Explorations in Targeting Low Intensity Focused Ultrasound for Neuromodulation

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy in Biomedical Engineering

by

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2014
ABSTRACT OF THE DISSERTATION

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Doctor of Philosophy in Biomedical Engineering

University of California, Los Angeles, 2014

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It has recently been demonstrated by several researchers that ultrasound at intensities well below the commonly accepted threshold for effect on humans is potentially capable of reversibly modulating neuronal circuits and function. However, several critical barriers exist to translating these theories and techniques to the clinic as a non-invasive transcranial therapeutic or neural research tool.
The primary goal of thesis is to enable detailed exploration of the relationship between the nature of the ultrasonic stimulus and a neuromodulatory outcome by exploring the hypothesis that low intensity ultrasound can be discretely targeted to neural structures to induce neuromodulation. This work is undertaken with the ultimate goal of advancing safe, controllable ultrasonic neuromodulation as a treatment for human neurologic diseases through an exploration of targeting – the engineering challenge of applying known quantities of ultrasound to known locations.

An experimental assay of the effect of hypothalamic sonication in the Göttingen minipig was developed to explore targeted neuromodulation in the brain of a larger animal model. A transducer, coupling system, and surgical procedure were developed that allowed for spatially accurate stereotactic transcranial sonication of deep-brain structures with known acoustic intensities. A pilot study assaying the effect of ultrasonic stimulation targeted to the hypothalamus in the minipig yielded preliminary evidence that a set of ultrasonic pulsing parameters exist that can induce a statistically significant rise in heart rate (>5%) coincident with ultrasonic stimulation.

Targeting was also explored through the development of an invasive neurostimulation paradigm. Novel <1.8 mm diameter low frequency ultrasonic microtransducers were developed, micro fabricated, and characterized for this effort. The targeting potential for this paradigm was demonstrated in a feasibility study in the rat. Targeting was further explored through the development of an algorithm for effectively sampling the ultrasonic parameter space by nesting the
prime drivers of bioeffects into a matched-pair sampling framework of acoustic parameters.

With these results, this work provides an engineering foundation for future systematic studies of ultrasonic neuromodulatory effects. Through the exploration of targeting and parameter selection enabled by this work, rigorous assays for ultrasonic neuromodulation can now be conducted.
The dissertation of Amit Prasanna Mulgaonkar is approved.

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This dissertation is dedicated to my Mom, Dad, loving sister Ashwini, and grandparents. Without your unquestioning encouragement and support, across countries and continents, none of this would have been possible. You have inspired me by example, and set the standard to which I try to live and work.
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LIST OF ACRONYMS

CNS – Central Nervous System
BBB – Blood Brain Barrier
HIFU – High Intensity Focused Ultrasound
LIFU – Low Intensity Focused Ultrasound
DBS – Deep Brain Stimulation
VNS – Vagus Nerve Stimulation
tDCS – Transcranial Direct Current Stimulation
TNS – Trigeminal Nerve Stimulation
rTMS – Repetitive Transcranial Magnetic Stimulation
FUS – Focused Ultrasound Surgery
SONAR – Sound Navigation and Ranging
CW – Continuous Wave
PRF – Pulse Repetition Frequency
PD – Pulse Duration
PRP – Pulse Repetition Period
PRF – Pulse Repetition Period
N – Number of Cycles per Pulse
DF – Duty Factor

f - Frequency

I_{SPTA} – Intensity, Spatial Peak, Time-Average

I_{SPPA} – Intensity, Spatial Peak, Pulse-Average

AIUM – American Institute of Ultrasound in Medicine

NEMA – National Electrical Manufacturers Association

FDA – Food and Drug Administration

ODS – Output Display Standard

TcMRgFUS – Transcranial MRI-Guided Focused Ultrasound Surgery

MRgFUS – MRI-Guided Focused Ultrasound Surgery

FWHM – Full-Width at Half Maximum

P_r – Peak Rarefaction Pressure

PII – Pulse Intensity Integral
ACKNOWLEDGEMENTS

The following dissertation could not have been completed without the support, guidance, assistance, and mentorship, of many advisors, colleagues, collaborators, friends, and family over the years.

I would like to thank my primary academic advisor, Dr. Warren Grundfest for his unyielding support, guidance, and instruction in the art of biomedical engineering over my graduate course of study. I am very thankful for his help in shaping this thesis, and am fortunate to have had the opportunity to have learned from such a master on a variety of different projects. My deepest appreciation also goes to Dr. Martin Culjat, my primary engineering advisor at the Center for Advanced Surgical and Interventional Technology (CASIT), the laboratory in which much of this work was completed. More than anyone, he has been responsible for keeping the lights on for my various research endeavors, and the completion of this thesis stands as a testament to his guidance, patience, mentorship, and support over the years. Much of my growth as an engineer, researcher, and person since I first walked in the lab door can be attributed to his influence, and for that I am eternally grateful. My sincerest thanks also go to Dr. William Melega, my primary advisor on the bio/neuroscience aspects of this project, and under whose guidance and expert hand the in vivo aspects of this project were designed and conducted. This work could not have been successful without his sustained mentorship, input, and time, and I thank him greatly for
opening my engineer eyes to a brand new sphere of research and what it means to conduct true scientific inquiry.

I am also grateful for the input of my committee members Dr. Nader Pouratian and Dr. Wentai Liu, whose willingness to provide critical feedback and allowing me to harness their vast knowledge and experience in the field helped push the project forward and shape the overall work.

I am deeply indebted to Dr. Alexander Bystritsky for introducing me to the field, and his initial guidance, as well as research and financial support for the project. I also owe a deep debt of gratitude to Dr. Rahul Singh, under whose guidance I learned the foundations of ultrasonics which underpins this work, and who taught me the benefits of the “sparkle factor”, and to not fear electronics.

Bioengineering, and this research subject in particular is a highly interdisciplinary and collaborative affair, and a number of other individuals played important roles in the completion of this research. I would like to thank the rest of the project research team: Meghedi Babakhanian for being my fellow ultrasonic compatriot and grunt over all these years, Dr. George Saddik for his contribution to the system electronics and transducer matches, and Dr. Bryan Nowroozi for his experimental design and statistical advice. I would also like to thank Dr. Ken Roos, Dr. Maria Jordan, Tyrone Wong, and Shawn McGill for their assistance and expertise for the in vivo portions of this work, Matt Weisbart for his CNC mastery, as well as Dr. James Sayre and Jim Garritano for their invaluable help with the statistical analysis of the data. I would also like to acknowledge the invaluable contribution of my apartment mate, desk mate, and
lab mate for so many years, Dr. Shyam Natarajan for his help in reviewing and editing this manuscript.

My deepest appreciation also goes to my grad student coworkers, colleagues, and friends without whom this journey would not have been the same: Richard, Ken, Scotty, Qing, Chris, Pria, Zach, Omeed, Ji, Jun, Alan, Neha, Dave, Justin, Dr. Dutson, Holly, Stephanie, Kitty, Sarah, Eric, John, Shaunak, Jason, Antoine, the WDS, JARS, and ID. Without you all, I would have gone insane.

I would also like to acknowledge the funding support during the completion of this work from the UCLA Department of Bioengineering, UCLA Graduate Division, the Stein/Oppenheimer Endowment, the John H. Bent Foundation, the Friedman Family Foundation, the Koch Foundation, the Army Telemedicine and Advanced Technology Research Center (Grant W81XWH-07-1-0672 for Project 6 – “Low Intensity Focused Ultrasound for Transcranial Neuromodulation”), and the Advancing Bioengineering Innovations program of the UCLA Business of Science Center.

In addition, Section 3.3.2 has been adapted from the manuscript from “Focused Ultrasound for Noninvasive Neuromodulation”, by M.O. Culjat, R.E. Fan, M. Babakhanian, A.P. Mulgaonkar, R.S. Singh, W.S. Grundfest, and W.P. Melega appearing in The Textbook of Nanoneuroscience and Nanoneurosurgery, B. Kateb and J. D. Heiss, Eds., CRC Press, 2014.
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Chapter 1: INTRODUCTION

The development of effective therapies for the treatment of central nervous system (CNS) disorders and diseases has historically been a challenging problem due, in part to the presence of the blood brain barrier (BBB) that restricts passage of most therapeutic drugs and agents from the systemic circulation into the brain, and to the inherent invasiveness and associated risk of interventional surgical procedures. Furthermore, existing CNS drug therapy regimes often are often limited by adverse systematic effects, and effective neurosurgical approaches are often not applicable to a large population of patients. As a result, there is currently great research interest in designing new noninvasive CNS therapies without the limitations of traditional techniques.

The use of ultrasound – oscillating pressure waves with frequencies above the threshold of human hearing, has long been an established part of the modern practice of medicine. Ultrasound imaging, most commonly associated with prenatal examination and fetal-imaging has found use in applications as diverse as prostate imaging and intracranial pressure monitoring. Using the same basic physical principles that underlie ultrasound imaging – the transmission, reflection, or absorption of acoustic pressure waves through body tissues – modern ultrasonic therapeutic techniques, such as lithotripsy, high intensity focused ultrasound (HIFU), bone fracture healing, and rehabilitative hyperthermy have been developed. This growing interest in ultrasound as a
therapy is due in part to its relatively low cost, established gross safety thresholds, and lack of ionizing radiation.

While modern ultrasonic medical imaging is considered to be quite safe, it has long been established that ultrasound at very high intensities can have a biological and bio-active impact on living systems. In fact some of the very early experimentation with primitive sonar-derived ultrasonic imaging systems accidentally yielded crude bio-safety and dose thresholds. However, it has recently been demonstrated by several researchers that even at much lower intensities, well below the commonly accepted thresholds for high intensity effect on humans, ultrasound is potentially capable of reversibly and safely modulating neuronal circuits and function.

It has been hypothesized that lower intensity ultrasound, with proper tuning and focusing of the ultrasonic acoustic energy, can be delivered from outside the head to targeted regions of the brain to effect neuromodulation.

The objective of this work is to advance these early observations of ultrasound-mediated CNS effects, and to test the hypothesis that low intensity focused ultrasound (LIFU) can be discretely targeted to neural structures to induce neuromodulation. This work was originally motivated by a desire to harness LIFU to create a new therapeutic modality for neural pathologies, and began as a collaboration with clinical and industry partners to produce a novel treatment for epilepsy.
1.1 Acute Need for New Neuromodulation Strategies

There currently exists a pressing medical need for new neuromodulatory strategies, both to address the growing burden of neurological CNS pathologies and to probe and explore brain function in greater detail. Neuromodulation can be used to treat conditions in fields as diverse as urology (voiding dysfunction) [2], medically refractory chronic pain [3], and depression [4]. For a review of the functional role neurotransmitters and their effect on neuromodulation, please see [5].

The need for new therapeutic modalities is acute, as the burden of CNS pathologies with currently limited treatment options, such as such as epilepsy, is expected to grow. Epilepsy is a devastating disorder affecting at least 50 to 100 million people [6, 7], or approximately 1% of the population worldwide [8]. Epilepsy is the second most common chronic neurologic disease after cardiovascular disorders [9], with a much higher incidence in the developing world [10-12]. Epilepsy accounts for a worldwide illness burden similar to that of lung cancer in men, and breast cancer in women [13]. There are at least 2 million people with epilepsy in the U.S.A. [8]. Chances of experiencing at least one seizure in a normal lifetime are 10%, and one-third of those who have had a seizure will develop epilepsy [8]. In the military community, epilepsy is a significant problem after traumatic brain injury and may occur in over 50% of soldiers following head trauma [14, 15].
While the global burden of epilepsy [16, 17] is growing, treatment options have not advanced to match. Cumulative drug therapy, the first line of treatment, is unsuccessful in controlling seizures in over 40% of patients [18, 19], and 80% of the cost of epilepsy in the United States is accounted for by such medically intractable patients [20]. Medical management frequently is ineffective either because the pharmaceuticals fail to completely suppress seizures or because the side effects of the drugs, which are uniformly toxic at high doses, can become intolerable at even normal clinical dosages [18]. Over 50% of participants in a six-year study of anti-epileptic drugs for partial and generalized epilepsy suffered unacceptable adverse events, and around 20% discontinued treatment based on these events [21].

Patients with medically refractory seizures are at high risk for significant morbidity [22-24], enduring social and psychological adverse consequences [25, 26], and premature death [27-29]. Non-surgical therapeutic options for patients with refractory epilepsy are typically limited to clinical trials with newly developed antiepileptic drugs. However, the likelihood of achieving freedom from seizures after two or more anticonvulsant drugs have previously failed is low [19].

The management of pharmacoresistant seizure disorders is increasingly performed using resective surgeries which, despite their obvious attendant liabilities, are preferable to further medical treatment [30]. With modern diagnostic and surgical technology, the morbidity and mortality risk is low, [31], with outcomes for resective surgical procedures that can be superior to those for some medical therapies in terms of control of seizures, and quality of life [30].
However, surgery is expensive and its availability is limited, especially in developing countries where studies indicate 80% of those with epilepsy are thought to live [32].

Other neurosurgical interventions for Epilepsy such as Deep Brain Stimulation (DBS) and Vagus Nerve Simulation (VNS) [33] are invasive and have an associated morbidity risk [34-36]. Nonetheless, DBS is now an established clinical procedure. These invasive approaches, however, require extensive neurosurgical expertise and, due to their cost and invasive nature, are not envisioned to have wide applicability to large global patient populations. A variety of new non-invasive therapies such as transcranial direct current stimulation (tDCS) [36], Trigeminal Nerve Stimulation (TNS) [37], and repetitive transcranial magnetic stimulation (rTMS) [38-40] are being explored as treatments for Epilepsy, but are still in the research phase [40-42].

Consequently, there is a pressing need for more effective treatments for brain pathologies such as Epilepsy, which may also be applicable to conditions such as Alzheimer’s disease, Parkinson’s disease, and neuropathic pain management. In particular, methods that can provide non-invasive interventions to treat diseases in the brain are of great interest, including CNS applications of ultrasound.

This present work was motivated in part by early reports in the literature demonstrating a potential interaction between low-intensity ultrasound and Epilepsy [43, 44]. As such, ultrasound applied to the CNS is envisioned as a
potentially low-cost, non-invasive technique that would allow for the targeted treatment of neurologic conditions.

1.2 Advancing Ultrasonic Neuromodulation

The primary goal of this present work is to enable detailed exploration of the relationship between the nature of the ultrasonic stimulus (pressure, intensity, frequency, temporal parameters) and a neuromodulatory outcome by exploring the hypothesis that low intensity ultrasound can be discretely targeted to neural structures to induce neuromodulation. This hypothesis will be explored through the development of purpose-built ultrasonic transducers, systems, tooling, and techniques for precise, accurate, targeted delivery of known ultrasound doses to intact neural structures.

This work is undertaken with the ultimate goal of advancing safe, controllable ultrasonic neuromodulation as a treatment for human neurologic diseases by approaching ultrasonic neuromodulation from an engineering perspective.

Chapter Two is intended to serve as an introduction to ultrasound and the field of ultrasonic neuromodulation. The basic physics underlying ultrasonic waves and the resulting biological effects of acoustic energy on living tissues is discussed. The concept of ultrasonic intensity, which will be used as a measure of acoustic dosage is introduced and used to frame a discussion of the current state of the art of ultrasonic neuromodulation. Chapter Three introduces the critical barriers to progress in the field of ultrasonic neuromodulation that must be
addressed prior to advancing ultrasonic neuromodulation to the clinic, and identifies many of the gaps in our current knowledge of this phenomenon. The overall focus of this thesis and the experimental work described herein is to addresses some of these identified issues.

To allow for targeted exploration of ultrasonic neuromodulation, a number of different purpose-built ultrasonic transducers and systems were needed. Chapter Four details the design, development, and testing of a family of ultrasonic transducers and systems that will subsequently be used. A framework and protocol for the measurement and quantification of ultrasonic fields is also introduced. Chapter Five details the development of, and acoustic engineering for a large-animal model of transcranial ultrasonic neurostimulation. The resulting work on transcranial targeting using the previously validated transducer systems is used to address some of the critical barriers to progress discussed in Chapter Three. The engineering work was experimentally validated with a pilot trial of hypothalamic sonication in the Göttingen minipig, which is detailed in Chapter Six.

This work is subsequently extended to ultrasound applications in small-animal rodents. Chapter Seven introduces a novel invasive ultrasonic neuromodulation paradigm, and details the design, development and testing of a new class of microtransducer.

Chapter Eight details an algorithm and paradigm for effective sampling of the ultrasonic parameter space. A framework was developed for exploring the sample space of the ultrasonic parameters that uses matched pairs of values to
establish the critical parameter, or relative contribution of the individual pulse components to the final neuromodulatory outcome. A pilot study using this algorithm was conducted in the rat model in part to attempt to replicate the results from the large-animal hypothalamic sonication trials.

Chapter Nine concludes with a discussion of outstanding critical barriers to progress in the field of ultrasonic neuromodulation.
Chapter 2: Introduction to Ultrasonic Neuromodulation

Ultrasound has been in active clinical use since the 1950s, most commonly as a modality for diagnostic imaging in medicine. However, indications of possible ultrasound-induced bioeffects were identified as early as 1917 [45], with the first review of more detailed experimentation with crude early ultrasound systems published in 1930 establishing the potential for a variety of ultrasound-induced effects on biological materials [46, 47].

These early reports led to interest in the use of ultrasound as a therapeutic modality. The pioneers in this field observed that effects of ultrasound in the body were highly variable, with the observed effects depending on the intensity magnitude of insonation, region of exposure, and on the method of ultrasound delivery. It was later observed in the 1950’s that through acoustic focusing, the spatial distribution of ultrasound-induced bioeffects could be altered. This discovery inspired a field of research that came to be known as Focused Ultrasound Surgery (FUS) which used highly intense ultrasound to produce destructive focal lesions in tissues for therapy. These high-intensity techniques were subsequently explored for neurosurgical and neurological research [48].

Historically, clinical uses of therapeutic ultrasound have predominantly focused on the gross tissue level, and made use of highly intense ultrasound to cause irreversible/destructive tissue bio effects. The techniques and resulting
effects are relatively well studied, and have been demonstrated in the clinical setting through procedures such as lithotripsy or targeted ablation of tumors or fibroids. More recent efforts have focused on applying less intense ultrasound for therapy, and have found use clinically in physical therapy and fracture repair.

Current research efforts have demonstrated that ultrasound, at even lower intensities, may have reversible effects at the cellular level. This has led to interest in the therapeutic use of ultrasound for applications such as sonoporation, sonothrombolysis, gene therapy, and drug delivery. More recent efforts have demonstrated the possibility of using lower intensity ultrasound to directly stimulate neural structures. It is the potential for lower intensity ultrasound to directly stimulate neural structures that motivates this present work.

A detailed discussion of the current applications of ultrasound to the CNS, along with an introduction to the physical principles of ultrasound and ultrasonic bioeffects that underlie these applications follows.

### 2.1 Fundamentals of Ultrasound

Ultrasound waves are pressure waves that propagate through a medium with a frequency greater than 20 kHz. The audible range for humans is 20 Hz to 20 kHz; therefore, ultrasound falls immediately above the range of human hearing, with infrasound falling below. The first major application of ultrasounds came in the early 20th century in the form of SONAR (SOund Navigation and
Ranging), a tool for detecting icebergs and submarines, motivated by the Titanic disaster and the advent of submarine warfare in World War I. Diagnostic medical ultrasound, or medical sonography, was first explored following World War II, using surplus Naval sonar equipment [49]. For a more comprehensive history of the development of ultrasound and acoustic imaging, please refer to [50, 51].

2.1.1 WAVE-NATURE OF ULTRASOUND

Ultrasound relies on the formation of alternating zones of tension and compression in the elastic/viscoelastic molecular bonds of a material’s constituent particles in response to a stimulus as the particles vibrate about their resting position. When the direction of vibration is parallel to that of wave propagation, these waves are said to be propagating longitudinally.
When the bonds between particles are shortened relative to their resting state, zones of higher particle density are formed in the medium. When bonds are lengthened, periods of lower density occur. These periods of high density are known as “compression” and the periods of low density as “rarefaction”. These periods correspond to zones of higher and lower pressure within the medium (Figure 2-1).

One full cycle, corresponding to a period of compression and rarefraction

Figure 2-1: Schematic of a longitudinal ultrasonic pressure wave. Oscillation of molecular bonds around their equilibrium length create zones of higher and lower particle densities, and therefore pressures. The linear distance between subsequent minima or maxima is the wavelength.
is the wavelength $\lambda$. The wavelength is defined as the length of the space over which one cycle occurs, and is the ratio of the velocity of the wave in a medium and the frequency of the wave. The wavelength ($\lambda$) is related to the fundamental frequency of the wave ($f$) and the speed of sound in the medium ($c$) by Equation (2.1).

$$f = \frac{c}{\lambda}$$  \hspace{1cm} (2.1)

The waveform frequency ($f$) is the number of cycles that occur in one second, measured in Hertz (1 Hz = 1 cycle/s). The time elapsed for each cycle ($\tau$) is defined as the period, which is mathematically defined as the inverse of the frequency.

$$\tau = \frac{1}{f}$$  \hspace{1cm} (2.2)

For a continuous wave (CW), one without interruption until its ultimate cessation, in a medium of known speed of sound ($c$), the wave can be fully described by the frequency ($f$) and duration, along with the amplitude of the wave. However, in imaging and therapy, the acoustic energy is commonly pulsed, with zero acoustic energy being generated between the pulses. In the pulsed application of ultrasound, also known as pulsed-CW, discrete pulse packets consisting of a finite number of cycles ($N$) of frequency ($f$) are transmitted. The rate at which the pulse packets occur is the pulse repetition frequency (PRF).
Figure 2.2: Continuous Wave (CW) versus Pulsed Ultrasound.
These critical parameters can also be defined in terms of time and duration. The pulse duration (PD), the time required for a single pulse packet is equal to the period of one cycle (τ) multiplied by the number of cycles in the pulse (N). The time between the start of two successive pulses is the pulse repetition period (PRP), which is the inverse of the PRF.

\[ PD = N \times \tau \quad (2.3) \]
\[ PRF = \frac{1}{PRP} \quad (2.4) \]

The relative amount of time for which the ultrasound is on versus off in pulsed ultrasound is known as the duty factor (DF). The duty factor can be represented as a fraction of time that the pulsed ultrasound is on relative to pulse repetition period, or as a percentage of the same. This is given mathematically by Equation (2.5). A CW signal, by virtue of being always on would have a duty factor of 100%. With other parameters being held constant, duty factor increases as pulse duration, and therefore N increases, or with increasing PRF.

\[ DF = \frac{PD}{PRP} \quad (2.5) \]

As a consequence of Equation (2.1), for a given speed of sound the wavelength will be inversely proportional to the frequency. Therefore, as the frequency increases, the wavelength decreases. For a homogenous material, the speed of sound is a function of the material's density (\( \rho \) – mass/volume) and its bulk modulus (\( \beta \)), or its resistance to uniform compression. Due to the
relationship between density and bulk modulus, an increase in density usually results in an increase in the speed of sound.

\[ c = \sqrt{\frac{\beta}{\rho}} \]  

(2.6)

More generally and presented without derivation [52] from the Navier-Stokes equations, the propagation of an ultrasonic wave in one dimension and the evolution of acoustic pressure \((P)\) as a function of position \(z\) and time \(t\) can be described by the acoustic wave equation given by:

\[ \frac{\partial^2 P}{\partial z^2} - \frac{1}{c^2} \frac{\partial^2 P}{\partial t^2} = 0 \]  

(2.7)

The most general solution for a wave traveling in one direction is given by:

\[ P = P_0 \sin(\omega t - kz) \]  

(2.8)

Where \(\omega\) is the angular frequency and \(k\) is the wave number.

\[ \omega = 2\pi f \]  

(2.9)

\[ k = \frac{2\pi}{\lambda} \]  

(2.10)

2.1.2 INTENSITY

Acoustic intensity is defined as the rate at which energy passes through a unit area, and depends on the above parameters as well as the properties of the medium or material of propagation. The intensity is equal to the power of an acoustic wave divided by the area of which that power is spread. The instantaneous intensity \((I_i)\) is given by Equation (2.11) and the average intensity by Equation (2.12). The peak intensity is proportional to the square of the
pressure amplitude \( (P) \), so if the amplitude is doubled the total intensity is quadrupled.

\[
I_i = \frac{P_i^2}{\rho c} \\
I = \frac{P^2}{2\rho c}
\] (2.11, 2.12)

### 2.1.3 Reflection

There exists a complex resistance to the flow of the acoustic wave through a medium known as the acoustic impedance \((Z)\). The characteristic acoustic impedance of a medium \((Z_0)\) is inherent material characteristic dependent on the density \((\rho_0)\) and speed of sound \((c_0)\) in a given unperturbed material (2.13).

\[
Z_0 = \rho_0 c_0
\] (2.13)

Characteristic impedance is usually reported in the units of Rayls, which has differing definitions in both the MKS and CGS unit systems (2.14).

\[
\text{Rayl (MKS)} = \frac{Pa}{m^2/s} = \frac{kg}{m^2 \cdot s} = \frac{N \cdot s}{m^3} \\
\text{Rayl (CGS)} = \frac{dyn \cdot s}{cm^3}
\] (2.14)

Ultrasonic acoustic pressure waves are reflected at material boundaries where there exists an impedance mismatch, or a difference in the acoustic impedances on either side of the boundary. The greater the impedance mismatch, the larger the reflection.
The pressure amplitude reflection coefficient \((R_p)\) is the ratio of the incident \((P^i)\) and reflected \((P^r)\) pressure amplitudes at an interface. For a wave with an incident angle of \(\theta_i\) and a transmission angle of \(\theta_t\), the pressure amplitude reflection coefficient is given by Equation (2.15).

\[
R_p = \frac{P^r}{P^i} = \frac{Z_{0,2} \cos \theta_i - Z_{0,1} \cos \theta_t}{Z_{0,2} \cos \theta_i + Z_{0,1} \cos \theta_t}
\tag{2.15}
\]

As intensity is the square of pressure (2.11), it follows that the intensity reflection coefficient, \(R_I\), defined as the ratio of the time-averaged intensity magnitudes of the reflected and incident waves, is equal to \((R_p)^2\).

