 20 ms of US waveform transmission. This time course for the emergence of direct US-induced effects on baseline brain activity is consistent with previous electrophysiological and imaging observations made in rodents [10, 11, 15] and rabbits [16]. In the present study we aimed to determine if tFUS could be used for targeted cortical modulation in humans by monitoring its influence on sensory-evoked brain activity. Based on the stability of SEP amplitudes recorded across tFUS trials (Figure 3.5), we conclude there were no cumulative effects of tFUS on brain activity as studied here. Rather we found the acute effects of tFUS on brain activity to be short-lived (< 1 sec). Both shorter and longer lasting effects of US on brain activity have been described depending on numerous factors including the US waveform characteristics implemented, as well as the anatomical
and physiological features of the brain region targeted [10, 11, 14-16]. In chronic pain sufferers for example, a transcranial US waveform (8 MHz for 15 sec) transmitted through the temporal window to the human posterior frontal cortex led to a reduction in pain ratings and improved mood for up to 40 minutes [39]. Unraveling the spatial and temporal complexities underlying the ability of US to modulate brain activity will require additional efforts.

### 3.5.2 Safety of tFUS

Ultrasound has not caused tissue damage in studies implementing its non-thermal bioeffects to modulate neuronal activity at acoustic intensities below those recommended for safe use in diagnostic imaging [5, 9-11, 15, 16]. However, appropriate precautions and procedures must be followed to ensure the safe use of US for modulating human brain activity. To avoid the generation of standing waves we followed the recommendations by O’Reilly et al (2010) and used a broadband, sharply focused US transducer operating in a pulsed wave mode [40]. Others have also shown the rate of tissue (cranium, skin, and soft tissue) heating is slower and the likelihood of transient cavitation is reduced when using pulsed waves versus continuous wave US [40-44]. With respect to acoustic power, the spatial-peak pulse-average intensity (\(I_{\text{SPPA}}\)) of the tFUS waveform we used (23.87 W/cm\(^2\)) was below the 190 W/cm\(^2\) maximum recommended limit for diagnostic imaging applications [41-43]. We additionally used short duration (500 ms) tFUS waveforms as stimuli since this is not enough exposure time for relatively low-intensity pulsed US to produce significant tissue heating. Ultrasound at high
intensities or during long exposures can cause irreversible tissue damage like any energy source, so caution should be used when implementing it to modulate brain activity.

### 3.5.3 Physiological mechanisms underlying the effects of tFUS

Given the influence of tFUS on sensory-evoked brain activity ([Figure 3.4](#) and [Table 3.2](#)), we naturally questioned whether it could affect sensory discrimination behavior. The psychological and neurobiological mechanisms underlying simple decisions [45] and sensory discrimination behaviors [46] are complex. Not surprisingly, it is therefore difficult to relate sensory evoked physiology to stimulus discrimination behaviors. In the present study we found tFUS enhanced sensory discrimination performance on two-point and frequency discrimination tasks ([Figure 3.7](#)) without altering task attention or decision bias ([Figure 3.6](#)). This improvement of sensory discrimination behaviors under tFUS treatment conditions may seem paradoxical since tFUS produced a reduction in the amplitude of SEP complexes, but several mechanistic possibilities exist which can explain these observations.

The focal volume of the ellipsoid acoustic beam we implemented was approximately 0.21 cm$^3$ at half maximum field intensity. Within this volume the 500 ms pulsed acoustic pressure wave may locally shift the balance of excitation and inhibition by acting on mechanically sensitive components of the brain including cell membranes, ion channels, and synaptic vesicle cycles [47]. Our physiological observations suggest tFUS transiently shifts the balance of neuronal activity to favor local inhibition. Short-latency evoked gamma activity has been related to the N20 component of MN-elicited SEPs and is thought to represent cortical activity responsible for the initial encoding of a
sensory stimulus [48, 49]. Thus, one hypothesis consistent with the reduction of short-latency evoked gamma activity we observed during tFUS treatment is that the pulsed acoustic pressure waves dampen excitation or increase local interneuron firing and perhaps modulates the activity of fast-spiking interneurons. Stated differently, the same amount of incoming sensory activity from a MN stimulus could be acting upon populations of neurons, which are under the influence of increased local inhibition triggered by tFUS.

As described above the rendering of cortex less sensitive to sensory encoding thalamocortical activity also explains the reduction in SEP amplitudes we observed during tFUS treatment. Increased local inhibition produced by tFUS might serve as a filter by reducing the spatial spread of cortical excitation in response to MN stimulation or during sensory discrimination tasks. Such actions could theoretically result in more spatially restricted population activation patterns thereby yielding improvements in the cortical representation of tactile stimuli. This hypothesis helps to explain the enhancement of somatosensory discrimination we observed in response to tFUS treatment. Several other mechanistic explanations certainly exist, so it is difficult to draw any definitive conclusions. Gaining a better understanding of how pulsed US affects the balance of inhibition and excitation in targeted brain regions, as well as how it influences the activity of local circuits versus long-range connections will advance our ability to implement tFUS in the study and mapping of human brain circuits.
3.5.4 tfUS for functional brain mapping

One of the most enticing applications of transcranial focused ultrasound (tFUS) is its emerging utility for non-invasive, functional brain mapping in humans. Here, tFUS provides a highly focused energy source capable of non-invasively producing changes in human brain activity. In neurosurgical applications, transcranial high-intensity focused ultrasound (HIFU) was recently combined with MR-thermometry (MRgHIFU) to heat and destroy the ventral intermediate nucleus of the thalamus for the successful treatment of essential tremor in awake, behaving patients [50]. During MR-guided stereotactic targeting of HIFU beams, it was observed that sub-ablative heating of ventroposterolateral thalamic regions induced sensory effects, such as paresthesia of the lips and fingers in some patients. However, sub-ablative sonication events targeting the ventral intermediate region of the thalamus produced transient suppression of postural tremor thereby providing a functional confirmation of the ablation target prior to lesioning with HIFU [50]. This functional mapping of deep-brain nuclei in humans with transcranial MRgHIFU enabled the active refinement of lesion coordinates such that ablation of the ventral intermediate nuclei could be achieved without destroying adjacent sensory regions of the thalamus.

It is important to recognize that the neuromodulation produced by MRgHIFU was elicited by focally heating deep-brain nuclei to around 48° C for about 10 sec during transcranial transmission of 0.65 MHz continuous wave US at intensities < 550 W/cm² from 1024 transducers operating in a phased array [50]. In the present report, we describe an approach where relatively low-intensity US (23.87 W/cm²) transmitted from a single-element 0.5 MHz FUS transducer for 500 ms can be used to transiently modulate brain
activity in the cortex of humans. Taken together these observations highlight the powerful potential of using tFUS for modulating and mapping brain function in both laboratory and clinical settings. Further studies are needed to validate and refine the thermal and non-thermal neuromodulation potential of tFUS. These rapidly evolving capabilities of tFUS should encourage changes in the way we study human brain function and support the exploration of new approaches to treating brain disorders. Thus, we anticipate many exciting advances for neuroscience when extending the capabilities of tFUS for non-invasively modulating human brain circuits.

3.6 References


3.7 Tables

**Table 3.1:** Acoustic field properties of FUS and tFUS.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>FUS (no skull)</th>
<th>tFUS (through skull)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure Max (MPa)</td>
<td>0.912011</td>
<td>0.441755</td>
</tr>
<tr>
<td>Pressure Min (MPa)</td>
<td>-0.795180</td>
<td>-0.410011</td>
</tr>
<tr>
<td>ISPPA (W/cm²)</td>
<td>23.865</td>
<td>5.895</td>
</tr>
<tr>
<td>FWHM X (mm)</td>
<td>4.329</td>
<td>4.556</td>
</tr>
<tr>
<td>FWHM Y (mm)</td>
<td>4.476</td>
<td>4.894</td>
</tr>
<tr>
<td>FW20% X (mm)</td>
<td>7.240</td>
<td>6.790</td>
</tr>
<tr>
<td>FW20% Y (mm)</td>
<td>7.780</td>
<td>7.513</td>
</tr>
<tr>
<td>HWHM Z (mm)</td>
<td>20.4</td>
<td>18.0</td>
</tr>
<tr>
<td>HW20% Z (mm)</td>
<td>47.6</td>
<td>43.2</td>
</tr>
</tbody>
</table>

**Table 3.2:** Mean amplitudes of SEP complexes recorded from C3 when tFUS beam was targeted to S1.

<table>
<thead>
<tr>
<th>SEP Complex</th>
<th>Mean Amplitude ± SEM (µV)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>tFUS</td>
</tr>
<tr>
<td>P27–N20</td>
<td>0.83 ± 0.15</td>
<td>0.38 ± 0.09</td>
</tr>
<tr>
<td>N33–P27</td>
<td>-1.66 ± 0.15</td>
<td>-0.99 ± 0.13</td>
</tr>
<tr>
<td>P50–N33</td>
<td>3.12 ± 0.50</td>
<td>2.72 ± 0.44</td>
</tr>
<tr>
<td>N70–P50</td>
<td>-2.21 ± 0.40</td>
<td>-2.42 ± 0.62</td>
</tr>
<tr>
<td>P100–N70</td>
<td>1.54 ± 0.48</td>
<td>2.66 ± 0.85</td>
</tr>
<tr>
<td>N140–P100</td>
<td>-1.72 ± 0.46</td>
<td>-1.71 ± 0.72</td>
</tr>
<tr>
<td>LP</td>
<td>3.78 ± 0.85</td>
<td>2.87 ± 0.85</td>
</tr>
</tbody>
</table>

* indicates a significant difference $P < 0.05$

**Table 3.3:** Mean amplitudes of SEP complexes recorded from C3 when tFUS beam was targeted +1 cm anterior of S1.

<table>
<thead>
<tr>
<th>SEP Complex</th>
<th>Mean SEP Amplitude ± SEM (µV)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>tFUS</td>
</tr>
<tr>
<td>P27–N20</td>
<td>1.00 ± 0.73</td>
<td>1.57 ± 1.22</td>
</tr>
<tr>
<td>N33–P27</td>
<td>-1.15 ± 0.79</td>
<td>-1.40 ± 0.34</td>
</tr>
<tr>
<td>P50–N33</td>
<td>2.06 ± 1.96</td>
<td>1.77 ± 1.71</td>
</tr>
<tr>
<td>N70–P50</td>
<td>-3.77 ± 3.89</td>
<td>-3.29 ± 2.98</td>
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<tr>
<td>P100–N70</td>
<td>4.22 ± 3.05</td>
<td>4.44 ± 2.56</td>
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<tr>
<td>N140–P100</td>
<td>-2.24 ± 1.46</td>
<td>-2.27 ± 1.58</td>
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<tr>
<td>LP</td>
<td>4.47 ± 1.92</td>
<td>3.96 ± 3.03</td>
</tr>
</tbody>
</table>
**Table 3.4:** Mean amplitudes of SEP complexes recorded from C3 when tFUS beam was targeted - 1 cm posterior of S1.

