

Non-invasive bidirectional acoustoelectric neural interface

Jean Rintoul

**Interventional Systems Neuroscience Lab
Department of Brain Sciences
Imperial College London**

A thesis submitted for the degree of Doctor of Philosophy (PhD)

Clinical Medicine Research

2024

Abstract

The non-invasive sensing and stimulation of electrical signals in the brain, with high spatial and temporal specificity, has long been a strategic goal in neuroscience. Such a tool would open pathways to understanding the brain and its disorders. To date, the focal detection and stimulation of electrical signals in the brain has been impossible due to the inability to non-invasively reach deep brain areas with high spatial specificity. This thesis proposes a new hybrid modality capable of detecting and stimulating electrical signals in the brain with improved spatial specificity, through utilization of the acoustoelectric interaction.

The underlying physics of the acoustoelectric interaction is investigated, leading to a new mathematical model which encapsulates the vector nature of the interaction. This acoustoelectric mathematical model predicts that heterodyning will occur between the electric and acoustic field, enabling precise signal recovery using techniques previously established in radio communications. Simulation and acoustoelectric phantom characterization demonstrate how this technique can be used to create a focal low frequency electrical signal deep in the brain for neuromodulation and demodulate complex electrophysiological signals at the focus of the ultrasound for acoustoelectric neural recording. Moving to in vivo validation using electrophysiology in a rodent model, we acoustoelectrically demodulated visual evoked potentials around the carrier frequency of the ultrasound for the first time, with associated artefact tests. Finally, the first in vivo evidence of acoustoelectric neuromodulation is reported and an evidence-based argument developed as to why the acoustoelectric interaction is a contributing mechanism to ultrasound brain stimulation. Acoustoelectric neuromodulation has a clear mechanism of action, harnessing the focality of ultrasound and the well-understood direct electrical pathway developed by the Hodgkin-Huxley model, giving it a unique set of advantages to target deep areas of the brain.

Acknowledgements

First and foremost, I would like to thank my supervisor Dr. Nir Grossman. It has been a privilege to complete my PhD study under your mentorship. I appreciate your scientific guidance and continued support over the years. I would also like to thank my second supervisor Dr. Esra Neufeld, for his insightful guidance and interesting discussions.

Thank you to all the Grossman Lab members, particularly Dr. Xiaoqi Zhu, Dr. Edward Rhodes, Dr. Jon Howard, Dr. Patrycja Dzialecka, Dr. Maria Garcia-Garrido, Dr. Nawal Zabouri, Dr. Sofia Peressotti, Ketevan Alania and Rachel Law. You made the ISN lab a great and supportive environment to work at.

I had been wanting to study further in this area for a long time. I am incredibly grateful to my husband Dr. Geoffrey Irving for supporting me in taking a crazy career turn from consumer electronics into an academic direction in a foreign country, just as we were growing our family. Luckily this all coincided with the Covid-19 pandemic. I am also grateful to my daughters Mae and Io for keeping me firmly grounded in the day to day and their constant curiosity about mice and ultrasound.

Thank you to my mum and architect Judith Rintoul, for being an example of a career driven woman with a kid – I grew up not having any other expectations. Thanks to my grandparents for being born in Scotland and England – without you we would not have been eligible for the UK Ancestry Visa which has made the move easier.

Statement of Publications

Chapter 2,3,4 covering the mathematics, simulation and phantom characterization of the acoustoelectric effect are published in Nature Communications Physics –

Rintoul, J.L., Neufeld, E., Butler, C., Cleveland O. R., Grossman N.. Remote focused encoding and decoding of electric fields through acoustoelectric heterodyning. *Communication Phys* **6**, 79 (2023). <https://doi.org/10.1038/s42005-023-01198-w>

Chapter 5 is being prepared for publication.

Chapter 6 is being prepared for two publications.

Statement of Originality

I declare that all work contained within this thesis is my own, unless otherwise stated, and conforms to the rules and guidelines set out by Imperial College London. I chose to use the third person plural, as this is traditionally used in manuscripts submitted to academic journals, although all work except that described below was done by myself.

Dr. Esra Neufeld provided the original mathematical derivation insight into the gradient dependence of the acoustoelectric field, as well as advice on modifying a thermal diffusion solver in Sim4Life to solve the resulting acoustoelectric equation.

The bipolar isolation transformer was designed by Dr. David Bono from MIT. The circuit diagram for this transformer is included in Appendix 1.

I also wish to also acknowledge Dr. Patrycja Dzialecka, who developed the original mouse surgery protocol for *in vivo* Calcium Imaging, which I have adapted for use with electrophysiology.

Copyright Declaration

The copyright of this thesis rests with the author. Unless otherwise indicated, its contents are license under a Creative Commons Attribution-Non-Commercial 4.0 International Licence (CC BY-NC).

Under this licence, you may copy and redistribute the material in any medium or format. You may also create and distribute modified versions of the work. This is on the condition that: you credit the author and do not use it, or any derivative works, for a commercial purpose.

When reusing or sharing this work, ensure you make the licence terms clear to others by naming the licence and linking to the licence text. Where a work has been adapted, you should indicate that the work has been changed and describe those changes.

Please seek permission from the copyright holder for uses of this work that are not included in this license or permitted under UK Copyright Law.

List of abbreviations

ARF	Acoustic Radiation Force
ASD	Amplitude Spectral Density
CAD	Computer Aided Design
CSF	Cerebrospinal Fluid
dB	Decibel
FEM	Finite Element Method
HH	Hodgkin-Huxley
LED	Light Emitting Diode
MUA	Multi-unit activity
Pt-Ir	Platinum Iridium
PVDF	Polyvinylidene difluoride film
PWM	Pulse Width Modulation
SEM	Standard error of the mean
TI(B)	Thermal Index Bone
V1	Primary Visual Cortex
WT	Wild type

Table of Contents

Chapter 1: Introduction and background	14
1.1 Introduction.....	14
1.2 Background.....	15
1.2.1 The Hodgkin-Huxley model of the neuron.	15
1.2.2 Tools for non-invasive neuromodulation and their limitations.....	15
1.2.3 Tools for non-invasive neural recording and their limitations	18
1.2.4 Discussion	21
1.3 The acoustoelectric effect	22
1.3.1 Rationale for selecting the acoustoelectric effect.	22
1.3.2 History of the acoustoelectric effect	23
1.4 Non-invasive bidirectional acoustoelectric neural interface concept overview	25
1.4.1 Acoustoelectric neuromodulation concept	25
1.4.2 Acoustoelectric neural recording concept.....	26
1.5 Aims and outline of this thesis	26
1.5.1 Acoustoelectric theory and simulation.....	26
1.5.2 Physiological phantom experiments on the acoustoelectric field.	27
1.5.3 <i>In vivo</i> acoustoelectric neural recording.....	27
1.5.4 <i>In vivo</i> acoustoelectric neuromodulation	28
1.6 Risks and challenges	28
Chapter 2: Theory and simulation of the acoustoelectric effect	30
2.1 Introduction.....	30
2.2 Physical description of the acoustoelectric effect	31
2.3 Mathematical derivation of acoustoelectric field in electrolytic liquids and soft tissues.....	32
2.3.1 Small Term Assumption	35
2.3.2 Special Case 1: Homogeneous medium	36
2.3.3 Special Case 2. Infinite planar transducer	36
2.4 Simulation Methods	37

2.4.1 K-space homogeneous simulation of the acoustoelectric field	37
2.4.2 Finite element heterogeneous simulation of the acoustoelectric field	38
2.4.3 Finite element model simulation validation	40
2.5 Results	46
2.5.1 Computational Investigation of the acoustoelectric field	46
2.5.2 Characterization of the applied and generated fields in the computational model	49
2.5.3 Acoustoelectric safety simulations	51
2.5.4 Mechanism and focality comparisons between acoustoelectric neuromodulation and temporal interference stimulation.	54
2.5.5 Computational investigation of acoustic focality limitations in a mouse	56
2.6 Discussion	58
Chapter 3: Materials and methods	60
3.1 Overview	60
3.2 Methods: Acoustoelectric phantom	60
3.2.1 Development of low-cost open-source 3D XYZ phantom	61
3.2.2 Water degassing treatment	64
3.2.3 Impedance adapter for monitoring voltage and current concurrently	65
3.2.4 Low pass differential filter design	67
3.2.5 High pass differential filter design	68
3.2.6 Gradient descent based current source.	70
3.2.7 Acoustoelectric phantom instrumentation overview	72
3.2.8 Characterization of the applied fields	75
3.3 Methods: Animal experiments	77
3.3.1 <i>In vivo</i> electrophysiology surgery	78
3.3.2 Anesthesia selection rationale for acoustoelectric <i>in vivo</i> experiments	79
Chapter 4: Acoustoelectric phantom	81
4.1 Introduction	81
4.2 Results	81
4.2.1 Verification the acoustoelectric effect is not an artefact	81
4.2.2 Acoustoelectric heterodyning experimental evidence.	87
4.2.3 Acoustoelectric directional dependence experimental evidence	89

4.2.4 Acoustoelectric neuromodulation feasibility.....	92
4.2.5 Acoustoelectric neural recording feasibility	93
4.2.6 Temperature dependence in the acoustoelectric interaction.	95
4.2.7 Conductivity and the acoustoelectric interaction.....	96
4.3 Discussion.....	98
Chapter 5: Acoustoelectric neural recording.....	100
5.1 Introduction.....	100
5.2 Methods	103
5.2.1 Acoustoelectric neural recording instrumentation.....	103
5.2.2 Acoustoelectric method for calibrating ultrasound focal location.....	105
5.2.3 Acoustic and acoustoelectric cone characterization.....	105
5.2.4 Recovery surgery for acoustoelectric neural recording.	106
5.2.5 Experimental procedure for <i>in vivo</i> acoustoelectric neural recording.....	107
5.3 Results.....	108
5.3.1 Acoustoelectric heterodyning is the mechanism behind ultrasound current source density imaging... 108	
5.3.2 <i>In vivo</i> acoustoelectric neural recording of visual evoked potentials.	117
5.3.3 Single-shot <i>in vivo</i> acoustoelectric neural recording of electrophysiological signals	120
5.3.4 Artefact tests	121
5.4 Discussion.....	125
5.4.1 Safety profile	125
5.4.2 Summary	126
Chapter 6: Acoustoelectric neuromodulation: a contributing mechanism to ultrasound brain stimulation.	128
6.1 Introduction.....	128
6.2 Methods	130
6.2.1 Acoustoelectric neuromodulation instrumentation	130
6.2.2 Acoustic and acoustoelectric cone characterization.....	132
6.2.3 Experimental procedure for <i>in vivo</i> acoustoelectric neuromodulation	135
6.2.4 Data exclusion criterion	137
6.3 Results.....	139
6.3.1 Ultrasound induces electrical frequency mixing products in the electrophysiological range.	139

6.3.2 An acoustoelectric interaction is driven by the acoustic field and electric artefact from the ultrasound.	142
6.3.3 Confirming the source of the electrical artefact at 500kHz	145
6.3.4 <i>In vivo</i> ultrasound stimulation is correlated to the measured acoustoelectric difference frequency amplitude in the brain.	147
6.3.5 <i>In vivo</i> ultrasound stimulation is amplitude dependent on the electric field present in the medium ...	149
6.3.6 <i>In vivo</i> acoustoelectric neuromodulation: stimulation frequency is dependent on both the acoustic and electric field.	154
6.3.7 <i>In vivo</i> acoustoelectric neuromodulation is not dependent on the temperature dissipation envelope.	159
6.4 Discussion	160
<i>Chapter 7: Discussion and conclusions</i>	164
7.1 Overview	164
7.2 Key advances	164
7.2.1 Chapter 2: Acoustoelectric theory and simulation	164
7.2.2 Chapter 4: Acoustoelectric phantom characterization	165
7.2.3 Chapter 5: <i>In vivo</i> Acoustoelectric neural recording	167
7.2.4 Chapter 6: <i>In vivo</i> Acoustoelectric neuromodulation	167
7.3 Future directions	168
7.3.1 Future improvements to further understand the physics of the acoustoelectric interaction	168
7.3.2 Future improvements to acoustoelectric neural recording	169
7.3.3 Future improvements for acoustoelectric neuromodulation	173
7.4 Implications	175
<i>References</i>	177
<i>Appendix 1: Isolation Transformer by David Bono</i>	194

List of Figures

Figure 1-1 Diagram of temporal interference stimulation.....	18
Figure 1-2 Comparative wavelengths of electromagnetic and acoustic spectrums.....	22
Figure 1-3 Diagram of acoustoelectric neuromodulation and neural recording concepts.....	25
Figure 2-1 The acoustoelectric field.....	31
Figure 2-2 Model schematic.....	32
Figure 2-3 Finite Element Model Simulation.....	39
Figure 2-4 Instantaneous pressure amplitude.....	41
Figure 2-5 Computation of the acoustoelectric effect in weak electrolytes.....	46
Figure 2-6 Simulation predictions of the acoustoelectric effect in weak electrolytes.....	48
Figure 2-7 Characteristics of the applied acoustic fields & acoustoelectrically generated electric field obtained through computational modelling.....	50
Figure 2-8 k-wave temperature simulation.....	53
Figure 2-9 Safety and electrical neuromodulation feasibility map.....	54
Figure 2-10 Focality comparison with temporal interference stimulation.....	55
Figure 2-11 Finite element simulation of propagating 500kHz acoustic field through a mouse.....	56
Figure 2-12 Acoustic reflection study.....	57
Figure 3-1 Customization of 3D delta printer to precision XYZ stage for acoustoelectric characterization study.....	61
Figure 3-2 Custom CAD design of 3D printed parts.....	63
Figure 3-3 Automated XYZ phantom for mapping acoustic and electric fields.....	64
Figure 3-4 Water degassing and treatment.....	65
Figure 3-5 Impedance adapter.....	65
Figure 3-6 Low pass differential filter.....	67
Figure 3-7 High pass differential filter.....	69
Figure 3-8 Gradient descent based current source.....	71
Figure 3-9 Instrumentation Design.....	72
Figure 3-10 Characterization of the applied acoustic and electric fields.....	76
Figure 4-1 Acoustoelectric characterization the transducer electrical and acoustoelectric field.....	82
Figure 4-2 Acoustoelectric timing and dependence on applied fields.....	84

Figure 4-3 Acoustoelectric statistical artefact tests	86
Figure 4-4 Measurements of acoustoelectric frequency mixing in a tissue phantom.	87
Figure 4-5 Measurements of acoustoelectric spatial characteristics in a physiological phantom.	89
Figure 4-6 Acoustoelectric neuromodulation feasibility.....	92
Figure 4-7 Acoustoelectric neural recording feasibility.	93
Figure 4-8 Temperature dependence in the acoustoelectric interaction.....	95
Figure 4-9 Acoustoelectric dependence on conductivity.....	96
Figure 5-1 Acoustoelectric neural recording concept.....	100
Figure 5-2 Acoustoelectric instrumentation and position calibration.....	103
Figure 5-3 Acoustic and acoustoelectric cone characterization.....	106
Figure 5-4 Advantages of acoustoelectric heterodyning in signal recovery.....	108
Figure 5-5 In vivo acoustoelectric neural recording at low frequencies can be confounded by VEP harmonic artefacts.	112
Figure 5-6 Continuous ultrasound changes the visual evoked potential waveform.	115
Figure 5-7 <i>In vivo</i> acoustoelectric neural recording of electrophysiological signals.	117
Figure 5-8 Single-shot in vivo acoustoelectric neural recording of spontaneous neural activity.....	120
Figure 5-9 Acoustic isolation and LED shield test.....	122
Figure 5-10 Two-tone test.....	124
Figure 6-1 Acoustoelectric neuromodulation concept.....	129
Figure 6-2 Acoustoelectric instrumentation.....	130
Figure 6-3 Acoustic and acoustoelectric cone characterization.....	133
Figure 6-4 Data exclusion due to depth of anaesthesia.	137
Figure 6-5 Data exclusion due to electrochemical DC offset effects at high voltages.	138
Figure 6-6 Ultrasound induces electrical focal frequency mixing products.	139
Figure 6-7 Ultrasound undergoes an acoustoelectric interaction induced from the transducer electrical artefact. ..	143
Figure 7-1 Improving the dynamic range of the preamplifier measurement through attenuation of the electrical ultrasound artefact.	170
Figure 7-7-2 Transmission advantages over EEG.....	171
Figure 7-7-3 Electrode surface area	172

Chapter 1: Introduction and background

1.1 Introduction

Brain disorders are a massive burden to society and increasing in prevalence as our population ages¹. To decrease this burden, major improvements to both diagnostic brain imaging and therapeutic tools are required.

To enable therapeutic neuromodulation to be more broadly applicable, we need less invasive methods than surgical intervention, that also have high spatial focality. Invasive Deep Brain Stimulation (DBS) has had great impact helping treatment-resistant patients with disorders such as Parkinson's disease² and obsessive–compulsive disorder³, with great potential for other disorders such as depression⁴ and Alzheimer's disease⁵. Being a surgical procedure, DBS bears the risks for serious complications which limit its therapeutic impact⁶, reaching far fewer patients than it could if it were less invasive.

Conversely, we are limited in our ability to accurately probe and diagnose neurological disease. Epileptic foci are currently found using EEG⁷ which is limited to diffuse cortical estimations of electrical activity – in ill posed inverse problem⁸. MRI detects slow changes in blood flow through the brain⁹, and MEG can only detect cortical electrical activity¹⁰. We need to improve non-invasive tools to enable the spatially specific detection of electrophysiological activity in deep areas of the brain.

In this PhD thesis, we seek to extend our understanding of physics in service of providing new tools to reach the goals outlined: non-invasive focal neural recording and stimulation.

1.2 Background

1.2.1 The Hodgkin-Huxley model of the neuron.

The Hodgkin–Huxley model of the neuron is a set of nonlinear differential equations that approximates the electrical engineering characteristics of excitable cells such as neurons and muscle cells. It is our most accurate model to date for explaining brain dynamics, highlighting the importance of the electrical aspects of brain signals.

Alan Hodgkin and Andrew Huxley first published their findings in 1952 to explain the action potential firings in the squid giant axon¹¹ and they received the 1963 Nobel Prize in Physiology or Medicine for this work. The Hodgkin-Huxley model is considered an accurate description to this day, central to our understanding of ion channel dynamics, spike timing and circuit level computational neuroscience.

Since this electrical model is the best model we have to date of brain function, developing a bidirectional neural interface that can read and write electrical signals to the brain with high spatial specificity, would be the most direct method to accurately interface with neural activity.

1.2.2 Tools for non-invasive neuromodulation and their limitations

Non-invasive tools for neuromodulation have been in existence for the last century, slowly becoming more spatially precise. By looking at previous approaches and their limitations we can determine what a better next iteration might be based on.

Electroconvulsive Therapy (ECT)

Electroconvulsive therapy (ECT) was the first non-invasive neuromodulation and was invented in the 1930s¹². During ECT up to 1 ampere is applied between two electrodes, intentionally triggering a seizure. There is evidence ECT causes changes in brain chemistry, and it can quickly reverse symptoms of mental health conditions such as bipolar disorder, severe depression and schizophrenia, though its mechanism is poorly understood. This is a global and non-focal technique meaning it may change parts of the

brain that do not require intervention. While still in use today, it is often seen as a last resort, as it carries associated risks of amnesia and learning difficulties.

Transcranial direct and alternating current stimulation (tDCS and tACS)

Transcranial direct and alternating current stimulation have come to the forefront of non-invasive stimulation in recent years, providing non-focal means to stimulate only cortical brain regions¹³. Though promising, these techniques have most of their energy routed around the skull and only reach the brain in very low amplitudes¹⁴. Direct current stimulation is ionizing and non-focal¹⁵, whilst alternating current stimulation is less likely to induce ionization making it a safer choice, although recent studies show that even AC stimulation can generate a DC offset as an artefact thought to be induced through the electrode chemistry¹⁶. Without control of focality however, it is difficult to precisely affect specific brain targets. However, oscillatory synchronization of neurons has been determined *in vivo* at electric field amplitudes as low as 0.5 V/m¹⁷.

Transcranial Magnetic Stimulation (TMS)

TMS uses electromagnetic induction via a coil applied to the scalp to generate an electric current in the brain and was invented in 1986¹⁸. A coil of wire is held next to the skull producing a varying magnetic field and pulsed. The changing magnetic field then induces an electric current in the brain that activates nearby nerve cells at the cortical surface.

Deep TMS (dTMS) stimulates wider and deeper areas of the brain and can reach up to 6cm into the brain and has been FDA approved to treat obsessive compulsive disorder, migraines and addiction. The path of the current can be difficult to model using simulation tools because the brain is irregularly shaped with variable internal dielectric properties, leading to a nonuniform magnetic field strength and conduction¹⁹ throughout its tissues²⁰.

Ultrasound Neuromodulation

Ultrasound neuromodulation has been shown to work in humans^{21–23} and mice²⁴, though interestingly the specific thresholds are highly variant, with responses seen between 0.2 MPa²⁴ through to 3.6 MPa²⁵ in studies conducted with different protocols in different labs.

Though neuromodulation works, its mechanism is not yet understood, and there are many conflicting reports of results²⁶, making interpretation of generic thresholds and limitations hard to quantify in a repeatable way. However, the promise of a highly focal form of neuromodulation able to reach deep brain regions keeps this nascent technology alluring to many labs and technology start-ups.

A variety of theories exist as to how ultrasound neuromodulation stimulates neurons. Most recently, Yoo and Shapiro²⁷ found that ultrasound excites cortical neurons via mechanosensitive calcium accumulation and ion channel amplification. The concept of mechanosensitive²⁸ ion channels would change our current understanding of how the neural cell works, opening the Hodgkin-Huxley model to new horizons. Other mechanism concepts include the acoustic radiation force (ARF)²⁹, as ultrasound neuromodulation appears to roughly follow the envelope of the applied acoustic field, or even temperature effects induced from thermal dissipation from the ultrasound³⁰. Thermal dissipation has been relatively clearly disproven by Yoo and Shapiro, and currently the mechanosensitive ion channel model is favoured.

Most commercially available ultrasound systems output square waves to reduce signal generation complexity, pulsed at the resonant frequency of the ultrasound transducer. Typically, the pulsed square waveform used in ultrasound neuromodulation is below 1MHz and up to 300ms in length²⁵. This square-wave pulse has a broad-spectrum distribution in Fourier space. The electrophysiological response is characterized by an onset effect and a refractory period after stimulation. Furthermore, most rodent studies are done under either ketamine/xylazine or isoflurane anaesthesia which has been reported to greatly change the EMG response amplitude³¹. There are many excellent reviews on ultrasound neuromodulation²⁵, delving into the highly variant parameter space, yet showing a clear causal stimulation from ultrasound.

Ultrasound Neuromodulation^{32–34} is very promising as it can reach higher focality than electromagnetic based neuromodulation techniques, though its mechanism of action is poorly understood which limits its application and advancement²⁵.

Temporal Interference Stimulation (TI)

Temporal interference stimulation³⁵ works by superimposing two fields of slightly different frequencies so that the difference frequency is in the typical range of neural oscillations (**Fig 1-1**) and spatially located where both fields overlap. The difference frequency appears when the two applied signals multiply together via natural means in non-linear media such as the brain³⁶. It has been experimentally verified that neurons will sympathetically oscillate with the difference frequency. The difference frequency location can be controlled by adjusting the amplitude of the applied electric fields giving it greater focality than tDCS/tACS.

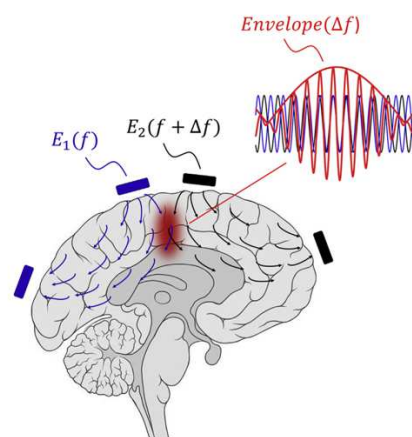


Figure 1-1 | Diagram of temporal interference stimulation.

Two electric fields are applied with a difference frequency (Δf). The area of superposition shown in red where both fields are present induces a multiplication of the two fields resulting in the sum and difference frequencies occurring at this overlapping region.

1.2.3 Tools for non-invasive neural recording and their limitations

The neuroimaging methods listed below have all opened doors to understanding the brain and are transformative diagnostic tools. However, they have limitations, and these can be informative in determining better approaches to non-invasively detecting electrical activity with high spatial precision in the brain.

Electroencephalography (EEG)

EEG measures electrical activity in the brain using electrodes that are attached to the scalp in an array arrangement. The size of the signals is in the microvolt range and

diffusion and attenuation occur through the scalp. The signal measured at the electrodes is a mix of all the electrophysiological signals around it. EEG does not provide perfect source localization due to the difficulty of the inverse problem with limited signal-to-noise ratio based on the limits of contact impedance and number of electrodes that are feasibly distributed over the scalp. EEG poorly measures areas of the brain below the cortex and often requires intense interpretation and averaging to determine neural activity correlates. However, it is safe to deploy and a well-developed field producing numerous impactful insights into brain dynamics.

Functional magnetic resonance imaging (fMRI)

Functional magnetic resonance imaging (fMRI) measures brain activity by detecting changes in the blood-oxygen-level dependent (BOLD) signal over a timescale of seconds. Whilst extremely valuable, it cannot measure electrical neural activity at this timescale. Separately, MRI is an expensive imaging method requiring dedicated staff to run it and large amounts of space to house the required magnets and helium quenching chambers making experimentation and improvement of this tool a slow and expensive process.

DIANA sequence fMRI

Recent work has announced a new MRI sequence with temporal resolution fast enough to directly capture neuronal activity³⁷, though the mechanism has thus far remained unclear. A new preprint recently published (May 29, 2023) failed to replicate the original DIANA study and the authors reported that the only way that they could get a DIANA-like signal was by cherry-picking data³⁸. Even if we later find a way to replicate the DIANA study or determine its mechanism, there is room for more than one solution to high time and spatial resolution neural imaging of electrical activity.

Magnetoencephalography (MEG)

Magnetoencephalography (MEG) is a neuroimaging technique for mapping the magnetic fields produced by electrical currents occurring naturally in the brain, using highly sensitive magnetometers³⁹. This technique is very sensitive to noise, needing to be

performed in a shielded room. Like EEG, this is also an ill-posed inverse problem making source localization difficult.

Magnetic fields are less distorted than electric fields by the skull and scalp, which results in the better spatial resolution of MEG when compared to EEG. However, the decay of magnetic fields as a function of distance is more pronounced than for electric fields. Therefore, MEG is most sensitive to superficial cortical activity, and unable to detect or localize electrical activity deeper in the brain.

Functional near infrared spectroscopy (fNIRS) and Diffuse Optical Tomography (DOT)

Functional Near Infrared Spectroscopy (fNIRS) typically uses two wavelengths of light (780nm and 2500nm) and their relative difference in spectrums to determine blood oxygenation. This can be used as a functional imaging technique when paired with tomography. In adults, diffusion and absorption limits the depth this technique can image due to the thickness of the skull. fNIRS has advantages in that it can be made portable and at a low cost and be used on infants whose skulls are comparatively thin⁴⁰. Apart from the limitation of measuring blood flow changes instead of electrical activity, a fundamental constraint of optical imaging is light diffusion, which limits the spatial resolution in deep-tissue imaging⁴¹.

Photoacoustic Tomography (PAT)

Photoacoustic tomography⁴² is based on the photoacoustic effect, where sound waves are emitted when light is absorbed in a medium. Non-ionizing laser pulses are delivered into biological tissues and part of the energy is absorbed and converted into heat, leading to transient thermoelastic expansion and thus wideband ultrasonic emission. The generated ultrasonic waves are detected by ultrasound transducers and then analysed to produce images⁴³. Optical absorption is closely associated with physiological properties, such as haemoglobin concentration and oxygen saturation; thus photoacoustic imaging can produce similar images to fNIRS, though with higher resolution at shallow depths.

Electrical Impedance Tomography (fast neural EIT)

Electrical impedance tomography (EIT) is a non-invasive form of medical imaging where a spatial tomographic image reconstruction is inferred from surface electrode measurements by sending small alternating currents through a conductive body such as the head to measure impedance changes^{44,45}. Its attractive for functional brain imaging as it can both spatially localize sources and be acquired at a time resolution sufficient to monitor fast electrical changes. Electrical conductivity varies considerably among biological tissues (typically used in absolute EIT) or the movement of fluids and gases within tissues (difference EIT). Thus, the frequency of the applied current can be used to differentiate material properties using bioimpedance spectroscopy (BIS).

Recently fast neural EIT was developed^{46,47}, which is based on the idea that as ion channels open in the brain, current will route through the cell wall, whereas when ion channels are closed, they cannot route through the cell wall yielding small detectable changes in impedance. Though exciting, broader limitations of the technique include changing contact impedance of the electrodes⁴⁸ leading to spurious estimations, algorithmic challenges for modelling capacitive properties in the medium, and the very high signal to noise ratio required to measure such small impedance changes through the skull making it impractical for non-invasive neuroimaging of electrical activity.

1.2.4 Discussion

In all the purely electromagnetic based stimulation techniques there are limitations due poor penetration as current routes around the skull, and poor focality due to the inhomogeneity of dielectric properties throughout the head. This same problem limits the focality of purely electrical neural imaging techniques such as EIT and EEG, which are also ill-posed inverse problems due to the limited number of electrodes on the scalp, differing contact impedances and cortical mixing of electrophysiological activity which obfuscates any information deeper in the brain.

Although our goal is to stimulate and detect electromagnetic activity in the brain, all the electromagnetic-only techniques share a common set of failure modes limiting their

focality to the principle of superposition, all unable to take advantage of interferential focusing at the length scales of the human brain. Hence it would be ideal if there was a physics-based interaction which let us convert from a non-electromagnetic field with more focal field properties at the length-scales of the brain, to electromagnetic activity.

1.3 The acoustoelectric effect

1.3.1 Rationale for selecting the acoustoelectric effect.

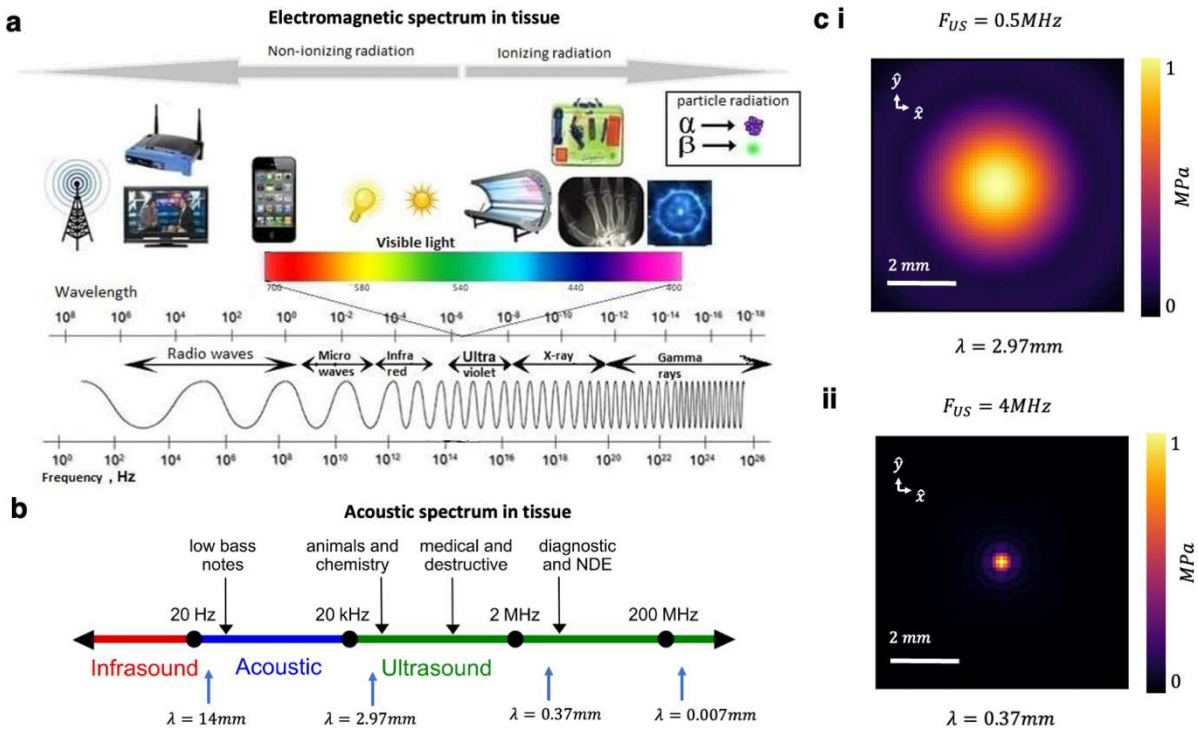


Figure 1-2 | Comparative wavelengths of electromagnetic and acoustic spectrums.

a, The electromagnetic spectrum **b**, The acoustic spectrum. **c**, i) 0.5MHz acoustic focal size ii) 4Mhz acoustic focal size.

In biomedicine, a long-standing goal has been to non-invasively and focally manipulate and record electrophysiological signals at depth. To date, this has been limited by the long wavelength of electric fields at the relevant frequencies, which exceeds anatomical length

scales⁴⁹ (e.g. at 100 kHz, the wavelength in tissue is in the order of hundreds of meters), thereby preventing spatial interferential focusing due to the diffraction limit. Higher frequencies with wavelengths within the anatomical length scales (e.g., at 10 GHz, the wavelength in tissue is in the order of tens of millimetres) have a low penetration depth in tissue (**Fig 1-2 a**). Remote focality has also been limited by the high dielectric heterogeneity of the tissue. For example, the cerebrospinal fluid in the brain leads currents away, preventing flexible targeting and precise focusing. In contrast to electric fields, acoustic fields have much shorter wavelengths at the same frequencies and a much better penetration, enabling spatial interferential focusing using established techniques such as beamforming at biological length scales⁵⁰. In addition, the acoustic properties of tissues have smaller variability than dielectric properties. For example, in the brain, focusing is achievable if skull aberration can be corrected for⁵¹. Hence, it would be advantageous to harness acoustic waves' focusing ability for both recording and stimulating electrophysiological signals (**Fig 1-2 b**).

1.3.2 History of the acoustoelectric effect

The electrolytic acoustoelectric effect was discovered by Herzfeld and Rock in 1946 when they observed conductivity changes under the influence of an acoustic field in salt solutions⁵². This project originated due to a Navy contract where they were enlisted to research the effects of acoustic waves in sea water.

In the year 2000, Lavandier and Jossinet^{53–56} quantified and extended this work putting the initial observations into an Ohm's Law based scalar derivation based on the adiabatic compressibility and ionic mobility of the electrolytic medium. Their in-depth analysis of ionic mobility gave the existence of the acoustoelectric effect a solid foundation. They report that in the acoustoelectric effect⁵⁷, local mechanical compressions modulate the ion density, thereby changing the spatial distribution of the conductivity. According to Ohm's law, the mechanical modulation of conductivity perturbs the electric potential thereby inducing a small current.

Ultrasound current source density imaging (UCSDI)

More recently in 2008, Olafsson and Witte^{58–60}, applied the work of Herzfeld, Rock, Lavandier and Jossinet to a new measurement technique called current source density imaging in biological tissue, updating the mathematical derivation with a lead-field reciprocity-based estimate to calculate the current density distribution in tissue. They have obtained acoustoelectric measurements from an *ex vivo* lobster nerve and rabbit heart extending its application to biological imaging. Berthon and Tanter⁶¹, also investigated the technique showing it could be used to create images with a high time resolution. These findings all struggle with a precise understanding of the underlying physics and the signal to noise of the measured acoustoelectric field which has thus far limited its translational potential to larger electrophysiological signals such as those coming from the heart⁶².

Acoustoelectric Brain Imaging (AEBI)

In 2022, 2 years after I started this PhD project articles in a lab led by Dong Ming in China^{63–66} emerged claiming the acoustoelectric effect has been used to decode neural signals in rats. This work uses a different signal reconstruction technique to the other labs that have tried ultrasound current source density imaging, specifically using a low pulse repetition rate of 80Hz for the decoding of the acoustoelectric envelope. They obtain an acoustoelectric efficiency ratio⁶⁶ of 1:24, and this work is investigated in depth in **chapter 5** which is dedicated to acoustoelectric neural recording, and these experiments are recreated in a saline phantom and *in vivo*. The results of this investigation, suggest that unless they provide a spectral specificity confound test, it is impossible to distinguish the demodulated signal from VEP harmonics.

Acoustoelectric opportunities identified for investigation

There is an opportunity to provide a better understanding of the mathematics and physics underlying the acoustoelectric interaction, as the derivation to date is based on the scalar quantities in Ohm's law. It would also be valuable to provide clear evidence in a physiological phantom that the acoustoelectric effect is not confounded by other artefacts, and any factors that can increase and decrease its amplitude investigated. Any new physics findings can then be translated into an *in vivo* setting, ideally showing large signal

to noise improvements from previous work to gauge the future translational potential for diagnosing and treating neurological disorders.

1.4 Non-invasive bidirectional acoustoelectric neural interface concept overview

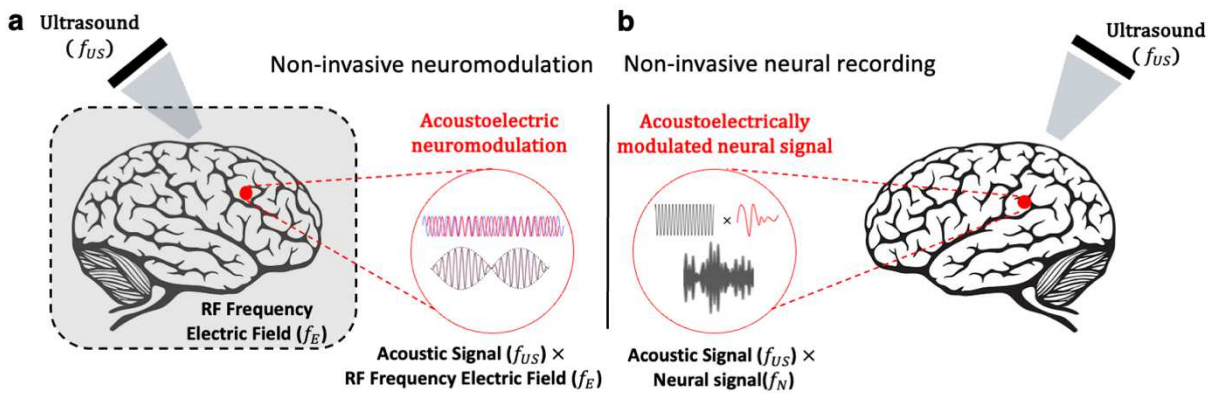


Figure 1-3 | Diagram of acoustoelectric neuromodulation and neural recording concepts.

a, Acoustoelectric neuromodulation. A high frequency electric field and ultrasound are used to create a focal electric field at the difference frequency. **b**, Acoustoelectric neural recording. Heterodyning occurs between an endogenous electric field and the acoustic focus such that the modulated high frequency signal can be detected on an external measurement electrode.

1.4.1 Acoustoelectric neuromodulation concept

The core innovation of this new stimulation approach involves using ultrasound to control the focal size of an electric field through use of the acoustoelectric effect. Ultrasound has a much smaller wavelength than electric fields, enabling it to have higher focality at depth⁶⁷. Acoustoelectric neuromodulation is hypothesized to occur when an applied electric field mixes with an acoustic field (**Fig 1.3 a**). The resultant electric field will have the shape of the ultrasound focus and manifest as the sum and difference frequencies between the applied acoustic and electric field. In this way, we can apply high frequency fields which are beyond the frequencies known to stimulate the brain and elicit a low difference frequency neuromodulatory response within the electrophysiological range.

1.4.2 Acoustoelectric neural recording concept

Acoustoelectric neural recording (**Fig 1.3 b**) is achieved when ultrasound mixes with endogenous brain signals at the acoustic focus. The resultant high frequency sum and difference frequency electric signals can be detected outside the head as there are no other naturally occurring signals in this range (other neural signals or thermal based Johnson noise), enabling high signal to noise ratios in signal recovery. This is different to the work in ultrasound current source density imaging to date as it does not make use of the information that the acoustoelectric interaction involves heterodyning. By harnessing this information in a neural decoding algorithm, we aim to reach much high signal-to-noise ratios making neural decoding far more viable.

There are two advantages of this technique over existing neuroimaging techniques. Firstly, we may be able to decode neural activity at only the focal area of the ultrasound, providing both spatial specificity and greater depth than existing electrical techniques which can access only the cortical areas. Secondly, at the modulated frequency, the electric signal travels further since the impedance is lower, conveying transmission advantages through the skull.

1.5 Aims and outline of this thesis

1. Acoustoelectric theory and simulation.
2. Physiological phantom experiments on the acoustoelectric field.
3. Acoustoelectric neural recording *in vivo*.
4. Acoustoelectric neuromodulation *in vivo*.

1.5.1 Acoustoelectric theory and simulation

The hitherto existing model of the acoustoelectric effect assumes a scalar interaction, limiting the accuracy of the predictions to a planar transducer where the acoustic field has a singular direction. Since increasing the conversion efficiency of the acoustoelectric interaction would be advantageous, a more accurate mathematical model and simulation would allow us to explore this interaction in more detail. For instance, if we could

accurately understand the impact of the medium properties, applied field amplitudes and frequencies, we could optimize the acoustoelectric magnitudes.

This results chapter explores acoustoelectric theory with a new mathematical derivation, inclusive of the resultant predictions of orientation dependence and heterodyning, followed by a homogeneous k-space simulation, and an inhomogeneous finite element simulation in the mouse brain. From these simulations we can determine whether the resultant amplitudes are within the electrophysiological range for neuromodulation, and whether the technique can be performed within relevant established safety parameters for ultrasound. Additionally, we then compare the predicted focality against the most focal electromagnetic neuromodulation to date i.e. temporal interference, proposing that acoustoelectric neuromodulation can provide an order of magnitude improvement.

1.5.2 Physiological phantom experiments on the acoustoelectric field.

The second results chapter is based on the development of an automated tank phantom used to map the acoustoelectric field and to verify and validate the predictions made in derivation and simulation, ensure the acoustoelectric field is not an artefact, and test the acoustoelectric neural recording and neuromodulation concepts in saline. Further studies are performed to understand the ionic mobility term in the derivation in terms of controlled temperature and salinity experiments.

1.5.3 *In vivo* acoustoelectric neural recording

By moving to an *in vivo* rodent model for experimental verification, we develop an experiment to measure visual evoked potentials whilst under the influence of continuous ultrasound stimulation. We then interpret the heterodyne findings of the acoustoelectric interaction in terms of the ultrasound current source density literature, unveiling the underlying mechanism. Through recreation of prior literature showing acoustoelectric signal recovery at very low pulse repetition frequencies, we show an extra artefact test is necessary to separate these findings from VEP harmonics. With this background, we acoustoelectrically demodulate visual evoked potentials *in vivo* around the carrier frequency of the ultrasound for the first time utilizing heterodyning, with artefact tests

proving it is not an artefact. Furthermore, single trial spontaneous neural activity can be acoustoelectrically decoded without the use of averaging, suggesting this technique could become a useful real-time tool.

1.5.4 *In vivo* acoustoelectric neuromodulation

The first *in vivo* evidence of acoustoelectric neuromodulation is reported and an evidence-based argument developed as to why the acoustoelectric interaction is a contributing mechanism to ultrasound brain stimulation. Acoustoelectric neuromodulation has a clear mechanism of action, harnessing the focality of ultrasound and the well-understood direct electrical stimulation pathway developed by the Hodgkin-Huxley model, giving potentially unique advantages to target deep areas of the brain with a highly controllable mechanism of action.

1.6 Risks and challenges

Ideally good science should be about taking calculated bets to push our understanding of the natural world forward. However, we should be strategic in where we place our efforts, to optimize for the best outcomes. Below, I lay out an array of risks and challenges, which are then tackled in the forthcoming Chapters.

Firstly, the electrolytic acoustoelectric effect has not enjoyed much popularity to date in scientific literature compared to the piezoelectric effect, making it questionable that this interaction even exists. The solid-state acoustoelectric interaction induced via acoustic surface waves in solids has also received for more attention^{68–70}. If it can be verified to exist, the acoustoelectric interaction in electrolytes has unique properties that make it highly advantageous over established neuromodulation and neural recording techniques as outlined in our review of existing techniques. I will endeavour to perform thorough tests to ensure measurements are not artefactually confounded.

The theoretical basis of our understanding of the acoustoelectric effect has thus far limited the signal to noise and amplitude of the detected acoustoelectric interaction. If we cannot

push forward to new theoretical and physically verified predictions, showing that this effect has the potential to reach greater signal to noise ratios, it may not be worth pursuing further.

Below is a breakdown of risks for *in vivo* translation which can be added on top of any theoretical, simulation and tank phantom characterization risks:

- Too small: The evoked difference frequency may be too small to induce neuromodulation, or to detect for neural recording.
- Too big: The required amplitude acoustic or electric fields may cause tissue damage.
- We have little understanding of the electrophysiological repercussions of applying high frequency and high amplitude voltages to brain tissue.
- There is a potential confound with direct ultrasound neuromodulation. However, we expect stimulation at the onset only with ultrasound neuromodulation and would not expect to see the emergent frequency mixing components, hence it should be differentiable.

Chapter 2: Theory and simulation of the acoustoelectric effect

2.1 Introduction

In biomedicine, a long-standing goal has been to non-invasively and focally manipulate and record brain signals for diagnostic and therapeutic purposes and a better understanding of the brain. It would be advantageous to harness acoustic waves' focusing ability for recording and manipulating electrophysiological signals, whilst affecting the neural tissue directly through the well understood Hodgkin-Huxley^{71,72} model which describes electrical brain interaction. In other words, the acoustoelectric interaction⁵² is an ideal candidate.

The previously accepted model of the electrolytic acoustoelectric phenomenon^{73,74,75} has been based on Ohm's law which does not account for the vectorial and spatially dependent nature of the underlying physics. Thus, there is potential to further our understanding of the interaction by developing a mathematical model that captures these key characteristics using Gauss theory⁷⁶.

To further understand the emergent characteristics of the acoustoelectric interaction, a homogeneous simulation of the mathematical result for the acoustoelectric effect was developed using k-space approximations. This fast-running simulation enabled safety and amplitude estimations, and comparison against existing electrical stimulation techniques.

The new vector based mathematical model was then solved in an inhomogeneous tissue model of a mouse, using finite element modelling. An acoustic, quasistatic electric field

and a thermal solver are modified to solve the equation in inhomogeneous media, with thorough convergence tests throughout to ensure accuracy.

The new acoustoelectric interaction proposed in this chapter enables two key predictions, one of angular dependence between the incident electric and pressure fields due to the introduction of the acoustic gradient term, and the second that heterodyning will occur between the electric and acoustic field. Simulation of the acoustoelectric effect enabled safety predictions for acoustoelectric neuromodulation, and a comparison with an existing focal electrical neuromodulation technique - temporal interference, predicting an order of magnitude improvement in focality.

Lastly, finite element modelling of the acoustic field only is used to predict how ultrasound reflections in a mouse head alter the focality of stimulation for a 500kHz transducer with parameters matching the later experimental ultrasound chapters. We see that the focality is affected by reflections and the duration of the ultrasound pulse, and that the skull is relatively transparent compared to the air/head interface which causes the bulk of acoustic reflections.

2.2 Physical description of the acoustoelectric effect

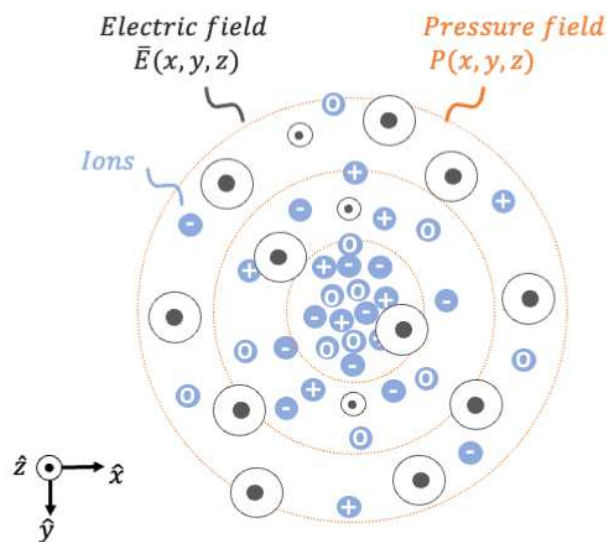


Figure 2-1 | The acoustoelectric field.

The acoustoelectric interaction is the modulation of conductivity induced by the pressure field during periods of compression and rarefaction, as the density of ions in solution⁵² oscillates. When a current is applied to the ionic solution, the localized conductivity change from the acoustic field will induce a localized potential difference due to the ion density modulation.

2.3 Mathematical derivation of acoustoelectric field in electrolytic liquids and soft tissues

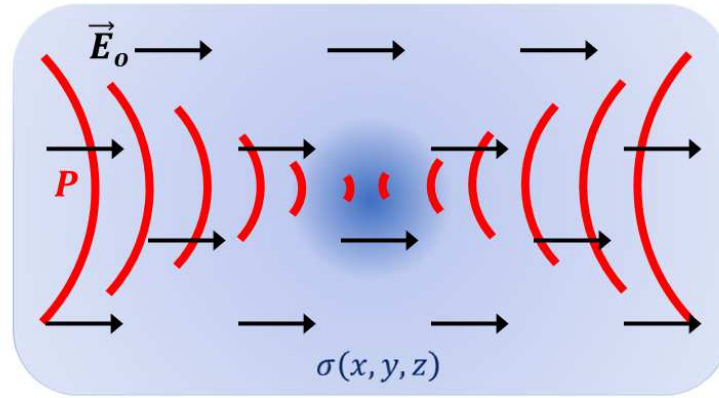


Figure 2-2 | Model schematic

We assume an external electric field $\vec{E}_o(x, y, z)$ that induces ionic currents with a density $\vec{J}(x, y, z)$ in an electrolytic liquid (or soft tissue) with a conductivity distribution $\sigma(x, y, z)$, **Figure 2.2**. According to Gauss's theory, the integral of the normal component of the current vectors over a closed surface is equal to the integral of the current source density within the enclosed volume⁷⁶. Thus, if the current source is external to the volume of interest, the divergence of the current density is zero at any location within the volume of interest.

$$\nabla \cdot \vec{J} = 0 \quad (1)$$

According to Ohm's law, the current density equals the product of the electric field and conductivity⁷⁶.

$$\vec{J} = \sigma \vec{E}_o \quad (2)$$

Combining **Eq. 1** and **2** states that the divergence of the product of the conductivity and electric field is zero within the volume of interest.

$$\nabla \cdot (\sigma \vec{E}_o) = 0 \quad (3)$$

If an acoustic pressure field $P(x, y, z)$ is concurrently applied to the electrolytic medium, it creates a conductivity perturbation $\sigma_{ae}(x, y, z)$ due to the electrolyte's compression and rarefaction in space and the associated changes in ion concentration. This conductivity perturbation creates an electric field modulation $\vec{E}_{ae}$ such that Gauss's law of net-zero flux is again satisfied.

$$\nabla \cdot ((\sigma + \sigma_{ae})(\vec{E}_o + \vec{E}_{ae})) = 0 \quad (4)$$

Expanding we get

$$\nabla \cdot (\sigma \vec{E}_o) + \nabla \cdot (\sigma \vec{E}_{ae}) + \nabla \cdot (\sigma_{ae} \vec{E}_o) + \nabla \cdot (\sigma_{ae} \vec{E}_{ae}) = 0 \quad (5)$$

The first term equals zero due to **Eq. 3**, leaving:

$$\nabla \cdot (\sigma \vec{E}_{ae}) + \nabla \cdot (\sigma_{ae} \vec{E}_o) + \nabla \cdot (\sigma_{ae} \vec{E}_{ae}) = 0 \quad (6)$$

The last term is a product of two small quantities and can be neglected as explored at the end of this section (see **Section 2.3.1** small term assumption).

Eq. 6 is then reduced to

$$\nabla \cdot (\sigma \vec{E}_{ae}) + \nabla \cdot (\sigma_{ae} \vec{E}_o) = 0 \quad (7)$$

Exploring the relationship between conductivity and pressure

The conductivity σ of electrolytic solutions as shown above, is governed by the concentration and mobility of the dissolved ions. In an electrolyte, the conductivity can be expressed as

$$\sigma = F \gamma_{\pm} k_d c (z_+ \mu_+ + z_- \mu_-) \quad (8)$$

where F is the Faraday constant, $\gamma_{\pm}$ is the mean activity coefficient, k_d is the dissociation constant, c is the solute molar concentration (i.e., molarity), z_+ and z_- are the charge

number (i.e., valence) of the cation and anion, respectively, and μ_+ and μ_- are the electrical mobility of the cations and anions, respectively⁷⁷. Physiological saline is considered in this study as a strong electrolyte with a low molar concentration of single valence ions resulting in $\gamma_{\pm}$ and k_d values of ~ 1 . Thus, the conductivity is governed by the concentration and mobility of the dissolved ions. In saline $z_+ = z_- = 1$, and $\mu_+ \approx \mu_- = \mu$ hence

$$\sigma \approx 2Fc\mu \quad (9)$$

The partial derivative of conductivity in terms of molar concentration and mobility results in a relative change in conductivity proportional to the sum of the relative change in molar concentration and relative change in mobility

$$\frac{\partial \sigma}{\sigma} = \left(\frac{\partial c}{c} + \frac{\partial \mu}{\mu} \right) \quad (10)$$

The relative change in molar concentration c is exerted by the pressure induced compression and rarefaction of the solution's volume (V) with fixed number of ions. Under adiabatic conditions, the relative volume change is related to the pressure P by

$$\frac{dV}{V} = -\beta P \quad (11)$$

where β is the medium's adiabatic compressibility. Adiabatic compressibility is a measure of how much a material's volume changes when pressure is applied, and how it relates to the material's density ($\rho = \frac{m}{V}$) i.e.:

$$\frac{dP}{P} = \rho \beta \quad (12)$$

However, in an ionic solution, we use the molar concentration c to understand the concentration of ions under pressure instead of density. Since

$$c = \frac{n}{V} \quad (13)$$

where n is the ions amount in moles, the derivative of the molar concentration in terms of volume results in a relative change in molar concentration equal to the product of the pressure P and the medium's adiabatic compressibility β .

$$\frac{\partial c}{c} = \beta P \quad (14)$$

The relative change in mobility μ is exerted via the pressure-induced change to the solution's temperature and viscosity⁷³ controlled by Van der Waals interactions. The change in viscosity is negatively proportional to the pressure at temperatures higher than 4°C⁷⁸. The change in temperature is positively proportional to the pressure via the medium's volumetric (cubic) thermal expansion coefficient⁷⁹. Thus

$$\frac{\partial \mu}{\mu} = \alpha P \quad (15)$$

where α is a constant.