For the case of normal incidence (Figure 2-3), the pressure amplitude reflection and transmission coefficients \((R_p, T_p)\) and the intensity reflection and transmission coefficients \((R_I, T_I)\) are as follows:

\[
|R_p| = \frac{Z_2 - Z_1}{Z_2 + Z_1}
\tag{2.16}
\]

\[
|T_p| = 1 + |R_p| = 1 + \frac{Z_2 - Z_1}{Z_2 + Z_1} = \frac{2Z_2}{Z_2 + Z_1}
\tag{2.17}
\]

\[
R_I = \left[ \frac{Z_2 - Z_1}{Z_2 + Z_1} \right]^2
\tag{2.18}
\]

\[
T_I = 1 - R_I = 1 - \left[ \frac{Z_2 - Z_1}{Z_2 + Z_1} \right]^2 = \frac{4Z_1Z_2}{[Z_2 + Z_1]^2}
\tag{2.19}
\]
2.1.4 ATTENUATION

During the passage of an ideal plane acoustic wave though a viscoelastic medium, the wave will lose energy through conversion into other forms of energy (absorption), or the redirection of small fractions of the energy due to inhomogeneities in the material (scattering) [53]. The effects of both can be lumped together in the amplitude attenuation coefficient ($\alpha$). The total attenuative loss during travel through a medium is a function of the distance traveled and commonly expressed in dB/cm as follows, where $x$ is the distance traveled [50].

\[
\alpha_{\text{dB/cm}} = \frac{10}{x} \log \left( \frac{I_0}{I(x)} \right) \quad (2.20)
\]

\[
\alpha_{\text{dB/cm}} = \frac{20}{x} \log \left( \frac{\rho_0}{\rho(x)} \right) \quad (2.21)
\]

The attenuation of a material is also usually a function of frequency and temperature. In biologic materials, the frequency dependence exhibits a power-law relationship as given in Equation (2.22), with the coefficient of frequency
dependence $n$ generally ranging from 1 to 2 [50]. In almost all cases, increased frequency leads to higher attenuation.

$$\alpha = \alpha_0 \cdot f^n$$  \hspace{1cm} \text{(2.22)}$

2.2 Harnessing Acoustic Bioeffects for Therapy

2.2.1 Ultrasonic Bioeffects

As previously mentioned, an ultrasonic wave has the ability to transfer energy as it propagates, generally known as the acoustic power. The transfer of energy to tissues during the transit of an ultrasonic wave can cause a variety of effects within the tissues. These bioeffects can be classified into two major categories: thermal, and non-thermal (mechanical).

The conversion of acoustic energy to heat due to the absorption of an ultrasonic wave can cause heating within the tissues. Non-thermal effects, such as cavitation, acoustic streaming, or low intensity mechanical oscillation are also possible within tissues. Cavitation is the expansion, contraction, and bursting of micro-bubbles, often causing acoustic shock waves that disrupt or damage cells. Acoustic streaming is a localized flow of liquid around the bubbles, causing shear stresses at the cell surfaces, and varying the transport of ions and molecules into cells.

Historically, of most concern is typically the potential for intense heating (thermal) of the tissues, and acoustic cavitation (mechanical) within the tissues.
due to their potential to cause irreversible tissue damage. Ultrasonic bioeffects are a function of the properties of the insonated tissue, as well as the ultrasound exposure level. Higher levels of ultrasonic exposure generally correlate to a greater magnitude of bioeffects. For a more detailed treatment of ultrasonic bioeffects and their implications, the reader is directed to [54, 55].

2.2.2 MEASURES OF ACOUSTIC EXPOSURE

Understanding of the ultrasonic exposure is critical for establishing the potential for bioeffects within tissues. More precisely, ultrasonic bioeffects are a function of the spatial and temporal distribution of the pressure, and therefore intensity fields within the tissues. The critical parameter for pressure is the peak-negative (rarefaction) pressure $P_r$.

Several different methods exist for reporting the intensity of an acoustic beam. In modern medical ultrasound, intensity is commonly measured at the spatial point of the highest intensity along the beam axis, and is reported as either $I_{SPPA}$ (Intensity, Spatial Peak, Pulse-Average) or $I_{SPTA}$ (Intensity, Spatial Peak, Time-Average). $I_{SPPA}$ provides a measure of the average intensity of each pulse, while $I_{SPTA}$ provides a time-averaged intensity of all the delivered pulses.

Time-averaged and pulse-averaged intensities are related to each other mathematically by the duty factor. The smaller the duty factor or the shorter amount of time the ultrasound is on relative to off, the smaller the time-averaged intensity ($I_{SPTA}$) relative to the pulse-averaged intensity ($I_{SPPA}$). As the duty factor
approaches 100% and therefore becomes more CW-like, the $I_{SPTA}$ converges towards the $I_{SPPA}$.

$$I_{SPTA} = DF \cdot I_{SPPA}$$  \hspace{1cm} (2.23)

Broadly, $I_{SPTA}$ is generally an indicator of ultrasound induced thermal effects, while $I_{SPPA}$ is a good indicator of mechanical bioeffects such as cavitation [56].

While current voluntary best-practice standards [57, 58] encourage reporting acoustic intensity in terms of $I_{SPTA}$ and $I_{SPPA}$, historically other measures such as maximum intensity, output beam intensity, and spatial-average intensity have been used interchangeably and without specification in the literature. Without knowledge of specifically which measure of intensity was used, it can be difficult or impossible to compare historical to current values. When referring to intensity values reported in the literature, this present work lists intensity values as they appear in the original report.

### 2.2.3 Acoustic Exposure and Bioeffect Regulatory Standards

As the scientific understanding of the biological effect ultrasonic fields have on body structures has grown, the regulatory standard used to judge what is a “safe” intensity and pressure dose of ultrasound has evolved [59, 60].

The current measurement standards are a joint effort between the AIUM, NEMA, and FDA and most recently published in 1998 [61], and known colloquially as the output display standard (ODS). In an effort to more
realistically reflect the intensity measured within the tissue and account for the effects of tissue attenuation, the intensity values are reported as an in-situ, or derated value (denoted by a subscript “0.3”), which in the ODS paradigm assumes an acoustic attenuation factor of 0.3 dB/cm-MHz [62]. The limits on ultrasound output adopted by the FDA (“Track 3 Limits”) are listed in Table 2-1.

\[
I_{SPTA,3}(z) = I_{SPTA}(z) \cdot \exp(-0.069 f_c z)
\]  

(2.24)

<table>
<thead>
<tr>
<th>Imaging Application</th>
<th>ISPTA,3 (mW/cm^2)</th>
<th>ISPPA,3 (W/cm^2)</th>
<th>MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal</td>
<td>720</td>
<td>190</td>
<td>1.9</td>
</tr>
<tr>
<td>Cardiac</td>
<td>720</td>
<td>190</td>
<td>1.9</td>
</tr>
<tr>
<td>Peripheral Vascular</td>
<td>720</td>
<td>190</td>
<td>1.9</td>
</tr>
<tr>
<td>Ophthalmic</td>
<td>50</td>
<td>-</td>
<td>0.23</td>
</tr>
</tbody>
</table>

The current FDA limits include a unitless measure known as the mechanical index (MI), which is intended to be a measure of the risk of inertial cavitation [63]. The MI can be calculated as:

\[
MI = \frac{\rho_{c,3} \sqrt{\text{MHz}}}{\sqrt{f_c} \text{ MPa}}
\]  

(2.25)

It is worth noting that the FDA limits presented in Table 2-1 are for 510(k) regulatory submissions aiming to show substantial equivalence to ultrasound systems that predate the 1976 Medical Device Amendment to the Food, Drug, and Cosmetic Act. These values are based on historical instrumentation data and observations of safety, and do not directly account for the actual mechanisms by which biological effects are produced [64]. While values under this threshold can
be considered to be conservatively safe, values above are not necessarily inherently dangerous. The actual values produced by modern ultrasound equipment can in many cases be substantially higher as shown in Table 2-2. Indeed, measured values for clinical Pulse Doppler imaging have been reported as high as $I_{	ext{SPTA}} = 9,080 \, \text{mW/cm}^2$ and $P_r = 3.5 \, \text{MPa}$ [65].

<table>
<thead>
<tr>
<th>Pressure (MPa)</th>
<th>Diagnostic</th>
<th>Pulse Doppler</th>
<th>Therapy</th>
<th>Lithotripter</th>
<th>HIFU [53]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 8</td>
<td>1 - 5</td>
<td>0.1 - 0.7</td>
<td>10 - 100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ISPTA (mW/cm²)</td>
<td>1 - 30</td>
<td>300 - 5000</td>
<td>20 - 10000</td>
<td>10 - 100</td>
<td>(1 - 10) x 10^6</td>
</tr>
<tr>
<td>Total Power</td>
<td>1 - 100</td>
<td>20 - 250</td>
<td>100 - 15000</td>
<td>1 - 50</td>
<td></td>
</tr>
<tr>
<td>Frequency (MHz)</td>
<td>2 - 10</td>
<td>2 - 10</td>
<td>0.75 - 3.0</td>
<td>0.2 - 0.9</td>
<td>1 - 10</td>
</tr>
<tr>
<td>Pulse Duration (us)</td>
<td>2 - 10</td>
<td>0.3 - 10</td>
<td>cw/ms</td>
<td>0.2 - 10</td>
<td></td>
</tr>
</tbody>
</table>

### 2.2.4 Applications of Acoustic Bioeffects: Therapeutic Ultrasound

Although the imaging and monitoring applications of ultrasound are best known, there are also many applications of ultrasound that aim to harness the bioeffects that result from an acoustic wave’s transit through tissues for therapy. For an excellent overview of the current state of the art of therapeutic ultrasound, please see [67]. For a more detailed review of the neural applications of focused ultrasound, the reader is directed to [68] by this author.

Therapeutic ultrasound is used to treat a specific disease or condition in the body, most commonly by heating tissues via absorption of focused acoustic waves. Because tissues in the body have different absorption coefficients, it is possible to selectively heat certain tissues while leaving others unaffected. Many
procedures also depend on the non-thermal effects of therapeutic ultrasound. Both thermal and non-thermal effects are often desired, although in many cases they cannot be differentiated. Frequencies between 700 kHz and 3.3 MHz are most common in therapeutic ultrasound, with maximum energy absorption occurring at depths between 2 and 5 cm [69].

Therapeutic ultrasound can generally be categorized as either low intensity or high intensity. Low intensity therapeutic ultrasound is performed most frequently for physical therapy of conditions such as osteoarthritis, carpal tunnel syndrome, and tennis elbow using either unfocused or focused transducers. Tissues are usually warmed to ~38°C for 10 min at intensities in the 1 W/cm² range [70]. Fracture healing can also be accelerated using low intensity therapeutic ultrasound, typically in the 50 mW/cm² range [71-73]. Another low intensity application is hyperthermia, in which tumors are heated during radiation therapy to ~43°C for 1-2 hours at roughly 10 W/cm² [70].

Common uses of high intensity ultrasound include HIFU and shockwave lithotripsy of kidney stones into tissues and generally use focused transducers or arrays. During a HIFU procedure, tissue is typically heated to temperatures higher than 70°C for 1-3 seconds at intensities in the 1 kW/cm² range [70], effectively burning and ablating tissues.

Recently, other high intensity yet non-thermal potentially therapeutic effects of ultrasound have generated excitement in various medical fields. It has recently been discovered that focused ultrasound could be used to produce temporary and reversible disruption of the blood brain barrier disruption for
drug delivery [74-79]. Ultrasound energy has also been shown to enhance effects of thrombolytic agents, transdermal drug delivery, anticancer drugs, and gene therapies [80-83]. In addition to the pressure oscillation experienced by cells and tissue caused by ultrasound, secondary effects such as heat and cavitation can induce strong physical and biological effects in tissues as well [84].

### 2.3 Focused Ultrasound Surgery of the Brain

The field of focused ultrasound ablation is the best known application of therapeutic ultrasound in the brain, and the researchers in this field have led the development of noninvasive transcranial focused ultrasound technologies. Focused ultrasound ablation is generally associated with HIFU, due to the high intensities required to ablate tissues. HIFU was suggested many years ago for use as a tool to target and ablate subcutaneous tissue volumes by localized heating, specifically as a potential therapy tool for deep-seated soft tissue tumors. The application of HIFU technology was first described for various applications in the 1950’s and 1960’s [85-89], through work by Fry et al. to develop four-transducer systems that produced lesions in animal models. HIFU is now FDA-approved for the heating and ablation of uterine fibroids in the U.S.A., and is used for the treatment of prostate and bone cancers in several countries. After decades of research, the technology has evolved from a topic in research laboratories to one that is undergoing clinical trials [81].

The mechanism by which HIFU systems can ablate tissues is most commonly attributed to thermal effects [90]. The absorption of focused
ultrasound energies is used to raise the temperature of a selected tissue volume to over 55 °C, a temperature threshold identified with leading to coagulative necrosis and immediate cell death [90-92].

The primary impediment to use of ultrasound in the brain, whether for therapeutic or imaging purposes, has been the cranium. Early attempts at producing lesions in the brain through the intact skull bone were unsuccessful [93, 94]. Propagation through the cranium results in significant distortion and attenuation of the acoustic beam, thereby limiting accuracy of the procedures and also increasing potential health risks to the patient.

The bulk of prior ultrasound research efforts have therefore been performed invasively through a window in the skull following craniotomy. Parkinson’s patients were among the first to be treated with HIFU performed through a craniotomy [95]. Although the treatment helped to ameliorate some Parkinson’s symptoms, this research was not further pursued because of the requirement to remove a section of the skull and difficulties in obtaining accurate targeting. Commercial neurosurgical HIFU systems now exist, such as the ExAblate (Insightec, Haifa, Israel), that combine the visualization of soft tissues, temperature sensitivity, and guidance of MRI with the use of phased-array focused transducers to provide compensation for ultrasonic distortion through the skull [96].

Recent research has now demonstrated the clinical feasibility of transcranial MRI-guided focused ultrasound surgery (TcMRgFUS or MRgFUS) using the ExAblate system and others [97]. In 2010, it was reported that
therapeutic ultrasound could be non-invasively focused effectively through an intact skull to target a glioblastoma. The feasibility of transcranial targeting with non-invasive focused ultrasound ablation was also demonstrated, although clinical outcomes were mixed due to insufficient power [98]. More recently, an ultrasonic thalamotomy through an intact human skull has been demonstrated [99, 100]. Much work is still required, however, both in the further development of high power systems for effective ablation at different tissue depths and also in defining safety margins to deliver energy selectively without overheating the skull.

2.4 Ultrasonic Neuromodulation

In 1950, Fry et al. reported the first effects of ultrasound targeted directly on neural tissues, demonstrating the temporary suppression of nervous conduction through the ventral nerve cords of a crayfish [101]. Later demonstrating neuromodulation in the intact brain of a cat [102], Fry’s early work signaled the ability of targeted focused ultrasound to reversibly modulate both the peripheral, and central nervous systems. While the ultrasonic exposure levels of these early works [48, 87, 89, 101] were neither well characterized nor understood, they are considered to be high by modern standards [45].

These initial efforts have been expanded upon with a variety of studies on different neural structures conducted at varying acoustic intensities and stimulation durations. Initial trials were generally conducted at higher acoustic
intensity levels with the effects judged to be thermal in nature. However, current efforts are focused on stimulating using low intensity, non-thermal pulses.

While the specific mechanism underlying the neuromodulatory effects of focused ultrasound has not yet been fully elucidated, the current body of evidence suggests that the application of ultrasound, especially in the case of non-thermal low intensity stimulation, achieves its effects through mechanical interaction with neural tissues [103]. Prior studies indicate that many voltage-gated ion channels show mechanosensitive properties that render their gating kinetics sensitive to transient changes in lipid bilayer tension [104]. Systematic studies of the underlying molecular mechanisms and the role that specific acoustic pulse parameters play in effecting neuromodulation are currently underway in this author’s lab [105].

2.4.1 Ultrasonic Neuromodulation in the Periphery

The potential for ultrasound to inhibit peripheral nerve conduction with the therapeutic goal of pain management [106] was recognized early on, with Young and Henneman demonstrating selective effects on cat saphenous nerve bundles soon after [107]. Some of the earliest studies on focused ultrasound stimulation were conducted by Russian researchers in the 70’s [108-114] who observed modulation of neuronal firing in mammals and human subjects.

More recently, a permanent nerve block on a rabbit sciatic nerve in vivo was demonstrated using high intensity (>1.5 kW/cm²), 30 second continuous sonication [115]. These studies, however, were confounded by temperature
elevations that are accompanied by the delivery of high-intensity ultrasound. Since excessive heat and pressure associated with high-intensity ultrasound may damage the brain tissue, lower-energy pulsed application of focused ultrasound sonication has been suggested as an alternative. Modulation (both up and down regulation) of bullfrog sciatic nerve conduction has also been demonstrated with low energy sonication [116, 117].

2.4.2 ULTRASOUND-INDUCED EX VIVO CNS NEUROMODULATION

Studies with ex vivo experimental set-ups have provided supporting evidence that focused ultrasound may activate and modulate CNS neuronal activity [118, 119]. Tyler et al. reported that low-intensity (pulse average intensity < 3 W/cm²) low-frequency (440 and 670 kHz) ultrasound pulses can induce reproducible excitation of neuronal circuits in ex vivo mouse hippocampal neurons [120]. In their experiment, short bursts (25 μs – 75 ms) of low-intensity ultrasound were delivered every 100 ms for 5 minutes, inducing activation of the voltage-gated sodium and calcium channels of the hippocampal neurons. In addition to modifying calcium signaling and neuronal electrical activity, sonication altered central synaptic transmission mediated by synaptic vesicle exocytosis. These experiments demonstrated the ability of low intensity, low frequency ultrasound to remotely modulate brain circuit activity in ev vivo preparations.
2.4.3 Low and Lower Intensity Ultrasound-Induced In Vivo CNS Neuromodulation

There is a growing body of evidence that ultrasound at low, and lower intensities can be used for CNS neurostimulation in intact brains. Drawing from the initial findings in the laboratories of Tyler [120] and Yoo [121], recent efforts by a number of investigators have demonstrated the ability to stimulate in vivo brain circuits in a variety of small animal models. A large range of pulse parameters, intensities, stimulation durations, and targeting paradigms have been reported. Key reports are summarized below using the pulse and intensity terminology as detailed by the original investigator. For groups with multiple pulsing schemes, each pulse reported as critical is assigned a unique identifier for reference, in the form of Investigator_Date_Pulse#.

In the intact mouse, Tyler and colleagues were able to demonstrate general motoric activity with motor cortex stimulation as well as evoke synchronized oscillations by deep targeting of the hippocampus using unfocused transducers [122, 123]. Local field potentials (LFP) and multiunit activity (MUA) were recorded in the primary motor cortex with pulsed ultrasonic stimulation (350 kHz, 80 c/p, 1.5 kHz PRF, 100 pulses – Tyler_2010_1) at an intensity of 36.20 mW/cm² ISPTA. Bilateral targeting of the primary motor cortex triggered tail twitches and EMG activity in the lumbosacrocaudalis dorsalis lateralis muscle (500 khz, 100 c/p, 1.5 kHz PRF, 80 pulses, 64.53 mW/cm² ISPTA – Tyler_2010_2) and the contralateral triceps brachii muscle (350 khz, 80 c/p, 2.5 kHz PRF, 150
pulses, 42.90 mW/cm² $I_{SPTA}$ – Tyler_2010_3). Lower frequencies were found to produce more robust EMG responses, and no measureable BBB disruption was observed during the course of the study. When transmitted to the hippocampus, an LFP was reliably triggered with a stimulation of 84.32 mW/cm² $I_{SPTA}$ (250 kHz, 40 c/p, 2 khz PRF, 650 pulses – Tyler_2010_4) and produced a significant increase in spike frequency.

Small animal *in vivo* lower intensity focused ultrasound has also been demonstrated in the laboratory of Yoo, using large geometrically focused (6-10 cm diameter, 7-8 cm radius of curvature) air-backed transducers in multiple animal models.

In the rabbit model, the group has demonstrated the ability to induce motoric activity with focused ultrasonic stimulation (690 khz, 50 ms PD, PRF 10 Hz, duration > 1s), and the ability to both activate and suppress changes in blood oxygenation level dependent (BOLD) fMRI signals [124]. Using the above pulse parameters, BOLD stimulation was observed at an acoustic intensity (AI) of 3.3 W/cm² $I_{SPTA}$ and 6.4 W/cm² $I_{SPPA}$ (Yoo_2011_1) with a higher intensity (6.3 W/cm² $I_{SPTA}$ and 12.6 W/cm² $I_{SPPA}$ – Yoo_2011_2) causing visible muscle motion and measurable EMG activity.

The same study demonstrated the ability to reversibly suppress the response of the visual cortex to light stimulation without causing damage to the blood brain barrier or brain tissue [124]. The p30 component of the visual evoked potential (VEP) was also decreased for approximately 7 minutes when stimulated for 7-8 seconds at a fundamental frequency of 690 kHz using shorter pulses (0.5
ms PD, 200 Hz PRF) at acoustic intensities of 3.3 and W/cm$^2$ ISPPA (Yoo_2011_3). Using similar parameters but at an acoustic intensity of 160 mW/cm$^2$ ISPTA and 3.3 W/cm$^2$ ISPPA (Yoo_2011_4), cortical activity in the visual area was suppressed.

Using similar pulse parameters, but a higher intensity (650 kHz, 0.5 ms PD, 100 Hz PRF, AI = 300 mW/cm$^2$ ISPTA and 6 W/cm$^2$ ISPPA – Yoo_2011B_1), Yoo was able to demonstrate that pulsed ultrasound focused to the thalamus significantly reduces the time to emergence from intraperitoneal ketamine/xylazine (80:10 mg/kg) anesthesia in rats [125].

In further experiments in the rat model, Yoo demonstrated the ability to stimulate the abducens nerve and produce eyeball movement using pulses of 350 kHz ultrasound (0.36 ms PD, 1.5 kHz PRF, 200 ms duration) at an AI of 4.6 W/cm$^2$ ISPTA and 8.6 W/cm$^2$ ISPPA (Yoo_2012_1) [126]. Notably, no effect was observed using the same pulse parameters at a fundamental frequency of 650 kHz tested over an intensity range of 0.5 to 20 W/cm$^2$ ISPPA. A separate study at a fundamental frequency of 350 kHz demonstrated the ability to elicit tail movements in the rat (0.5 ms PD, 1 kHz PRF, 300 ms duration, repeated every 2 s, AI of 4.5 W/cm$^2$ ISPTA and 9.1 W/cm$^2$ ISPPA - Yoo_2012B_1) [127].

King and colleagues have confirmed that ultrasound can be used to induce in vivo ultrasonic transcranial neurostimulation of the mouse somatomotor response, using unfocused continuous wave ultrasonic stimulation at a fundamental frequency of 500 kHz [128]. A reliable EMG response was observed at an acoustic intensity of 16.8 W/cm$^2$ ISPPA applied for 80 ms. They report that
continuous wave sonication is as or more effective than pulsed delivery, and that
the probability of sonication success increases with intensity and stimulation
duration. The EMG response was found to be all an all-or-nothing effect, and
corresponds with stimulus onset. A strong relationship between anesthesia level
and stimulus was also observed.

Based on the protocol set forth by Tyler [123], Younan et al. have recently
reported the ability to produce a transient ultrasound-evoked motor responses in
over 60% of experimental sonications in the rat at a 320 kHz fundamental
frequency, using a focused pulse of 230 µs PD, 2 kHz PRF, and a total duration of
250 ms repeated every 10 seconds, corresponding to an AI of 7.5 W/cm² I_{SPPA}
[129]. They have also reported observing a pressure and intensity threshold
under which no stimulation occurs, as well as confirmed the necessity of higher
ultrasound levels to induce stimulation under strong levels of anesthesia.

The first potential application of low intensity focused ultrasound for the
treatment of neural disorders has recently been demonstrated by Yoo et al. An in
vivo study in a rodent model of pentylenetetrazol (PTZ) induced epileptic
seizures has demonstrated selective modulation of regional neural activity in
brain by the suppression of ictal activity [43, 130]. The PTZ induced epileptic rats
treated with low intensity focused ultrasound (690 kHz, 0.5 ms PD, 100 Hz PRF,
P_r = 0.27 MPa, AI = 130 mW/cm² I_{SPTA} and 2.6 W/cm² I_{SPPA}) demonstrated less
severe epileptic behavior based on a Racine score. Tyler has also demonstrated
the ability to disrupt kainic acid (KA) induced seizures in the mouse model using
both continuous wave and repeated applications of pulsed ultrasound [123].
Taken together, these reports would seem to indicate that ultrasound at intensities below the thresholds normally monitored for bioactive effect is capable of inducing neuromodulation. However, these early research reports differ significantly in test parameters, methodology, and outcome [131]. As a result, many of the critical factors involved in ultrasonic neuromodulation still remain unknown. These challenges are discussed in Chapter Three.
Chapter 3: Critical Barriers to Effective Transcranial Ultrasonic Neuromodulation

While recent advances in the published literature continue to provide additional support for the concept of low-intensity ultrasonic neuromodulation, several critical barriers exist to translating these theories and techniques to the clinic as a non-invasive transcranial therapeutic or neural research tool. These roadblocks exist both in our current theoretical understanding of the underlying mechanism(s) involved in the interaction of ultrasound energy with neural structures and circuitry, and in the practical aspects of using ultrasound for non-invasive transcranial ultrasonic neuromodulation.