<table>
<thead>
<tr>
<th>SEP Complex</th>
<th>Mean SEP Amplitude ± SEM (µV)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sham</strong></td>
<td><strong>tfUS</strong></td>
<td></td>
</tr>
<tr>
<td>P27–N20</td>
<td>0.95 ± 0.39</td>
<td>0.73 ± 0.30</td>
</tr>
<tr>
<td>N33–P27</td>
<td>-1.39 ± 0.74</td>
<td>-1.44 ± 0.71</td>
</tr>
<tr>
<td>P50–N33</td>
<td>2.56 ± 3.81</td>
<td>2.27 ± 2.33</td>
</tr>
<tr>
<td>N70–P50</td>
<td>-4.20 ± 3.90</td>
<td>-3.58 ± 3.41</td>
</tr>
<tr>
<td>P100–N70</td>
<td>3.26 ± 1.87</td>
<td>4.25 ± 1.97</td>
</tr>
<tr>
<td>N140–P100</td>
<td>-2.36 ± 1.29</td>
<td>-3.37 ± 1.21</td>
</tr>
<tr>
<td><strong>LP</strong></td>
<td>5.56 ± 2.39</td>
<td>6.18 ± 2.81</td>
</tr>
</tbody>
</table>
3.8 Figures

Figure 3.1 Ultrasound can be focused through human skull bone. Acoustic intensity fields emitted from a 0.5 MHz focused ultrasound (FUS) transducer measured in free space (no skull, A) and following transcranial transmission through hydrated human cranial bone (tFUS, B). The white-lines (z = 30 mm) on the three-dimensional acoustic intensity maps indicate the focal plane for cross-sections of the beam profiles shown (right). The pseudo-color look-up tables show the acoustic intensity scales (W/cm²) for each experimental condition. (C) Line plots illustrate the lateral (X; left) and vertical (Y; middle) peak normalized acoustic intensity profiles for the acoustic beam in the focal plane of 0.5 MHz FUS transmitted into free space (no skull; black-line) and through human cranial bone (red-line). Also illustrated are line plots (right) showing the axial (Z) peak normalized intensity profiles of the FUS field for both the free space (black-line) and transcranial (red-line) experimental condition.
Figure 3.2 Schematic outline of experimental approach. (A) The FUS transducer is shown placed at EEG electrode site CP3 on a realistic finite element model of a human head (left). A top-down schematic (right) illustrates transducer and electrode positioning at international 10-20 EEG electrode site sites C1, CP1, CP5 and P3. (B) The timing strategy for delivering transcranial focused ultrasound (tFUS) or sham prior to, during, and following median nerve stimulation (MN stim) is illustrated. (C) A schematic illustrates the pulsed ultrasound (US) waveform transmitted from the focused ultrasound transducer. The acoustic frequency of the waveform was 0.5 MHz and the pulse duration was 360 usec (black). The pulse repetition frequency (PRF; red) was 1 kHz for the stimulation duration of 500 msec.
Figure 3.3  Transcranial focused ultrasound can be targeted to spatially discrete regions of human cortex. Top-down (A) and coronal cut-away (B) show the acoustic intensity field of the tFUS beam projected from EEG site CP3 into a realistic FEM model of the brain derived from whole head structural MR images. Projection of the tFUS acoustic field clearly illustrates the targeting of primary somatosensory cortex (S1) with reference to the primary motor cortex (M1) and the central sulcus (CS). (C) Coronal MR slices showing projections of the measured tFUS fields from EEG electrode site CP3 further illustrate the spatial specificity of 0.5 MHz tFUS in the crown of the post-central gyrus (S1) and posterior wall of the central sulcus. Coronal slices are shown along the anterior-posterior axis of the beam corresponding to the center of the beam (top) and 2.0 mm posterior of the beam center (bottom) to show the acoustic intensity drop off as a function of tFUS beam width.
Transcranial focused ultrasound targeted to human somatosensory cortex modulates sensory-evoked brain activity. *(Top)* Grand average (N = 10) somatosensory evoked potentials (SEP) for electrode site (C1, CP1, CP5, and P3) as a result of right median nerve stimulation (MN stim; 100 trials) are shown for sham *(black)* and transcranial focused ultrasound (tFUS; *(red)*) treatment conditions. N20, P27, N33, P50, N70, P100, N140, and LP components of SEPs are illustrated across the different electrode sites. Grey vertical bars indicate regions where there were significant differences (P < 0.025) in the peak-to-peak amplitudes of SEP complexes between sham and tFUS conditions. *(Bottom)* Time-frequency plots illustrate the spectral power of evoked brain oscillations in the alpha (α, 7 – 12 Hz), beta (β, 13 – 30 Hz), and gamma (γ, 30 – 55 Hz) frequency bands in relation to the onset of tFUS (dashed vertical line) and MN stimulation (solid vertical line) for sham and tFUS treatment conditions. Statistical difference plots are also shown for each frequency band where maroon regions indicate a significant difference (P < 0.025) between sham and tFUS treatment conditions. A pseudo-color look-up table is shown at the bottom of the figure to illustrate the power of each frequency band examined.
Figure 3.5  The influence of transcranial focused on brain activity is not cumulative and remains stable across time. Plots of the trial mean amplitudes for N20 and N33 components of SEPs recorded from C3 during tFUS treatment are plotted as a function of trial number. The tFUS trial data are plotted against the grand average mean ± SEM of the N20 and N33 SEP amplitudes recorded during sham treatments. These data show there were no cumulative effects of tFUS across trial number. Error bars represent SEM.
Figure 3.6  Transcranial focused ultrasound does not affect task attention or response bias on somatosensory discrimination tasks. (A) Data acquired under sham (white) and tFUS (grey) treatments during two-point discrimination testing. The average percent correct for control catch trials (one pin) are illustrated as histograms (top). The criterion (c) data are illustrated as box plots (bottom) where the central line is the median and the edges of the box are the 25th and 75th percentiles with whiskers extending to the extreme data points. (B) Data acquired under sham (white) and tFUS (grey) treatments during frequency discrimination testing. The average percent correct for control catch trials (no frequency difference between paired air puff stimuli) are illustrated as histograms (top). The criterion (c) data are illustrated as box plots (bottom) where the central line is the median and the edges of the box are the 25th and 75th percentiles with whiskers extending to the extreme data points.
Figure 3.7  Transcranial focused ultrasound targeted to somatosensory cortex enhances sensory discrimination abilities in humans. (A) The data illustrated were acquired under sham (black) and tFUS (red) treatments during two-point discrimination testing. The group average (N = 12) cumulative d’ data show the pin distance needed to achieve a threshold d’ of 1 was lower for tFUS versus sham. (B) The data illustrated were acquired under sham (black) and tFUS (red) treatments during frequency discrimination testing. The group average (N = 12) cumulative d’ data show the frequency separation to achieve a threshold d’ prime of 1 was lower for tFUS treatment compared to sham. Data from these psychophysical studies show tFUS treatment significantly lowered sensory discrimination thresholds. Error bars represent SEM.
Figure 3.8 Transcranial focused ultrasound produces differential effects on sensory-evoked activity as a function of the brain region targeted. (A) Top-down view of the brain shows the spatial location of tFUS beams targeting the crown of the post-central gyrus (S1), and sites 1 cm anterior (+1 cm, A) and posterior (−1 cm, P). (B) (Top) SEP traces recorded from electrode C3 showing the grand average (N=8) responses to right median nerve stimulation (MN stim; 100 trials) for sham (black) and tFUS (red) for 1 cm anterior (left) or 1 cm posterior (right) of S1. There were no significant peak-to-peak amplitude differences between sham and tFUS conditions when transducers were offset 1 cm from S1. (Bottom) Time-frequency plots illustrate the power of evoked brain oscillations in the alpha (α, 7–12 Hz), beta (β, 13–30 Hz), and gamma (γ, 30–55 Hz) frequency bands in relation to the onset of tFUS (dashed vertical line) and MN stimulation (solid vertical line) for sham and tFUS treatment conditions. Statistical difference plots are also shown for each frequency band where maroon regions indicate a significant difference (P < 0.025) between sham and tFUS conditions. A pseudo-color look-up table is shown at the bottom of the figure to illustrate the power of each frequency band examined.
4 Effects on EEG Phase

*Ultrasound influence of phase aspects of cortical activity*

Work presented in this chapter has been published as:

4.1 Abstract

Background: The integration of EEG recordings and transcranial neuromodulation has provided a useful construct for noninvasively investigating the modification of human brain circuit activity. Recent evidence has demonstrated that focused ultrasound can be targeted through the human skull to affect the amplitude of somatosensory evoked potentials and its associated spectral content.

Objective/hypothesis: The present study tests whether focused ultrasound transmitted through the human skull and targeted to somatosensory cortex can affect the phase and phase rate of cortical oscillatory dynamics.

Methods: A computational model was developed to gain insight regarding the insertion behavior of ultrasound induced pressure waves in the human head. The instantaneous phase and phase rate of EEG recordings before, during, and after transmission of transcranial focused ultrasound (tFUS) to human somatosensory cortex were examined to explore its effects on phase dynamics.

Results: Computational modeling results show the skull effectively reinforces the focusing of tFUS due to curvature of material interfaces. Neurophysiological recordings show that tFUS alters the phase distribution of intrinsic brain activity for beta frequencies, but not gamma. This modulation was accompanied by a change in phase rate of both beta and gamma frequencies. Additionally, tFUS modulated phase distributions in the beta-band of early sensory-evoked activity but did not affect late sensory-evoked activity, lending support to the spatial specificity of tFUS for neuromodulation. This spatial specificity was confirmed through an additional experiment where the ultrasound transducer was moved 1 cm laterally from the original cortical target.
Conclusions: Focused ultrasonic energy can alter EEG oscillatory dynamics through local mechanical perturbation of discrete cortical circuits.

4.2 Introduction

Transcranial focused ultrasound (tFUS) has been demonstrated as a feasible method for transcranial neuromodulation in humans [1]. We have previously showed tFUS can alter the amplitude of somatosensory evoked potentials with a concomitant change in tactile sensory perception. It is not clear however, what the effect of tFUS is upon oscillatory neural dynamics. The integration of electroencephalographic (EEG) recordings and transcranial neuromodulation has provided a useful, noninvasive construct for investigating the alteration of neural dynamics, including effects on EEG phase dynamics [2, 3]. Oscillations in the electric potential of neuronal assemblies are the result of increases and decreases at regular intervals of the extracellular voltage of the neuronal population. The responsiveness of neurons can vary depending on whether this synchronous extracellular voltage oscillation is in a lower or higher stage (phase), and the influence of this oscillating phase on neuronal processing and cognitive function has long been recognized [4, 5]. The application of tFUS for neuromodulation is an emerging field, and the mechanisms underlying ultrasonic neuromodulation of neural tissue are only beginning to be understood [6]. We have previously explored the effects of tFUS on the amplitude of sensory evoked potentials and the event related spectral content of sensory evoked brain oscillations [1]. In the present study, we focused on investigating the feasibility and effects of tFUS on both intrinsic and evoked phase dynamics. Specifically, we were interested studying how tFUS affects both the phase and phase rate
of beta and gamma oscillations that have been previously identified to be modulated by other non-invasive neuromodulation methods like transcranial magnetic stimulation [7] and transcranial alternate current stimulation [8]. Here, we report tFUS to preferentially modulate the phase of beta activity in intrinsic EEG signals but to affect both beta and gamma activity in evoked EEG responses. Lateral movement of the ultrasound transducer 1 cm from its original position ameliorated these effects, lending support for its high spatial specificity. These findings support the hypothesis that tFUS stimulation modulates EEG oscillatory dynamics similar to existing technologies and may provide a complimentary and more spatially specific form of transcranial neuromodulation.

4.3 Materials and methods

4.3.1 Computational modeling and acoustic field mapping

To gain insight regarding the intracranial spatial patterns and resolution of US induced pressure waves, a simple finite element method (FEM) model was constructed using COMSOL Multiphysics software (COMSOL, Inc., Burlington, MA). The modeling domain consisted of a circle (r = 9 cm) to approximate the brain encompassed by a 5 mm thick annulus representing the skull, and a larger annulus (r = 15 mm) outside the skull to provide an outer boundary of skin and acoustic coupling gel. This simple 2D geometry approximates the head as a cylinder and is valuable for developing an understanding of the basic insertion behavior of US as it propagates across model tissue layers (skin and skull) into the brain. The density (ρ) of brain was specified as 1,030 kg/m³ and the speed of sound (c) was 1550 m/sec [9]. For the skull, ρ was set to 1,912 kg/m³ and c was
estimated as 2,300 m/sec based on previous empirical observations [10]. The outermost annulus for skin and ultrasound gel was specified to have the material properties of water.