Substituting from **Eq. 10**, the relative change in conductivity is proportional to the applied pressure

$$\frac{\partial \sigma}{\sigma} = (\beta + \alpha)P = kP \quad (16)$$

where k is a constant incorporating the pressure dependent changes in relative molar concentration and ionic mobility.

Substituting **Eq. 16** into **Eq. 7**

$$\nabla \cdot (\sigma \vec{E}_{ae}) + \nabla \cdot (\sigma kP \vec{E}_0) = 0 \quad (17)$$

Using the divergence product rule ($\nabla \cdot (a\vec{b}) = a(\nabla \cdot \vec{b}) + \nabla a \cdot \vec{b}$) we get

$$\nabla \cdot (\sigma \vec{E}_{ae}) + \nabla(kP) \cdot (\sigma \vec{E}_0) + kP \nabla \cdot (\sigma \vec{E}_0) = 0 \quad (18)$$

Since $\nabla \cdot (\sigma \vec{E}_0) = 0$ in **Eq. 7**, we get

$$\nabla \cdot (\sigma \vec{E}_{ae}) = -\nabla(kP) \cdot (\sigma \vec{E}_0) \quad (19)$$

Since the dot product of two vectors provides information about the angle between them, it can be seen in **Eq. 19** that the amplitude of the resultant acoustoelectric field depends on the relative orientation of the pressure gradient to the electric field.

2.3.1 Small Term Assumption

We aim to compare the magnitude of $\nabla \cdot (\sigma \vec{E}_{ae})$ and $\nabla \cdot (\sigma_{ae} \vec{E}_0)$ with that of $\nabla \cdot (\sigma_{ae} \vec{E}_{ae})$ in **Eq. 6**. σ_{ae} is smaller than σ by a factor kP and, based on **Eq. 19**, so is $\vec{E}_{ae}$ compared

to $\vec{E}_0$. However, a careful comparison also must consider that the product of $\sigma_{ae}\vec{E}_{ae}$ is the product of two distributions that both contain frequency components with spatial frequencies of $2\pi cf$, where c is the acoustic velocity in the medium of interest and f is the frequency. Thus $\sigma_{ae}\vec{E}_{ae}$ contains spatial frequencies up to twice that spatial frequency (multiplication in direct space corresponds to convolution in Fourier space), which leads to an additional factor of up to 2 when applying the gradient. As a result, the magnitude of $\nabla \cdot (\sigma_{ae}\vec{E}_{ae})$ is in the order of $2kP$ smaller than that of $\nabla \cdot (\sigma\vec{E}_{ae})$ and $\nabla \cdot (\sigma_{ae}\vec{E}_0)$.

Assuming the acoustic change in conductivity is based on the change in molar concentration and ionic mobility^{80,81} i.e., $k = 1 \times 10^{-9}[1/Pa]$ and that the peak pressure is in the order of $10^6[Pa]$, $2kPa = 0.002 \ll 1$, thus confirming **Eq. 7** is at least a self-consistent approximation.

2.3.2 Special Case 1: Homogeneous medium

If the region of interest has a constant adiabatic compressibility and conductivity, we get

$$\nabla \cdot \vec{E}_{ae} = -k \nabla P \cdot \vec{E}_0 \quad (20)$$

2.3.3 Special Case 2. Infinite planar transducer

In the case of an ideal infinite planar transducer, pressure travels in one direction, and if the electric field is oriented in the same direction, or shows the same translational symmetry, the acoustoelectric model can be reduced to a scalar one. In Cartesian coordinates, **Eq. 19** can be written as

$$\frac{\partial E_{ae,x}}{\partial x} + \frac{\partial E_{ae,y}}{\partial y} + \frac{\partial E_{ae,z}}{\partial z} = -k \frac{\partial P}{\partial x} E_{0,x} - k \frac{\partial P}{\partial y} E_{0,y} - k \frac{\partial P}{\partial z} E_{0,z} \quad (21)$$

where $E_{ae,j}$ and $E_{0=0,j}$ are the components of the generated acoustoelectric field and applied electric field in the $\hat{j}$ direction ($\hat{j} = \hat{x}, \hat{y}, \hat{z}$), respectively. If the acoustic field is applied via an infinite planar transducer and the electric field is parallel to the acoustic field propagation (or shows the same $\hat{y}\hat{z}$ translational symmetry)

$$\frac{dE_{ae,y}}{dy} = \frac{dE_{ae,z}}{dz} = k \frac{dP}{dy} E_{0,y} = k \frac{dP}{dz} E_{0,z} = 0 \quad (22)$$

Thus, we get a one-dimensional equation:

$$\frac{dE_{ae}(x)}{dx} = -k \frac{dP(x)}{dx} E_0(x) \quad (23)$$

Integrating over x , using the integration by parts rule ($\int f'g = fg - \int fg'$)

$$E_{ae}(x) = -kP(x)E_0(x) + k \int_0^x P(x) \frac{dE_0(x)}{dx} dx + c \quad (24)$$

The constant c must be zero because there is no E_{ae} without E_0 and P . If the electric field is homogenous such that $\frac{dE_0(x)}{dx} = 0$, we get:

$$E_{ae} = -kPE_0 \quad (25)$$

The amplitude of the acoustoelectric field under these special conditions is proportional to the multiplication of the pressure and the background electric field. This equation thus reduces to the previously employed and published equation⁸², which is revealed to be a special case of a more general model.

2.4 Simulation Methods

2.4.1 K-space homogeneous simulation of the acoustoelectric field

The simulations of the acoustoelectric modulation generated by the applied electric and acoustic fields were performed by solving **Eq. 19** for the induced electric potential ϕ_{ae} , with $\vec{E}_{ae} = -\nabla\phi_{ae}$, assuming a homogeneous substrate. The solution can be obtained by solving the Poisson equation i.e. $\Delta \cdot (\phi_{ae}) = \nabla(\beta P) \cdot (\vec{E}_0)$ with a source term $S = -\nabla(\beta P) \cdot (\vec{E}_0)$, using a custom Python script. To compute the source term, the applied acoustic field P was computed with an analytic solution for an ideal spherically focused ultrasound transducer using a summation of Bessel functions⁸³. The applied electric field $\vec{E}_0$ was assumed to be homogenous (constant orientation and magnitude). The dot product of ∇P and $\vec{E}_0$ was computed and then multiplied by the constant k (i.e. $1\text{e-}9 \text{ Pa}^{-1}$)^{77,81}, which is readily available from material datasheets. To solve the Laplacian in the Poisson equation for the electric potential ϕ_{ae} , we implemented a Fast-Poisson-Solver⁸⁴ (since σ was

homogeneous), leading to a diagonal system in Fourier-space (k-space). The Fourier transformation of the source term S resulted in $k^2 \widehat{\phi_{ae}}(k) = -\hat{S}(k)$, where $\hat{f}(k)$ denotes the spatial Fourier transformation of $f(x)$. Then $\widehat{\phi_{ae}}(k) = -\hat{S}(k)/(k^2)$ and ϕ_{ae} were obtained through an inverse Fourier transformation. $\vec{E}_{ae}$ was obtained as the negative gradient of ϕ_{ae} or by multiplying with $-i\vec{k}$ before inverting the transformation component-wise.

The k-space solution, installation and running instructions are published online on Github⁸⁵.

2.4.2 Finite element heterogeneous simulation of the acoustoelectric field.

To solve the acoustoelectric equation for the more general case of inhomogeneous media with arbitrary electrode geometries, the finite element modelling tool Sim4Life (IT'IS Foundation, Zurich) was used, as it has been rigorously tested to meet the MQS001AA201507 verification benchmark. I created geometry using parallel plates to create a constant electric field, and a spherically focused transducer. Since the acoustoelectric equation has not been solved in inhomogeneous media previously, no single solver encapsulated the mathematics. Hence, the 3-solver pathway shown (**Fig. 2.3 a**) first solves for the acoustic pressure field using a LAPWE solver, then the quasi-electrostatic solution for electric field before exporting the individual field results into Python to compute the source term for the diffusion equation. The final step of solving the diffusion equation to obtain the acoustoelectric potential is done by modifying a thermal solver.

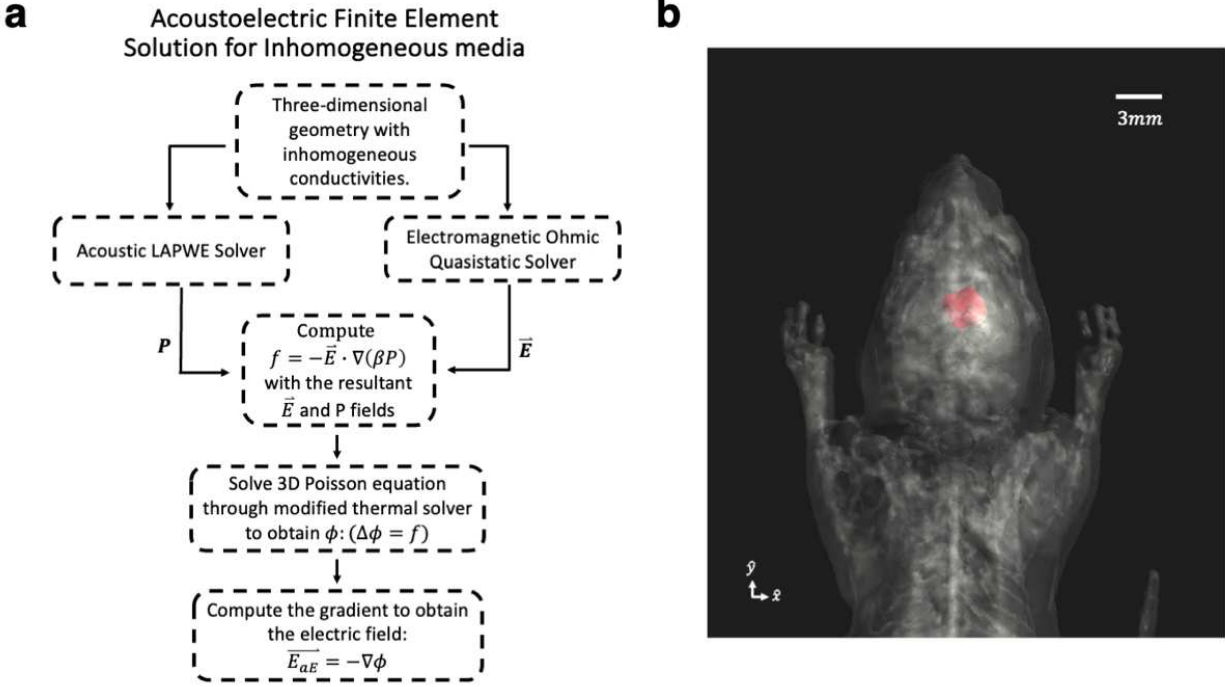


Figure 2-3 | Finite Element Model Simulation.

a, Simulation pathway under a constant electric field generated with parallel plates and a spherical focused transducer. **b**, Acoustoelectric field cross-section solved deep inside brain of heterogeneous mouse model showing intensity -3dB contour of 1MPa 500kHz acoustic wave.

The acoustoelectric effect can be expressed in terms of Poisson's equation:

$$\Delta\phi_{ae} = f \quad (26)$$

where $\Delta\phi_{ae}$ is the Laplacian of the acoustoelectric potential and the source term

$f = -\vec{E}_0 \cdot \beta \nabla \delta P$. Since most FEM solvers do not contain an explicit Poisson solver, we modified Penne's Bioheat Equation which is implemented in the thermal solver in Sim4Life⁸⁶. The Penne's Bioheat Equation states that:

$$\rho c \frac{\delta T}{\delta t} = \nabla \cdot (k \nabla T) + \rho Q + \rho S - \rho_b c_b \rho \omega (T - T_b) \quad (27)$$

where k is the thermal conductivity, Q denotes the specific metabolic heat generation rate, S is the specific absorption rate, and ω represents the perfusion rate. ρ is the density of the medium, ρ_b , c_b and T_b are respectively, the density, the specific heat capacity, and the temperature of blood. Metabolic heat generation, heat capacity, thermal conductivity, density, and perfusion coefficients are all tissue-specific quantities. The term $\rho_b c_b \rho \omega$ is

often lumped together and called the heat-transfer rate. To modify this into the diffusion equation we set the heat generation rate Q to 0, k to 1, ρ to 1, and the change in temperature with respect to time and initial conditions to 0. This results the Penné's Bioheat Equation now being of the form:

$$\nabla \cdot (\nabla T) = -S \quad (28)$$

or the Poisson equation. Here T will be replaced by ϕ_{ae} and $-S$ will be the calculated source term. We can now perform the diffusion in Sim4Life by importing our computed source term, using the thermal solver modified as per the above description. The diffusion is solved independently for the real and imaginary parts of the source term, to keep the time domain phasor properties intact.

The final acoustoelectric field (V/m) is calculated by taking the negative gradient of the solution generated by the diffusion solver. This simulation pathway can be used for both homogeneous and heterogeneous media, though is limited by the assumption that the adiabatic compressibility is a constant and found from a material database^{57,87}[NO_PRINTED_FORM], and conductivity is a constant⁸⁸ which is only true in a homogeneous medium. This pathway lets us solve for arbitrary geometries and makes an approximation for heterogeneous media as shown in our mouse tissue solution (Fig 2.3 b).

The heterogeneous finite element solver pathway and relevant Python code joining the solvers together, installation and running instructions are published online⁸⁹ on Github with associated readme files describing solver functionality and pathway.

2.4.3 Finite element model simulation validation

The k-space simulation and heterogeneous finite element model are the first simulation solutions to the new derivation, so it was necessary to validate the accuracy of the simulation before making any scientific inferences. Error could be introduced through poor convergence, discretization, or computational errors in the implementation. By removing the uncertainty in these error sources, greater weight could be made on the simulation

solutions to enable modelling and exploration of physical parameters such as safety, focality and neuromodulation feasibility.

2.4.3.1 Acoustic validation

The accuracy of the acoustic finite element simulation was ascertained by comparing the Sim4Life acoustic solution for a spherical transducer to an analytic solution of Bessel equations in a homogeneous medium. The analytic solution can be calculated without a finite element model, using a Matlab solution written by Robin Cleveland, Professor of Biomedical Engineering Science at Oxford. This solution is based on a numerically convergent solution of the pressure distribution in terms of a summation of Bessel functions⁹⁰. Through this analytic convergent solution, the ultrasonic transducer planned for experiments was simulated in a homogeneous medium accurately and then compared to the Sim4Life acoustic transducer solution.

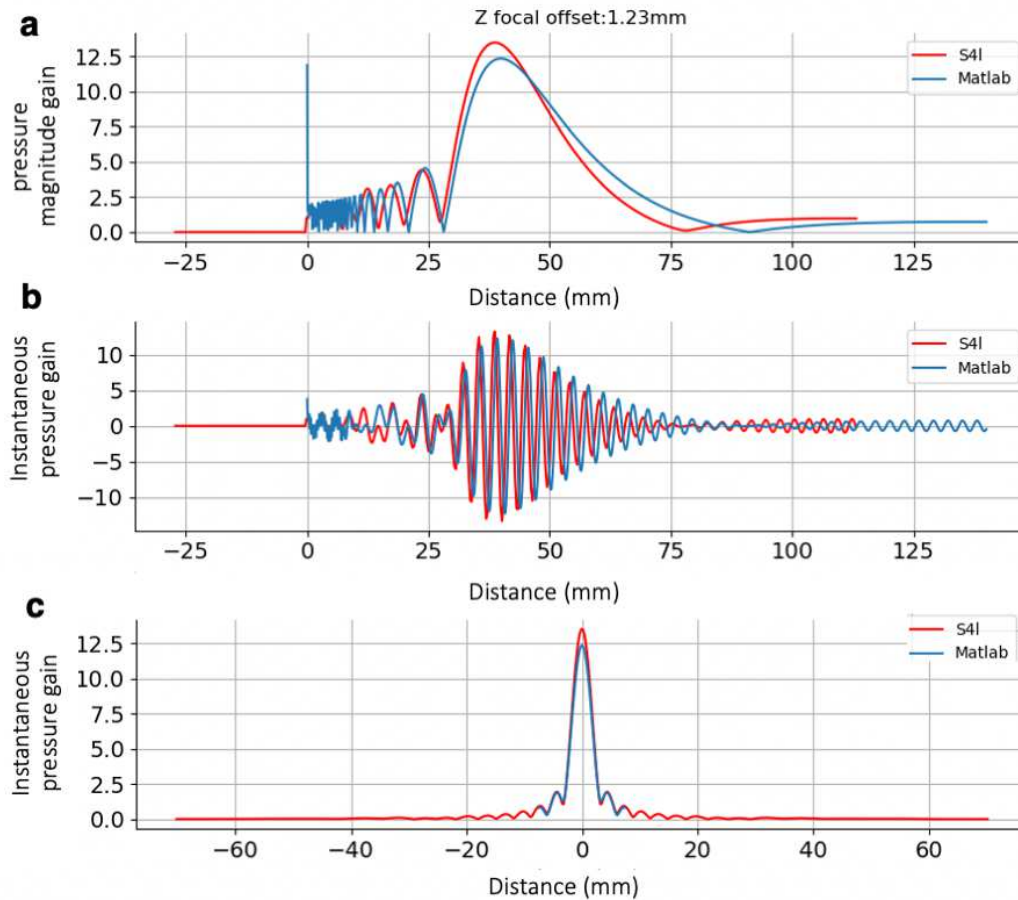


Figure 2-4 | Instantaneous pressure amplitude.

Comparison between analytic matlab solution (Blue) and Sim4Life FEM simulation (Red). **a, Axial comparison between FEM solver and analytic Matlab solution of the pressure magnitude - x axis starts at base of transducer moving outwards past the pressure focus, b, axial view of the instantaneous pressure c, radial view of the pressure magnitude, thus comparing the analytic summation of Bessel function solution with the finite element model in Sim4Life.** The transducer is modelled as a 0.5MHz transducer with a radius of curvature of 43mm and aperture width of 44mm and the speed of sound in water is 1488m/s.

In **Figure 2.4** there is a small axial magnitude and position offset. The focal offset is less than a wavelength, showing a reasonably accurate solution at the current grid resolution of 0.3mm. To increase accuracy the grid resolution can be increased, although this also increases the duration of calculation which makes any simulation development and testing far slower to iterate. The grid size for the finite element solution shown is 0.3mm which can be compared to the wavelength of the transducer to estimate of potential error margins. At 0.5MHz, knowing that $f = \frac{c}{\lambda}$ where f is transducer frequency, c is the speed of sound in the medium, and λ is the wavelength, the wavelength is 2.976mm. With this grid resolution and wavelength there are 9.9 grid points per wavelength. The offset or error between the two simulations shown in **Fig 2.4** is 1.23mm. The number of grid points per wavelength is the causes a small discretization error. 12 points per wavelength would yield better accuracy in the FEM solvers. However, at higher grid resolutions the computational limits of the server were reached, and the simulation would stall. This scale discretization error was considered sufficient in the FEM simulation for estimating the size and feasibility of the acoustoelectric effect for neuromodulation.

2.4.3.2 EM Simulation Validation

The electromagnetic field has a larger wavelength at around 80m, so the grid parameters for this simulation in a homogeneous medium are far less important than for the acoustic simulation. The electrostatic potential between two point-charges is:

$$\phi(r) = \frac{1}{4\pi\epsilon_0} \frac{3(p \cdot \hat{r})}{r^2} \quad (29)$$

where p is the dipole moment, ϵ_0 is the permittivity of free space, and r is the distance between the point-charges. The electric field due to a dipole field in a homogeneous medium with point charges can be computed as:

$$E = -\nabla\phi = \frac{1}{4\pi\epsilon_0} \frac{3(p \cdot \hat{r})\hat{r} - p}{r^3} \quad (30)$$

where ϕ is the potential, and E the electric field. These equations are well known, and the Sim4Life Low Frequency Quasistatic solvers have been used in numerous studies for FDA approved medical devices based on its solutions, as well as the independent verification by an external company Thess S.A. who verified these solvers meet the MQS001AA201507 verification benchmark.

2.4.3.3 Gradient Calculation validation

To validate each of the calculated quantities in the finite element modelling/Python joint pathway, the gradient operator was assessed using numerical differentiation as it is used to both calculate the gradient of the pressure field and once again to determine the gradient of ϕ when making the final electric field estimation of the acoustoelectric field:

$$\Delta\phi_{ae} = (\nabla \cdot \nabla)\phi_{ae} = \nabla E_{ae} = E_0 \cdot \beta \nabla P \quad (31)$$

The goal of the gradient validation is to determine that the gradient calculation can handle complex numbers as ϕ is a complex number, can compute over an irregular grid, and lastly compute accurately over three dimensions. The gradient calculation tested was the implementation in Numpy 1.19. To verify Numpy's gradient calculation, it was compared to the numerical differentiation approach where gradient is defined as:

$$f'(x) = \lim_{h \rightarrow 0} \left(\frac{f(x+h) - f(x)}{h} \right) \quad (32)$$

Here x is the grid position and h is the change in position. Equivalently, for grid positions with elements on either side, an accurate way to compute the derivative is using the central difference formula:

$$f'(x) = \lim_{h \rightarrow 0} \left(\frac{f(x+h) - f(x-h)}{2h} \right) \quad (33)$$

Higher order derivatives can be estimated with higher order Taylor expansions and the edges of a volume can be calculated with the forward or backward differences. To test Python's numerical computing Numpy library gradient implementation, a second order estimation of the derivative was taken using the central difference theorem shown above. Since the pressure field can be extracted from the acoustic finite element solver as well as the grid points used in the finite element model, a gradient comparison could be made with Numpy 1.19 calculated using the central difference formula. The Euclidean norm

between the two calculated gradients was assessed. There was a small difference in the Euclidean distances due to wavelength/grid mismatches, otherwise known as discretization error. During the finite element modelling pathway simulation, several grid interpolations are performed to go from one simulation grid to another, always interpolating the larger grid into the smaller grid to minimize these errors. If the grids and wavelength points are not precisely aligned, this error will always be present. No FEM solver is completely accurate, though the discretization can be sufficiently small compared to the expected length scales of the modelled physical interaction enabling verification of the physics model in simulation.

2.4.3.4 Poisson solver validation

The finite element modelling pathway requires the discrete solution to the Poisson equation in three dimensions. To prove that accurate convergence occurs in the highly modified Sim4Life thermal solver, the central difference theorem was used to compute the Laplacian operator of the resultant voltage field ϕ , and this value was compared to the original source term of the Poisson simulation. Thus, the numerical accuracy of the solution can be validated by back-calculating a source term and comparing it to the finite element modelling computed source term.

The Laplacian of a function ϕ is the sum of all the unmixed second partial derivatives:

$$\Delta\phi = \sum_{i=1}^n \frac{\delta^2\phi}{\delta x_i^2} \quad (34)$$

Using the central difference theorem from numerical methods the second derivative was estimated with respect to x as:

$$\frac{\delta^2\phi}{\delta x_i^2} = \frac{\phi_{i+1j} - 2\phi_{ij} + \phi_{i-1j}}{(\nabla x)^2} \quad (35)$$

This technique was implemented on the resultant ϕ to back compute the original source term. The numerical methods calculation of ϕ was then subtracted from the source term and the Euclidean distance computed to determine the similarity between quantities. They match within an accuracy of the expected grid discretization error.

2.4.3.5 Simulation validation summary

Through this validation, the physics/mathematical model is implemented correctly in the finite element modelling pathway, though grid discretization errors were easy to introduce and there was a clear tradeoff between accuracy and processing time. This is the first finite element simulation in heterogeneous media of the new acoustoelectric derivation. The more efficient k-space simulation is an analytic solution with no possibility of grid discretization errors, making it both fast and accurate for calculations in homogeneous solutions. In explorations and results described henceforth, I use only the k-space simulation.

2.5 Results

2.5.1 Computational Investigation of the acoustoelectric field

Next, we used electromagnetic and acoustic simulations to explore the spatiotemporal properties of the acoustoelectrically generated field in physiological saline solution, as saline closely mimics both acoustic and electromagnetic tissue properties^{91,92}. We applied a continuous focal acoustic field and a homogenous electric field at various frequencies and amplitudes. We modelled the transducer's acoustic fields analytically, using a summation of Bessel functions⁸³. Based on previous experimental studies^{93,73,80}, we set the adiabatic compressibility constant k to $1e^{-9} \text{ Pa}^{-1}$. To estimate the acoustoelectrically generated electric field, we computed **Eq. 19** for the induced electric potential ϕ_{ae} , with $\vec{E}_{ae} = -\nabla\phi_{ae}$, using a fast Poisson solver operating in the Fourier-space (i.e., k-space); see **Methods 2.3** for more details on the computation procedure.

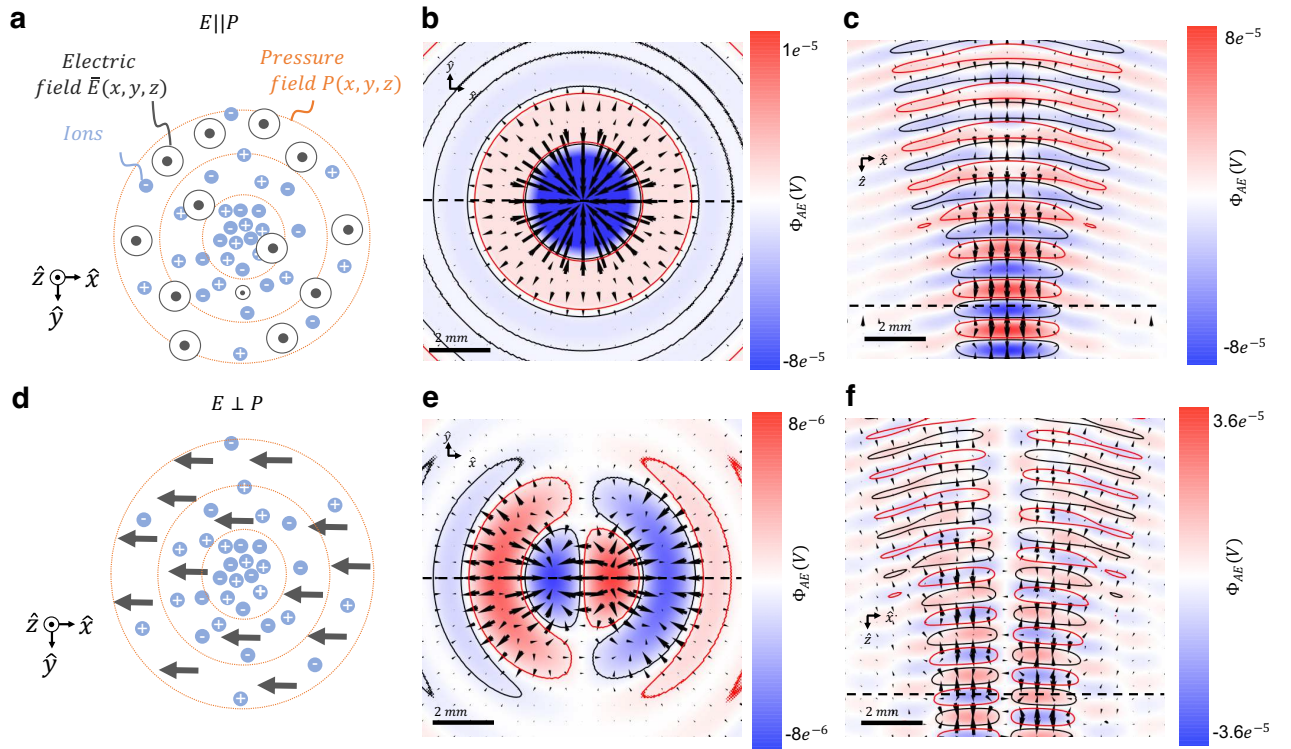


Figure 2-5 | Computation of the acoustoelectric effect in weak electrolytes.

Characteristics of the electric field $\vec{E}_{AE}$ generated by focal acoustic field $P(x, y, z)$, here $f_A = 500 \text{ kHz}$ and peak amplitude 1 MPa, and homogenous electric field $\vec{E}(x, y, z)$, here $f_E = 0 \text{ Hz}$ (i.e., DC) and amplitude 3.2

kV/m **a**, Schematic of the computational model in which $\vec{E}$ is applied parallel to the propagation direction of the acoustic field P . **b**, Spatial distribution of the acoustoelectrically generated electric field in the focal plane of the ultrasound. Radial plane $\hat{x}\hat{y}$ distribution along the black dashed line in **c**; shown are $\vec{E}_{AE}$ vector lines (arrows) and the corresponding electric potential Φ_{AE} maps (colour scale, with contour lines indicating equipotential). **(c)** Axial plane $\hat{x}\hat{z}$ distribution, showing the same as **(b)** but note the different scale. **d**, Same as **(a)** but with $\vec{E}$ perpendicular to the propagation direction of P . **e,f**, Same as **(b-c)** but with $\vec{E}$ perpendicular to the propagation direction of P .

We found that when the electric field was applied in parallel with the direction of the acoustic field propagation (**Fig. 2.5a**), the generated electric field had a three-dimensional topology with a concentric ring dipole in the radial direction (**Fig. 2.5b**) and a periodic linear multipole in the axial direction (relative to the acoustic field propagation) with the ultrasound wavelength (**Fig. 2.5c**). The amplitude of the generated electric field was proportional to the amplitudes of the applied acoustic field and electric field. For example, application of an acoustic field with a peak amplitude of 1 MPa via a spherical 500 kHz transducer (63.2 mm radius of curvature, ROC) and an electric field with an amplitude of ~ 3 kV/m, generated an electric field of ~ 0.17 V/m, corresponding to an acoustoelectric field's coupling factor c_{ae} of approximately 55 pPa^{-1} where c_{ae} is the ratio between the peak amplitude of the acoustoelectrically generated electric field and the product of the applied electric field and peak acoustic field.

When the electric field was applied perpendicular to the direction of acoustic field propagation (**Fig. 2.5d**), it changed the topology and strength of the generated electric field. Specifically, the concentric ring geometry in the radial direction changed to a dipole (**Fig. 2.5e**, see also **Fig. 2.6a** for an amplitude distribution comparison). The amplitude of the generated field decreased as the acoustic propagation direction and electric field alignment dropped (**Fig. 2.6b**). We found that the acoustoelectric generated electric field was four times larger when the applied fields were in parallel as compared to perpendicular.

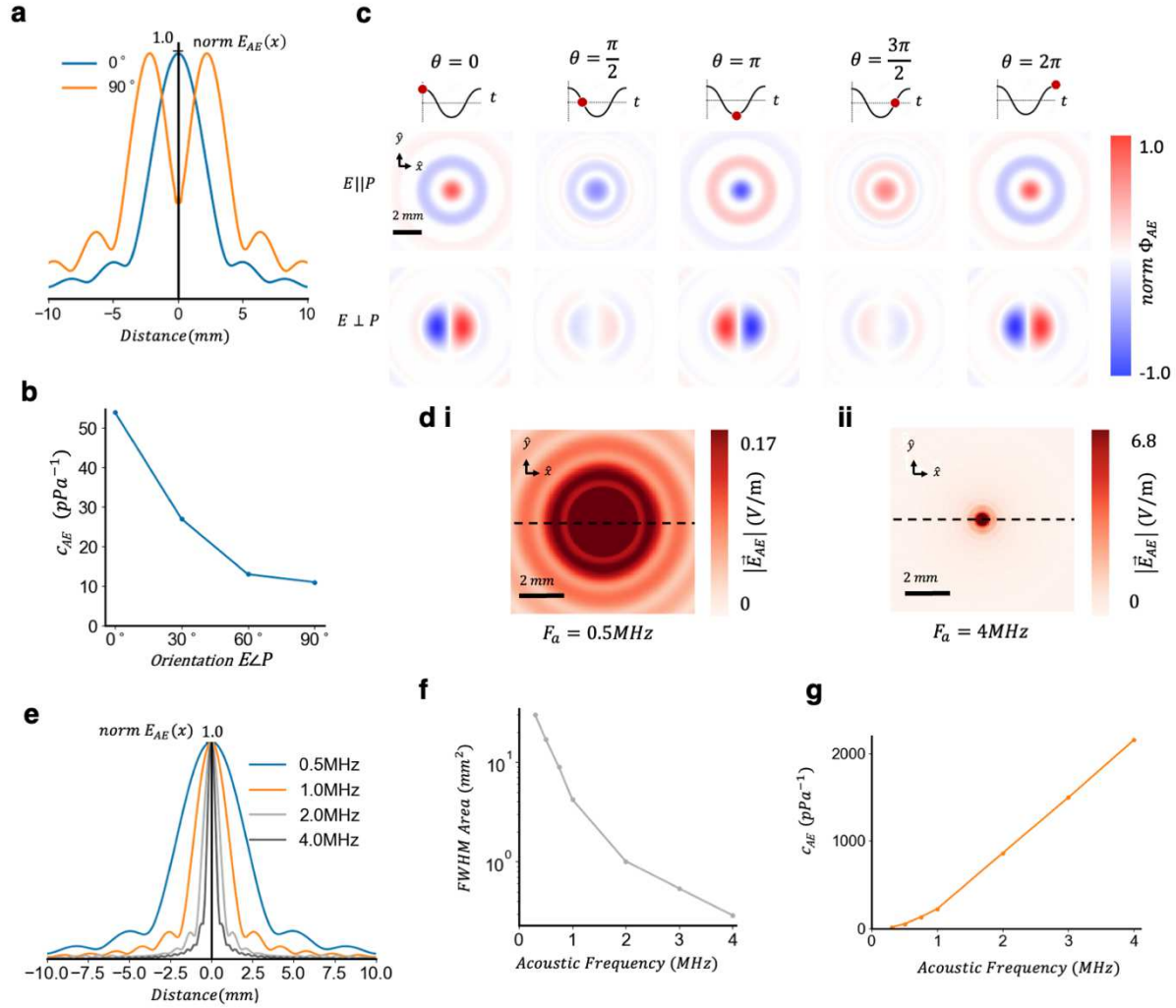


Figure 2-6 | Simulation predictions of the acoustoelectric effect in weak electrolytes.

a, Line plot comparing the normalized amplitude of generated field $|\vec{E}_{ae}|$ along the black dashed lines in **Fig. 2.5(b)** and **(e)**. **b**, Acoustoelectric coupling factor c_{ae} vs. angle between the applied electric field and acoustic propagation direction. **c**, Evolution of the generated electric field over time shown at the same point in space (focus) as time progresses over an acoustic wavelength. Shown are Φ_{AE} maps in the radial plane $\hat{x}\hat{y}$ at different phases of the ultrasound transducer period for parallel and perpendicular electric field orientation; θ , instantaneous phase of the applied acoustic field. **d**, Effect of the applied acoustic field frequency on the generated electric field. Electric and acoustic fields were applied as in **Fig. 2.5a** but at different acoustic frequencies. Representative $|\vec{E}_{AE}|$ maps in the radial plane $\hat{x}\hat{y}$ with acoustic field at $f_A=500$ kHz and $f_A=4$ MHz, respectively. **e**, Normalized $|\vec{E}_{AE}|$ along the $\hat{x}$ direction vs. applied acoustic frequency. **f**, Full width half maximum (FWHM) area of the generated field vs. applied acoustic frequency decreases as frequency increases. **g**, Acoustoelectric coupling factor vs. applied acoustic frequency increases as frequency increases.

When a DC electric field was applied, the polarity of the generated electric field changed periodically at the acoustic frequency (**Fig. 2.6c**). However, if the electric field was applied at a frequency different from the acoustic frequency, the generated electric field changed periodically with components at the difference and sum frequencies of the electric and acoustic fields. The instantaneous amplitude of the generated electric field was maximal/minimal when its components at the sum and difference frequencies were superimposed constructively/destructively. The maximum amplitude of the sum and difference frequency envelope was twice that of the single frequency when a DC field was applied, i.e. c_{ae} was 110 pPa^{-1} .

Applying ultrasonic fields at higher frequencies reduced the diameter of the generated electric field focus due to the smaller pressure foci (**Fig. 2.6 d-e**). The smaller acoustic foci resulted in generation of a stronger electric field due to the higher acoustic field gradients (**Fig. 2.6 f-g**). For example, c_{ae} was 55 pPa^{-1} with a 500 kHz acoustic frequency and 2000 pPa^{-1} with a 4 MHz acoustic frequency, while peak pressures remained constant at 1 MPa.

2.5.2 Characterization of the applied and generated fields in the computational model

The gradient of the pressure field, otherwise known as force density, affects the acoustoelectric generated electric field. The components of this pressure-based vector are shown in Figure **2.7-b**. In the radial plane (i.e., perpendicular to the acoustic field propagation), the force density changes direction periodically in a concentric ring's geometry while in the axial plane, the force density changes direction periodically on a wavelength scale governing the shape of the acoustoelectrically generated electric field seen in **Fig. 2.5**. The components of the acoustoelectrically generated electric field x,y,z orientations are governed by the product between the applied force density and electric field in those orientations. Although force density may be well understood in classical

mechanics, considering it as a source of electromagnetic oscillation mediated by the impedance of the medium is unexplored.

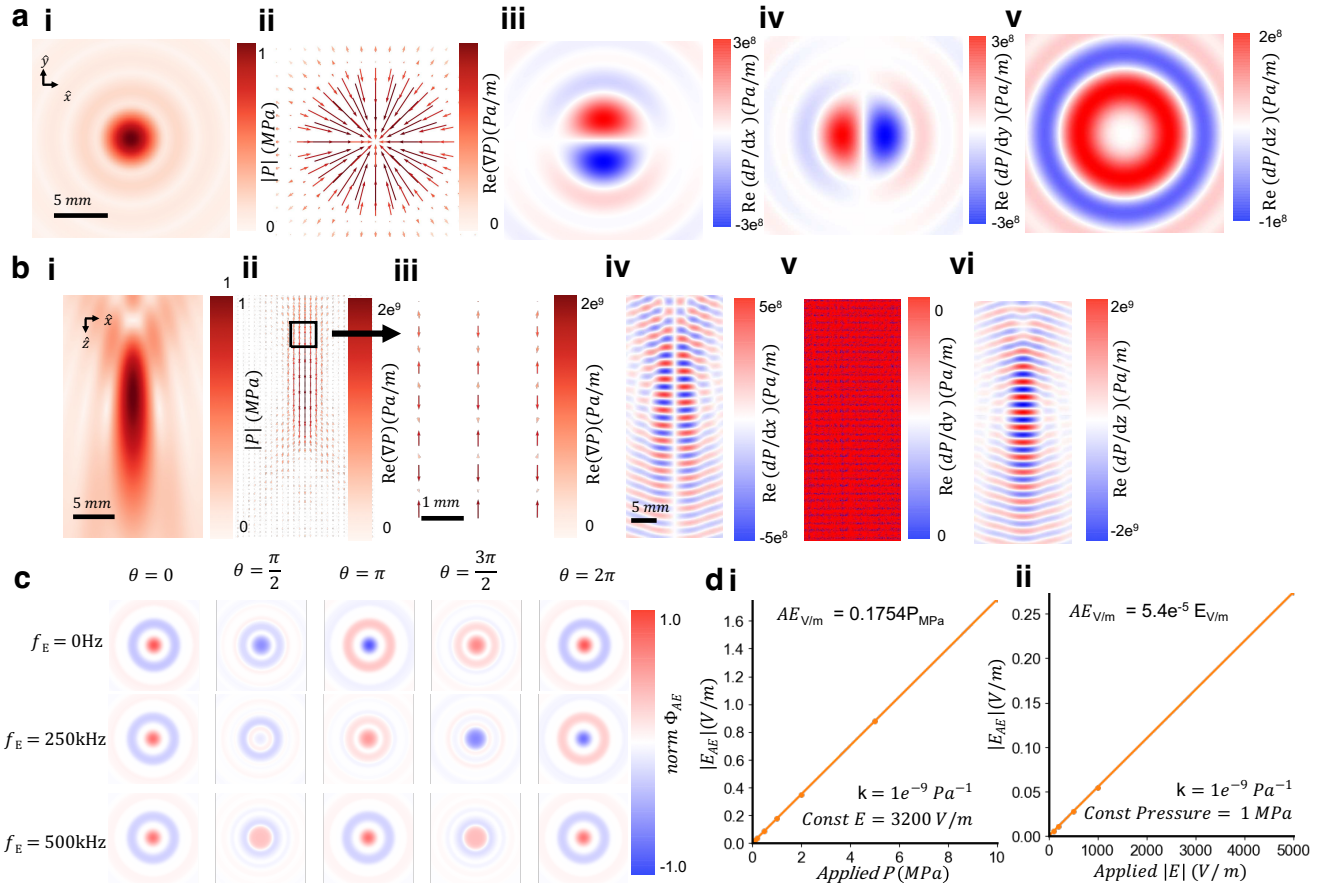


Figure 2-7 | Characteristics of the applied acoustic fields & acoustoelectrically generated electric field obtained through computational modelling.

a, Acoustic pressure distribution in the radial plane $\hat{x}\hat{y}$ of the ultrasound transducer with $f_A = 500$ kHz and peak amplitude 1 MPa propagating in the $\hat{z}$ direction. (i) Map of pressure amplitude. (ii) Vector map of pressure gradient. (iii), Map of $\hat{x}$ component of the pressure gradient. (iv) same as (iii) but $\hat{y}$ component. (v) same as (iii) but $\hat{z}$ component. **b**, Same as (a) but in the axial plane $\hat{x}\hat{z}$. **c**, Evolution of the acoustoelectrically generated electric field via focal acoustic field as in (a) and a homogeneous electric field of 3.2 kV/m parallel to the propagation of the acoustic field propagation. Shown are normalized Φ_{AE} maps in the radial plane $\hat{x}\hat{y}$ at different instantaneous phase θ of the ultrasound transducer period ($1/f_A$). **d**, Peak amplitude of the acoustoelectrically generated electric fields versus (i) peak amplitude of applied acoustic field with applied electric field amplitude fixed (here at 3.2 kV/m), (ii) amplitude of applied electric field with fixed acoustic field amplitude (here at 1 MPa at the peak); electric field was parallel to the propagation of the acoustic field propagation.

The frequency mixing property of the acoustoelectric effect results in generated electric fields that oscillate at the sum and difference frequencies. Hence, the temporal spatial pattern of the combined fields depends on the frequencies of the applied fields. **Figure**

2.7c shows example time evolutions of the generated field for different applied frequencies. The spatial distribution changes dependent on the relative phase difference between the sum and difference frequencies.

Figure 2.7d shows the relationship between the amplitudes of the applied electric and acoustic fields and the amplitude of the generated electric field when the applied fields are parallel, demonstrating a linear relationship between the generated electric field amplitude and the applied electric field amplitude, consistent with previous studies⁸⁰. Similarly, it shows a linear relationship between the generated electric field amplitude and the applied acoustic field amplitude, consistent with previous studies⁹⁴.

2.5.3 Acoustoelectric safety simulations

Ultrasound is a type of energy created by pressure waves, and its effects on living tissues are heating (thermal) and mechanical (non-thermal) effects. Two commonly accepted safety metrics derived from these bioeffects are the thermal index (TI) and mechanical index (MI).

Mechanical Index (MI):

The acoustic power, or the amount of acoustic energy per unit time shows the amplitude of the pulse pressure. The two types of mechanical non-thermal bioeffects of the acoustic field in liquids are cavitation and streaming. Cavitation is the expansion and contraction or collapse of bubbles because of the acoustic pressure and is dependent on the negative, not positive, pressure. Streaming is the movement of complex fluids because of the acoustic radiation force and is a smaller effect than cavitation. The safety index related to mechanical effects is the mechanical index (MI) which is defined as peak negative pressure divided by the square root of the frequency of the ultrasound wave.

$$MI = \frac{P_-}{\sqrt{f}} \quad (36)$$

where P_- is the peak negative pressure (MPa) and f is the frequency in MHz. P_- is the peak rarefaction pressure of the ultrasound wave (MPa), derated by an attenuation factor to account for in-tissue acoustic attenuation⁹⁵. The higher the transducer frequency, the higher pressure can go without risking cavitation. The ideal value of the MI should be less

than 1.9 to avoid bioeffects and this is the FDA approved maximum threshold for diagnostic imaging at 500kHz. Using a transducer at 500kHz, and allowing a maximum MI of 1.9, the maximum allowable pressure is 1.345MPa. To apply this in the k-space simulation, the result was scaled so that the maximum negative pressure was 1.345MPa.

It should be noted that there are further techniques that can be applied to maximize the positive pressure and minimize the negative pressure, such as use of specific types of unipolar waveforms, or pulse width modulation of the ultrasound beam to average the power.

Thermal Index (TI):

The thermal index (TI) is intended as a measure of an ultrasound beam's thermal bioeffects. Absorption of sound waves may cause heating in tissue. The thermal index depends on:

- a measure of time-averaged acoustic power
- assumptions of the properties of the tissue being heated
- assumptions about the path of the ultrasound beam

According to Pennes' bioheat transfer equation, the tissue temperature increases with this heat energy⁹⁶.

$$TI = \frac{W_P}{W_{deg}} \quad (37)$$

- W_P is the relevant (attenuated) acoustic power at the depth of interest
- W_{deg} is the estimated power necessary to raise the tissue temperature by one degree Celsius.

If the acoustic power of the ultrasound beam is enough to raise the tissue temperature by 1.0°C, then the thermal index is 1.0. Since the Thermal Index is dependent on the power distribution through space and the material the beam propagates through, it is best estimated through a thermal transient solver such as the one in Sim4Life which has solutions for both CEM43 (cumulative equivalent minutes at 43°C, a thermal dose parameter that predicts tumor cell death and tissue damage) and the Arrhenius tissue

damage⁹⁷. Another useful tool used for thermal simulations is the software package k-Wave⁹⁸, where the pressure induced thermal increase can be simulated under a continuous 1MPa 500kHz acoustic wave in water (**Figure 2.8**). It should be noted that the timescale of temperature change over seconds is outside that of neuromodulation which we expected to occur between 1-10Hz, making it less likely to be an artefactual confound should neuromodulation induced EMG muscle movements be detected in experiments.

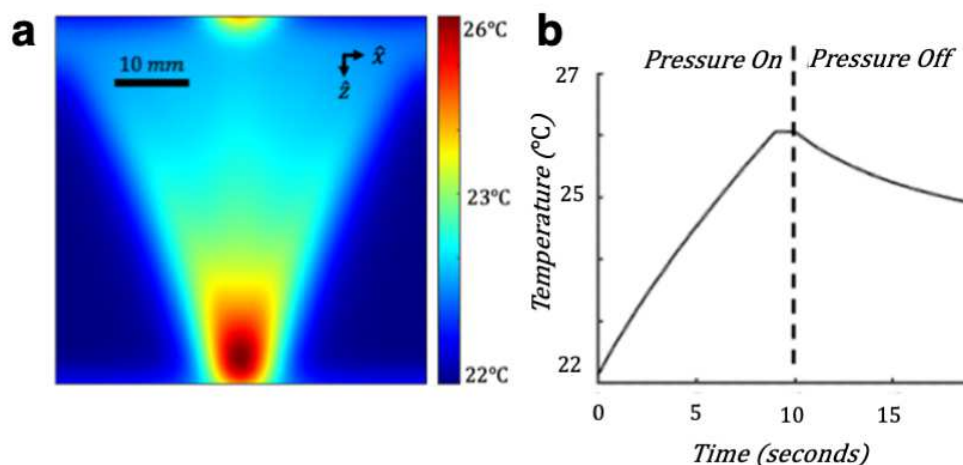


Figure 2-8 | k-wave temperature simulation.

Characterisation of pressure induced thermal increase. Simulation of thermal changes using k-wave⁹⁹. **a**, Temperature map **b**, Temperature dissipation over time at the ultrasound focus, with 10 second continuous pressure wave and maximum focal pressure 1 MPa.

Safety result above the stimulation threshold

To determine if my acoustoelectric simulation is feasible, the resultant acoustoelectric generated field was scaled such that the maximum pressure field was set to an MI of 1.9 as per FDA guidelines. The resultant generated acoustoelectric field is greater than 1V/m which is the minimum threshold for successful neuromodulation³⁵. The acoustoelectric neuromodulation paradigm appears feasible based on simulation parameters and within safe limits of ultrasound imaging.

Acoustoelectric neuromodulation feasibility map

Changing the electric or pressure field scales the acoustoelectrically generated field linearly. In **Figure 2.9** the applied acoustic and electric fields are shown relative to the emergent acoustoelectric generated field. There is a large area that is safe and above the electrical stimulation threshold for neuromodulation, and without the acoustic field safety

thresholds. Acoustoelectric modulation could be made both effective and safe for neuromodulation applications based on this modelling.

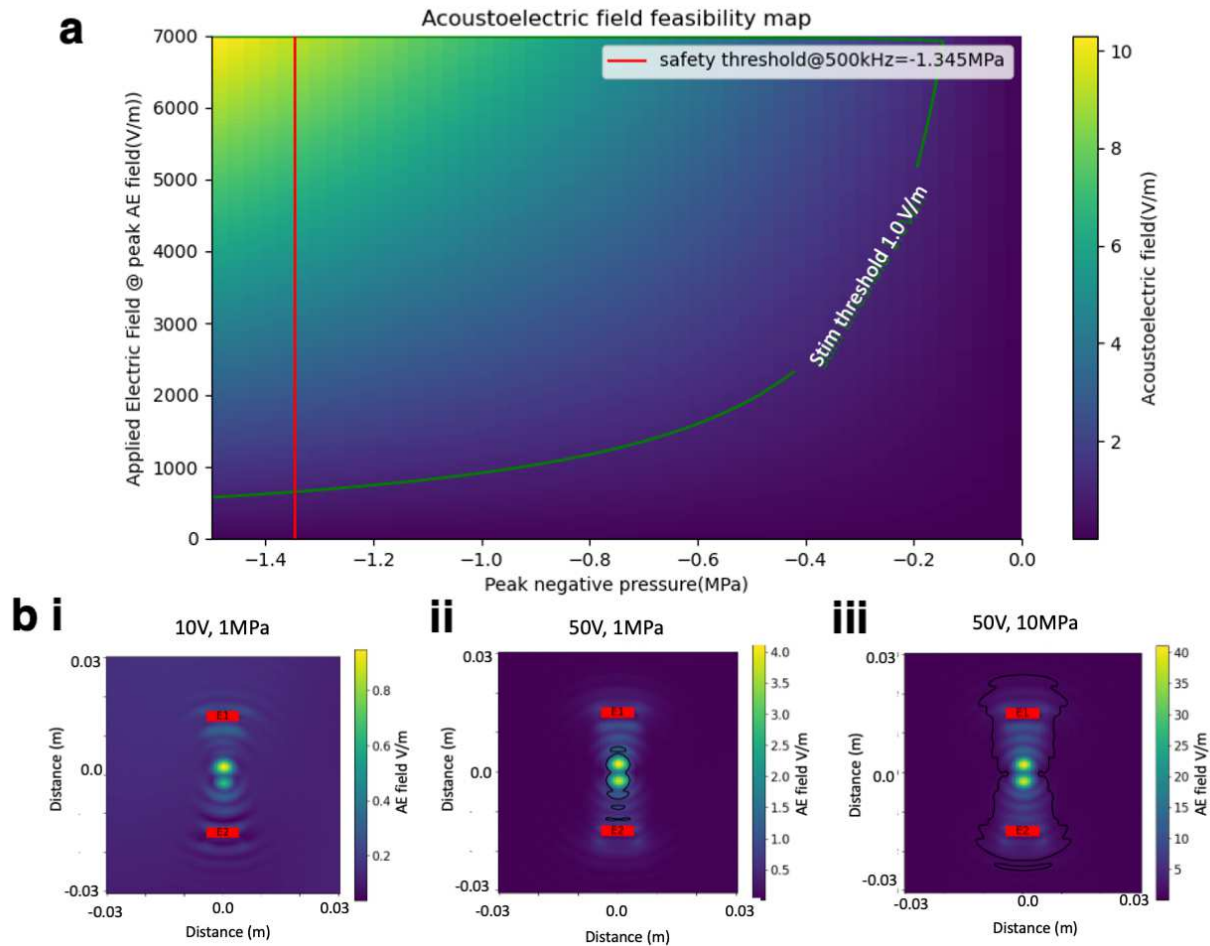


Figure 2-9 | Safety and electrical neuromodulation feasibility map.

a, Feasibility map showing the safety threshold Mechanical Index in red, and the stimulation threshold as the green line. **b, i)** 10V 1MPa, no area above 1V/m stimulation threshold. **ii)** 50V 1MPa, 67:2mm² above 1 V/m stimulation threshold **iii)** 50V 10MPa, 796:3mm² above 1 V/m stimulation threshold.

2.5.4 Mechanism and focality comparisons between acoustoelectric neuromodulation and temporal interference stimulation.

Comparing acoustoelectric neuromodulation focality to the existing temporal interference³⁵ results reported by Dr. Nir Grossman, both paradigms were simulated using similar electrode size and spacing to the homogeneous acoustoelectric neuromodulation

simulation. The results of both simulations were normalized to compare the areas calculated from the half-width half maximum of the resultant field as shown in **Figure 2.10**.

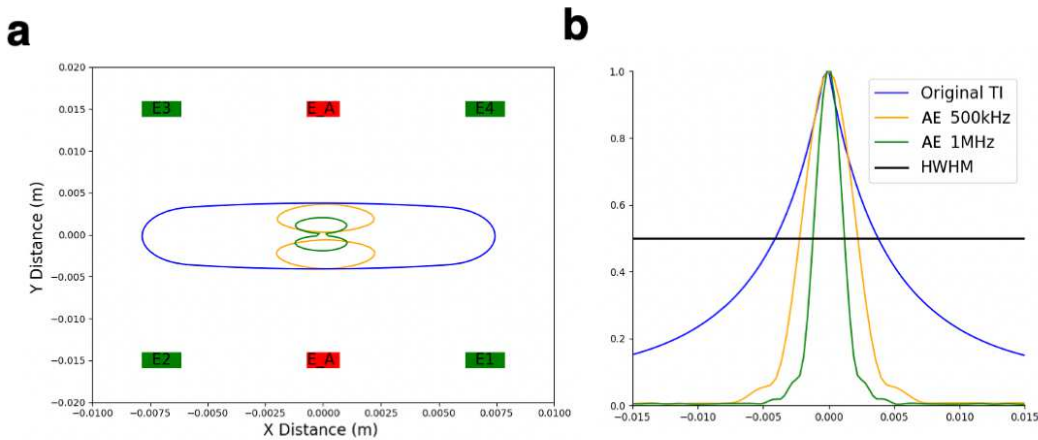


Figure 2-10 | Focality comparison with temporal interference stimulation.

a, Acoustoelectric field focality contours. **b**, One dimensional acoustoelectric field cross-section.