This chapter discusses several of the gaps in knowledge of our current understanding of low-intensity ultrasonic neuromodulation based on the current state of the art. Progress must be made in improving our understanding of these areas to help push the field forward. With new research reports in the literature, many of these unknowns are being slowly filled in. The experimental work described in this thesis specifically aims to address and provide clarity on many of these unknown elements.
3.1 Identification of Critical Ultrasonic Parameters

Currently, there is no clear relationship between the character of an ultrasonic wave directed to a neural structure, and the likelihood and nature of a resulting neuromodulatory effect. As defined in Section 2.1.1, the parameters that are necessary to fully define an ultrasonic sonication pulse are the center frequency, pressure amplitude, number of acoustic cycles in the pulse, and pulse repetition rate. Varying these parameters alters the pressure field experienced within the tissues, as well as the temporal energy deposition (intensity) at, and surrounding the target.

Investigators have previously managed to demonstrate successful sonication using both continuous wave and pulsed ultrasound, as well as both low- and high- duty cycle pulsing schemes over widely varying stimulation durations. Furthermore, these effects have been observed over a wide range of pressures, intensities, and center frequencies.

The potential sample space for ultrasonic sonication parameters is very broad. A better understanding of the role each of these following parameter aspects plays in ultrasonic neuromodulation and the relative criticality of each to the overall effect is necessary to advance the field. The potential contribution of various aspects of the ultrasound parameters on the neuromodulatory outcome are discussed below.
3.1.1 Frequency

A wide range of sonication center frequencies have been demonstrated to be effective in a range of models. While generally below 1 MHz due to skull attenuation effects, different groups have had success with a variety of frequencies, with most experimentation bounded between 250 kHz (Tyler et al.) and 690 kHz (Yoo et al). The sonication center frequency is an important parameter which has a large interplay with the physics of the actual sonication wave:

- **Acoustic Focusing Limit**: As a general rule, the lower the frequency, the wider the diameter of the acoustic focus due to wavelength diffraction limits. Conversely, the higher the frequency, the tighter the possible focus spots.

- **Depth of Penetration**: Tissue attenuation and scattering is also frequency dependent. The lower the frequency, the deeper ultrasonic wave is able to penetrate into the tissue depth of tissue penetration.

- **Energy Transfer over Time**: For a constant stimulation time, more energy is transferred at a higher frequency than at a lower, due to a larger number of cycles fitting into a given time at a higher frequency.

- **Tissue Heating**: The energy lost to attenuation and scattering manifests itself as tissue heating. Therefore, stimulation center frequency impacts local tissue heating and bioeffects.
• **Resonant Effects**: Neural structures such as protein membranes, channels, or neurotransmitter vesicles are known to have certain resonant behaviors based on their chemical structure as well as molecular interactions with their fluidic and molecular microenvironment [132-135]. Ultrasound has further been postulated to act on these viscoelastic properties [103]. Conceivably, these interactions may be frequency dependent, and acoustic center frequency may have an important interplay with the inherent resonance of neural structures.

While recent experimental evidence in the literature [44, 126, 128] has seemed to indicate that lower stimulation center frequencies are more effective neuromodulators than higher frequencies at stimulating a robust motor response in a small animal model, observation has not yet been expanded to other physiologic outcomes, nor a theoretical basis for this observation established.

### 3.1.2 Intensity, Pressure, Sonication Scheme, and Duration

Many different sonication schemes have been attempted and documented in the literature. These can roughly be broken down into high and low duty cycle pulses, continuous-wave, and short and long total stimulation durations. Pulse durations reported in the literature range from 0.05 s (Tyler et al.) to 27 s (Yoo et al.)
As described in Section 2.1.2, a wide variety of inter-related, yet distinct parameters can be used to quantify the energy-output of the ultrasonic sonication. Most commonly used are the peak rarefraction pressure \((P_r)\), the spatial-peak, time-averaged intensity \((I_{SPTA})\), and the spatial peak, pulse averaged intensity \((I_{SPPA})\). Ultrasonic pulses can be constructed that maximize one of these parameters at the expense of the other.

It has not yet been established which of these three parameters, if any, is the critical parameter at building a neuromodulatory ultrasound pulse. Tsui et al., in a model of ultrasonic neuromodulation of peripheral nerve action potential amplitude and velocity, has found that shorter duration pulses of ultrasound seem to activate, while long pulses seem to inhibit \([117]\). However, an opposite relationship was shown by Yoo et al. in the rabbit model \([124]\). This confusion makes it difficult to intelligently construct ultrasonic pulses for neuromodulation.

The insonation pattern has several potential effects. For a pulsed sonication scheme, the lower the duty cycle (smaller \(I_{SPTA}\) relative to \(I_{SPPA}\)), the more heat can be carried away through convection and conduction by blood. The longer the duration, the more total heat energy deposited. As the duty cycle approaches unity, behavior of the pulse tends towards that of a continuous wave.

In addition, one can calculate an effective total ultrasound dose for a given sonication pattern. One question that has not yet effectively been explored is if it is this total dose that is important, or the way in which that dosage was reached.

A physical analogy to this is the filling of an empty pool. If the end goal is a certain volume of water, the pool can be filled at high pressure for short bursts of
time, low pressure bursts for longer periods of time, or a continuous trickle. The sum volume is the same in each case, but the mechanical effects on the pool, piping, and required tubing experienced with each filling scheme is different.

3.2 Neuromodulatory Specificity

Given a set of ultrasonic parameters which is capable of producing neuromodulation, the physiologic domain over which those parameters work is still unclear. Differences in targeting methodology, target specificity, and even the fundamental meaning of targeted stimulation are evident in the literature. This work aims to explore some of these considerations.

It is critical to have an understanding of the extent of neuromodulation experienced. Understanding exactly what is being modulated, and where, is highly important for advancing the state of the art. Two primary paradigms for targeting claims are primarily discussed in the literature based on the level of claimed target specificity, roughly based on assumptions of the stimulus acting evenly on all neural targets, or being particularly tuned to certain circuits. Many claims of targeting might ultimately be hollow as other structures and circuits not actively being monitored might be concurrently stimulated.

3.2.1 TARGETING OF DISCRETE STRUCTURES AND CIRCUITS

If it is assumed that a given set of ultrasonic parameters stimulate all brain structures and regions evenly, some sort of control over radiation pattern of a
stimulatory sonication is necessary. In the lower-intensity neurostimulatory literature, such control has taken the form of spherical focusing (Yoo et al), or as columnar focusing (Tyler et al).

Spherical focusing assumes that the energy outside the area of interest is sub-threshold, and therefore non-stimulating. Columnar focusing assumes a constant acoustic energy throughout the column, but without attendant lateral stimulation. In columnar focusing, it is conceivable that structures both above and below the target are being activated based on the trajectory of the beam. For example, a beam targeting the deep neural structures such as the thalamus would pass through more superficial brain structures with the same intensity.

It is also worth noting that in many experimental animal models, even mechanical focusing has limitations. Especially in small animal models, the physical width of the ultrasonic beam may be on the order of the size of the brain, and even when focused, may serve to evenly sonicate a percentage of the brain.

![Figure 3-1: Schematic illustration of three different focusing paradigms. Left: Columnar focusing, where the energy is assumed to be focused through a column. Center: Spherical focusing, where a discrete roughly cigar-shaped focus point is assumed to exist in space. Right: No focusing, where the entire brain is sonicated evenly.](image-url)
volume.

Alternatively, it is possible that the ultrasonic parameters (intensity, pulse scheme) necessary to achieve a neuromodulatory outcome are specific to the neural circuit under investigation. Many researchers have chosen not to use any sort of focusing paradigm in their work. This has not yet been rigorously demonstrated in the literature.

3.2.2 *Interspecies Variability*

At this time, it is unclear if a set of sonication parameters that yield a neuromodulatory outcome in a given animal or circuit holds valid for other species. No experiment uniformly targeting the same neural structure and physiologic endpoint using the same transmission methods and parameters has been attempted.

3.3 *Experimental Barriers*

In addition to the open scientific issues facing ultrasonic neurostimulation, several challenges with experimental design exist which further confound investigation of neuromodulatory effects.

3.3.1 *Role of Anesthesia*

The effect of anesthesia, which effectively serves to blunt neural responses to stimuli, would logically serve as a confounding factor in *in vitro* studies of the
neurological effect of ultrasounds, and has long been identified as a point of concern in the field. While the effect of anesthesia on ultrasonic neuromodulation was first experimentally described by Yoo [125] and was recently confirmed by King [128], anesthesia remains a vital and ethically necessary component of most animal protocols for ultrasonic experimentation.

A variety of different approaches, including both interperitoneal bolus injections (ketamine/xylazine, urethane) and constant induction/inhalation (isofluorane/oxygen) have been used in the literature. As a result, the exact conditions for an experiment must be taken into account, and it must be understood that the anesthesia plane, especially in cases of bolus injection may vary over time. Ultimately, more detailed investigation of the effect of these different anesthesia methods, as well as research into conscious-animal preparations must be undertaken. Experiments subsequently described in this work make use of inhaled isoflurane, with effort taken to maintain the subject at a low, but constant anesthesia plane in an attempt to overcome many of the shortcomings in anesthesia protocols in the literature.

3.3.2 Transcranial Stimulation

The primary impediment to the use of ultrasound in the human brain, whether for therapeutic or imaging purposes, has been the cranium. As mentioned earlier, the cranium significantly impacts the transmission of ultrasound between the transducer and the desired region of the brain.
First, the high density and speed of sound of the cranium results in large acoustic impedance mismatches both at the skin/cranium and cranium/dura interfaces. One consequence is that these mismatches can result in refraction of the beam that modifies the position and shape of the beam focus. The mismatches also cause large reflections of the acoustic beam (> 40% of the energy at each interface) that both reduce the energy transmitted into the brain and cause reverberations that clutter the acoustic signal.

When using continuous wave energy, reverberations are especially problematic, since the energy is not given time to dissipate. Reverberations are not only problematic at the beam’s entry point into the brain, but by all internal surfaces of the cranium, which effectively form an echo chamber. As the ultrasound beam passes through the focal zone, the beam continues and reflects off of the far side of the cranium, causing further interference at the desired focal zone.

Second, the heterogeneity, variable topography, and nonuniform thickness of the cranium refract the acoustic beam as it passes through the skull, making it difficult to precisely target specific regions while also broadening the beam focus. Third, the cranium can cause significant attenuation of the acoustic beam. Attenuation in cortical bone is approximately 6.9 dB/cm MHz, and results in a further reduction of energy traveling into the brain through both absorption and scattering [136]. However, of greater concern than the loss of energy is the absorptive conversion of acoustic energy into thermal energy, which can lead to heating of the skull and underlying tissues in the brain, especially at high
intensities. Finally, acoustic coupling of the ultrasound transducer using gels or liquids is difficult, due both to gravity and the presence of hair on the head.

Together, these challenges have created significant barriers to the adoption of ultrasound for noninvasive transcranial applications. The effects of refraction can be mitigated by using one of several insonation windows, or regions of the skull with thinner or flatter walls, such as the temporal region, the back of the head, below the jaw, or the eyes.

3.3.3 LACK OF STANDARDIZED REPORTING SCHEME

One of the primary impediments and confounding factors to further exploration in the field is the lack of a standardized set of measurements and parameters for reporting ultrasonic parameters. With so many different parameters to report and so many ways to report them, many investigators have used their own schemes to describe their work, making meaningful comparison of acoustic intensities difficult, and complicating efforts at replication. A standardized set of parameters should be reported to ensure that work can be critically analyzed [57, 58]. This work addresses these issues by proposing a standard set of terms to be used to describe the components of a sonication pulse.
Chapter 4: Transducer Characterization

Being able to quantify and control the output of an ultrasonic probe is important for being able to perform controlled trials of targeted ultrasonic neuromodulation. A number of custom built transducers and systems were designed, fabricated, and tested as part of this work. While the basic theory, physics, and operation of ultrasonic transducers has long been understood [66, 137, 138], many of the specific practical details involved in transducer design, construction, and testing are not openly published and freely available.

Furthermore, accurately quantifying the acoustic output of a transducer is not straightforward, and acoustic measurements can be challenging without the proper equipment and knowhow. A variety of systems and protocols exist to take these measurements, but implementations can vary greatly. This chapter describes the characterization and verification of the ultrasonic transducers and systems which will be used as the foundation for the experimental efforts to be described in subsequent chapters, as well as the measurement protocol used to make that determination.

4.1 Acoustic Measurement

The goal of acoustic measurement is to measure the spatial and temporal distribution of acoustic output. There are four primary high-level measures of acoustic output:
1. **Acoustic Pressure** – Relates the degree of compression/rarefraction a given material undergoes when transited by a sound wave, and is a measure of the forces acting on the material.

2. **Acoustic Power** – A measure of the energy transfer of an ultrasonic wave as it propagates, and a function of the total beam.

3. **Acoustic Intensity** – The power passing through a unit area in a particular direction, usually specified for a particular point in the field.

4. **Acoustic Focus** – The spatial distribution of the above.

Practically, acoustic measurement is performed to quantify the acoustic output field of transducers to determine the spatial contour of the maxima of the acoustic field, generally known as the focus of the transducer. The width of this contour measured parallel to the face of the transducer and perpendicular to the direction of propagation will vary as a function of the axial distance from the transducer. If reported singularly, the focus width is usually measured across the plane intersecting the point of maximum axial intensity. For the purposes of this work, acoustic radiation and propagation is assumed to be linear, in that the beam shape and spectral content of the pressure waveform are independent of the applied source power \[139\]. As a consequence, the peak positive and peak negative pressures for a sinusoidal waveform are therefore equal.

A number of different broad families of techniques exist to measure and quantify acoustic output, which can be roughly segmented into techniques based on measurements of acoustic pressure, acoustic displacement, and acoustic
radiation force. For a more detailed treatment of various ultrasound test methods, the reader is directed to [53] and Chapter 13 in [66]. In this work, measurements taken using a piezoelectric hydrophone scanned through an acoustic field in a water tank are used as the basis for acoustic output characterization.

**4.1.1 NEED FOR SPATIAL CHARACTERIZATION**

For generic planar piston source of radius \( a \) undergoing sinusoidal excitation at frequency \( c/\lambda \), the resulting field distribution is complex, with many maxima and minima in the near field zone, and a diverging beam width in the far field. In the near field, a number of different minima and maxima can be found, given by:

\[
Z_{\text{max}} = \frac{4a^2 - \lambda^2 (2m + 1)^2}{4\lambda(2m + 1)} \quad (4.1)
\]

\[
Z_{\text{min}} = \frac{a^2 - \lambda^2 m^2}{2m\lambda} \quad (4.2)
\]

With the transition from near-field to far field occurring at:

\[
Z_o = \frac{4a^2 - \lambda^2}{4\lambda} \simeq \frac{a^2}{\lambda} \quad [\text{For } \lambda \gg a] \quad (4.3)
\]

For a flat planar source, the beam is roughly collimated in the near field, but diverges in the far field. For a focused source, the mathematical focus width, given a focal length \( F \) is given by:

\[
\text{Focus, FWHM} = 0.71\lambda \frac{F}{a} = 0.71\frac{cF}{fa} \quad (4.4)
\]
4.1.2 Acoustic Measurement Protocol

A measurement protocol based on scanning a piezoelectric hydrophone through an acoustic field in water was developed as the basis for characterizing the acoustic output of a transducer. Specifically, the acoustic test methods used to quantify the output of an acoustic beam in this present work are based those described in [53] and are adapted for use with the Sonora Acoustic Measurement System (Acertara Acoustic Laboratories, Longmont, CO), an acoustic scanning system designed for performing FDA regulatory submission measurements (Figure 4-1).

Briefly, the protocol relies on translating a piezoelectric hydrophone through the acoustic field of a transducer transmitting into a large water tank and spatially sampling the pressure field with the hydrophone. These measurements of pressure are turned into instantaneous intensities via Equation (2.11) and temporally integrated over the entire pulse to yield the Pulse Intensity Integral (PII), given by Equation (4.5).

\[ PII = \int \frac{p^2}{\rho c} dt \]  \hspace{1cm} (4.5)

From the PII, \( I_{\text{SPTA}} \) is the product of PII and PRF, while \( I_{\text{SPPA}} \) is the PII divided by the PD, where the PD is specifically defined as 1.25 times the time between 90% of the maximum PII, and 10% of the maximum PII.

\[ I_{\text{SPTA}} = PII \cdot PRF \]  \hspace{1cm} (4.6)
\[ I_{\text{SPPA}} = \frac{PII}{PD} \]  \hspace{1cm} (4.7)
\[
PD = 1.25 \cdot (t_{0.9PII} - t_{0.1PII})
\tag{4.8}
\]

The acoustic measurement system first scans the hydrophone through the field to determine the correct axial orientation of the transducer under test, and then performs axial and radial scans to spatially map the field. Custom software written in MATLAB (MathWorks, Natick, MA) translates individual axial 2D beam slice information into a full 3D spatial intensity profile of the beam.

All spatial acoustic dimensions reported in this work are derived from measurements of maximum rated intensity derived from peak negative rarefaction pressure, and all focus dimensions as the full-width half maximum (FWHM, -3 dB intensity, -6 dB pressure) contour of the acoustic field. Specifically, the focal point is considered to be the axial location of maximum rated intensity, the focus width to be the x- and y-axis FWHM diameter in terms of intensity, and the focal length to be the axial FWHM intensity from the axial location of maximum intensity.

The developed acoustic output measurement protocol was validated by comparing beam profiles generated using the protocol against scans taken from first principles using a home-built manual scanning setup, and in comparison to a commercial pulse-echo beam scan of a 0.5” steel bead.

The developed test protocol used in this work involves the measurement of acoustic pulses at the transducer center frequency using a pulsing scheme that corresponds to a 20% duty cycle. This ratio was chosen to allow for the clean acquisition of a single well developed pulse, the basis for calculations of intensity using the methods described by [53].
Unless otherwise noted, all measurements reported in this work have been taken using this pulsing scheme. Furthermore, the uniform use of this scheme allows for rapid comparison of acoustic output and time- and pulse-averaged intensities between different transducers and driving systems. However, this protocol needed to be adapted for use with high duty cycle pulses, as it can become more and more difficult to catch a single discrete pulse at higher duty cycles as described in the next section. This difficulty illustrates some of the practical difficulties experienced in the accurate measurement and quantification of acoustic output.
Figure 4-1: Acoustic scanning tank as used as part of an acoustic output quantification protocol. A protocol was developed based on the Sonora Acoustic Measurement System (Acertara Acoustic Laboratories, Longmont, CO), consisting of piezoelectric hydrophone mechanically scanned in three axes through an acoustic field. The test tank is a precision scanning system mounted on a water tank that allows for the measurement and mapping of acoustic fields in liquids. Custom software was written in to turn 2D slice measurements into 3D beam profiles.
**EXPLORATION OF HIGH DUTY CYCLE PULSE MEASUREMENTS**

A set of measurements was taken at various PRFs with the same number of cycles per pulse (N) to explore the effect the duty cycle has on the measurement of acoustic output using the developed test protocol. Acoustic measurements at the axial focus of a 6 cm diameter 7 cm radius of curvature air-backed single element transducer at different PRF values were taken while sweeping the driving frequency over the range $V_{in} = 0.01 - 0.05 \ V_{p-p}$.

As expected, peak rarefaction pressure ($P_r$) increases linearly with voltage, and remains relatively constant for selected PRFs (Figure 4-2). However, the tank system exhibits difficulty distinguishing the start and end of distinct pulses. As a result of this, the measured PII for the three cases varies between the relatively high duty cycle (PRF = 25 kHz) case and the lower duty cycle cases when it should remain constant for all three measurements. This is best shown by inhomogeneity of the pulse durations calculated for each of the three PRFs (Figure 4-3). As a consequence of an inaccurate PII measurement, $I_{SPTA}$ values will be reported inaccurately (Figure 4-4 and Figure 4-5). Since the PII scales in proportion to the pulse duration, the effect on reported $I_{SPPA}$ should not be as extreme. An additional complication with tank measurements is that the system does not automatically recognize changes in PRF. $I_{SPTA}$ values are reported at an assumed PRF of 1 kHz, and must be manually corrected to reflect the actual PRF (Figure 4-5).
Figure 4.2: Tank measurements of $P_r$ scale linearly with voltage, as expected.

Figure 4.3: Inhomogeneity in the reported pulse durations from low relative to high duty cycles.
Figure 4-4: $I_{SPTA}$ values must be manually corrected to reflect the actual PRF used. This figure further demonstrates the effect of a poor measurement window at high duty cycle. The $I_{SPTA}$ at PRF = 25 kHz should be substantially higher than that at PRF = 1 kHz.

Figure 4-5: Tank reports of $I_{SPTA}$ do not accurately account for changes to the actual PRF.
4.2 Power Systems

Piezoelectric crystals generate ultrasonic acoustic energy in response the application of a time-varying voltage potential applied across their terminals. While there are a variety of methods for generating this voltage potential, most commonly used is a linear voltage amplifier driven by a controllable arbitrary function generator. Monitoring mechanisms can be put to provide the user with real-time feedback, and tolerance for varying transducer input electrical impedance can be engineered.

A variety of different drive systems have been used in the course of this work. While they all maintain the same basic system architecture, that of a user-controllable function generator driving an amplifier (Figure 4-6), these systems differ in the components used, maximum power output, and tolerance for impedance mismatch.
4.2.1 System 1

System 1 was a robust, high power transducer drive system provided under a material transfer agreement between BrainSonix Corporation and UCLA as the BrainSonix Pulser BX1001 (Figure 4-7). An Agilent 33220A function generator was used to drive an Electronics + Innovation 240L high-power, impedance mismatch tolerant amplifier. A Werlatone C5948-10 dual-directional coupler placed after the amplification stage allowed for the measurement of forward and reverse applied powers by an Agilent E4419B power meter with Agilent 8482H power heads. The amplifier was capable of generating 200 W of power from 10 kHz to 12 MHz, with a gain of 50 dB.
Figure 4-7: LIFU System 1. System core is a 200 W, 50 dB ENI 240 L amplifier.

Figure 4-8: LIFU System 2. System core is a 0.8 W, 25 dB Mini-Circuits ZHL-32A amplifier.
4.2.2 System 2

LIFU System 2 was a compact, lower power transducer drive system consisting of a medium power ZHL-32A amplifier (Mini-Circuits, Brooklyn, NY) driven by either an Instek SFG-830 30 MHz arbitrary function generator or an Agilent 33220A 20 MHz arbitrary function generator (Figure 4-8). The amplifier is rated to have a gain of 26.67 dB and a maximum power output of 0.8 W at 500 kHz.

4.2.3 System 3

System 3 is a high-powered drive system that couples a Kalmus 1000HLMP amplifier (AR Modular, Bothell, WA) to a computer console-controlled USB function generator that was produced specifically for exploration of ultrasonic neuromodulation. Depicted in Figure 4-9 the system is capable of producing 1 kW output with a nominal 60 dB gain into a 50 Ω load.

The output of the system was characterized sequentially, first by measuring the output voltage and behavior of the USB function generator in response to an input of a known pulsed signal (PD = 5 µs, PRF = 5 kHz) while varying the voltage. The signal from the USB function generator was subsequently fed into the power amplifier, and the resulting amplified voltage measured. An Agilent DSO3202A oscilloscope was used to capture and measure all voltage waveforms, with a high-powered 50 Ω oil-cooled load of known attenuation used for the high-powered measurements. Measurements of output
at various stages of the system relative to the system input are tabulated in Table 4-1.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>0.013</td>
<td>7.44</td>
</tr>
<tr>
<td>0.02</td>
<td>0.024</td>
<td>16.6</td>
</tr>
<tr>
<td>0.03</td>
<td>0.036</td>
<td>27.8</td>
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<td>0.04</td>
<td>0.048</td>
<td>44.41</td>
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<td>0.05</td>
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<td>61.82</td>
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<td>0.06</td>
<td>0.072</td>
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<tr>
<td>0.08</td>
<td>0.095</td>
<td>141.5</td>
</tr>
<tr>
<td>0.09</td>
<td>0.107</td>
<td>175.89</td>
</tr>
<tr>
<td>0.1</td>
<td>0.122</td>
<td>202.45</td>
</tr>
<tr>
<td>0.15</td>
<td>0.18</td>
<td>304.76</td>
</tr>
<tr>
<td>0.2</td>
<td>0.242</td>
<td>422.31</td>
</tr>
<tr>
<td>0.25</td>
<td>0.3</td>
<td>509.39</td>
</tr>
<tr>
<td>0.3</td>
<td>0.36</td>
<td>552.93</td>
</tr>
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<td>0.35</td>
<td>0.42</td>
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<tr>
<td>0.4</td>
<td>0.48</td>
<td>626.94</td>
</tr>
<tr>
<td>0.45</td>
<td>0.536</td>
<td>700.95</td>
</tr>
<tr>
<td>0.5</td>
<td>0.596</td>
<td>766.26</td>
</tr>
</tbody>
</table>

The measured output of the function generator was found to be linear relative to the console-selected input level. However, the voltage gain of the amplification stage was found to vary slightly based on input, averaging approximately 64 dB. The relationship between voltage input selected for the function generator, and measured output voltage after the amplification stage is depicted shown in Figure 4-10.
Figure 4-9: LIFU System 3 with block diagram. System core is a 1 kW, 60 dB Mini-Circuits Kalmus 1000HLMP amplifier.