A plane wave incident pressure field of 100 Pa across a range of acoustic frequencies from 5 kHz to 2 MHz was used to represent stimulation from the US transducer. We extracted the pressure profile along a radius perpendicular to the planar acoustic waves in the FEM model to model the intracranial wavelength of US (Figure 4.1A). The spatial resolution for a particular US frequency was calculated as the average distance between the peaks of the extracted pressure profiles. Assuming a linear homogenous media, the theoretical resolution can be calculated as the wavelength (\(\lambda\)), which is dependent on the speed of sound in the material and the wave frequency (\(f\)), by \(\lambda = \frac{c}{f}\). The simulated wavelengths were compared to the theoretically expected wavelengths (Figure 4.1B) to validate the model for visualizing the diffraction patterns of US in brain tissue (Figure 4.1C).

The acoustic intensity profile of the transcranial focused ultrasound waveform was measured using a calibrated hydrophone and then projected into a realistic head FEM model as previously described [1]. Briefly, the hydrophone, US transducer, and rehydrated skull fragment were positioned in a water tank, and the position of the hydrophone manipulated using a three-axis stage and an assortment of optomechanical components. The measured tFUS acoustic field was then projected from EEG site CP3 into a three-dimensional FEM model of the head created from magnetic resonance images to estimate the acoustic field distribution in the brain during US stimulation in subjects.
4.3.2 Subjects

Two separate subject groups were used in experiments. The first experiment included eighteen volunteer participants (11 male, 7 female, age 18-54, mean age = 29.62 ± 10.9), which performed the experimental task with the US transducer placed at CP3. A separate sample of seven volunteer participants (5 Male, 2 Female, age 22-57, mean age = 28.8 ± 11.6), performed the experimental task identically with the exception that tFUS was projected from transducers placed at a site 1 cm lateral of CP3. All participants provided written informed consent to voluntarily participate in the study. None of the participants reported current drug use (prescription or otherwise) or a history of neurological impairment and all were self-report right hand dominant. Participants received remuneration for participation. The Institutional Review Board at Virginia Tech approved all procedures.

4.3.3 Experimental procedures

Participants were seated in a desk chair and instructed to view a cross on a computer screen in front of them. A total of 120 ultrasonic stimuli (0.5 sec) were delivered at an ISI of 6 sec with a positive randomization of 4 sec from the 10-20 EEG site CP3. The tFUS treatment condition involved acoustically coupling the active face of the US transducer to the scalp, while the sham condition involved flipping the transducer such that the inactive face made contact with the scalp. In this manner, ultrasonic energy was not transmitted into the head and the active buzzing sound of the transducer was identical for both the sham and tFUS condition. Both sham and tFUS treatment were run in a single session counterbalanced across subjects. Total collection time was approximately one hour.
Additionally, a separate experiment with identical procedures was conducted with the transducer displaced 1 cm laterally on the scalp as a control for the spatial specificity of tFUS.

Electroencephalography (EEG) data were acquired using four 10 mm gold-silver cup electrodes placed at sites C3, CP1, CP5, and P3 referenced to the left mastoid and grounded to the left ulnar styloid process. Somatosensory evoked potentials (SEPs) were elicited in response to right median nerve stimulation delivered through a bar electrode affixed to the wrist. Amplitude was set to elicit a small but noticeable thumb twitch. In each treatment condition a total of 120 median nerve stimuli were delivered, time-locked to occur 100 ms after the onset of tFUS (Figure 4.2).

4.3.4 tFUS waveform

The transcranial ultrasonic neuromodulation waveform used in these experiments has been previously described [11, 12]. Briefly, transcranial ultrasonic waveforms were generated using a two-channel, 2-MHz function generator (BK Precision Instruments). Channel 1 delivered US at a pulse repetition frequency (PRF) of 1.0 kHz and channel 2 drove the transducer at a 0.5 MHz acoustic frequency (\( A_f \)) with channel 1 serving as an external trigger for channel 2. The pulse duration (PD) of the waveform was set to 0.36 ms by adjusting the number of cycles per pulse (c/p) on channel 2 to 180, and the stimulus duration (0.5 s) was set by adjusting the number of pulses (np) on channel 1 to 500. The output of channel 2 was sent through a 40-W linear RF amplifier (E&I 240L; Electronics & Innovation) before being sent to a custom-designed focused ultrasound transducer (Blatek, Inc., State College, PA) having a center frequency of 0.5 MHz, a
diameter of 30 mm and a focal length of 30 mm. The waveform employed for tFUS stimulation had the following parameters: $A_f = 0.50$ MHz, PD = 360 $\mu$s, PRF = 1.0 kHz and np = 500. This produced a stimulus duration of 0.5 s yielding a peak rarefractional pressure of 0.80 MPa, a mechanical index of 1.13 and a spatial-peak pulse-average intensity ($I_{SPPA}$) of 23.87 W/cm$^2$ before transcutaneous and transcranial transmission. We have previously verified this waveform does not produce heating of the skin or skull bone. The transducer was coated with acoustic coupling gel and placed on the scalp at the 10-20 electrode location CP3 before being secured in place with athletic pre-wrap bandaging.

4.3.5 Data processing and calculations

All offline processing and analyses were done with Matlab v8.0 (The Mathworks, Inc., Natick, MA) using custom scripts and EEGLAB [13]. All data analyses were performed on channel CP5 as it provided the best signal to noise ratio of the electrodes. Prior to analysis, data were band-pass filtered (1-90 Hz) and notch filtered at 60 Hz using a Hamming windowed finite impulse response filter. Data were inspected for artifact and any contaminated epochs were removed from further analysis. 90 random EEG trials were used for each subject, as this was the greatest number of trials available from all subjects due to loss of data as a result of artifact rejection. Data was segmented into epochs of 2 seconds (-1000 ms to 1000 ms) around the onset of median nerve stimulation. To avoid phase distortion, processing was done with zero-phase digital filtering using linear finite impulse response filters that had 60 dB attenuation at the specified frequency bands and a minimal filter order. Power spectra before ultrasound stimulation (-500 to -
200 ms), during tFUS but before MN stimulation (intrinsic; -100 to 0 ms), and during tFUS but after MN stimulation (evoked; 1 to 400 ms) was calculated to ensure the EEG data had spectral content in particular frequency bands for later analyses. Power spectra were calculated by determining the fast Fourier transform of each trial, and then averaging across subjects for the time periods of interest. The shorter intrinsic time period was zero padded for increased frequency interpolation.

For each subject and each trial, phase of the EEG signal was calculated by first bandpass filtering the data with zero-phase digital filtering using linear finite impulse response filters into beta (13-30 Hz) and gamma (30-55 Hz) frequency bands. Instantaneous phase was then calculated using the Hilbert transform to first transform the real valued signal into a complex signal, whose argument then gives the instantaneous phase. Phase rate was calculated by unwrapping the instantaneous phase and calculating its slope [14]. Analyses were conducted on epochs prior to any stimulation (baseline; -200 to -100 ms), during tFUS but before MN stimulation (intrinsic; -100 to 0 ms), and during tFUS after median nerve stimulation (early and late evoked periods; Figure 4.2). For the analysis of the effect of tFUS on evoked neural dynamics, two time epochs were selected after median nerve stimulation to represent early and late somatosensory activity respectively according to general timings of somatosensory early and late evoked potentials [15] such that the early epoch was specified as 17-70 ms and the later epoch was 71-260 ms. Previous literature has identified potentials up to 80 ms to be generated in primary somatosensory area [15].

Spectral content was also calculated from the trial average EEG response using the short-time Fourier transform with a window size slightly larger than the period of the
average frequency of the beta (13-30 Hz, window size of 50 ms) and gamma (30-55 Hz, window size of 25 ms) frequency bands, and an overlap of half the window duration. The total power was then calculated as the sum of the spectral power within each of the time epochs of interest, calculated for each of the frequency bands separately.

4.3.6 Statistical analyses

The phase of EEG activity is reported to be an important consideration when delivering stimuli, as the current phase during stimulus presentation can inform us on the consequent processing of the stimuli [16]. This was not controlled during our experiments however, and prevented averaging of a subject’s phase over trials, as the trial averaged phase is approximately zero due to trials not being temporally aligned by phase. Thus, to compare the instantaneous phase between stimulation conditions for each time epoch, the distributions of instantaneous phase, collected over time and trials, were tested for differences using a two-sample Kolmogorov-Smirnov test (K-S test) with Bonferroni’s post hoc correction for the number of time epochs (p < 0.0167).

Phase rate is useful in determining differential effects on EEG dynamics independent of temporal alignment by phase. To compare the phase rate between conditions, phase rate values were averaged across each subject’s trials and the distribution of mean trial values for subjects tested for significance using a two-sample Kolmogorov-Smirnov test (K-S test) with Bonferroni’s post hoc correction (p < 0.0167). Additionally, phase rate was averaged for each subject across trials and time to obtain an average phase rate value used in a paired sample t-test with Bonferroni’s post hoc correction (p < 0.0167), except for comparisons where the US transducer was displaced 1
cm laterally, which used two-sample t-tests, as subjects were not identical to those with the US transducer placed at CP3.

Independent of phase dynamics, differences in spectral power were assessed using the total spectral power over time of the trial averaged EEG along with a paired sample t-test and Bonferroni’s post hoc correction (p < 0.0167), except for comparisons where the US transducer was displaced 1 cm laterally, which used two-sample t-tests, as subjects were not identical to those with the US transducer placed at CP3.

4.4 Results

4.4.1 Spatial resolution and diffraction of transcranial ultrasound

Following distortion of the pressure waves transmitted through the model skull (located at axial distance zero), acoustic waves continued to propagate into and through the model brain having a wavelength dependent on their acoustic frequency (Figure 4.1A). With increasing acoustic frequency, the wavelength of the intracranial sound pressure decreases yielding increased spatial resolutions for US, shown for our model and with a comparison to theoretical sound pressure wavelengths in brain tissue in Figure 4.1B. Additionally, due to the mismatch of material properties and curvature of material interfaces, incident sound pressure waves bend and refract as they are transmitted across the layers, producing a slight focusing effect on incident waves of planar ultrasound (US). Resultant diffraction patterns of planar US were modeled to illustrate this natural focusing effect (Figure 4.1C). This focusing effect in the model held for the use of focused ultrasound as well. Transcranial mapping of the focused US transducer revealed peak intensities located about 20 mm from the face of the transducer, which drops off
sharply laterally over approximately 2 mm from the center of peak effects \textbf{(Figure 4.3A)}. This profile of tFUS was found to effectively target S1 in the realistic head model in \textbf{Figure 4.3B} when projected from site CP3 on the scalp.

\textbf{4.4.2 Pre-stimulus baseline}

The power spectra of the pre-stimulation, intrinsic, and evoked, periods are shown in \textbf{Figure 4.4}. These results show that there was power within the frequency bands of interest (beta and gamma) recorded by the EEG for further analyses. Comparing the phase distributions during this time period, no statistically significant differences were found for both beta ($D = 3.31e^{-3}$, $p = 0.33$) and gamma ($D = 2.38e^{-3}$, $p = 0.74$) frequency bands. Regarding the phase rate, the trial and time averaged phase rate of subjects indicated no significant effects between tFUS and sham conditions for beta ($t(17) = -2.14$, $p = 0.047$) or gamma ($t(17) = 0.80$, $p = 0.44$). There were also no baseline differences for beta phase rate ($t(6) = 0.86$, $p = 0.42$) or gamma phase rate ($t(6) = -0.66$, $p = 0.53$) between the separate groups for the laterally displaced (1 cm) transducer. Similarly, the total spectral power indicated no effects between tFUS and sham conditions for beta ($t(17) = 0.23$, $p = 0.82$) or gamma ($t(17) = 0.37$, $p = 0.71$). There were also no differences in baseline power between the two groups for the separate groups in experiment 2; for beta ($t(6) = 0.77$, $p = 0.47$) or gamma ($t(6) = -0.07$, $p = 0.95$).
4.4.3 Modulation of intrinsic neural dynamics with tFUS

4.4.3.1 Phase

The distribution of instantaneous phase during the 100 ms time epoch, when tFUS was active prior to MN stimulation demonstrated a statistically significant difference between tFUS and sham stimulation for the beta frequency band (D = 7.97e-3, p = 6.08e-5) though no statistical difference in gamma phase was found (D = 3.23e-3, p = 0.36; Figure 4.5A).