Using a 500kHz ultrasound transducer, acoustoelectric neuromodulation is predicted to have a 4.5 times focality improvement on temporal interference, and with a 4MHz ultrasound transducer a 15 times improvement on the focality of temporal interference is expected. Based on theory, increasing the acoustic frequency beyond this should enable even higher focality.

The gradient around the focal spot is far steeper indicating that field changes due to inhomogeneity in the medium will not affect the size of the focal spot as much as regular TI, and it should work with a similar focality for a greater range of field magnitudes¹⁰⁰.

2.5.5 Computational investigation of acoustic focality limitations in a mouse

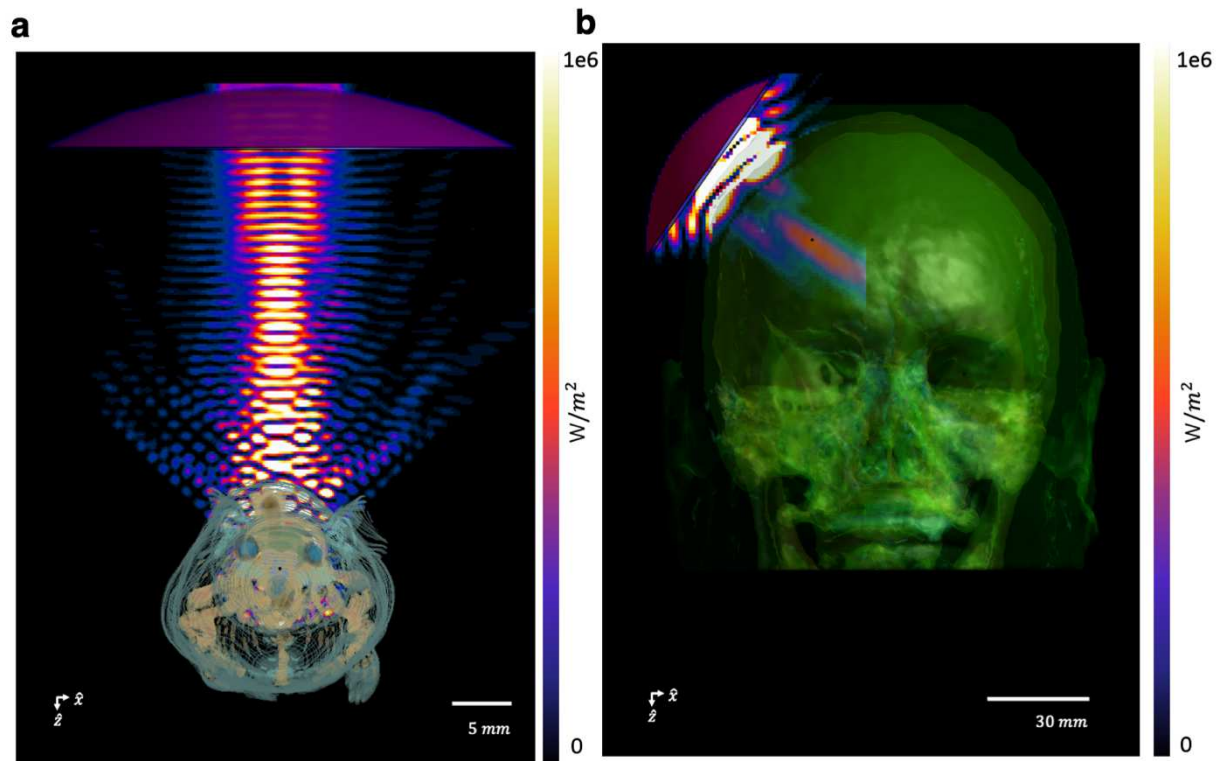


Figure 2-11 | Finite element simulation of propagating 500kHz acoustic field through a mouse.

a, Geometric model of inhomogeneous mouse surrounded by water. **b**, Attenuation in a human skull model with water interface with skull.

In a human skull, the length scales are appropriate to use a 500kHz ultrasound transducer, which has a focal spot approximately 6.3cm from the surface of the transducer as the focus can reach deep into the brain even though much of the acoustic waves amplitude is reflected off the skull surface. This 500kHz frequency transducer has also been used in many studies in ultrasound neuromodulation successfully and is able to be positioned such that the focal position can be detected with 1mm increments as its wavelength is 3mm, making its use practical and attractive for both a phantom and an *in vivo* mouse study. However, in a mouse head the wavelength of acoustic propagation is not attenuated sufficiently before it reflects off the opposite surface, meaning a lot of internal acoustic reflections will occur. These internal reflections may change the overall focal profile, so we investigated the expected distribution of reflections through simulation using the acoustic solver in Sim4Life.

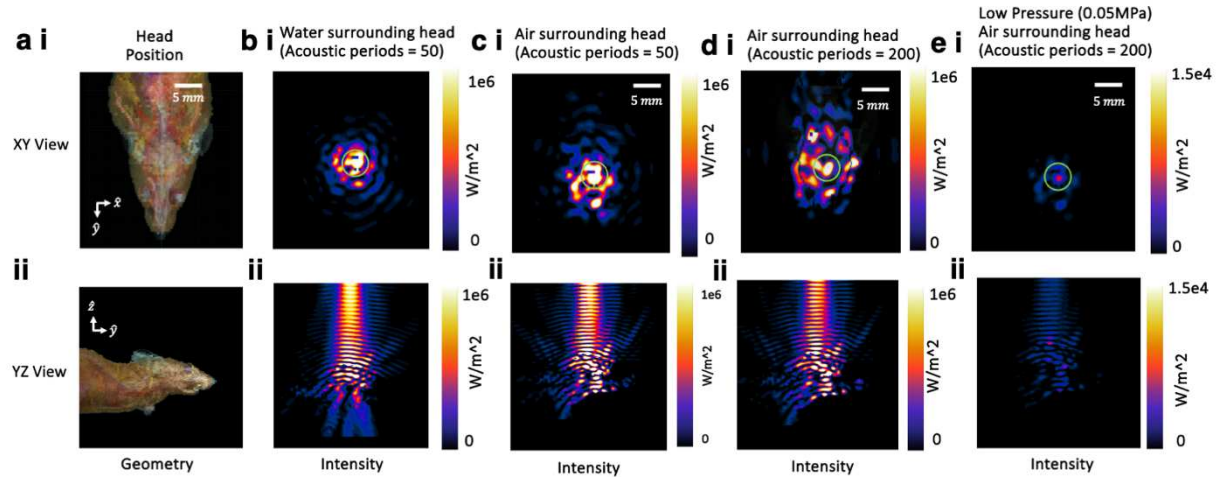


Figure 2-12 | Acoustic reflection study.

All pressures at 1MPa unless otherwise stated. **a**, i) XY View geometry ii) YZ view Geometry and position. **b**, i) XY view of reflections when water is surrounding head to reduce impedance difference at air/head interface. ii) YZ view of i) **c**, i) XY view of reflections when air is surrounding head. ii) YZ view of i) **d**, i) Acoustic simulation run for more periods shows further reflections and areas of constructive interference. ii) YZ view of i) **e**, i) Low pressure 0.05MPa XY view shows clearer focal point. ii) YZ view of i).

From simulation we see that the mouse skull is approximately transparent to ultrasound (**Fig 2.12 b i,ii**), and the air interface causes many internal reflections in the mouse brain (**Fig 2.12 c i,ii**) which are not at the focus. Furthermore, for longer duration acoustic signals such as when the number of acoustic periods exceeds 200 (60cm of acoustic signal at $\lambda \approx 3mm$, where the mouse head is only 1,5cm wide) the amplitude of the reflections increases due to constructive interference (**Fig 2.12 d i,ii**). We can see that the reflection pattern changes over time as other peaks occur as the simulation runs longer and areas of constructive rarefaction and compression develop. By decreasing the pressure but keeping the number of periods high, we can also reduce the total internal reflections within the mouse head (**Fig 2.12 e i,ii**) as attenuation diminishes off target superpositions.

To obtain higher focality we could use an ultrasound system with a higher transducer frequency (shorter wavelength) or move to a larger head (i.e. a human). Although in larger animals the skull thickness presents a barrier to the propagation of acoustic fields, the mouse skull is comparatively thin¹⁰¹ with the thin skull being modelled as acoustically transparent. If the mouse head is surrounded by water the pressure can dissipate into the

water which has a similar acoustic impedance to physiological tissue, whereas when air surrounds the mouse head, we get far more internal reflections due to the acoustic impedance mismatch between the air and physiological tissue interface. Simulating the multiple reflections generated in the murine head would be impossible, as each mouse has a slightly different head shape making accurate predictions impossible in translational studies.

Based on this simulation of *in vivo* experiments with a 500kHz transducer and mouse, we should be aware of constructive interference maxima away from the focal point, and not be concerned with the thickness of the mouse skull causing reflections. Running shorter pulses with fewer acoustic cycles will possibly also enhance focality and decrease constructive interference within the mouse geometry.

2.6 Discussion

Through theoretical derivation we come to a new understanding of the interaction between acoustic and electric fields in electrolytes which encompasses the vector nature of the two fields, enabling key predictions on the emergent acoustoelectric field. Since this solution is based on the gradient of the acoustic pressure field, the amplitude of the acoustoelectric field will increase with transducer frequency, and the vector multiplication between the pressure gradient and the applied electric field means that the emergent acoustoelectric field will be proportional to the angle between the fields, being maximum when the acoustic and pressure fields are aligned and minimum when they are perpendicular to each other. Furthermore, multiplying between electric and acoustic fields leads to sum and difference frequency products in the emergent acoustoelectric generated field through the product to sum trigonometric identity.

To further explore the characteristics of the generated field, two simulations were created. The first simulation utilizes Fourier space (k-space) methods to solve acoustoelectric equation quickly and analytically but is limited to homogenous media. The second finite element modelling pathway consists of an acoustic, quasistatic electric field and modified

thermal solver to enable the equation to be solved in heterogeneous media. Though the finite element pathway has been tested accurate for modelling purposes, it is more complex and slower to run than the k-space solution. The k-space solution is then used to explore safety and feasibility of acoustoelectric neuromodulation.

Within safe electric field and acoustic parameters, the generated acoustoelectric electric field were simulated to be above the threshold to recruit neural activity (1V/m). The stimulation locus is 5-15 smaller than the stimulation locus achieved with TI stimulation. Since the focal area is dependent on the gradient of the acoustic field, it is less likely to recruit surrounding tissue which is not at the target as the field change is so steep.

Acoustoelectric neuromodulation is a feasible approach for performing neuromodulation at depth, at least based on physics and simulation, and it has the potential to give far greater spatial focality than established methods of any electrical based neurostimulation. Similarly acoustoelectric neural recording is also feasible, having more flexible parameters than the acoustoelectric neuromodulation paradigm. With this information we move on to explorations in a physiological phantom.

Chapter 3: Materials and methods

3.1 Overview

The experiments in this Chapter describe acoustoelectric phantom hardware development and *in vivo* electrophysiology surgery procedures. While this spans a variety of techniques, they are common methods across multiple Chapters. The acoustoelectric phantom descriptions are adapted from the manuscript published in Nature Communication Physics¹⁰² with additional detailed information on hardware design. The developed equipment was used in the acoustoelectric saline phantom characterization study described in **Chapter 4**, then moved to the animal testing facility and adapted for the *in vivo* studies in **Chapter 5** and **Chapter 6**.

3.2 Methods: Acoustoelectric phantom

To ascertain the properties of the acoustoelectric interaction, a custom-built phantom was developed to which both electric and acoustic fields could be applied while moving a probe around on an XYZ stage through saline solution, measuring the emergent signals with a preamplifier, and monitoring all input signals simultaneously. This system comprised both electrical design of components and custom designed 3D printed parts to convert a low-cost 3D delta printer into a precision acoustoelectric XYZ stage, able to do time synced mapping of all acoustic, electric, and acoustoelectric fields through three dimensional spatial coordinates with an accuracy of 0.1mm. All aspects of this development process have been made open source as a low-cost XYZ phantom for mapping acoustic and electric fields may be a generally useful tool for others.

3.2.1 Development of low-cost open-source 3D XYZ phantom

AN XYZ stage that can perform precision scans in a tank of water was required to map the acoustic, electric, and acoustoelectrically generated fields in XY and XZ dimensions. There are commercially available solutions which can do this though the tank/stage alone can cost upwards of 30K at 2021 prices which limits accessibility to this extremely useful tool. Since acoustoelectric measurements are not commonplace, extensive customization would have been needed on top of any commercially available acoustic tank solution to map both electric and acoustic fields. Here, a low-cost and open-source tank phantom measurement solution is presented appropriate for acoustic and electric field measurements with 0.1mm resolution in X, Y and Z dimensions (see **Fig 3-1**). Electric field measurements were made when the hydrophone was not attached, so that the ground plane of the hydrophone did not affect the electric measurements. All software and hardware modifications have been made open source, so that others can easily recreate this useful piece of equipment for studies in electric or acoustic fields.

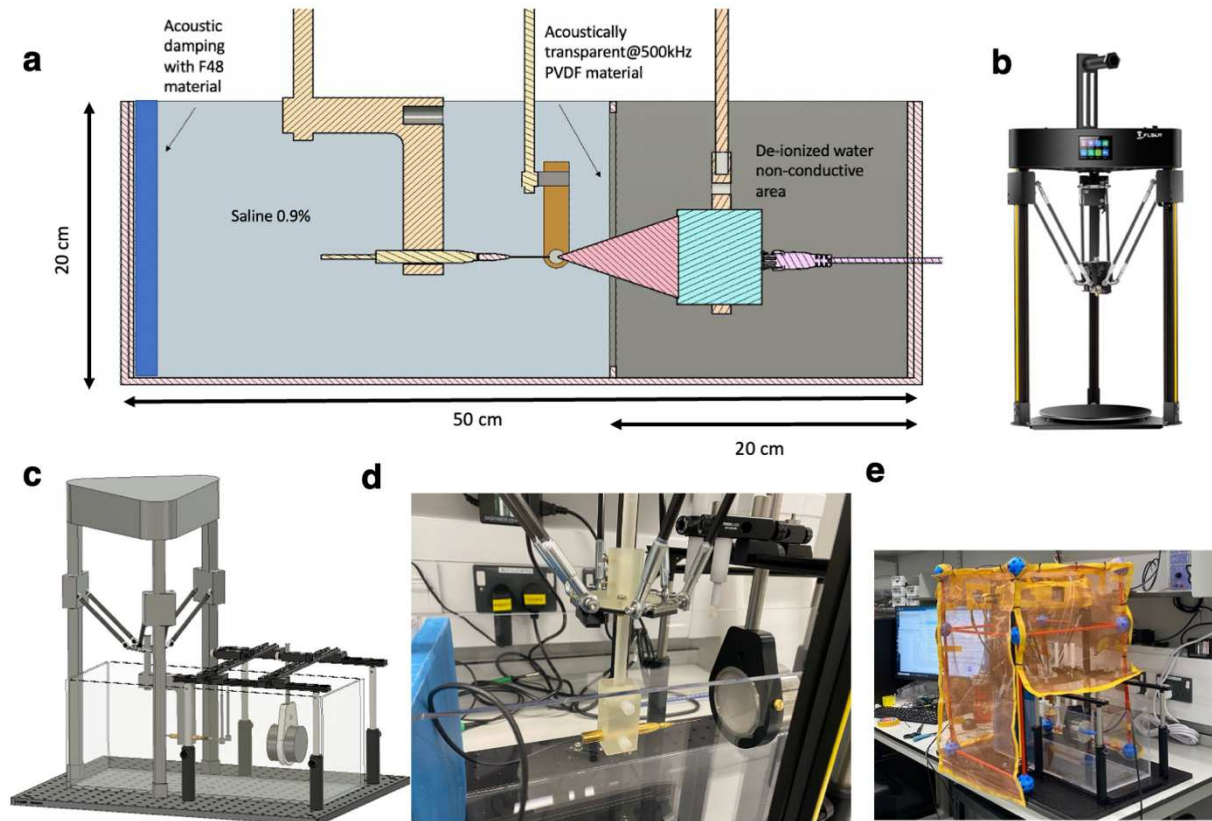


Figure 3-1 | Customization of 3D delta printer to precision XYZ stage for acoustoelectric characterization study.

a, Cross-section of tank phantom 3D CAD model. Tank is 50x20x20cm, with inner portion of tank 20x20x20cm. **b**, FLSUN Q5 delta printer in original form. **c**, Mount made from Thor labs components for ultrasound transducer and hydrophone **d**, photo of completed phantom tank with acoustic backing material **e**, Computer, home-made Faraday cage and modified 3D printer with tank all put together.

Recently, innovations in 3D printing technology have made XYZ stages much cheaper. 3D printers typically operate in Cartesian coordinates, such that one arm moves in 2 coordinate planes and the stage moves in the third coordinate plane. These printers form the bulk of the 3D printer market but are inappropriate for use in a tank phantom, as having the tank of water sit on a moving stage would cause movement of the liquid inside it. Delta printers offer an alternative. Delta printers convert Cartesian coordinates into polar coordinates and are typically less accurate, though they do have a central arm which can move in all XYZ dimensions making it a sensible choice for an acoustic phantom stage. The FLSUN Q5 is a readily available 3D printer with open-source firmware and a community of users with forums and Facebook help groups and it sells on Amazon for only \$200, a good price point for a 3D stage with refined and accessible software that enables direct interfacing via G-code commands. The printer comes in parts to be assembled, which means I could leave parts out that weren't needed such as the extruder on the moveable XYZ central arm which usually controls the plastic deposition. After removing the extruder, a bare XYZ stage remained.

I designed parts to replace the extruder and hold the hydrophone and electrode probes (**Fig 3-2**), using the free online CAD tool ONSHAPE. All designed parts are listed online in the Github repository Open 3D Phantom¹⁰³ and were printed through the online 3D printing company SGD3D in Rigid Resin 4000. The electrodes are made from Platinum Iridium wire (Alfa Aesar, diameter 0.25mm) with white nail polish creating an insulating barrier around their length, so that only a small portion of the wire is exposed in the medium.

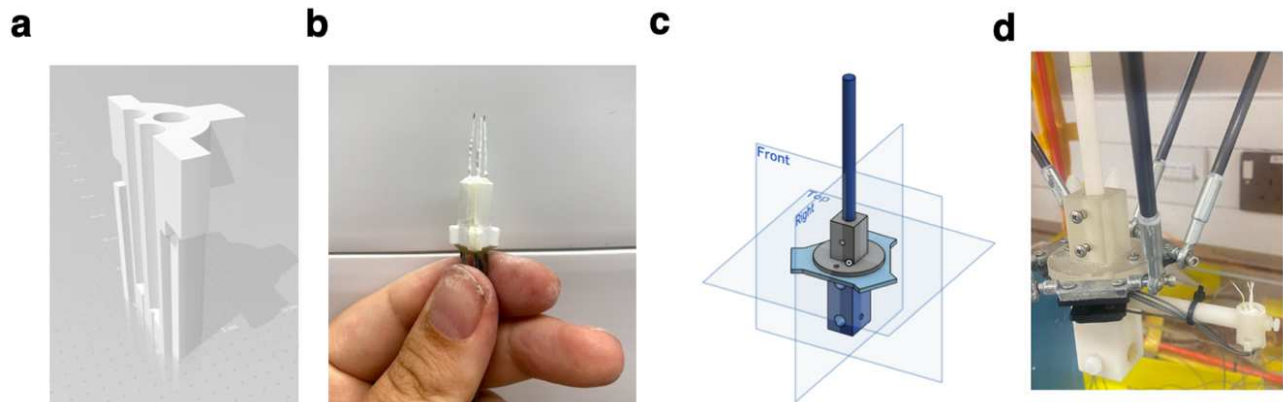


Figure 3-2 | Custom CAD design of 3D printed parts.

a, 3D rendering of electrode holder part, which was symmetrical and could be glued together with Platinum Iridium electrodes inside. **b**, completed electrodes, insulated with white nail polish so that only 0.5mm wire ends were exposed. **c**, 3D rendering showing both the electrode mount point, and part to hold electrode mount onto the extruder base. **d**, completed part mounted onto the extruder, showing measurement electrodes able to be moved in XYZ coordinates.

G-code is the standard for position commands for 3D printers, mills, lathes, and other automated factory machinery. To make programming the printer easier, I created a Python library to interface with the printer API so that knowledge of G-code was abstracted and basic X,Y,Z coordinates could be input. The XYZ stage unfortunately emitted electrical broadband noise from its motor when it was on so a relay was added to turn off the stage when measurements were taken. A Faraday cage was fashioned out of copper mesh wire, around a frame. The tank was custom made to fit inside the delta printer stage. The ultrasound was kept stationary and mounted to a frame made from standard photonic lab components (Thorlabs) listed in the table contained in the Open3D phantom Github repository¹⁰³.

The hydrophone could also be mounted on the central arm a set distance apart from the electrodes. This fixed distance allowed for accurate positioning of the stimulate/measure electrodes or the hydrophone at the pressure focus. This enabled calibration of the acoustic focus such that the evoked electric fields and pressure could be measured in the same location.

The delta printer made an effective XYZ stage able to map both acoustic and electric fields in solution. The acoustic field from a 500kHz spherical focused transducer mapped in XY (**Fig 3-3 a**) and XZ (**Fig 3-3 b**) dimensions produced a clear and accurate map, the small periodic pattern in the axial direction likely caused by imperfect absorption of the acoustic field by the damping material at the back of the tank. Electric fields can also be mapped (**Fig 3-3 c,d**), although it should be noted that since the stimulating electrodes were nearby, they create impediments to the moving sensor probe too close.

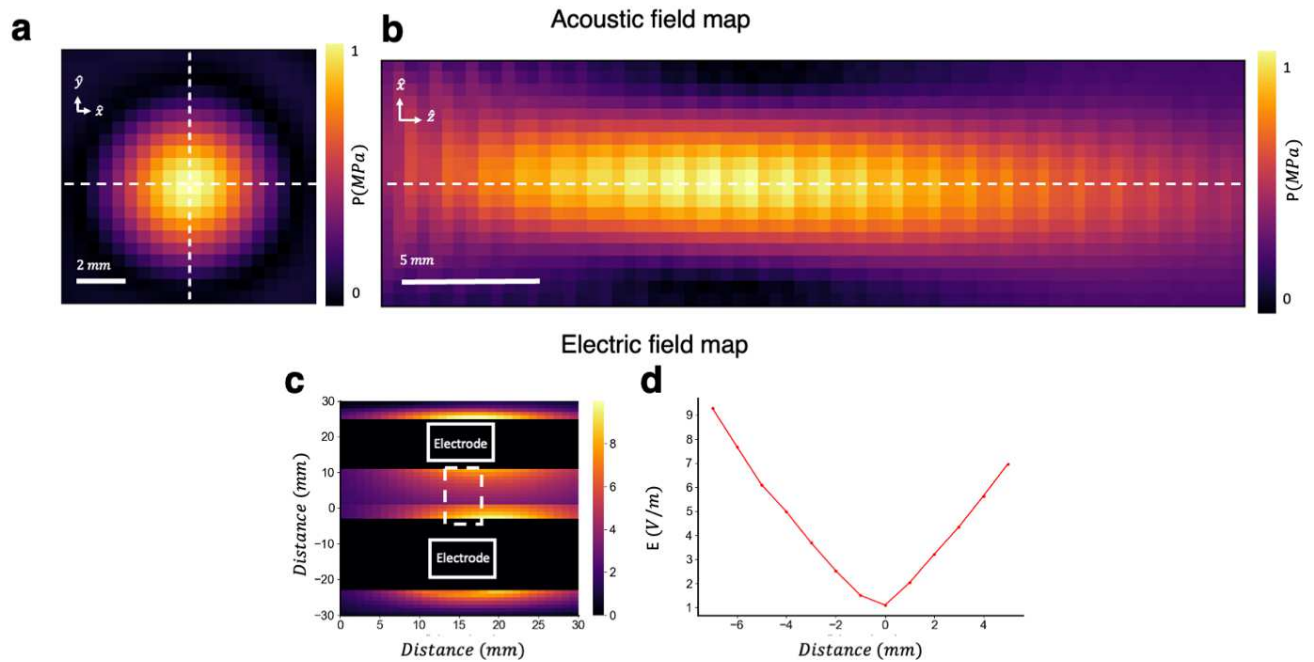


Figure 3-3 | Automated XYZ phantom for mapping acoustic and electric fields.

a, Map of acoustic pressure amplitude in the radial plane $\hat{x}\hat{y}$ (left), i.e., perpendicular to the acoustic field propagation direction, measured with 0.2 mm hydrophone (sensitivity = 52 mV/MPa). **b**, Same as in (a) but in the axial plane $\hat{x}\hat{z}$, i.e., parallel to the acoustic field propagation. **c**, Characterization of the applied electric field between a dipole stimulator. 2D map of voltages measured by moving dipole between probes, black areas were too close to the electrodes to obtain a measurement without the moving probe bumping the dipole. **d**, 2D zoom view of the area outlined in c.

3.2.2 Water degassing treatment

Most acoustic tank phantoms use de-ionized and degassed water to minimize effects of cavitation bubbles. We created our own degassing treatment for de-ionized water using a vacuum chamber (BACOENG 12 Liters Vacuum Chamber Kit with 3 CFM Single Stage Vacuum Pump, Amazon, UK). The process for degassing the water was to fill the vacuum chamber with enough de-ionized water to fill the tank (20L), then place a magnetic stirrer

into the bottom of the vacuum chamber, closing the lid and placing the whole chamber on top of the magnetic stirrer. By turning on the pump and the magnetic stirrer the air bubbles are extracted from the water. Use of the magnetic stirrer to agitate the water accelerates the degassing process. When the pressure needle stops moving the pump can be turned off. The entire process took about 20 minutes (**Fig 3-4**).

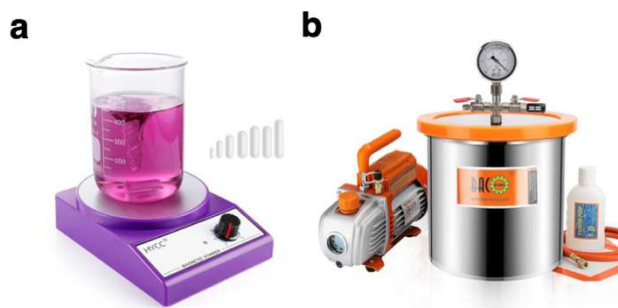


Figure 3-4 | Water degassing and treatment. a, Magnetic stirrer **b,** vacuum degassing chamber

Although many publications use degassed water¹⁰⁴, we found minimal difference between using regular water and degassed water. Furthermore, degassing only lasts a short period of time before both air and conductive particles in the air naturally re-enter the water, or stick to the parts submerged in the tank. To minimize bubbles on the submerged sensor assembly I used a water filled pipette to clear the bubbles away once it was submerged. Unless there is a continuous de-gassing and de-ionizing procedure throughout experimentation, water will not truly be degassed or de-ionized.

3.2.3 Impedance adapter for monitoring voltage and current concurrently

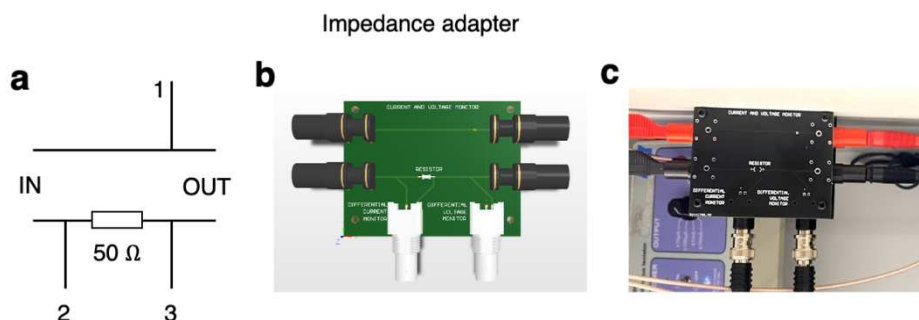


Figure 3-5 | Impedance adapter.

a, Circuit diagram of the impedance adapter. **b,** 3D-model of the printed circuit board made in Altium **c,** Final impedance adapter.

To monitor the current and voltage simultaneously across a phantom load from either the voltage source, or the measurement electrodes which are in solution I needed a plug and play adapter which enabled the signals to both pass through and be measured (**Fig 3-5**) so that both voltage and current flow could be continuously monitored.

The impedance adapter is a very simple two parallel lines, which enable the differential measurement of the voltage between them (**Fig 3.5 a, 1 and 3**), and then with a 50 Ohm resistor the current can be measured in parallel (**Fig 3.5 a, 2 and 3**). Both the measurement of the voltage and current need to be differential, so as not to accidentally ground one side or the other. If the impedance adapter is to be used in a single-ended arrangement, the GND line should be on the side of 1 in the diagram so that the current can still be measured, and the voltage output will not be shorted to ground. To calculate the current, Ohm's Law ($V=IR$) is employed for the known load (50 Ohms), and since we can measure the voltage across it we can then recover the current through $I=V/R$.

Whenever there is a non-linearity in the medium, whether it be the electrochemical interface between the electrodes and the saline solution, or capacitive coupling in the cabling, the lag between the current and voltage will be affected, and we could measure this lag using the adapter shown. Furthermore, when injecting large currents, its easy to enter the non-linear response regime as electrodes reach their charge injection limit (CIL)¹⁰⁵, which can lead to distortions in the measured voltage. Being able to see how both the voltage and current were affected under these conditions, was very helpful to both understanding and debugging various charge injection scenarios. For example, across a saline load often the voltage would appear linear as a voltage source was used, but the current would not be. Having the ability to observe the non-linear behaviour easily, as both current and a voltage were recorded simultaneously was extremely informative. This technique only works when the output impedance of the function generator is far greater than 50 Ohms, which is standard to minimize signal distortion.

3.2.4 Low pass differential filter design

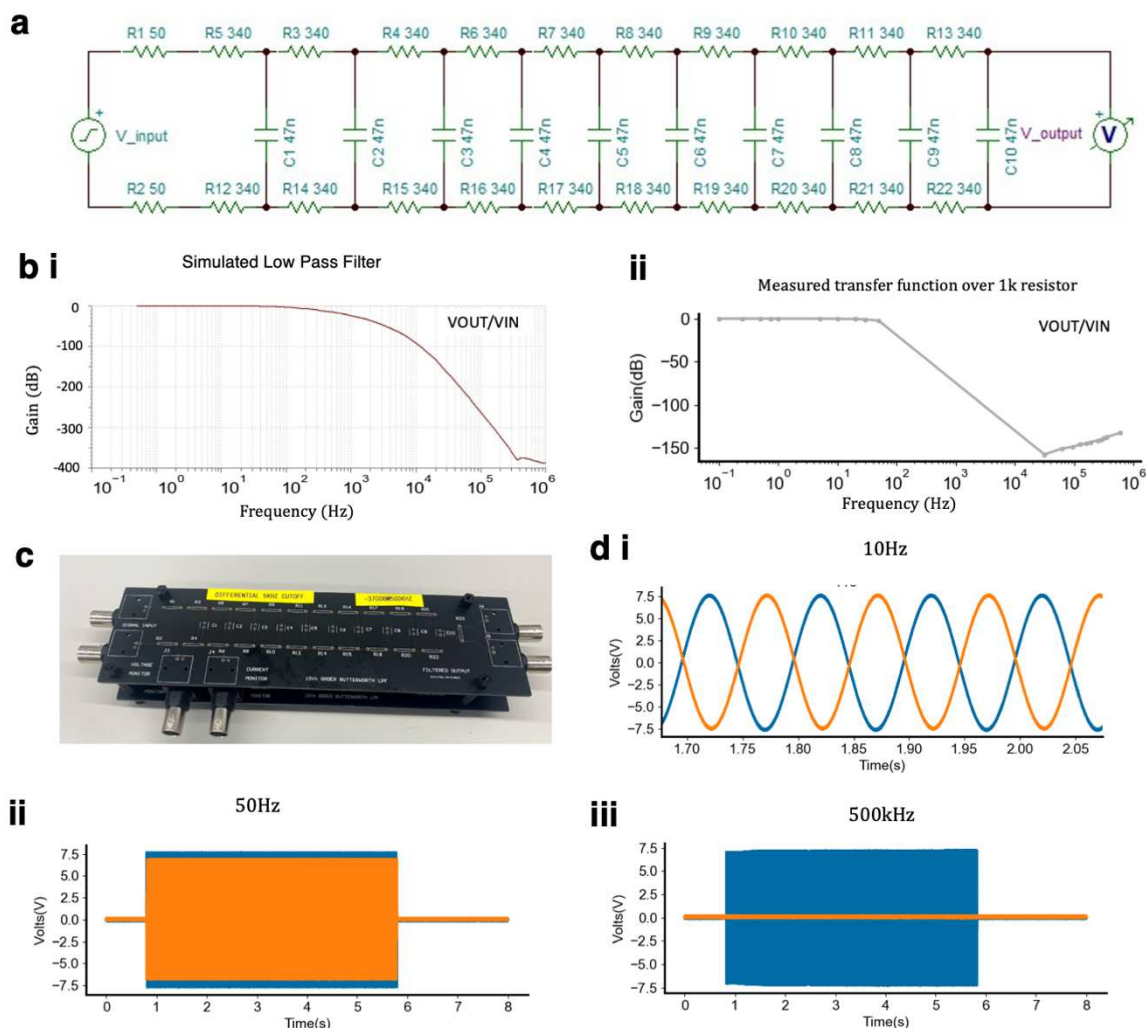


Figure 3-6 | Low pass differential filter.

a, Circuit for low pass differential filter. **b, i)** simulated transfer function **ii)** Real transfer function of the low pass filter based on the real components over 1 kOhm resistor. **c**, photo of the completed filter. **d, i)** When 10Hz is applied(blue) the output lets 10Hz pass through without attenuation(orange). **ii)** When 50Hz is applied(blue) there is a very small attenuation at the output of the filter(orange) **iii)** When 500kHz is applied (blue) none of it appears at the output of the filter (orange).

To limit the delicate preamplifier from overloading from the high frequency applied electric field, a highly attenuating low-pass differential filter was designed and built to remove the unwanted signals and detect the difference frequency (**Fig 3-6**).

Differential circuits can provide better performance over single-ended circuits as they provide higher linearity, immunity to common-mode interference signals, and have higher signal to noise. The advantages of performing measurements using such a differential filter were sufficient to warrant implementing a differential filter design instead of the more common single-ended filter design. However, most software packages only support the design of single-ended filter schemes, making it difficult to easily design a differential filter and simulate it in software before investing time building it. To design a differential filter and obtain a simulated transfer function I first needed to design a single-ended filter in pSPICE, then convert into a differential filter¹⁰⁶. A single-ended low-pass filter can be converted into a differential filter by repeating and folding the design around the GND plane to create the V_{in+} and V_{in-} required for the differential measurement. Hence, the GND is removed, and the centre capacitor values can be recalculated as half the original value due to the rule of sums for two series capacitors and the load resistor doubled due to the sum of two series resistances.

To optimize the design to fit available resistor, capacitor and inductor values, differential conversion was performed and matching components from manufacturers were identified. When I found values that were within the right range for the initial differential filter design, halved their value and recomputed the transfer function for the pSPICE simulation to determine if I had a stable filter response. I selected different available product values and recomputed the transfer function of the single-ended filter again until finding an attenuation curve that was correct. The final filter implementation used only resistors and capacitors. The final filter attenuated over 300dB (**Fig 3-6 b i**) which of course could not be measured in the real filter implementation where this signal level was below the noise floor (**Fig 3-6 b ii**).

3.2.5 High pass differential filter design

A high-pass passive differential filter was designed for use with applied low frequency electric fields. When a high amplitude electric field was applied at low frequencies, it was important to attenuate this low frequency much as possible to avoid overloading the

SR560 preamplifier so that it could still detect very small amplitudes around 500kHz. The intent for this filter was to allow the application of a signal in the neural range which would be up modulated via the acoustoelectric effect, thereby enabling testing of the acoustoelectric neural recording concept.

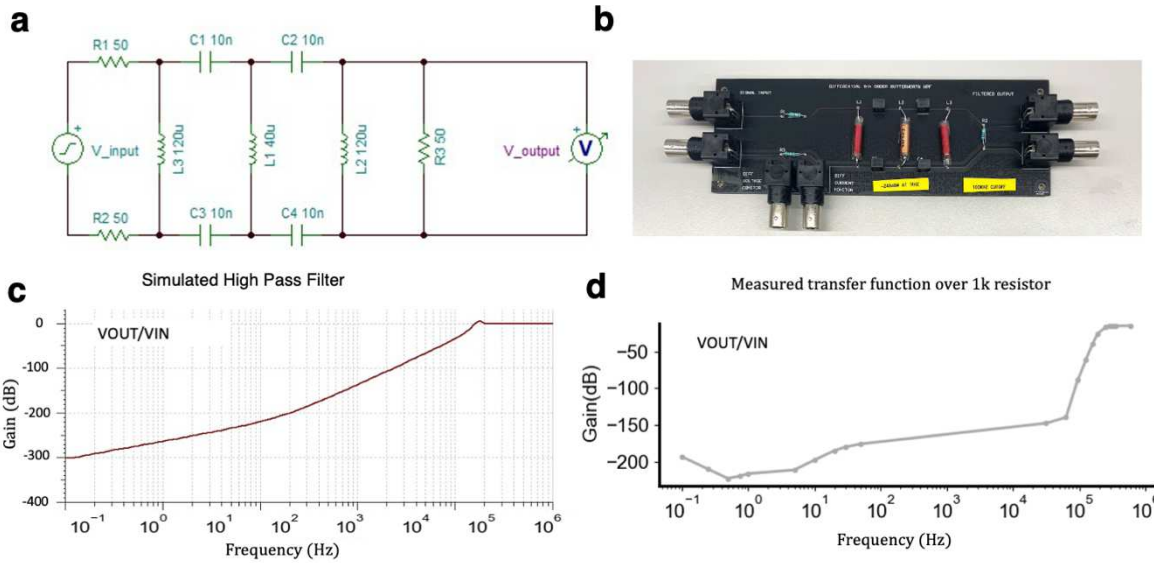


Figure 3-7 | High pass differential filter.

a, high-pass filter circuit design. **b**, completed physical filter **c**, simulated transfer function up to 1MHz. **d**, Real transfer function of the physical hardware filter.

The high pass differential filter has a 100kHz -3dB cut-off and was designed using pSPICE as a single-ended filter before being converted into a differential design similar to the low-pass filter described previously (**Fig 3-7**). Unlike the resistor/capacitor implementation of the low-pass filter, this inductor-based filter unfortunately introduced a non-linear distortion in saline, despite performing precisely over a linear load such as a 1 kOhm resistor (**Fig 3-7 d**). The non-linear saline response suggests that the ionic interactions with the applied electric field are more complex. This was outside the scope of the acoustoelectric study, though certainly an interesting topic for electrochemists, which would require isolation from the electrode interface which also may induces non-linear electrical responses.

3.2.6 Gradient descent based current source.

Current sources are used predominantly over voltage sources in the field of biological electrical stimulation to enable more precise control of energy deposition and control for changes in contact impedance of the electrodes applied to the skin. An entire industry has bloomed around the development of accurate current sources to be used in electrical stimulation, though these efforts have all been developed in labs using linear resistive loads. Current sources are generally able to work over a load range given a known resistance and capacitance and are only linear within a small range of frequencies as their design is based on intrinsically non-linear transistors, capacitors and operational amplifiers – the most famous and frequently used being the Howland current pump¹⁰⁷. This load range is limiting in the context of ionic solutions such as saline or biological tissue, as typically the electrode interfaces and the non-linear properties of tissue will range outside the limits of the electrical current source design. These design limitations also limit the frequency range of stimulation we have access to as the impedance of the medium changes with frequency. Due to the requirement to apply a field at frequency of 500kHz, a different approach was required to develop a flexible and accurate current source more generally applicable to the field of current source design for biomedical stimulation applications.

In electrical stimulation, changes in current delivery are required over timescales of seconds or longer as the electrode interface changes slowly. This non-instantaneous requirement opens the door to simple computational feedback techniques to be implemented to deliver a constant current, such as a gradient descent algorithm which can adjust the output current based on the use of the impedance adapter to measure applied voltage and current over a known load in the circuit instantaneously. The current is then calculated and the voltage output changed if it is above or below the target. When the output voltage delivers a current within the acceptable margins of the goal current, the gradient descent algorithm stops adjusting the output. In this way, we have developed a software controlled current source that can be used over any impedance and frequency.

Using gradient descent we can control the learning rate, decimal places of accuracy required in the result and the maximum number of iterations. In the implementation below the precision is set to 0.01 (**Fig 3-8**).

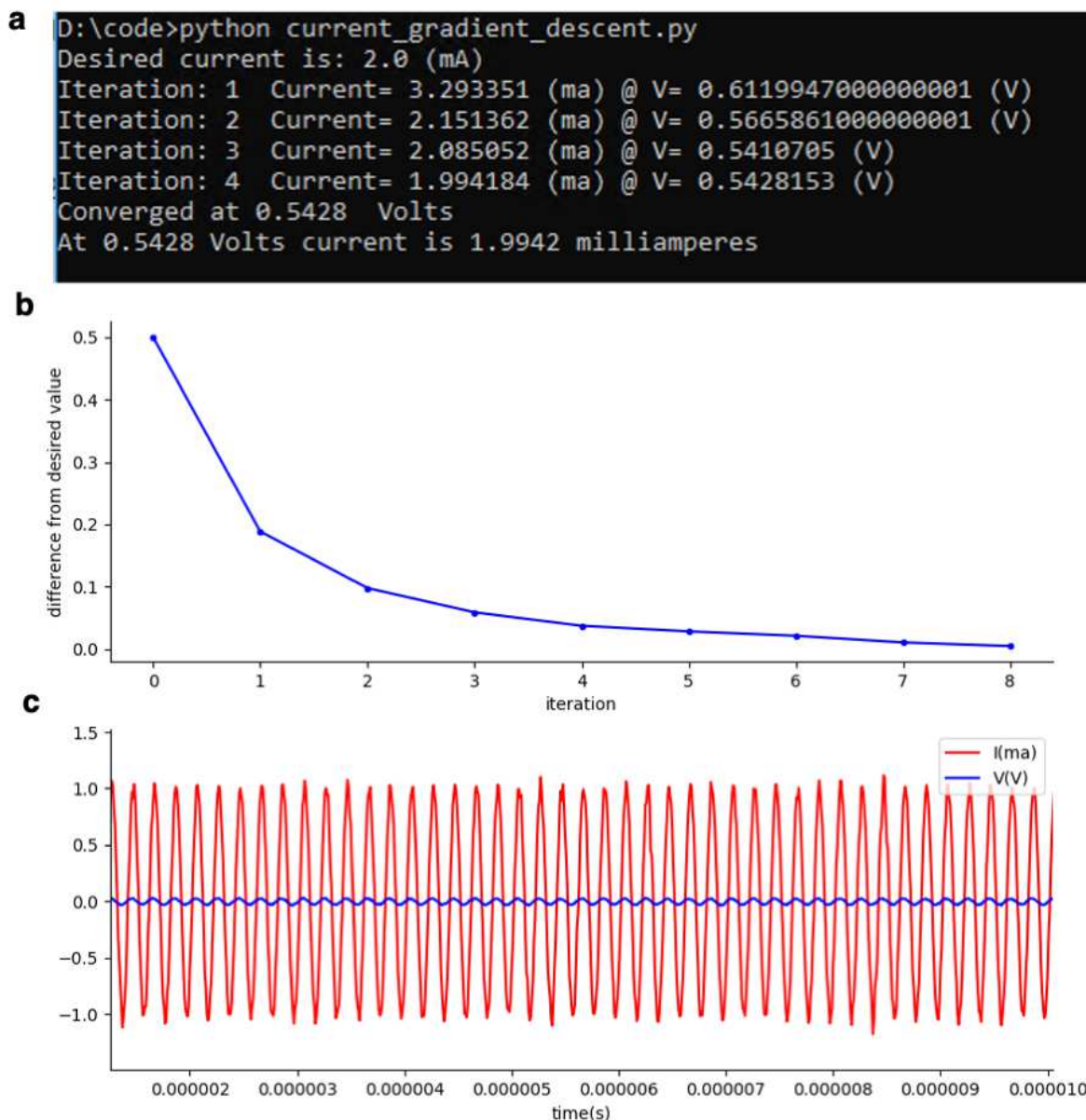


Figure 3-8 | Gradient descent based current source.

a, Command line example of each iteration of the gradient descent algorithm getting closer to the 2mA target at 500kHz. b, Number of iterations versus difference from desired current c, Output current showing 2mA output peak to peak with voltage value calculated from gradient descent algorithm.

The gradient descent algorithm was an effective means to control the current delivered in the saline phantom at any chosen frequency with any load parameters. This has a far

broader capability to work dependably over highly variant loads and frequency ranges than any traditional current source and reduces electrical design complexity. Its only downside is that it has a non-instantaneous response.

In both current source and voltage source implementations the function generator needs to be isolated from the non-linear load so that non-linearities generated in the load from the applied stimulus do not feed back into the waveform generation part of the circuit – which would introduce further non-linear distortion. This isolation can be achieved using a shielded transformer which was provided by Dr. David Bono **Appendix 1**.

3.2.7 Acoustoelectric phantom instrumentation overview

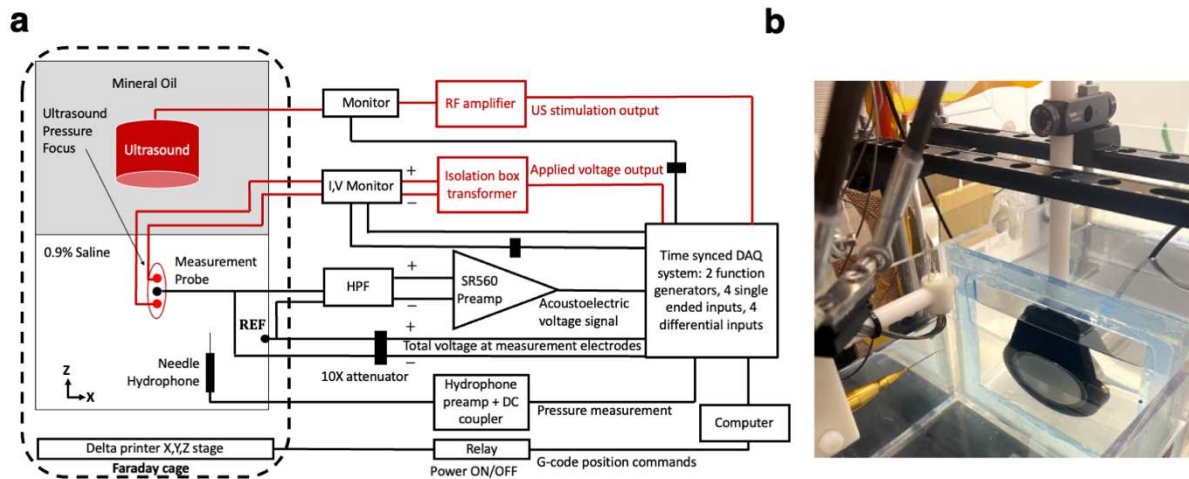


Figure 3-9 | Instrumentation Design.

a, Schematic of instrumentation set up to enable monitoring of all signals while applying known pressures and electric fields. Stimulation connections shown in red, measurement instrumentation shown in black. b, Image of the hydrophone and probe mounted into XYZ stage delta printer, with custom 3D printed probe¹⁰⁸, PDVF saline/mineral oil interface between transducer and measurement area.

Phantom. The measurements of the acoustoelectric modulation of the applied electric fields were done in a custom-built acrylic tank (50cm x 20cm x 20cm) divided into two parts by 12 μ m thick acoustically transparent Polyvinylidene difluoride film (PVDF, Precision Acoustics, UK). In one part of the tank, the ultrasound transducer was mounted and immersed in nonconductive mineral oil to minimise electromagnetic interference from the transducer to the recording area. In the second part of the tank, the stimulation electrodes, recording electrodes, and hydrophone were placed and immersed in 0.9%

saline. An acoustic damping material (Aptflex-36, Precision Acoustics) was attached to the back of the tank to minimize acoustic reflections, though I decided not to add it to the sides of the tank to minimize the costs of this comparatively expensive material. The phantom tank was surrounded by a Faraday cage to reduce electromagnetic interference. See **Fig 3-9** for a diagram of the experimental set up.

Probe scanning. The stimulation electrode-pair, recording electrode, and recording hydrophone were mounted using a 3D printed adapter on an XYZ stage¹⁰⁹ (Q5 Delta 3D Printer, FLSUN) and scanned across the ultrasonic focal area in 0.5 mm increments. The scanning was controlled via G-Code commands sent through a custom Python 3 interface.

Data acquisition. All applied signals were monitored at 5MS/s, and all signals were time synced using a combined multiple interface (CMI) for instrument synchronization between the function generators and oscilloscopes with trigger inputs. The applied voltages, monitor channels and electric potentials were logged using data acquisition hardware (WiFiScope WS5, TiePie engineering, Netherlands, WiFiScope WS6 DIFF). The logged signals were streamed from the data acquisition hardware to a workstation PC using custom C code which utilized the Tiepie SDK to control the triggers, channels, and function generator output. The compiled C code which called the oscilloscope commands was in turn called within a Python wrapper, which also sent G-code commands to the XYZ stage and controlled a relay to turn off all equipment in the Faraday cage during recording, including the motor of the stage which emitted low level electromagnetic interference. In each measurement, the pressure focus was automatically found using a custom script that searched for the peak pressure in the radial plane first and then along the axis.

Acoustic fields application. The acoustic field was applied using a spherically curved ceramic (PZT) 500 kHz ultrasound transducer (60 mm diameter, 63.5 mm acoustic path length; Precision Acoustics Ltd, UK). The ultrasound transducer was driven by an arbitrary function generator (Handyscope HS5, TiePie engineering, Netherlands) and a 40 W linear power amplifier (240L, Electronics & Innovation Ltd, USA).

Electric fields application. The electric field was applied using a pair of 98% platinum iridium wire electrodes (0.25mm cross-section diameter, 1.4 mm length exposed tips; Alfa Aesar, Netherlands) with 3mm inter-electrode spacing. The electrodes were driven by a second arbitrary function generator (Handyscope HS5, TiePie engineering, Netherlands) and a custom-made high-frequency voltage source made of glass core transformers (Hitachi Metals, USA) with a maximum ± 20 V amplitude at an effective 3dB bandwidth range of 5kHz to 2MHz. In a subset of measurements in which frequencies lower than 5kHz were applied, the electrodes were driven directly from the arbitrary function generator.

Pressure recordings. The acoustic field was measured using a 0.2 mm needle hydrophone (52mV/kPa at 500 kHz calibration; Precision Acoustics Ltd) and a DC-coupled preamplifier (Precision Acoustics Ltd). Hydrophone recordings were made in saline, and the hydrophone was removed and cleaned with deionized water immediately afterwards to minimize corrosion.

Electric potential recordings. The electric potentials were measured through a recording electrode similar to the stimulation electrodes, which was placed between the two stimulation electrodes. The recorded potential was amplified using a low-noise differential amplifier (SR560, Stanford Research Systems) and filtered using a custom-built^{110,111} passive differential high or low-pass filter to filter out the signal at the frequency of the applied electric field while allowing the sum and difference frequencies to reach the amplifier unattenuated. The electric potential recordings were made when the hydrophone was not attached to avoid any interference between the two.

Signal Processing and Analysis. Custom Python 3.8.4 scripts were created to create the data analysis toolchains using the numerical analysis libraries Numpy, Scipy, OpenCV2 and Pandas libraries.

Time domain analysis. To isolate the heterodyned high frequency acoustoelectric signal, in the case of an applied 8kHz electric field and 500kHz acoustic field, a 17th-order Chebyshev II digital bandpass filter was applied from 100Hz below the difference frequency, up to 100Hz above the sum frequency. A similar filter was applied when the applied electric field was at different frequencies, whereby the pass band started 100Hz below the difference frequency and stopped 100hz above the sum frequency.

Frequency domain analysis. A 1-D discrete Fourier Transform¹¹² with Flat Top^{113,114} window to optimize amplitude accuracy was computed, as we report amplitude spectral density (ASD) instead of power spectral density (PSD) to easily read the average peak to peak amplitude of each measurement. After the Fourier transform was computed, the two-sided amplitude spectrum was multiplied by 2, and half the array was taken – converting it into its single-sided form. The units of the single-sided amplitude spectrum then give the mean peak amplitude of each sinusoidal component making up the time-domain signal.