Figure 4-10: Amplifier gain from LIFU System 3. Voltage gain was found to average 64 dB, but vary based on the input.
4.3 Ultrasonic Transducers

Fundamentally, an ultrasonic transducer probe transforms electrical energy into mechanical energy, or vice-versa. When a charge is applied to a piezoelectric material, it deforms, producing mechanical stress. When the driving electric field is varied over time, the mechanical deformation also varies in concert with the applied field, producing a deformative wave which can then be coupled to, and transmitted into a medium. The resulting acoustic wave is a convolution of the electrical excitation signal and the impulse response of the transducer [66].

In its simplest embodiment, an ultrasound transducer of the type used for neurostimulation experimentation consists of a piezoelectric element mounted in sealed housing with an air-backing (Figure 4-11). The piezoelectric element is generally held in place around its circumference by being potted into the transducer housing using an elastomeric material such as silicone. For a more thorough treatment of transducer design principles and considerations, the reader is directed to Chapter 5.4 of [66] and Chapter 6 of [50].

A number of different transducers were designed, tested, and/or characterized as part of this overall work. The detailed design and analysis of the transducer family whose use around which Chapter 5 is predicated, and subsequently detailed in Chapter 6 is presented below. The methods described are similar to those used for other transducers which are subsequently detailed.
Figure 4-11: Basic schematic of a simple air-backed ultrasound transducer. The piezoelectric element is potted within a sealed housing, backed by air. This approach is generally used in applications where high power is preferred, and short pulse tones are not necessary.
Figure 4-12: Other transducers explored as part of this work. *Top Left:* 500 kHz 1 in diameter focused probe (Olympus V301-SU-F1.25), *Top Right:* 500 kHz 1 in diameter unfocused probe (Olympus V301-SU), *Bottom:* Experimental 1 MHz 1 cm probes
4.3.1 LARGE HIGH-POWER 650 kHz TRANSDUCER PROBES

A series of high-powered air-backed transducers operating at 650 kHz were designed and characterized as part of this work, and commonly referred to as the ‘LIFU Large’ series. Each of these transducers was composed of a spherically curved piezoelectric element labeled A through D. The probe’s element dimensions and acoustic focus measurements are summarized in Table 4-2.

<table>
<thead>
<tr>
<th>Transducer</th>
<th>Element Diameter [mm]</th>
<th>Element ROC [mm]</th>
<th>Axial Focus Distance [mm]</th>
<th>Focus Width [mm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>60</td>
<td>70</td>
<td>65</td>
<td>4.18</td>
</tr>
<tr>
<td>B</td>
<td>40</td>
<td>70</td>
<td>56</td>
<td>6.03</td>
</tr>
<tr>
<td>C</td>
<td>60</td>
<td>70</td>
<td>62</td>
<td>4.27</td>
</tr>
<tr>
<td>D</td>
<td>60</td>
<td>70</td>
<td>63</td>
<td>4.00</td>
</tr>
</tbody>
</table>

Transducers A and B were adapted from transducers provided under a material transfer agreement with BrainSonix Corp, and used 6 cm and 4 cm diameter PZT-5H ceramic from Channel Industries respectively (Figure 4-13 and Figure 4-14). The initial design, dimensions, and frequency were based on those successfully used by Yoo et al. These transducers were exclusively used in conjunction with System 1.
Figure 4-13: LIFU Large Probe A. Probe is composed of an air-backed 6 cm diameter 7 cm radius of curvature air-backed element in an acrylic housing. *Left*: Transducer backside showing active electrical connection. *Right*: Transducer front face showing ground electrode.

Figure 4-14: LIFU Large Probe B. Probe is composed of an air-backed 4 cm diameter 7 cm radius of curvature air-backed element in an acrylic housing. Probe disassembled showing wire management and cable strain-relief.
Transducers C and D were designed, fabricated, and tested to replicate the performance characteristics of Transducer A, and are formed from of 6 cm diameter 7 cm radius of curvature spherical elements made from fine-lapped PZT-5H with electroless nickel electrodes on both sides (Boston Piezo Optics, Bellingham, MA).

Transducer bodies were 2.75 inches in diameter and constructed from a cylindrical (hollow) stainless steel tube. The end of the body was capped with a stainless-steel plug that was secured to the body around the periphery with screws. The transducer element was potted into the body using RTV 118 Silicone (GE). Electrical connection to the drive system was through an SMA bulk-head adaptor. The hot (positive) lead attached to the hot electrode face of the transducer using magnet-wire and conductive epoxy. Probe bodies were constructed from stainless steel, and served as the electrical ground return path, with a bridge of conductive epoxy and solder applied between the ground face of the transducer and the body over the silicone potting. A rubber-water proof gasket was placed between the transducer body and top cap, and all screws were sealed with cyanoacrylate adhesive to maintain water-tight integrity.

A detailed characterization of the acoustic output of the probes was performed. A representative 3D beam pattern from a series of raster scans conducted along the transducer axis showing the spatial layout of the beam as well as the location of intensity minima and maxima is shown in Figure 4-16.

Sweeps of electrical drive system input while measuring acoustic output were conducted to characterize the overall system’s electro-acoustic profile for
later experimental use were also. As measurements cannot safely be taken in all acoustic power/intensity domains due to risk of damage to the hydrophone, this characterization allows for prediction of acoustic output outside of the measurement domain. Representative measurements of Probe C driven by LIFU System 3 demonstrating the relationship between system input voltage and acoustic output in terms of intensity and pressure is shown in Figure 4-17 and Figure 4-18 respectively.

This thorough characterization allows for the delivery of known amounts of ultrasound to known locations
Figure 4-15: Design schematics and fabrication of LIFU Large Probes C and D. Probes were designed out of a stainless steel housing and capped by a stainless steel plug with a rubber gasket (not shown) screwed into the transducer body. The transducers use a 650 kHz 6 cm diameter spherically curved element with a 7 cm radius of curvature.
Figure 4-16: 3D raster beam intensity profile from ‘LIFU Large’ series of transducers. Transducer has a 6 cm diameter and a 7 cm radius of curvature. Areas of higher intensity are depicted in red (hot) while areas of lower intensity are depicted in blue (cold). As the transducer is spherically focused, a locus of higher intensity is visible around the natural focus point of the transducer, along with a number of intensity local maxima and minima, as discussed in Section 4.1.1. Figure created through use of a custom Matlab script that reinterprets acoustic scan tank slice output.
Figure 4-17: Intensity measurements as a function of input voltage to LIFU System 3 at for Probe C v2 at the measured acoustic focus. $f = 650$ kHz, PD = 200 us, PRF = 1 kHz.
Figure 4-18: Relationship between measured pressure and intensity for LIFU Large Transducer C being driven by LIFU System 3.
Chapter 5: Development of a Large-Animal Assay of Low Intensity Transcranial Ultrasonic Neurostimulation

While ultrasonic neurostimulation has been demonstrated in a variety of small animal models (Section 2.4.3), an intermediate step is necessary to help push the neurostimulatory theory and techniques to humans. An *in vivo* model of large animal transcranial neurostimulation was developed to assay the effect of ultrasound on the intact brain of a Göttingen minipig.

This chapter details the engineering development and characterization of a method to deliver targeted insonation to the brain of the Göttingen minipig, specifically focusing on development and testing of an acoustic coupling system, and acoustic characterization of the minipig skull. Surgical procedures, ultrasound coupling techniques, as well as ultrasound transducers, pulsing systems, and instrumentation were developed as part of work, and used to explore targeted delivery of ultrasound to deep brain structures in the minipig.

A pilot feasibility study of transcranial neurostimulation of the minipig using the tools and techniques developed is detailed in Chapter Six.
5.1 Motivation

The Göttingen minipig was chosen to help address some of the limitations in previous small-animal studies detailed in Chapter Three. At the outset of this work, *in vivo* low-intensity ultrasonic stimulation had only been demonstrated in rodent and lagomorpha models. The brain of the gyrencephalic Göttingen minipig more closely resembles the human brain in anatomy, growth, and development than the brains of more commonly used small laboratory animals [140-143]. Furthermore, the relatively large size of the minipig brain allows for targeting of both cortical and subcortical structures with MRI imaging [144, 145]. The challenges inherent in transcranial neurostimulation of the minipig are also a more realistic analog to those that would be encountered in humans.

This effort was intended to help bridge the gap between exploratory small animal work and what would be needed for future clinical studies. This work was also initially motivated by the early reports from Tyler [44] and Yoo [121] on low-intensity ultrasonic neuromodulation and its potential interaction with Epilepsy [43], and by a request from clinical collaborators for a translational model for ultrasonic neurostimulation.

5.2 Goals

The primary goal of this undertaking is to develop a model of low-intensity ultrasonic neurostimulation that will provide a better analogue to humans than
small animals and rodents, as well as to develop translational tools and techniques.

The challenges of transcranial ultrasound in animals with larger cranial thicknesses (such as humans) are well established. Many of the approaches used involve multi-element phased arrays, which can be impractical in clinical settings. We hypothesize that careful targeting of ultrasound through the minipig cranium is possible with a single-element transducer.

**5.3 Experimental Surgical Design**

Many challenges were involved in the development of the Göttingen minipig model of ultrasonic neuromodulation. The primary tasks involved in the development of this minipig model of ultrasonic neuromodulation can be broken into mechanical and acoustic tasks. A method was needed to accurately position the transducer in 3D space relative to the target location while simultaneously maintaining acoustic coupling. A method of setting up and taking down this system while interoperating with the surgical procedure was also necessary. Furthermore, a value corresponding to the loss of acoustic energy from the transducer to the brain target relative to measured water tank values was needed to convert applied acoustic fields to acoustic fields experienced at the target.
5.3.1 Considerations for targeted acoustic coupling in the larger animal

Ultrasonic energy cannot be easily transmitted through the air, and therefore biological soft tissues must be directly coupled to the transducer in order for ultrasonic energy to be transmitted to them. Accurately delivering ultrasonic energy from a piezoelectric transducer to a specific brain/soft-tissue target location in known quantities with an understood profile is non-trivial.

Prior to our efforts, there were no suitable examples in the literature of couplers through which the transducer and the tissue target could be connected to provide a ready transmission path for the acoustic energy. Traditional methods of inverting the animal over a large tank of water such as those used by Yoo et al. at Harvard are too bulky and cumbersome to be used with a large animal such as the minipig, and do not allow for fine positional control of the transducer and therefore focus point within the animal. Collimated coupling methods such as those used by Tyler et al. at Arizona State, while allowing for a measure of path planning, do not provide a true constrained focus.

Other necessary considerations for the coupler included integration with the surgical installation procedure. In light of number of sharp items in the surgical space that could potentially damage the coupler during its installation and use, coupling mechanisms such as water bags were ruled out. In addition, in order to generate sufficient acoustic power, relatively large (multi-cm) scale transducers must be used. However there is limited space in the otherwise tightly
packed surgical field, constraining coupler dimensions. Furthermore, the coupler must be able to accommodate subject to subject variability in fit and sizing.

As a result of all these complicating factors, the acoustic coupler, surgical procedure, and installation techniques that will be subsequently detailed had to be developed in parallel.

5.3.2 Targeting Approach

Targeting was accomplished by adapting a human neurosurgical stereotactic setup to the minipig. Briefly, by co-registering the stereotactic setup to MRI-derived brain anatomy, the stereotactic assembly can be configured in such a way to generate a vector axis of known orientation that precisely intersects the target brain anatomy of known location.

This stereotactic setup consists of two parts, a head mounting ring, and a micromanipulator system. A circumferential ring mounts around the head of the subject to provide mechanical support and serve as the basis for the target coordinate system. The micromanipulator system is subsequently attached to this ring, and through translation in the x, y, z axis as well as angular rotation is capable of generating a vector pointing to the target (Figure 5-1).
Figure 5-1: Schematic of human stereotactic neurosurgical instrumentation used to target the transducer. Human stereotactic instrumentation (grey) sets the targeting axis, while the transducer is mounted inside a coupler assembly (yellow).
5.3.3 Acoustic Cranial Access: Strategies for Transcranial Sonication

The cranium of the Göttingen minipig has a dual-layer construction, with a trabecular space sandwiched between and inner and outer cranial layer. As the trabecular space is air-filled, and therefore effectively prevents ultrasound transmission, a strategy was needed to bridge between the subject’s brain tissue and the piezoelectric ultrasound transducer. Two surgical strategies were explored to bridge this gap, full and partial craniotomy.

**Full Craniotomy**

Initially, a full craniotomy was proposed to allow for ultrasound access. The entire skull over the target site was to be removed, directly exposing the dura of the brain. A coupler consisting of a sealed conical water-filled cavity attached to an acoustic gel-filled conformal enclosure that mounted flush to the top dural surface of the brain was designed and fabricated (Figure 5-2). The transducer was mounted directly to the stereotactaic instrument via a common mount shaft, and the coupler was able slide freely over this shaft to account for variations in animal head size. Once positioned against the dural surface, the coupler allowed for axial repositioning of the transducer. This technique had the advantage of providing a direct low-impedance acoustic path between the transducer and the brain target. Very low acoustic losses were expected, and the acoustic targeting was expected to coincide with the natural water-tank focus of the transducer.
Experimental validation testing was performed to validate this approach. Acoustic testing demonstrated the feasibility and acoustic functionality of the coupler mechanism. However, \emph{ex vivo} experiments on Göttingen minipig cadavers revealed several limitations to this approach. It was observed that there was a chance of puncturing the dura and brain during the drilling of the pilot bore-hole through the skull. Furthermore, the craniotomy procedure frequently left jagged cuts in the skull. There was further concern as to how large an opening can be practically performed, compared to how large an opening is necessary for unimpeded acoustic transmission. All these factors combined to make it tough to guarantee a perfect seal of the acoustic coupler onto the brain surface. As a result, acoustic gel leakage into the brain cavity was also observed to be a significant concern.

More significantly, it was also further discovered that without mechanical support from the brain case, the brain itself is highly deformable after a full craniotomy.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5-2.png}
\caption{Schematic of an acoustic coupler mechanism designed to integrate with a full craniotomy. Coupler features a water-filled cavity with an acoustic gel (in blue)-filled insert that mounted flush to the brain of the subject. Experimental validation of the coupler demonstrated successful acoustic transmission. However, cadaver testing demonstrated the physiological limitations of this approach.}
\end{figure}
Contact pressure from the coupling mechanism was observed to noticeably deform the brain anatomy. Even a few ounces of gel alone, with the weight of the coupling mechanism fully supported served to deform the brain. Such a deformation can move the orientation of the various brain structures of interest relative to their un-deformed locations. For these reasons, the full craniotomy approach was rejected.

**Partial Craniotomy**

To eliminate the complications inherent with both a full craniotomy and the air-filled trabecular space in-between the two skull layers, a partial craniotomy procedure was developed whereby the top layer of the skull and trabeculae are removed, leaving the lower skull intact and maintaining brain-case patency. A coupler was designed for this partial craniotomy procedure which consisted of a water-filled cavity capped with an acoustically transparent membrane that mounts flush to the upper surface of the lower skull (Figure 5-3). In this iteration of the coupler design, the coupler was mounted to the stereotactic frame via a mount shaft, with the transducer mounted to a separate piston-like shaft fitting within the mount shaft. This allowed for dynamic axial repositioning of both the transducer and the coupler. Furthermore, this enabled greater flexibility in changing the axial focus target of the transducer relative to the assembly, while still being capable of being used on subjects of varying head size.
However, even with this partial craniotomy technique, limitations still exist with the maximum skull opening, and therefore the diameter of the coupler where it makes contact with the lower skull. Surgically, a smaller opening (on the order of 0.5 in/12.7 mm) was recommended by the surgeons both in terms of subject trauma, and the physical space required for clearance of the mounting screws which are used to secure the localizer frame. Acoustically, a larger opening is preferable to allow a larger aperture for the ultrasonic beam to propagate unimpeded and prevent diffraction artifacts. The interaction between these aperture considerations, the partial craniotomy technique, and the resulting effects on ultrasound propagation will be explored in subsequent sections.

### 5.4 Design and Testing of a Modular Large-Animal Transcranial Acoustic Coupler

The maximum surgical diameter of the partial craniotomy governs the

![Diagram](image_url)

**Figure 5-3:** Coupler mechanism designed to integrate with a partial craniotomy approach. The coupler assembly allows for axial translation of the transducer, and makes contact with the lower lamina of the skull using an acoustically transparent membrane.
diameter of the aperture through which the acoustic beam exits the coupler and enters the subject. As the acoustic energy emanating from a spherically curved transducer is roughly conical in shape, the maximum aperture needed to cleanly transmit the beam is also a function of the depth of the acoustic target. A more superficial target would demand a narrower acoustic beam width through the craniotomy. Conversely, the deeper the target, the wider the beam will be when it crosses the skull. Based on the predicted theoretical geometric conical profile of the beam, it was calculated that an approximately 50.8 mm aperture would be needed to be able to account for a fully range of target depths.

5.4.1 ACOUSTIC MODELING

Acoustic finite-element modeling using PZFlex acoustic modeling software (Weidlinger Associates, Washington DC) was used to refine the predicted geometric model, and to explore the interaction between the diameter of the coupler aperture and the position of the transducer relative to the aperture, on the transmitted acoustic beam. The simulations modeled a 650 kHz spherically curved piezoelectric transducer with a diameter of 6 cm and a radius of curvature of 7 cm.

Assuming a 12.7 mm diameter aperture, corresponding to the minimum guaranteed partial craniotomy diameter, two boundary condition scenarios were evaluated, corresponding to deepest and shallowest target depth, and therefore the widest and narrowest expected beam width at the aperture. The target depth boundary conditions were calculated based on the upper- and lower-bounds of
the expected target depth within the brain, and thickness of the skull, as the transducer focus. The resulting offset necessary to place the transducer focus on the acoustic target could be used to calculate the beam width at the aperture.

The wide-beam width deep-focus scenario was calculated to have an offset in which the transducer was separated from the aperture by 17 mm, and that of shallow focus scenario having an offset 35 mm (Figure 5-4).

When the coupler was placed only 17 mm away from the aperture, significant degradation of focus was observed. The focus point appeared to shift up (closer to the transducer), while both the focus length and width grew. Internal reflections within the coupler cavity are were also evident, leading to a less spatially and temporally confined acoustic pulse. When the transducer was

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**Figure 5-4:** Boundary condition analysis of focus depth with varying transducer positions. Approximate best and worst case dimensions for the acoustic target and skull thickness shown. Values derived from prior work in minipigs and experimental measurements of skull thickness.
separated from the aperture by 35 mm, which corresponded to an absolute best case positioning scenario, much less alteration of the acoustic beam was observed. In both these cases, a high-duty cycle pulse was modeled.
Figure 5-5: PZFlex boundary condition simulation of the impact separation distance between the transducer and a 25 mm aperture has on the resulting acoustic wave. *Left:* 17 mm separation (lower bound). *Right:* 35 mm separation (upper bound) Top images depict the model, where green denotes air, red - piezoceramic, pink - acrylic, and light blue - water. Bottom images depict heat maps of the simulated pressure fields from uniform excitement of the piezoelectric element. Hot colors depict higher pressures, while cool colors depict lower pressures.
Further refinement work was carried out with additional PZFlex simulation modeling and experimentation with ex vivo minipig skull samples. Ultimately, a coupler piece with an aperture diameter of 1.75 in (44.45 mm) was designed, evaluated, and found to have an acceptable acoustic performance through a wide range of possible experimental configurations while simultaneously providing good interoperability with the surgical procedure. Lathed from a single billet of acrylic, the coupler has a 10 mm shank to bridge the gap between the outer skull and the inner skull/gel interface point (Figure 5-6). A PZFlex simulation of the pressure field through this coupler is shown in Figure 5-7.

Figure 5-6: Schematic representation of the acrylic coupler piece with dimensions. This piece acoustically connects the coupler assembly to the lower-skull of the minipig. Primary dimensions are listed in inches, with secondary dimensions in millimeters.
Figure 5-7: PZFlex simulation of the effect on the pressure distribution field of a spherically curved piezoelectric element. Element with a 6 cm diameter and 7 cm radius of curvature from a coupler with the dimensions depicted in Figure 5-6. No significant abnormality of the pressure field is observed in this model. Hot colors depict higher pressures, while cool colors depict lower pressures.
5.4.2 COUPLER ACOUSTIC TESTING AND VALIDATION

A thin acoustically transparent 0.7 mm polypropylene self-adhesive membrane (Scotch 341, 3M, Saint Paul, MN) was used to close the aperture and maintain the coupler in a watertight state. An experiment in the acoustic test tank was conducted to directly measure the impact the coupler has on the shape, pressure, and intensity of the acoustic beam. The pressure, intensity, and beam shape in space was first measured for the transducer by itself. Subsequently, the coupler assembly was assembled around the transducer. Measurements were taken at points corresponding to an increasing offset ($\Delta Z$) between the front face of the transducer, and the membrane sealing the acoustic coupler. The coordinate system was held constant, with the $Z_+$ direction away from the front face of the

![Diagram](image)

Figure 5-8: Schematic depicting the relative position of the transducer, the coupler, and the acoustic focus. The spacing between the transducer face and the acoustically transparent window covering the coupler aperture ($\Delta Z$) was varied and the acoustic focus, focus width, and resulting pressure and intensity measured.
transducer pointing towards the acoustic and geometric focal point of the piezoelectric element (Figure 5-8). In such a coordinate system, if the coupler has no effect, the Z-axis position of the acoustic focus should remain constant.

The spacing between the front of the coupler and the transducer was varied from a $\Delta Z$ of 30 mm to 45 mm in 5 mm increment’s, with the first 30 mm trial corresponding to the position of the transducer when it is as far forward in the coupler as possible. All trials were conducted using ‘LIFU Probe C’ powered by LIFU System 3 at the standard test parameters of $\text{VinIpad} = 0.01 \text{ V}_{pp}$, $\text{PD} = 200 \mu\text{s}$, $\text{PRF} = 1 \text{ kHz}$, $f = 650 \text{ kHz}$.

The coupler was found not to have a deleterious impact on acoustic performance, with the results of this study tabulated in Table 5-1. As the separation between the transducer and the coupler aperture grew the measured point of acoustic focus relative to the transducer remained constant. No axial shift in the focus was observed.

<table>
<thead>
<tr>
<th>Membrane Position, $z$ (mm)</th>
<th>Focus, $z$ (cm)</th>
<th>Focus Width, $X$ (cm)</th>
<th>Focus Width, $Y$ (cm)</th>
<th>$\text{Pr}$ (kPa)</th>
<th>$\text{Ispta}$ (mW/cm$^2$)</th>
<th>$\text{Isppa}$ (W/cm$^2$)</th>
<th>MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free-Water</td>
<td>6</td>
<td>0.417</td>
<td>0.418</td>
<td>124.4</td>
<td>82.12</td>
<td>0.42</td>
<td>0.13</td>
</tr>
<tr>
<td>30</td>
<td>6</td>
<td>0.396</td>
<td>0.392</td>
<td>136</td>
<td>98.02</td>
<td>0.5</td>
<td>0.15</td>
</tr>
<tr>
<td>35</td>
<td>6</td>
<td>0.406</td>
<td>0.405</td>
<td>126.8</td>
<td>87.14</td>
<td>0.45</td>
<td>0.14</td>
</tr>
<tr>
<td>40</td>
<td>6</td>
<td>0.407</td>
<td>0.403</td>
<td>121</td>
<td>81.14</td>
<td>0.42</td>
<td>0.131</td>
</tr>
<tr>
<td>45</td>
<td>6</td>
<td>0.406</td>
<td>0.398</td>
<td>125.5</td>
<td>85.35</td>
<td>0.44</td>
<td>0.14</td>
</tr>
</tbody>
</table>

While the focus point remained constant relative to separation distance, other acoustic differences were observed based on the separation, with marked
changes relative to the no coupler control measured at $\Delta Z = 30$ mm, and a measurable yet slight changes occurring at $\Delta Z = 35$, 40, and 45 mm (Table 5-2). The diameter of the acoustic focus was found to shrink by approximately 5% when the coupler was located at $\Delta Z = 30$ mm, with $\Delta Z = 35$-45 mm showing diameter decreases of approximately 3-5%. Significant differences in pressure and intensity relative to the no coupler case were also measured for the $\Delta Z = 30$ mm position. Peak rarefaction pressure was observed to increase by nearly 10%, while intensity increased by nearly 20%. This compares to the approximately 2% change in pressure and <8% change in intensity observed for the other cases.

The most likely explanation for the differences observed in the $\Delta Z = 30$ mm is the formation of a resonance inside the coupler cavity, either between the acoustic membrane and the transducer, the side walls and the transducer, or a combination of both. This makes intuitive sense as the transducer is closest to these reflecting surfaces in the $\Delta Z = 30$ mm position, and based on the PZFlex simulation (Figure 5-7) of a coupler with similar geometry, some interaction between the sloping walls of the coupling piece and the acoustic wave is observed. While the increase in measured intensity and pressure is consistent with the existence of a node, the apparent decrease in focus diameter, while ultimately still slight, is unexpected.
Table 5-2: Percent Change in Acoustic Values Relative to Coupler Position

<table>
<thead>
<tr>
<th>Membrane Position, z= (mm)</th>
<th>Focus, z (cm)</th>
<th>Focus Width, X (cm)</th>
<th>Focus Width, Y (cm)</th>
<th>Pr (kPa)</th>
<th>Ispta (mW/cm²)</th>
<th>Isppa (W/cm²)</th>
<th>MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free-Water</td>
<td>6</td>
<td>0.417</td>
<td>0.418</td>
<td>124.4</td>
<td>82.12</td>
<td>0.42</td>
<td>0.13</td>
</tr>
<tr>
<td>30</td>
<td>6</td>
<td>94.96%</td>
<td>93.78%</td>
<td>109.32%</td>
<td>119.36%</td>
<td>119.05%</td>
<td>115.38%</td>
</tr>
<tr>
<td>35</td>
<td>6</td>
<td>97.36%</td>
<td>96.89%</td>
<td>101.93%</td>
<td>106.11%</td>
<td>107.14%</td>
<td>107.69%</td>
</tr>
<tr>
<td>40</td>
<td>6</td>
<td>97.60%</td>
<td>96.41%</td>
<td>97.27%</td>
<td>98.81%</td>
<td>100.00%</td>
<td>100.77%</td>
</tr>
<tr>
<td>45</td>
<td>6</td>
<td>97.36%</td>
<td>95.22%</td>
<td>100.88%</td>
<td>103.93%</td>
<td>104.76%</td>
<td>107.69%</td>
</tr>
</tbody>
</table>

5.5 Quantification of Effect on Acoustics from Transcranial Sonication

Accurate targeting and dosage of acoustic energy onto the target requires an understanding of the acoustic effect the lower-lamina of the skull has on the ultrasound wave. The large impedance mismatch between the portions of the skull bone that must be transited to deliver the acoustic energy to the brain serves to drastically reduce the effective acoustic energy at the target site relative to what is delivered by the transducer outside of the skull. Furthermore, the curved shape of the lower-laminal layer can possibly induce lensing effects, skewing the focus of the beam in-vitro and in-vivo relative to water tank measurements of beam profiles. A series of theoretical and ex-vivo experiments were conducted to address these issues.