4.4.3.2 Phase rate

Phase rate provides a measure of the modulation of instantaneous phase, or phase velocity. The trial and time averaged phase rate indicated no effect between tFUS and sham stimulation for beta (t(17) = -1.01, p = 0.33) or gamma frequency (t(17)=0.70, p = 0.49). However, statistically significant differences in phase rate distributions were found between tFUS and sham stimulation in both the beta (D = 0.095, p = 1.50e-7) and gamma (D = 0.069, p = 3.45e-4) frequency bands (Figure 4.5B).

4.4.3.3 Spectral power

The total spectral power within the beta and gamma frequency bands in the 100 ms time epoch prior to MN stimulation showed no effects due to tFUS or sham stimulation (beta: t(17) = 0.70, p = 0.49; gamma: t(17) = 0.23, p = 0.82).
4.4.4 Effect of tFUS on evoked neural dynamics

4.4.4.1 Phase

Based upon previous results for an effect of tFUS upon the amplitude of specific potentials of the somatosensory evoked potential (SEP) [1], we examined instantaneous phase in specific time bins according to early and late SEP potential latencies. For the early SEP epoch a statistically significant difference in phase was found between tFUS and sham stimulation in the beta band (D = 0.010, p = 1.92e-4) but not for gamma frequency band (D = 5.99e-3, p = 0.086). For the late SEP epoch no statistical differences in beta (D = 1.98e-3, p = 0.59) or gamma phase were found (D = 9.32e-4, p = 1.00; Figure 4.6A).

4.4.4.2 Phase rate

The trial and time averaged phase rate indicated no effect between tFUS and sham stimulation on evoked neural dynamics in the early epoch for beta (t(17) = -0.77, p = 0.45); or gamma frequencies (t(17) = 1.15, p = 0.27). There were no differences for the late epoch for both beta (t(17) = -0.99, p=0.33) or gamma (t(17) = 0.50, p = 0.62). The analysis of phase rate distributions for the early SEP epoch found a statistical difference between tFUS and sham in the gamma band (D=0.090, p = 7.60e-4) but not the beta band (D = 0.055, p = 0.11; Figure 4.6B). For the late SEP epoch statistical differences were found in both the beta (D=0.080, p = 6.03e-10) and gamma (D = 0.048, p = 7.33e-4) frequency bands (Figure 4.6B). Thus, differences in phase rate were not captured by the overall mean value.
4.4.4.3 **Spectral content**

There were no statistically significant effects in the early SEP epoch for beta (t(17) = 0.28, p = 0.78) or gamma (t(17) = 0.47, p = 0.64) and no statistically significant differences in the late epoch for beta (t(17) = -0.49, p = 0.63) or gamma power (t(17) = -0.31, p = 0.76).

4.4.4.4 **Spatial specificity**

To test the spatial specificity of tFUS effects on evoked neural dynamics, the US transducer was positioned 1cm lateral from the original position. For the early SEP epoch statistical differences in phase distributions were found between tFUS delivered at scalp site CP3 and scalp site 1cm lateral in the beta band (D = 0.012, p = 2.56e-3) but not in the gamma band (D = 5.46e-3, p = 0.46; **Figure 4.7A**). For the late SEP epoch, no statistical differences were found in either the beta (D = 3.68e-3, p = 0.19) or gamma band (D = 2.60e-3, p = 0.61; **Figure 4.7A**).

The trial and time averaged phase rate showed an effect due to placement of the US transducer in the gamma band for both the early (t(23) = -5.00, p = 4.67e-5) and late (t(23) = -8.25, p = 2.53e-8) epochs, but no effects upon the beta band for early (t(23) = 0.25, p = 0.80) or late (t(23) = 0.12, p = 0.91) epochs (**Figure 4.8A**). Regarding phase rate distributions, the early SEP epoch did not have significant statistical differences between tFUS delivered at scalp site CP3 and scalp site 1cm lateral in the beta band (D = 0.065, p = 0.19), but was significantly different in the gamma band (D = 0.66, p = 4.49e-104). For the late epoch, significant statistical differences were found in both the beta (D = 0.071, p = 1.28e-4) and gamma bands (D = 0.78, p=0.01; **Figure 4.7B**).
The total spectral power showed no effect of US transducer location in the early SEP epoch for beta (t(23) = 0.88, p = 0.39) or gamma (t(23) = 1.02, p = 0.32). There was an effect in both the beta (t(23) = -4.50, p = 1.63e-4) and gamma (t(23) = -3.62, p = 0.0014) frequency bands of the late SEP epoch (Figure 4.8B).

4.5 Discussion

In this study we examined the effects of tFUS on intrinsic and evoked EEG oscillatory dynamics. Computational modeling results provided insight into the frequency dependence of intracranial pressure diffraction patterns and show the skull effectively reinforces the focusing of tFUS. Our EEG recordings show that tFUS preferentially affected the phase of beta-band but not gamma-band frequencies of intrinsic brain activity. Interestingly, tFUS did modulate the phase rate of both beta and gamma intrinsic activity. We found tFUS affected phase distributions in the beta-band of early sensory-evoked activity but had no effects on late sensory-evoked activity, lending support to the spatial specificity of tFUS for neuromodulation. This spatial specificity was confirmed through an additional experiment in which we moved the ultrasound transducer 1 cm laterally from the original cortical target.

4.5.1 Spatial resolution of tFUS

Our computational FEM model revealed the 0.5 MHz transducer used here conferred a lateral resolution of approximately 3.1 mm that is ideal for targeting specific locations within individual gyri. The models further demonstrated the skull produced dense diffraction patterns of acoustic waves, but that the skull curvature provided a slight
improvement in the resolution of focal acoustic fields. These results generalize to the anatomical geometry of an actual skull as well, where the curvature would provide some additional focusing of incoming waves of focused acoustic waves specifically within the US beam focal zone. The combination of small lateral and vertical resolution using tFUS, plus controlled axial resolution, allows for neuromodulation of discrete cortical circuits superior to transcranial magnetic stimulation (TMS) for example, which produces electric fields in the cortex spanning several centimeters [17] and is presently constrained by a depth-focality trade off [18].

4.5.2 Neural dynamics

The phase of the on-going intrinsic EEG has been associated with various cognitive functions [19] and the coupling or temporal synchrony is considered a critical mechanism for these functions. It is not clear in these results why acoustic energy preferentially affected the phase of beta but not gamma frequencies, but this may be due to the focus of mechanical energy preferentially affecting the resonance of pyramidal cells and/or ascending pathways in layer five that have been demonstrated to largely contribute to beta oscillations [20]. Despite this hypothesis, tFUS altered the distribution of phase rate in all time epochs and frequencies of interest. The phase of the ongoing EEG may be considered to be the oscillation of the electric potential generated by temporally aligned excitatory post-synaptic potentials from pyramidal cells of a large neural mass. The precise mechanisms underlying phase rate are not explicitly understood, however, phase rate changes may be the result of local recurrent inhibitory mechanisms that serve to keep the balance between excitation and inhibition. Indeed, there is evidence for pyramidal cell
mediated activation of inhibitory cells in the rat somatosensory cortex that serve to maintain the balance between excitation and inhibition [21]. As such, changes in phase rate may represent activity of these circuits for the facilitation of signal transmission between populations with similar or resonant oscillatory phase characteristics. Within previous literature, phase rate is framed as a means of self-organization [22, 23] and as a useful indicator of transitions in states. Freeman and colleagues posit that variations in phase rate is evidence that cortex is unstable in the sense that it jumps between states, yet conditionally stable in that neurons self-organize their activity, which is not readily evident in the ongoing EEG signal and in small changes in phase [22]. Inspection of the trial and time averaged phase rate may also not reflect the finer changes in oscillatory activity due to the loss of information from averaging, as reflected in our analyses of variation of phase rate indicating no effects between tFUS and sham stimulation. Phase rate was also previously implicated in cumulative changes in neural activity due to prolonged single-pulse TMS [24].

The SEP elicited by median was used to introduce coordinated temporal and spatial activity into the EEG to inspect for changes in phase dynamics localized to areas of the cortex according to evoked potential latencies [25]. We found significant differences in the instantaneous phase distributions due to tFUS in the beta frequency bands corresponding to the early and late SEP components. Furthermore, these phases were unique from those due to tFUS when the transducer was displaced 1 cm laterally, suggesting tFUS stimulation is uniquely able to modulate the phase activity of the beta frequency band of SEP components dependent upon spatial positioning. The effect upon phase was modest and selective, while the effect of tFUS upon phase rate was rather
robust across frequencies and time points, suggesting that phase rate may be a more sensitive parameter for exploring modulation in EEG phase dynamics, or that the mechanical bio-effects of tFUS are particularly effective upon the neural circuitry involved in maintaining phase but does not necessarily directly contribute to the phase of the measured signal.

Interestingly, differences in total spectral power were absent for tFUS modulation between the transducer locations during the early components of the SEP, but were present during the later time periods. We observed that tFUS modulation at the original scalp position resulted in lower total sum power than tFUS did upon moving the transducer laterally, and may be from a weaker effect on SEP neuronal activity due to stimulation being located further from S1. We find it not surprising that differences can be found in phase dynamics independent of differences in spectral information. The exact roles of spectral magnitude and phase dynamics on, for example, the generation of event-related potentials remains largely conjectured in literature, as evidenced by the continued debate between the evoked and oscillatory models of event-related potential generation [26-28]. Nevertheless, depending upon spatial location and the timing of delivery, this work adds to the recent mounting evidence [1, 29-31] that focused ultrasound can be targeted to discrete cortical circuitry at spatial resolutions superior to existing non-invasive electrical and electromagnetic techniques to affect certain behaviors [1, 29] in response to acute neuromodulation.