3.2.8 Characterization of the applied fields.

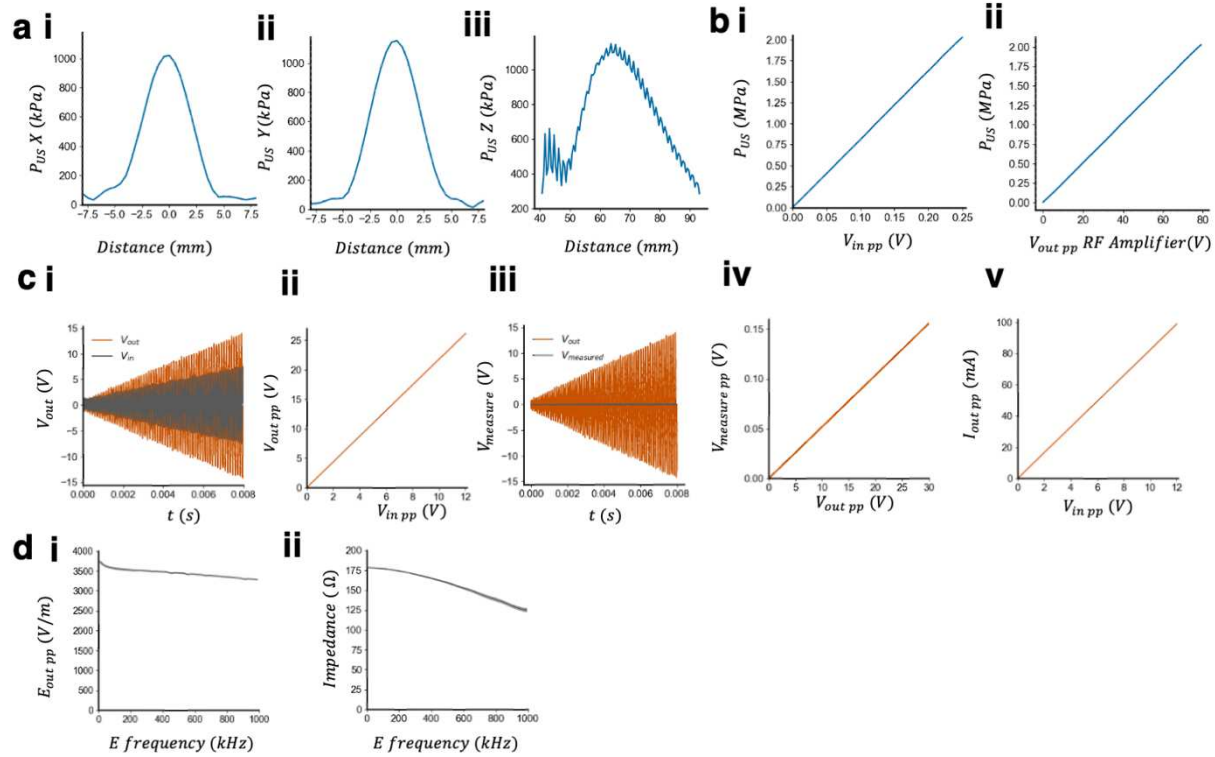


Figure 3-10 | Characterization of the applied acoustic and electric fields.

a, Line plot of the measured acoustic pressure amplitude along the (i) $\hat{x}$ direction, (ii) $\hat{y}$ direction, and (iii) $\hat{z}$ direction. Note, the recording in the $\hat{z}$ direction (i.e., axial) shows small standing waves effect, as the damping material at the back of tank potentially may not eliminate all reflections. **b**, Measured acoustic pressure amplitude vs amplitude of the driving voltage to the ultrasound transducer (i) before the RF amplifier (ii) after the RF amplifier. **c-e**, Characterization of the applied electric field. **c**, Electric voltage output. (i), Ramped sine wave used to characterize the electric field generated in saline via the platinum iridium electrode probe. (ii), Relationship between the function generator voltage (V_{in}) and the output of the voltage source (V_{out}), i.e., the isolation transformer with x2 gain; shown values are peak-to-peak (pp) amplitudes. (iii), Amplitude ramp function delivered to saline showing output of isolation transformer and measured electric potential in the solution. (iv), Measured output of isolation transformer vs. voltage at the measurement electrodes; shown values are pp amplitudes. (v), Output current amplitude, measured across a resistor in series vs. measured function generator output voltage; shown values are pp amplitudes. **d**, Electric field output. (i) Applied electric field measured across the stimulation electrodes (3 mm spacing) in saline vs applied frequency. (ii) Computed impedance across the stimulation electrodes vs. applied frequency.

We characterize the pressure field such that the RF amplifier voltage, or function generator output can easily be translated into a maximum output pressure at the focus of the 0.5MHz ultrasound transducer in saline and mineral oil, with a PVDF film placed between the two liquids as per the acoustoelectric phantom design (**Fig 3-9 b**). Notably, in our 0.008 second measurement a standing wave develops in the axial direction (**Fig 3-**

10 iii), despite having Aptflex F-36 damping material absorbing the acoustic wave at the back of the tank.

The applied electric field can be characterized by applying a ramping voltage through the function generator and simultaneously measuring the output voltage of the transformer, the current output across the stimulation electrodes, and the voltage across the measurement electrodes. Our Platinum Iridium probe has stimulation electrodes 3mm apart and measurement electrodes 1mm apart in a bioimpedance configuration as shown in **Section 3.2.3**, and since it is a dipole the output electric field falls off with $\frac{1}{radius^3}$ ¹¹⁵ making the voltage received across the differential measurement electrodes comparatively small (**Fig 3-10 c**). We also characterize the output electric field with respect to frequency, ramping the electric field function generator from 10kHz through to 1MHz in 10kHz increments, showing a reasonably constant output (**Fig 3-10 d i**), as well as measuring the decreasing impedance with respect to frequency (**Fig 3-10 d ii**). **Fig 3-10 e i and ii** show the output voltages at 500kHz and 8kHz.

3.3 Methods: Animal experiments

The surgery procedure described below was common between the acoustoelectric neural recording and the acoustoelectric neuromodulation experiments detailed in **Chapter 5** and **Chapter 6**. An animal fitted with a head bar and electrode implant was then allowed to recover and could participate in both types of experiments. This was in line with the 3R's (replacement, reduction, and refinement) of animal research, meaning each animal made a maximal contribution to scientific research and the number of animals was kept to a minimum.

It took a significant amount of time to refine the surgery below such that an animal recovered quickly and was able to participate in many repeated experiments whilst maintaining good signal quality. Once this surgery was refined it was a superior technique to non-recovery electrophysiology experiments as animals were under anaesthesia for a shorter amount of time, meaning the electrophysiological signals were higher amplitude than they would be if a measurement took place directly after a lengthy surgery procedure.

As an extra precaution to maintain mouse well-being, mice were given at least one rest day between experiments. The original recovery surgery procedure was based on Dr. Patrycja Dzialecka's recovery surgery technique developed for Calcium Imaging during Temporal Interference stimulation¹¹⁶ and modified to suit the requirements of acoustoelectric neural recording and neuromodulation.

3.3.1 *In vivo* electrophysiology surgery

All mice were wild type C57BL/6 male and female mice, aged between 3-6 months. Mice were housed in standard cages in Imperial College London animal facility, with ad libitum food and water in a controlled light-dark cycle environment, with standard monitoring by veterinary staff. The Imperial College of London's Animal Welfare and Ethical Review Body approved all animal procedures, and all experiments were performed in accordance with relevant regulations/according to the United Kingdom Animals (Scientific Procedures) Act 1986.

Mice were anaesthetized using 1-3% (vol/vol) isoflurane in oxygen and subcutaneously administered Vetergesic (0.1mg/kg) and Carprofen (5mg/kg) to inhibit pain and inflammation and saline for hydration (10ml/kg/hour). A head plate was attached to the mouse using clear dental cement (PalaXpress Dental Cement, AgnTho's AB, Sweden) and a craniotomy performed with a drill (diameter 0.5mm) at the intended electrophysiological measurement sites. The position of the craniotomy relative to bregma was anteroposterior 0.0 mm, mediolateral -2 mm for the motor cortex electrode and anteroposterior -3.5mm, mediolateral 2.25mm for the visual cortex electrode used to measure visual evoked potentials.

The Pt-Ir electrode implant (0.5mm diameter wire, VWR, Lutterworth, UK) was positioned over the drill holes such that the end of the wire was touching the brain, and dental cement used to secure it to the back of the head bar. Low toxicity silicone sealant (Kwik-Cast, World Precision Instruments, UK) was then poured around the electrodes to seal moisture around the craniotomy site and over the exposed skull, whilst also stabilizing the electrode

position. We tested the silicone for acoustic transparency in a petri dish test phantom, obtaining equivalent results with and without the use of silicone.

Nail polish was then applied to the Pt-Ir wire to form a non-conductive exterior, such that only the portion of wire in the brain is exposed. This ensures measurements were from the brain and not above the brain in the ultrasound gel which would later be placed over the skull. The mouse was then recovered in a warming chamber and administered Carprofen (5mg/kg) for three days after surgery with daily weight checks, then allowed to recover for one week before experiments commenced.

3.3.2 Anesthesia selection rationale for acoustoelectric *in vivo* experiments.

The experimental procedures for acoustoelectric neural recording and neuromodulation are detailed separately in the Methods sections of **Chapter 5** and **Chapter 6**, as they involved different instrumentation arrangements and types of anaesthesia. These Chapters, though complete in their own descriptions, do not provide the comparative rationale between the anaesthesia choices for each set of experiments which we discuss here.

0.5% (vol/vol) isoflurane in oxygen was chosen for the acoustoelectric neural recording experiments as this anaesthesia level could be kept constant for a precisely controlled period (1-2 hours), and the anaesthesia depth easily changed throughout the experiment if need be. At this low anaesthesia level, the mouse was sedated and not in deep anaesthesia. Even with this constant low-level application of Isoflurane, the sedation level experience by the mouse changed slowly over time with the largest amplitude visual evoked potential signals being present at the beginning of the experiment, and gradually diminishing as the experimental time continued. At higher levels of Isoflurane anaesthesia, the visual evoked potential was very small or no longer present.

Ketamine/Xylazine was used for the acoustoelectric neuromodulation experiments. This anaesthesia was administered via a single subcutaneous injection at the beginning. This means that as the body load of anaesthesia slowly changes the depth of anaesthesia,

and the mouse will remain sedated as the anaesthesia level became very light. It is this light anaesthesia period where ultrasound brain stimulation evoked an EMG response. This window of time in which the acoustoelectric neuromodulation experiments could be undertaken is in line with reports on how to perform ultrasound brain stimulation, which is typically only reported when the mouse is awake or in extremely light anaesthesia^{23,34,117,118}. We utilized an experimental anaesthesia depth where the mouse was not moving, which is deeper than many of the reported experiments. This is also reflected in the applied pressure fields which are on average slightly higher than those reported when the mouse is awake.

Chapter 4: Acoustoelectric phantom

4.1 Introduction

The acoustoelectric interaction has uniquely promising characteristics making it a good candidate for focused non-invasive neuromodulation and focused detection of neural signals. Theory and simulation have proven it is a viable direction to explore further and begin testing in the real world. To ascertain if the predictions made in **Chapter 2** are true, a physiological tissue mimicking phantom was developed to experimentally verify and explore the characteristics of the acoustoelectric interaction in **Chapter 3**. In this Chapter, we experimentally verify the theoretical predictions and perform feasibility tests in the saline phantom determining that the acoustoelectric bidirectional neural interface meets the criteria to move to *in vivo* testing in mice.

4.2 Results

4.2.1 Verification the acoustoelectric effect is not an artefact.

Though the acoustoelectric effect has been reported since 1953⁵² it has not received as much attention as the highly influential field of piezoelectrics, suggesting it is worth investigating the existence of the acoustoelectric interaction itself. Alternate causes of the effect could include electrical transmission from the transducer into the measurement area, as the RF amplifier produces large voltages. Its also possible that the acoustic field vibrates the measurement electrodes at the focal point of the transducer causing some non-linear electrical artifacts. Mixing frequencies could also be introduced through non-linear electrochemical effects at the electrode interface such as dual layer capacitance/Helmoltz capacitance¹¹⁹, generated on the electrodes in conductive solution,

or the Debye-Falkenhagen effect¹²⁰ which predicts non-linear response of an electrolyte as electric field frequency increases¹²¹. Lastly, the mixing may be occurring in the measurement instrumentation itself. If we can show the acoustoelectric interaction exists independent from these possible confounds, it becomes a far more plausible area of research.

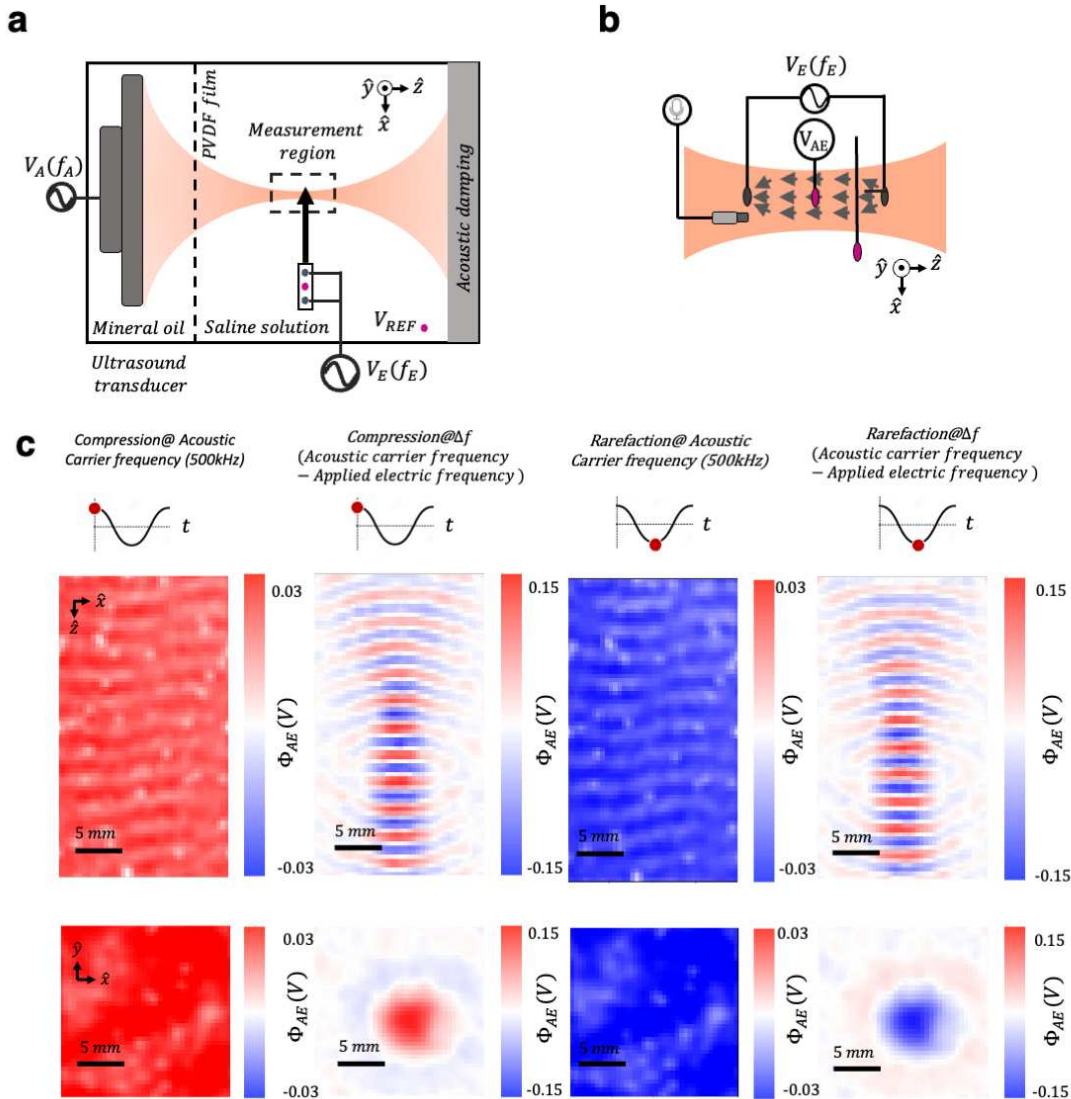


Figure 4-1 | Acoustoelectric characterization the transducer electrical and acoustoelectric field.

Time-frequency characteristics of the electric field generated by simultaneous application of a focal acoustic pressure field $P(x, y, z)$ and an electric field $\vec{E}(x, y, z)$ to a saline filled tank. **a**, Schematic of experimental setup, showing setup overview, **b**, zoomed view of the boxed measurement region in (a); V_A , applied electrical potential from RF power amplifier driving the ultrasound transducer at a frequency f_A ; V_E , applied electrical potential from arbitrary waveform generator and custom-made voltage source to the electrodes at a frequency f_E ; V_{AE} , measured acoustoelectrically generated electric potential. **c**, $\hat{x}\hat{y}$ and $\hat{x}\hat{z}$ spatial

maps of acoustoelectric difference frequency and acoustic carrier frequency for both a compression and rarefaction.

To experimentally verify the acoustoelectric phenomenon, experiments with an ultrasound source and an electric field were conducted in physiological saline. A 500 kHz focused ultrasound transducer (ROC=63.2 mm) was used to create the acoustic field, and a pair of Pt-Ir electrodes (0.25 mm diameter, 3 mm spacing) were used to create the constant electric field (8kHz) which was moved around with the measurement electrode. The acoustoelectrically generated field was measured by a third electrode (0.25 mm diameter) relative to a remote reference electrode (see **Chapter 3 Fig 3-1** for a schematic representation of the experimental setup). The saline had a conductivity of 1.6 S/m similar to biological tissues¹²².

If the acoustoelectric effect was an artifact due to the electric field distribution caused by the RF amplifier, its shape would be a point source distribution emanating from the transducer, similar to the point source distribution of a single charge and the measured acoustoelectric field would not follow the shape of the pressure field. To elucidate the acoustoelectric focality phenomenon we created time-synced recordings with spatial mapping in $\hat{X}\hat{Y}$ and $\hat{X}\hat{Z}$ directions in 0.9% saline. In these experiments, the electric field is aligned with the propagation direction of the acoustic field. We observe that the frequency mixing products only occur within the focal distribution of the transducer, and that they are not present at the carrier frequency (500kHz) indicating there is no linear piezoelectric effect in solution, only heterodyning (**Fig 4-1 d**). Furthermore, we have electrically isolated the transducer with mineral oil so that electric signals cannot travel from the transducer into the saline measurement area of our phantom, so that this field cannot be due to the electrical artefact from powering the transducer.

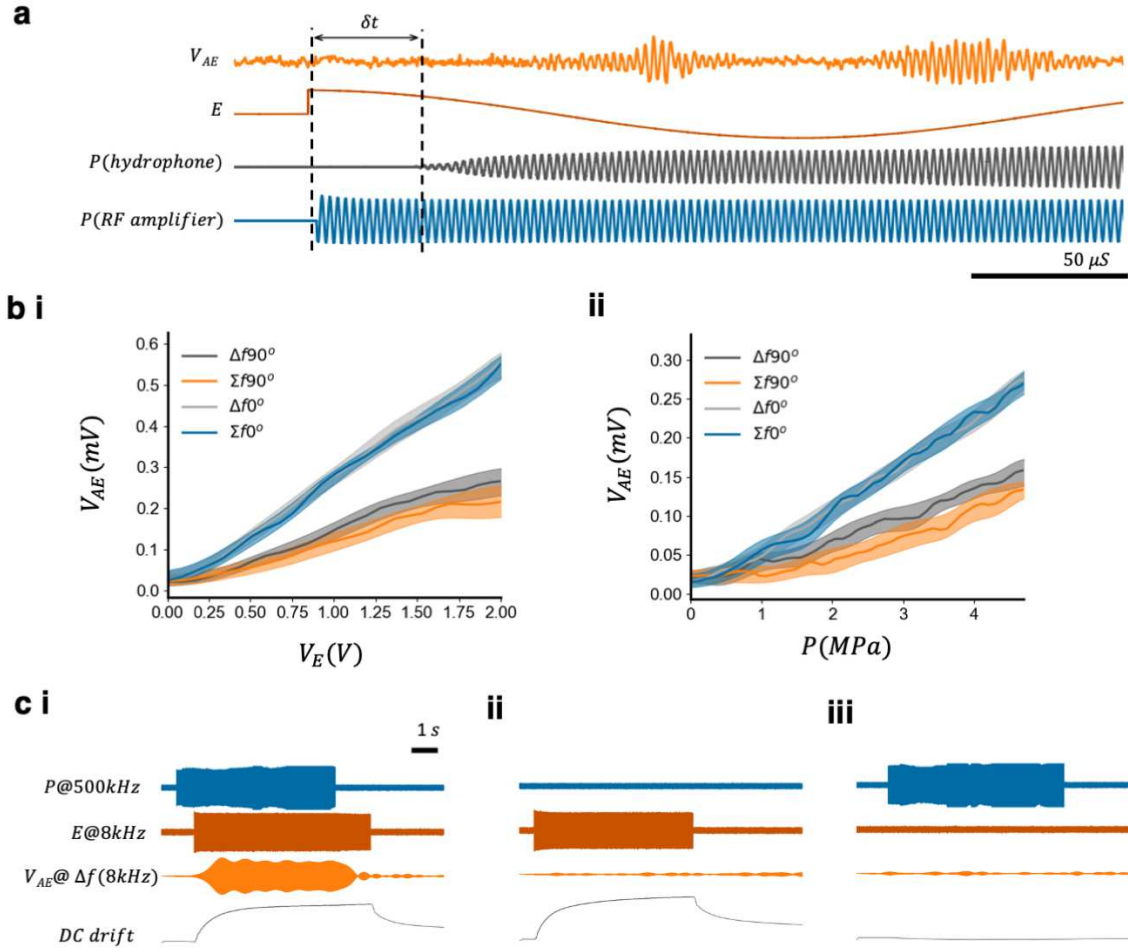


Figure 4-2 | Acoustoelectric timing and dependence on applied fields.

a, View of the onset of the acoustoelectrically generated electric potential, showing the traces of the applied RF amplifier input to the ultrasound transducer (blue), resulting acoustic field at the phantom's measurement region (grey) as measured by hydrophone, applied electric field (red), generated acoustoelectric potential (orange). The onset of acoustoelectric generated field mixing occurs after the hydrophone measurement of the arriving ultrasonic field. **b**, Amplitude of the acoustoelectrically generated electric potential vs. (i) amplitude of the applied acoustic field with the amplitude of the applied electric field fixed (here at 3.2kV/m), (ii) amplitude of applied electric potential with the amplitude of the applied acoustic field fixed (here at 1 MPa at the peak). Shown are mean $\pm$ st.d. values at the difference frequency Δf and sum frequency Σf when the fields are parallel (0°) or perpendicular (90°); $n=50$ measurements; light grey, 0° at Δf ; blue, 0° at Σf ; dark grey, 90° at Δf ; orange, 90° at Σf ; Here the acoustic field frequency $f_A=500$ kHz and electric field frequency $f_E=8$ kHz. **c**, Representative time-synced recordings of the applied acoustic field P (blue), applied electric field E (red), acoustoelectrically generated electric field (orange), and extraneous DC drift (grey) when (i) both the acoustic and electric fields were applied (ii) only the electric field was applied (iii) only the acoustic field was applied.

Next, we measure the onset timing differences between the acoustic and electric fields (Fig. 4-2 a) showing the onset of the acoustoelectric mixing frequencies only after the

pressure wave reaches the focal area, and the acoustoelectric effect is not measurable before this when the faster electrical signals from the RF amplifier are turned on. The focality difference between the carrier and the mixing frequencies and timing diagram provide evidence the acoustoelectric effect is not due to electromagnetic radiation from the transducer.

The acoustoelectric effect may still be due to pressure induced vibration on the stimulation and measurement electrodes. If this is the case, changing the applied electric field amplitude while the vibration inducing pressure is kept constant, should not change the amplitude of the acoustoelectric field. However, when the applied electric field is gradually increased, the acoustoelectric field amplitude also changes (**Fig 4-2 b i**) approximately linearly in line with the derivation solution, proving the effect is not due to vibrations on the stimulation or measurement electrodes. Furthermore (**Fig 4-2 c iii**) we show there is no acoustoelectric field generated when pressure is applied alone.

If the acoustoelectric effect is due to dual-layer capacitance, or even the Debye Falkenhagen effect, we would expect to still see the mixing of frequencies when only voltage was present. However, increasing pressure approximately linearly increases the emergent difference frequency (**Fig 4-2 b ii**), providing evidence this interaction is not solely due to the stimulation electrodes and their electrochemical effects. We can see that when voltage is present only, there is no mixing effect based on (**Fig 4-2 c i,ii**), thus it is not caused by the voltage stimulation alone or any of the electrochemical effects related to it.

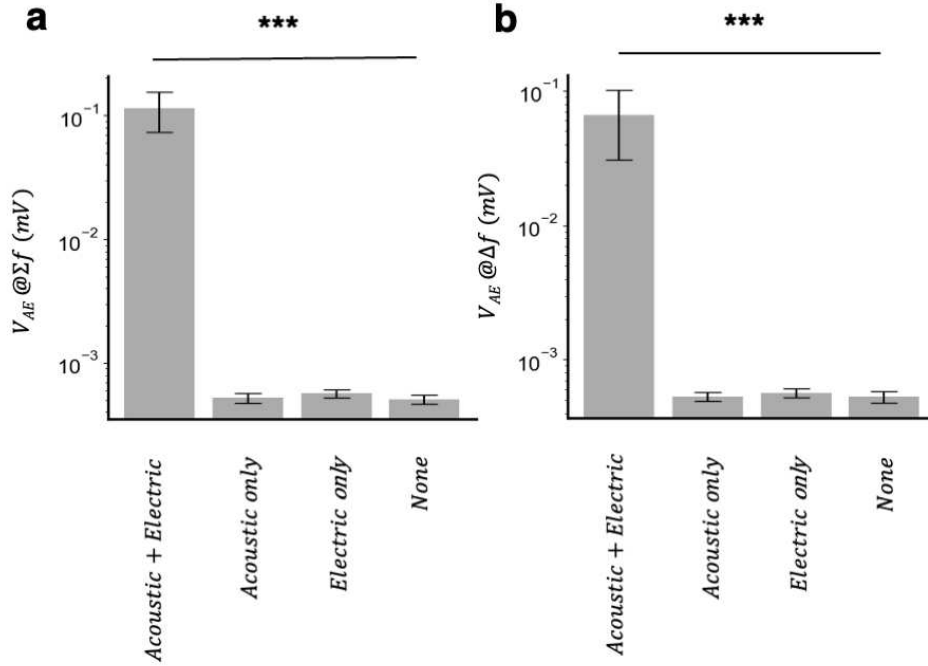


Figure 4-3 | Acoustoelectric statistical artefact tests.

Amplitude of recorded V_{AE} at **a**, the sum frequency Σf , **b**, the difference frequency Δf comparing when both electric and acoustic fields were applied, only acoustic field was applied, only electric field was applied, no fields were applied; values are mean $\pm$ st.d.; *** indicates $p < 10^{-20}$; significance was tested with Tukey's honest significance test corrected for multiple comparisons, $n=50$ measurements.

To isolate the acoustoelectric effect from non-linearities in our instrumentation, we used Tukey's honest significance test corrected for multiple comparisons, comparing pressure and electric field together, pressure or current only, and no pressure or electric field applied in saline, all with $N=50$ measurements yielding $P < 0.001$ or extreme significance at 99.9% that the acoustoelectric effect is not caused by non-linearities in the instrumentation or electrode saline interface (**Fig 4-3 a,b**). In each case, all signals were injected through the instrumentation at the same amplitude and amplified with the RF amplifier/transformer system and disconnected individually at the point of contact with saline when deactivated to ensure a true instrumentation representation.

With the above tests, isolating the shape of the focal acoustoelectric field as compared to the carrier frequency, the linear scaling and dependence on both the applied acoustic and electric fields, and the instrumentation test to determine the heterodyning effect is

occurring in the medium and is dependent on both fields being present, we determine the acoustoelectric interaction is not confounded by artefacts.

4.2.2 Acoustoelectric heterodyning experimental evidence.

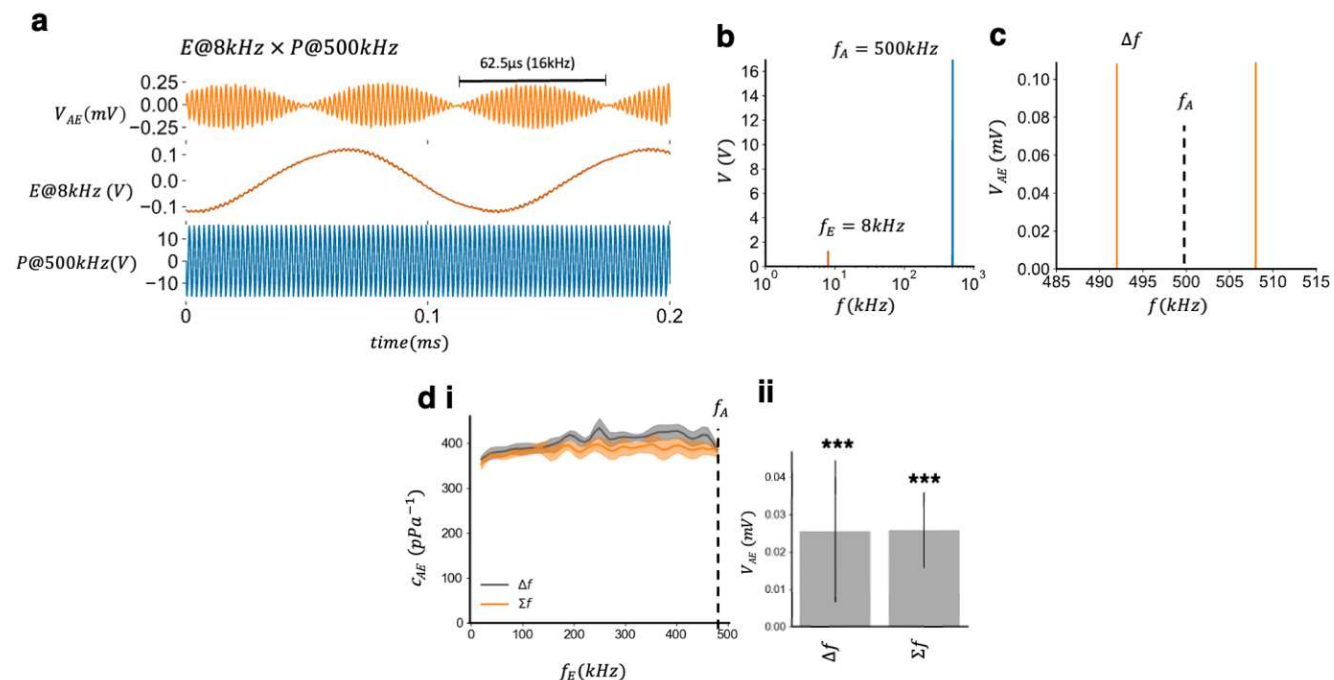


Figure 4-4 | Measurements of acoustoelectric frequency mixing in a tissue phantom.

a, Representative recordings when the electric field is applied at a frequency $f_E=8$ kHz and the acoustic field at a frequency $f_A=500$ kHz. Shown are traces of applied acoustic field P (blue), applied electric field V_E (red), and generated electric potential V_{AE} (orange); V_{AE} was filtered before logging (high-pass filter, cut-off frequency 100 kHz). The superposition of the sum frequency ($\Sigma f=508$ kHz) and difference frequency ($\Delta f=492$ kHz) is manifested in V_{AE} as a periodic modulation of the envelope amplitude at their difference frequency $\Sigma f-\Delta f=16$ kHz. **b**, Amplitude spectral density (ASD) of the signal traces in (a) showing the applied V_A and V_E traces, **c**, ASD of recorded V_{AE} trace with peaks at the frequency mixing products Δf and Σf but not at the acoustic frequency f_A . **d**, Effect of the applied electric field frequency. (i) Acoustoelectric coupling factor c_{ae} at Σf and Δf vs. applied electric field frequency f_E with applied acoustic frequency f_A fixed at 500kHz, the acoustoelectric field amplitude is estimated by the amplitude of the ASD at the relevant frequency; (ii) mean $\pm$ st.d. recorded V_{AE} amplitude across the frequency range in (i). *** indicates $p<10^{-20}$; significance was tested with pairwise t-test against the condition in which no fields were applied; $n=200$ measurements.

To experimentally verify frequency mixing we first applied an electric field ($f_E=8$ kHz) parallel to the acoustic field ($f_A=500$ kHz) propagation direction and measured the electric fields at the acoustic field focus. We found that the combined fields generated electric field components oscillating at the difference and sum frequencies, i.e., $\Delta f=492$ kHz and

$\Sigma f = 508$ kHz, (**Fig 4-4 a,b,c**) as was predicted by our mathematical model (**Chapter 2 Eq. 19**). There were no electric field oscillations at the difference and sum frequencies when the acoustic field or the electric field was applied individually.

We then applied the electric field, sweeping through a broad range of frequencies between 10 kHz and 500 kHz while keeping the acoustic frequency fixed at 500 kHz. We found a consistent generation of electric field components at the difference and sum frequencies across the applied frequencies range (**Fig 4-4 d i**). To obtain the acoustoelectric interaction amplitude with respect to electric field frequency, no analog filters could be used as this would affect the amplitude of the results. This also means that the preamplifier could not be used as the applied electric field would saturate the preamplifier obscuring the acoustoelectrically generated sum and difference frequencies. Therefore, the measurements were made without amplification, which was possible due to the continuous, single frequency characteristic of the applied signals that yields high SNR sum and difference frequencies when averaged over an 8 second period.

To determine whether heterodyning was the generalized effect, we took the sum and difference frequency amplitudes of the applied fields and compared to the baseline amplitude across our spectral data (**Fig 4-4 d ii**) obtaining an extremely significant ($p < 10^{-20}$; $n=200$ measurements) result in a pairwise t-test for both sum and difference frequencies, providing strong evidence that the acoustoelectric interaction is a heterodyning interaction.

4.2.3 Acoustoelectric directional dependence experimental evidence

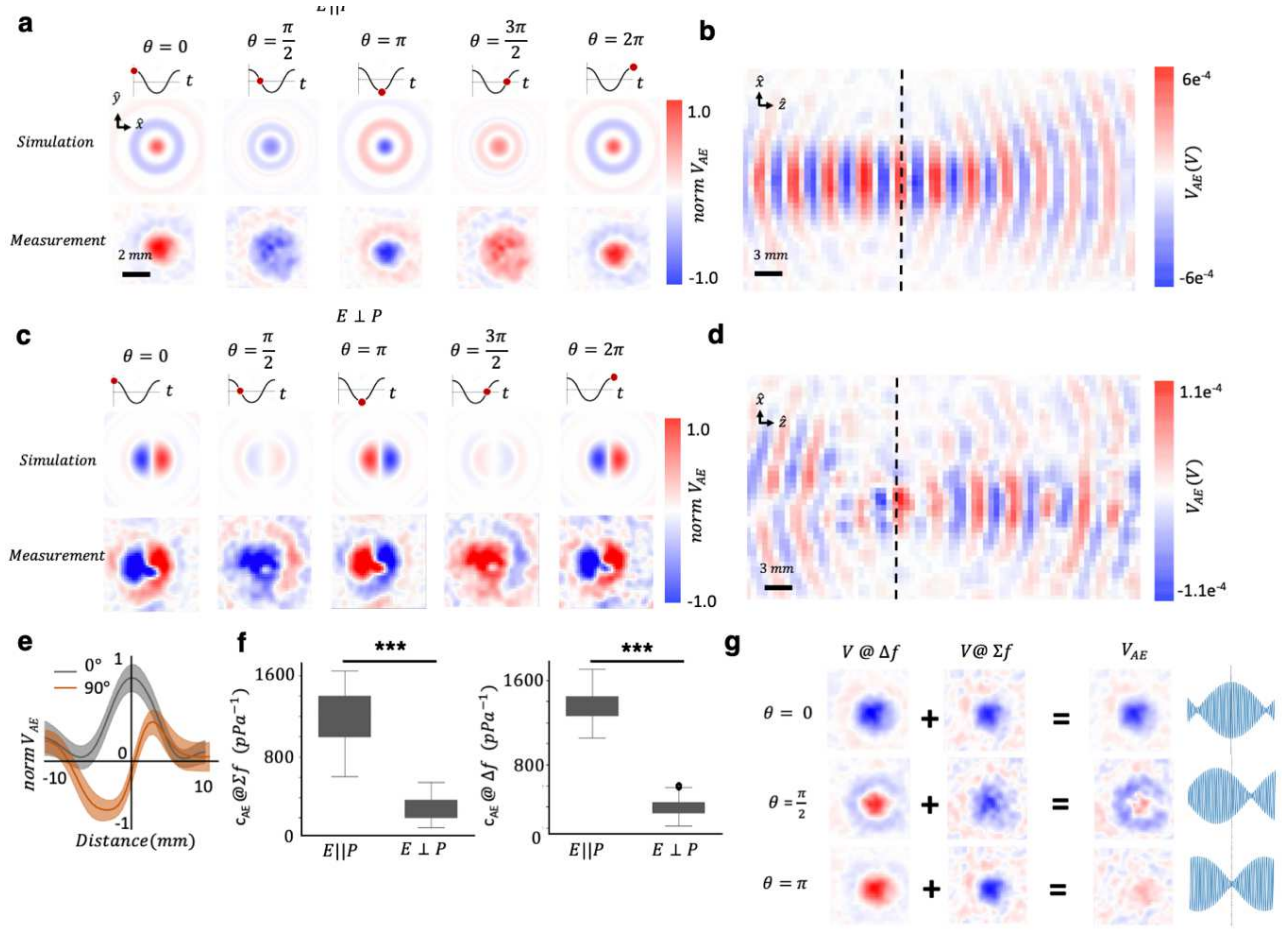


Figure 4-5 | Measurements of acoustoelectric spatial characteristics in a physiological phantom.

Spatiotemporal characteristics of the electric field generated by simultaneous application of a focal acoustic pressure field $P(x, y, z)$ at a frequency $f_A = 500$ kHz propagating in the $\hat{z}$ direction and an electric field $\vec{E}(x, y, z)$ at a frequency $f_E = 8$ kHz. Measurement probe scanned across the volume. **a-b**, The applied electric field is parallel to the propagation direction $\hat{z}$ of the applied acoustic field. **a**, Spatial distribution of the measured electric potential V_{AE} (normalized to max value) in the radial plane $\hat{x}\hat{y}$ at the black dashed line in **(b)** shown at different phases θ of the acoustic period ($1/f_A$), together with the simulated distribution. **b**, Spatial distribution of the measured electric potential V_{AE} in the axial plane $\hat{x}\hat{z}$. **c-d**, The applied electric field is perpendicular to the propagation direction $\hat{z}$ of the applied acoustic field, shown as in **a-b**. **e**, Line plot comparing the measured electric potential V_{AE} normalized to max value along the black dashed lines in **(b)** and **(d)**. **f**, Acoustoelectric coupling factor at the sum frequency Σf , and the difference frequency Δf , shown values are mean $\pm$ st.d.; *** indicates $p < 10e^{-20}$; significance was assessed using paired t-test; $n=50$ measurements. **g**, Spatial distribution of the measured electric potential V_{AE} at the difference frequency Δf and sum frequency Σf and their superposition (normalized to max value) in the radial plane $\hat{x}\hat{y}$ at different phases θ of the acoustic period ($1/f_A$).

After confirming that the acoustoelectric effect multiplies the acoustic and electric fields yielding an electric field with components that oscillate at the fields' sum and difference frequencies, we explored the spatiotemporal characteristics of the generated electric field by repeating the electric field measurements across the acoustic field region to build a time-synced two-dimensional distribution. We found that when we applied the electric field parallel with the direction of the acoustic field propagation the generated electric field had a concentric ring dipole in the radial direction (**Fig 4-5 a**) and a periodic linear multipole in the axial direction (**Fig 4-5 b**), as was predicted by our computational modelling. Shaping the topology of an electric field with an acoustic field, is potentially a way to create an electrostatic lens^{123,124}.

When we applied the electric field perpendicular to the direction of acoustic field propagation, the concentric ring geometry in the radial direction changed to a linear dipole (**Fig 4-5 c-d**), revealing an orientation dependent field distribution as was predicted in our computational modelling. **Fig 4-5 e** shows a comparison of the normalized field amplitude profile along a radial axis (see **Chapter 2 Fig 2.6a** for corresponding computation). The ratio between peak amplitudes when comparing the parallel and the perpendicular orientation was 4.12 ± 0.36 (mean $\pm$ st.d.; $n=50$ measurements; **Fig 4-5 f**), also predicted by our computational modelling (see **Chapter 2 Fig 2.6 b**). Thus, we have experimentally validated the new orientation dependent derivation. The generated electric field distribution was a superposition of the components oscillating at the sum and difference frequencies, resulting in an envelope minimum (as measured at the local waveform maxima) when the two sine wave components were in antiphase relationship and a maximum when they were in phase (**Fig 4-5g**). This reveals the amplitude of the acoustoelectric interaction is dependent on this frequency mixing time evolution.

The amplitude of the generated electric field was proportional to the applied acoustic and electric fields. When the acoustic field was applied with a peak amplitude of 1 MPa, the ratio between the generated and applied electric fields was 1 to 250 when the maximal value was taken across the 0.1 second recording span, corresponding to an acoustoelectric field coupling factor c_{ae} of approximately 2000 pPa⁻¹, i.e., much larger

than the simulation prediction of 110 pPa^{-1} . For example, an application of an acoustic field with a peak amplitude of 1 MPa and an electric field with an amplitude of 250 V/m generated an electric field that oscillates at the sum and difference frequencies with a peak amplitude of 1 V/m . The higher coupling factor can be attributed to a change in the ionic mobility, which would affect the constant k since the adiabatic compressibility at 20°C in water is well established at 1 MPa to be $4.6 \times 10^{-10} \text{ Pa}^{-1}$ ¹²⁵, and all the other parts of the equation are experimentally verified. However, a larger effect than simulation predicts is welcome for the purposes of acoustoelectric neuromodulation.

Measurement techniques:

2D field image reconstruction. After the filtered acoustoelectric data was compiled into a large three-dimensional array (X,Y, time), a 2D low-pass image filter was applied with Gaussian Convolution kernel of 5 pixels to smooth the resulting images. Since the pixel resolution was limited by the 0.5 mm increment resolution of the delta printer we used for scanning, the averaging filter (particularly in the $\hat{x}\hat{z}$ compiled data) would bias the minima and maxima to zero as it averaged over the periodic dipole (500 kHz ultrasound wavelength $\approx 3 \text{ mm}$, of similar width to the convolution kernel), so that we then rescaled the filtered image by the minima and maxima range of the unfiltered image to obtain an accurate representation of amplitude, whilst also denoising the resultant 2D measurement image.

Fig. 4-5 a-b. For the spatial 2D maps, electrodes were placed as in **Fig 4-1**. For the 2-dimensional mapping, a recording was made for each pixel. For each recording, the electric field was ramped at the beginning and end to avoid voltage spike transients in the preamplifier. The previously described high pass filter was used as an 8 kHz applied electric field was applied. A half-amplitude, two wavelength duration acoustic signal was applied at the beginning of each recording, and that marker was used to time synchronize the measurement data. After applying digital filtering to isolate the sum and difference frequencies in the recorded electric signals, they were combined into a single three-dimensional array (one time and two spatial dimensions). The reconstructed $\phi_{ae}(t)$ was used to visualize the time evolution ($\hat{x}\hat{y}$ and $\hat{x}\hat{z}$ views). A viewing tool is provided along

with the code and data in the online repository to scroll through the reconstructed transient $\hat{x}\hat{y}$ and $\hat{x}\hat{z}$ views⁸⁵.

Fig 4-5 c-d. All parameters were the same as in **Fig. 4-5 a-b**, and all electrodes were located as in **Fig. 4-5**, except for the reference location, which was moved closer and into the null area shown in the simulation prediction. This produced a clearer spatial map, potentially due to minimized edge diffraction/reflection interferences with the smaller amplitude acoustoelectric signals.

4.2.4 Acoustoelectric neuromodulation feasibility

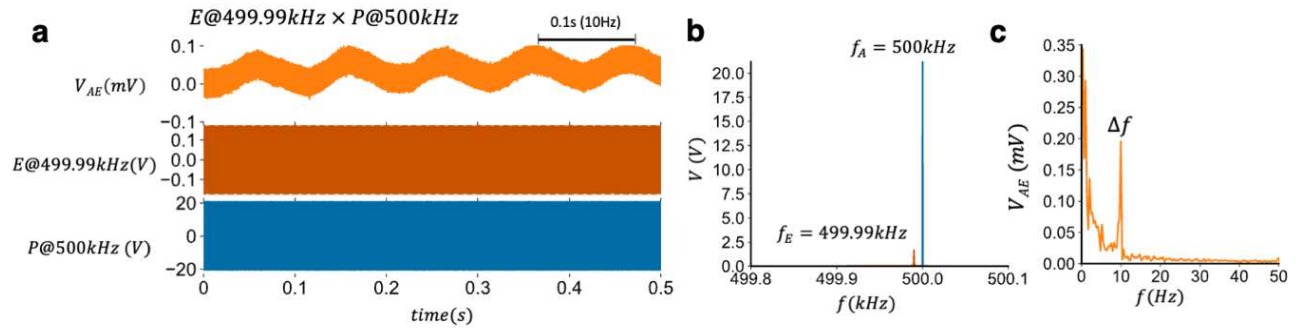


Figure 4-6 | Acoustoelectric neuromodulation feasibility.

a, (i) Same as (4-4 a-c) but for an electric field at $f_E=499.99$ kHz (i.e., $f_E \approx f_A$). V_{AE} was filtered before logging (low-pass, -3dB cut-off frequency 5 kHz) to remove the high frequency electric field, which also removes the sum frequency. An oscillation at the difference frequency $\Delta f=f_A-f_E=10$ Hz is evident. **b**, Amplitude spectral density (ASD) of the signal traces showing the applied V_A and V_E traces, **c**, ASD computed from V_{AE} trace in (a).

The feasibility of acoustoelectric neuromodulation was tested by applying an acoustic and electric field with a small difference frequency of 10Hz (**Fig 4-6 a**), which generated an electric field that oscillates at a low frequency within the electrophysiological spectrum^{126,127}, whilst the applied high frequency field is outside the electrophysiological relevant range¹²⁸, showing how electric fields can be encoded at depth. A low-pass filter (5kHz -3dB cut-off frequency) was incorporated inline such that the high frequency (499.99kHz) electric field was removed before the signal enters the preamplifier, so that the low frequency signal can be measured at sampling rate 5MHz. The design for the differential low-pass filter is documented and characterized in **Chapter 3**. In an *in vivo*

neuromodulation translation experiment, the pressure and electric field could be adjusted independently until a neuromodulation threshold was reached.

4.2.5 Acoustoelectric neural recording feasibility

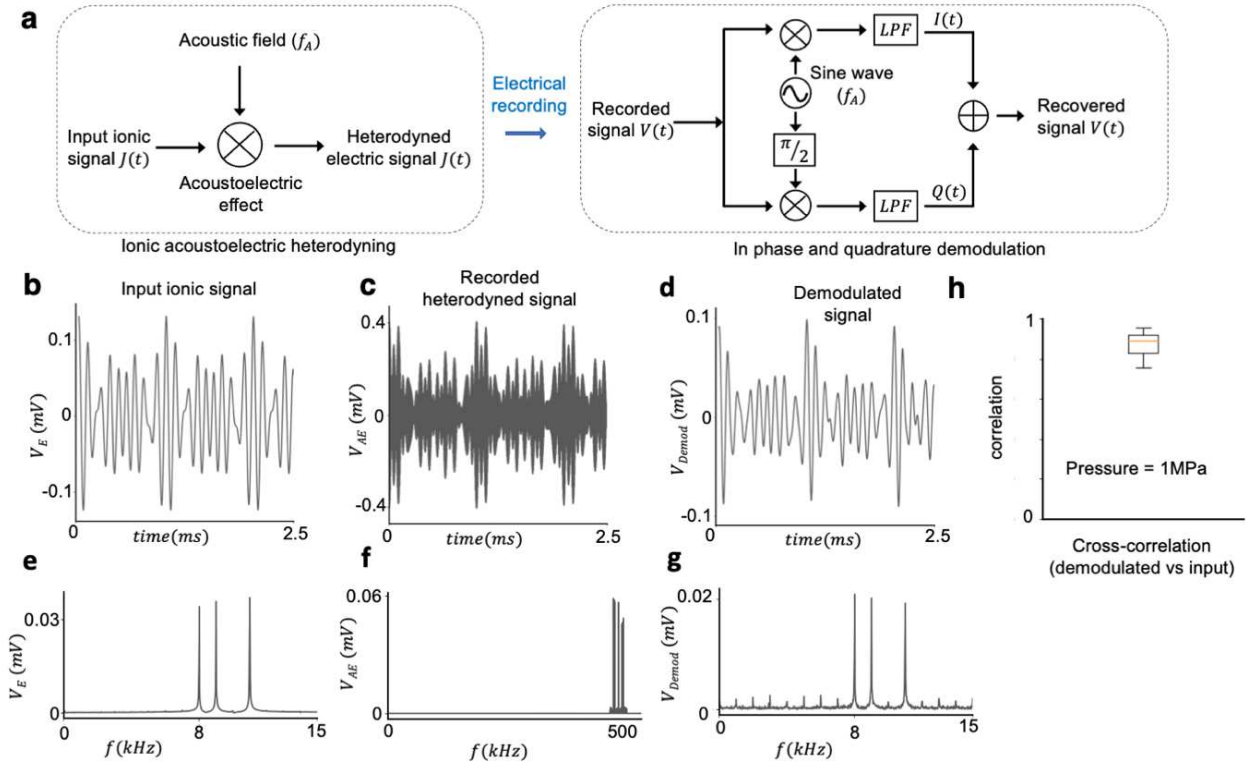


Figure 4-7 | Acoustoelectric neural recording feasibility.

a, Schematic of the acoustoelectric heterodyning and demodulation procedure. An ionic current signal in the target location is focally mixed with a sinusoidal acoustic signal via the acoustoelectric effect to generate an electric signal with the original information but now modulated up to the acoustic frequency f_A . The electric potential of the modulated signal is recorded and computationally demodulated to recover the original signal via in-phase and quadrature (IQ) demodulation. In IQ demodulation, the signal is multiplied by two sinusoidal waveforms at the known frequency f_A and with $\pi/2$ phase difference. The resultant signals are low pass filtered (LPF) to yield the $I(t)$ signal and the $Q(t)$ phase shifted signal from which the instantaneous amplitude and phase of the demodulated signal are recovered. **b-g**, Representative traces of the ionic acoustoelectric heterodyning and demodulation, shown are **b**, input ionic signal, **c**, recorded potential of the acoustically heterodyned electric signal, high-pass filtered in software to remove the applied ionic signal, **d**, computationally demodulated signal, **e**, amplitude spectrum density (ASD) of the signal in (b), **f**, ASD of the signal in (c), **g**, ASD of the signal in (d), the small extra peaks are caused by intermodulation – where the sum and difference frequencies interfere with the original applied fields¹²⁹. **h**, Cross-correlation between the demodulated signal and the input signal; $n=10$ measurements.

After confirming that the acoustoelectric effect multiplies the acoustic and electric fields, we explored whether this principle could be utilized to remotely and focally heterodyne electric signals in liquid electrolytes and detect them without losing information, in the same way amplitude modulated (AM) radio transmission operates¹³⁰. Shifting a low frequency electric signal to a higher one, provides powerful signal-to-noise advantages in noisy environments such as the body, as signals at focal spatial locations of interest can be isolated via frequency modulation¹³¹ from the surrounding neural signals. We applied a complex electric signal (consisting of a superposition of 8 kHz, 9 kHz, and 11 kHz sine waves) to the acoustic field focus and recorded the acoustoelectrically generated electric potentials as before. To recover the original signal from the generated higher frequency potential, we deployed an in-phase and quadrature (IQ) demodulation strategy^{132,133}. See **Fig 4-7a** for a schematic of the acoustoelectric heterodyning and demodulation procedure.

For the IQ demodulation signal recovery (**Fig 4-7**), a hardware high-pass filter (3rd order differential Butterworth 100 kHz -3dB cut-off) was applied as before to ensure we completely removed the original low-frequency signal so that it did not enter the preamp. We performed IQ demodulation on this recorded signal. This hardware filter innately introduced a time lag between the complex ionic input signal and the recorded signal, which we overcame with rolling cross-correlation to find the optimal phase offset, as well as the maximal point of correlation using Pearson's correlation coefficient^{134,135}, as it is not amplitude dependent when using normalized inputs. This coefficient was reported over a range of recordings with mean and standard deviation.

IQ demodulation was carried out on a heterodyned recorded signal (8 + 9 + 11kHz sine wave). To demodulate the recorded signal, we multiply it by a simulated 500kHz sine wave carrier, and a 90° phase offset signal. We add these together to return a demodulated signal. To ensure we reduce spurious harmonics¹³⁶ we then low-pass filter the result using a 17th-order Chebyshev II digital lowpass filter with 20kHz cut-off frequency.

Fig 4-7 b-d shows representative traces of the input ionic current signal, the acoustoelectric heterodyned signal, and the recovered demodulated signal, with **Fig 4-7e-g** showing their respective spectral components. We found that the original electric signals can be accurately recovered from the acoustoelectrically generated electric fields (**Fig 4-7h**). The cross-correlation between the recovered and input signals was 0.87 ± 0.063 (mean $\pm$ std.; $n=10$ measurements).

4.2.6 Temperature dependence in the acoustoelectric interaction.

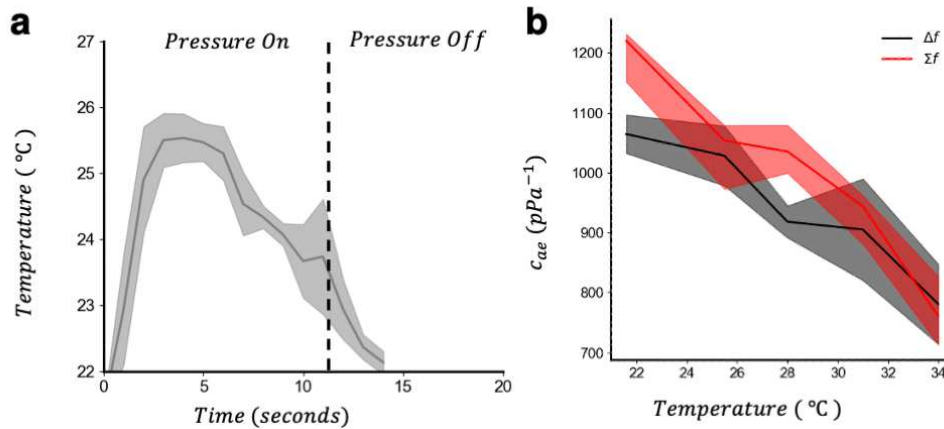


Figure 4-8 | Temperature dependence in the acoustoelectric interaction.

a, Physiological phantom measurements of thermal changes at the ultrasound focus. Temperature dissipation over time at the ultrasound focus, with 10 second continuous pressure wave and maximum focal pressure 1 MPa. **b**, Acoustoelectric coupling factor at focal region vs. the saline temperature as phantom is heated.

A temperature probe is placed at the ultrasound focus and the change in temperature over time is measured when a continuous 1MPa 500kHz continuous ultrasound signal is applied (**Fig 4-8 a**). There is a sharp rise when pressure onset occurs, then thermal dissipation while continuous ultrasound persists for 8 seconds. Comparing the temperature envelope to the difference frequency (**Fig 4-2 c i**), these two quantities show no obvious similarity. Furthermore, the time scale of temperature change develops over seconds compared to the timescale of the emergent acoustoelectric sum (508kHz) and difference (492kHz) frequencies. To discover the effect on the acoustoelectric interaction when the saline is independently heated, we heated the entire 20L tank of saline with an

aquarium heater and saw that the acoustoelectric amplitude and conversion efficiency decreased as temperature increased (**Fig 4-8 b**).