5.5.1 Theoretical Transmission Coefficient of Minipig Skull

As an ultrasonic wave transitions through a series of mediums, it undergoes reflection as a function of the impedance mismatch, and attenuation
as a function of distance traveled within the various media. The total loss in energy and intensity over the transit is also a function of the angle of incidence of the wave on the interfaces.

A simplified transmission model of the transit of an acoustic wave across a thin section of bone section was considered (Figure 5-9). The incident wave was assumed to be in the form of a uniform plane harmonic wave acting perpendicular to the bone section, with Snell’s law and wave-mode conversion effects ignored. The attenuation effects of the mediums surrounding the bone, and that of the thin bone section itself were assumed to be negligible.

Based on the acoustic properties from Table 5-3 and assuming that the lower lamina is comparable to human cortical bone, two cases were considered:

Case 1: Water to Cortical Bone to Brain, corresponding to an *in vivo* measurement within the minipig.
Case 2: Water to Cortical Bone to Water, representing an *in vitro* trial of skull attenuation in a water tank.

The effect on the acoustic wave of each material transition for both cases is tabulated in Table 5-4.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Velocity [m/s]</th>
<th>Density [kg/m^3]</th>
<th>Attenuation [dB/(cm MHz)]</th>
<th>Acoustic Impedance [MRayl]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>330</td>
<td>1.2</td>
<td></td>
<td>0.0004</td>
</tr>
<tr>
<td>Bone, Cortical</td>
<td>3476</td>
<td>1975</td>
<td>6.9</td>
<td>7.38</td>
</tr>
<tr>
<td>Bone, Trabecular</td>
<td>1886</td>
<td>1055</td>
<td>9.94</td>
<td>1.45</td>
</tr>
<tr>
<td>Brain</td>
<td>1560</td>
<td>1040</td>
<td>0.6</td>
<td>1.62</td>
</tr>
<tr>
<td>Average Soft Tissue</td>
<td>1561</td>
<td>1043</td>
<td>0.54</td>
<td>1.63</td>
</tr>
<tr>
<td>Blood</td>
<td>1584</td>
<td>1060</td>
<td>0.2</td>
<td>1.68</td>
</tr>
<tr>
<td>Water</td>
<td>1480</td>
<td>1000</td>
<td>0.0022</td>
<td>1.48</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Pressure [R_P]</th>
<th>Pressure [T_P]</th>
<th>Pressure [α_db]</th>
<th>Intensity [R_I]</th>
<th>Intensity [T_I]</th>
<th>Intensity [α_db]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water to Cortical Bone</td>
<td>0.67</td>
<td>1.67</td>
<td>-4.43</td>
<td>0.44</td>
<td>0.56</td>
<td>2.54</td>
</tr>
<tr>
<td>Bone to Brain</td>
<td>-0.64</td>
<td>0.36</td>
<td>8.87</td>
<td>0.41</td>
<td>0.59</td>
<td>2.29</td>
</tr>
<tr>
<td>Water to Bone to Brain</td>
<td>0.57</td>
<td>4.83</td>
<td></td>
<td>0.33</td>
<td></td>
<td>4.83</td>
</tr>
<tr>
<td>Water to Cortical Bone</td>
<td>0.67</td>
<td>1.67</td>
<td>-4.43</td>
<td>0.44</td>
<td>0.56</td>
<td>2.54</td>
</tr>
<tr>
<td>Bone to Water</td>
<td>0.67</td>
<td>0.33</td>
<td>9.52</td>
<td>0.44</td>
<td>0.56</td>
<td>2.54</td>
</tr>
<tr>
<td>Water to Bone to Water</td>
<td>0.56</td>
<td>5.09</td>
<td></td>
<td>0.31</td>
<td></td>
<td>5.09</td>
</tr>
</tbody>
</table>

Based on this simple model, I hypothesize that 33% of the intensity of an incident acoustic wave would be expected to transmit through the bone in the
first Water to Bone to Brain case, and 31% in the second Water to Bone to Water case. This corresponds to a loss of 4.83 dB in Case 1, and 5.09 dB in Case 2.

As expected, the overall acoustic transmission values in both cases are broadly similar. As brain is predominately water, and therefore acoustically comparable to water, similar results were expected. However, the slightly higher impedance of brain results in a marginally better match with bone, resulting in a slight increase in the transmission coefficient in the first case.

In both cases, a little over half of the incident acoustic intensity was lost at the first interface, between water and the bone, and similarly a little over half the remaining acoustic intensity was lost transiting out of the bone into the third medium.

When the attenuation through the thickness of the bone layer is considered, an additional 0.69 dB/MHz per millimeter of bone thickness is expected. The frequency dependence on attenuation of bone can be considered close to unity at low frequencies (approximately 0.2 – 1 MHz) [146]. This would add an additional 0.5175 dB and 0.67275 dB for a transition through 1.5 mm of bone at 500 kHz and 650 kHz respectively. Assuming the attenuation values for human cortical bone is similar to that of the lower lamina of the Göttingen minipig, one would need to transition approximately 8.69 mm of the lower lamina for a 500 kHz wave to lose half its intensity through attenuative loss within the bone itself.
In a more thorough study of transcranial acoustics in human samples, Fry calculated a loss between 6 -8 dB, and measured a loss of approximately 10 dB [147].

5.5.2 Measurement of Acoustic Transmission Through Göttingen Minipig Skull-Lower Lamina Samples

Two samples of the lower lamina of the Göttingen minipig skull were acquired and their acoustic transmission properties measured. Samples were excised from minipig cadavers. Tissue was dissected away and the lower lamina extracted through use of rongeurs. Sample #1 was intact through the area of the expected beam path but had a notch on the side, adjacent to this area. Sample #2 (Figure 5-10) was later mechanically smoothed by hand to remove the trabecular ridges and provide a more uniform transmission surface.

Acoustic Test Methods

In-vitro experiments on excised minipig skulls were performed both manually and using the AMS. The samples were placed in a custom enclosure inside the AMS scanning tank in between the transducer and a calibrated membrane hydrophone (Sonora Medical Systems, Model 804 SN S4-315). All electronics were encapsulated for immersion into the water scanning medium. The skull samples were insonated using the 650 KHz 6 cm LIFU Large Transducer v1 driven by LIFU System 1 at f = 650 kHz, PRF = 1 kHz, N = 25 cycles at a system input voltage Vin = 0.01V_{rms}. Acoustic measurements were
taken without the skull, with the skull holder, and with the skull mounted to the skull holder. Both z-scan (depth), and in-plane acoustic scans were captured and analyzed, with the focus point, intensity, and focus size measured. Once the focus point was established, measurements were taken approximately a half centimeter above and below the measured focus.

**RESULTS AND DISCUSSION**

Insonation of minipig sample skull #1 revealed severe temporal and spatial distortion of the acoustic pulse and a large attenuative effect on the transmitted intensity based on the specific orientation of the skull to the transducer.

![Figure 5-10: Sample Göttingen minipig skull lower lamina #2. **Left:** Prior to smoothing the trabeculae. **Right:** Subsequent to smoothing and removal of the trabeculae. Rostral (R) axis to the image left, caudal (C) axis to the right. Medial (M) axis pointing towards image top, lateral (L) towards image bottom.](image)

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The results of an initial scan to find the acoustic focus, as defined as the axial location of maximum intensity, conducted without tight control of the angle of the skull relative to the axis of the transducer are tabulated in Table 5-5. The scan showed large shifts in acoustic pulse shape and geometric focus with the skull in the acoustic path, in comparison to a no skull-control.

The axial location of the focus point was found to have shifted downwards by 1.29 cm away from the transducer. The width of the acoustic beam at the focus point was also found to have non-uniformly widened, approximately doubling in one axis, and nearly quadrupling in the other.

When the maximum intensities for both cases are compared, a large acoustic loss of 21.71 dB, corresponding to the transmission of less than 1% of the incident acoustic intensity is demonstrated.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (No Skull)</th>
<th>Translaminal</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Axial Position, Z (cm)</td>
<td>6.5</td>
<td>7.792</td>
<td>19.88</td>
</tr>
<tr>
<td>ISPTA (mW/cm2)</td>
<td>23.292</td>
<td>0.157</td>
<td>-99.33</td>
</tr>
<tr>
<td>ISPPA (W/cm2)</td>
<td>0.66</td>
<td>0</td>
<td>-100.00</td>
</tr>
<tr>
<td>Pr (kPa)</td>
<td>0.1433</td>
<td>0.01312</td>
<td>-90.84</td>
</tr>
<tr>
<td>X-Axis Focus Width (cm)</td>
<td>0.4188</td>
<td>0.957</td>
<td>128.51</td>
</tr>
<tr>
<td>Y-Axis Focus Width (cm)</td>
<td>0.4248</td>
<td>1.9616</td>
<td>361.77</td>
</tr>
<tr>
<td>MI</td>
<td>0.154</td>
<td>0.014</td>
<td>-90.91</td>
</tr>
<tr>
<td>Measured Center Freq (MHz)</td>
<td>0.649</td>
<td>0.642</td>
<td>-1.08</td>
</tr>
<tr>
<td>Pulse Duration (us)</td>
<td>35.487</td>
<td>47.062</td>
<td>32.62</td>
</tr>
</tbody>
</table>

When measured intensities of the no-skull control and with-skull translaminal cases were compared at the same axial locations, an average acoustic loss of 20.0 dB ± 4.85 was calculated. The relative loss was found to
decrease with increasing depth, indicating a possibly non-linear effect of the skull properties or orientation on the acoustic beam.

Further analysis of the measured acoustic pulse of the focus reveals a noticeable distortion of the pulse shape, with a widening of the pulse duration and ringing (Figure 5-11), potentially indicative of measured point of maximum intensity no longer residing on the transducer's center axis. Furthermore, a non-uniform widening of the focus diameter was observed, seeming to indicate that

![Figure 5-11: Measured acoustic transmission of a pulse through Skull #1. Both control and with skull measurements taken at axial position of maximum intensity. The skull causes a large signal attenuation, and distortion of the beam. Through-skull signal is also distorted relative to the control.](image)
the beam is skewed relative to the normal axis of the tank and measurement.

Several possible explanations for this axial skewing of the beam may exist, including the presence of the notch in the skull sample, angular misalignment of the transducer to the skull, a non-uniform lensing effect of the skull, or the impact of the trabecular ridges on the surface of the lower lamina.

Analysis of second, more intact lower lamina sample revealed a difference in acoustic transmission properties of the skull based on where the ultrasonic sonication was targeted, but not as strong of a dependence on the axial measurement location (Table 5-6). Measurements of the magnitude and axial location of the point of maximum intensity show that approximately 0.14% of the incident acoustic intensity was transmitted through the middle of the skull, versus approximately 6.4 percent through the forward portion of the skull. This translates to an average loss over three axial measurement locations of 28.4 dB ± 0.362 for the mid skull, and 11.9 dB ± 0.443 for the forward skull, both substantially larger than then calculated theoretical loss value of 5.09 dB.

As expected, the attenuative effect of the skull in intensity is constant relative to axial location. However, variation in the pressure attenuation based on the axial location, and in comparison to the intensity attenuation was observed. The pressure losses were calculated to be 13.8 dB ± 7.15 for the mid skull, and 7.00 dB ± 6.05 for the forward skull. Pressure losses were lowest when measured below the expected focus. No obvious explanation for this discrepancy is apparent.
While the forward skull appears to attenuate less, it appears to shift the focus up, with the axial position of maximum intensity of the forward skull occurring at $z = 4.4$ cm, compared to $z = 6.0$ cm for the control and $z = 6.1$ cm for the mid skull. Furthermore, radial spread of the pulse energy is much less when targeted to the forward skull, with the beam focus through the forward skull measured as being roughly twice that of the control, significantly less than that through the mid skull.

Measurements of the lower lamina fragments reveal the potential for wide shifts in acoustic transmission and focus based on the orientation and configuration of the skull relative to the transducer and measurement setup, and the significant limitations of relying on these measurements and fragments for comprehensive understanding of the skull’s impact on the stimulation acoustics. Furthermore, without the surrounding anatomy, potential in-skull reflections and reverberations that might occur in a patent brain case due to high reflection coefficient of the skull cannot be adequately analyzed and addressed. As demonstrated by the variability between the two samples, and the differences in measured acoustic losses between the samples and the simple theoretical model presented earlier, such a simple model does not provide good fidelity at recreating the effect of the skulls on the acoustics.
Table 5-6: Measurements of Acoustic Pulse and Focus Properties Through Göttingen Minipig Lower-Lamina Sample #2

<table>
<thead>
<tr>
<th></th>
<th>Measured Values</th>
<th>% Change From Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (No Skull)</td>
<td>Mid Skull</td>
</tr>
<tr>
<td><strong>Axial Position, Z (cm)</strong></td>
<td>6</td>
<td>6.1</td>
</tr>
<tr>
<td>ISPTA (mW/cm2)</td>
<td>26.8</td>
<td>0.039</td>
</tr>
<tr>
<td>Pr (kPa)</td>
<td>155.4</td>
<td>21.5</td>
</tr>
<tr>
<td>X-Axis Focus Width (cm)</td>
<td>0.418</td>
<td>5.233</td>
</tr>
<tr>
<td>Y-Axis Focus Width (cm)</td>
<td>0.427</td>
<td>1.133</td>
</tr>
<tr>
<td><strong>Above Focus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Axial Position, Z (cm)</td>
<td>5.5</td>
<td>5.5</td>
</tr>
<tr>
<td>ISPTA (mW/cm2)</td>
<td>22.75</td>
<td>0.03</td>
</tr>
<tr>
<td>Pr (kPa)</td>
<td>146.3</td>
<td>17.1</td>
</tr>
<tr>
<td>X-Axis Focus Width (cm)</td>
<td>0.41</td>
<td>4.61</td>
</tr>
<tr>
<td>Y-Axis Focus Width (cm)</td>
<td>0.421</td>
<td>4.48</td>
</tr>
<tr>
<td><strong>Below Focus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Axial Position, Z (cm)</td>
<td>6.5</td>
<td>6.5</td>
</tr>
<tr>
<td>ISPTA (mW/cm2)</td>
<td>19.28</td>
<td>0.03</td>
</tr>
<tr>
<td>Pr (kPa)</td>
<td>37.9</td>
<td>19.9</td>
</tr>
<tr>
<td>X-Axis Focus Width (cm)</td>
<td>0.435</td>
<td>5.119</td>
</tr>
<tr>
<td>Y-Axis Focus Width (cm)</td>
<td>0.459</td>
<td>5.342</td>
</tr>
</tbody>
</table>
5.5.3 Measurement of Acoustic Transmission Through Stereotactically Addressed Intact Göttingen Minipig Skull

In an attempt to rectify the shortcomings of acoustic testing using only the lower lamina of the minipig discussed previously, a pair of experiments on intact Göttingen minipig skulls were conducted. Effort was made to mimic the experimental delivery of ultrasound to the \textit{ex vivo} skull as closely as possible to the surgical \textit{in vivo} delivery of ultrasound. The transducer was placed within the surgical acoustic coupler, and the entire assembly aimed at the appropriate stereotactic coordinates.

\textbf{Materials and Methods}

\textbf{Skull Preparation:} Two intact skull samples were acquired from Göttingen minipig cadavers corresponding to experimental test subjects \#4 (Figure 5-12) and \#5 (Figure 5-13). Please refer to Section 6.5 for additional information on the subjects.

Samples were skeletonized by manual gross dissection of flesh, and subsequent exposure to a dermestid beetle colony. Boiling and other liquid maceration techniques were avoided to prevent bone shrinkage and possible alteration of acoustic properties. Samples were degreased by soaking in a dilute hydrogen peroxide mixture until no fatty remnant was observed in the solution. The initial soak was performed with 3\% hydrogen peroxide, with subsequent washes using 1.5\% solution. Skulls were later sliced using a fine band saw in the
rostral direction from the posterior aspect of the skull to approximately the hard palate, exposing the cranial cavity (Figure 5-14).
Figure 5-12: Intact skull from subject #4

Figure 5-13: Intact skull from subject #5

Figure 5-14: Exposed cranial cavity of subject #5. Left: Dorsal view. Right: Anterior view.
**Skull Mounting:** A variety of methods to mechanically mount and secure the skulls were experimentally explored, including constructing a frame around the skull, clamping around the snout, and securing bolts into the anterior aspect of the skull (Figure 5-15). These methods were ultimately rejected due to inability to secure a steady mount, and the possible risk of damage to the skull specimen.

Skulls were secured though use of a clamp consisting of two opposed right-angle brackets (‘CL 5 General Purpose Table Clamp’, Thorlabs) clamped against the inside of the nasal passageway (Figure 5-17) and the hard pallet, tensioned using a retaining nut. The clamp end inserted into the nasal passageway was pushed as far forward as possible, while maintaining alignment with the center plane of the skull (Figure 5-16).

**Skull Positioning:** Skulls were held in a custom designed assembly which

![Figure 5-15: External skull mounting methods. Left: CAD model of an external mounting for the skull. The external frame, which was secured to the skull through ¼-20 screws, forms a gimbal around which the skull can be rotated and the orientation of the acoustic beam to the skull altered. Experiments demonstrated that this method cannot securely constrain the skull without slippage. Right: Photo of an around-snout mounting technique.](image-url)
mounted the transducer into the acoustic coupler, and positioned the entire assembly in the correct stereotactic orientation and configuration. The assembly was capable of translating the transducer in the z-axis relative to the coupler and skull, while maintaining the proper angular acoustic trajectory. The skull, transducer, and coupler were mounted inverted, with the direction of acoustic propagation facing up out of the AMS tank to allow for visual inspection of the skull cavity and to avoid trapping bubbles within the cavity.

Stimulator Systems: Trials were conducted using 650 kHz 6 cm Probe C v2, being driven by LIFU System 3 at a center frequency of 650 kHz, with a PRF
of 1 kHz, and pulse duration of 200 µs through Probe C Match v4, into Cable Green.

**Measurement Protocol:** All Measurements were taken using an Onda HNR-1000 needle hydrophone, serial number 1610 and taken in the Sonora AMS tank system. All measurements taken with the axis of acoustic propagation facing towards the top of the tank (inverted orientation).

With only the transducer mounted to the rest rig, z-axis alignment of the hydrophone was performed, with the aligned acoustic center determined. Measurements at 0.1 mm intervals were taken to establish the location of
maximum pressure and intensity on the z plane. This z-axis sweep was repeated for a variety of system input voltages, with measurements of the actual input voltages taken. A raster scan at the z-axis focus location was performed to determine the planar contour of the acoustic beam at the focus.

Without re-centering the beam, the protocol was repeated after placement of the coupler, and subsequently placement of the skull over the coupler.

**RESULTS AND ANALYSIS**

The effects on measured pressure, intensity, mechanical index, and z-axis shift of the intact skull, averaged over all trials and input voltages are tabulated in Table 5-7 for the intact skull from Subject #4, and Table 5-8 for the intact skull of Subject #5. Three loss and transmission zones were considered, that of the transition from the transducer to the coupler (T -> C), the transition from the output of the transducer and coupler complex through the skull (T+C -> S), and the overall transmission and acoustic loss from the transducer face to the physiologic target, after passing through the coupler and skull (T -> C -> S). Raw measurement values for all voltage and pulse duration trials can be found in Appendix C. Results are summarized below.
Table 5-7: Averaged Effect of Intact Skull, Subject #4

<table>
<thead>
<tr>
<th></th>
<th>T -&gt; C</th>
<th>T+C -&gt; S</th>
<th>T -&gt; C -&gt; S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>StdDev</td>
<td>Mean</td>
</tr>
<tr>
<td>Pr [MPa]</td>
<td>0.9233</td>
<td>0.0301</td>
<td>0.3917</td>
</tr>
<tr>
<td>Transmission Coefficient</td>
<td>0.6965</td>
<td>0.2879</td>
<td>8.1442</td>
</tr>
<tr>
<td>Attenuation (dB)</td>
<td>0.1001</td>
<td>0.3465</td>
<td>-0.4501</td>
</tr>
<tr>
<td>ISPTA [mW/cm²]</td>
<td>0.8646</td>
<td>0.0693</td>
<td>0.1595</td>
</tr>
<tr>
<td>Transmission Coefficient</td>
<td>0.6422</td>
<td>0.3452</td>
<td>7.9745</td>
</tr>
<tr>
<td>Attenuation (dB)</td>
<td>0.1501</td>
<td>0.2887</td>
<td>-0.5251</td>
</tr>
<tr>
<td>ISPPA [W/cm²]</td>
<td>0.8646</td>
<td>0.0693</td>
<td>0.1566</td>
</tr>
<tr>
<td>Transmission Coefficient</td>
<td>0.6422</td>
<td>0.3452</td>
<td>8.0555</td>
</tr>
<tr>
<td>Attenuation (dB)</td>
<td>0.1501</td>
<td>0.2887</td>
<td>-0.5251</td>
</tr>
<tr>
<td>Pii [µJ/cm²]</td>
<td>0.8646</td>
<td>0.0693</td>
<td>0.1595</td>
</tr>
<tr>
<td>Transmission Coefficient</td>
<td>0.6422</td>
<td>0.3452</td>
<td>7.9745</td>
</tr>
<tr>
<td>Attenuation (dB)</td>
<td>0.1501</td>
<td>0.2887</td>
<td>-0.5251</td>
</tr>
<tr>
<td>MI</td>
<td>0.9275</td>
<td>0.0368</td>
<td>0.3878</td>
</tr>
<tr>
<td>Transmission Coefficient</td>
<td>0.6591</td>
<td>0.3494</td>
<td>8.2315</td>
</tr>
<tr>
<td>Attenuation (dB)</td>
<td>0.1001</td>
<td>0.3465</td>
<td>-0.1251</td>
</tr>
</tbody>
</table>
Table 5-8: Averaged Effect of Intact Skull, Subject #5

<table>
<thead>
<tr>
<th></th>
<th>T -&gt; C</th>
<th></th>
<th>T+C -&gt; S</th>
<th></th>
<th>T -&gt; C -&gt; S</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>StdDev</td>
<td>Mean</td>
<td>StdDev</td>
<td>Mean</td>
<td>StdDev</td>
</tr>
<tr>
<td>Pr [MPa]</td>
<td>0.9936</td>
<td>0.0632</td>
<td>0.3485</td>
<td>0.0173</td>
<td>0.3462</td>
<td>0.0277</td>
</tr>
<tr>
<td>Transmission Coefficient</td>
<td>0.0680</td>
<td>0.5626</td>
<td>9.1631</td>
<td>0.4289</td>
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<td>0.0981</td>
<td>0.1220</td>
<td>0.0031</td>
<td>0.1219</td>
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<tr>
<td>Transmission Coefficient</td>
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<td>0.4354</td>
<td>9.1384</td>
<td>0.1095</td>
<td>9.1509</td>
<td>0.4020</td>
</tr>
<tr>
<td>Attenuation (dB)</td>
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<td>0.1802</td>
<td>-0.1833</td>
<td>0.1256</td>
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<tr>
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<td></td>
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<tr>
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<td>0.1220</td>
<td>0.0031</td>
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<td>0.0110</td>
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<tr>
<td>Transmission Coefficient</td>
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<tr>
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<td>0.0278</td>
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<td>0.4033</td>
<td>9.2608</td>
<td>0.7011</td>
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<td>0.1801</td>
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<td>0.2927</td>
<td>-0.1335</td>
<td>0.1152</td>
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<tr>
<td>Z Shift (cm)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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</tbody>
</table>

**ATTENUATION**

Averaged over all trials and input voltages, the total attenuative acoustic loss from the transducer to the physiologic target, after passing through the acoustic coupler and the lower lamina of the skull into the skull cavity was found to be 64.85% ± 1.15 (8.72 dB ± 0.51) with respect to transmitted pressure, and
86.48% ± 0.87 (8.46 dB ± 0.34) with respect to intensity for the skull from Subject #4, and 65.27% ± 2.77 (9.23 dB ± 0.70) and 87.81% ± 1.1 (9.15 dB ± 0.4) for pressure and intensity respectively for Subject #5. Averaged over both samples, the average transmission coefficient for intensity measures was found to be $T_I = 0.1298 ± 0.0099$ and that for pressure based measurements to be $T_P = 0.3536 ± 0.00212$.

As expected, similar loss figures in dB were measured for both intensity and pressure. Notably, the transmission and attenuative loss values are also broadly consistent for both samples of the intact skull. No correlation between transducer driving voltage and the attenuative loss, or pulse duration and loss was observed.