4.6 References


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Figure 4.1 Model of transcranial US transmission. (A) Acoustic pressure levels simulating transcranial transmission of planar ultrasound waves in the brain for the acoustic frequencies 0.05, 0.25, and 0.50 MHz. Pressure profiles within the brain region become more erratic due to interactions with neighboring pressure waves as a result of curvature of the interfaces with the skull. (B) The FEM simulated and theoretical spatial resolutions of acoustic waves in the brain are plotted as a function of acoustic frequency. (C) The spatial diffraction patterns of transcranial planar US modeled using FEM simulations are illustrated for the acoustic frequencies 0.05, 0.25, and 0.50 MHz.
Figure 4.2  Schematic of the timing of events. Baseline refers to the timing of EEG before tFUS stimulation (< -100 ms). Intrinsic refers to the timing of EEG (-100 to -1 ms) during tFUS but before median nerve (MN) stimulation. Evoked refers to EEG after MN stimulation. Periods of interest include Early epoch (17 to 70 ms) and Late epoch (71 to 260 ms).
Figure 4.3 Transcranial transmission of focused ultrasound. (A) Pseudo-color map of the acoustic intensity field emitted by the 0.5 MHz transducer after transcranial transmission through hydrated human cranial bone ($Z = 10$ mm). (B) Modeled projection of the mapped acoustic intensity field from EEG scalp site CP3 into a realistic FEM model of human brain.
Figure 4.4  Power spectra of EEG data. Average power spectra recorded from electrode CP5 and calculated for the pre-tFUS, intrinsic, and evoked time periods for Sham (blue) and tFUS (red) stimulation, as well as a baseline (black) condition where subjects did not receive ultrasonic stimulation. Shown are the average power spectra for ultrasonic stimulation delivered from EEG electrode site CP3 (A, N = 18) and 1 cm laterally (B, N = 7).
Figure 4.5  Effect of tFUS on baseline intrinsic EEG. Group (N = 18) normalized histograms of instantaneous phase (A) and phase rate (B) for the 100 ms epoch prior to MN stimulation. tFUS (white) and sham stimulation (grey) recorded at CP5 overlaid for the beta (β) and gamma (γ) frequency bands. Note the difference in profiles for the cases with significant differences. An asterisk (*) denotes a statistically significant (p < 0.05) difference between tFUS and sham.
Figure 4.6  Effect of tFUS on evoked EEG. Normalized group (N = 18) histograms of evoked dynamics between tFUS (white) and sham (grey) stimulation recorded at CP5. (A) Normalized histograms of instantaneous phase for the evoked early epoch and late epoch for beta (β) and gamma (γ) frequencies. (B) Normalized group (N = 18) histograms of instantaneous phase rate for beta (β) and gamma (γ) frequencies. An asterisk (*) denotes statistical significance p < 0.05.
Figure 4.7  Effect of US transducer movement on EEG. tFUS stimulation (white, N = 18) and tFUS displaced 1 cm laterally (grey, N = 7) recorded at electrode site CP5. (A) Instantaneous phase for the evoked early epoch and late epoch for beta (β) and gamma (γ) bands. (B) Normalized group histograms for comparison of instantaneous phase rate for beta (β) and gamma (γ) frequencies. An asterisk (*) denotes statistical significance p < 0.05.
Figure 4.8  Effect of movement of US transducer on total spectral power. (A) Group average phase rate for both the early and late evoked time bins for beta (β) and gamma (γ) frequency bands for placement of the ultrasound transducer at the original location (CP3, white, N=18) and moved 1cm laterally (1 cm L, grey, N = 7). (B) Group average total spectral power observed following tFUS stimulation at original scalp location (CP3, white, N=18) and 1 cm laterally (1 cm L, grey, N=7) for beta (β) and gamma (γ) bands. An asterisk (*) denotes statistical significance p < 0.05.
5 Computational Exploration

*Sensitivity of the ultrasound beam profile to the intracranial space*

Work presented in this chapter has been published or submitted as:


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5.1 Abstract

Objective. While ultrasound is largely established for use in diagnostic imaging and heating therapies, its application for neuromodulation is relatively new and crudely understood. The objective of the present study was to investigate issues specific to the use of ultrasound for neuromodulation. Approach. A computational model of transcranial-focused ultrasound was constructed and validated against bench top experimental data. The models were then incrementally extended to address and investigate a number of issues related to the use of ultrasound for neuromodulation. These included the effect of variations in geometry of skull and gyral anatomy and the effect of multiple tissue and media layers, such as scalp, skull, CSF, and gray/white matter on ultrasound insertion behavior. In addition, a sensitivity analysis was run to characterize the influence of acoustic properties of intracranial tissues. Finally, the heating associated with ultrasonic stimulation waveforms designed for neuromodulation was modeled. Main results. The insertion behavior of a transcranially focused ultrasound beam is subtly influenced by the geometry and acoustic properties of the underlying tissues. Significance. These issues are critical for the refinement of device design and the overall advancement of ultrasound methods for noninvasive neuromodulation.

5.2 Introduction

Transcranial-focused ultrasound (tFUS) is an emerging technology for non-surgical stimulation of the human brain. tFUS offers a superior millimeter resolution compared to existing technologies like transcranial magnetic stimulation (TMS) which influences areas of the cortex spanning several centimeters [1, 2]. Recently, it has been
demonstrated that tFUS directed over the somatosensory cortex in humans affects EEG amplitude, power, phase, and tactile behavior [3, 4]. The intracranial manifestation of mechanical and thermal effects by tFUS depends on the insertion behavior of ultrasound across the various layers of tissue. Additionally, it is still not clear how the neuronal response couples to the exertions of ultrasound on neural tissue. An understanding of both the insertion behavior of ultrasound across the tissue layers in the context of neuromodulation and the coupled neuronal response is key to the continued advancement of ultrasound stimulation methods. The objective of the present study was to investigate the insertion behavior of tFUS for neuromodulation across the human head and quantify its sensitivity to tissue domains, their parameters, and their geometry.

Focused ultrasound has previously been investigated for such applications as brain tumor ablation, blood-brain barrier opening, and thrombolysis [5]. In these applications, it is advantageous to deliver the desired level of ultrasound energy through an intact human skull to the prescribed locations, especially for deeper subcortical regions. The intact skull though represents the primary barrier to ultrasound. The high attenuation, diffusion, and refraction of ultrasound waves in cranial bone compared to the neighboring tissues results in a significant loss of energy and distortion of the transmitted ultrasound beam, and is the primary barrier to high resolution transcranial ultrasound imaging [6]. To an extent, adaptive focusing techniques are able to account for the defocusing effect of the skull [7], and is critical to the application of high intensity focused ultrasound. In the context of neuromodulation though, despite bone absorbing ultrasound almost 90 times more efficiently than soft tissue [8], the skull does not pose such a dire obstacle to the transmission of sufficient energy for low intensity focused
ultrasound applications. In addition to the effect of tissue properties on ultrasound, it is also important to demonstrate that ultrasound for neuromodulation does not heat the tissue. At low intensities over short exposure times, ultrasound does not generate appreciable tissue heating, and the mechanical effects of ultrasound used in neuromodulatory capacities has not been reported to cause tissue damage [9-11]. Thus, as the safety of ultrasound has been extensively investigated, and the insertion behavior of ultrasound characterized in the context of various other applications, there is a need to explore the insertion behavior of tFUS and the heating characteristics for the purposes of neuromodulation beyond the barrier of the skull. We developed a computational model of tFUS for neuromodulation and used this model to explore the insertion behavior of the ultrasound beam in the intracranial space. We evaluated several paradigms to explore the sensitivity of focused ultrasound to tissue layers, their acoustic properties, and their geometry.

5.3 Methods
We developed computational models of the human skull and superficial cortical layers, including CSF, white matter, and gray matter, to evaluate the insertion behavior of tFUS across the skull and the resultant intracranial maps of intensity and heating. The finite element method models were constructed in COMSOL Multiphysics v4.3 (COMSOL, Burlington, MA) to calculate pressure, intensity, and heat generation. By these methods, we were able to investigate the subtle influence that various aspects of human biology impart on the behavior of tFUS.
The initial computational model recreated quantitative acoustic field mapping of focused ultrasound transmitted through a hydrated fragment of human cranium, which has been detailed previously [3]. Briefly, a calibrated hydrophone mounted on a motorized stage was used to measure the acoustic intensity profile from the ultrasound transducer coupled to a skull fragment in a 58 L acrylic water tank at a 400 µm spatial resolution. The ultrasound transducer is a custom designed single-element focused transducer (Blatek, Inc., State College, PA) having a center frequency of 0.5 MHz, a diameter of 30 mm, and a focal length of 30 mm. The transcranial ultrasonic neuromodulation waveform used has been previously described [12, 13], and has an acoustic frequency of 0.5 MHz, a pulse duration of 360 µs, and consists of 500 pulses delivered at a pulse repetition frequency of 1.0 kHz, resulting in a stimulus duration of 0.5 sec. As reported in Legon et al. 2014, we observed that transcranial transmission using this setup results in a spatial-peak pulse-average intensity ($I_{SPPA}$) of 5.90 W/cm$^2$ [3].

To recreate this experiment in the computational environment, a two-dimensional geometry with axial symmetry was created as shown in Figure 5.1A. The left most edge was specified as the axis of rotation, and the bottom most circular edge was specified as the single element of the focused transducer which serves as the ultrasound source. The transducer element is shaped with a focal length of 30 mm and an aperture diameter of 30 mm, simulating the transducer used in bench top experiments within the water tank, and is similarly driven at a frequency of 0.5 MHz. The normal displacement of the transducer element was specified as 13 nm, based on calculations involving the piezoelectric constant of the transducer element materials and the ultrasonic
neuromodulation waveform. Above the transducer element is a plane of skull 5 mm thick, and the space between the transducer and skull was specified as water. Beyond the skull layer the space was specified as water again, to recreate the conditions of the bench top measurements. The material properties specified in each domain are detailed in Table 5.1, and geometries detailed in Table 5.2. The sound velocity, density, thermal conductivity, and heat capacity of the water domain is derived from the default material properties for water in COMSOL.

To more finely resolve the pressure gradients in the focal area of the transcranial domain, the mesh size was specified as 1/6 of the wavelength within an elliptical region enclosing the focal point. A coarser mesh of 1/4 of the wavelength was specified in all other regions. Additionally, the model is bounded along the top and sides by cylindrical perfectly matched layers to absorb the outgoing ultrasound waves and prevent their reflection back into the modeling domain. The model solved for the stationary acoustic field to determine the acoustic intensity distribution in the materials with the further assumptions that acoustic wave propagation is linear and that the amplitude of shear waves are nominal compared to those of the primary, compressive waves. Shear waves are greatly attenuated by tissue compared to the longitudinal waves in an ultrasound beam [14, 15].

The acoustic intensity magnitude was then used to calculate the heat source for thermal simulations. Material properties were assumed to not change with temperature and that cooling due to blood perfusion was negligible in model layers of biological tissue. For the computational models recreating bench top experiments in the water tank, all domains were assigned an initial temperature of 294 K, corresponding to room
temperature. For computational models investigating the stimulation and heating of biological tissue, all tissue domains were assigned an initial temperature of 310 K, corresponding to body temperature. The time course of application of the heat source was specified to mimic the transcranial ultrasonic neuromodulation waveform. Heating was applied in 360 µs durations repeated at 1.0 kHz for 0.5 sec. No heating was applied for an additional 0.5 sec to observe cooling of the tissue, bringing total simulation time to 1 sec. The max time step of thermal simulations was set at 1/7 of the pulse duration, to resolve the heating and cooling between each application of an ultrasound pulse.

Following comparison of the experimental acoustic field mapping to the representative computational model, the model was then expanded to investigate how features such as geometry and material properties influence the insertion behavior of transcranially-focused ultrasound in humans. First the transcranial domain was given material properties of brain tissue, as specified for gray and white matter in Table 5.1. This offers a simplified model of the effects of transcranial-focused ultrasound in humans and a baseline for comparisons to later models. Next the skull layer was curved (Figure 5.1B), given a radius of 17 cm, to deviate from the idealized straight plane of the previous models, and decrease the effective mechanical coupling between the transducer and skull.

Tissue layers for cerebrospinal fluid (CSF), gray matter, and white matter were then added above the skull layer (Figure 5.1C) with the thicknesses and material properties stated in Table 5.1 and Table 5.2. The thickness of layers was based on computational models of electrical epidural motor cortex stimulation [16] and is specified in Table 5.2. The thickness of the CSF layer was derived from the sum of the thicknesses
of the dura mater and CSF layers from previous models of the precentral gyrus [17], as we were unable to find the relevant acoustic parameters for the dura mater in literature for our models. Additionally, the CSF was assumed to have the material properties of water, due to the lack of literature characterizing the parameters of interest for our models. Sensitivity analyses were then run with the layered tissue model to further explore the influence of these additional layers. Models scaling the attenuation coefficient of white and gray matter by 0.1, 0.2, 0.5, 1.0, 1.4, 2.0, 5.0, and 10.0 were solved to inspect its influence on the ultrasound beam profile and tissue heating. The scaling factor 1.4 was included as the attenuation coefficient of white matter has been reported to be 1.4 times that of gray matter [18]. Additionally, the thickness of the CSF layer was scaled by 0.1, 0.2, 0.5, 1.0, 2.0, and 5.0 to inspect the influence of the material’s presence as CSF volume is known to vary, such as due to age related loss of cortical volume [19]. Furthermore, sensitivity analyses scaling the density and speed of sound properties of the gray and white tissue layers were run, as the mechanical properties of cortical tissue have been shown to vary with age and disease [20-22].