To understand the effect of temperature in terms of the constant in the homogeneous acoustoelectric derivation which should match our homogeneous experiment, we note that temperature affects the ionic mobility (α), a term in the acoustoelectric constant k:

$$v_d = \alpha E$$

Here the drift velocity (v_d) is proportional to the ionic mobility (α) multiplied by electric field. As the temperature (T) rises, ions move faster (v_d increases), causing more collisions such that the ionic mobility under the same electric field will decrease.

$$\alpha \propto \frac{1}{T}$$

4.2.7 Conductivity and the acoustoelectric interaction

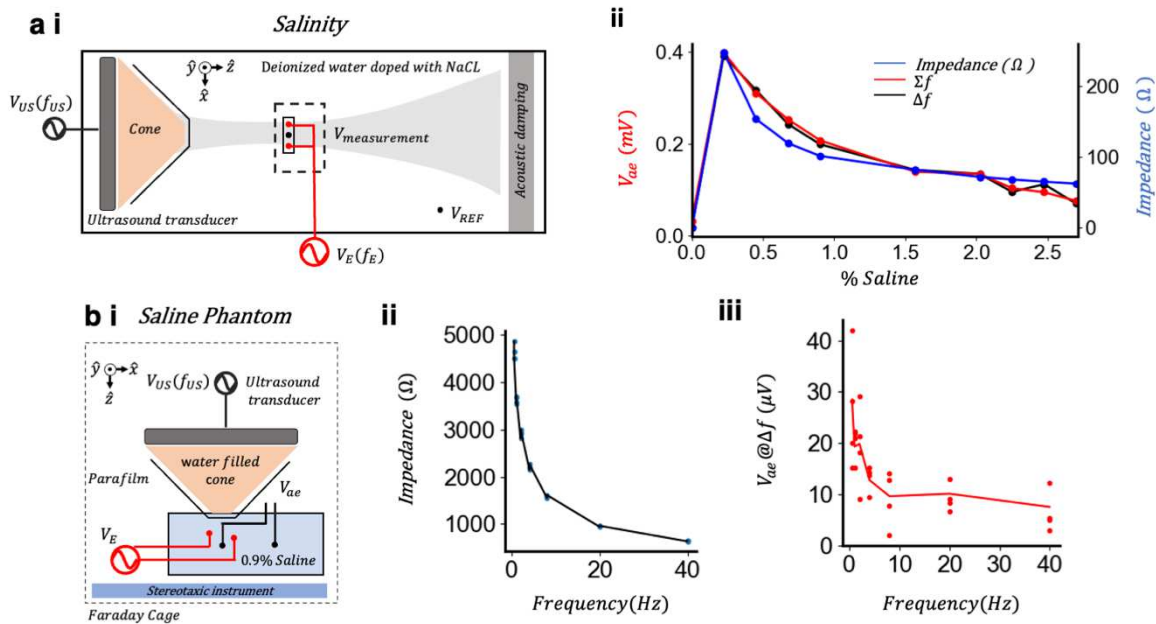


Figure 4-9 | Acoustoelectric dependence on conductivity.

a, i) Experimental arrangement for varying the salinity of de-ionized water in tank phantom. **ii)** Acoustoelectric field amplitude (V_{ae} (mV)) at $\Delta f = 500\text{kHz} - 10\text{kHz}$ with 500kHz acoustic field and 10kHz electric field, versus salinity percentage in de-ionized water, shown alongside impedance versus grams of NaCl in de-ionized water calculated by applying a 10kHz 24V peak-peak electric field between two

electrodes 7mm apart and recording V and I using the impedance adapter described in Chapter 3 **b**, i) Experimental arrangement for testing acoustoelectric difference frequency change with frequency. ii) Phantom test showing impedance versus applied voltage frequency calculated by applying a current across two electrodes spaced 7mm apart and measuring with the impedance adapter described in Chapter 3. iii) Phantom measurement in 0.9% saline, $F_s=5\text{MHz}$, Pressure=1MPa, duration=6 seconds, 4 measurements at each frequency, and low-pass filtered to remove high frequency applied voltage (5V pp) at 500kHz - Δf amplitude from the acoustoelectric interaction decreases with frequency.

To experimentally determine the acoustoelectric relationship with the conductivity of the medium, non-conductive de-ionized water was gradually doped with NaCl, and the impedance measured by transmitting a 10kHz electric field and measuring the voltage, and current using the impedance adapter described in **Chapter 3**, and then applying Ohm's Law ($V=IR$) to recover the impedance. The impedance in saline decreases with increasing salinity (**Fig 4-9 a i ,ii**). An acoustic waveform was then added (1MPa calibrated at the focal point), and the acoustoelectric sum and difference frequency amplitudes measured at each salinity concentration, and it was found that they follow the same curve as the impedance (**Fig 4-9 a ii**). As more ions are added in the form of NaCl, ionic mobility is decreased, and the Van der Waals forces between the ions start to inhibit the acoustoelectric interaction. This relation is important to understand in terms of the acoustoelectric amplitude in higher and lower impedance media, for instance we can now predict that in the higher impedance medium of the mouse brain we should expect a higher acoustoelectric amplitude (White matter conductivity = 0.46S/m, compared to 0.9% saline conductivity of 1.6 S/m).

Electrochemistry is dominated by electrode interface effects, and these are worth considering in this salinity experiment where the impedance changes. Here, the electrodes remained constant while only the salinity was changed. While the higher concentration of ions may interact differently with the electrode interface, this is an effect due to the chemistry of the medium itself, and not just the electrode interface which is constant.

Next, we investigated the acoustoelectric amplitude relationship with the difference frequency at low difference frequencies, by first calculating the impedance spectrum at low frequencies (**Fig 4-9 b i,ii**) by applying a direct low frequency sine wave and

measuring both the current and voltage simultaneously to recover the impedance via Ohm's Law. We then moved to the acoustoelectric paradigm where a 1MPa acoustic field was applied, and a high frequency electric field applied near 500kHz to generate a low frequency difference frequency. We find again that the acoustoelectric amplitude trends with the impedance, noting that at very low frequencies there is very high impedance, where the acoustoelectric interaction yields the highest amplitudes (**Fig 4-9 b iii**). This relationship could possibly be exploited to yield larger mixing amplitudes for acoustoelectric neuromodulation.

4.3 Discussion

In this Chapter we show experimentally for the first time that the acoustoelectric phenomenon involves frequency mixing such that the sum and difference frequencies are created in the resultant electric field. The acoustoelectrically generated electric field has a unique spatial distribution that depends on the relative fields' orientation, varying from periodic concentric rings to a dipole source. We demonstrate how these unique properties can be utilised to encode and decode complex electric signals remotely and focally.

Firstly, some very basic questions on the acoustoelectric interaction were investigated, such as whether it truly exists, and if there could possibly be another artefactual explanation for its reported measurement. The theoretical predictions on directional dependence between the two fields are then verified, validating the new vector-based derivation, as well as clear evidence that the interaction is indeed based on a heterodyning interaction producing clear sum and difference frequencies.

Acoustoelectric neuromodulation is simulated in the phantom by applying a high frequency electric field and acoustic field with a small difference frequency between them and seeing a low frequency signal in the electrophysiological range emerge at amplitudes known to be effective at electrical stimulation. Acoustoelectric neural recording was performed by inserting an electrical signal made up of a superposition of three sine waves at the focus of the ultrasound and measuring the acoustoelectrically up modulated signal around the carrier. The original signal was then successfully recovered through IQ

demodulation. Both acoustoelectric neuromodulation and neural recording were shown to be viable neural interfacing techniques within the limitations of the saline phantom.

Lastly, we investigate the effect of temperature and conductivity on the acoustoelectric interaction, showing that increasing the temperature decreases the amplitude of the acoustoelectric mixing products. Increasing the conductivity by changing the salinity of the medium decreases the acoustoelectric magnitudes, showing that the acoustoelectric interaction is dependent on the impedance of the medium. Higher impedances lead to higher acoustoelectric magnitudes, and this extends to frequency such that low frequency acoustoelectric mixing products are far larger than higher frequency mixing products, suggesting that low frequency acoustoelectric neuromodulation will have the greatest amplitudes, ideal for translation into a low frequency electrophysiological stimulation paradigm in high impedance mouse brains.

In summary, in this Chapter we have shown how the spatial precision of ultrasound can enable focal heterodyning of electric fields. Given the ubiquitous use of signal heterodyning in solid-state electronics, these findings could open frontiers in remote electrochemical, bioelectronics and medicine, with the potential for developing devices that are less invasive or completely non-invasive.

Chapter 5: Acoustoelectric neural recording

5.1 Introduction

While we have tools to measure diffuse cortical electrical activity through the skull (EEG) or blood flow throughout the brain (fMRI), we are unable to measure electrical neural activity with high spatial specificity or in deep brain regions. If we could measure neural signals with high spatial specificity throughout the brain, our ability to diagnose neural disorders and understanding of the brain would be transformed. By harnessing the heterodyne characteristics of the acoustoelectric interaction, we present a technique to measure electrical signals in a rodent brain, limited by the focusing ability of ultrasound.

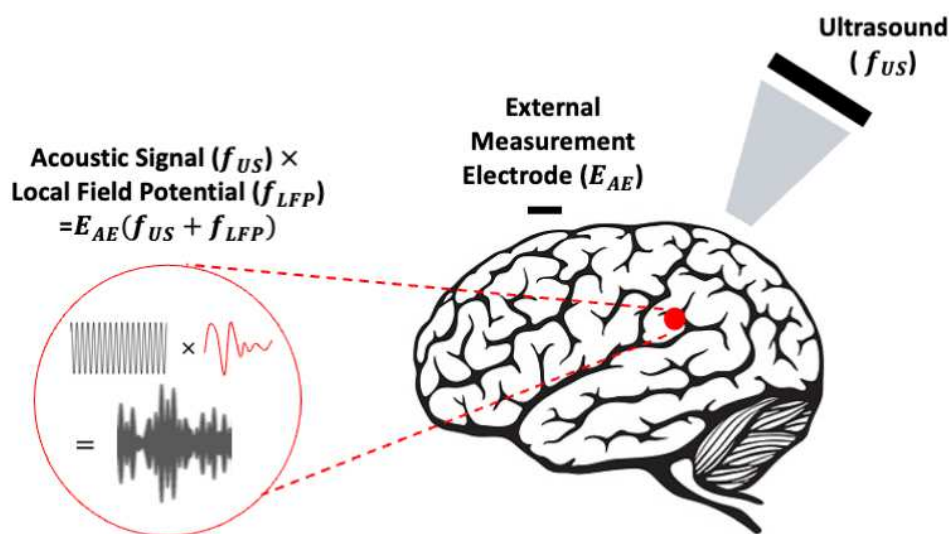


Figure 5-1 | Acoustoelectric neural recording concept.

Acoustoelectric neural recording - A high frequency electric field mixes with a highly focal acoustic signal. The sum and difference frequencies appear around the acoustic carrier signal through acoustoelectric heterodyning, where they can be recovered using amplitude demodulation.

Acoustoelectric neural recording multiplies the low frequency electrophysiological signals at the ultrasound focus by the acoustic carrier frequency via the acoustoelectric

interaction¹⁰². This results in a modulated electric signal near the carrier frequency of the ultrasound which can be recovered with traditional amplitude demodulation techniques used in radio communications (**Fig 5-1**). This is advantageous for detecting electric fields with high spatial specificity as ultrasound has improved focusing ability over electric fields, utilizing established beam forming¹⁴⁶ and tomographic focusing methods¹⁴⁷.

In the work presented in this Chapter, we investigate the previously published work on ultrasound current source density imaging by the Ultrasound and Neural Imaging Laboratory (EUNIL) in Arizona^{58,82,148–152}, revealing its mechanism to be acoustoelectric heterodyning. This gives insight into how to optimize signal recovery as more narrowband techniques can be utilized. With this knowledge, we can potentially attempt demodulating smaller amplitude signals than^{58,82,148–152} *ex vivo* ECG measurements.

We then perform both phantom and *in vivo* experiments recreating Tianjin Universities reported results^{64,66,153,154} in acoustoelectric brain imaging (AEBI), finding an alternate possible explanation for them unless an additional artefact test is added. Through repeating their technique we can recreate the reported results recovering the visual evoked potential with or without ultrasound, indicating there is an alternative mechanism at the low frequencies this test was performed at that does not depend on the acoustoelectric interaction. Through a series of tests demodulating the VEP alone, we find that the VEP harmonics can be demodulated to recover the low frequency signal, providing an interesting alternative explanation. This source of artefact can be overcome by performing a spectral specificity test, ensuring that the specific sum and difference frequencies of the expected neural signal rise above the noise floor.

Through *in vivo* rodent experiments, we then show acoustoelectric neural recording to work for the first time, by recovering the time averaged visual evoked potential successfully via Hilbert demodulation, repeating for two different frequency visual evoked potentials, and performing an accompanying spectral specificity test with statistically significant results. While visual evoked potentials are usually averaged to enable a better view of the underlying neural signal, we then show we can demodulate a single trial

through acoustoelectric demodulation and compute an accurate correlation with the original VEP in portions of the recorded signal. This is significant as it indicates this technique has the potential to be used in the real-time recovery of spatially specific neural signals. The variance in correlation accuracy throughout the trial may be attributed to the varying acoustic wave interference in the mouse brain, induced by reflections which we model through heterogeneous simulation of a mouse head surrounded by air with an ultrasound transducer at 500kHz to match experimental parameters. Selecting both a transducer frequency and mammal skull size to reduce reflections, could overcome these issues.

To further prove the results are not confounded, we perform a VEP shield test to ensure the VEP is not due to electrical artefacts induced by the LED, an acoustic isolation test where we show that the electrical artefact from the transducer is not responsible for the modulation, and a two-tone test providing evidence the modulation is not occurring in the cables or preamplifier front end.

The conversion between the neural signal and the acoustoelectric signal has a ratio of approximately 1:200, making the acoustoelectric signal very small, and is the limiting factor in the translational potential of this technique. To improve the amplitude and efficiency of the acoustoelectric modulation, we propose various improvements including increasing the dynamic range of the preamplifier by blocking the electric artefact from the ultrasound transducer, increasing the surface area of the measurement electrodes, or quantifying how high frequency electric fields attenuate less than low frequency electric fields, enabling them to travel through the skull with fewer losses.

This Chapter is focused on acoustoelectric neural recording proof-of-concept and is not a safety optimization, hence further translational studies should be done to meet the established safety standard for diagnostic ultrasound imaging. Acoustoelectric neural recording is a promising new route to spatially specific electrophysiological measurements, and this Chapter provides the first *in vivo* evidence of its efficacy.

5.2 Methods

5.2.1 Acoustoelectric neural recording instrumentation

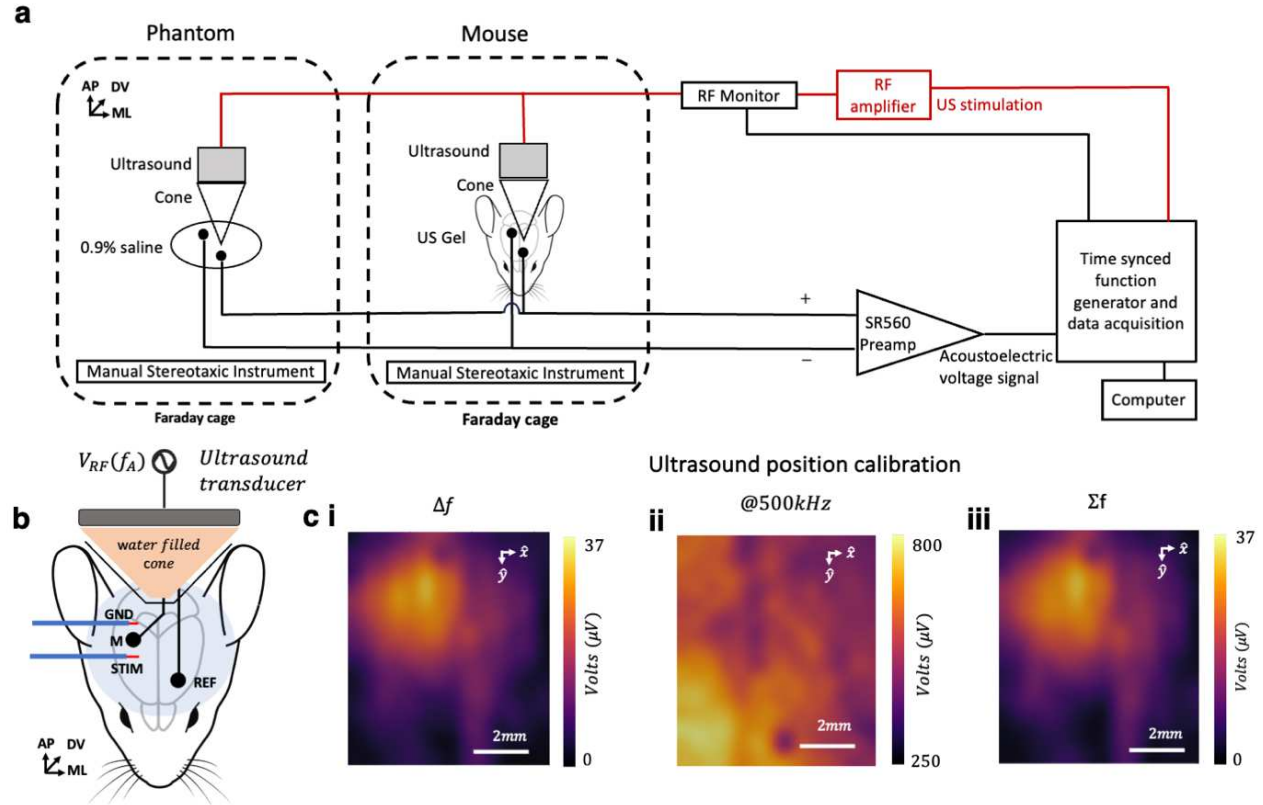


Figure 5-2 | Acoustoelectric instrumentation and position calibration.

a, Acoustoelectric neuromodulation instrumentation for both phantom and mouse experiments, measurement pathway shown in black and stimulation shown in red. **b**, Stimulation and measurement arrangement for position calibration in a mouse to target the visual cortex. **c**, Spatial calibration of stimulation electrode located at visual cortex with 1MPa 500kHz acoustic wave, and 1V output at 8kHz electrical signal on stimulation electrodes directly from function generator, using manual stereotaxic movement to recreate spatial image. i) Δf amplitudes over a 1cm square showing maxima at focus location. ii) Carrier frequency amplitude over same space has no focality. iii) Σf amplitudes over a 1cm square showing maxima at focus location.

Data acquisition. All applied signals were generated at 5MHz, and monitored at 2MHz, time synced using a CMI interface between the function generators and oscilloscopes with trigger inputs. The applied voltages, monitor channels and electric potentials were logged using data acquisition hardware (WiFiScope WS5, WiFiScope WS6 DIFF and Handyscope HS5, TiePie engineering, Netherlands). The logged signals were streamed from the data acquisition hardware to a workstation PC using custom C code which

utilized the TiePie SDK to control the triggers, channels, and function generator output. The compiled C code which called the oscilloscope commands was in turn called within a Python wrapper.

Electrophysiology brain recordings. The electrophysiological brain recordings were made by connecting a preamplifier (SR560, Stanford Research Systems) to the implant in the mouse brain.

Phantom. The measurements of acoustoelectric modulation of the applied fields were done in a small plastic petri dish (4cm diameter and 2cm depth) filled with 0.9% saline located where the mouse would otherwise be.

Mouse. The measurements of acoustoelectric neuromodulation were made according to the experimental procedure outlined in **Section 5.2.5**.

Pressure recordings. The acoustic field was measured using a 0.2 mm needle hydrophone (52mV/kPa at 500 kHz calibration; Precision Acoustics Ltd) and a DC-coupled preamplifier (Precision Acoustics Ltd).

Acoustic field application. The acoustic field was applied using a curved ceramic (PZT) 500 kHz ultrasound transducer (60 mm diameter, 63.5 mm acoustic path length; Precision Acoustics Ltd, UK). The ultrasound transducer was driven by an arbitrary function generator (Handyscope HS5, TiePie engineering, Netherlands) and a 40 W linear power amplifier (240L, Electronics & Innovation Ltd).

Electric fields application. The electric field described in phantom simulations was applied using a pair of 98% platinum iridium wire electrodes (0.25 mm diameter, Alfa Aesar). The electrodes were driven by a second arbitrary function generator (Wi-FiScope WS5, TiePie engineering, Netherlands).

5.2.2 Acoustoelectric method for calibrating ultrasound focal location

Position calibration was achieved via acoustoelectric frequency mixing at the contralateral electrode to the side of LED stimulation with visual cortex coordinates AP -3.5mm, ML 2.25mm. An 8kHz 1V amplitude peak-to-peak electrical sinusoid was applied at this electrode location and the 500kHz ultrasound focus was moved in 0.5mm increments over the head (or through 0.9% saline in the case of phantom testing) (**Fig 5-2 b**). The acoustoelectric mixing amplitudes reached a maximum when the ultrasound focus was positioned over the voltage stimulation source (**Fig 5-2 ci, iii**). In the saline phantom, detailed calibration maps could be made like the ones shown; however when calibrating in a mouse a simpler and faster linear traversal crosshair method was undertaken to find the focus so that fewer points were needed to localize the focal point as time was limited while the mouse was under anaesthetic sedation.

The carrier frequency at 500kHz (**Fig 5-2 c ii**) remained uncorrelated to the 8kHz electric field source, suggesting its origin was due to the electrical artefact from the transducer and not the acoustoelectric interaction which follows the shape of the ultrasound focal area.

5.2.3 Acoustic and acoustoelectric cone characterization

Before moving to the animal facility, an acoustoelectric study was undertaken in a homogeneous saline tank phantom with the ultrasound cone covered with stretched parafilm to understand the resulting focality, and acoustoelectric amplitudes of the resultant fields in the pure homogeneous environment without the reflective aspects of the mouse/air interface.

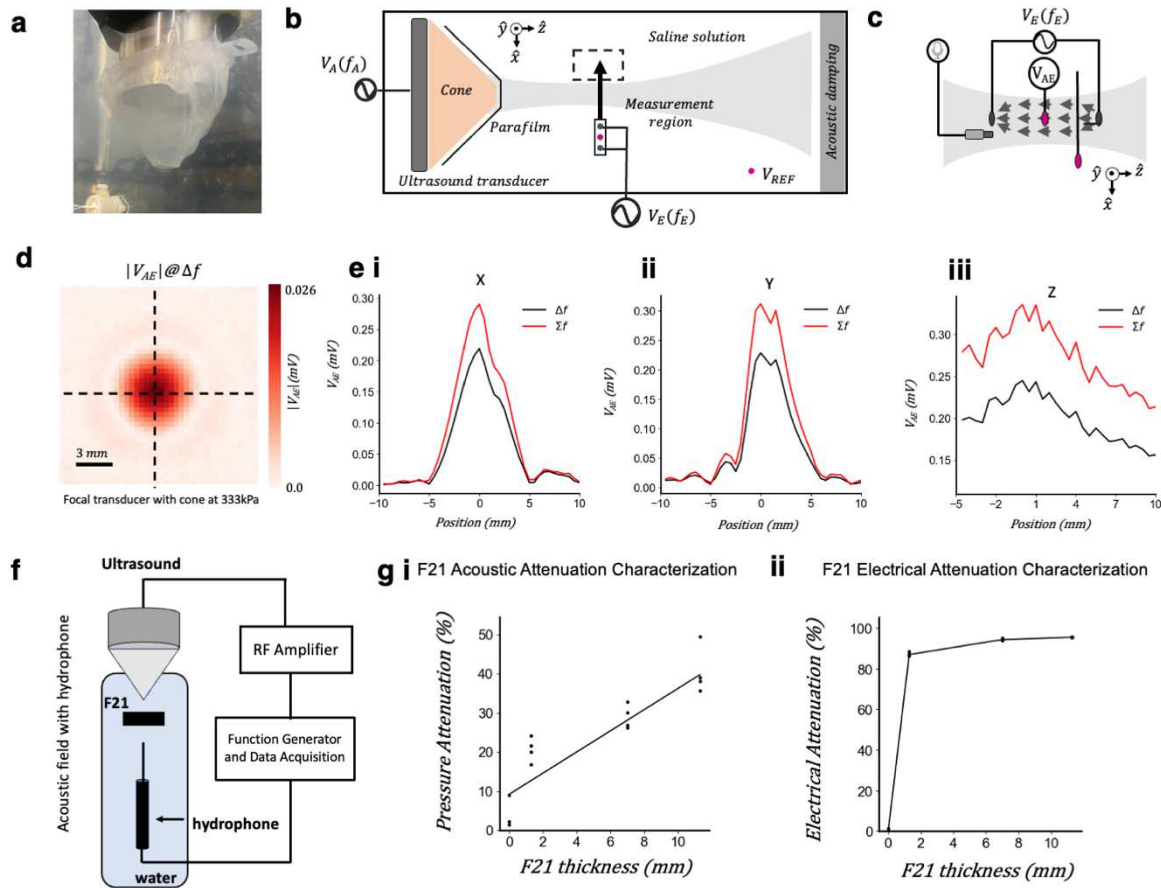


Figure 5-3 | Acoustic and acoustoelectric cone characterization.

a, 500kHz ultrasound transducer with cone, covered in parafilm. **b**, Phantom tank for XYZ scanning of electric fields generated through the acoustic focal area. **c**, Hydrophone and electrical measurement electrodes for measuring acoustoelectric effect. **d**, XY spatial distribution map of acoustoelectric difference frequency generated with 500kHz at 333kPa, and 20V peak-peak 8kHz signal on voltage electrodes aligned with the ultrasound. 0.1s duration at 5MHz sampling rate, for each pixel. Data band filtered around difference frequency of 492kHz. **e**, Similar parameters to d, except showing X, Y and Z axis sum (508kHz) and difference frequencies and their distribution for each axis. **f**, Hydrophone and transducer arrangement for F21 characterization. **g**, i) F21 material acoustic attenuation versus thickness. 8 second duration recordings, 5MHz sampling rate repeated 4 times at each F21 thickness. Regression line: Attenuation (%) = 2.7 * thickness (mm) + 9.2 ii) F21 material electrical attenuation versus thickness. Regression line: Attenuation (%) = 6 * F21 thickness (mm) + 3.5.

5.2.4 Recovery surgery for acoustoelectric neural recording.

Described in Chapter 3, as the techniques pertain to both Chapter 5 and Chapter 6.

5.2.5 Experimental procedure for *in vivo* acoustoelectric neural recording

Anaesthesia of mice was achieved at a constant 0.5% (vol/vol) Isoflurane in Oxygen. Eye lubricant (Vaseline) was placed on the eyes to prevent them from drying out. The anesthetized mouse was placed onto a thermal mat (Thermostar, RWD, China) to maintain homeostasis and head clamp (Neurotar standard clamp, Finland) to enable precise stereotaxic localization of coordinates. The electrode implant was connected to the SR560 preamplifier (Stanford Research Systems, USA) via a 1.27mm header at the back of the head.

The ultrasound transducer was mounted into a pre-characterized (**Fig 5-3**) water filled cone. Parafilm was stretched over the end of the cone to seal the water in, while the transducer was positioned via stereotaxic instrument above the mouse head covered in ultrasound gel (Anagel, UK) providing an acoustic connection between the cone and the top of the mouse skull.

Ultrasound was then applied in 30 second trials at 1MPa, while an LED was flashed in the eye contralateral to the visual cortex electrode implant. The experiment was continued for an hour. After this time, despite the low anaesthesia level we found the VEP amplitude started to decrease so the experiment was stopped, and the mouse recovered so that it could undergo testing another day. After recovering in the warming chamber, the mouse was given water saturated food pellets and returned to its home cage.

5.3 Results

5.3.1 Acoustoelectric heterodyning is the mechanism behind ultrasound current source density imaging

Ultrasound current source density imaging (UCSDI) has been reported *ex vivo*^{151,155} and in physiological phantoms⁸² without a clear understanding of the underlying mechanism, limiting the technique's reach to larger electrophysiological signals such as *ex vivo* ECG¹⁵¹. Due to the acoustoelectric heterodyning discovery¹⁰² (described in **Chapter 4**), it is worth revisiting the published literature, to determine if we can clarify how UCSDI works and determine how to improve the signal recovery process.

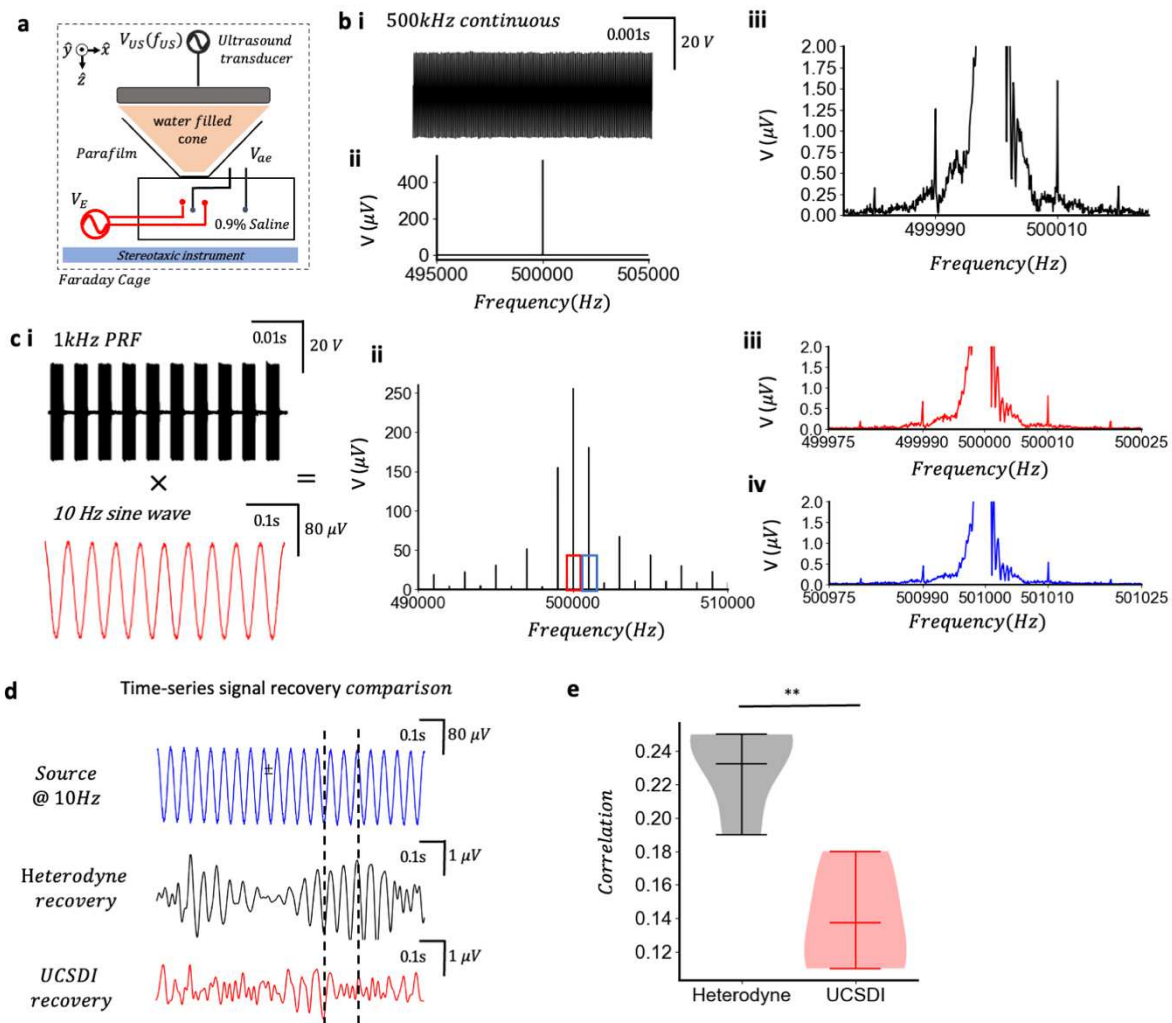


Figure 5-4 | Advantages of acoustoelectric heterodyning in signal recovery

a, Saline phantom with ultrasound cone positioned above 0.9% saline in petri dish, $80\mu V$ 10Hz sinusoid is applied separately by a voltage source (RED), and two platinum-iridium electrodes detect electric field in solution, sampling rate 5MHz, pressure output 1MPa in all recordings. **b**, i) continuous 500kHz 1MPa waveform monitored from output of RF amplifier. ii) Amplitude spectral density plot of electrical artefact from transducer measured in saline. iii) zoom view of mixing products at sum and difference frequency of the 10Hz and 500kHz fields. **c**, i) typical ultrasound current source density imaging waveform with 1kHz pulse repetition frequency (PRF) of 500kHz square wave, showing original acoustic waveform and 10Hz sine wave ii) amplitude spectral density (ASD) of the recorded signal showing how the amplitude of the electrical artefact from the transducer dominates. iii) zoom view of ii) showing the sum and difference frequency of the 10Hz multiplication around the 500kHz carrier iv) zoom view of ii) at 501000Hz showing sum and difference around the secondary harmonic of the PRF wave. **d**, comparison between the original source signal and the two methods to recover the source through demodulation. **e**, Group correlation analysis using 4, 12 second recordings with 1 second correlation windows shows T-Test $p = 0.005$, with Heterodyne signal recovery correlation mean $\pm$ st.d = 0.23 ± 0.02 , and UCSDI PRF signal recovery correlation mean $\pm$ st.d = 0.13 ± 0.02 .

First, we calibrated the position of the ultrasound transducer over platinum-iridium electrodes as described in **Methods 5.2.2** and injected a voltage at $80\mu V$ peak-to-peak in amplitude between the two measurement electrodes which detected both the applied source signal, using a gain of 5000 when a 1MPa acoustic field was applied. A continuous waveform (**Fig 5-5 b i, ii**) was used to confirm the acoustoelectric heterodyne interaction in the petri dish with frequency mixing products shown in **Fig 5-5 b iii**.

A typical signal application strategy in UCSDI is to pulse a square wave ultrasound signal at 500kHz with a pulse repetition frequency at 1kHz^{62} , while generating a low frequency signal in the electrophysiological range (e.g. 10Hz) which was at the same amplitude as our continuous wave experiment (**Fig 5-5 c**). The UCSDI signal recovery is then calculated as the broadband envelope of the measured electric signal between 0.3-0.6MHz, which may also include any electric artefacts from the ultrasound which dominate the recorded signal (**Fig 5-5 c ii**), making it particularly susceptible to electrical noise. Other strategies used to date include chirping the ultrasound pulse to induce an even more spectrally broadband signal¹⁵⁶ but at higher instantaneous amplitudes, with all these technique variants originating from the EUNIL lab in Arizona. By applying the PRF waveform, we noted sum and difference frequency products induced around the broadband peaks of the pulsed signal (**Fig 5-5 c iii, iv**), revealing acoustoelectric heterodyning is in fact taking place. While the heterodyned sum and difference frequency peaks are present, they are smaller in amplitude than when a single continuous wave is

used and decrease in amplitude as the sinc function of the square PRF signal moves away from the carrier signal.

We then proceeded to recover the UCSDI signal using the broadband method reported, filtering between 0.3-0.6MHz, and taking the Hilbert transform of the resulting signal. We then band-pass filtered this signal between 5-40Hz to see the resulting demodulated signal. We performed this same procedure on the continuous wave data, except with a narrower filter band so that we only incorporated the sum and differences frequencies (1kHz around the singular central 500kHz carrier). Comparing the time-series recovered signals to the original applied source signal (**Fig 5-5 d**), the result of acoustoelectric heterodyne narrowband signal recovery is both larger in amplitude and has more clearly aligned peaks to the original source signal than the UCSDI method. As we are not blocking reflections in the petri-dish it is also likely that some phase shifts are due to these reflections from the bottom of the dish.

Moving to a correlation analysis (**Fig 5-5 e**) between the two signal recovery methods, and using a 1 second correlation period across 4 12 second recordings in each group, we see a highly significant difference between the continuous wave and UCSDI waveform ($p = 0.005$), where the correlation is always higher in the continuous wave group where all of the modulation energy is distributed into the minimal number of spectral bins compared with the PRF method which spreads the modulation over a broadband space. Since the energy is now narrow band, electrical noise is greatly reduced and the transducer artefacts can be filtered out, enabling both the frequency and phase of the modulated signal to be recovered.

This comparison study shows the mechanism behind previous UCSDI studies to be acoustoelectric heterodyning, and the broadband acoustic input signal strategy previously used to be sub-optimal for signal recovery as it also includes electrical artefacts. The continuous wave signal recovery method optimizes the modulation energy into a known narrow band enhancing correlation in the recovered signal, as compared to the previously reported broadband signal recovery methods. However, future advantages might be

gained by combining the pulsed method to reduce energy dissipation in the tissue to meet safety requirements for thermal dissipation, whilst utilizing an algorithm that optimizes specifically for narrowband heterodyne products.

Acoustoelectric brain imaging and VEP harmonic confounds

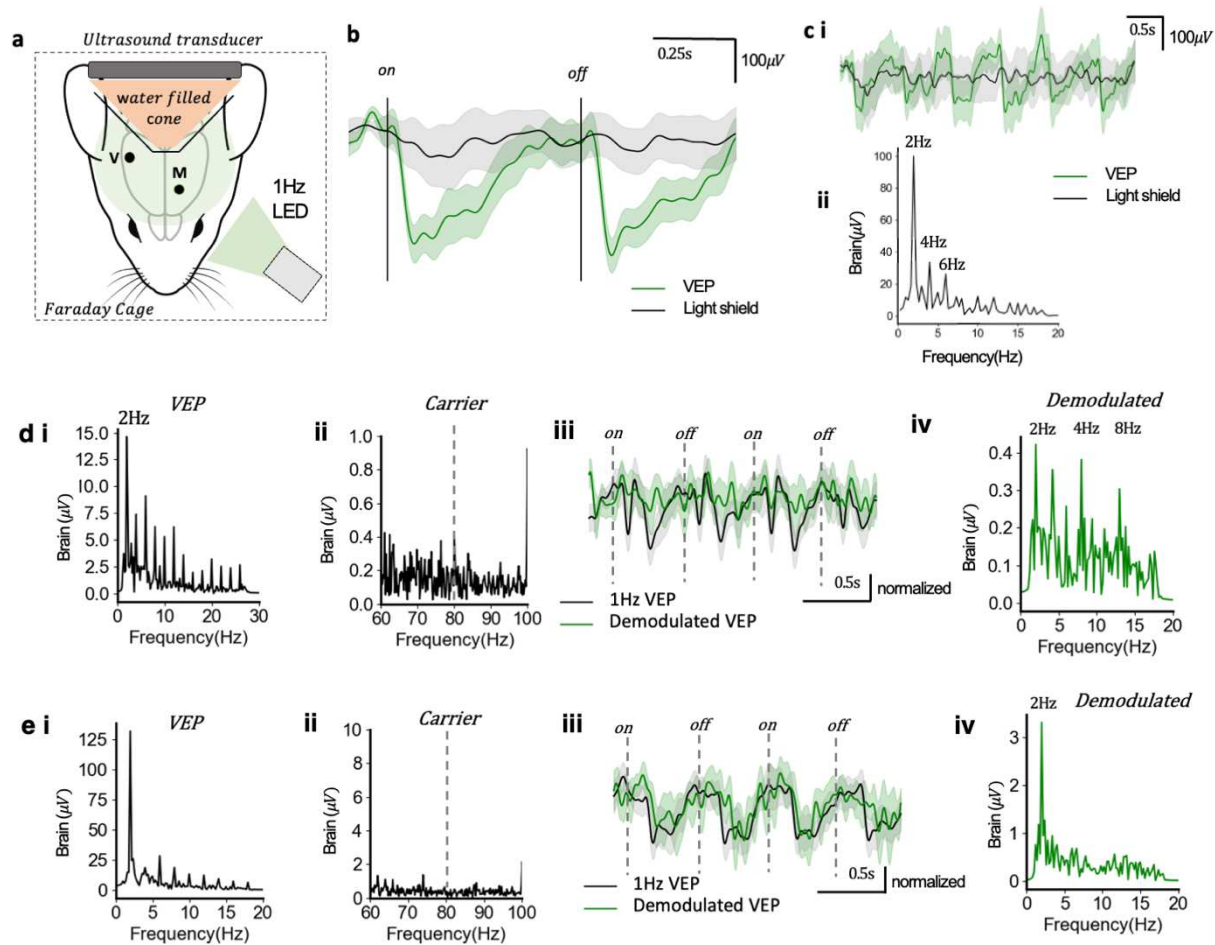


Figure 5-5 | In vivo acoustoelectric neural recording at low frequencies can be confounded by VEP harmonic artefacts.

a, i) *In vivo* experiment for acoustoelectric brain imaging of the visual cortex with contralateral green LED stimulation. Duration 8s, $F_s = 5\text{MHz}$, Gain = 500 **b**, Single cycle of LED showing visual evoked potential when green LED flashes, and when green LED flashes behind aluminium foil shielding screen (grey), data filtered 0-20Hz, $n=60$ events. Error bar of 95% confidence interval. **c, i)** response to 3 LED cycles, $n=16$ repeats of segment with 95% CI **ii)** ASD of **i)** **d**, Acoustoelectric brain imaging performed with 80Hz PRF, 1MPa ultrasound. **i)** ASD of VEP with SNR = 40.9dB **ii)** ASD of PRF carrier frequency (80Hz) and $\pm 20\text{Hz}$ **iii)** 2 cycles of original VEP (black line) averaged over 20 repeats (95% CI) and Hilbert demodulated signal (green line) using bandwidth shown in **ii)** **iv)** ASD of demodulated VEP shows peaks at 2,4,6Hz similar to original VEP. SNR = 21.8dB **e**, Same as **d** except with no ultrasound applied **i)** ASD of VEP with SNR = 46.8dB, **ii)** ASD of PRF carrier frequency (80Hz) and $\pm 20\text{Hz}$ **iii)** VEP compared with demodulated signal. **iv)** ASD of demodulated VEP shows peaks at 2Hz similar to original VEP. SNR = 28.2dB

Acoustoelectric brain imaging (AEBI) has been reported around an 80Hz PRF acoustic signal⁶⁶. The technique used to perform AEBI, is to take the Hilbert transform around the 80Hz PRF, and this envelope signal is reported to contain the acoustoelectric brain signal.

The mechanism is not well understood, though numerous reports have now been published^{63,64,66,157–160}.

To further understand the previous *in vivo* claims of acoustoelectric brain imaging^{157–159,161}, we repeated their VEP experiment. A recovery surgery was carried out as per **Methods 5.2.3**. First, we ensured we could measure a VEP without electrical artefacts by pulsing a green LED at 1Hz (duty cycle 50%) while the mouse was under light anesthesia 0.5% (vol/vol) Isoflurane in Oxygen (**Fig 5-6 a**). A light shield was placed between the mouse and the LED, while all the same signals were applied, and we found we could only measure the electrophysiological VEP signal when the LED light was visible to the mouse (**Fig 5-6 b**), providing evidence that the VEP is not an electrical artefact. Looking at the ASD of the VEP we can see a typical¹⁶² response to both the ON and OFF conditions leading to a spectral maximum at 2Hz (**Fig 5-6 c i, ii**), with typical harmonics at 4Hz and 6Hz. These high frequency harmonics, which continue up in the frequency spectrum, is key to understanding how these VEP harmonics could be confused with an acoustoelectric signal.

We then applied the reported 80Hz PRF ultrasound signal at 1MPa and repeated the 1Hz LED protocol. By taking the Hilbert envelope of a 40Hz bandwidth centered around the 80Hz PRF and low pass filtering the resulting envelope below 20Hz, we found we could demodulate and recover the VEP signal (**Fig 5-6 d iii**). By taking the ASD of the demodulated signal, we can see the original VEP frequencies clearly repeated at 2,4,8Hz with an SNR of 21.8dB (**Fig 5-6 d iv**), calculated as $20\log_{10}\left(\frac{\text{signal}}{\text{noise}}\right)$ where the noise floor is the mean of all signals under 20Hz, and the signal is taken as the amplitude of the fundamental (i.e. 2Hz). The ratio between the VEP and the recovered signal is 1:24 which is very similar to the reported ratio in the AEBI literature⁶⁶, which is better than any acoustoelectric conversion ratio measured to date.

We then repeated the same experiment without any ultrasound at all and found we could still recover the signal via Hilbert envelope demodulation and found the VEP frequencies in the ASD of the demodulated signal (**Fig 5-6 e i-iv**). The ratio of the original VEP to the

recovered signal was still 1:24. However, this cannot be explained via acoustoelectric brain imaging as there is no acoustic field present. However, when measuring VEPs we see harmonics of the VEP frequency ¹⁶³, though these harmonics get smaller as the frequency increases, they are still very much present at 80Hz, and any periodic signal within the bandwidth of the Hilbert transform will appear in its envelope as the fundamental (**Fig 5-6 c ii**). We conclude that the published works to date on *in vivo* acoustoelectric brain imaging (AEBI) could be confounded by these harmonics, particularly since there is no reported control. To prove results are not confounded in future work a spectral specificity test could be implemented which checks that the SNR at the expected signal sum and difference frequencies is significantly above the noise floor around the carrier, as the presence of the sum and difference frequencies shows that acoustoelectric heterodyning has taken place.

A confound to the visual cortex spatial maps reported using AEBI

The spatial map that is recreated in the reported AEBI *in vivo* work⁶⁶ which show time-based changes in the visual cortex, may be due to the ultrasound modulating the VEP amplitudes directly in the reported mapped 4mm x 4mm region. For instance, we found that when a continuous ultrasound signal was applied, the waveform of the VEP changed dramatically (**Fig 5-7 a**). Moving the ultrasound focus around will mean that the VEP response itself is changed in shape and amplitude as the acoustic field amplitude changes over the visual cortex, thereby also changing the shape and amplitude of the modulated signal and its harmonics. Since there is no normalization for this change in VEP amplitude as the focus moves across the cortex, the reported acoustoelectric map cannot be correct and is instead a representation of the VEP amplitude changes due to the applied acoustic field.

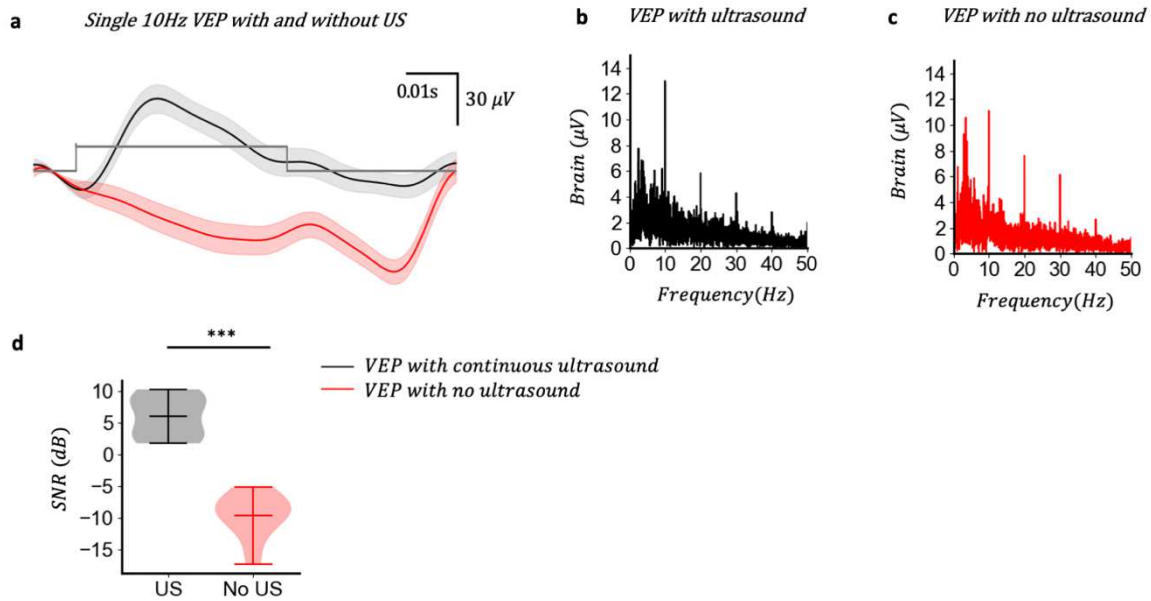


Figure 5-6 | Continuous ultrasound changes the visual evoked potential waveform.

a, $n = 260$ repeats of single 10Hz VEP, displayed in 0.1s interval, shown with 95% confidence interval error bar. All signals filtered between 8-80Hz. Sample rate of recording = 2MHz. Black line VEP under influence of 1MPa ultrasound, red line shows VEP response with no ultrasound present. **b**, ASD of VEP when 500kHz continuous ultrasound is applied. **c**, ASD of VEP when there is no continuous ultrasound applied. **d**, Mean deflection across 11 30 second trials in each group, each with 260 VEPs. Alternating in recording order to minimize confounds due to anaesthesia duration induced VEP amplitude changes. T-test $p = 8.7e-10$. US group mean $\pm st.d = 6.01 \pm 3.17$, no US group mean $\pm st.d = -9.68 \pm 3.36$.

Sidenote: Effect of ultrasound on the visual evoked potential waveform amplitude

A mouse under light 0.5% (vol/vol) isoflurane in oxygen anesthesia was exposed to a 10Hz flashing green LED for 30 seconds, with and without 1MPa continuous 500kHz sinusoidal ultrasound stimulation over the visual cortex. Averaging over 258 individual LED flashes, we see that the VEP with continuous ultrasound (**Fig 5-7 a**) has a more pronounced upward deflection than the VEP when no ultrasound was applied, which has a negative deflection. The ASD of the VEP with ultrasound (**Fig 5-7 c**) has a similar spectrum to the VEP with no ultrasound, and both are clearly responding to the LED frequency, albeit with differences in their shape and deflection direction.

To quantify the change statistically, each 30 second measurement was repeated 11 times at the same anesthesia level, alternating between with and without ultrasound stimulation to average out any time dependent anesthesia changes, and a deflection direction

calculated from the mean amplitude of the VEP extracted from each trial. An extremely significant difference was present between the ultrasound and no ultrasound group ($p = 8.7e-10$ **Fig 5-7 d**).

5.3.2 *In vivo* acoustoelectric neural recording of visual evoked potentials.

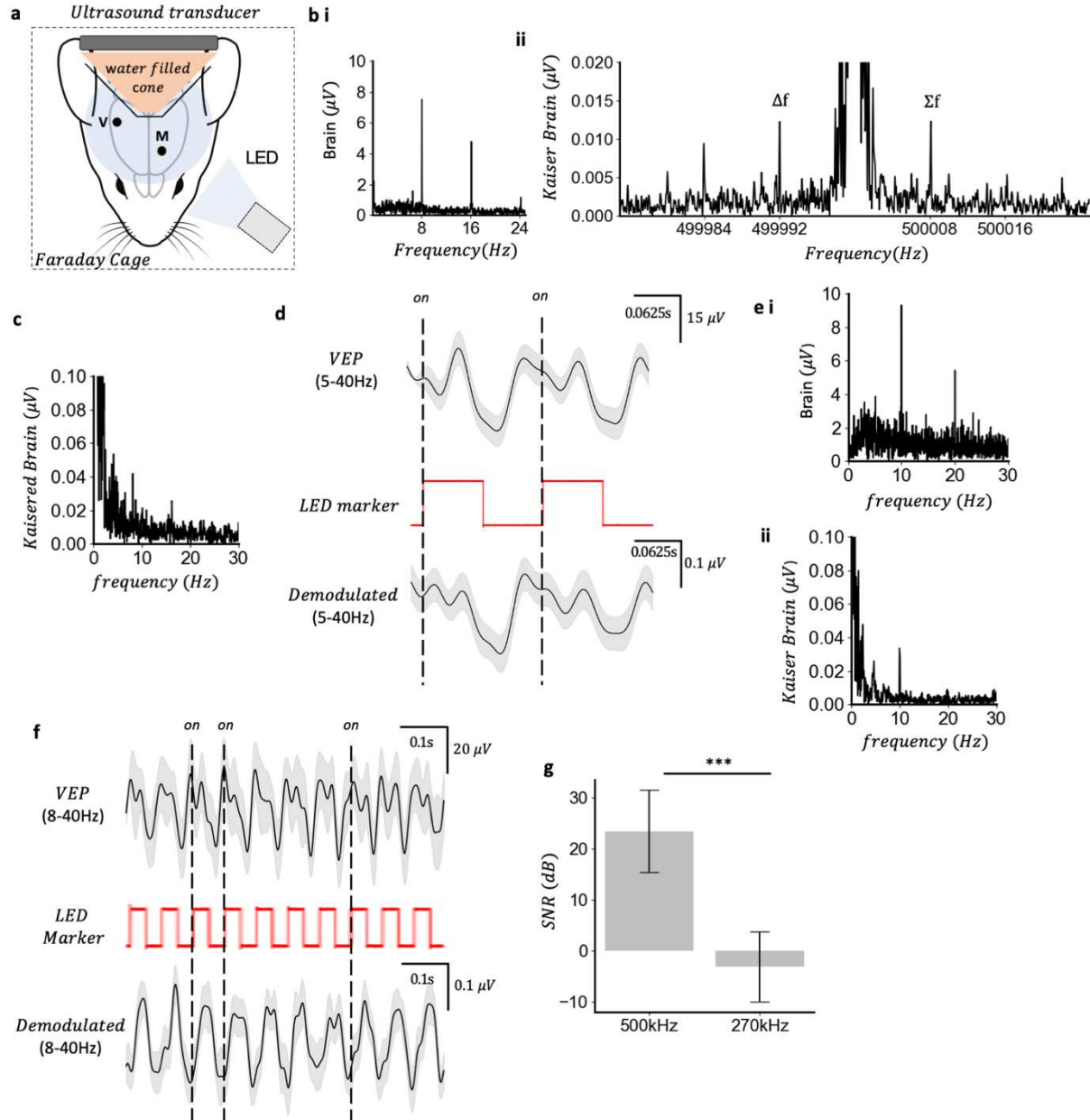


Figure 5-7 | *In vivo* acoustoelectric neural recording of electrophysiological signals.

a, *In vivo* instrumentation showing 500kHz transducer positioned over a mouse which has undergone a recovery surgery to implant two measurement electrodes. Head is covered in gel forming acoustic connection with water filled ultrasound cone. **b**, Acoustoelectric neural recording at sampling rate 2MHz, 30 second duration, 500kHz sine wave and 1MPa, averaged over 20 recordings to reduce noise floor, preamplifier gain = 5000. **i**) ASD of the visual evoked potential (VEP) shows peaks at 8Hz and harmonics. **ii**) Kaiser windowed spectrum (beta = 14), showing modulation around the carrier frequency (500kHz). Sum and difference frequency peaks have SNR = 30.5dB. **c**, Demodulated Kaiser windowed spectrum showing large deviation around 0Hz, and clear 8 and 16Hz peaks. **d**, Time series 8Hz signal showing 2 LED cycles, averaged over 105 repeats of a 0.25 second segment, band-filtered between 5-40Hz, with 95% confidence

interval shown in light grey. Time correlated LED marker channel shown in red. Hilbert demodulated signal, with filtering matching the LFP signal shown at the bottom. Amplitude ratio between the LFP and demodulated signal 150:1 **e**, i) ASD of local field potential shows 10Hz visual evoked potential and 10Hz harmonics ii) Kaiser windowed spectrum (beta = 14) of the demodulated signal. **f**, Time series 10Hz signal, averaged over 26 repeats of a 1 second segment, band-filtered between 8-40Hz, with 95% confidence interval shown in light grey. Time correlated LED marker channel shown in red. Hilbert demodulated signal, with filtering matching the LFP signal shown at the bottom. Amplitude ratio between the LFP and demodulated signal 200:1. **g**, Bar chart signal to noise ratio (dB) compared at two different carrier frequencies, comparing the mixing products to the noise floor. At acoustic signal carrier (500kHz) SNR (dB) $\text{mean} \pm \text{st. d} = 23.38 \pm 8.0$, and randomly selected carrier at 270kHz SNR (dB) $\text{mean} \pm \text{st. d} = -3.14 \pm 6.87$. n=5 mice, 20 trials of 30 second duration for each mouse, T-test statistic p=0.001.