As further expected, the vast majority of the loss in acoustic energy occurs at the transition across the skull, with roughly 8.5 dB of energy lost at this junction. On average, approximately 14% of the acoustic intensity incident on the skull is transmitted through.

Compared to the theoretical values calculated in Section 5.5.1 for the simplified Water to Bone to Water model ($T_I = 0.31, \alpha_{dB} = 5.09$), the measured average transmission coefficient for both skulls is more than half that of the theoretical transmission coefficient. This means more energy than expected was lost transitioning the lower laminal interface.

This is not surprising, as the simple theoretical values calculated earlier do not account for attenuative and resonant losses within the thickness of the laminal layer or wave-mode conversion. The critical angle of incidence from
normal for transverse wave propagation predominating relative to longitudinal at the water/bone interface is approximately 21.85° [50]. Based on the curved nature of the lower lamina, it is possible that some portion of the acoustic energy is meeting the skull at greater than this critical angle of incidence, producing transverse waves within the skull which do not reach the target. Further exploration and modeling would be needed to explore this possibility.

**AXIAL ACOUSTIC PARAMETER MAXIMA**

In both skulls, the z-axis position of maximum pressure and intensity with the intact skull and coupler in the acoustic path relative to no skull was found to shift up slightly (closer to the transducer). However, this shift, in the case of intensity ranging from -3.75 mm ± 0.050 mm to -1.335 mm ± 0.116 mm for both skulls was well within the length of the acoustic focus and not expected to have a significant effect of beam targeting.

This variability in calculated axial shift is most likely an artifact of the measurement error of the AMS system when measuring axial acoustic fields without much variation. As shown by Figure 5-19, measurements taken through the transducer + coupler + skull complex exhibit less variability and do not have the central notch that control measurements of the transducer, and the transducer with coupler do.

The location of the z-axis maximum was found to be independent of the driving voltage or pulse duration used. No correlation between these measures and the resulting position of the maximum was found.

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The lack of an appreciable z-axis shift in the location of maximum pressure and intensity from these intact skulls measured using experimentally-analogous targeting is more compelling than the shifts measured in the lower-lamina fragments.

**FOCUS WIDTH**

Raster scans were performed at the axial position of maximum intensity with and without the intact skull from Subject #4 to ascertain the effect of the

**Effect of Coupler/Skull on Axial Measurements**

Figure 5-19: Measured intensity (Pii) along the Z-Axis for the transducer only, the transducer and coupler, and the transducer-coupler-skull acoustic pathway. Measurements taken at an input voltage of 0.08 V. Skull used is the intact skull from Subject #4.
intact skull on beam focus width. The geometry of the skull from Subject #5 did not allow for a raster to be performed safely within the confines of the braincase. The normalized raster in the X-Y plane of the Transducer and Coupler is depicted in Figure 5-20 and that of the transcranial Transducer-Coupler-Skull pathway in Figure 5-21.

The acoustic focus was considered according to the FWHM criteria (width over which the peak intensity drops by half). Focus diameters, as well as the positive and negative extent of the focus in both the x- and y-axis relative to the beam center axis are tabulated in Table 5-9. Measured values of the focus diameter were within half a millimeter for the with- and without-skull cases. The location of the acoustic focus along the axis of wave-propagation was also found to have shifted by two millimeters.

<table>
<thead>
<tr>
<th></th>
<th>Transducer + Coupler</th>
<th>Transducer + Coupler + Skull</th>
</tr>
</thead>
<tbody>
<tr>
<td>z @ Max Intensity (cm)</td>
<td>6.1</td>
<td>6.3</td>
</tr>
<tr>
<td>-x (mm)</td>
<td>-2.35</td>
<td>-1.6</td>
</tr>
<tr>
<td>+x (mm)</td>
<td>1.3</td>
<td>1.7</td>
</tr>
<tr>
<td>x-axis Focus Diameter (mm)</td>
<td>3.65</td>
<td>3.3</td>
</tr>
<tr>
<td>-y (mm)</td>
<td>-1.76</td>
<td>-1.5</td>
</tr>
<tr>
<td>+y (mm)</td>
<td>1.45</td>
<td>1.9</td>
</tr>
<tr>
<td>y-axis Focus Diameter (mm)</td>
<td>3.21</td>
<td>3.4</td>
</tr>
</tbody>
</table>
Figure 5-20: Isoline representation of measured X-Y raster (transducer and coupler) at the axial location of maximum intensity (z = 6.1 cm) normalized to the peak intensity in the plane. The focus diameter (FWHM) is 3.65 mm in the x-axis, and 3.21 mm in the y-axis.
Figure 5-21: Isoline representation of measured X-Y raster (transducer, coupler, skull) at the axial location of maximum intensity (z = 6.3 cm) normalized to the peak intensity in the plane. The focus diameter (FWHM) is 3.3 mm in the x-axis, and 3.4 mm in the y-axis. Isoline representation depicts a tight, narrow focus of the acoustic energy, and faster fall-off relative to the no-skull case.
Chapter 6: Pilot Feasibility Study of

Ultrasonic Stimulation of the Hypothalamus

A pilot feasibility study (n = 5) in the Göttingen minipig has been performed to evaluate the targeting approach, and acoustic characterization detailed in Chapter Five. This limited study has provided preliminary evidence demonstrating and validating that our systems and techniques, in combination with MRI-guided brain targeting may be used to elicit ultrasound-evoked physiological responses. A variety of acoustic pulses of varying parameters were targeted to the dorsomedial hypothalamus of the anesthetized minipig. Changes in heart rate (HR) and blood pressure (BP) were recorded coincident with targeted hypothalamic ultrasound stimulation. This effort is the first published lower-intensity transcranial ultrasonic stimulation in a larger animal model [148].

6.1 Hypothesis and Metrics

This goal of this engineering validation study will be to attempt the transcranial ultrasonic stimulation of the hypothalamus.

The hypothalamus is the body’s autonomic control center and as such controls several vital cardiovascular physiologic body functions, and the body’s
cardiovascular response to stress [149]. It stands to reason that hypothalamic stimulation would alter these vitals.

Furthermore, studies of hypothalamic stimulation in the vervet model [150] and minipig have demonstrated changes in heart rate and blood pressure with DBS. It was found that dorsal stimulation of the hypothalamus resulted in increases in heart rate and blood pressure above baseline ranging between 4-23% and 10-72% respectively [150]. This response was found to be dependent on stimulation voltage amplitude. Based on the similarity of early reported ultrasound stimulatory effects compared to DBS, it was hypothesize that ultrasound can be used to stimulate the hypothalamus.

Several assumptions underlie this study of hypothalamic neurostimulation. The hypotheses embodied in these assumptions and the metrics for testing the hypotheses are as:

Hypothesis 1: Ultrasonic stimulation of the hypothalamus will lead
to a temporally correlated change in heart rate and/or blood pressure.

Metric: Vitals will change by 5% from baseline in response to stimulation.

Hypothesis 2: Heart rate and blood pressure will increase with
hypothalamic stimulation.

Metric: Change in vitals will be positive (vitals will elevate).

Hypothesis 3: Heart rate and blood pressure change will be
reversible
Metric: Subsequent to cessation of stimulation, vitals will return to baseline.

Hypothesis 4: Ultrasound induced vital change will occur at low intensity.

Metric: Low intensity will be defined as an $I_{SPTA} < 500 \text{ mW/cm}^2$.

Hypothesis 5: Targeted transcranial ultrasound stimulation is possible through a large animal cranium using a focused single-element transducer.

Metric: Shift in acoustic focus location will be $<0.5 \text{ cm}$ on all axes.

Hypothesis 6: Stimulatory ultrasound parameters will be below the threshold for tissue damage.

Metric: Stimulus parameters will have a mechanical index lower than the FDA threshold of 1.9 [151].

6.2 Experimental Materials and Methods

Five Göttingen minipig subjects (referred to as Subject #1-5) were enrolled in this study of hypothalamus-targeted ultrasonic neurostimulation. One subject experienced a failure of the stimulation equipment (Subject #3), while another failed to achieve stable physiologic baseline and yielded limited data (Subject #5) and were excluded from analysis. All experiments were conducted under university-approved protocols and in accordance with all animal health, safety,
and welfare guidelines and regulations. Animal methods and techniques were performed using previously validated experimental procedures [150, 152-155].

6.2.1 Subject Population:

Sexually mature adult female Göttingen minipigs (Marshall BioResources, North Rose, NY) at the age of 7 months and weighing 25 – 30 kg were entered into the study. Animal care was provided in accordance with the Guide for Care and Use of Laboratory Animals: 8th Ed. 2011 (National Institutes of Health Publication. Bethesda, MD), and all procedures will be preapproved by the UCLA Chancellor's Animal Research Committee. Full veterinary care was provided by UCLA Department of Laboratory Animals staff.

6.2.2 Stereotactic Target Determination:

Prior to the experiment, an MR scan to identify brain structures was performed. A Radionics MRIA-LF localizer (9 axial fiducials) was attached to the animal prior to MR scanning (Siemens Symphony, 1.5 Tesla). T1 weighted coronal, axial, and sagittal MR sections were obtained throughout the hypothalamus region and a coronal reconstruction generated. Targeting of the hypothalamic region was accomplished according to regional landmarks identified by eye in a published stereotactic brain atlas of the Göttingen minipig species. A corresponding trajectory was planned using the iPlan 2.6 software
(BrainLab, Feldkirchen, Germany), and translated to the localizer coordinate system.

6.2.3 FRAME MOUNTING:

Animals were initially anesthetized with ketamine (20-40 mg/kg i.m.) and maintained on 1-3% isoflurane in 100% oxygen. Arterial and venous catheters were placed in the femoral artery and vein for blood pressure recording and fluid administration. Body temperature, oxygen saturation, pCO₂, and heart rate, and blood pressure were monitored continuously throughout the procedures. A stereotactic head mounting ring is mounted to the animal with four screw pins, with two secured against the dorsal surface of the subject’s skull, and two against the malar bones against either the temporal or zygomatic process. Lidocaine (20 mg/ml) was injected locally at incisions and locations of frame mounting points.

A CT-compatible fiducial localizer is fit to the head mounting frame ring, and a CT scan is performed (GE Signa 1.5T) to register the frame position to the prior MR scan and pre-planned brain trajectory (Figure 6-1). A commercially available human stereotactic device (Cosman-Robert-Wells (CRW), Radionics, Inc.) is subsequently mounted to the head mounting ring and adjusted to correspond to the desired target axis.
Figure 6-1: CT fiducial localizer attached to head mounting ring. Fiducial localizer is the cage-like structure that surrounding the subject head that is mounted to the head-frame. Head mounting ring is secured by four pins, the lower two against the malar bones, and the upper two against the dorsal surface of the skull.
6.2.4 **PARTIAL CRANIOTOMY:**

An incision was made into the scalp exposing the top layer of the skull. A drill incision was made in the outer layer of the trabecular porcine skull, to the level of the lower skull. The lower skull wall was left intact so as to maintain the integrity of the brain cavity. A circular burr-hole approximately 2-in (50.8 mm) in diameter, centered on the axis of the sonication pathway and the transducer was opened. The top surface of the lower laminal layer was smoothed of trabecular ridges using a high-speed rotating surgical bone cutting tool.

6.2.5 **TRANSDUCER POSITIONING AND MOUNTING:**

The transducer was mounted in a custom acoustic coupler, described previously (Section 5.4), and attached to the CRW frame. Degassed ultrasound conductivity gel (Pharmaceutical Innovations Ultra/Phonic Brand) was evenly applied to the bottom of the coupler, and degassed scanning gel

![Figure 6-2: Diameter of partial craniotomy opening through the outer layer of the skull. An opening two inches wide was made to fit the coupler and provide an unimpeded acoustic transmission pathway.](image-url)
(Pharmaceutical Innovations Ultra/Phonic SG Brand) was deposited on the lamina. The coupling assembly was placed into the burr hole and placed against the lamina, therefore ensuring that the transducer was acoustically coupled to the lamina of the skull.

6.2.6 STIMULATOR SYSTEMS

Subjects were stimulated using air-backed 6 cm aperture and 7 cm radius of curvature lead-zirconate-titanate (PZT) ultrasonic transducers driven by a high-power RF amplifier system. Subjects # 1 and # 2 were stimulated using LIFU System 1 (Brainsonix BX1001) and Subjects # 3 – 5 with LIFU System 3.

Subjects # 1 and # 2 were stimulated using Probe A, which was measured as having an in-water focus at 5.6 cm and a -3 dB focus diameter of 5.6 mm.

Subjects # 3 – 5 were stimulated by LIFU Large - f = 650 kHz, D = 6 cm, ROC = 7 cm, [C] V3 and LIFU Large - f = 650 kHz, D = 6 cm, ROC = 7 cm, [D] V3, which were electrically matched as appropriate to the stimulator system, which were measured as having a in water focus at 6 cm and a focus diameter of 4.2 mm.

6.2.7 VITAL RECORDING

For the first three subjects, vitals were documented through transcription of the heart rate and blood pressure as displayed from a commercial veterinary physiologic monitor. For Subjects # 4 and # 5, a custom Labview script was used
to digitize and automatically record the vitals being measured by a different commercial veterinary monitor (SurgiVet ADVISOR, Smiths Medical) over a serial data link at 1 Hz. The capability to record real-time ECG waveforms was added for Subject # 5 by recording and digitizing raw ECG voltage traces from a second ADVISOR monitor at 1 kHz.

6.3 Results of Stimulatory Trials

Targeted ultrasound stimulation using the surgical stereotactic targeting described above was observed to have a temporally correlated neuromodulatory effect on three out of the five subjects exposed to targeted ultrasonic stimulation.

Exploratory trials were conducted on the first subject at a center insonation frequency of 650 kHz. A set of parameters (25 kHz PRF, N c/p = 10, 15.38 µs PD) that induced ultrasound-correlated gross motor motion was discovered. Based on this initial subject, a pulsing scheme corresponding to a PRF of 25 kHz and 25 cycles per pulse (PD = 38 µs) was discovered that appeared to lead to temporally correlated changes in vitals. Subsequent subjects were insonated using the same pulsing scheme, with the only variable being the power applied to the transducer, and therefore the applied intensity. Results from the successful trials subjects are discussed below.
6.3.1 Subject # 1

Two sets of parameters were discovered that appeared to cause temporally correlated qualitative gross motor motion in the subject. All motion was observed at an isoflurane concentration of 1.5%.

<table>
<thead>
<tr>
<th>Parameter #</th>
<th>Vin (Vrms)</th>
<th>f (kHz)</th>
<th>PRF (kHz)</th>
<th>N (c/p)</th>
<th>PD (us)</th>
<th>Duty Factor</th>
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<td>0.15</td>
<td>650</td>
<td>25</td>
<td>10</td>
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<td>0.38</td>
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<td>650</td>
<td>25</td>
<td>10</td>
<td>15.38</td>
<td>0.38</td>
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Using parameter P1_1 at a focus depth of z = 35 mm, oscillatory movement of the hind legs was observed, with the right hind leg moving first, followed by the left. The parameter and depth combination was repeated three times, with hind leg motion commencing between approximately 90 – 138 s into a 150 s stimulation train. Shaking appeared to cease approximately 95 – 150 s following the end of stimulation.

Using parameter P1_2 at a focus depth of z = 35 mm, corresponding to the expected location of the hypothalamus, three trials of 150 s stimulation duration were attempted. Similar gross motoric motion was observed in each of these trials, with motion commencing after approximately 120 seconds of stimulation, and ending approximately 118 seconds after stimulation cessation.

Two trials each were conducted using P1_2 at a focus depth of z = 30 mm, and z = 40 mm, bracketing the focus depth previously used. No visible motoric motion was observed at either of these focus depths.
Another series of three stimulation trials in quick succession was attempted using P1_2 at a focus depth of $z = 35$ mm and a stimulation duration of 150 s. Similar to the initial set, the right leg was observed to start oscillating, followed by the left leg. When the stimulations were provided in quick succession, the time to observable motoric motion appeared to decrease. A final 180 s long stimulation trial was attempted, resulting in strong hind-limb oscillatory motion, and defecation, and a marked increase in heart rate. Movement stopped following the cessation of sonication.

Stimulation was observed to have a qualitative effect on the heart rate. The low fidelity of heart-rate measurement in this subject precludes a quantitative analysis. The subject’s resting, non-stimulated (control) vitals were considered highly stable, with average control heart rate, systolic, diastolic, and mean blood pressure values of 90 BPM ± 1, 62 mmHg ± 1.7, 49 mmHg ± 1.3, 55 mmHg ± 1.6 respectively were measured. Ultrasonic stimulation was observed to gradually raise the heart rate, with these changes subsiding after the cessation of stimulation. Gross motoric motion only appeared to occur after the heart rate crossed the threshold of roughly 94 BPM. Averaged over all the successful trials, heart rate was measured as being 94 BPM ± 1.4, with systolic, diastolic, and mean blood pressures of 59 mmHg ± 4.3, 47 mmHg ± 4.7, 52 mmHg ± 3.9 respectively. However, the low temporal fidelity of vitals measurements does not allow for finer-grain analysis, and in most cases, the magnitude of change observed was small, generally being less than 3 BPM.
6.3.2 Subject # 2

A set of pulse parameters and targeting parameters (P2_2 - Vin = 275 mV_{p-p}, 25 cycles per pulse, 25 kHz PRF) were identified that appeared to cause reversible and repeatable neuromodulation in the minipig subject at a target depth of z = 30 mm. Upon LIFU delivery to the hypothalamus region for dose durations of 90 s, sustained increases in blood pressure and heart rate were observed. Vitals were observed to return to baseline subsequent to stimulation cessation.

<table>
<thead>
<tr>
<th>Parameter #</th>
<th>Vin (Vrms)</th>
<th>f (kHz)</th>
<th>PRF (kHz)</th>
<th>N (c/p)</th>
<th>PD (us)</th>
<th>Duty Factor</th>
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<td>25</td>
<td>25</td>
<td>38.46</td>
<td>0.96</td>
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<tr>
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<td>25</td>
<td>10</td>
<td>15.38</td>
<td>0.38</td>
</tr>
</tbody>
</table>

Figure 6-3 represents repeat 90 second total dose stimulation trials using parameter set P2_2 at a target depth of z = 30 mm. Similar results were obtained for five successive trials. When each stimulation trial was compared to a control using a t-test, the null hypothesis of no ultrasonic effect can be rejected for all trials at the p <0.05 level, indicating that ultrasound had a statistically significant effect on measured vitals. The parameter set P2_2 increased the heart rate on average by 9.4 bpm ± 2.88, corresponding to a change of 5.78% ± 1.8. The average control (non-stimulated) heart rate for this period was 161 bpm ± 1.64%. Blood pressure was observed to obey roughly the same relationship.
Figure 6-3: Repeated stimulation trials using parameter P2_1, at a target depth of $z = 30$ mm resulted in statistically significant increases in heart rate. Top and Bottom plots are two runs out of five sequential trials that resulted in a heart rate rise >5% Starts at 0 seconds, and runs for 90 seconds (denoted by the blue shaded area).
A trial at parameter set P2_3 at a the same target depth of \( z = 30 \text{ mm} \), with a smaller N was attempted, and demonstrated no change in heart rate or blood pressure (Figure 6-6). This trial also demonstrated the stability of the vitals without ultrasonic stimulation (Mean HR = 160 bpm ± 1.72, Mean BP = 64 mmHg ± 1.0).

A longer stimulation trial of 300 s duration was attempted using the same P2_2 and \( z = 30 \text{ mm} \) set of parameters and target depth (Figure 6-4). A temporally correlated rise in vitals was again observed to occur, appearing

![Graph](image.png)

**Figure 6-4:** 300 second stimulation trial of Minipig Subject #2 using parameter set P2_2 at a target depth of \( z = 30 \text{ mm} \). Vitals were found to increase with stimulation, but plateau. Vital parameters returned to baseline at the conclusion of the trial. Dip in vitals at the 120 second mark occurs both in heart rate and blood pressure, and is unlikely to be sampling error.
initially similar in character to that of the 90 s stimulation trials. However, following 90 s of stimulation, vitals dropped somewhat, only to rise again and plateau. Vitals subsequently returned to resting baseline at the cessation of stimulation.

Using P2_2 targeted to a depth of $z = 40$ mm, elevation of heart rate and blood pressure ($\Delta = 13$ bpm, 20 mmHg) were also observed to occur, following a similar vital rise pattern as that observed at a target depth of $z = 30$ mm (Figure 6-5).

A single increase in vitals was also observed for parameter set P2_1, but was not repeatable.
Figure 6-5: Stimulation trial using parameter set P2_2 at a target depth of $z = 40$ mm. Similar vital rise pattern was observed as $z = 30$ mm target depth.

Figure 6-6: Trial of parameter set P2_3, targeted to $z = 30$ mm. No appreciable rise in vitals was observed. Time averaged intensity delivered was roughly half that at of the successful parameter set P2_2, with similar pulse-averaged intensity and peak negative pressure.
### 6.3.3 Subject # 4

#### Table 6-3: Subject # 4 Parameters of Interest

<table>
<thead>
<tr>
<th>Parameter #</th>
<th>Vin (Vpp)</th>
<th>f (kHz)</th>
<th>PRF (kHz)</th>
<th>N (c/p)</th>
<th>PD (us)</th>
<th>Duty Factor</th>
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</thead>
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<td>24.7</td>
<td>38</td>
<td>0.95</td>
</tr>
<tr>
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<td>450</td>
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<td>15.75</td>
<td>38</td>
<td>0.875</td>
</tr>
<tr>
<td>P4_3</td>
<td>0.11</td>
<td>650</td>
<td>25</td>
<td>24.7</td>
<td>38</td>
<td>0.95</td>
</tr>
<tr>
<td>P4_4</td>
<td>0.13</td>
<td>650</td>
<td>25</td>
<td>24.7</td>
<td>38</td>
<td>0.95</td>
</tr>
</tbody>
</table>

Several temporally correlated rises in heart rate were observed at 650 kHz with the PRF set to 25 kHz, and the pulse duration set to 38 µs. When parameter P4_1 was used in conjunction with electrically matched Probe C V2 at an anesthesia level of 1.75% isoflurane, heart rate was measured to rise over 5% twice in four back-to-back periods of stimulation (rises of 5.07% and 6.20% respectively). Notably, out of the four trials, stimulation failure (<5% rise in heart rate) occurred immediately following successes. When corrected to account for a gradual linear shift in the vital baseline ($\chi^2$ test: $p < 0.01$), the standard deviation of the entire test period was found to be 2.25% of the mean. When this test period is analyzed using a non-parametric Mann-Whitney Rank-Sum test, the null hypothesis that the medians of the on and off periods are equal can be rejected (uncorrected: $p < 0.01$, corrected $p = 0.0017$).

A similar increase of 7.76% was observed using similar pulse parameters, but a center frequency of 450 kHz using the unmatched Probe D v2 (Parameter P4_2) at an isoflurane level of 1.5% (Figure 6-7). When the ultrasound stimulation portions of this test segment are grouped and compared with the
grouped control (no ultrasound) portions, a significant difference) between the means (paired t-test, p < 0.001 and medians (Mann-Whitney, p <0.001) of the two groups is revealed. A repeated attempt following the successful trial, but subsequent to an anesthesia transient was not successful.

Increases in heart rate were also observed using Probe D v2 with an electrical match using parameters P4_3 and P4_4 at an anesthesia level of 1.75 % isoflurane (Figure 6-8). Parameter P4_3 induced a 5.46% change in heart rate. Using parameter P4_4, two trials were conducted which both demonstrated

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**Figure 6-7:** Test segment of Subject #4 subjected to insonation at f = 450 kHz, PRF = 25 kHz, and PD = 38 µs (P4_2). A 7.77 % change in heart rate (denoted by the label “effect”) was observed. A repeated subsequent attempt showed no such change in heart rate, but was confounded by an anesthesia transient.
increases in heart rate greater than 5% (9.65% and 5.21% respectively). Parameter P4_4 corresponds to the highest power test run conducted on Subject 4, and is the only parameter set in this subject to result in repeatable heart rate increases using the same parameters.

### 6.4 Discussion

In this study, temporally correlated cardiovascular vital elevation with ultrasonic neurostimulation transcranially targeted to the hypothalamus was observed. This observation, along with the effects of acoustic energy, pulse
character, frequency, and anesthesia on this and other observations, and their repeatability are discussed below.