To gain insight on the influence of gyral geometry on the behavior of tFUS, we constructed two-dimensional models of the precentral gyrus, including two adjacent sulci and two neighboring gyri based on computational models of electrical epidural motor cortex stimulation [16]. In addition to models with the sulci oriented perpendicularly to the skull (Figure 5.1D), other models with sulci slanted thirty degrees were constructed (Figure 5.1E). Maximum element size within the gyral anatomy model domains was restricted to 1/6 the wavelength. Additionally, bounding perfectly matched layer domains surrounded the modeling area on all sides as ultrasound intensity profiles were solve for
0, 3, 6, 9, and 12 mm transducer offsets from the center line of the model. This modeling geometry assumes the plane of the model is the plane of symmetry, representing the middle plane of an infinite slab. Attempts to extend the model to three dimensions proved exceedingly computationally intensive and thus the model was kept two-dimensional. While this planar, infinite slab, geometry is not representative of the true geometry of the focused ultrasound transducer included on the bottom of the models, the resultant pressure field is qualitatively representative of the beam profile at the center plane of symmetry for the true geometry of the ultrasound transducer, and thus intensity profiles examined as normalized quantitates. Furthermore, to allow fair comparison to a model without gyral anatomy, another computational model similar to that of the layered cortex in Figure 5.1C was constructed but without a plane of symmetry, thus resembling the geometries of Figures 5.1D-E, but without the sulci.

To quantify the area of stimulation by the focused ultrasound beam, the geometry of the root mean squared intensity (I_RMS) solved for from the FEM model was characterized for intensities greater than the half maximum. This thresholding resulted in an elliptical profile that served as the proxy for stimulated neural tissue in the computational model from which the area was calculated, excluding any area that was not contained in the gray or white matter (e.g. the CSF and skull). Additionally, the centroidal principal axes of the area moment of inertia of the thresholded intensity profile was calculated to determine their principal angles to characterize any deformation of the ultrasound beam. The length of the centroidal principal axes bounded by the thresholded profile was also determined to help characterize the geometry of the proxy for stimulated neural tissue.
5.4 Results

A computational model recreating quantitative acoustic field mapping of focused ultrasound (US) transmitted through a hydrated fragment of human cranium was constructed and then extended to evaluate the insertion behavior of tFUS across the skull and the resultant intracranial profiles of intensity and heating. The effects due to tissue properties and geometry were quantified and compared.

5.4.1 Computational model of acoustic water tank measurements

Calculations of intensity from a computational model were compared to experimental measurements. A close up of experimental measurements in the region of focus and minimally offset from the intracranial surface of the skull are shown in Figure 5.2A and the intensities for a similar area in the computational model shown in Figure 5.2B. Characterization of the half maximum intensity profile for both the computational and experimental models is shown in Figures 5.2C-D. To allow comparisons between the experimental and computational fields, calculations of intensity within the skull layer were removed, as in Figure 5.2B, and then the experimental and computational models aligned according to the location of maximal intensity. The absolute difference and relative error between the two data sets were then calculated, and is shown in Figure 5.3. The greatest absolute differences are in a small region immediately adjacent to the skull, while all other regions, particularly at the focal region of the ultrasound transducer, are notably low. These absolute differences are further reflected in the calculations of relative error, Figure 5.3B, where the errors are minimal solely in the focal region. The increase
in relative error outside the focal region is attributable to intensity values approaching zero in the denominator of relative error calculations once outside of the focal region. Beyond the few differences in intensity profiles between experimental and computational models likely due to differences between ideal simulations and non-ideal observations, the qualitative similarity, and particularly the good quantitative agreement within the region of focus, between the computational and experimental profiles is reassuring of the model.

The computational model also allows visualization of the profile of heat generation by tFUS, which is not as readily observed in experimental preparations, and is shown in Figure 5.4. The heat generated in the skull is several orders of magnitudes greater than that generated in the water domain, and follows the profiles of intensity from tFUS. The time course of temperature change is shown in Figure 5.4C. During US stimulation the temperature steadily rises with a rate highly dependent on the spatial location relative to the focus of the ultrasound transducer and the properties of the material exposed to ultrasound, with skull tissue heating up considerably more than the transcranial water domain.

5.4.2 Extension to homogenous brain tissue and curved skull surfaces

The transcranial water domain was then given material properties of brain tissue (white and gray matter of Table 5.1) to compare how the profiles of intensity and temperature change based on the transcranial domain material. The intensity and heating of the cranial domain changed minimally as a result of this material change. As shown in Figure 5.5, the general shape of both the intensity and heat profiles changes negligibly. Additionally,
the maximum intensities and their locations between water and brain transcranial domains with a planar skull differ minimally (Table 5.3). However, the maximal change in temperature in the transcranial domains differ by orders of magnitude, and their y-coordinates differ by a few millimeters as well, due to the large difference in attenuation coefficients between the two materials. The temperature increase due to the change of the transcranial domain from water to brain tissue was about 50 fold, similar to the 46 fold increase in attenuation coefficient in Table 5.1. Additionally, changing the material properties of the transcranial domain slightly altered the magnitude of intensity effects in the cranial domain though it did not alter its heating behavior (Table 5.3).

The skull layer was then changed to a circular arc to simulate a curved region of the skull and alter the coupling with the ultrasound transducer face in a manner that could similarly occur when placing the transducer on human skull. The resultant intensities and heat generation following curvature of the skull layer are shown in Figure 5.6, where the range of intensities and temperature increases have been decreased in comparison to previous models with a planar skull layer. Additionally, the maximum intensity was no longer located in the skull layer, though the maximum temperature increase still occurred in the skull layer (Table 5.3). The location of the maximum intensity in the brain domain moved down approximately 0.5 mm following curvature of the skull, though interestingly the location of maximum temperature increase did not change. Contours of the intensity profiles were analyzed and are shown in Figure 5.7. The curvature of the skull results in a more compact region of high intensities at the ultrasound transducer’s region of focus. The half maximum contour of intensity for the planar skull enclosed an area of 47.4 mm², while the curved skull decreased the enclosed area to 45.0 mm². Additionally, the planar
skull produced a longer, thinner half max contour compared to the curved skull. Overall, the curved skull further focused the US beam to produce a more compact region of maximal effects, albeit at a lower magnitude compared to the planar skull.

5.4.3 Extension to layered cortical tissue and sensitivity analyses

Layers for CSF, gray matter, and white matter were added following a planar skull layer to further investigate the modulation of human cortex using focused ultrasound. The profiles of intensity and temperature rise are shown in Figure 5.8, which did not change in overall shape compared to the previous homogenous brain model. The half maximum intensity contours are also very similar, with the homogenous brain model only having a slightly longer elliptical profile (by 0.19 mm) than the layered cortical model. Interestingly the maximum intensity in the skull layer decreased while the transcranial maximum increased in the layered cortical model compared to the homogenous brain (Table 5.3). Between the cortical and homogenous models, the max temperature increases were very similar, with the only difference being that the cortical model’s location for max temperature increase was elevated 0.67 mm along the Y-axis. Thus, the addition of a CSF layer between the skull and brain domains subtly influences the magnitude of intensities in both the skull and brain domains, but does not alter the magnitude of heat generation in either domain.

As we found sparse literature on the differing acoustical properties of the cortical layers, sensitivity analyses were run to explore their impact on model behavior. The attenuation coefficient of white matter has been reported to be 1.4 times that of gray matter by Kremkau and colleagues [18], and after running simulations at a range of
scaling factors we found that only for factors greater than 2.0 for the white matter attenuation coefficient did the half maximum intensity proxy for stimulation change noticeably. Overall, with increasing white matter attenuation coefficient, the elliptical profile decreases in area (Figure 5.9A). The maximum intensity and temperature rise values in the skull and CSF did not change, but in the cortical tissue the maximum intensity decreased while the heat generation increased, albeit only slightly across the entire range (Figure 5.9B). Interestingly, the elliptical profile of intensity did not change though the profile of temperature rise did (Figures 5.9C-E). The location of maximum temperature increase was located in the lower gray matter layer for the lowest two values of white matter attenuation coefficient simulated, after which the location of the maxima elevated into the white matter with a variation of about 1 mm as the attenuation coefficient continued to increase. The elliptical profile of temperature increase also returned for these higher values of attenuation coefficient. Overall, changes to the white matter attenuation coefficient between half and double that of the gray matter minimally changes the profiles of intensity and temperature change in the layered model of cortical tissue. Scaling of the attenuation coefficient of the gray matter (Figure 5.10) had even less of an impact on the intensity profile of ultrasound compared to scaling white matter’s coefficient (Figure 5.10A), and produced a bimodal distribution of heat generation for high values of attenuation coefficient in the gray matter (Figure 5.10E).

The initial inclusion of a CSF layer between the skull and brain domains subtly influenced the profiles of intensity and temperature increase in both the skull and brain domains. To further explore the effect of CSF layer presence, a sensitivity analysis of CSF layer thickness was run to explore the impact on model behavior. Regarding the area
enclosed by the half maximum intensity contour (Figure 5.11A), the thickness of the CSF layer minimally impacted the area enclosed, except for the case when the CSF was 15.5 mm thick. During this case of a very thick CSF layer, intensities within the half maximum intensity threshold were present within the CSF layer as well, reducing the area contained in brain tissue layers. The maxima intensity and temperature increases in the skull were most sensitive to the thickness of the CSF layer (Figure 5.11B), with the skull domain having a greater range of values (0.82 W/cm² and 0.02°C) compared to the brain domain (0.40 W/cm² and 3.2e-4°C). The increase of maximal intensity within the CSF with scaling factor is attributed to more of the focal region of ultrasound being contained in CSF with increasing layer thickness. Additionally, the thickness of the CSF layer readily influenced the location of maxima, with maxima increasing in elevation as the CSF thickness increased (Figures 5.9C-E).

Sensitivity analyses of the density and sound velocity properties of the gray and white matter layers revealed an interplay between all the model layers of skull, CSF, and gray/white matter influencing all measurements of interest (Figure 5.12). The sound velocity of the white matter layer readily influences the ultrasound beam profile, as well as the maximal intensity in the skull (Figure 5.12A). Varying the density of white matter caused similar trends in the change of maximal intensity and temperature values as the velocity, but influenced the half maximum intensity contour much less (Figure 5.12B). The sound velocity of the gray matter layer also readily influences the ultrasound beam profile (Figure 5.12C), with increasing half maximum contour area with increasing sound velocity, an opposite trend compared to the white matter sound velocity. Varying the density of gray matter (Figure 5.12D) also caused a similarly oppositely sloped trend
affecting the half maximum intensity contour compared to varying the density of white matter.