Mice underwent a recovery surgery to implant two Platinum Iridium electrodes (**Methods 5.2.3**), to enable the recording of visual evoked potentials. Once recovered, the mouse was lightly anesthetized with Isoflurane as per the experimental procedure described in **Methods 5.2.4**. A green LED was flashed at 8Hz and 0.5 duty cycle, while a 1MPa 500kHz continuous pressure signal was applied for 30 seconds (**Fig 5-8 a**), and the *in vivo* electric signal recorded.

The amplitude spectral density of the visual evoked potential (**Fig 5-8 b i**) shows the fundamental LED flashing frequency and harmonics, and the Kaiser windowed spectral density around the carrier frequency (**Fig 5-8 b ii**) reveals the modulation sum and difference frequency products. Since the amplitude of the carrier frequency is far higher than the small, modulated sum and difference frequency signals, a Kaiser window¹⁶⁴ is used to reduce spectral leakage. Though the Kaiser window does successfully reduce spectral leakage it also introduces amplitude distortion, reducing the computed amplitude of both the carrier and the modulation products.

To recover the time series signal, a single 30 second trial is undertaken. The signal from the implanted brain electrodes is band filtered 1kHz on either side of the 500kHz carrier frequency, to remove all trace of the low frequency VEP from the recorded signal whilst leaving sufficient spectral space for filter ripple to be far from the tiny modulation products. The Hilbert transform and its absolute value are then computed to recover the analytic signal. Since there was a lot of noise close to the 500kHz carrier, this appears as noise below 5Hz in the demodulated signal (shown in spectral density form in **Fig 5-8 c**), thus the resulting signal is filtered between 5Hz and 40Hz. This demodulated signal is then

segmented and averaged throughout the trial to see VEP trends more clearly and a 95% confidence interval error bar computed over the 105 repeats present in the trial (**Fig 5-8 d**). Similarly, the original VEP signal is filtered between 5-40Hz to match the demodulated signal and segmented. The original VEP signal has a clear 8Hz as does the demodulated signal (**Fig 5-8 d**). We obtain a high Pearson correlation of 0.94 between the VEP and the demodulated signal for the time averaged LED flash cycle segment.

Similarly, a 10Hz LED was flashed in front of a mouse (**Fig 5-8 e i**) and demodulated (**Fig 5-8 e ii**). This time the final time series signals were filtered between 8-40Hz (**Fig 5-8 f**) and averaged over 1 second segments (26 segment repeats per trial), revealing both the VEP and the demodulated signal. Though there is a clear 10Hz repeating pattern in the demodulated signal, it is not in phase or the same shape as the 8Hz VEP signal leading to a low correlation of -0.06. The LFP shape and amplitude are known to change shape and amplitude with depth¹⁶⁵, and one explanation for this could be that the signal averaged by the volume of the ultrasound in this trial and the specific region the electrode is located have imperfect overlap.

Lastly, we perform a frequency specificity test to ensure there is no confound induced from neural VEP harmonic artefacts, by calculating the signal to noise ratio of the 10Hz mixing products compared with the noise floor around both the real ultrasound carrier and a randomly selected low frequency carrier(SNR noise floor taken as mean of 5-40Hz on either side of the carrier, signal as the mean of the sum and difference frequency, with $SNR = 20\log_{10}(\frac{signal}{noise})$). We find that over n=5 mice, with 20 repeats of 30 second trials for each mouse, there is an extremely significant difference (p=0.001) between the SNR at the ultrasound carrier (500kHz) with $mean \pm st. d = 23.38 \pm 8.0$, compared to the selected 270kHz SNR (dB) $mean \pm st. d = -3.14 \pm 6.8$, an SNR below the noise floor (**Fig 5-8 g**).

5.3.3 Single-shot *in vivo* acoustoelectric neural recording of electrophysiological signals

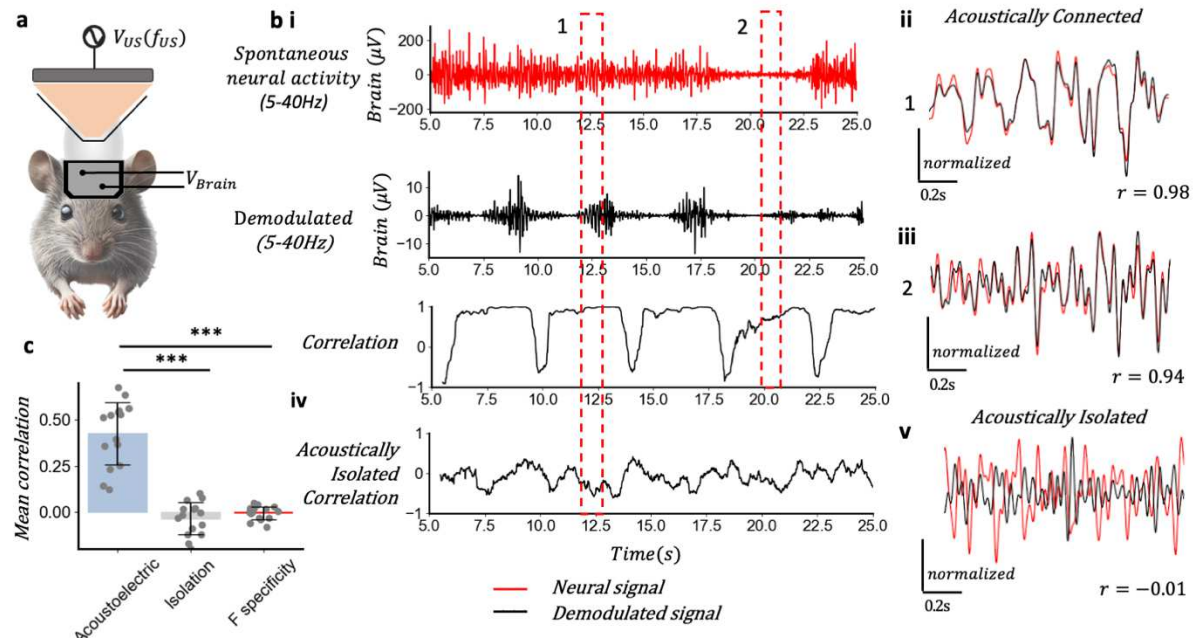


Figure 5-8 | Single-shot *in vivo* acoustoelectric neural recording of spontaneous neural activity.

a, experimental arrangement for recording of spontaneous neural activity. **b, i**) Spontaneous neural activity filtered between 5-40Hz under light Isoflurane anaesthesia so that burst activity can be seen (see amplitude decrease between 18-22s). On/Off ramp of ultrasound remove from trial so that the DC offset induced from the ultrasound doesn't induce filter ringing from the high pass filter. Demodulated activity shown below, with the Pearson correlation between the spontaneous activity and IQ demodulated signal computed over a 1M (0.5s) sample rolling window. **ii**) When the ultrasound is acoustically connected, high demodulation correlations can be seen throughout the file. Shown here is a 1 second zoom of timepoint 1 highlighted in **i**). Pearson correlation $r = 0.98$. **iii**) Demodulation result ($r = 0.94$) at timepoint 2 in **i**). **iv**) Acoustically isolated correlation shows no strong trend compared to **iv**) **v**) zoom view of timepoint 1 during acoustic isolation test, shows low Pearson correlation ($r = -0.01$) between demodulated and spontaneous neural signal. **c**, Mean correlation of spontaneous signal with demodulated signal computed during acoustoelectric neural recording, acoustically isolated and frequency specificity test at 270KHz. ANOVA $F(2,13) = 73.95$, $P = 1.73e-14$, post hoc comparisons using the Tukey HSD test, (500kHz-ISO, $P = 2.7e-6$; 500kHz-270kHz, $P = 9.8e-7$; 500kHz-270kHz, $P = 0.76$), $n = 7$ mice with 2 trials from each mouse;

While averaging is usually employed to overcome the natural neural based trial-to-trial variation in visual evoked potential measurements¹⁶⁶, single-trial demodulation of spontaneous neural activity would enable acoustoelectric neural recording to be used as a real-time tool, without the need for post-hoc averaging to recover the neural signal. Using a similar methodology to the visual evoked potential experiment, we recorded spontaneous neural activity under low level Isoflurane sedation at 0.5% (vol/vol) Isoflurane in Oxygen such that spontaneous activity could be measured (**Fig 5-8 a**) and

recovered the signal through IQ demodulation when ultrasound was applied. The Pearson correlation was then computed over the duration of continuous ultrasound (**Fig 5-8 b i**) revealing a high correlation ($r = 0.98, 0.94$ **Fig 5-8 b ii, iii**), compared to when the acoustic isolation test was performed ($r = -0.01$, **Fig 5-8 b iv, v**). The Pearson correlation during acoustoelectric decoding of spontaneous activity, shows periodic phase shifts (switching from 1 to -1) which can occur when multi-path acoustic signals change the dominant carrier phase at the location of the modulation. Within the mouse head, the 500kHz transducer (2.97mm wavelength) will create standing waves, reducing focality and making multi-path changes to the carrier amplitude more likely.

To ensure the correlation in the demodulation was due to acoustoelectric heterodyning we employed both the frequency specificity and acoustic isolation test over 7 mice with 2 trials for each mouse, finding the mean correlation between the spontaneous activity and the demodulated signal throughout the trial to be extremely significant (ANOVA $F(2,13) = 73.95$, $P = 1.73e-14$) when compared to the acoustic isolation and frequency specificity mean correlation (**Fig 5-8 c**), providing evidence acoustoelectric neural decoding can be performed without averaging.

Accurate single-shot acoustoelectric neural decoding without the need for multi-trial averaging eliminates the need for experimental time lag in accurate acoustoelectric signal recovery as demodulation techniques can be performed in hardware in real-time.

5.3.4 Artefact tests

To determine whether acoustoelectric neural recording is confounded we performed 4 artefact tests:

1. Frequency specificity test shows mixing is not due to broadband VEP harmonics.
2. Acoustic isolation test proves that an acoustic field must be involved to induce the modulation effect. i.e. the RF electrical artefact is not inducing mixing.
3. LED shield test provides evidence the VEP is not electrical noise from the LED.
4. Two-tone test shows that mixing is not occurring due to non-linearity in the preamplifier.

Frequency Specificity Test

The broadband frequency specificity test, addresses the VEP harmonic confound found in prior work, where no specific mixing products were identified, yet the signal could still be demodulated via Hilbert transform, and has been addressed with results for $n=5$ mice shown in **Fig 5-7 g**.

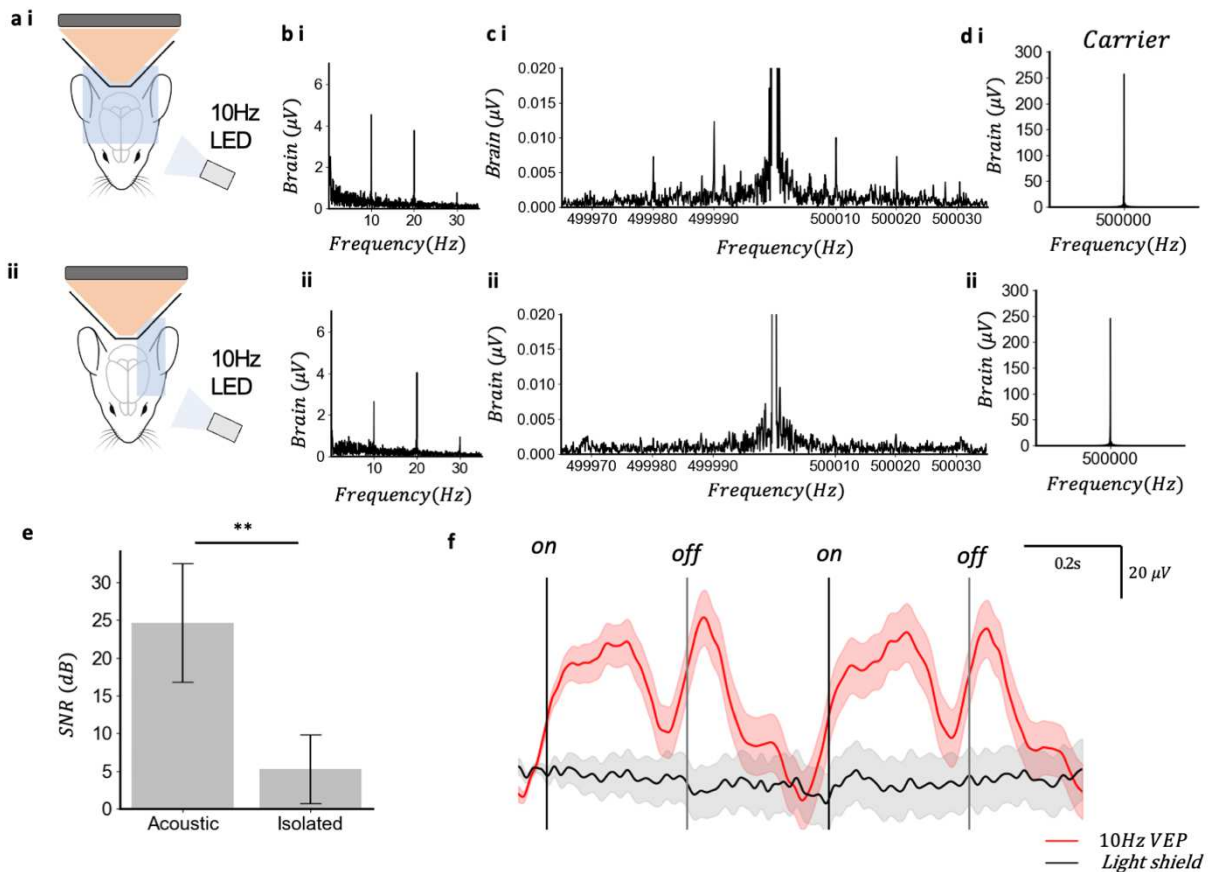


Figure 5-9 | Acoustic isolation and LED shield test.

a, Experimental arrangement showing gel with and without acoustic connection. **b**, Rectangular window amplitude spectral density (ASD), average of 20 recordings, 30 seconds each trial, gain=5000, 1MPa focused ultrasound for i) acoustically connected ii) acoustically isolated **c**, Kaiser windowed ($\beta = 16$) spectrum around carrier frequency 500kHz showing acoustoelectrically modulated spectral peaks. i) acoustically connected ii) acoustically isolated **d**, Carrier amplitudes showing the artefactual electric signal remains the same amplitude as a proof point that mixing is not occurring electrically i) acoustically connected ii) acoustically isolated **e**, Comparison between acoustically connected and isolated groups for $n=5$ mice with 20 trials for each mouse. T-test statistic $p=0.0027$. Acoustically connected mean $\pm$ st. $d = 26.64 \pm 7.85$ dB, Acoustically disconnected mean $\pm$ st. $d = 5.27 \pm 4.56$ dB **f**, LED shield test, showing LED flashing into eye (red line) and aluminium shielding in front of LED, but all control signals the same (black line) averaged over $n=300$ 10Hz VEP events with 95% CI.

Acoustic Isolation Test

Next, we performed an acoustic isolation test to determine if an acoustic signal is needed to induce the modulation effect, with the alternative being that the electrical artefact from the transducer induces electrical mixing with the VEP. We connected the ultrasound to the mouse via conductive gel and then compared this to the case where the gel still enabled an electrical connection but provided an air gap between the transducers acoustic output and the mouse head, thereby blocking the acoustic signal (**Fig 5-9 a**). We then compared the modulation amplitudes when the same 10Hz LED signal (**Fig 5-10 b**) was applied and the electrical artefact from the transducer was the same amplitude (**Fig 5-9 d**). The modulation products were only present when the ultrasound was acoustically connected to the mouse (**Fig 5-9 c**). We repeated this for $n=5$ mice, with 20 30 second trials for each mouse, finding a highly statistically significant difference ($p = 0.0027$) between the mixing product SNR between groups, and the acoustically connected group with a much higher SNR ($\text{mean} \pm \text{st.d} = 26.6 \pm 7.85\text{dB}$) compared to the acoustically isolated group ($\text{mean} \pm \text{st.d} = 5.27 \pm 4.56\text{dB}$) (**Fig 5-9 e**).

The acoustic isolation test also provides proof that the electric mixing is not happening due to capacitive coupling or non-linearities in the preamplifier front end, since the carrier electrical artefact is the same in both acoustically connected and disconnected groups (**Fig 5-9 d i,ii**).

LED shield test

The visual evoked potential signal may be an electrical artefact alone from flashing the LED close to the mouse. To ensure this was not the case, and we were in fact measuring the neural signal, we performed an LED shield test where a 10Hz flashing LED was shielded with aluminium foil so that the light was shielded from the mouse eye, while all other trigger signals remained the same (**Fig 5-9 f**). VEP averaging was performed over 300 LED flash events, enabling the VEP to be clearly seen while the LED was exposed, with no neural signal present when the LED flash was shielded from the mouse. This provides evidence that the VEP itself was not an electrical artefact of the stimulation and measurement system.

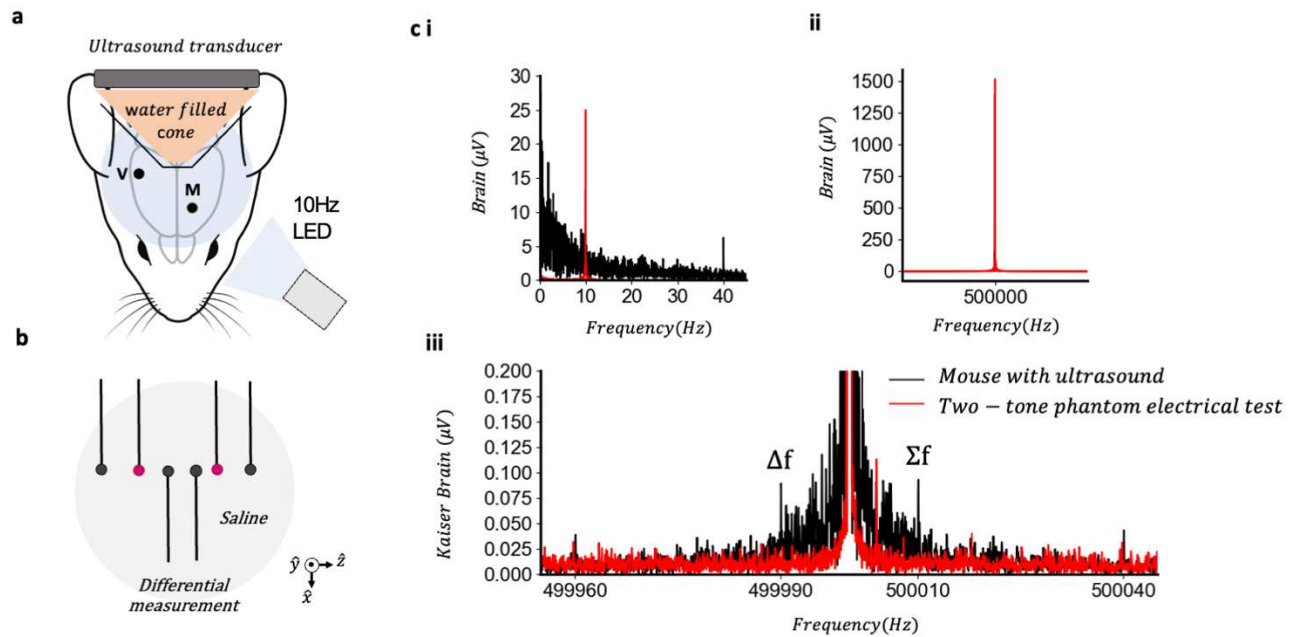


Figure 5-10 | Two-tone test.

a, in vivo experimental arrangement with electrophysiological signal and electrical artefact from acoustic field overhead. **b**, Phantom two-tone test, where the same amplitude signals as the in vivo experiment are applied with the same preamplifier settings. Red dot indicates stimulation electrode. **c**, i) Rectangular window amplitude spectral density (ASD) of visual evoked potential, averaged over 20 recordings, 30 seconds each, gain=2000, 1MPa focused ultrasound. ii) ASD of carrier amplitude where two-tone amplitude is larger than mouse electrical artefact. iii) Modulated signal spectrum view with Kaiser windowed (beta = 16) spectrum around carrier frequency 500kHz showing acoustoelectrically modulated spectral peaks.

Two-tone test

Since we have two electrical signals, an electrical signal at the ultrasound carrier (500kHz) due to the electrical artefact from the ultrasound and the 10Hz neural signal, it is possible that these signals are electrically mixing in the preamplifier front end, and the modulation products are caused by this. To determine if the modulation products are due to non-linearities in the preamplifier front end we performed a two-tone test where signals that match the amplitudes of the experimental signal of interest were injected into the system and the mixing product amplitudes were measured¹⁶⁷, with the experimental arrangement shown in (**Fig 5-10 a,b**).

The neural signal amplitude was artificially recreated with a 10Hz sine wave in saline, at an amplitude larger than the VEP amplitude in the mouse (**Fig 5-10 c i**), as was the electrical artefact carrier amplitude (**Fig 5-10 c ii**). The modulation products were then measured using a preamplifier gain of 2000 for both recordings, and the 10Hz modulation product was larger in the case where the ultrasound was applied (20.5dB), than the electrical signals alone (-2.28dB). Whilst it is possible to induce non-linear mixing at higher gains, particularly when the preamplifier is close to saturating, at the realistic amplitudes which we used in our experiments, the preamplifier non-linearity was below the noise floor.

As a secondary check for electrical mixing occurring elsewhere in the recording signal path, the acoustic isolation test can be used as it also indicates that the mixing was due to the acoustic field, and not simply due to electrical mixing either in solution, the cabling or the preamplifier front end.

A tertiary check for preamplifier mixing is that the demodulation correlation reported in **Fig 5-9** changes over time despite the applied acoustic field remaining constant, providing some evidence that the modulation itself is not occurring in the preamplifier, as the correlation would then be uniform over the trial.

5.4 Discussion

5.4.1 Safety profile

The safety limits for acoustic fields involve the MI and TI. The ideal value of the MI should be less than 1.9 at 500kHz to avoid the cavitation bioeffect on the rarefaction cycle of the waveform and this is the FDA approved maximum threshold for diagnostic imaging⁹⁶. Estimating the mechanical index for a continuous wave 500kHz ultrasound transducer, the maximum allowable pressure is 1.345MPa, a threshold which we are currently below as all recordings in this Chapter are performed at 1MPa.

To ascertain the thermal index (TI), we use our safety simulations reported in **Chapter 2**, of a continuous wave at 500kHz and 1MPa which will raise temperature in tissue 4°C, and a thermal index of 4 which is above the recommended safety threshold of 1. Hence the current continuous ultrasound scheme is outside the safety limit for thermal dissipation, making it outside the safety parameters for use in humans. Despite the debate on the accuracy of the thermal index measure³⁰ it would certainly be advantageous to be far below this limit, which seems quite possible with the listed improvement strategies. For example, pulsing the transducer would reduce heating, though lower the modulation SNR. The solution would be to use an array of transducers to distribute the heating at the skull more evenly, use of electrically insulating material to enable higher preamplifier gain, and large surface area electrodes. These types of improvement seem well within reach to make translation to humans a possibility.

Anecdotally, I found mice recovered well even when larger pressures were used, which surpassed both the MI and the TI. I did no histology examinations to assess this in further detail and can only report the observation that they recovered quickly, resumed normal eating and grooming behavior and were able to undergo repeat experiments on subsequent days.

5.4.2 Summary

In the work prepared in this Chapter, *in vivo* proof-of-concept for acoustoelectric neural recording is presented for the first time, inclusive of single-shot signal recovery, a proof point which would enable this technique to become a new spatially focused real-time electrophysiological recording tool. We perform 4 confound tests, to prove that acoustoelectric neural recording is not due to the electrical artefact from the transducer or mixing in the preamplifier. Hence, we can report acoustoelectric neural recording *in vivo*, for the first time.

We review the prior work on ultrasound current source density imaging, revealing its mechanism to be the acoustoelectric heterodyne interaction, describing how it can be further optimized to retain both frequency and phase information, so that accurate signal

recovery can be performed. Previous work claiming acoustoelectric brain imaging was reproduced *in vivo* at low frequencies, overlap with the location of VEP harmonics. Unless an additional artefact test is performed, it would not be possible to isolate the cause of the demodulated signal to be these VEP artefacts versus an acoustoelectric interaction.

The acoustoelectric conversion ratio between the recovered signal and the original VEP is about 1:200, which may pose problems if it were to be applied to a mammal with a thicker skull such as humans. To translate these *in vivo* findings in rodents to humans, a series of optimizations would need to be performed ranging from improvements in waveform specificity through tomographic techniques, improving the dynamic range of recording by blocking the electrical artefact from the ultrasound transducer, electrode surface area optimization, and frequency optimization to enable the highest SNR acoustoelectric signal to be detected through the skull.

From a safety perspective, no optimizations to adhere to the diagnostic ultrasound guidelines have been performed in this study, though they will be required for further translation to humans. Continuous wave ultrasound is used at 1MPa, which is below the mechanical index (MI) for safe use, but above the thermal index (TI). To minimize thermal dissipation, the ultrasound waveform could be pulsed, and this could be the subject of further studies.

Development of a tool which can provide spatial targeting at depth and detect electrical neural signals would enable the detection of epileptic foci, OCD and other disorders, using the native electrical neural signal instead of slower derivatives such as the BOLD signal or Calcium changes, enabling previously inaccessible insights into how we understand the brain.

Chapter 6: Acoustoelectric neuromodulation: a contributing mechanism to ultrasound brain stimulation.

6.1 Introduction

Stimulation of the brain via ultrasound has potential as a therapeutic tool due its high spatial focality and ability to target deep brain regions. However, its impact has been limited due to our lack of understanding of its underpinning mechanism. In this Chapter, we present the first *in vivo* evidence of acoustoelectric neuromodulation and investigate how it is a contributing mechanism to ultrasound stimulation.

Through physiological phantom experiments we show that a focal electric signal is created via acoustoelectric mixing between the acoustic field and the electric artefact created from powering the piezoelectric crystal in the ultrasound transducer. The high frequency electric field from the transducer artefact is capacitively coupled to the ionic medium via a water filled ultrasound cone.

Through *in vivo* rodent experiments, we provide evidence that the low frequency electric signal in the brain when ultrasound is applied is correlated with the evoked neural response measured through electromyography (EMG). The low frequency evoked electric signal in the brain can be increased in amplitude by varying an independently applied electric field when ultrasound is applied and decreased in amplitude by blocking the high frequency electric artefact field with an electrically insulating and acoustically transparent

material. Thus, the amplitude of ultrasound brain stimulation is dependent on both an acoustic and an electric field being present in the medium – an acoustoelectric interaction. *We then move to applying an independent electric field at a small difference frequency from the acoustic field. In vivo* acoustoelectric neuromodulation occurs when the electric and acoustic field are at different frequencies, such that the difference frequency between them induces a low frequency difference frequency electric field capable of evoking an EMG response (**Fig 6-1**). Evoked muscle signals are correlated to the acoustoelectric difference frequency present in the brain, demonstrating acoustoelectric neuromodulation for the first time. Evoking a neural response at the difference frequency between the applied acoustic and electric field is evidence that ultrasound brain stimulation is not due to the acoustic radiation force or acoustic field cavitation effects, as the envelope of the ultrasound is different from the low frequency electric signal evoked in the brain at the acoustoelectric difference frequency.

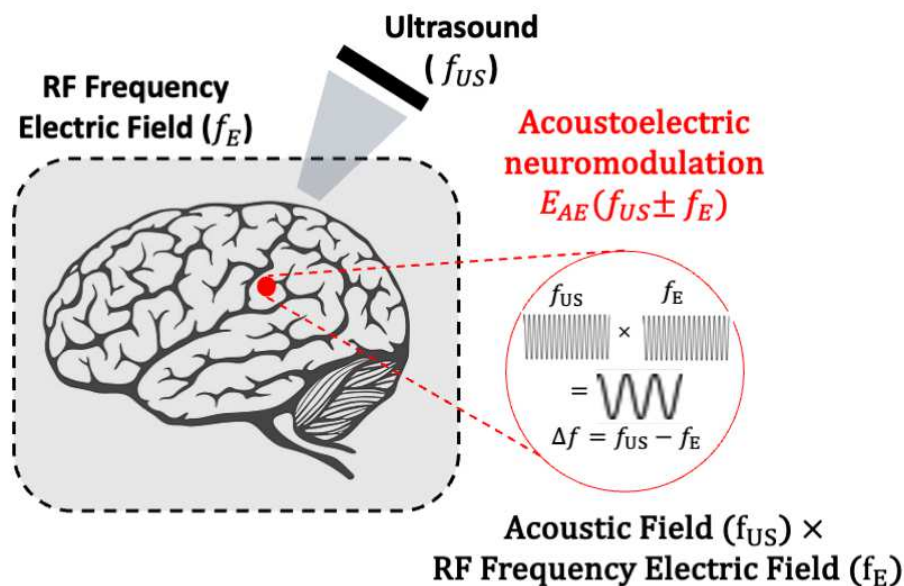


Figure 6-1 | Acoustoelectric neuromodulation concept.

A focal acoustic field and non-focal high frequency electric field are applied so that they mix via the acoustoelectric interaction, creating a focal electric signal at the difference frequency between the two applied fields.

Lastly, we provide evidence that acoustoelectric neuromodulation is not due to the temperature envelope from the applied acoustic energy, as the envelope of the low frequency brain signal and the temperature are at different frequencies.

Acoustoelectric neuromodulation opens the gateway to new targeted therapeutics, with a clear mechanism of action which can be precisely controlled.

6.2 Methods

6.2.1 Acoustoelectric neuromodulation instrumentation

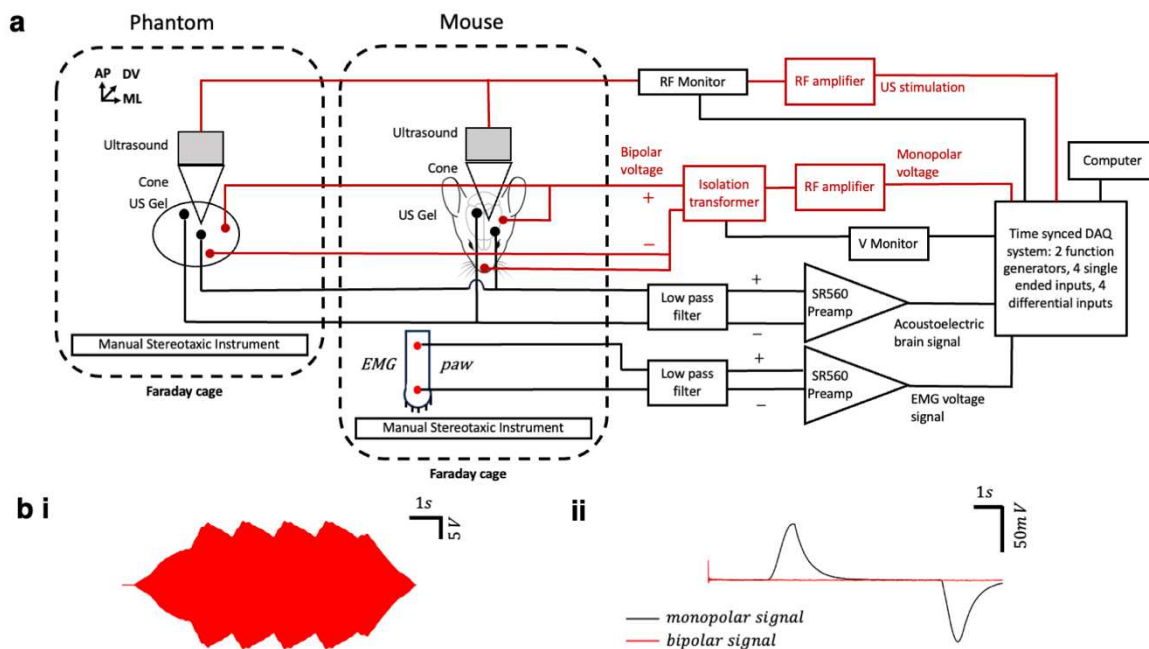


Figure 6-2 | Acoustoelectric instrumentation.

a, Instrumentation for acoustoelectric neuromodulation in both saline phantom and mouse with stimulation instrumentation shown in red and measurement system shown in black. **b**, i) Stimulation signal monitored during 70V peak-to-peak 500kHz voltage signal applied via the Platinum Iridium electrodes used for stimulation in the mouse. ii) Differential measurement at electrodes in 0.9% physiological saline phantom, sampling rate 5MHz, 12 seconds duration. Since the DC offset was so large, I introduced a front end 0.3-1MHz filter on the preamplifier (gain =1) so that the measured signal now looks like peaks/troughs at the onset and offset, showing monopolar DC offset changes (black line) and bipolar signal (red line).

Data acquisition. All applied and monitored signals were recorded at 5MS/s, and were time synced using a CMI interface between the function generators and oscilloscopes with trigger inputs. The applied voltages, monitor channels and electric potentials were

logged using data acquisition hardware (WiFiScope WS5, WiFiScope WS6 DIFF and Handyscope HS5, TiePie engineering, Netherlands). The logged signals were streamed from the data acquisition hardware to a workstation PC using custom C code which utilized the TiePie SDK to control the triggers, channels, and function generator output which I wrote, with advice from TiePie's excellent email support. The compiled C code which called the oscilloscope commands was controlled through a Python wrapper script.

Electrophysiology recordings. The electrophysiological brain recordings were made by connecting a preamplifier (SR560, Stanford Research Systems) to the implant in the mouse brain (**Section 6-2-3**) via a low pass filter (5kHz -3dB frequency) whose response and schematic are described in **Chapter 3 Section 3.2.4**. This passive differential low pass filter was used whenever a high frequency electric field was applied, so that low frequency electric fields <1kHz could be measured without overloading the preamplifier. In some recordings (**Section 6.3.3, 6.3.4**) where only ultrasound was applied, no low pass filter was used as the *in vivo* raw voltages measured between the two brain electrodes (7mm spacing) did not surpass the $\pm 1V$ voltage limits of the preamplifier. In both cases the low frequency acoustoelectric brain signal was present providing additional evidence this low frequency brain signal is not a non-linear recording artefact induced from the front-end low pass filter.

Electromyography (EMG) recordings. EMG was measured similarly to the brain recordings using a second low-noise differential amplifier (SR560, Stanford Research Systems) and filtered using a custom-built^{110,111} passive differential low-pass filter to filter out the signal at the frequency of the applied electric field.

Mouse. Measurements of acoustoelectric neuromodulation were made according to the experimental procedure outlined in **Section 6.2.4**.

Pressure recordings. The acoustic field was measured using a 0.2 mm needle hydrophone (52mV/kPa at 500 kHz calibration; Precision Acoustics Ltd) and a DC-coupled preamplifier (Precision Acoustics Ltd).

Acoustic fields application. The acoustic field was applied using a curved ceramic (PZT) 500 kHz ultrasound transducer (60 mm diameter, 63.5 mm acoustic focal length; Precision Acoustics Ltd, UK). The ultrasound transducer was driven by an arbitrary function generator (Handyscope HS5, TiePie engineering, Netherlands) and a 40 W linear power amplifier (240L, Electronics & Innovation Ltd).

Electric field application. The electric field was applied using a pair of 98% Platinum Iridium (Pt-Ir) wire electrodes (0.25 mm cross-section diameter, Alfa Aesar) with shape and positioning described in **Section 6.2.4**. The electrodes were driven by a second arbitrary function generator (Wi-FiScope WS5, TiePie engineering, Netherlands). To achieve output voltages which surpassed the limited 12V function generator range a second 20W linear power amplifier was used (325LA, Electronics & Innovation Ltd). The output of the power amplifier was monopolar, such that only one of the two electrodes would apply voltage whilst the other was GND. Since this configuration is not charge balanced, it would induce a large DC offset via electrochemical effects at the electrode interface^{119,171} (**Fig 6-2 b**), hence an isolation transformer (a custom-made high-frequency voltage source made of glass core transformers (Hitachi Metals) with an effective -3dB bandwidth range of 5 kHz to 2 MHz) (Schematic in **Appendix 1**) was added to provide a balanced bipolar output, charge balancing the electrodes to successfully minimize ionizing (DC) offsets, as well as isolate the mouse/phantom from the electric field generation hardware to decrease the effects of total harmonic distortion (THD)¹⁷².

6.2.2 Acoustic and acoustoelectric cone characterization

Before moving to the animal facility, an acoustic and acoustoelectric characterization study was undertaken with the ultrasound cone and parafilm attached in a 0.9% saline tank phantom to mimic physiological conditions, to understand the resulting focality and acoustoelectric amplitudes. These calibration curves are referenced in numerous places throughout the results.

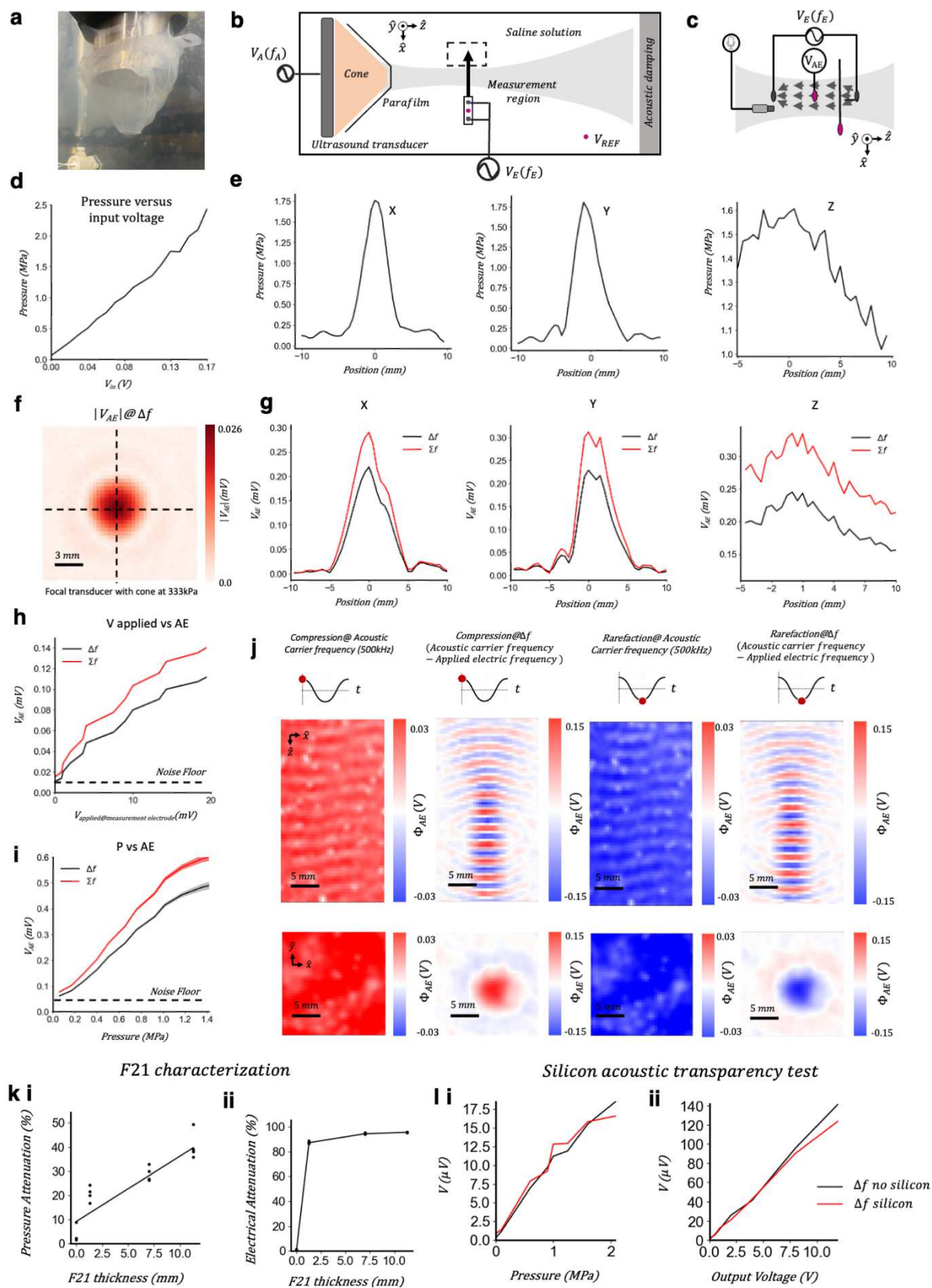


Figure 6-3 | Acoustic and acoustoelectric cone characterization

a, 500kHz ultrasound transducer with cone, covered in parafilm. **b**, Phantom tank for XYZ scanning of electric fields generated through the acoustic focal area. **c**, Hydrophone and electrodes for measuring acoustoelectric effect. **d**, Input voltage to the E&I 240L RF amplifier versus output pressure measured at the focal spot of the ultrasound transducer in 0.9% saline. **e**, XYZ mapping of the acoustic amplitude measured with a hydrophone. Input voltage to RF amplifier at 0.13V @ 500kHz. Z depth axis is made irregular by reflections off the back of the tank, despite the use of acoustic damping material. **f**, XY spatial distribution map of acoustoelectric difference frequency generated with 500kHz pressure at 333kPa, and 20V peak-peak 8kHz on voltage electrodes aligned with the ultrasound. 0.1s duration at 5MHz sampling rate, for each pixel. Data band filtered around difference frequency of 492kHz. **g**, Similar parameters to **f**, except showing X, Y and Z axis sum (508kHz) and difference (492kHz) frequencies and their distribution for each axis. **h**, Ramping amplitude of applied 8kHz voltage with 500kHz pressure constant amplitude at 0.4MPa. Shown are sum and difference voltage amplitudes calibrated to the focal point of the transducer. **i**, Ramping pressure of 500kHz ultrasound transducer with constant 12V output 8kHz voltage on electrodes. Shown are the sum and difference voltage amplitudes calibrated to the focal point of the transducer. **j**, XY and XZ spatial maps of acoustoelectric difference frequency and acoustic carrier frequency for both a compression and rarefaction. **k**, i) F21 material acoustic attenuation versus thickness. 8 second duration recordings, 5MHz sampling rate repeated 4 times at each F21 thickness. Regression line: Attenuation (%) = $2.7 * \text{F21 thickness (mm)} + 9.2$ ii) F21 material electrical attenuation versus thickness. Regression line: Attenuation (%) = $6 * \text{F21 thickness (mm)} + 3.5$. **l**, Measurements of the difference frequency, with and without a 1mm piece of silicone used for skull and electrode moisture insulation. Varying both pressure and voltage in a petri dish phantom versus acoustoelectric difference frequency.

To elucidate the acoustoelectric focality phenomenon we created time-synced recordings with spatial mapping in XY and XZ directions in 0.9% saline. The electric field is aligned with the propagation direction of the acoustic field. We observe that the frequency mixing products only occur within the focal acoustic field of the transducer, and that they are not present at the carrier frequency (500kHz) indicating there is no linear piezoelectric effect in solution, only heterodyning (**Fig 6-3 j**).

F21 was chosen to attenuate the electric field from the transducer, as a mineral oil filled cone was found to deteriorate the Parafilm film that covered the transducer cone, making it impractical to use without it leaking. I also tried using a conductive mesh, but this didn't reduce the electric field from the transducer sufficiently, and had unpredictable effects on the acoustic field. F21 attenuated the electric field, whilst being close to transparent for the acoustic field and could be inserted and removed at will during the same experiment making it an effective and flexible choice.

6.2.3 Experimental procedure for *in vivo* acoustoelectric neuromodulation

Anaesthesia of mice was achieved via subcutaneous injection of 75 mg/kg Ketamine and 10 mg/kg of Xylazine, as opposed to the Isoflurane anaesthesia used in the neural recording experiments. Eye lubricant (Vaseline) was placed on the eyes to prevent them from drying out. The anesthetized mouse was placed onto a thermal mat (Thermostar, RWD, China) to maintain homeostasis and head position stabilized with head clamp (Neurotar standard clamp, Finland) to enable precise stereotaxic localization of coordinates over the head. The electrode implant was connected to the SR560 preamplifier (Stanford Research Systems, USA) via a 1.27mm Dupont header at the back of the head, while stainless steel EMG electrodes (0.25mm diameter, Arbitter, UK) were placed in the front paw¹⁷³. The EMG electrodes were then connected to a second SR560 preamplifier to enable simultaneous brain and front paw EMG recordings.

The electric field was applied using a pair of 98% platinum iridium wire electrodes (Alfa Aesar, 0.25mm diameter), which were formed into loops to increase the exposed surface area, further decreasing electrochemical induced offsets when electric fields were applied. One was inserted into the mouth, and the second shaped into a halo above the head and placed between the ultrasound cone and mouse head within the gel interface using masking tape (Fisher Scientific) to hold the top electrode in place at the back of the electrode implant.

The ultrasound transducer was mounted in a cone which was pre-characterized (**Fig 6-3 d-j**) and filled with water. Parafilm was stretched over the end of the cone to seal the water in, while the transducer was positioned via stereotaxic instrument above the mouse head. Ultrasound gel (Anagel, UK) was applied on top of the mouse head, surrounding all electrodes, and providing an acoustic connection between the end of the cone and the mouse skull.

Acoustic and electric fields were then applied according to the instrumentation description (**Section 6-2-1**). Measurements of EMG could only be made 45 minutes to 1.5 hours after administration of anaesthesia dependent on the mouse. Before 45 minutes the mouse

would be completely non-responsive to any form of acoustic or acoustoelectric stimulation. After the anaesthesia lightened and small spontaneous movements started to occur, the mouse was given Antisedan (1 mg/kg) subcutaneously to hasten Xylazine recovery and moved to a warming chamber. After recovering in the warming chamber under observation, the mouse was given water saturated food pellets and returned to its home cage.

Voltage amplitude. To determine the voltage level at the electrodes for each experiment after positioning the halo and mouth electrode, a calibration was done to turn the voltage up to a level as high as possible, whilst minimizing non-linear electrochemical effects due to the electrode interface. If a DC offset was induced greater than 1mV, the voltage was decreased. This calibration was done when the mouse was in a deep anesthesia plane (<45minutes into ketamine/xylazine) as it is purely electrochemical and not dependent on evoked neural signals.

Pressure amplitude. The pressure amplitude was minimized to avoid large onset effects from the transducer and decreased as the anaesthesia depth lightened such that it was just above the EMG threshold. Pressure amplitude ranged between 0.5-2.5MPa as this was where the most reliable EMG responses were seen whilst mice were under light anaesthesia. This was determined by testing ultrasound stimulation alone, and ramping the pressure until a response was observed.

Most of the *in vivo* rodent experiments performed to date were done when the mouse is fully awake^{117,174–176}, requiring lower pressure thresholds to evoke EMG and often with spontaneous movements also present. I chose to perform the experimental measurements when the anaesthesia was light, and there were no spontaneous movements which required pressure magnitudes that were slightly higher than when the mouse was awake. No recordings reported here were performed when the mouse was awake or producing spontaneous movements.

6.2.4 Data exclusion criterion

Some data were excluded from the statistics and the criteria for exclusion are described below:

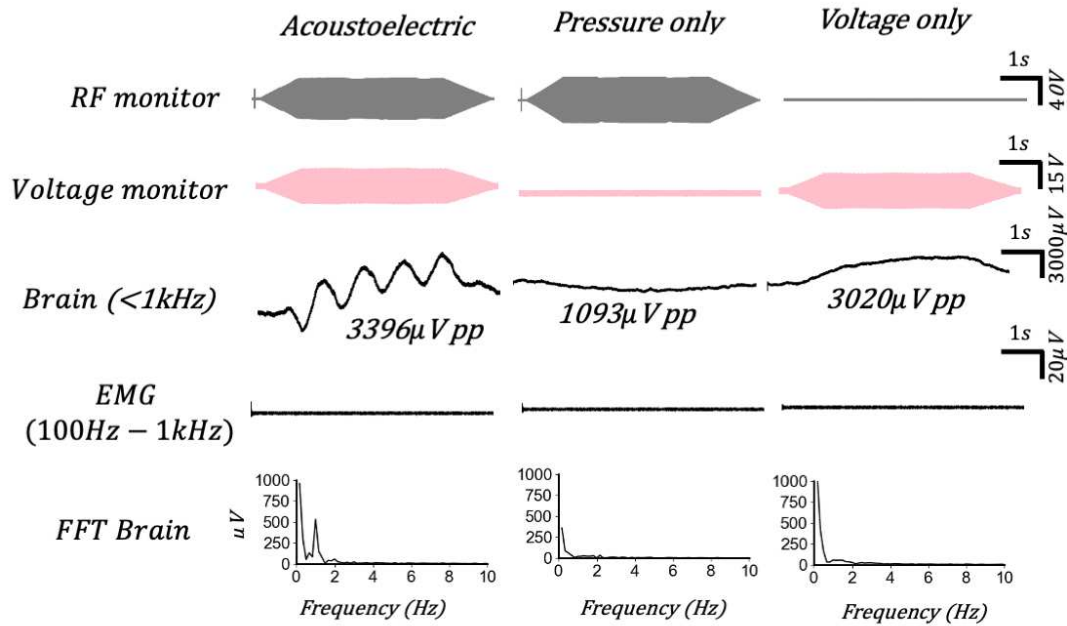


Figure 6-4 | Data exclusion due to depth of anaesthesia.

Leave one out experiment example performed when anaesthesia was too deep to evoke any EMG response, despite $\Delta f=1\text{Hz}$ being present in the brain. $v=30\text{Vpp}$ at the stimulation electrodes, distance between electrodes = 7mm. $E=0.71\text{ V/m}$ which we found to be above threshold for muscle movements.

For all experiments, only recordings within the time window of EMG response were included in the data set. Recordings where the anaesthesia was too deep to evoke an EMG response were excluded (**Fig 6-4**). If spontaneous movements were present, the experiment was stopped, and the mouse recovered.

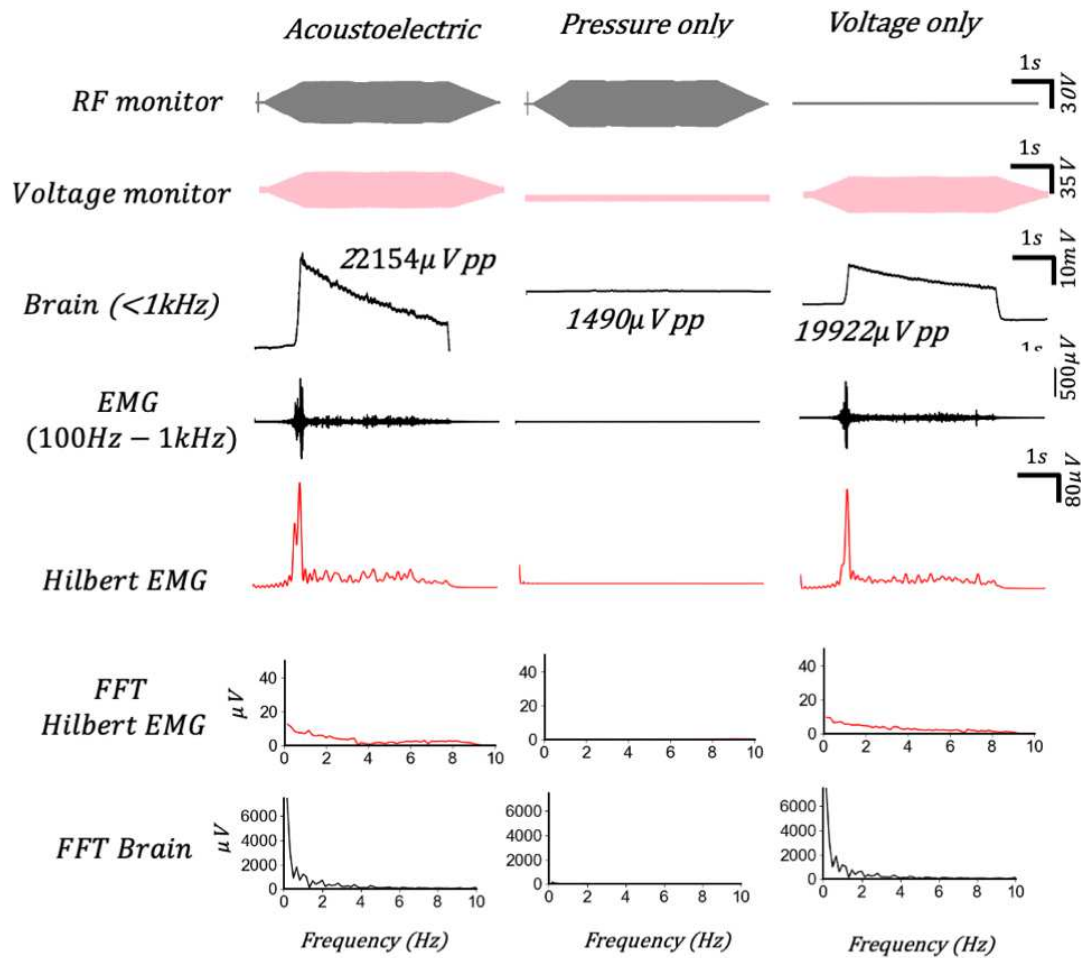


Figure 6-5 | Data exclusion due to electrochemical DC offset effects at high voltages.

$\Delta f = 1\text{Hz}$ $P = 1.5\text{MPa}$, $V = 70\text{Vpp}$. At very high voltages non-linear electrochemical effects at the electrode interface obfuscate the acoustoelectric difference frequency in the brain signal. The ASD plots are all shown with the same y-axis so the amplitude can be directly compared.

In the case above where a 70V peak-to-peak voltage was applied at the electrodes, we see there is a low frequency (filtered <1kHz) signal evoked when voltage alone is applied, and this electrochemically induced signal can trigger an EMG response. Though a bipolar source is used to generate a balanced voltage output, any asymmetry between the surface area of the halo and mouth electrodes will induce non-linear electrochemical DC offsets¹⁷⁷ at these high voltages capable of surpassing the EMG threshold. Since the electrochemical effect due to the electrode interface dominates in this high voltage case, the acoustoelectric 1Hz cannot be seen in the signal measured in the brain meaning we should not expect the mouse to respond to 1Hz as the EMG threshold was surpassed by the DC offset already. When no difference frequency could be seen in the evoked brain

signal as shown above in the acoustoelectric brain signal on the left, the data was not included in the statistics. This means that the remaining files had a clear electrical difference frequency which produced a low frequency sine wave in the brain.