Based on acoustic tests of intact excised minipig skulls detailed in Section 5.5.3, the overall transmission coefficients between the transducer and the in-brain physiologic target was found to be 0.1298 ± 0.0099 for pressure and 0.3536 ± 0.00212 for intensity. Based on water tank measurements of the free-water output of the transducer and the average attenuation of the acoustic beam by the skull calculated, in-brain on target values for acoustic intensity, pressure, and mechanical index for the critical parameters identified in Section 6.3 were calculated, and are tabulated in Table 6-4, Table 6-5, and Table 6-6.
Table 6-4: Subject # 1 Calculated Acoustic Intensities

<table>
<thead>
<tr>
<th>Parameter #</th>
<th>ISPTA (mW/cm²)</th>
<th>ISPPA (W/cm²)</th>
<th>Pr (MPa)</th>
<th>MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1_1</td>
<td>160,221.90</td>
<td>416.58</td>
<td>3.98</td>
<td>4.32</td>
</tr>
<tr>
<td>P1_2</td>
<td>243,715.31</td>
<td>633.66</td>
<td>4.91</td>
<td>5.32</td>
</tr>
<tr>
<td>P1_3</td>
<td>31,634.25</td>
<td>82.25</td>
<td>1.74</td>
<td>1.88</td>
</tr>
</tbody>
</table>

Table 6-5: Subject # 2 Calculated Acoustic Intensities

<table>
<thead>
<tr>
<th>Parameter #</th>
<th>ISPTA (mW/cm²)</th>
<th>ISPPA (W/cm²)</th>
<th>Pr (MPa)</th>
<th>MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>P2_1</td>
<td>39,242.51</td>
<td>102.03</td>
<td>1.97</td>
<td>2.14</td>
</tr>
<tr>
<td>P2_2</td>
<td>168,237.80</td>
<td>174.97</td>
<td>2.58</td>
<td>2.80</td>
</tr>
<tr>
<td>P2_3</td>
<td>67,295.12</td>
<td>174.97</td>
<td>2.58</td>
<td>2.80</td>
</tr>
</tbody>
</table>

Table 6-6: Subject # 4 Calculated Acoustic Intensities

<table>
<thead>
<tr>
<th>Parameter #</th>
<th>ISPTA (mW/cm²)</th>
<th>ISPPA (W/cm²)</th>
<th>Pr (MPa)</th>
<th>MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>P4_1</td>
<td>65,970.00</td>
<td>69.44</td>
<td>1.59</td>
<td>1.73</td>
</tr>
<tr>
<td>P4_3</td>
<td>9,643.23</td>
<td>11.02</td>
<td>0.77</td>
<td>1.05</td>
</tr>
<tr>
<td>P4_3</td>
<td>55,342.68</td>
<td>58.26</td>
<td>1.47</td>
<td>1.60</td>
</tr>
<tr>
<td>P4_4</td>
<td>77,296.80</td>
<td>81.37</td>
<td>1.74</td>
<td>1.89</td>
</tr>
</tbody>
</table>
6.4.1 Vital Elevation

Several parameters were identified that caused a measurable and significant change in heart rate of 5% or above. When a vital change was observed with ultrasonic stimulation, the heart rate was observed to rise in the positive direction.

Parameter set P2_2, which induced reversible and repeatable changes in heart rate and blood pressure was calculated as having an intensity at the target of 21.8 W/cm\(^2\) \(I_{\text{SPTA}}\) and 22.71 W/cm\(^2\) \(I_{\text{SPPA}}\), with a peak rarefaction pressure of 0.91 MPa. This corresponds to a calculated MI of 0.99. Similarly, parameter set P4_4 was observed to cause a similar reversible and repeatable increase in heart rate.

A single increase in vitals was also observed for parameter set P2_1 (\(I_{\text{SPTA}} = 5.01\) W/cm\(^2\), \(I_{\text{SPPA}} = 13.24\) W/cm\(^2\), \(P_r = 0.70\) MPa), and P4_3 (\(I_{\text{SPTA}} = 7.18\) W/cm\(^2\), \(I_{\text{SPPA}} = 7.56\) W/cm\(^2\), \(P_r = 0.56\) MPa). Out of four successive trials, P4_1 (\(I_{\text{SPTA}} = 8.56\) W/cm\(^2\), \(I_{\text{SPPA}} = 9.01\) W/cm\(^2\), \(P_r = 0.56\) MPa) was able to cause a significant rise in heart rate on alternating trials.

While the intensity levels found to cause stimulation are higher than some reported values, namely the intensities in the mW/cm\(^2\) range reported by Tyler, they are within the range reported by others, namely King, (AI < 16.8 W/cm\(^2\)) [128], and Yoo (\(I_{\text{SPTA}} \sim 4.5 - 9.1\) W/cm\(^2\), W/cm\(^2\) \(I_{\text{SPPA}} \sim 8.6 - 12.6\) W/cm\(^2\)) [126, 156] for some of their experiments.
Furthermore, all in-brain parameters used in this study are below the FDA standard for maximum allowable MI of 1.9. Parameter P2_2 was calculated as having an on-target MI of 0.99, while parameter P4_4 was calculated as having an MI of 0.67. These values indicate that the risks of ultrasound-induced damage to the brain tissue of the animal are minimal.

The 5% rise in heart rate threshold was initially an arbitrarily defined metric based on the low-end of observed changes in heart-rate with DBS stimulation and the observed short-term variability in a stable subject’s vitals. However, a greater than 5% change was observed and determined to statistically significant at several points during the present study. It is worth noting though, that this is a relative measurement – the vital change requires an initial reference point from which to determine the change. Even in the stable subjects, gradual shifts in vitals (~ 30 bpm) were observed over the entire hours-long course of the experiment. Vital baselines to calculate percent change were therefore drawn from temporally close or paired control periods immediately preceding or following the stimulation.

Even with vital drift, the measured variability of the controls can be considered significant. For example, the average measured changes in heart rate induced by parameter set P2_2 in Subject #2 was 5.78% ± 1.8 with the standard deviation of the unstimulated controls from that period being 1.64%. The average change in heart rate is significant, lying outside of 2σ from the control. Each of these trials was also individually t-tested against a control and were individually all significant at the p < 0.05 level.
The 5% change in heart rate metric also appears to be valid and significant in Subject #4, as the standard deviation, when corrected to account for the linear drift in vitals was found to lie just outside the $2\sigma = 4.50\%$ level of variation from the control. Various tests of means and medians were also used to provide additional evidence that the vital changes observed were significant.

6.4.2 Relationship Between Acoustic Energy and Vital Elevation

The occurrence of, and repeatability of the stimulation appear to be tied to the acoustic energy delivered to the focus.

When the pulsing parameters were kept constant and only the voltage input to the transducer, and therefore the output intensity and pressure was varied, effects did not appear to be on a continuum; instead they appeared to be all or none past a certain threshold. This is similar to King’s observations regarding the occurrence of an ultrasound-modulated EMG response [128]. The existence of an ultrasonic threshold, either pressure or intensity, would be consistent with the observations of Younan et al. [129]. Increasing the acoustic energy delivered was observed to increase the likelihood of seeing a neuromodulatory effect, also consistent with the observations of several investigators [44, 128, 156]. However, it must be noted that these comparisons assume equal ultrasonic effect across brain regions, which has not yet been explored or demonstrated.
6.4.3 Pulse Character and Vital Elevation

For the pulses in Subjects #2 and #4 that demonstrated a neuromodulatory outcome, the duty cycles tended to be high and as a result, the pulse-average and time-average intensities are nearly comparable and the stimulation wave behaves similarly to a continuous-wave signal.

An experiment using parameter set P2_3 targeted to the same location as P2_2, a stimulation parameter set that yielded repeatable results, and delivering the same pulse-averaged intensity and peak negative pressure ($I_{SPPA} = 22.71$ W/cm$^2$, $Pr = 0.91$ MPa) but less than half the $I_{SPTA}$ (8.73 vs 21.84 W/cm$^2$) demonstrated no significant change in heart rate and blood pressure for the lower $I_{SPTA}$ pulse.

Two possible conclusions can be drawn from this, first that the occurrence of a modulatory effect is dependent on the duty cycle, and second that a critical threshold exists for the spatial peak, pulse average intensity ($I_{SPTA}$). The first conclusion would seem to be supported by the work of King who observed a greater likelihood of neuromodulation at higher duty cycles tending towards continuous-wave stimulation [128] and Yoo, who observed that stimulatory pulses tend towards higher duty cycles [156]. Further testing, comparing short duty cycle and long duty cycle pulses generating the same $I_{SPTA}$ will be necessary to shed light on, and draw conclusions from this observation.
6.4.4 Effect Frequency Dependence

A series of lower frequency 450 kHz sonications was conducted on Subject #4 using the same PRF and PD as the rest of the subject trials, which were conducted at 650 kHz. Parameter P4_2, a trial at 450 kHz induced a significant 7.77% change in heart rate at a substantially lower intensity (1.25 W/cm² ISPTA, 1.43 W/cm² ISPPA) than the other successful stimulation trials in the same subject conducted at 650 kHz. The time-averaged intensity at 450 kHz is ~12.5% lower than the lowest intensity that resulted in a heart rate change at 650 kHz.

This observation is consistent with the notion that ultrasonic neuromodulation is dependent on fundamental frequency. There is growing consensus in the literature that ultrasonic neuromodulation is more likely to occur, and be induced by lower intensities at lower frequencies. King has reported that higher stimulation intensities are required at higher frequencies [128], and Yoo has demonstrated effects that only occur at certain frequencies (350 kHz) that cannot be induced at others (650 kHz), even when substantially higher intensities are used [126].

While transcranial transmission should be nominally higher at lower frequencies, the width of the focus point will also widen, resulting in energy deposition over a larger area. Without further analysis, it is unclear which, if any of these physical effects, or some sort of actual physiologic link to a certain frequency is the source of this observation.
6.4.5 ANESTHESIA

The role of anesthesia is a major, yet unavoidable confounding factor in this study. Anesthesia has a known and direct impact on the cardiovascular vital measures being considered. However, each animal’s tolerance to anesthesia was found to be variable. In this present study, subjects #1 and #2 were found to tolerate a minimum isoflurane anesthesia level of 1.5% while Subject #4 was maintained at 1.75%.

The animal must be kept comfortably sedated on humanitarian grounds for purposes of pain management. However, there is now strong evidence in the literature on the adverse effect of anesthesia on observing neuromodulatory outcomes. King has conclusively demonstrated the impact of anesthesia on ultrasound induced neuromodulation and that higher anesthetic doses can blunt neuromodulatory effects which are observable at lower concentrations [128]. Yoo has also demonstrated that ultrasonic neurostimulation impacts the effect of the anesthesia, and can induce quicker recovery from a fixed anesthetic dose [125]. This effect has also been noted by Tyler [44, 123]. This may explain the instability observed in the animals under ultrasonic neurostimulation and the difficulty experienced in keeping them at a steady baseline.

Maintenance of a stable, yet light anesthetic plane was one of the major challenges in the study. Significant time per subject was spent trying to achieve such a plane. Even with anesthesia maintained at a constant level, subject vitals were observed to gradually fluctuate over the many-hour course of the
experiment. Possible alternate methods, contingent upon institutional review-board approval include the use of different anesthetic agents, the use of interperitoneal anesthetic delivery, and/or the simultaneous delivery of analgesic agents.

6.4.6 Visible Motion

Parameter set P1_2, which was observed to cause repeated strong motoric motion (oscillation of the hind limbs, followed by the body) in the first subject, was calculated as generating on-target intensities of approximately $I_{SPTA} = 31$ W/cm$^2$, $I_{SPPA} = 82.25$ W/cm$^2$ and a maximum rarefaction pressure of $P_r = 1.74$ MPa. While higher than the intensities reported in the LIFU literature to date and the highest reported in this present study, this parameter set does not exceed the FDA index for mechanical damage ($MI = 1.88 < FDA \ MI = 1.9$). Even at the intensities the brain is subject to using P1_2, cavitation damage is judged to be unlikely. Future post-mortem histological analysis should be conducted to confirm this judgment.

There are several possible explanations for the observed motion with hypothalamic targeting, and the fact that it occurred in only a single subject. It is possible that the acoustic beam was off-target, and was actually impinging upon different neuroanatomy. Notably, the visible motion in Subject #1 only occurred at a specific target depth ($z = 35$ mm), and not when the ultrasound was bracketed above ($z = 30$ mm), and below ($z = 40$ mm). Even if the beam was correctly targeted, the acoustic path passes through the motor cortex. At the
intensities used, it is conceivable that enough acoustic energy impinged upon the motor cortex to cause motor stimulation.

While the defecation and other qualitative animal behaviors demonstrated by this animal are similar to those documented in trials of hypothalamic DBS stimulation \[150\], the parameters used for this animal are notably higher than other recent studies considered to be part of the low-intensity ultrasonic neuromodulation paradigm. It is possible that more subtle changes in heart rate were observed in this animal at lower intensities, but were lost due to the low sampling rate of vital collection, and that motor stimulation is only observed at higher intensities. Yoo has reported that higher intensities are necessary to see gross motor motion in comparison

It is also conceivable that the observed motion is the result of induced seizure activity. This might explain why the motor motion appeared to begin in roughly the same location every time (hind legs), and the progress to the rest of the body with continual stimulation. There is preliminary evidence in the literature that ultrasound, especially at higher duty cycles/CW can trigger seizure activity \[123\]. This potential finding should be confirmed with neurological recording of brainwave/electrical activity.

### 6.4.7 Repeatability

While P2_1, P4_1, and P4_3 seem to cause a measurable heart rate, this change does not appear to be immediately repeatable at intensities below \~10 W/cm². These results would also seem to provide corroboration to the
observations by Tufail that not all neuromodulation parameters work every single time [44], and put in context the neuromodulatory outcome success rate of 60% reported by Younan [129]. No clear explanation for this documented and observed lack of repeatability is apparent.

When both Subject #2 and #4 were sonicated at such intensities, changes in heart rate were generally observed the first time the parameter at that power level was used, but not with an immediately repeated trial. Subject #4 (P4_1) seemed to indicate that if sufficient time is given between stimulation trials at these power level, a measurable rise in heart rate returns. This may be indicative of some sort of biological relaxation, acclimatization, or homeostatic process at work and is worth further study.

6.5 Conclusion

This study has provided preliminary pilot data that ultrasound may be able to induce neuromodulation in the Göttingen minipig. We have observed that the heart rate may be induced to increase with transcranial ultrasonic stimulation targeted to the hypothalamus of the Göttingen minipig at a fundamental frequency of 650 kHz using a high-duty cycle pulsing scheme with a PRF of 25 kHz and pulse duration of 38 µs (N = 25), when delivered through the surgical procedure and acoustic coupler whose development was detailed in Chapter Five. However, the low sample size of this study, the potential for variability within the test protocol, and the preliminary nature of these results should be kept in mind.
Revisiting the hypotheses underlying this study, ultrasound sonication parameters have been identified that appear to lead to a temporally correlated statistically significant increase in heart rate, in excess of 5% with transcranial ultrasonic stimulation. However, these parameters are in excess of the established threshold of $I_{SPTA} < 500 \text{ mW/cm}^2$, yet still within the FDA limits on mechanical index.

While a neuromodulatory effect in the large animal Göttingen minipig model has been demonstrated, this model suffers from several shortcomings. Measurements on additional subjects to build up statistical power were not possible due to the high cost per subject for this large-animal surgical procedure. While this study has demonstrated the ability to accurately target brain regions through the lower lamina of the skull in the minipig following a partial craniotomy, there is no guarantee that the target brain region is actually responsible for the observed effects. Furthermore, the transcranial process adds significant burden to the stimulator system, as roughly 87% of the sonication intensity generated by the transducer is lost transitioning the skull. Furthermore, the invasive nature of the partial craniotomy requires additional surgical time and skill, adding to study costs. Furthermore, this procedure is highly traumatic to the animal, and can make finding a stable vital baseline difficult.

Using heart rate or blood pressure change as a metric for hypothalamic stimulation has several limitations. The hypothalamus also functions as the central nervous system’s link to the endocrine system, and as such has important hormone secretion functions. Hypothalamic stimulation may cause the secretion
of a large number of hormones which might have unanticipated up- or down-regulation effects or trigger cardiovascular compensatory mechanisms, obscuring changes in heart rate or blood pressure. Indeed, DBS stimulation of the hypothalamus has also been shown to have an impact on complicated measures such as hunger and satiety [150, 157, 158].

While this study has demonstrated the ability to perform transcranial stimulation in a large animal, ultimately this model of ultrasonic neuromodulation is not suited for detailed exploration of the role pulse parameters play in the neuromodulatory outcome. A model with a more direct outcome measure, and one not as sensitive to changes in anesthesia level would be desirable as a medium to follow up on, and further explore the various observations from this present work.
Chapter 7: Design and Construction of an Invasive Microtransducer for Ultrasonic Neuromodulation

Much of the promise for ultrasonic neuromodulation lies in the potential for the technique to be implemented non- or minimally-invasively. However, such approaches suffer from many limitations, as demonstrated by the previously detailed work on the transcranial minipig model. In particular, the difficulty in accurately targeting and focusing known quantities of ultrasonic energy on specific neural structures from outside the skull can complicate even basic exploration of ultrasonic neuromodulation.

The ability to deliver ultrasound directly to target neural structures from within the brain would eliminate many of the complications of transcranial targeting by simplifying the acoustic transmission path. In this chapter, a novel, invasive targeted ultrasonic neuromodulation paradigm is described. This approach was enabled through the development of a new class of low-frequency microtransducer. The design and testing of a prototype series of such transducers is detailed. The invasive targeting approach was experimentally validated in a the rat model.
7.1 Invasive Approach to Targeted Neuromodulation

In comparison to ultrasonic delivery from outside the brain, an invasive approach has several unique advantages and disadvantages. Directly apposing the probe to the neural structure in question may allow for very precise targeting. In addition, an invasive approach should be less demanding from a transducer energy delivery perspective. As the highly reflective transcranial transition step is eliminated and the attenuative path length between the transducer and target is decreased, total acoustic loss from the transducer to the target should also decrease. This would require less acoustic energy to be generated by the transducer to have an equivalent effect at the target. This lower intensity requirement may allow for improved specific in the targeting of neural structures.

However, there are also several limitations inherent to an invasive approach. There is a risk of brain tissue trauma due to the presence of the probe within the brain. Considering the approximately centimeter size of most small-animal brains, even small, millimeter-scale probes would disrupt a relatively large amount of tissue. This potential risk of brain trauma thus requires any prospective invasive ultrasound probe to be as small as possible. This size restriction can limit the maximum acoustic power of the probe, as the effective radiating area is decreased.

Furthermore, the potential challenges in coupling a probe directly into the brain are not well established. While the brain is known to have similar acoustic properties to water [136], an ex-vivo experiment attempting to study brain-probe
coupling in a water-tank was inconclusive because of the difficulty of maintaining brain position relative to the probe due to the buoyancy of the brain in the water environment (Figure 7-1).

7.2 Invasive Probe for Ultrasonic Neurostimulation

No commercial or research probes were available that met the dual requirements of both small diameter, and low frequency robust sonication necessary for invasive ultrasonic neurostimulation. Furthermore, there is no precedent in the literature for a transducer that would be suitable for this proposed use. As a result, a new class of ultrasonic microtransducer was designed and developed for this application.
Desired specifications included a center frequency between 450 and 750 kHz, a diameter less than 1.5 mm, and the ability to generate pulsed and CW ultrasound with an intensity up to 150 mW/cm$^2$ $I_{SPTA}$, radiated in a narrow, uniform pattern.

Traditional microtransducers typically operate at high frequencies, as there is an inverse relationship between the thickness of the piezoelectric element and its fundamental resonant frequency. Fabrication techniques were developed to adapt microtransducer layouts to the thicker piezoelectric elements necessary for lower frequency use.

7.2.1 Initial Concepts

PZT-based piezoceramic was chosen as the active element material due to its ready availability and desirable mechanical properties. As piezoceramic would need to be on the order of 2-3 mm thick to resonate around the 600 kHz range. The resulting element would be taller than its width, in contrast to most other applications which use short but wide element geometries. This narrow, but tall element geometry cannot be reliable packaged or produced using traditional methods of transducer fabrication, thus requiring the development of new probe fabrication techniques.

Early work focused on the impact various piezo-element packaging structures/configurations would have on the width of the transducer. Packing of the element is necessary to provide both the mechanical support and electrical connections to the element. Two primary approaches to mounting the element
were considered, the internal mounting of the piezoelectric element within a hollow housing (Figure 7-3), and the external mounting of the element on top of a structure (Figure 7-2).

A probe with an internally mounted element was judged to be easier to fabricate, but would require the use of a smaller-diameter element to account for the thickness of the housing, while still maintaining overall probe dimensions with specifications. An externally mounted element, while theoretically allowing for an element very close to the maximum diameter of the probe was judged to require a complicated fabrication method due to the tight tolerances involved.

Other approaches considered included variations of acoustic waveguides and the use of non-standard piezoelectric element geometries, but were rejected after bench top experimental testing revealed poor acoustic radiation patterns.

Using inspiration from the work of Kirk Shung and colleagues [159-161], conductive matching and backing layers were considered for use. A number of different conductive paints, conductive epoxies, and conductive adhesives were considered for forming the ground and hot electrical connections necessary to drive the piezo element. Early concepts focused on “casting” the electrical backing/matching layers to the correct shape. Different methods of casting and assembly of the various components to the piezo were explored, included heat shrink wrapping, compression taping, and jig alignment.

A protocol for producing piezo micro-elements from bulk was developed. First, conductive, acoustically lossy epoxy (E-Solder 3022, Von Roll, New Haven, CT) was spin-coated on one side of a piezoelectric element and cured, serving as a
conductive backing layer. The resulting stack was subsequently diced to the correct dimensions using a wafer cutter (Figure 7-4).

The resulting elements with conductive contacts on both faces were subsequently mounted on top of a 19 Ga flat-tipped syringe (1.067 mm OD, 0.686 mm ID) or inside a 1.8 mm OD glass micropipette tube (Figure 7-5 and Figure 7-6). Engineering proof-of-concepts of both approaches were produced, and subsequently refined and connectorized.
Figure 7-2: Piezoelectric element externally mounted to support structure.

Figure 7-3: Schematic diagram of a piezoelectric element mounted inside a hollow conductive tube.
Figure 7-4: Piezoelectric element fabrication workflow. PZT elements with an epoxy contact on only a single side was coated with a conductive acoustically lossy epoxy. The epoxy was spin-deposited, and cured to the element. This stack was subsequently diced to size.
Figure 7-5: Piezoelectric element mounted on top of a flat-ground 19 Ga needle. Contacts to the element are made using conductive epoxy traces.

Figure 7-6: Piezoelectric element mounted within a glass micro-pipette tube.
7.2.2 Externally Mounted Piezo Proof of Concept

A matched pair of two transducers with the piezoelectric element mounted external to a support were produced (Figure 7-7). First, the piezoelectric element was mounted to the end of a 19 Ga dispensing syringe tip using conductive epoxy. Next, a wire was soldered to the inside of the conductive syringe body, forming the hot (+) contact for the element. The entire assembly was wrapped in an insulating layer of Kapton polyimide film, and coated with a thin layer of conductive epoxy that contacted the front of the transducer to create a ground trace. The ground trace and inner hot wire were connected to an SMA bulkhead connector, attached to a custom fabricated aluminum transducer body. The hollow transducer body was threaded to accept a ¼-20 thread on one end, and an 8-32 thread on the other. Finally, the transducer body was connected to the dispensing tip using a conductive luer-lock to 8-32 adaptor, allowing for the entire assembly to be mounted onto an optical post. This allowed for the orientation of the transducer to be accurately controlled and positioned. This construction method uses the entire body of the transducer as a ground. As constructed, transducer diameters using this approach ranged between 1.3 - 1.5 mm.

Electrical connectivity was verified using a Vector Network Analyzer, and the transducer design was found to be functional, with a measurable acoustic output. The FWHM intensity focus was measured as being roughly ovoid in shape, 3 mm wide by 4.3 mm tall. Unlike a focused radiator, energy decreased as a function of distance from the tip of the transducer. The radiation pattern was
even, and significant side lobes were not observed. Electrical matching of the transducer input impedance to the driving source was found to nearly quadruple the output acoustic intensity.
Figure 7-7: A matched pair of two transducers with the piezoelectric element mounted external to a support were produced
Figure 7-8: 3D Intensity raster scan of the output of the needle-mounted invasive probe, driven with the low-power LIFU System 2. The transducer exhibits a long, even, columnar radiation pattern, without measurable side lobes.

Figure 7-9: This transducer layout has a roughly ovoid focus, approximately 3 mm in width by 4.3 mm in height, FWHM in terms of intensity.
7.2.3 Internally Mounted Piezo Proof of Concept

A transducer using a diced piezoelectric element mounted inside a glass capillary tube was also fabricated. A metal rod placed inside the capillary tube served as the hot contact, and a thin conductive epoxy trace from the front face of the transducer, down the side of the capillary tube provided a ground connection. The electrical connections were spliced into a SMA cable, with the cable’s ground sheath connecting to the conductive epoxy ground on the capillary tube, and the internal hot contact connecting to the metal rod. This transducer approach was easier to fabricate, requiring less precision assembly. However, it could only use a smaller element than the external approach. The maximum width of this transducer was 1.8 mm, slightly larger than the target of 1.5 mm.

This transducer was also functional and capable of producing acoustic energy in response to an electrical drive signal. The FWHM intensity profile was measured as being approximately 3.5 mm wide by 5 mm tall. Notably, the beam pattern is slightly wider and less confined than that of the externally mounted transducer discussed previously, yet the maximum intensity for the same input is slightly higher. This may be due to a number of factors including the geometry of the transducer, the element, or this probe having an inherently slightly better electrical match to the source due to the nature of its construction. Further exploration will be required to analyze these findings further. Electrical matching
was again found to increase output intensity by a factor of approximately four.
Figure 7-10: Intensity radiation pattern for invasive microtransducer constructed from a piezoelement placed inside a hollow tube. Probe displays an even, confined acoustic pattern that radiates in a confined column, without side lobes.

Figure 7-11: Intensity normalized to peak value, measured along the YZ plane. Full representation (left) and isoline representation (right)
7.3 Experimental Validation of Invasive Probes in the Rat Model

An experimental validation trial was conducted to develop techniques necessary for invasive neurostimulation, and to explore surgical placement of the invasive probes. The basic surgical protocol developed from this effort work is described in more detail in Section 8.3). Invasive probe placement with the tip of the probe adjacent to the rat dorsomedial hypothalamic nucleus (DMD) was attempted in three subjects under isoflurane anesthesia. A cranial access burr-hole was drilled above the target to allow for insertion of the probe. Stereotaxic targeting was accomplished through use of a Kopf rat stereotaxic apparatus guided by a published rat brain atlas [1].

A slightly angled (~15-20°) approach to the target anatomy relative to vertical was determined to be ideal to avoid vascular neural trauma. Probe implantation was successful and appeared to be tolerated in two of the three subjects. Histological analysis of the track left by the probe seemed to indicate that the actual probe trajectory matched the intended plan and was on target.