5.4.4 Influence of gyral geometry on the US beam

Transcranial transmission of the focused US beam results in an elliptical focus that can be deformed by the inclusion or proximity of sulci. These deformations are more qualitatively apparent in the models of the slanted sulci, as at certain horizontal offsets the beam focus spans across the CSF of sulci into the brain regions on either side (Figure 5.13B). The quantification of the half maximum intensity contours is shown in Figure 5.14 to allow comparison of the deformations to the US beam by gyral anatomies, as well as to a baseline comparison point derived from a model with no sulci (‘NS’ in figures). Overall, the more the beam focus is localized to a sulcus, the smaller the thresholded area and axes lengths compared to other cases in models including sulci. This is especially notable in the model with perpendicular sulci, where a horizontal offset of 6 mm, aligning the transducer well with a sulcus, results in a drop of area of about 40 mm$^2$ compared to the rest of the offsets (Figure 5.14A).

Particularly noteworthy though, is that the absence of sulci in the model can result in a decreased area of stimulation compared to models with sulci. This is shown well in the comparison of the US beam focus in the model with no sulci to the beam focus in the model with perpendicular sulci. Except for the case when the region of focus is centered on a sulci itself, when the region of focus is either between two sulci or near one it results in greater areas of stimulation compared to the model with no sulci (Figure 5.14A). Also particularly noteworthy is that the gyral anatomy did not typically result in sufficient
deformation of the US beam focus to rotate the centroidal principal moment axes by more than one degree. Only one particular offset with rotated sulci (9 mm) managed to rotate the principal moment axis by more than one degree (Figure 5.14C).

5.5 Discussion

Transcranial focused ultrasound is an appealing approach for noninvasive neuromodulation of cortical tissue for a wide variety of applications, including those with deeper cortical targets. However, the improvement and adoption of ultrasound methods for neurostimulation is greatly dependent on furthering our understanding of ultrasonic mechanisms, including its insertion behavior across the skull. We developed a computational model of the resultant intensity profiles of transcranially focused ultrasound based on acoustic tests in a water tank, and extended the model to solve for the heating by the ultrasonic neuromodulation waveform. We used the model to then explore the effect of tissue properties and model geometries on the behavior of the ultrasound beam. To quantify the model response of ultrasound, we characterized the ultrasound beam using half maximum intensity contours and their corresponding area moments of inertia. While the relationship between ultrasound intensity and stimulation of neural tissue is not established, the half maximum intensity contours provided a quantitative measure of the model response to estimate the influence of tissues and geometry on the region of effects by tFUS. By beginning to investigate and consider these issues in the context of neuromodulation, we can advance the utility of focused ultrasound methods for human neuromodulation.
To ensure that the computational models would be relatively accurate and credible, we began with construction of a computational model recreating acoustic testing of tFUS in a water tank. While it is possible to adjust the computational model to have an identical maximum intensity value as that observed in the experimental measurements, obtaining an identical profile of intensities is more difficult. Most notable in the difference between the computational and experimental profiles of intensity in Figure 5.2 is the warped region of moderately high intensities near the inner surface of the skull and below the maxima. This may be largely attributed to differences between the experimental approach and the idealized computational model, namely the inhomogeneous, anisotropic, and slightly curved human skull fragment used in the experimental tests. Unlike the idealized skull layer in the computational model, human skull has an inhomogeneous curved structure with a varying density and thickness that is compensated for in applications requiring very precise control of the transcranial distribution of ultrasound [23, 24]. Outside of the region near the inner skull surface, at the focal region and far field locations, the intensity profiles between the experimental and computational models are qualitatively similar and we deemed the computational model an acceptable recreation of the experimental observations for the purposes of this investigation.

Using the computational model we were also able to calculate the intensities within the skull layer, and simulate the heat generation from focused ultrasound in both the cranial and transcranial domains. The majority of heating takes place in the skull layer, largely due to the fact that the attenuation coefficient of the skull is much higher than that of the water. In fact, the model overestimates the heating of the transcranial
water domain, as we used a value of 0.02 Np/m, while the attenuation coefficient of water at room temperature is closer to 6e-3 Np/m based on reported data [25]. We used this larger value due to our representing CSF with the same parameter set in later models, as the density and sound velocity of water were found to be similar to that of CSF according to one source [26], and the CSF containing proteins and other compounds likely increases the attenuation coefficient to some degree.

Transcranial magnetic stimulation is another form of noninvasive neuromodulation that passes unimpeded through skull and whose manifestation of effects (electric fields) is influenced by the geometry of neural tissue [2]. As reflected in the simulations of this work though, tFUS seems to be manipulated in an opposite manner compared to TMS; the skull is the barrier to transmission of energy by ultrasound and the geometry of neural tissue only influences the manifestation of effects due to ultrasound (i.e. intensity and heating) subtly. As the properties of the skull (e.g. thickness, density, curvature) can vary over the expanse of the cranium [24], this implies that the transcranial effects of US can vary with transducer placement on the skull. Indeed, curvature of the skull layer in the computational model resulted in a 62% drop in maximal transcranial intensity, and a 56% drop in transcranial heat generation. The drop in effects by ultrasound within the skull layer was of an even greater scale, though they are not of concern in regards to neuromodulation but merely as a safety check and possible means of secondary effects. The influence of tissue geometry on the effects of US were quantified using the half maximum intensity contours, and was found to have subtle effects. Overall, the region of effects by US stayed at the focus of the ultrasound transducer, with CSF in sulci being the source of subtle influence on the geometry of the
intensity contours. This translates into the targeting of US for neurostimulation not being variable with the intracranial geometry and thus not being a significant concern for the design of an ultrasound transducer’s region of effects. In comparison to TMS where the induced electric fields are on the scale of centimeters and greatest at the gyral crown, but the effective electric field for neuronal stimulation is for elements within the gyral walls [27], one can focus design efforts of an ultrasound transducer to have a region of focus at the desired depth after accounting for the placement of the transducer on the cranium. Additionally, differences in tissue properties between white and gray matter could have a more substantial influence on the ultrasound beam, if the properties between the two differ greatly due to developmental or pathological changes. Thus placement and targeting of the ultrasound transducer are the primary factors of concern when applying tFUS, especially as the scale of effects by tFUS are in the range of millimeters.

Overall, the intracranial manifestation of effects by US (intensity, heating) is more readily controlled than the effects of TMS and other electromagnetically based noninvasive neuromodulation methods. The profile of these manifestations though depends on the insertion behavior of US across the various layers of tissue. As US offers the advantages of finer spatial resolution and variable depths of stimulation compared to noninvasive electromagnetic methods though, it is an appealing alternative to electromagnetic methods for a number of possible applications. However, it is still not clear how the neuronal response couples to the exertions of ultrasound on tissue. Using a computational model to systematically investigate parameters of interest, we found that the profiles of intensity produced by tFUS is relatively insensitive to the geometry of intracranial tissue, that the material properties of the intracranial tissue can influence the
intensity profile more substantially, and that the skull is a major source of influence on
the ultrasound beam profile. An understanding of both the insertion behavior of
ultrasound across the skull and the coupled neuronal response is key to the continued
advancement of ultrasound stimulation methods.

5.6 References

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5.7 Tables

**Table 5.1: Material parameters.**

<table>
<thead>
<tr>
<th>Material</th>
<th>Speed of sound (m/sec)</th>
<th>Density (kg/m³)</th>
<th>Attenuation Coefficient (Np/m)</th>
<th>Thermal Conductivity (W/K/m)</th>
<th>Heat Capacity (J/kg/K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>1,483</td>
<td>999.5</td>
<td>0.02</td>
<td>0.595</td>
<td>4,186</td>
</tr>
<tr>
<td>Skull [28]</td>
<td>2,300</td>
<td>1,912</td>
<td>21.5 [29]</td>
<td>0.43 [30]</td>
<td>1,440</td>
</tr>
<tr>
<td>CSF [28]</td>
<td>Water</td>
<td>Water</td>
<td>Water</td>
<td>Water</td>
<td>Water</td>
</tr>
<tr>
<td>Brain - Gray matter [31]</td>
<td>1,550</td>
<td>1,030</td>
<td>0.92 [30]</td>
<td>0.528 [30]</td>
<td>3,640</td>
</tr>
<tr>
<td>Brain - White matter [31]</td>
<td>1,550</td>
<td>1,030</td>
<td>0.92 [30]</td>
<td>0.528 [30]</td>
<td>3,640</td>
</tr>
</tbody>
</table>

**Table 5.2: Model geometry.**

<table>
<thead>
<tr>
<th>Object</th>
<th>Dimensions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Axial model span</td>
<td>50 mm radial</td>
</tr>
<tr>
<td></td>
<td>80 mm height</td>
</tr>
<tr>
<td>Gyral model span</td>
<td>100 mm width</td>
</tr>
<tr>
<td></td>
<td>80 mm height</td>
</tr>
<tr>
<td>Skull</td>
<td>5 mm thick [17]</td>
</tr>
<tr>
<td></td>
<td>3.1 mm thick</td>
</tr>
<tr>
<td>Gray matter</td>
<td>2.5 mm thick [16]</td>
</tr>
<tr>
<td>Precentral gyrus</td>
<td>12 mm wide [17]</td>
</tr>
<tr>
<td></td>
<td>1 mm wide</td>
</tr>
<tr>
<td>Central sulcus</td>
<td>16 mm deep [17]</td>
</tr>
<tr>
<td></td>
<td>1 mm wide</td>
</tr>
<tr>
<td>Precentral sulcus</td>
<td>16 mm deep [17]</td>
</tr>
</tbody>
</table>

**Table 5.3: Transcranial maxima and locations in computational models.**

<table>
<thead>
<tr>
<th>Model</th>
<th>Irms</th>
<th>Temperature Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Skull Max (W/cm²)</td>
<td>Trans Max (W/cm²)</td>
</tr>
<tr>
<td>Plane Skull - Water</td>
<td>6.81</td>
<td>5.22</td>
</tr>
<tr>
<td>Plane Skull - Brain</td>
<td>6.53</td>
<td>5.22</td>
</tr>
<tr>
<td>Curved Skull - Brain</td>
<td>1.64</td>
<td>3.20</td>
</tr>
<tr>
<td>Plane Skull - Layered Cortex</td>
<td>6.51</td>
<td>5.25</td>
</tr>
</tbody>
</table>
Geometries of computational models of transcranial focused ultrasound.
(a) Water tank model. (b) Curved skull model. (c) Layered brain model. Separate layers account for the skull, CSF, gray matter, and white matter. (d) Perpendicular sulci model. (e) Slanted sulci model. Sulci are rotated $30^\circ$ from the perpendicular.