6.3 Results

6.3.1 Ultrasound induces electrical frequency mixing products in the electrophysiological range.

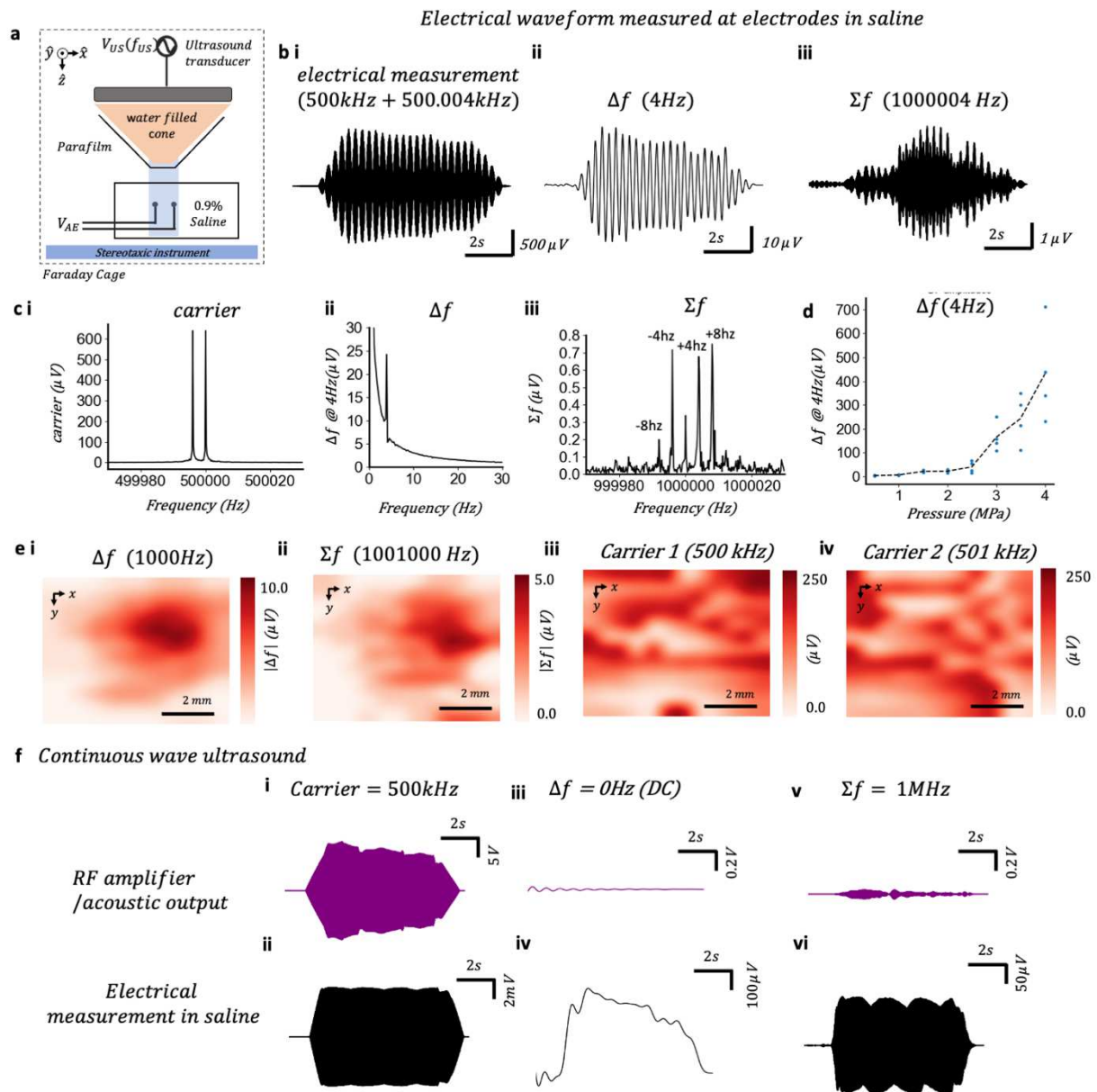


Figure 6-6 | Ultrasound induces electrical focal frequency mixing products.

a, Instrumentation for measuring electric field generated at the focus of an acoustic field. **b**, Representative electrical waveform as measured in saline at the focal point of the ultrasound, maximum pressure 1MPa. Recording duration 8s, Sample rate, 5MHz recording i), electrical measurement unfiltered dominated by the acoustic frequencies ii) 100Hz low-pass filtered to show difference frequency at 4Hz iii) high pass filtered (1MHz) to reveal the sum frequency (1MHz + 4Hz). **c**, Amplitude spectral density (ASD) plot of i) the two applied signals ii) Δf iii) Σf **d**, The output pressure is ramped in 0.5MPa increments using the same waveform as in (b) and (c), and the amplitude of the difference frequency at 4Hz measured, repeated 4 times at each pressure. **e**, 1MPa pressure, with a dual 501+500kHz waveform applied, each pixel shown calculated from the amplitude spectral density of the recorded electrical signal (amplifier gain = 500) calculated over duration 4 seconds at the frequency described in the image in 0.5mm increments. **f**, Continuous wave ultrasound i) RF amplifier output @500kHz ii) phantom electrical measurement in saline band filtered around 500kHz iii) 10Hz low pass filter on RF amplifier output to observe difference frequency at 0Hz (DC) iv) 10Hz low pass filter on phantom measurement electrodes to expose DC offset difference frequency v) band filtered RF amplifier measurement showing the sum frequency. vi) phantom electrode measurement of the band filtered sum frequency (1MHz).

To investigate the electrical properties of ultrasound alone, we delivered an acoustic signal formed from the superposition of two sine waves (500kHz and 500kHz + 4Hz) with peak amplitude 1MPa into a petri dish of 0.9% saline with instrumentation detailed in methods (**Fig 6-2 a**) and Platinum Iridium measurement electrodes spaced 7mm apart as shown in **Fig 6-6 a**. The superposition of acoustic fields created both an electrical signal in saline at the applied acoustic frequencies (**Fig 6-6 bi**), and the mixing sum and difference products (**Fig 6-6 b ii, iii**). Looking at the Fourier transform of the measured electrical signal present in saline from the ultrasound shows the dominant applied frequencies (**Fig 6-6 c i**), a clear difference frequency (**Fig 6-6 c ii**), and at a factor of 25 less than the difference frequency we see the sum frequency (**Fig 6-6 c iii**) and further intermodulation products¹⁷⁸.

To determine if the resulting low frequency voltage is based on pressure alone, we ramped the pressure in 0.5MPa increments (**Fig 6.3 i**) and noted that the increase in the voltage suggests a quadratic (x^2) relationship (**Fig 6-6 d**). This non-linear increase could be explained via the acoustoelectric effect where the pressure multiplies with a voltage present in the medium which is also increasing as the pressure increases.

We then used a stereotaxic instrument to move the ultrasound in 0.5mm increments through an 8mm square, measuring the amplitude of the emergent sum and difference frequencies (**Fig 6-6 e i, ii**), and the two applied carrier signals (**Fig 6-6 e iii, iv**), this time

with carrier frequencies separated by 1kHz. The sum and difference frequencies are focal with the acoustic field while the carrier frequencies are not focal with the acoustic field similar to acoustoelectric characterization measurements (**Fig 6-3 j**) where an electric field was applied independently at a second frequency to evoke a difference frequency.

Since a sum frequency exists, this focal electric field cannot be induced by the acoustic radiation force (ARF)¹⁷⁹ which only occurs at the low frequency amplitude envelope of the acoustic wave. The focal sum and difference envelope are also not able to be produced through electrical interactions alone, as these would not evoke an electric field with the focality of ultrasound. Hence, we have a heterodyne interaction, which could be explained by either the acoustoelectric heterodyning effect¹⁰², or non-linear acoustics¹⁸⁰ if we assume a yet to be reported ionic solution based piezoelectric effect.

We then moved to a single continuous wave and measured both sum and difference frequencies multiplying with itself in the physiological saline phantom at the difference frequency (DC) and sum frequency (1MHz) (**Fig 6-6 f**). The low frequency part (DC) component of the evoked electrical signal is in the electrophysiological range to induce neural stimulation^{35,181}, thereby becoming a potential explanation for the mechanism of ultrasound neuromodulation. For instance, should an ultrasound pulse occur, which is the common strategy used for ultrasound stimulation delivery²⁵, an electrical DC offset would be induced at the acoustic focus which is potentially capable of inducing neural stimulation.

To determine whether the amplitude of this DC offset is large enough to induce neuromodulation, we calculated the electric field amplitude. At DC the induced electrical offset at the focus at 1MPa is $500\mu V$ (**Fig 6-6 f iv**), and the focal maxima to minima distance is half the wavelength (1.5mm) since $\lambda = 2.97\text{mm}$ at 500kHz. To estimate the acoustoelectrically generated electric field, we divided the measured DC offset amplitude by the focal distance yielding 0.33V/m. While this is below the generally accepted threshold to induce neuromodulation^{182,183}, we performed an additional phantom study providing evidence the impedance of the ionic medium effects the acoustoelectrically

generated amplitudes (see **Fig 4-9**). Since mouse brain has an impedance 8 times higher than 0.9% saline, this would now place the acoustoelectrically generated amplitude at 2.66V/m which is above the threshold for electrical neurostimulation found in previous studies^{35,184}. Recent studies also point to the activation function (gradient of the electric field) being the key indicator for electrical brain stimulation efficacy^{185,186}, suggesting the induced electric field gradients from the focal electric field may be more important than the electric field amplitude alone in determining stimulation efficacy. This induced focal electrical field now becomes a viable candidate for the mechanism behind ultrasound brain stimulation.

6.3.2 An acoustoelectric interaction is driven by the acoustic field and electric artefact from the ultrasound.

Next, we explored whether the origin of the electrical mixing products is due to non-linear acoustics, or the acoustoelectric interaction. If the acoustoelectric interaction is the cause of the focal electric field, there must be a large high frequency electric field that is mixing with the acoustic field, which we do find in all our measurements in the form of the RF electrical artefact from powering the piezoelectric element in the ultrasound transducer. As the transducer emits a high frequency and high-power electrical RF signal, it will capacitively couple to any ionic body in its path forming an RF monopole antenna^{187,188}.

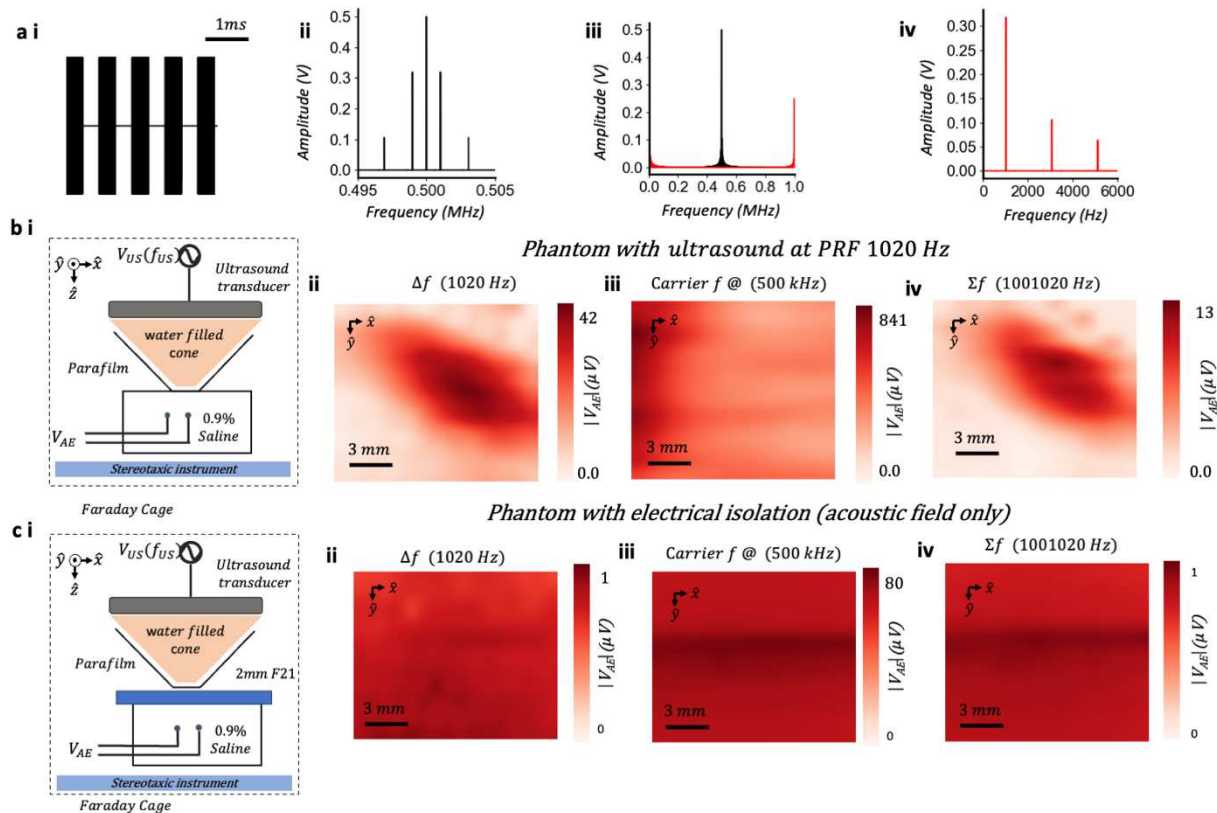


Figure 6-7 | Ultrasound undergoes an acoustoelectric interaction induced from the transducer electrical artefact.

a, i) Acoustic field time series with pulse repetition frequency 1020Hz, chosen to avoid mains harmonics **ii)** amplitude spectral density (ASD) of the timeseries waveform shown in **i)**, **iii)** Multiplying the timeseries waveform by itself, then taking the ASD shown in red, comparison with original waveform in black. **iv)** PRF*PRF at Δf . **b, Ex vivo** phantom electrical measurements sum and difference components of applied acoustic signal show resulting focal field where PRF = 1020Hz, carrier frequency = 500kHz, Pressure maximum = 1MPa, with stereotaxic instrument moved in 0.5mm increments over area. **i)** experiment arrangement **ii)** difference frequency map **iii)** carrier frequency map **iv)** sum frequency map. **c, i)** Phantom measurements as in **b**, with 2mm thick F21 acoustically transparent and electrically isolating material between saline and transducer cone. **ii)** Difference frequency shows no focal area. **iii)** Carrier frequency **iv)** sum frequency shows no focal area.

To test if the transducer was supplying the electric field required to induce an acoustoelectric interaction, we applied a pulsed 500kHz waveform at 1020Hz pulse repetition rate (PRF) (**Fig 6-7 a i**), with ASD shown in **Fig 6-7 a ii**, to obtain sum and difference frequencies (**Fig 6-7 a iii, iv**). We selected the pulse repetition rate of 1020Hz to avoid any mains harmonics confounding results. When the 500kHz PRF signal is multiplied with itself we see that the odd harmonics at 1020Hz, 3060Hz, 5100Hz now decrease sequentially in amplitude. Previously Darvas et al.¹⁸⁹ has also reported a 1kHz

difference frequency when applying a 1kHz pulsed ultrasound signal. Then, by moving the stereotaxic instrument in 0.5mm increments, we mapped the focal sum and difference frequencies in XY space, as well as exposing the comparatively high amplitude and non-focal electrical carrier frequency at 500kHz (**Fig 6-7 b**). If the mixing interaction was due to an ionic piezoelectric effect whereby the acoustic wave induces a direct electrical response at the applied acoustic frequency, we would have expected the carrier frequency to also have the focality of ultrasound.

We then used an acoustically transparent and electrically insulating material (2mm thick F21, Precision Acoustics, UK) characterized in **Fig 6.3 k i, ii**, to determine if shielding the medium from the RF electric field emanating from the transducer decreases the focal mixing amplitude at the sum and difference frequencies. In **Fig 6-7 c**, we can see the amplitude of the carrier is diminished by the F21, and the focal area at the sum and difference frequency have disappeared. Thus, the emergent acoustoelectric field is dependent on the electrical output of the ultrasound transducer and the focality of the acoustic field. This is evidence that a focal electric field is generated via acoustoelectric mixing from the RF antenna generated by powering the ultrasound transducer and the propagating acoustic field and is not due to non-linear acoustics - since an interaction based only on non-linear acoustics would not be affected by changes in electric field amplitude.

Next, we investigated whether this emergent focal acoustoelectrically generated electric field is present in biological tissue, through *in vivo* ultrasound and acoustoelectric neuromodulation experiments.

6.3.3 Confirming the source of the electrical artefact at 500kHz

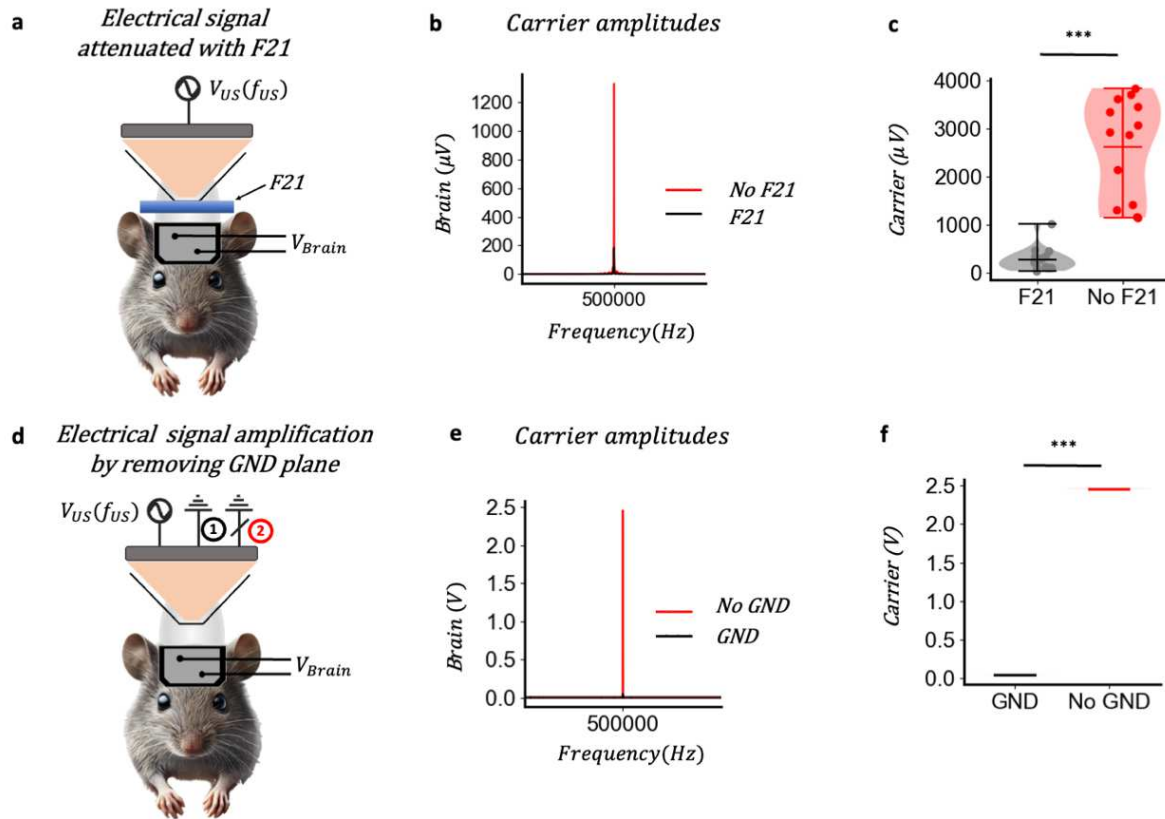


Figure 6-9 | Identifying the source of the RF electrical artefact at 500kHz, with 40Vpp, 1MPa acoustic sinusoid.

a, The electrical signal from the ultrasound transducer can be attenuated using 2mm thick F21(Precision Acoustics, UK). F21 has acoustic impedance water, making it acoustically transparent and electrically insulating. **b**, Carrier amplitude comparison between case where no F21 is between the transducer cone and the mouse, and when it is placed to attenuate the electric signal from the transducer. **c**, Carrier amplitude comparison; In the below group statistics, $n=4$ male mice were used in the light ketamine/xylazine anaesthesia window. Each mouse performed the with and without F21 test twice, with each recording of 6 second duration. t-test, two sided $t_{(16)} = -4.99$, $P = 1.86e-5$; F21 group (mean $\pm$ s.d. = $2003 \pm 1410 \mu V$); without F21 group (mean $\pm$ s.d. = $270 \pm 219 \mu V$). **d**, Electric signal amplification, by removing the GND plane of the ultrasound transducer. A switch was added to the transducer cable, which disconnected the GND plane entirely while sending through the same amplitude 1MPa signal. **e**, Representative view of large voltage 500kHz artefact when GND plane is removed from transducer. **f**, Carrier amplitude comparison with GND connected and disconnected; t-test, two sided $t_{(4)} = -1207$, $P = 1.47e-22$; No GND group (mean $\pm$ s.d. = $2.457 \pm 3.9 mV$); GND group (mean $\pm$ s.d. = $0.044 \pm 0.4 mV$).

To confirm the source of the 500kHz electrical artefact signal as being clearly from the transducer, F21 was used to attenuate the electric signal. **Fig 6-9** shows the voltage measurement in the mouse brain when a 1MPa (40Vpp) signal is output by the RF amplifier in all plots. The measurement electrodes embedded in the brain are affected by

the ultrasound transducers electrical signal, despite their being no direct conductive electrical connection, as non-conductive parafilm separates the transducer from the conductive acoustic gel and mouse head, due to capacitive coupling at these RF frequencies between all ionic media near the transducer⁴. This electrical artefact can be attenuated using an electrically insulating and acoustically transparent material called F21 (Precision Acoustics, UK) (**Fig 6-9 a, b, c**). There is an extremely statistically significant difference in the carrier amplitude when this electric shielding test was repeated over 17 recordings ($P = 1.86e-5$). Conversely, removing the GND connection of the ultrasound transducer will amplify the signal measured in the brain of the mouse, as more of the energy is transmitted through the air and into the environment when the GND plane is not available as a circuit return (**Fig 6-9 d, e, f**).

Combining the above evidence as to the amplitude and source of the 500kHz electric field, with the magnitude calculation required for stimulation in **Section 6.3.1**, we can see that the resultant acoustoelectric amplitude is well above the threshold necessary for neural stimulation – particularly at DC when the acoustic and electric field frequency are the same, due to the amplitude versus difference frequency trend being reliant on medium impedance(**Section 4.2.7**) . Furthermore, the amplitude of this resulting electric field may be dependent on the grounding of the transducer or subject.

6.3.4 *In vivo* ultrasound stimulation is correlated to the measured acoustoelectric difference frequency amplitude in the brain.

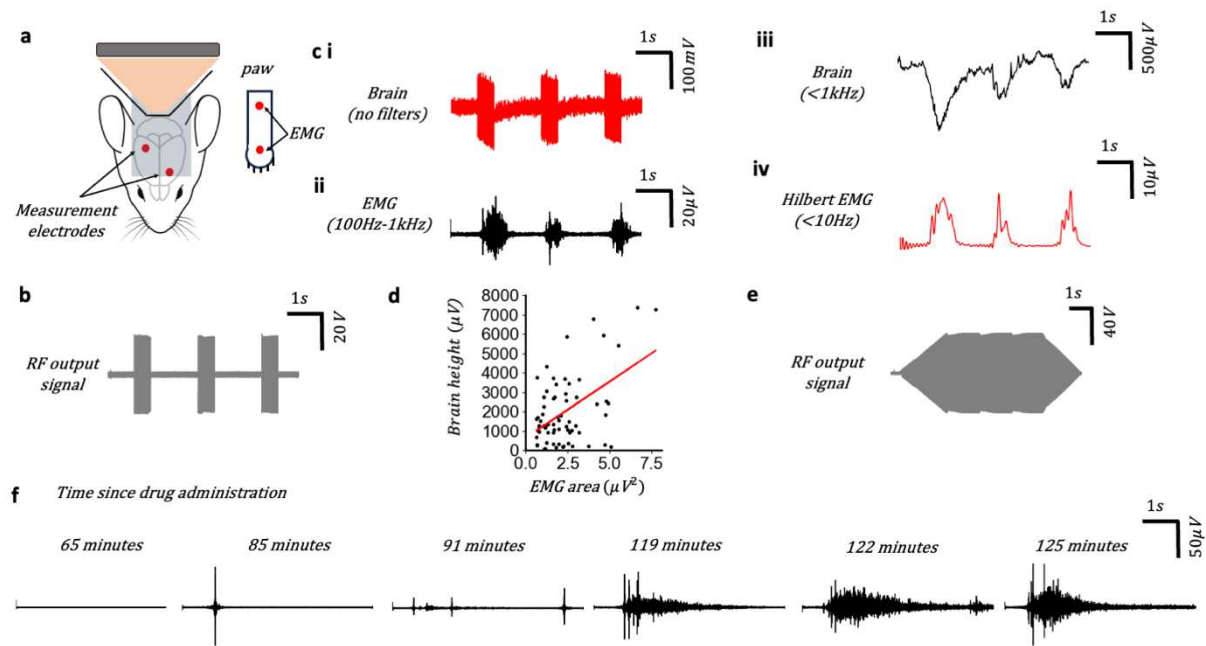


Figure 6-10 | *In vivo* ultrasound stimulation evokes a low frequency electrical signal at the difference frequency of the acoustic field.

a, 500kHz, 1MPa ultrasound applied to a mouse with two measurement electrodes in the brain detailed in Methods 6.2.4 and EMG measurement electrodes. Sampling rate 5MHz, duration 6seconds. **b**, 40V peak-peak signal output from the RF amplifier to the ultrasound transducer, calibrated to 1MPa based on Methods Fig 6.3, **c**, Raw signal without any filters measured in the brain at gain = 200. ii) Brain signal with 1kHz low pass filter applied. iii) EMG signal band filtered between 100Hz-1kHz. iv) EMG with applied Hilbert transform and low pass filter to expose the envelope of the EMG. **d**, Brain height versus EMG area with regression line brain height = $580 \times \text{EMG area} + 644$, $n=6$ mice over 24 recordings each with 3 pulses. **e**, RF monitor channel of 10 second duration 500kHz, 2MPa ultrasound applied continuously for 6 seconds, with a ramp at the beginning and end, $F_s = 5\text{MHz}$. **f**, Time series showing EMG traces when the same 2MPa acoustic signal is applied as in e, at increasing time since the beginning of drug administration.

Since we now have evidence that the ultrasound transducer can induce an acoustoelectric interaction in an ionic medium, it becomes a possibility that ultrasound neuromodulation works via this more direct electrical stimulation mechanism at the focal difference frequency. We performed a recovery mouse surgery (**Methods 6-2-3**) to enable brain and electromyography (EMG) measurements with concurrent pulsed ultrasound stimulation (**Fig 6-10 a**). Ultrasound pulses at 500kHz and 0.5 seconds in duration at 1MPa pressure are repeated at 2 second intervals throughout a 6 second 5MHz sample rate trial (**Fig 6-10 b**) while mice are under light ketamine/xylazine sedation (**Methods 6-**

3-4). We observed the 500kHz electrical artefact from the ultrasound transducer in the raw brain signal as a 200mV peak-to-peak signal at 500kHz (**Fig 6-10 c, i**) and note that EMG is elicited at the same pulse timings (**Fig 6-10 c, ii**). While the electrical artefact from the ultrasound transducer at 500kHz is commonly reported^{25,34}, when we low pass filter the signal recorded from the brain below 1kHz we observe a low frequency signal 1mV peak-to-peak amplitude DC offset at the onset of each pulse and it is this signal that is in the electrophysiological frequency range to induce neural responses¹⁹⁰ (**Fig 6-10 c iii**). Unlike the applied RF signal which remains at constant amplitude for each pulse, the low frequency signal varies in amplitude between ultrasound pulses, where the first pulse incurs a DC shift which is still waning when the second pulse starts, similar to the onset effect reported in ultrasound stimulation¹¹⁷. The measured pulse signal mirrors what we see in the phantom measurements of a continuous 500kHz wave inducing a difference frequency offset at 0Hz (DC) (**Fig 6-10 f**).

To determine whether the EMG response is correlated to the amplitude of the low frequency electric signal measured in the brain, we take the peak-to-peak pulse height of the low pass filtered brain signal and quantify the EMG spike train by taking its Hilbert transform, applying a 10Hz low pass filter and computing the area under each pulse (**Fig 6-10 c iv**). There is a weak correlation between the height of the brain signal and the EMG area, with an $r^2 = 0.25$. Hence the linear regression explains 25% of the variance in the data, leaving the rest of the variance to other biological factors such as when the mouse last ate and whether it is hydrated. r^2 values less than 50% are still considered good in biological data^{191,192}, though this suggests more experiments are required to show strong causal proof of interdependence.

Next, we investigated causes of variance in the EMG response to ultrasound stimulation, observing large EMG amplitude changes as the anaesthesia level lightened whilst the applied pressure remained constant at 2MPa (**Fig 6-10 e, f**). Anaesthesia lasted 1-2 hours and be too deep for the first period to evoke an EMG response. There would be a sudden onset whereby an EMG response could be evoked by ultrasound stimulation, from which point onwards the amplitude of the EMG response would increase until

spontaneous movements began. All mice were dosed similarly according to weight, though large time differences in the total time under anaesthesia were observed, and some mice had larger EMG responses at lower pressures than others. The variance in the EMG response amplitudes has been discussed in other works³¹. It is possible reflections inside the mouse head play a role also in the resultant evoked amplitude.

6.3.5 *In vivo* ultrasound stimulation is amplitude dependent on the electric field present in the medium

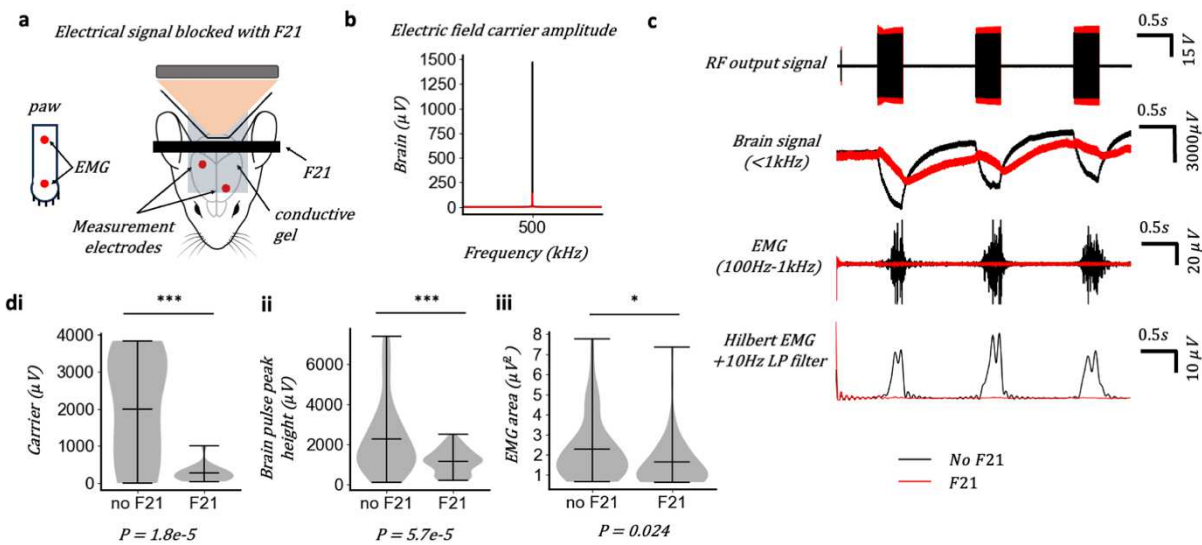


Figure 6-11 | *In vivo* ultrasound neuromodulation can be decreased in amplitude by blocking the electrical artefact from powering the ultrasound.

a, Mouse undergoing pulsed ultrasound with electrical artefact attenuated with F21. **b**, ASD of signal measured in the brain showing larger peak at 500kHz when no F21 in place (black), with comparison against electrical attenuation when F21 is in place (red). **c**, Representative signal without F21 (black) and with 2mm thick F21 (red) of the RF output signal, signal as measured by the electrodes in the brain (filtered <1kHz), EMG and Hilbert transformed low pass (10Hz) filtered EMG. Pressure without F21 = 2MPa, pressure with F21 = 2.4MPa to overcome acoustic attenuation **d**, n=5 mice, 20 trials in each group with each trial containing 3 pulses, where pressure with F21 is increased by 20% to overcome acoustic attenuation (Methods 6.3 k i, ii). i) Carrier amplitude comparison with and without F21 $p = 1.8 \times 10^{-5}$ ii) brain signal amplitude comparison $p = 5.7 \times 10^{-5}$ iii) EMG area comparison $p = 0.024$

To decouple the ultrasound electric artefact from the acoustic field *in vivo*, we repeated the F21 electrical shielding experiment performed in the physiological phantom (**Fig 6.7 b**) in the mouse (**Fig 6-11 a**) with the pulsed ultrasound stimulation protocol as described in **Section 6.3.4**. We alternately applied the acoustic signal without F21, then elevated

the transducer placing the 2mm thick F21 between the cone and the gel whilst minimizing air bubbles and repeating this protocol 4 times during the light anaesthesia window of ketamine/xylazine sedation, minimizing the effects of the changing anaesthesia depth by alternating the test case. To overcome the small acoustic attenuation (**Fig 6.3 k ii**) induced from the F21, we added 20% to the pressure output when the F21 was used to overcome any acoustic attenuation as shown in Fig 6.3 k.

F21 decreased the amplitude of the 500kHz ultrasound transducer electrical carrier artefact as measured in the brain (**Fig 6-11b**). We applied a 2MPa pulsed ultrasound signal without F21 (black traces **Fig 6-11 c**), and saw it produced a larger amplitude low frequency brain signal, despite having a higher output amplitude. The resulting EMG measured without F21 has clearly visible spikes, while F21 electrically shielding the transducer from the mouse did not evoke an EMG response (red trace **Fig 6-11c**). Looking at group dynamics over 5 mice, with 4 trials of each group per mouse, the electrical shielding properties of F21 are extremely significant (**Fig 6-11 d i**, $p = 1.8e-5$) with a factor of 8 difference between the 500kHz electrical amplitude with and without F21 in place (no F21 mean $\pm$ st.d = 2003 $\pm$ 1410, F21 mean $\pm$ st.d = 270 $\pm$ 219). This trend is similarly mirrored in the brain amplitude comparisons (**Fig 6-11 d ii**) with $p = 5.7e-5$, despite the amplitude of the acoustic signal being higher when F21 was used (no F21 mean $\pm$ st.d = 2247 $\pm$ 1823 , F21 mean $\pm$ st.d = 1823 $\pm$ 593).

Though there is a clear decrease in amplitude in the low frequency electric brain signal, the EMG decrease is less clear when comparing the with and without F21 groups (**Fig 6-9 d iii**) ($p = 0.024$, no F21 mean $\pm$ st.d = 2.27 $\pm$ 1.59 , F21 mean $\pm$ st.d = 1.6 $\pm$ 1.27). This is likely due to anaesthesia induced amplitude variance, as switching the F21 in and out from under the ultrasound cone took about 2 minutes, which is enough time to make a significant difference to the amplitude of the evoked EMG. Though the test order was alternated between F21/no F21 measurements, the EMG had high variance. Hence this experiment appears trustworthy from a physics perspective (i.e. the carrier and low frequency brain amplitude results) but does not provide strong EMG evidence due to both the difficulty and time required to place the F21 in the narrow lightening anaesthesia time

frame. However, the difference frequency amplitude trends measured in the brain provide evidence that the electrical artefact from the transducer affects the acoustoelectric difference frequency amplitude in the brain.

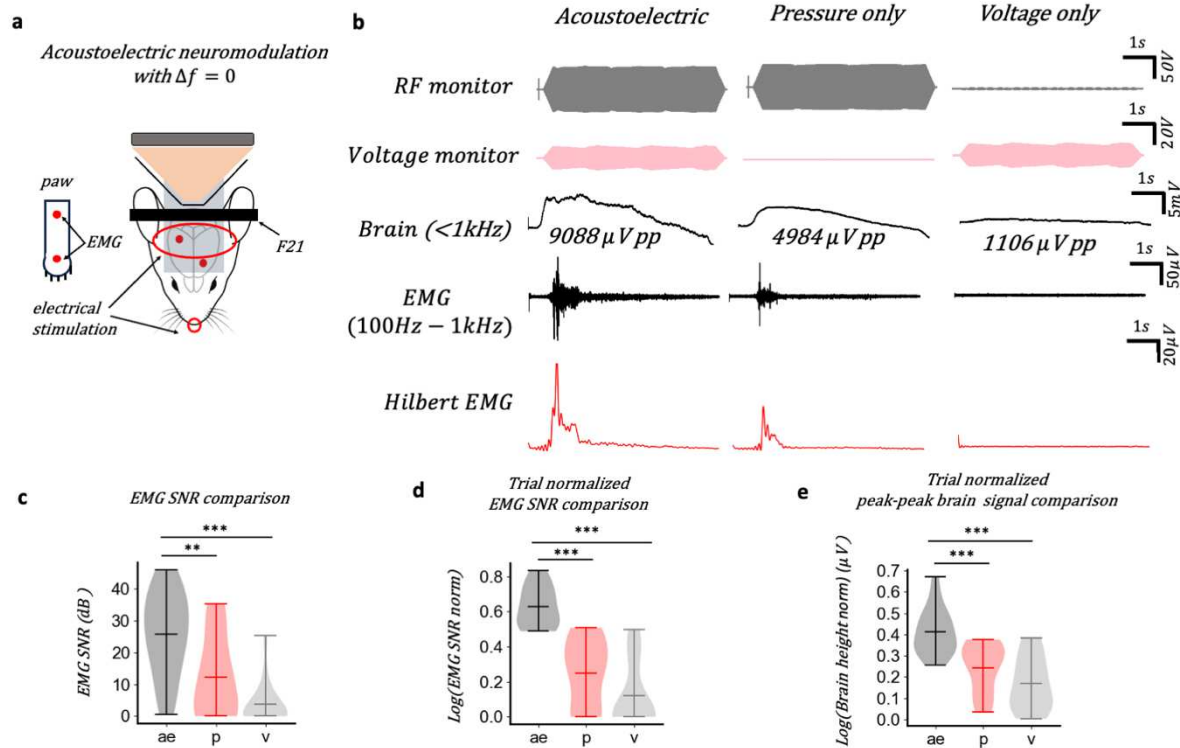


Figure 6-12 | *In vivo* ultrasound neuromodulation amplitudes are increased by applying a high frequency electric signal at the same frequency.

a, In vivo arrangement to apply ultrasound shielded via F21 and a bipolar electrical stimulus using a Pt-Ir halo and mouth electrodes, all recordings at $F_s=5\text{MHz}$ sampling rate, duration = 6 seconds. **b**, leave one out test with applied pressure 2.5MPa, voltage 40Vpp whilst brain and EMG signal are measured during acoustoelectric stimulation, pressure only, and voltage only. Peak-to-peak amplitude of acoustoelectric brain signal= $9088\mu\text{V}$, pressure only brain signal= $4984\mu\text{V}$ and voltage only brain signal= $1106\mu\text{V}$. **c**, $n=8$ mice, 2 leave one out trials for each mouse applied in randomized order to adjust for anaesthesia changes. Group comparison SNR comparison (dB) between acoustoelectric, pressure and voltage only field yields, ANOVA $F(2,13) = 15.01$, $p=1\text{e-}5$, post hoc comparisons using the Tukey HSD test, AE-P, $p = 0.005$; AE-V, $p = 6.25\text{e-}6$; P-V, $p = 9\text{e-}2$; **d**, Same data set as in d) with comparison of EMG SNR normalized for each leave one out test trio to adjust for anaesthesia changes. ANOVA $F(2,13) = 34.17$, $p=9.4\text{e-}10$, post hoc comparisons using the Tukey HSD test, AE-P, $p = 2.2\text{e-}6$; AE-V, $p = 9.58\text{e-}10$; P-V, $p = 6.9\text{e-}2$; **e**, Same data set as in d) brain signal peak-to-peak height comparison between acoustoelectric, pressure only and voltage only. ANOVA $F(2,13) = 19.97$, $p=1.8\text{e-}6$, post hoc comparisons using the Tukey HSD test, AE-P, $p = 5.02\text{e-}4$; AE-V, $p = 1.59\text{e-}6$; P-V, $p = 1.99\text{e-}1$;

To determine if the presence of an independent high frequency electric field at the same frequency as the ultrasound transducer can change the amplitude of acoustic

neuromodulation, we attenuated the electric field artefact coming from the ultrasound with F21 (**Methods Fig 6.3 k i,ii**) and added an independent bipolar electric field via a Pt-Ir halo and mouth electrode (**Fig 6-12 a**) at 500kHz. In each leave one out test pressure and the electric field were applied together at the same frequency, pressure alone and voltage alone, while all stimulation and recording traces were monitored simultaneously (shown in **Methods Fig 6-2**). To mitigate against EMG amplitude changes as the anesthesia depth lightened, each leave one out test was performed in randomized order. An additional low-pass filter was applied (**Chapter 3 Section 3.2.4**), to prevent the high amplitude high frequency electric signal from saturating the preamplifier.

When both an acoustic and an electric field are applied, we saw an onset response or DC offset at the difference frequency(0Hz) in the brain signal ($9088\mu V$) which was echoed in the EMG signal (**Fig 6-12 b**). Similarly, we see this same onset signal when pressure alone is applied ($4984\mu V$), though its amplitude is lower than when both an acoustic and electric field are applied. When only an electric field is applied, we see a much smaller brain signal deviation ($1106\mu V$) and no EMG response. The onset response is still present despite the use of F21, which only attenuates the electric signal from the ultrasound. By applying the waveforms at the same frequency, we ensure the mixing is at 0Hz (DC) which can be seen in all representative responses (**Fig 6-12 b**).

Looking at group responses we performed this test on $n=8$ mice with 2 leave one out trials for each mouse, where each leave-one-out trial of 3 recordings was performed in randomized order to account for time-based anesthesia changes. The EMG signal-to-noise was calculated by taking the Hilbert transformed, 10Hz low pass-filtered EMG amplitude at its maximum and the noise level estimated during the period before the fields were applied. Comparing the EMG signal-to-noise(dB) across acoustoelectric, pressure and voltage only groups we see an extremely significant difference in the ANOVA $F(2,13) = 15.01$, $p=1e-5$, with Tukey's HSD test returning very significant differences for the pairwise comparison AE-P, $p = 0.005$ and extremely significant for AE-V, $p = 6.25e-6$ (**Fig 6-12 c**). This strong effect is evidence that ultrasound stimulation is affected by the electric field present in the medium.

To account for anesthesia depth variance we normalized across each leave one out trial, computing the log normalized statistic comparison, yielding even higher significance (**Fig 6-12 d**) as the time-based effect of anesthesia depth has been minimized (ANOVA $F(2,13) = 34.17$, $p=9.4e-10$). Similarly, when we analyzed the group responses across mice for the acoustoelectrically generated signal in the brain, the acoustoelectrically generated brain signal was extremely significantly larger than pressure or voltage only cases (**Fig 6-12 e**) (ANOVA $F(2,13) = 19.7$, $p=1.8e-6$). The trends reported here provide additional evidence, alongside the phantom F21 decoupling findings, that non-linear acoustics cannot be the cause of ultrasound brain stimulation, as varying the electric field has such as significant effect on EMG amplitudes.

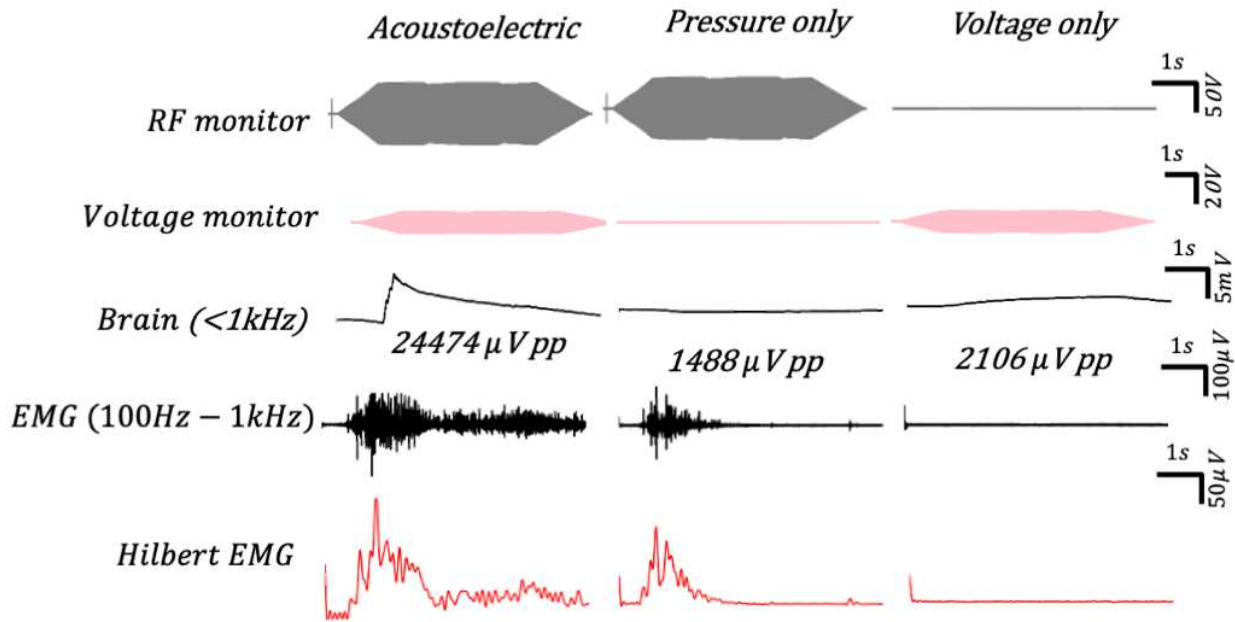


Figure 6-13 | *In vivo* acoustoelectric leave one out test with low anaesthesia @ $\Delta f = 0\text{Hz}$ (DC).

Representative leave one out test at same sample rate, duration, pressure and voltage amplitudes as Figure 6-12, with the only change being a lighter anaesthesia level ($P=2.5\text{MPa}$, $v = 40\text{Vpp}$). We observe a larger and longer duration EMG response to field onset. A small acoustic offset response can also be seen. The amplitude of the acoustoelectric brain signal is $24474 \mu\text{V}$, pressure only is $1488 \mu\text{V}$, and voltage only as $2106 \mu\text{V}$.

When the anesthesia depth is reduced the mouse becomes more responsive to both acoustic and electric fields (**Fig 6-13**), and we see a prolonged EMG response compared

to earlier stages of anesthesia (**Fig 6-12 b**). The anesthesia depth doesn't just affect the amplitude of the EMG response, it also affects its duration.

6.3.6 *In vivo* acoustoelectric neuromodulation: stimulation frequency is dependent on both the acoustic and electric field.

In the experiments described so far, the acoustic and electric frequencies were at the same frequency, enabling only amplitude-based evidence on the contribution of the electric field to ultrasound brain stimulation. To determine if the acoustoelectric interaction can also modulate the frequency of neural stimulation, we applied electric and acoustic fields at different frequencies in the same *in vivo* arrangement as **Fig 6-11**, using F21 to minimize the impact of the electrical signal emanating from the ultrasound transducer.

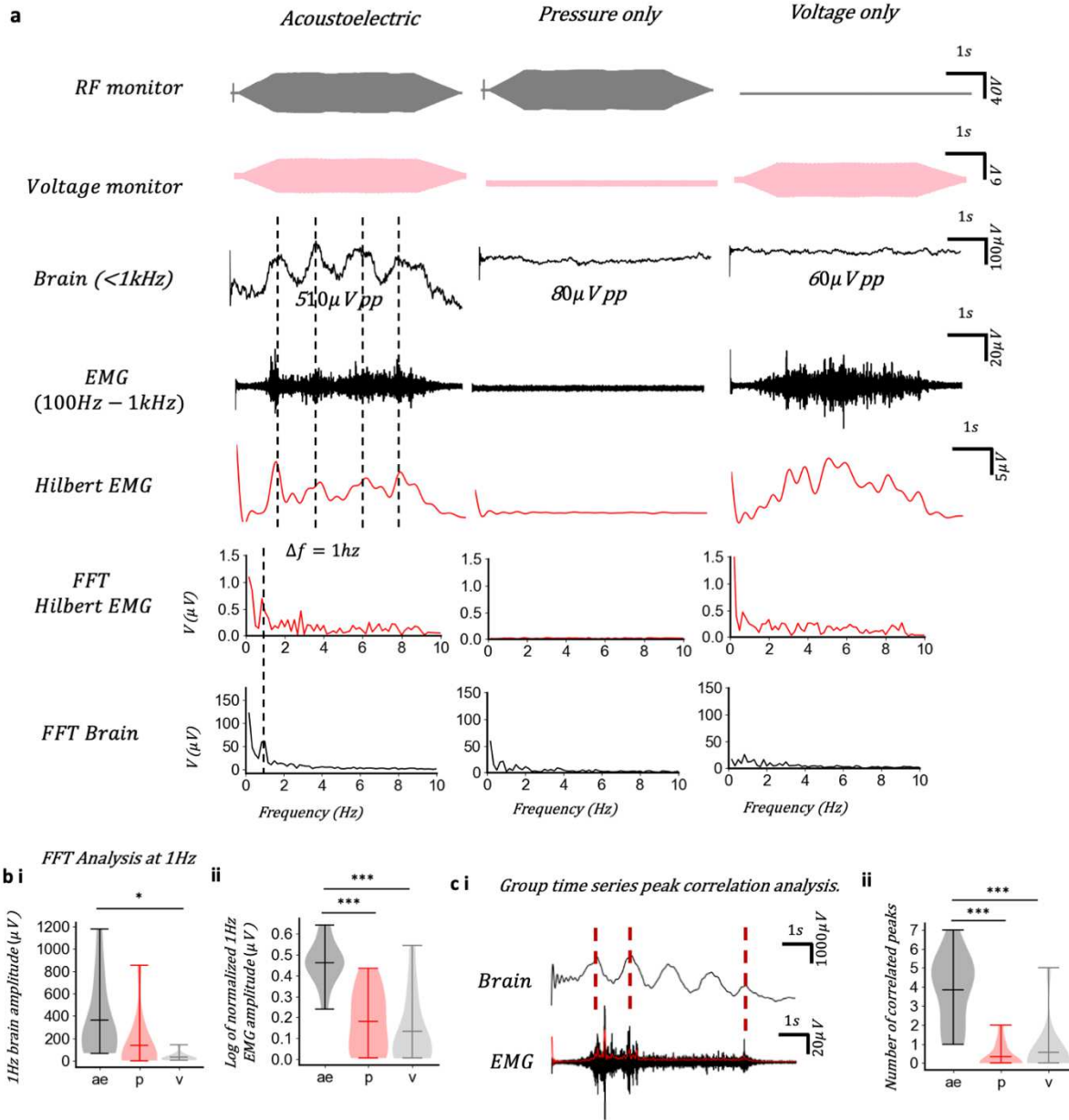


Figure 6-14 | In vivo acoustoelectric neuromodulation @ $\Delta f = 1 \text{ Hz}$.

a, 6 second duration, 5MHz sample rate leave one out test where the acoustic wave was applied at 500kHz 1.8MPa, and the bipolar voltage stimulus at 12Vpp, 500kHz +1Hz. The evoked difference frequency ($\Delta f = 1 \text{ Hz}$) can be clearly seen as measured via the brain electrodes and is mirrored in the Hilbert transform of the EMG signal (filtered <10Hz). The FFT of the EMG and brain signal, both have a peak at 1Hz. The peak-to-peak amplitude of the acoustoelectric signal is 510 μV , pressure only 80 μV , and voltage only 60 μV . **b**, i) The FFT amplitude at 1Hz was calculated for n=7 mice, 12 leave one out test performed when the anaesthesia was light enough to evoke an EMG response. ANOVA $F(2,9) = 5.57$, $p = 0.008$, post-hoc comparisons using the Tukey HSD test, AE–P, $p = 0.08$; AE–V, $p = 0.006$; P–V, $p = 0.55$; ii) The FFT 1Hz EMG amplitude was compared for all mice across acoustoelectric, pressure only and voltage only trials. ANOVA $F(2,9) = 17.9$, $p = 5.4 \times 10^{-6}$, post-hoc comparisons using the Tukey HSD test, AE–P, $p = 1.14 \times 10^{-4}$; AE–V, $p = 1.12 \times 10^{-5}$; P–V, $p = 0.71$; **c**, i) Group time series brain peaks that were time correlated to the Hilbert

transformed EMG peaks. Shown are 3 time-correlated peaks, demonstrating both the correlation and irregularity of the EMG response. ii) Time correlated peaks between the brain signal and EMG were counted across all trials and the acoustoelectric, pressure and voltage cases compared. ANOVA $F(2,9) = 22.4$, $p=7e-7$, post hoc comparisons using the Tukey HSD test, AE-P, $p = 2.7e-6$; AE-V, $p = 9.8e-7$; P-V, $p = 9e-1$);

The difference frequency ($\Delta f = 1\text{Hz}$) was clearly visible (**Fig 6-13 a**) in both the signal recorded in the brain, and the Hilbert transform of the EMG. We then repeated this across $n=7$ mice, with leave one out tests performed in randomized order to adjust for the anesthesia depth confound. We looked at the FFT amplitude measured in the brain across acoustoelectric, pressure and voltage only groups (ANOVA $F(2,9) = 5.57$, $p=0.008$), with a significant difference between the acoustoelectric and voltage only groups (**Fig 6-12 bi**). Since the EMG amplitude changed rapidly over time based on the decreasing anesthesia level, each leave one out trial was normalized against the EMG amplitude at 1Hz across all 3 groups, then the log of this was taken to determine the statistical relationship¹⁹³. We found extremely significant differences in the FFT analysis proving the acoustoelectric interaction has an extremely significant impact on the difference frequency amplitude of the resulting evoked EMG stimulation, when compared to pressure or voltage alone (ANOVA $F(2,9) = 17.9$, $p=5.4e-6$, post-hoc comparisons using the Tukey HSD test, AE-P, $p = 1.14e-4$; AE-V, $p = 1.12e-5$; P-V, $p = 0.71$).