Limited ultrasonic stimulation trials of up to a maximum acoustic intensity of 80 mW/cm² in CW were attempted. No obvious ultrasound induced effects were observed, and post-hoc statistical analysis revealed no statistically significant induced changes in HR/BP. However, it should be noted that the animals were deeply anesthetized.
7.4 Conclusion

This work has demonstrated the feasibility of constructing mm-scale low-frequency microtransducers and subsequently implanting them within the brain for invasive ultrasonic neuromodulation. First of their kind microtransducers were designed and fabricated that were capable of generating low-frequency ultrasound with a confined beam pattern. A procedure for implanting these probes within the brain of a rat was subsequently developed.

Enabled by the microtransducers, a new approach for targeted ultrasonic neuromodulation is made possible. However, the efforts reported here are only preliminary and intended as a proof of concept, rather than a full experimental evaluation of this approach.

While the prototype transducers are capable of producing acoustic output, their construction was limited by the available transducer fabrication resources. Further acoustic engineering of the transducer stack, with proper design and fabrication of the matching and backing layer will be necessary. With a reliable and repeatable mechanical design in hand allowing for consistent fabrication, further work will also be required to refine the electrical engineering of the transducers, eliminate electrical impedance mismatch issues, as well as to address possible capacitive coupling within the transducer design. In spite of these limitations, the fabricated prototype probes are currently in active use for other investigations of ultrasonic effects.
Additional refinement to the invasive animal experimental protocol will also be necessary to develop this approach into a valid experimental assay of ultrasonic neuromodulation. Specifically, the tolerance of the animal to the presence of the probe and the interaction of the resulting trauma with anesthesia must be further explored. Ultimately, the invasive approach is novel and promising enough to warrant further investigation.
Chapter 8: Matched-Pair Framework for Critical Value Exploration

As described in Section 3.1, a critical barrier to the advancement of ultrasonic neuromodulation is the lack of a clear relationship between the constituent acoustic parameters that compose an ultrasonic sonication pulse, and the potential for the resulting pulse to have a neuromodulatory effect. Five key parameters contribute to the ultimate character of a sonication pulse (Section 2.1.1), and a wide range of pulse constituent sets have demonstrated successful neuromodulatory effect in the literature. As a result, new experimenters are faced with a very large potential parameter space to explore, and no rational framework to do so efficiently.

In this chapter, a framework for the systematic exploration of the parameter space is presented, based on the relationship between a pulse’s constituent parameters and its resulting potential for bioeffects. Given the identification of successful parameter sets, the framework also allows for an analysis of the criticality of various pulse constituents.

Using this framework, a matrix of test parameters was created and a proof-of-principle trial of noninvasive hypothalamic stimulation in the rat model was performed.
8.1 Ultrasonic Parameters and Bioeffects

Section 2.1.1 and Section 3.1 characterized an ultrasonic sonication pulse by the following fundamental independent pulse parameters:

- Frequency = $f$ [Hz, kHz, MHz]
- Signal Amplitude = $V_{in}$ [mVrms, mVpp, Vrms, Vpp]
- Pulse Repetition Frequency = $PRF$ [Hz, kHz, MHz]
- Pulse Duration = $PD$ [$\mu$s, ms]
- Dose Duration = [ms, s, min]

Together, these parameters govern the pressure field as well as the energy deposition (intensity) at the target and surrounding tissues. Taken together, a set of these independent constituent ultrasonic parameters result in the dependent, time-averaged ($I_{SPTA}$) and pulse-averaged ($I_{SPPA}$) measures described earlier. Roughly, the $I_{SPTA}$ is used as an indicator of possible thermal interactions, and the $I_{SPPA}$ as an analogue for pressure, and as an indicator of mechanical interactions. Due to the nature of these averaged intensity quantities, multiple combination sets of independent pulse parameters can result in the same dependent derived averaged intensity.

At present it is unknown which of these descriptors – primary independent or dependent derived, are ultimately critical for a given pulse to have a neuromodulatory effect. In other words, it is unclear if it is the pulse parameters
that matter, the averaged intensities that result from those pulse parameters, or some combination of both.

Several possibilities for the critical parameter exist:

- A distinct and unique combination of primary independent parameters is necessary
- A range of primary independent values can have a neuromodulatory effect
- One or more independent parameters is critical
- A unique combination of $I_{SPTA}$ and $I_{SPPA}$ is necessary for neuromodulation
- A range of $I_{SPTA}$ and $I_{SPPA}$ values can cause neuromodulation
- $I_{SPTA}$ or $I_{SPPA}$ alone is the critical factor

As the $I_{SPTA}$ is mathematically equal to the product of the $I_{SPPA}$, PD, and PRF as given by Equation (2.23), the following quick relations govern the impact of the primary parameters on the derived intensities assuming evenly developed pulses:

- $I_{SPTA}$ increases linearly with PD and PRF, and quadratically with driving voltage amplitude
- $I_{SPPA}$ is roughly independent of PT and PRF, but also increases quadratically driving voltage amplitude
8.2 Matched-Pair Framework

As described in the previous section, full exploration of all possible combinations of the five parameters that define the sonication energy delivered to the target would be time consuming. Therefore, a new framework was developed to systematically explore the ultrasonic parameter sample space and conduct a structured search for potential neuromodulatory parameters, while potentially providing insight into the relative contribution of the independent pulse parameters and resulting dependent intensity values. This framework is based on the mathematical relationship between input parameters and the prime drivers of bioeffects, the time averaged ($I_{SPTA}$), and pulse averaged ($I_{SPPA}$) intensities, which can be used as indicators of thermal, as well as mechanical bioeffects respectively. For a given pulse, both of these values are related by the duty cycle. Compared to traditional linear search methods with an arbitrary seed, this framework makes use of matched-pairs of values to systematically test combinations of values.

Assuming an evenly developed pulse, for a given $I_{SPTA}$ and $I_{SPPA}$, a variety of different pulses can be constructed by varying the input power to the transducer, the PRF, and the PD. As long as the duty cycle remains constant, a wide variety of different PRF and PD combinations can be constructed to keep the relative ratio between the $I_{SPTA}$ and $I_{SPPA}$ constant. For example, for a given input voltage, a PD of 200 µs and a PRF of 1 kHz will have the same $I_{SPTA}$ and $I_{SPPA}$ as a PD of 100 µs and a PRF of 2 kHz.
Furthermore, for a given PD, $I_{\text{SPTA}}$ is a function of the PRF and the square of the driving voltage. Therefore, for a given PD, multiple input voltages and PRF combinations can be constructed to yield the same $I_{\text{SPTA}}$. For example, a PRF of 4 kHz at a $V_{\text{in}}$ of 1 mV will have the same $I_{\text{SPTA}}$ as a PRF of 1 kHz at a $V_{\text{in}}$ of 2 mV.

Based on these principles, a block-based matrix is presented that tests various combinations of both dependent $I_{\text{SPTA}}$ and $I_{\text{SPPA}}$ values while varying their constituent primary parameters. The values are structured such that matched overlapping pairs of values, both derived (intensity), and primary are tested within blocks, and between blocks. Each parameter has a matched-pair that appears again in the test series, but in combination with different combinations of other parameters. By approaching the construction of the ultrasonic pulse from both the constituent parameters and derived intensity perspective, this matrix provides the framework for rigorously testing, and then analyzing the relative contribution of each parameter.

For example, doubling the number of cycles in a pulse doubles $I_{\text{SPTA}}$ while maintaining the same $I_{\text{SPPA}}$. Similarly, doubling the PRF also doubles the $I_{\text{SPTA}}$ while maintain the same $I_{\text{SPPA}}$. If one of those sets of parameters works, while the others do not, we are provided with insight as to which is the critical parameter in the experiment.

One possible implementation makes use of each block having a fixed fundamental frequency, and driving voltage. Within blocks, pairs of PRF and PD pulse values, related by the duty cycle are tested. As a result of the constant driving voltage, each pair of pulse values experiences the same $I_{\text{SPPA}}$ and peak
rarefaction pressure. The $I_{SPTA}$ is repeatedly varied through a series of values based on the duty cycle, for multiple combinations of PD. Between each subunit of the block, the range of $I_{SPTA}$ tested is constant. As a result, multiple pairs of $I_{SPTA}$ and $I_{SPPA}$ values, derived from different constituent components are tested. Each of these combinations has the same $I_{SPPA}$ and $I_{SPTA}$, but varies in the way that they those values are reached. Between each block, the driving voltage increased yielding another set of matched $I_{SPTA}$ values, but this time varying in the $I_{SPPA}$.

The matrix of test-parameters formed through implementing this matched pair approach can be thought of as a multi-dimensional array with a number of different test axes iterating over the various ultrasound pulse parameter constituents, with the resulting propensity of each value-pair towards bioeffects encapsulated in the measures of intensity nested within.

By constructing a matrix of interrelated values derived from the proposed framework, a potential sample space of values can be systematically and intelligently tested, while providing insight into the potential relative contribution of each value. This matrix is modular, and the bounding box of parameters to test can be made as wide or narrow, or sampled as finely as desired.

8.2.1 MECHANISM FOR SAMPLE SPACE EXPLORATION

By varying both the constituent pulse parameter, and the resulting intensity, this framework provides a mechanism for systematically sweeping
through a volume of the full parameter space. The primary axis of exploration can altered based on the preference of the researcher. By using this framework, the summed and individual contributions of the various parameters can be tested, and by approaching each value from multiple axes, the risk of missing a successful value is minimized.

8.2.2 Critical Value Analysis

This matched-pair framework also allows for an analysis of the relative contribution, if any, of the tested values to the desired outcome. If a given pair of values is determined to be successful, tests can be run along all other parameter axes to determine their contribution. For example, if a successful outcome is observed for a given parameter pair, by testing along all the other axes and observing the outcome, it can be determined if they play a role in the ultimate effect. If traveling down each axis does not produce other notable results, this would indicate that only a discrete set of parameters has the desired outcome.

This method is most powerful when the outcome is judged on a discrete or binary scale. If such a study design is used, the matrix of test values can be subjected to a multi-way ANOVA to analyze the relative contribution of each parameter to the effect, assuming no refractory effects are present.
8.2.3 Constraints

A matrix of values derived from this framework is fundamentally limited by the extent of the parameter bounding box (extent of each axis), and step size of samples taken within that axis (sampling frequency per axis). The larger the bounding box and more test points per axis, the greater the number of trials that must be conducted. For example, one can test from $f = 500$ kHz to 1 MHz in 100 kHz increments, or from 500 kHz to 2 MHz in 50 kHz increments.

Assuming a set of ultrasonic parameters is capable of producing neuromodulation, the bounding box of the text matrix must encompass those parameters, and the sampling frequency within the box high enough to result in success. Furthermore, the narrower the range of parameters capable of causing neuromodulation, the finer the sampling period within the box must be. While this method provides a logical framework for testing various value pairs, it fundamentally cannot address the issue of large potential sample spaces.

8.3 Proof of Principle Trial of Matched-Pairs

Framework for Noninvasive Hypothalamic Stimulation in the Rat Model

A proof of principle trial of this block-based approach to the identification of critical ultrasonic parameters for inducing neuromodulation was attempted in a rat model of non-invasive hypothalamic neurostimulation. Based on prior work with ultrasonic hypothalamic neuromodulation in the minipig (Chapter 6) and
small-animal work from the literature (Section 2.4.3) we hypothesize that there is a set of ultrasonic parameters that will induce a neuromodulatory effect in the rat, specifically measurable heart rate/blood pressure change with hypothalamic sonication.

Prior to the inception of this work, no studies detailing ultrasonic neuromodulation in the rat model were available in the published literature. A matched pair-matrix centered on a fundamental frequency of 500 kHz was implemented and tested to hunt for an ultrasound-induced neuromodulatory effect. This preliminary work was also intended to provide supporting evidence for the results observed in the minipig, and serve as a basis for a higher-throughput platform for exploring ultrasonic hypothalamic stimulation.

8.3.1 MATCHED-PAIRS FRAMEWORK IMPLEMENTATION

Based on the above principles, a block-based matrix was constructed that tested a number of different ISPTA and ISPPA combinations while varying their constituent primary parameters. For a block of values, the Vin, and therefore the resulting ISPPA is held constant, and the duty cycle (and therefore ISPTA) is varied among three different combinations (0.2, 0.5, 0.8), spanning a 4x difference in duty cycle, and therefore ISPTA values.

Several different PD/PRF combinations were attempted. Each of these combinations has the same ISPPA and ISPTA, but varies in the way that they those values are reached. The PD/PRF range was selected such that they span the extent of values reported as neuromodulatory in the literature. The spacing of
parameter values was chosen to balance fidelity of sampling the parameter space with the time and number of trials required.

Between blocks, the driving voltage is doubled, quadrupling the $I_{SPPA}$ between blocks. Since the $I_{SPTA}$ within a block spans a 4x range, the highest $I_{SPTA}$ of one block is the same as the lowest $I_{SPTA}$ of a proceeding block, creating another overlapping pair.

The test matrix was conducted from the following independent pulse parameters:

- Frequency = 500 kHz
- Signal Amplitude (Transducer Vin) = [0.02, 0.04] Vin
- Pulse Duration (PD) = [20, 50, 100, 200, 400, 800, 1200] µs
- Pulse Repetition Frequency (PRF) = [0.17, 0.25, 0.42, 0.5, 0.63, 0.67, 1, 1.25, 2, 2.5, 4, 5, 8, 10, 16, 25, 40] kHz

These parameters resulted in the intensity combinations of $I_{SPTA}$ = [40, 100, 160, 400, 640] mW/cm$^2$ and $I_{SPPA}$ = [0.4, 0.8] W/cm$^2$, reported as free-water intensities. The parameters were selected to overlap with the range of values considered to be the most promising in the literature, and using $I_{SPTA}$ intensities around the order of magnitude of those reported in Tyler’s early work [44]. Using this framework to explore the nested intensity combinations, only 42 trials are needed to span the range compared to the 238 that would be necessary by pure combination.
8.3.2 TRANSUDER AND TARGETING

Stimulation was delivered using a commercially available focused ultrasound probe (V301-SU-F1.25, Olympus NDT) coupled to the rat through custom coupler assembly and targeted using a stereotactic frame. The probe, with a focal length of 38.6 mm, and a focus width of 2.057 mm was selected due to its use in previously published use in ultrasonic neuromodulation studies [123].

**COUPLING**

Two different coupling systems were developed and tested to couple the transducer to the subject, a series of fixed press-fit tubes to move the focus a known distance from the coupler face (Figure 8-1), and a variable slider that allowed for dynamic axial control of the probe focus (Figure 8-2). Both couplers were filled with degassed deionized water, and capped with a thin, acoustically transparent latex membrane. The membrane was tested and demonstrated excellent long-term stability, with no effects of aging on the measured acoustic properties. Based on prior reports in the literature and the relative thinness of the rat skull, the risk of beam deflection was considered minimal and an intensity transmission coefficient ($T_1$) of ~87% was assumed [162].

**TARGETING**

A KOPF Model 900 LS stereotaxic instrument (David Kopf Instruments, Tujunga, CA) was used for stereotaxic targeting of the animal (Figure 8-3). Subject was placed within the instrument and secured comfortably by placing the
ear bars into the ear canal. Bregma was identified, and used as the basis of the target coordinate system.

Anatomic targeting location was identified based on a published stereotactic atlas of the rat brain [1]. Based on this atlas, the target location for the dorsomedial hypothalamic nucleus (DMD) was identified as Bregma = -2.80 mm, x = 0.5 mm, and z = -9 mm (Figure 8-4). A trajectory orthogonal to the skull was designed such that the acoustic focus would overlap the desired target.

Based on this desired target depth, a fixed coupling tube 25 mm in length with a 20 mm offset from the transducer to the skull was selected. Using this coupler, the focus point was predicted to be 9 mm from the front of the coupler assembly, based on previous free-water measurements of only the transducer (Figure 8-5). When the transducer and coupler assembly were characterized together in the water tank, the focus was found to be 8 mm from the front of the coupler, in good agreement with the predicted location.
Figure 8-1: Fixed spacer-tube method of coupling. Precision machined spacers of known length allow for the acoustic focus to be accurately positioned at a known distance from the face of the coupler. Coupler is filled with degassed and deionized water and capped with an acoustically transparent membrane. Couplers sized to fit the Olympus V301 series of probes, and shown with a V301-SU-F1.25 (focused, left) and V301 (unfocused, right) probe.

Figure 8-2: Slider-style coupling mechanism developed for the Olympus V301 series prob. Mechanism allows for dynamic control of the axial focus location. Predicted beam pattern through the coupler, based on previous free-water measurements of only the transducer depicted in blue.
Figure 8-3: A KOPF Model 900 LS stereotaxic used for stereotaxic targeting of the animal. Subject was placed within the instrument and secured comfortably by placing the ear bars into the ear canal. Bregma was identified, and used as the basis of the target coordinate system. The transducer assembly was clamped to the stereotaxic frame for accurate spatial targeting.
Figure 8-4: Stereotactic brain atlas slice of the rat brain figure showing the location of the stimulation target, the dorsomedial hypothalamic nucleus (DMD) Image from [1]. The target was identified as being located at Bregma = -2.80 mm, x = 0.5 mm, and z = -9 mm relative to the midline and top of the skull.
Figure 8-5: Predicted beam pattern of focused V301-SU-F1.25 transducer using 25 mm length, 20 mm offset coupling fitting. Beam pattern predicted based on free-water measurements of transducer alone.
8.3.3 Stimulation Protocol

For each block, sonication was sequentially applied to each subject in a pattern of approximately 90 s on, and 90 s off. Therefore, for each sonication trial, there is a preceding (pre-) and following (post-) period, which ideally should represent a steady vital baseline and control against which to judge the sonication. ECG and blood pressure from an arterial line were measured and digitally recorded and in real time using NOTOCORD-hem Evolution (NOTOCORD SYSTEMS, Croissy Sur Seine, France), with corresponding on (stimulation) and off (no-stimulation) periods marked in the data record.

Test parameters that demonstrated a gross temporally correlated change in vitals by inspection during the experiment were repeated in sequence multiple times. Subjects were maintained on a mixture of 1.5% isoflurane and oxygen, inhaled through a nosecone to maintain a constant plane of anesthesia.

8.3.4 Subject Summary

Three male Sprague-Dawley rats weighing between 394.4 g – 402.6 g were enrolled into this within-subject study. Animals were initially anesthetized in an induction chamber using 3 – 4% isoflurane mixed with oxygen. Once a surgical plane of anesthesia had been reached, they were removed from the induction box and placed on a surgical table. Isoflurane anesthesia was maintained using a nose cone between 1.25 – 1.5%. One subject was removed from the trial due to failure to achieve a steady resting state.
8.3.5 Results

No apparent ultrasound induced change in either heart rate or blood pressure was observed with targeted hypothalamic stimulation at 500 kHz using the test protocol described above, spanning the range $I_{SPTA} = 40 - 640$ mW/cm$^2$ and $I_{SPPA} = 0.4 - 0.8$ W/cm$^2$, PD = 200 – 1200 µs with a 20 – 80% duty cycle.

In-depth post-hoc analysis of the recorded EKG waveform components using a number of different analysis techniques similarly yielded no statistically supportable basis for rejecting the null hypothesis that ultrasonic stimulation caused a measurable neuromodulatory effect at the $p < 0.05$ (two-tailed) threshold level.

Briefly, the difference in mean and median between a stimulus and its preceding unstimulated control, following unstimulated control, and between the preceding and following control periods was analyzed for a possible ultrasound induced effect over a number of different measured vital components. Of primary interest were the EKG and BP derived heart rate, the EKG derived PR duration, QT interval, P-wave magnitude, and PR duration, with the later values analyzed based on an initial analysis of potentially successful trials. Both two-sample t-tests and Wilcoxon rank-sum non-parametric tests were performed comparing unstimulated to stimulated periods for all trials and parameters. The non-parametric test was used in addition to the t-test to account for natural drift of the vitals measurements. Neither approach allowed for a statistically supported declaration of ultrasound-induced neuromodulatory effect.
Repeated measures ANOVA comparing control to sonicated periods for the same stimulus parameters over a number of repeated trials were conducted for a number of ultrasonic pulse parameters that demonstrated promise during testing, but also did not support rejecting the null.

All statistical analysis methods were developed and implemented in consultation with an expert biostatistician.

8.3.6 DISCUSSION

While intended as an initial proof-of-principle of the matched-pair parameter exploration paradigm as applied to the targeted non-invasive ultrasonic sonication of the rat hypothalamus, and not a rigorous trial, several experimental design factors may account for the lack of observed neurodmodulatory effect.

No prior published work has established suitability of hypothalamic stimulation as an assay for ultrasound-induced effect. More straightforward, it is unclear if ultrasound can even be used to stimulate the hypothalamus of the rat, and if such an effect does exist, would it result in a change in HR/BP large enough in magnitude to be identifiable. Even at rest, wide variations in measurements of rat heart rate, relative to mean and median values, were observed.

As discussed previously, the general interaction of anesthesia with ultrasonic neuromodulation is still being established and is a cause for concern.
Specific to this study, Yoo has demonstrated a relationship between ultrasonic sonication and anesthesia recovery time in rats [125]. However, Yoo administered anesthetic as an intraperitoneal ketamine/xylazine bolus, whereas this present study made use of constant maintenance levels of inhaled isoflurane in an effort to keep the subject’s anesthesia plane constant. Notably, the Stanford group’s efforts at using inhaled isoflurane made use of much lower levels of anesthesia (<0.5%), which were unachievable with the available equipment in this present study [128].

Ultimately, it is also possible the neurostimulation failure is due to the pulse-parameter protocol. The matched-pair framework for this study was setup specifically to explore a fixed range of parameters that was judged to be the most likely to induce neuromodulation based on reports in the literature. However, the intensity range was intentionally limited and selected to match those reported in the early work by Tyler in the mouse [44]. As the relationship between the stimulation parameters was selected to best cover the range of parameters that have demonstrated success in the literature, the most likely explanation is that the intensities attempted were subthreshold.

This contention may be supported by reports in the literature subsequent to this study that demonstrate a much higher critical ultrasonic intensity threshold relative to the values initially reported by Tyler in the mouse. More relevantly in the rat model, Younan et al. were very recently able to achieve motor response only at an acoustic intensity on the order 7.5 W/cm² I_{SPPA}, and further commented on the necessity for higher ultrasound levels under strong anesthesia.
For more context, the reader is referred to Section 2.4.3 for a full listing of intensity values reported in the literature, as well as the discussion of relationship between acoustic intensity and neuromodulatory effect in Section 6.4.

### 8.4 Conclusion

The matched-pair parameter sweep algorithm detailed here is adaptable and modulator enough for a wide range of ultrasonic investigations. While the trial provides a logical mechanism for testing a wide range of parameter combinations, the framework is most powerful when a set of successful parameters is identified, as it provides a mechanism for identifying and testing the criticality of the various parameters that are necessary to form an acoustic pulse. The failure of the pilot trial to identify a set of parameters that causes measurable hypothalamic stimulation with targeted sonication most likely due to experimental considerations or, as the framework is sensitive to its inputs, the range of parameters tested.

Further study will be necessary to draw strong conclusions on the efficacy of this framework, and of the hypothalamic stimulation model attempted in the pilot study. However, the framework presented here was found to be easy to setup and experimentally implement, and should serve as an effective tool for future researchers to more explore sample broad sample spaces for a variety of ultrasound-induced bioeffect applications.
Chapter 9: Conclusion

In this thesis, the hypothesis that low intensity ultrasound can be discretely targeted to desired neural structures to induce neuromodulation was explored. This exploration addressed several critical barriers to advancement in the emerging field of lower-intensity ultrasonic CNS neuromodulation. The engineering challenges of applying known quantities of ultrasound to known locations – targeting – were addressed to enable future detailed study of ultrasonic neuromodulation.

An experimental assay of the effect of hypothalamic sonication in the Göttingen minipig was developed to explore targeted neuromodulation in the brain of a larger animal model. A transducer, coupling system, and surgical procedure using human neurosurgical instrumentation were developed that allowed for transcranial sonication of deep-brain structures. A pilot study assaying the effect of hypothalamic ultrasonic stimulation on heart rate and blood pressure in the minipig yielded preliminary evidence that ultrasound can induce a statistically significant, repeatable rise in heart rate with stimulation at a fundamental frequency of 650 kHz, high pulse duty cycle, and in-brain intensities in excess of approximately 10 W/cm². Comparison between theoretical and ex-vivo measurements from intact skulls validated the ability to accurately estimate the target location, and acoustic intensity within the brain. This demonstrated
targeting accuracy serves as the foundation for future development of an accurate large-animal model of transcranial non-invasive neurostimulation.

Targeting was also explored through the development of an invasive neurostimulation paradigm, which makes use of an implantable microprobe placed directly adjacent to an addressed neural structure. In order to enable this stimulation paradigm, novel low frequency ultrasonic microtransducers were developed and characterized. These prototype transducers are the first reported devices in the available literature demonstrating low frequency operation in a compact millimeter-scale form factor. A feasibility study in the rat model demonstrated the potential for the proposed invasive targeting paradigm to be used experimentally.

An algorithm for effectively sampling the ultrasonic parameter space was then developed and detailed. By nesting the prime drivers of bioeffects into a sampling framework, issues of parameter specificity and targeting can be efficiently addressed. This algorithm and framework is intended to simplify the process of experimentally sampling the acoustic parameter space, and serve as a basis for future researchers to systematically identify, report and compare across experiments the ultrasonic parameters that are critical to neuromodulation.

With these results, this work provides an engineering foundation for future systematic and quantitative studies of ultrasonic bioeffects. Through the detailed understanding of targeting and parameter selection enabled by this work, rigorous assays for ultrasonic neuromodulation can be conducted. Our
hope is that this will accelerate the development of effective clinical therapies in the future.
REFERENCES


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