Figure 5.1
Figure 5.2  Experimental and computational models of tFUS into a water domain. (a) Experimental $I_{SPPA}$ measurements of transcranial focused ultrasound in a water tank. Skull border begins at a Y level of 0 mm. (b) Calculations of $I_{RMS}$ in a computational model recreating the experimental setup. Skull border begins at a Y level of 5 mm. (c) Half maximum intensity profile and characterization of the experimental measurements. Included on the plot is the area of the elliptical contour, the centroid of the contour, and the length of the centroidal axes bounded by the contour. (d) Half maximum intensity profile and characterization of the computational model.
Figure 5.3  Comparison of the intensity field between models. (a) Calculation of the difference between the intensities of the computational and experimental models. Note that at the region of focus the differences are lowest, while differences are greatest at an off center location close to the skull. (b) Relative error between the intensities of the computational and experimental models. Note that within the region of focus that the relative error is low, while outside of the region of focus the error increases. This is due to the denominator of the calculation being composed of low intensity values that outside the region of focus are less than one.
Figure 5.4 Calculations of heat generation in the computational model of tFUS into a water domain. (a) Rise in temperature due to US in the skull and transcranial water domain at the end of the US stimulation waveform. The horizontal line at \( y = 5 \) mm marks the border of the skull. Note that heat is primarily generated in the skull adjacent to the US transducer. (b) Rise in temperature due to US in the transcranial water domain. Note that the temperature increase is much less than 1/100\(^{th}\) of a degree. (c) Increase in temperature over time in the transcranial domain (upper plot) and intracranial domain (lower plot). Temperatures within the transcranial water domain are shown at the location of maximum transcranial intensity (black), and 1 and 2 mm lateral to that point (blue and red). The intracranial temperature is shown at the point of maximum intensity within the bone layer.
Figure 5.5  Model of tFUS into a brain tissue domain. (a) Intensity profile within both brain and skull layers. Note that peak intensities are within the skull layer, adjacent to the center of US transducer. (b) Intensity profile within the brain layer solely. (c) Heat generation by tFUS within the brain layer. Note that while heat generation is greater than in water, the temperature increase is still less than 1/100th of a degree. (d) Increase in temperature over time in the brain tissue domain at the location of maximum transcranial intensity (black), and 1 and 2 mm lateral to that (blue and red).
Figure 5.6 Model of tFUS through a curved skull layer and into a brain tissue domain. (a) Intensity profile within both the brain and skull layers. Note that the peak intensities are lower than for the model with a planar skull layer, and are no longer within the skull layer but are now within the focal region of the US transducer. (b) Profile of temperature increase within the brain and skull layers. Note that heating in the brain layer is still relatively low compared to that occurring in the skull. (c) Temperature increase within the brain domain. Note that the brain tissue at the interface with the skull is being heated at a similar level as that at the focal region of the US transducer. (d) Increase in temperature over time in the transcranial domain (upper plot) and intracranial domain (lower plot). Increases in temperature over time in the brain tissue domain are shown at the location of maximum transcranial intensity (black), and 1 and 2 mm lateral to that (blue and red). Note that there is less heat generation as compared to the model with a planar skull.
Figure 5.7 Comparison of intensity profiles between planar (left) and curved (right) skull layers. (a) Intensity contours of tFUS through a planar and curved skull layer. Note that the planar skull layer produced higher intensities and more elongated contours. (b) Half maximum intensity contours. Note that the planar skull layer produced a greater thresholded area with a longer major axis and an elevated centroid along the Y-axis. (c) Close up of half maximum intensity contours.
Figure 5.8  Model of tFUS through a planar skull and layered cortex. (a) Intensity profile within all layers with borders marked in white. Note that peak intensities are within the skull layer, adjacent to the center of US transducer. (b) Intensity profile within the transcranial layers solely. (c) Half maximum intensity contour. (d) Heat generation by tFUS within all layers. (e) Temperature increase in the transcranial layers from tFUS. (f) Increase in temperature over time in the transcranial domain (upper plot) and intracranial domain (lower plot). Temperatures within the cortex are shown at the location of maximum transcranial intensity (black), and 1 and 2 mm lateral to that point (blue and red). The intracranial temperature is shown at the point of maximum intensity within the skull layer.
Figure 5.9  Sensitivity analysis of the attenuation coefficient of white matter. (a) Characterization of half maximum intensity contours. Overall, the area of the elliptical profile decreased with increasing the attenuation coefficient. (b) Max intensities and max temperature increases in cortical layers (black), skull (blue), and CSF (red). Measurements in the skull and CSF were insensitive, but as the attenuation coefficient of white matter increased, the max cortical intensity decreased while heat generation increased. (c) Location of the maximum intensity (black) and maximum temperature change (blue). Note that the jump in location of max temperature change between the scaling factors of 0.2 and 0.5 is due to a change in location from the gray matter to the white matter. (d) Intensity profile (left) and rise in temperature (right) for a white matter scaling factor of 0.2. Note that the maximum rise in temperature happens in the layer of gray matter. (e) Intensity profile (left) and rise in temperature (right) for a white matter scaling factor of 0.5.
Figure 5.10  Sensitivity analysis of the attenuation coefficient of gray matter. (a) Characterization of half maximum intensity contours. Note that the intensity contour was insensitive to changes. (b) Max intensities and max temperature increases in cortical layers (black), skull (blue), and CSF (red). Metrics were largely insensitive. (c) Location of the maximum intensity (black) and maximum temperature change (blue). Note that the jump in location of max temperature change between the scaling factors of 2.0 and 5.0 is due to a change in location from the white matter to the gray matter. (d) Intensity profile (left) and rise in temperature (right) for a gray matter scaling factor of 0.5. (e) Intensity profile (left) and rise in temperature (right) for a white matter scaling factor of 5.0.
Figure 5.11  Sensitivity analysis of the CSF layer thickness. (a) Characterization of half maximum intensity contours. Overall, the area of the elliptical profile decreased with increasing the attenuation coefficient. Note though that at a scaling factor of 5.0, the half maximum intensity contour extends into the CSF layer. (b) Max intensities and max temperature increases in cortical layers (black), skull (blue), and CSF (red). Note that maximal values in the skull layer decreased slightly with increasing CSF layer thickness, while maximal values in the cortical layers were relatively less sensitive. (c) Location of the maximum intensity (black) and maximum temperature change (blue). Note that the increasing of location of maxima is namely due to the shift in CSF layer thickness. (d) Intensity profile (left) and rise in temperature (right) for a CSF thickness scaling factor of 2.0. (e) Intensity profile (left) and rise in temperature (right) for a CSF thickness scaling factor of 5.0.
Figure 5.12  Compilation of sensitivity analyses varying the density and speed of sound in the white and gray matter layers. (a) Sensitivity of the half maximum intensity contour (left), maximal values (center), and y-coordinate of maximal values in the brain layers (right) in models varying the speed of sound in the white matter. (b) Sensitivity of measures in models varying the density of the white matter. (c) Sensitivity of measures in models varying the speed of sound in the gray matter. (d) Sensitivity of measures varying the density of the gray matter.
Figure 5.13 Modeling focused ultrasound in simplified gyral anatomies. Normalized intensity profiles for perpendicular (a) and slanted (b) sulci, with 0, 3, 6, 9, and 12 mm transducer offsets from the x-origin (top to bottom respectively). Note that the transitions between CSF and neighboring tissue result in deformations of the focused US beam as compared to the previous planar, layered models.
Characterization of gyral geometry effects on the focused ultrasound beam. Following thresholding of the intensity profiles, the resultant elliptical profiles were characterized using area (a), axis length (b), and centroidal principal moment axis angle (c) to quantify the effect of perpendicular (black line) and slanted (blue line) gyral anatomy with varying transducer offsets on the focused US beam. These parameters are also compared to a similar layered cortical model with no sulci (labeled ‘NS’ along x-axis, red diamond in graphs) to establish a baseline free of gyral effects for comparison. Overall, when the ultrasound beam was focused in a sulcus, the thresholded area and axes lengths decreased. However the angle of the major axis was minimally perturbed by gyral anatomy.
6 Future Directions

Avenues for continued ultrasonic neuromodulation research
The research presented in the previous chapters suggests some possible lines of future academic enquiry.

6.1 **Eliciting motor evoked potentials**

Transcranial focused ultrasound (tFUS) is an emerging technology for non-surgical stimulation of the human brain. tFUS offers a superior millimeter resolution compared to existing technologies like transcranial magnetic stimulation (TMS) that stimulates areas of the cortex spanning several centimeters. It has been demonstrated that tFUS directed over the somatosensory cortex in humans affects EEG amplitude, power, phase, and tactile behavior. However, unlike TMS, there is as yet no evidence of tFUS effect on the human motor cortex using peripheral surface electromyography. This is important because measurement of an effect in the periphery via electromyography is direct empirical and quantifiable proof of cortical effect. The use of TMS for somatotopic motor mapping of cortical excitation and inhibition was predicated upon amplitude changes in the electromyographic motor evoked potential in different muscles of the periphery. Because of this, TMS is a proven ubiquitous form of non-surgical neuromodulation in humans. The influence of tFUS upon peripheral muscle activity in humans would represent a significant advancement in the utility of focused ultrasound methods for human neuromodulation. This will allow for 1) an empirically observable metric for tFUS cortical effect; 2) precise motor cortical mapping using tFUS; and 3) testing of tFUS parameter space for inducing both cortical excitability and inhibition.
6.2 Eliciting BOLD responses

Measuring blood oxygen level dependent (BOLD) signals using magnetic resonance imaging (MRI) is a functional neuroimaging technique to measure brain activity by detecting associated changes in blood flow in response to neuronal activity. This offers an alternative technique to obtaining quantifiable proof of cortical effect as compared to motor evoked potentials, albeit much more complicated to obtain than electromyographic methods. Additionally, monitoring BOLD responses allows observation of effects of stimulation in regions of cortex other than the motor cortex. Evidence showing effects of TMS on BOLD responses were accumulated as part of the mounting proof of the cortical effect of TMS on human cortex, and can similarly be done with tFUS. Advantageously, the integration of tFUS with MRI methods is more straightforward due to the mechanical mechanisms of tFUS as opposed the electromagnetic mechanisms of action of TMS, as well as the minimal size of stimulating hardware for tFUS compared to TMS. Figure 6.1 shows an analysis of early work done investigating the BOLD response of volunteers to tFUS of the primary motor cortex.

6.3 Functional brain mapping

Following proof of effects by tFUS in cortical areas beyond the motor cortex based on BOLD responses, focused ultrasound combined with functional MRI could be used for brain mapping studies to help recognize and diagnose functional disorders of the brain. For example, depression, obsessive-compulsive disorder, bipolar mania, autism, and other conditions could all benefit greatly from investigations into their pathological mechanisms and underlying neuronal circuits. The unique properties of a small
stimulation focus, mechanical mechanisms, and noninvasiveness afforded by tFUS, when coupled with real time feedback using magnetic resonance imaging, could improve understanding of brain function and targeting of treatments for mental and neurologic disorders. Figure 6.2 shows an illustration of the localization of the ultrasound beam profile based on the transducer’s positioning on the scalp, highlighting the possible utility of focally modulating neuronal circuits in real time to probe brain function.

### 6.4 Adaptation to previously established therapies

The treatment of neurologic disorders, such as Parkinson’s disease and chronic pain, may be possible using low intensity ultrasound methods, as it can focus into superficial and deep brain regions noninvasively. Invasive deep brain stimulation (DBS) treatment has shown promise as a therapy in a variety of mental and movement disorders. These therapeutic areas include chronic pain, epilepsy, obesity, and Parkinson’s. The disorders utilizing DBS, as well as other therapeutic methods, may all be performed with low intensity focused ultrasound. In the future, therapy with ultrasound may become established as a treatment option following initial medications, but before more invasive and permanent therapies like DBS and ablation. Thus, low intensity ultrasound could offer a noninvasive and reversible treatment option before resorting to strategies that result in permanent disruption of neuronal circuitry.

### 6.5 Cellular mechanisms

In part due to the fact that the physiological functions of nervous systems are primarily regarded as being regulated by electrical and chemical driving forces, the cellular
mechanisms of electrical and electromagnetic forms of neuromodulation are relatively well understood. This understanding of the exertions of electrical based stimulation methods on neuronal tissue facilitates its use in academic studies, as well as the continued refinement of electrical stimulation methods for therapeutic applications. However, it is still not clear how the neuronal response couples to the exertions of ultrasound on neuronal tissue. While diverse applications for ultrasonic stimulation are already emerging, and numerous studies into the safety of ultrasonic stimulation have been conducted, the biophysical transductions mechanisms underlying ultrasonic modulation of nervous tissue remains unclear. An understanding of the coupled neuronal response is key to the continued advancement of ultrasound stimulation methods, thus demanding further investigation.
Figure 6.1 Early investigation into the BOLD response from tFUS stimulation. Test statistics for an individual analysis revealing both focal increases (red) and decreases (blue) in BOLD activation in response to tFUS stimulation. The artifact produced by the ultrasound transducer hardware can also be seen in the image.
Figure 6.2 Illustration of the spatial profile and localization of tFUS during an MRI scan. Experimental measurements scaled and overlaid on MRI scans. Intensities at half maximum and greater of tFUS are shown.