Though the FFT analysis reveals a statistically significant trend, not all recordings had a clear 1Hz EMG pattern due to the onset effect induced by the ultrasound which causes a large DC offset (**Fig 6-12 c i**) as described in both (**Fig 6-4 f**), (**Fig 6-8**). To account for this, a higher sensitivity time series test was developed to correlate the peaks seen in the low frequency acoustoelectrically generated brain signal (see **Section 6.3.8**), with the timing of the Hilbert envelope peaks in the EMG response. In the typical representative signal (**Fig 6-12 c i**) we can see two time-correlated peaks at the beginning and one at the end. By counting the total number of time-correlated EMG and brain peaks we saw an even stronger statistically significant trend in the leave-one-out tests (**Fig 6-12 c ii**) providing further evidence acoustoelectric neuromodulation induces a neural response to the mixing frequency between the acoustic and electric field (ANOVA $F(2,9) = 22.4$, $p=7e-$

7, post hoc comparisons using the Tukey HSD test, AE–P, $p = 2.7\text{e-}6$; AE–V, $p = 9.8\text{e-}7$; P–V, $p = 9\text{e-}1$).

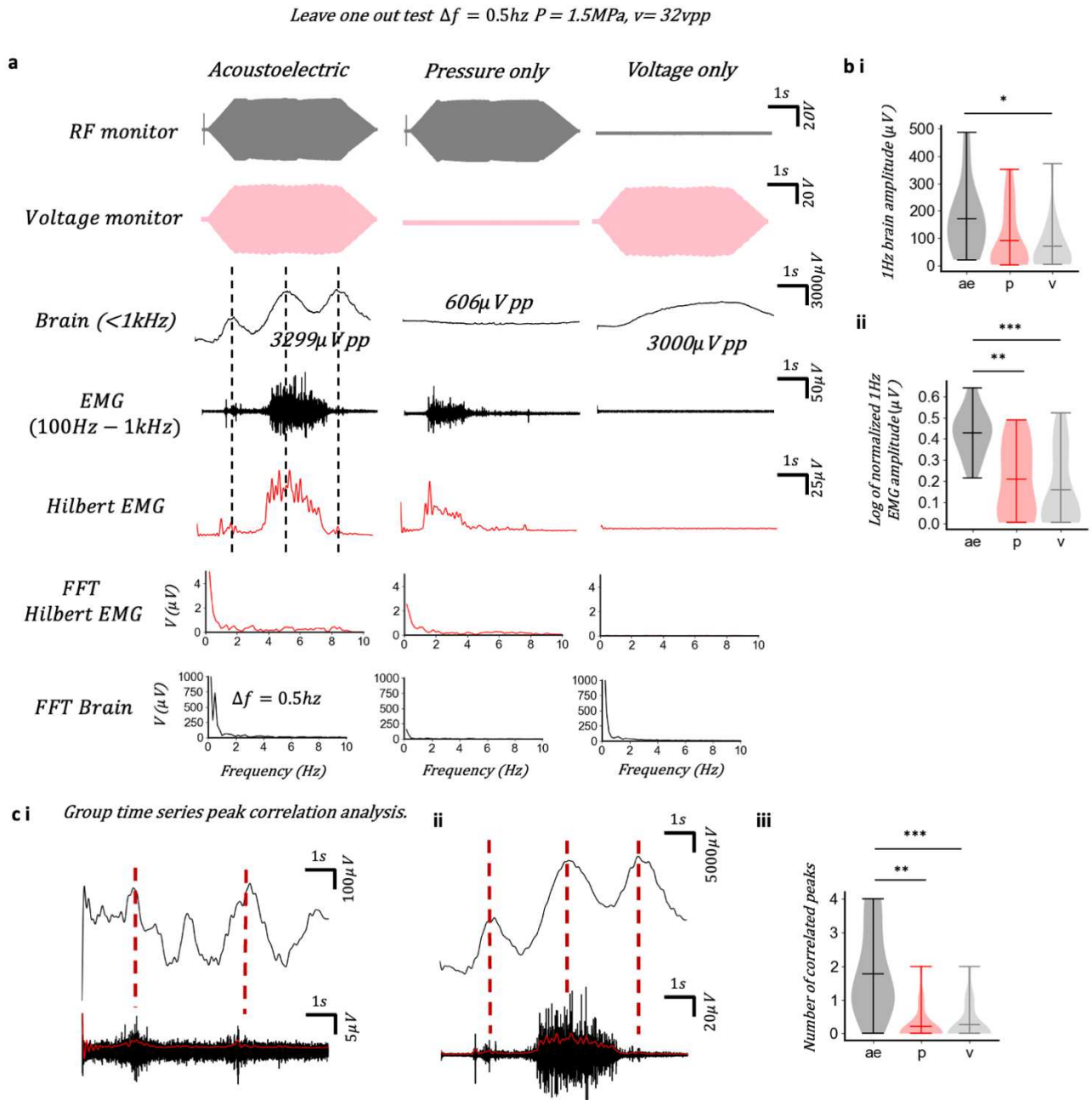


Figure 6-15 | *In vivo* acoustoelectric neuromodulation @ $\Delta f = 0.5\text{Hz}$.

a, 6 second duration, 5MHz sample rate leave one out test where the acoustic wave was applied at 500kHz 1.5MPa, and the bipolar voltage stimulus at 32Vpp, 500kHz +0.5Hz. The peak-to-peak amplitude of the acoustoelectric signal is 3299 μV , pressure only 606 μV , and voltage only 3000 μV . **b**, $n=5$ mice, 18 leave one out tests within anaesthesia selection criterion, randomized in recording order to avoid anaesthesia timing confound. Group FFT analysis same as in Fig 6-12 **b**, i) ANOVA $F(2,15) = 3.85$, $p=0.02$, post-hoc comparisons using the Tukey HSD test, AE–P, $p = 0.1$; AE–V, $p = 0.029$; P–V, $p = 0.83$; ii) ANOVA $F(2,15)$

= 14.77, $p=8.6e-6$, post-hoc comparisons using the Tukey HSD test, AE–P, $p = 3.47e-4$; AE–V, $p = 1.45e-5$; P–V, $p = 0.61$); **c**, Group peak correlation analysis same as in **Fig 6-14c**. i) Time series representation of brain signal and peak correlated EMG. Though the peaks are correlated with the EMG response for the two largest peaks, the brain amplitude of the acoustoelectric signal is slightly irregular. ii) Representative signal showing 3 correlated peaks, despite the second peak having the largest EMG response due a large DC offset induced in the signal from the second peak onwards. iii) ANOVA $F(2,15) = 17.9$, $p=1.25e-6$, post-hoc comparisons using the Tukey HSD test, AE–P, $p = 7.9e-6$; AE–V, $p = 1.53e-5$; P–V, $p = 0.98$);

In addition to the 1Hz acoustoelectric neuromodulation study, we repeated the acoustoelectric neuromodulation experiment with a difference frequency of 0.5Hz between the applied acoustic and electric field (**Fig 6-15 a**), with each leave one out trial being recorded in randomized order to mitigate against the anesthesia depth confound. Though the brain signal FFT amplitude test shows significance (**Fig 6-15 b i, ii**), it also includes the large adjacent DC offset induced from the onset of the two fields which may confound results. The peak correlation test has greater sensitivity and specificity for accounting for the irregular low frequency variation that was frequently seen in the 0.5Hz trials (**Fig 6-15 c i**) and the amplitude variations that they caused (**Fig 6-15 c ii**) i.e. the EMG appeared largest when a DC shift and 0.5Hz difference frequency coincided. 0.5Hz acoustoelectric neuromodulation was extremely significant with this more sensitive test, providing further evidence of acoustoelectric neuromodulation (ANOVA $F(2,15) = 17.9$, $p=1.25e-6$, post-hoc comparisons using the Tukey HSD test, AE–P, $p = 7.9e-6$; AE–V, $p = 1.53e-5$; P–V, $p = 0.98$).

A note on *in vivo* acoustoelectric neuromodulation analysis

The peak correlation analysis used in the acoustoelectric 1Hz and 0.5Hz tests was performed using the Python based `find_peaks` function on the Hilbert transformed EMG data which was then 10Hz low pass filtered to reveal the envelope of the EMG and the 10Hz filtered brain signal data. EMG data was considered a peak correlation match if it occurred within 0.2 seconds of the brain signal peak. We selected this range based on seeing that large deltas or high gradient high amplitude low frequency brain signals appeared most likely to induce EMG. The typical example of this is shown in (**Fig 6-15 c ii**), where there are 0.5Hz EMG signals but they are of very different amplitude, and the largest EMG spike burst is correlated with the largest delta in the low frequency brain signal. Typically, there were no time aligned peaks in the pressure or voltage data, which

were compared against the acoustoelectric brain signal in each case. Occasionally, there would be a single time aligned peak during the onset, and these are included in the statistics shown.

The peak correlation analysis has higher sensitivity and specificity than the FFT analysis, as there may only be time aligned peaks in segments of the recorded trial. Furthermore, it is robust to EMG noise due to electric field alone (**Fig 6-13 a**) as the EMG peaks in the electric field only data do not time align with those in the 1Hz data despite there being EMG activity in both the acoustoelectric and electric field only cases. When the pressure induced acoustoelectric field generated from the RF artefact from the ultrasound is above the EMG threshold also, the peak correlation analysis on the EMG is robust against false positives, as can be seen by the results in the statistics.

6.3.7 *In vivo* acoustoelectric neuromodulation is not dependent on the temperature dissipation envelope.

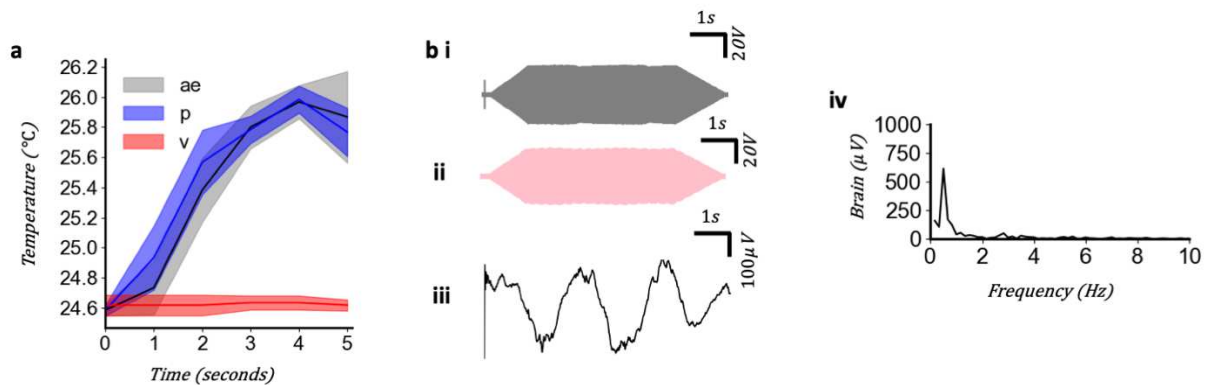


Figure 6-16 | *In vivo* acoustoelectric neuromodulation is not dependent on the temperature dissipation envelope.

1MPa pressure, 40V pp applied voltage signal, 6 second duration, repeated 6 times using a thermometer placed in the ultrasound gel just above the skull and below the ultrasound cone. Difference frequency of 0.5Hz. a, Temperature change with respect to time for each case of 0.5Hz acoustoelectric signal leave one out test, repeated 6 times per group. b, i) RF output monitor signal showing shape of pressure (1MPa) signal for each recording in a) ii) applied voltage signal (40Vpp). iii) Brain signal filtered <10Hz to reveal clear 0.5Hz oscillation. iv) Amplitude spectral density (ASD) FFT of brain signal showing clear peak at 0.5Hz.

A temperature probe was placed in the ultrasound gel above the skull and below the end of the cone, while a continuous 6 second acoustoelectric signal was applied. To determine

if temperature might be the cause of the neuromodulation, we applied an acoustic field at 500kHz and electric field at 500kHz + 0.5Hz to generate an acoustoelectric field at 0.5Hz. While applying continuous ultrasound at 1MPa does increase the temperature (**Fig 6-16 a**, shown in blue), this temperature increase is uncorrelated to the trajectory of the low frequency electrical signal generated in the brain when an acoustic field mixes with an applied electric field (**Fig 6-16 b iii**). Hence, temperature is not the cause of acoustoelectric neuromodulation or ultrasound brain stimulation. Further studies could place the thermoprobe in the brain of the mouse – I did not do this as I wished the mouse to recover.

6.4 Discussion

This Chapter provides the first *in vivo* evidence of a new form of neuromodulation induced through acoustoelectric heterodyning and forms an evidence-based argument as to why the acoustoelectric interaction may be a contributing mechanism to ultrasound brain stimulation.

Ultrasound alone induces electrical frequency mixing products at the sum and difference frequency of the applied waveform in a saline medium. These frequency mixing products are focal with the acoustic field and can be measured as an electrical signal. This suggests either non-linear acoustic mixing or the acoustoelectric interaction are responsible for this low frequency signal which is also in the electrophysiological range of stimulation. To separate non-linear acoustics effects from the acoustoelectric interaction, we partially blocked the source of the electric field – the RF antenna generated by electrically powering the piezo element in the transducer which capacitively couples to the ionic medium, using the acoustically transparent and electrically conductive F21 material. We find that the electric field artefact from powering the transducer does impact the focal electric field amplitude created at the mixing frequencies. Thus, an acoustoelectric interaction¹⁰² is driven by the acoustic wave and electric artefact from the ultrasound transducer.

Translating these phantom findings to *in vivo* experiments, we see the same low frequency electric signal in the brain of a mouse during standard pulsed ultrasound neuromodulation. Furthermore, the evoked EMG amplitude roughly correlates to the amplitude of this electric signal measured in the brain. However, the evoked EMG amplitude varies greatly based on anaesthesia depth, leading to a large degree of variance in a direct linear correlation.

To show amplitude dependence on both the electric and acoustic field in ultrasound brain stimulation, we attenuated the electric field from the transducer with the insulating F21 material and find that the difference frequency signal in the brain is significantly attenuated when using the insulating material. We then explore what happens when an independent high frequency electric field is applied at the acoustic frequency and find that the EMG evoked amplitudes during ultrasound brain stimulation have an extremely significant increase. Hence, ultrasound brain stimulation is amplitude dependent on both an acoustic field and the electric field generated by the piezo element electrical artefact, indicating non-linear acoustics or the acoustic radiation force (ARF) ¹⁹⁴ cannot be the cause of the electric difference frequency measured in the mouse brain. Recent literature has also ruled out the acoustic radiation force as it is too weak¹⁹⁵.

To show frequency dependence on the acoustoelectric interaction, we then apply an acoustic and electric field at a difference frequency of 1Hz and see that EMG spikes peak at the same 1Hz frequency providing the first evidence of acoustoelectric neuromodulation. This trend can be seen in both the FFT and by correlating the brain signal to the EMG spikes across 6 mice. We repeat this test and get similar results at 0.5Hz. In each case the electric brain signal may be irregularly shaped yet has a clear difference frequency trend. The onset of the ultrasound and electric field induce their own DC offset effects in all measured low frequency electric brain signals, such that the evoked difference frequency also has an accompanied changing DC component which affects the EMG threshold response. ¹⁷⁹¹⁹⁵Another potential mechanism is the temperature change due to heat dissipation from the acoustic field. By measuring the temperature and acoustoelectric envelope, we show that they are uncorrelated, evidence

that acoustoelectric neuromodulation is not due to thermal dissipation from the acoustic field.

Recent articles have shown calcium accumulation and ion channel amplification¹⁹⁶ when ultrasound stimulation is applied. While extracellular Ca^{2+} is shown to be the essential ionic initiator of ultrasonic brain stimulation, there are many other articles showing Ca^{2+} is also modulated by electric fields¹⁹⁷, in tACS and tDCS studies. Hence, the literature to date has not eliminated the possibility that ultrasound brain stimulation could be modulated by the more direct activation of voltage gated ion channels when undergoing acoustoelectric neuromodulation. It is likely that the measurement of the low frequency electric signal has not been reported because electrophysiological signals are typically recorded at or below 100kHz, and if no filters on the recording equipment are present the ultrasound high frequency artefact (500kHz) would dominate as random noise when sampled at a frequency below the ultrasound carrier, obscuring other embedded signals such as the smaller mixing frequency signals. By using a high recording sample rate of 5MHz and appropriate filtering, the low frequency electric brain signals are revealed.

Evidence provided in this Chapter shows that ultrasound brain stimulation is impacted by the surrounding electrical environment in the ionic medium. The heterodyning effect that occurs between the acoustic and the electric fields present in the medium creates an extremely significant amplitude and frequency difference to the neural evoked EMG signals. While the RF electric artefact from powering the piezo element is the dominant electrical contributor to acoustoelectric neuromodulation, it is also possible that endogenous electric fields or even acoustic only bioeffects contribute as well, and to what extent would require further exploration.

Many of the acoustoelectrically generated low frequency electric fields measured in the brain, had less than an ideal difference frequency response, affected by the DC offset of both the ultrasound and electric field. Acoustic reflections may also play a role in making the generated acoustoelectric field less than perfect within the mouse head as areas of constructive and destructive acoustic wave interference mix with the electric field. By

using a larger mammal and smaller transducer wavelength the internal reflections could possibly be minimized. Understanding which fields contribute to ultrasound stimulation opens doors to refining and optimizing this tool for more expansive therapeutic use in the future.

A further limitation is our understanding of the basic biophysics of electrical stimulation and its dependence on the axonal geometry with respect to the applied fields. For instance, if the neural stimulation effect is due to the activation function^{183,198} (the gradient of the electric field taken along the direction of the axon), then the orientation of the applied acoustic and electric field with respect to every neuron will affect stimulation amplitude, as well as the spatial focus of the evoked acoustoelectric field as this gradient will also guide the stimulation amplitude¹⁸².

Our understanding of the interaction between acoustic and electric fields in biological systems is in its infancy, whilst having potentially broad reaching implications on our ability to induce controlled and spatially focused stimulation of neural tissue.

Chapter 7: Discussion and conclusions

7.1 Overview

The non-invasive sensing and stimulation of electrical signals in the brain, with high spatial and temporal specificity, has long been a strategic goal in neuroscience. A highly focal bidirectional neural interface would enable better diagnostic and therapeutic tools, shedding new light onto epilepsy, Alzheimer's, OCD, depression, Parkinson's and further our understanding of brain dynamics.

The acoustoelectric interaction has potential to enable new bidirectional neural interfacing tools utilizing the focal specificity of ultrasound, while effecting and detecting neural signalling via direct electrical means – the native mode of interaction in neuron-to-neuron communication. However, this unique combination of advantages brings with it a similar set of challenges which deserve further research and development efforts. In this final Chapter the scientific advances made in this dissertation are summarised.

7.2 Key advances

7.2.1 Chapter 2: Acoustoelectric theory and simulation

The previously accepted model of the acoustoelectric interaction was based on Ohm's law which does not account for the vector nature of the underlying physics. To further our understanding of the phenomenon, we derive a new mathematical model using Gauss' theory. The new acoustoelectric mathematical model enables key new predictions, one of angular dependence between the incident electric and pressure fields due to the

introduction of the acoustic gradient term due to the vector multiplication, and that heterodyning will occur between the electric and acoustic field.

A k-space simulation was developed to solve the acoustoelectric equation in homogeneous media, enabling fast exploratory simulations of the basic physics. Then a heterogeneous simulation was developed using a finite element modelling pathway created from three different solvers: acoustic, quasistatic electric field and modified thermal diffusion solvers were all used to solve the acoustoelectric equation. We then validated this solver pathway as correct through cross-validation with the analytic solution.

Simulation of the acoustoelectric interaction enabled safety predictions for acoustoelectric neuromodulation, showing that it is feasible within both acoustic and temperature safety parameters.

Lastly, modelling just the acoustic field and its reflection between the mouse and air interface, show that reflections within the mouse head will cause constructive and destructive interference which may limit the focality of a 500kHz acoustic wave in a mouse model.

7.2.2 Chapter 4: Acoustoelectric phantom characterization

To ascertain if the predictions made through theory and simulation were true, a physiological tissue mimicking phantom was developed to experimentally verify and explore the characteristics of the acoustoelectric interaction. Both the acoustic gradient dependence and heterodyning were verified experimentally, validating the new mathematical derivation.

By frequency mixing an acoustic field with an applied electric field, a signal in the electrophysiological frequency range known to be effective in neuromodulation was generated at an amplitude predicted to evoke neuromodulation from the existing literature. The concept of acoustoelectric neural recording was then demonstrated in the physiological phantom, by applying a complex electric field mimicking an

electrophysiological signal, which was then up modulated to the sum and difference frequencies of the ultrasound transducer. This phantom test modelled acoustoelectric neural decoding being performed using IQ demodulation and the original electrophysiological range signal was recovered.

The acoustoelectric interaction was validated in saline as being a viable method for both encoding and decoding electric signals at depth, utilizing the spatial focality of ultrasound.

Earlier studies of the acoustoelectric phenomenon have helped us to understand the acoustoelectric coupling factor^{137,93} and started to explore applications such as current source density imaging^{138,139,140}. We have built on these original studies to further advance the understanding of the phenomenon by unveiling its frequency mixing property and complex field dependent shape. These findings provide a conceptual framework to design, interpret, and translate the electrolytic acoustoelectric phenomenon.

Given the broad use of electrostatic focusing lenses to focus electron beams with electric and magnetic fields^{123,124,141}, the potential translational prospects for acoustically controlled electric field lenses are exciting. The acoustoelectric heterodyning could enable non-invasive focal modulation and recording of electrophysiological signals at depth.

For example, remote, focal modulation of neural activities could be achieved by generating electric fields with a difference frequency Δf within the physiological range (<100 Hz) while keeping the frequency of the applied electric fields f_E sufficiently high (>1kHz) so it is attenuated by the low pass filtering of the cell membrane¹⁴². However, the low efficiency of the acoustoelectric conversion may render such focal neural modulation challenging. For example, generating an electric field of 1 V/m (a typical neuromodulation threshold¹⁴³) at Δf may require applying an electric field of hundreds V/m and an acoustic field of hundreds kPa that might be themselves strong enough to affect neural activity. Future studies in animal models will be able to test the feasibility of such an approach.

Remote, focal recording of neural activities could be achieved by applying a focal acoustic field to the target region to up-convert the endogenous neural signals to a higher frequency range (i.e., the sum and difference frequencies of the acoustic field), thereby rendering them electrically detectable remotely.

7.2.3 Chapter 5: *In vivo* Acoustoelectric neural recording

Next, we investigated the previously published work on ultrasound current source density imaging^{8,82,148–152}, revealing its mechanism to be acoustoelectric heterodyning for the first time. With this knowledge, we may be able to extend its sensitivity beyond *ex vivo* ECG measurements by using narrowband measurements to improve the signal-to-noise ratio.

We then performed both phantom and *in vivo* experiments recreating Tianjin Universities results^{64,66,153,154} in acoustoelectric brain imaging (AEBI) at low pulse repetition ultrasound frequencies. We showed that we can recreate the reported results recovering the visual evoked potential with and without ultrasound indicating an additional frequency specificity test is required to differentiate between the Hilbert demodulation of VEP harmonics and the heterodyne products of the acoustoelectric interaction.

Through *in vivo* rodent experiments, acoustoelectric neural recording was demonstrated, using an averaged visual evoked potential paradigm. To extend this into a tool that could one day be used for real-time recordings at depth, single shot recording of spontaneous neural activity is demonstrated with high correlation. To prove results were not artefactual four different confound tests are performed: an acoustic isolation test, a frequency specificity test, LED shield test and two-tone test. This study provides the first *in vivo* evidence of acoustoelectric neural recording.

7.2.4 Chapter 6: *In vivo* Acoustoelectric neuromodulation

This Chapter provides the first *in vivo* evidence of a new form of neuromodulation induced through acoustoelectric heterodyning and forms an evidence-based argument as to why the acoustoelectric interaction is a contributing mechanism to ultrasound brain stimulation.

A step-by-step series of *ex vivo* phantom and *in vivo* rodent investigations revealed that the amplitude of ultrasound brain stimulation was dependent on the electrical artefact from the ultrasound transducer as well as the emitted acoustic field. We consider temperature, the acoustic radiation force, non-linear acoustics, and electric only mixing, but none of these explain why the amplitude of ultrasound brain stimulation can be increased or decreased when an electric field in the medium changes in amplitude. However, the acoustoelectric interaction would explain our observations.

Acoustoelectric neuromodulation is demonstrated *in vivo*, with the difference frequency measured as peaks in the signal recorded in the brain as well as the electromyography response in the paw of the mouse. This frequency dependence on both the applied acoustic and electric field, is further evidence that ultrasound brain stimulation is not due to the acoustic envelope alone as the acoustic field alone cannot be responsible for the peaks at the difference frequency.

Acoustoelectric neuromodulation is new form of brain stimulation, which has a clear and controllable mechanism of action, harnessing the focality of ultrasound and the direct electrical stimulation pathway described by the Hodgkin-Huxley model, giving it a unique set of advantages to target deep areas of the brain with a tightly controlled mechanism of action.

7.3 Future directions

7.3.1 Future improvements to further understand the physics of the acoustoelectric interaction

Whilst progress has been made, our understanding of the interaction between acoustic and electric fields in biological systems is in its infancy.

The derivation could use further extension to better understand the impact ionic mobility has on the constant k in the heterogeneous version of the equation, as we have shown experimentally that the acoustoelectric amplitudes are higher when the ionic mobility is also higher (i.e. at higher impedances).

Increasing the ultrasound frequency was shown in simulation to massively increase the amplitude of the acoustoelectric effect as the gradient is far steeper at shorter wavelength high frequency acoustic fields. This information was not experimentally explored due to having only a single transducer and the limited spatial resolution of the XYZ stage, but simulation suggests that this would be an effective way to increase the amplitude conversion ratio between the applied acoustic and electric field and the resulting acoustoelectric field. Different valence ions, ionic mobility could all be explored further even in terms of a simple homogeneous phantom.

Other techniques such as transcranial magneto-acoustic stimulation^{199,200} (TMAS) using a static magnetic field an acoustic field, may also be related to the acoustoelectric effect, as TMAS is measured at the sum frequency of the two fields. Further investigation would need to be performed to explore the relationship between TMAS and the acoustoelectric interaction, and whether the findings in this neighbouring field are related to the acoustoelectric interaction.

7.3.2 Future improvements to acoustoelectric neural recording

Improvements in waveform specificity

The acoustoelectrically modulated waveform shown here is dependent on the integrated volume of the ultrasound focus, inclusive of both constructive and destructive regions induced in the mouse head. The reflections are a major problem because the transducer wavelength is the same order as the skull diameter, meaning any attempt at focusing within the mouse head at this wavelength has significant reflection problems. Being able to accurately target a specific waveform at a small area, would enable precise targeting, improving the signal-to-noise ratio as spurious non-target areas would not be included in

the acoustoelectrically modulated field. Techniques such as ultrasound tomography¹⁶⁸ might be particularly useful in this case as would moving to a mammal with a larger skull. The focality advantage of acoustoelectric neural recording is not well showcased in the mouse due to this focality limitation, though we know ultrasound can be focused to high spatial specificity in other contexts¹⁶⁹.

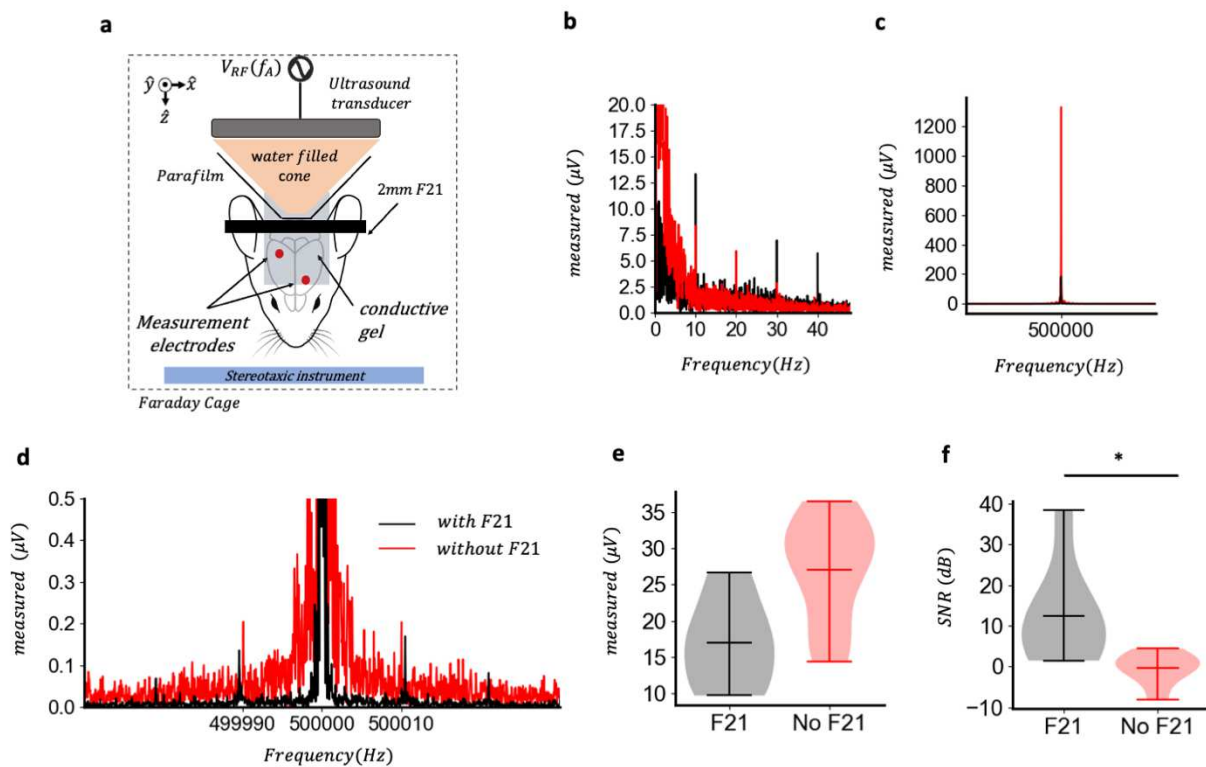


Figure 7-1 | Improving the dynamic range of the preamplifier measurement through attenuation of the electrical ultrasound artefact.

a, Experimental arrangement for attenuation of the electrical artefact from the ultrasound transducer. 1MPa was applied continuously for 30 seconds, while an LED was flashed at 10Hz, with sample rate = 2MHz. **b**, Visual evoked potential at 10Hz, duty cycle = 0.5, ASD measured with Isoflurane 0.5% (Vol/vol), pressure output 1MPa, where gain on preamplifier is 2000 for without F21 measurement (red line) and 10000(black line) for with F21 measurement. Sampling rate = 2MHz. More noise can be seen at the lower gain. **c**, Carrier amplitude ASD with F21(167 μV) and without F21 (1333 μV). **d**, A Kaiser window, beta = 10 is used to more clearly see the modulation amplitudes without the apodization error induced by spectral leakage from the high amplitude electrical carrier artefact. Note that the Kaiser window distorts the amplitude. SNR with F21/Gain 10000 = 48.8dB, without F21/Gain 2000 = 27.8dB. **e**, VEP amplitude comparisons for n=8 30second trials, with and without F21. **f**, Signal to noise ratio(dB) amplitude comparisons for n=8 30second trials, with and without F21, t-statistic p = 0.024.

Using F21 to improve the dynamic range of the preamplifier

A major limitation in the acoustoelectric neural recording measurements shown is the inability to use a high preamplifier gain due to the amplitude of the electrical artefact from the ultrasound transducer. To decrease the amplitude of electrical artefact at the carrier frequency, we could use the electrically insulating and acoustically transparent material (F21 from Precision Acoustics, UK) between the cone and the mouse head (**Fig 7-1 a**) which decreases the electrical carrier amplitude from $1333 \mu V$ to $167 \mu V$, an 8-fold decrease (**Fig 7-1 c**). This artefact attenuation allows the SR560 preamplifier gain to be increased without causing saturation. Increasing the gain on the preamplifier from 2000 to 10000 improves the SNR of the recorded modulated signal from SNR = 27.8dB, to 48.8dB when using F21 (**Fig 7-1 d**).

To calculate a group statistic, we repeated the test 8 times for each group while a mouse was kept at the same anesthesia level (0.5% Vol/Vol). We show both the average VEP amplitude (**Fig 7-1 e**) compared between the two groups, and the calculated signal-to-noise ratio with and without use of F21 material (**Fig 7-1 f**) yielding a t-statistic $p=0.024$, a significant difference between the two groups. Considering the VEP amplitude mean was smaller for the F21 group, the SNR difference would have been larger had the VEP amplitudes been the same.

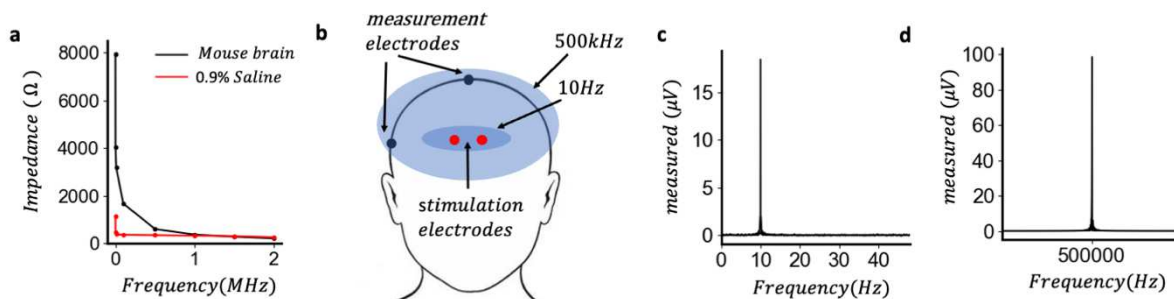


Figure 7-7-2 | Transmission advantages over EEG.

a, Impedance spectrum with frequency as measured in saline and mouse brain, where electrodes are 7mm apart in both cases. **b**, Physiological saline phantom showing equivalent amplitude contours at two different frequencies. Electrodes are kept in the same position, and the same amplitude ($V_{output} = 0.001V$), electrical sine wave stimulation is applied at two different frequencies, 10Hz and 500kHz. **c**, Saline distance traversal test at low frequency (10Hz) measured at a 1cm distant pair of electrodes is $18 \mu V$ and **d**, high frequency amplitude (500kHz) at the same distant electrode pair is $98 \mu V$, with the same applied voltage as in c.

Attenuation with distance advantages of the acoustoelectric interaction

By acoustoelectrically modulating up to a higher frequency (500kHz), we confer two benefits over traditional EEG. First, we move outside the Johnson noise ($1/f$) noise dominated spectral region which affects all EEG low frequency measurements¹⁷⁰, into a spectral region with a far lower noise floor. Secondly, we can take advantage of the decreasing amplitude of the dielectric impedance as the frequency increases (**Fig 7-2 a**), as high frequency signals will be attenuated less than low frequency electric signals (**Fig 7-2 b**). Hence the high frequency acoustoelectrically modulated signal will be less attenuated by the skull than the original low frequency scalp EEG. As a rough calculation metric, we applied the same amplitude voltage ($V_{output} = 1\text{mV}$) 10Hz in the electrophysiological frequency range and 500kHz sinusoid in 0.9% saline and measured the amplitude at electrodes located in the same place for both frequency recordings. The 500kHz signal was 5.35 times larger than the 10Hz signal, (**Fig 7-2 c,d**). Since brain tissue has an even higher impedance than saline (**Fig 7-2 a** where the 10Hz impedance is $8\text{k}\Omega$), if we were to modulate a signal up to 500kHz (impedance = 800Ω , inclusive of cable impedance) we would expect a 10 times improvement in the attenuation over the same distance. Hence, transmitting the acoustoelectric modulated signal through the skull at a higher frequency is advantageous, and is dependent on the impedance spectrum of the medium.

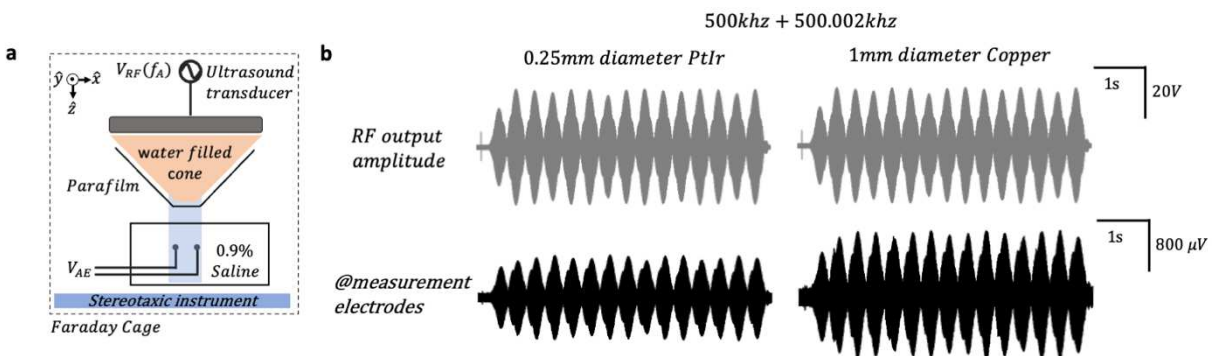


Figure 7-7-3 | Electrode surface area

a, Physiological phantom showing ultrasound positioned such that the ultrasound focus is calibrated to be located over two measurement electrodes, positioned 7mm apart. A 1MPa maximum amplitude waveform was sent through the RF amplifier, and recordings made at 5MHz for 8s duration of both the output RF signal, and the signal measured at the electrodes (gain = 2000). **b**, Comparison of 0.25mm diameter PtIr measurement electrodes with 1mm diameter copper electrodes shows that despite equivalent output signals, the signal measured is larger when the measurement surface area is also larger.

We compared the amplitude difference between two different surface area electrodes, one 0.25mm diameter Platinum Iridium, and the second 1mm Copper wire (**Fig 7-3 a**). The ultrasound transducer focal point was calibrated (**Methods Fig 7-3 c**) so that the focus was positioned directly above two electrodes spaced 7mm apart and kept in the same position for both tests. An RF amplifier output signal consisting of a 500kHz + (500kHz + with maximum output pressure 1MPa. The pair of Platinum Iridium electrodes with a smaller 0.25mm diameter wire picked up a smaller electrical signal, than the thicker (1mm Copper) electrodes (**Fig 7-3 b**) despite being positioned similarly. Both electrode material and electrode surface area can greatly change the measured amplitude of the signal, and further measurement advantages are likely to be conferred by increasing the surface area of the measurement electrodes.

When comparing acoustoelectric neural recording with a technique like EEG, EEG electrodes are limited in size as they will lose spatial information. However, an advantage of acoustoelectric neural recording is that it can use a much larger electrode as no spatial information needs to be conveyed from its size and position.

Bit-depth

Signals were recorded using a 14-bit sigma-delta ADC. Higher signal-to-noise ratio at these high frequencies could be obtained by shifting to a 24-bit ADC, though this would need to be balanced with the data transfer limitations of the recording hardware.

7.3.3 Future improvements for acoustoelectric neuromodulation

Acoustoelectric neuromodulation brings up many questions on the nature of ultrasound stimulation, and what part of it may be due to interaction of the acoustic field with either the artefact electric field from the transducer or the endogenous fields in tissue. Are mechanosensitive ions functioning through an acoustoelectric mechanism? Previous studies haven't documented this, indicating it may be an area of future scientific exploration. Further electrical isolation experiments could be done, as could a larger range of experiments in neurons using patch clamp electrophysiology, calcium and voltage imaging.

While it would be desirable to translate acoustoelectric neuromodulation to humans, the results when the applied frequencies were different were difficult to control, as there is a threshold for motor responses which the resulting low frequency sinusoid would either be above or below. This motor threshold was anaesthesia depth dependent, and the amplitudes of the applied fields at the focal area could only be roughly controlled, another area of improvement. However, across all experiments there was a strong amplitude effect when both an acoustic and electric field were present in the medium, whereby the acoustoelectric field was always larger in amplitude than acoustic and electric fields applied alone, evoking clearly larger EMG each time. A straight-forward clinical translation would be applying an acoustic and an electric field at the same frequency to control the amplitude of neuromodulation, enabling tighter control of neuromodulatory applications from both the perspective of administering an accurate therapeutic dose, as well as safety as each of the acoustic and electric field parameters can be controlled independently to be within safety guidelines.

Further technical optimization of the applied fields would improve the acoustoelectric neuromodulation technique, from precise control of the acoustic focal area through to use of interferential beamforming and phased arrays to enable more precise neuromodulation. It would also be helpful to have a more standardized system to apply the electric field, potentially even an electromagnet which the patient sits inside similar to an MRI as small geometric changes in the applied fields will affect the resultant acoustoelectric amplitudes.

From a safety perspective, to make this useful in humans the temperature index would need to be decreased from the continuous wave case, as the mechanical index is already within the safety guidelines. A simple way to decrease the TI would be to pulse the acoustic wave so less thermal energy is dissipated. This addition would require narrowband demodulation at the generated odd harmonics around the centre frequency which are generated by the square wave envelope of the proposed pulsed waveform. Though this would add complexity to the demodulation procedure, it is certainly feasible and would enable the temperature index to be more accurately controlled.

7.4 Implications

In this dissertation, proof-of-concept evidence is shown through derivation, simulation, saline phantom characterization and *in vivo* electrophysiology in rodents, that a spatially specific and non-invasive bidirectional acoustoelectric neural interface is feasible. Future translation of this technique to humans would open pathways to understanding the electrical signals of the brain and diagnose its disorders, producing a transformational impact on both neuroscience and healthcare.

In our *in vivo* studies, no attempt was made to optimize for safety as proof of concept was sufficient for a first pass. Further work would benefit from formalized safety investigations to ascertain the translational potential of this technique to humans, through to better focusing of the acoustic wave to account for skull aberrations and optimizing the signal-to-noise parameters of acoustoelectric neural recording by varying the frequencies, electrode surface areas and amplification techniques used.

Optimization of these techniques for acoustoelectric bidirectional interfacing, may have a potentially broad reaching and highly impactful application to human health and our understanding of the brain. While the first applications that come to mind are for non-invasive neural interfacing, nerve imaging and stimulation in soft tissue in any other part of the body would be even more feasible enabling a whole new class of human computer interaction devices. Since this technique is non-invasive, its advantage will lie in its ease of use without causing patient harm as it doesn't destroy tissue to reach deeper areas. If it can be shown to be tolerated by the body long term and without injury the range of potential diagnostics and therapeutic applications will massively increase.

Furthermore, since electric and acoustic fields can be applied with low cost and mass-manufactured hardware components, there are no size or cost limitations as there are for MRI. Since it's non-invasive, hardware iterations and upgrades are more possible making this technique a great candidate for mass manufacturing, enabling larger quantities of devices to be built with the potential for greater market penetration.

Future applications include non-invasive spatially targeted surgeries guided by closed loop feedback from the read and write capability of the acoustoelectric interaction, advances in prosthetic limb actuation guided by acoustoelectric imaging, alongside the insights to be made into neurodegenerative disease and other mental disorders by understanding the complex electrical networks of the brain.

References

1. Ding, C. *et al.* Global, regional, and national burden and attributable risk factors of neurological disorders: The Global Burden of Disease study 1990–2019. *Front Public Health* **10**, 952161 (2022).
2. Little, S. *et al.* Bilateral adaptive deep brain stimulation is effective in Parkinson ' s disease. 717–721 (2016) doi:10.1136/jnp-2015-310972.
3. Alonso, P., Cuadras, D., Gabriëls, L. & Denys, D. Deep Brain Stimulation for Obsessive- Compulsive Disorder : A Meta-Analysis of Treatment Outcome and Predictors of Response. 1–16 (2015) doi:10.1371/journal.pone.0133591.
4. Dreier, J. P. *et al.* Spreading convulsions, spreading depolarization and epileptogenesis in human cerebral cortex. (2012) doi:10.1093/brain/awr303.
5. Lozano, A. M. & Lipsman, N. Probing and Regulating Dysfunctional Circuits Using Deep Brain Stimulation. *Neuron* **77**, 406–424 (2013).
6. Walker, H. C. *et al.* Adverse Events Associated With Deep Brain Stimulation for Movement Disorders: Analysis of 510 Consecutive Cases. *Neurosurgery* **11**, 190–199 (2015).
7. Sadjadi, S. M., Ebrahimzadeh, E., Shams, M., Seraji, M. & Soltanian-Zadeh, H. Localization of Epileptic Foci Based on Simultaneous EEG–fMRI Data. *Front Neurol* **12**, 645594 (2021).
8. Lopez Rincon, A. & Shimoda, S. The inverse problem in electroencephalography using the bidomain model of electrical activity. *J Neurosci Methods* **274**, 94–105 (2016).
9. Turner, R. Uses, misuses, new uses and fundamental limitations of magnetic resonance imaging in cognitive science. *Philosophical Transactions of the Royal Society B: Biological Sciences* **371**, (2016).
10. Wens, V. Exploring the limits of MEG spatial resolution with multipolar expansions. *Neuroimage* **270**, 119953 (2023).
11. Hodgkin, A. L. & Huxley, A. F. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J Physiol* **117**, 500 (1952).

12. Suleman, R. A Brief History of Electroconvulsive Therapy. <https://doi.org/10.1176/appi.ajp-rj.2020.160103> **16**, 6–6 (2020).
13. Herrmann, C. S., Rach, S., Neuling, T. & Strüber, D. Transcranial alternating current stimulation: A review of the underlying mechanisms and modulation of cognitive processes. *Front Hum Neurosci* **7**, 46064 (2013).
14. Lopes, S., Davies, N., Toumazou, C. & Grossman, N. Theoretical investigation of transcranial alternating current stimulation using laminar model. *Proceedings of the Annual International Conference of the IEEE Engineering in Medicine and Biology Society, EMBS* 4152–4155 (2012) doi:10.1109/EMBC.2012.6346881.
15. Solomons, C. D. & Shanmugasundaram, V. Transcranial direct current stimulation: A review of electrode characteristics and materials. *Med Eng Phys* **85**, 63–74 (2020).
16. Franke, M., Bhadra, N., Bhadra, N. & Kilgore, K. Direct current contamination of kilohertz frequency alternating current waveforms. *J Neurosci Methods* **232**, 74–83 (2014).
17. Fröhlich David, F. and M. Endogenous Electric Fields May Guide Neocortical Network Activity. *Neuron* **23**, 1–7 (2010).
18. Horvath, J. C., Perez, J. M., Forrow, L., Fregni, F. & Pascual-Leone, A. Transcranial magnetic stimulation: a historical evaluation and future prognosis of therapeutically relevant ethical concerns. *J Med Ethics* **37**, 137–143 (2011).
19. Zangen, A., Roth, Y., Voller, B. & Hallett, M. Transcranial magnetic stimulation of deep brain regions: evidence for efficacy of the H-Coil. *Clinical Neurophysiology* **116**, 775–779 (2005).
20. Huang, Y. Z. *et al.* Consensus: New methodologies for brain stimulation. *Brain Stimul* **2**, 2–13 (2009).
21. Martin, E. *et al.* Ultrasound system for precise neuromodulation of human deep brain circuits. *bioRxiv* 2024.06.08.597305 (2024) doi:10.1101/2024.06.08.597305.
22. Mueller, J. K., Legon, W. & Tyler, W. J. Analysis of Transcranial Focused Ultrasound Beam Profile Sensitivity for Neuromodulation of the Human Brain. 1–32 (2015).

23. Mueller, J., Legon, W., Opitz, A., Sato, T. F. & Tyler, W. J. Transcranial focused ultrasound modulates intrinsic and evoked EEG dynamics. *Brain Stimul* **7**, 900–908 (2014).
24. Tyler, W. J. *et al.* Remote excitation of neuronal circuits using low-intensity, low-frequency ultrasound. *PLoS One* **3**, (2008).
25. Blackmore, J., Shrivastava, S., Sallet, J., Butler, C. R. & Cleveland, R. O. Ultrasound Neuromodulation: A Review of Results, Mechanisms and Safety. *Ultrasound Med Biol* **45**, 1509–1536 (2019).
26. Kamimura, H. A. S., Conti, A., Toschi, N. & Konofagou, E. E. Ultrasound neuromodulation: Mechanisms and the potential of multimodal stimulation for neuronal function assessment. *Front Phys* **8**, 1–9 (2020).
27. Yoo, S., Mittelstein, D. R., Hurt, R. C., Lacroix, J. & Shapiro, M. G. Focused ultrasound excites cortical neurons via mechanosensitive calcium accumulation and ion channel amplification. *Nature Communications* **2022 13:1** **13**, 1–13 (2022).
28. Haswell, E. S., Phillips, R. & Rees, D. C. Mechanosensitive channels: what can they do and how do they do it? *Structure* **19**, 1356 (2011).
29. Palmeri, M. L. & Nightingale, K. R. Acoustic radiation force-based elasticity imaging methods. *Interface Focus* **1**, 553–564 (2011).
30. Retz, K. *et al.* Measured acoustic intensities for clinical diagnostic ultrasound transducers and correlation with thermal index. *Ultrasound in Obstetrics & Gynecology* **50**, 236–241 (2017).
31. Yuan, Y., Wang, X., Yan, J. & Li, X. The effect of anesthetic dose on the motor response induced by low-intensity pulsed ultrasound stimulation Shanbao Tong, Junfeng Sun. *BMC Neurosci* **19**, 1–6 (2018).
32. Tyler, W. J. Noninvasive neuromodulation with ultrasound? A continuum mechanics hypothesis. *Neuroscientist* **17**, 25–36 (2011).
33. Tyler, W. J. Ultrasonic neuromodulation. *IEEE International Ultrasonics Symposium, IUS* (2017) doi:10.1109/ULTSYM.2017.8092717.
34. Tufail, Y., Yoshihiro, A., Pati, S., Li, M. M. & Tyler, W. J. Ultrasonic neuromodulation by brain stimulation with transcranial ultrasound. *Nat Protoc* **6**, 1453–1470 (2011).

35. Grossman, N. *et al.* Noninvasive Deep Brain Stimulation via Temporally Interfering Electric Fields. *Cell* **169**, 1029-1041.e16 (2017).
36. Luff, C. E. *et al.* The neuron mixer and its impact on human brain dynamics. *Cell Rep* **43**, (2024).
37. Toi, P. T. *et al.* In vivo direct imaging of neuronal activity at high temporospatial resolution. *Science (1979)* **378**, 160 (2022).
38. Choi, S.-H. *et al.* No Replication of Direct Neuronal Activity-related (DIANA) fMRI in Anesthetized Mice. *bioRxiv* 2023.05.26.542419 (2023) doi:10.1101/2023.05.26.542419.
39. Cohen, D. Magnetoencephalography: Evidence of Magnetic Fields Produced by Alpha-Rhythm Currents. *Science (1979)* **161**, 784–786 (1968).
40. Wolf, M., Muehlemann, T. & Haensse, D. Wireless miniaturized in-vivo near infrared imaging. *Optics Express, Vol. 16, Issue 14, pp. 10323-10330* **16**, 10323–10330 (2008).
41. Ansari, M. A., Erfanzadeh, M., Hosseini, Z. & Mohajerani, E. Diffuse Optical Tomography: Image Reconstruction and Verification. *J Lasers Med Sci* **5**, 13 (2014).
42. Grinvald, A., Frostig, R. D., Lieke, E. & Hildesheim, R. Optical imaging of neuronal activity. *Physiol Rev* **68**, 1285–1366 (1988).
43. Wang, X. *et al.* Noninvasive laser-induced photoacoustic tomography for structural and functional in vivo imaging of the brain. *Nature Biotechnology* 2003 21:7 **21**, 803–806 (2003).
44. Aristovich, K. Y. *et al.* Imaging fast electrical activity in the brain with electrical impedance tomography. *Neuroimage* **124**, 204–213 (2016).
45. Vongerichten, A. N. *et al.* Characterisation and imaging of cortical impedance changes during interictal and ictal activity in the anaesthetised rat. *Neuroimage* **124**, 813–823 (2016).
46. Hannan, S. *et al.* Imaging fast electrical activity in the brain during ictal epileptiform discharges with electrical impedance tomography. *Neuroimage Clin* **20**, 674–684 (2018).
47. Aristovich, K. Y., Santos, G. S. Dos, Packham, B. C. & Holder, D. S. A method for reconstructing tomographic images of evoked neural activity with electrical

- impedance tomography using intracranial planar arrays. *Physiol Meas* **35**, 1095–1109 (2014).
48. Kolehmainen, V., Lassas, M. & Ola, P. Electrical impedance tomography problem with inaccurately known boundary and contact impedances. *IEEE Trans Med Imaging* **27**, 1404–1414 (2008).
 49. Charles Proteus Steinmetz. Theory and calculation of transient electric phenomena and oscillations : Steinmetz, Charles Proteus, 1865-1923. <https://archive.org/details/theoryandcalcul2steigoog> (1920).
 50. Thomenius, K. E. Evolution of ultrasound beamformers. *Proceedings of the IEEE Ultrasonics Symposium* **2**, 1615–1622 (1996).
 51. Richards, L. A., Cleveland, R. & Stride, E. P. Creating uniform ultrasound fields in the brain without an array. *J Acoust Soc Am* **144**, 1699 (2018).
 52. Fox, F. E., Herzfeld, K. F. & Rock, G. D. The Effect of Ultrasonic Waves on the Conductivity of Salt Solutions. *Physical Review* **70**, 329 (1946).
 53. Lavandier, B., Jossinet, J. & Cathignol, D. Quantitative assessment of ultrasound-induced resistance change in saline solution. *Med Biol Eng Comput* **38**, 150–155 (2000).
 54. Lavandier, B., Jossinet, J. & Cathignol, D. Experimental measurement of the acousto-electric interaction signal in saline solution. *Ultrasonics* **38**, 929–936 (2000).
 55. Jossinet, J., Lavandier, B. & Cathignol, D. The phenomenology of acousto-electric interaction signals in aqueous solutions of electrolytes. *Ultrasonics* **36**, 607–613 (1998).
 56. Jossinet, J., Lavandier, B. & Cathignol, D. Impedance modulation by pulsed ultrasound. *Ann N Y Acad Sci* **873**, 396–407 (1999).
 57. Fox, F. E., Herzfeld, K. F. & Rock, G. D. The effect of ultrasonic waves on the conductivity of salt solutions. *Physical Review* **70**, 329–339 (1946).
 58. Olafsson, R., Witte, R. S. & O'Donnell, M. Measurement of a 2D electric dipole field using the acousto-electric effect. *Medical Imaging 2007: Ultrasonic Imaging and Signal Processing* **6513**, 65130S-65130S (2007).

59. Li, Q., Olafsson, R., Ingram, P., Wang, Z. & Witte, R. Measuring the acoustoelectric interaction constant using ultrasound current source density imaging. *Phys Med Biol* **57**, 5929–5941 (2012).
60. Witte, R., Olafsson, R., Huang, S. & Donnell, M. O. Imaging current flow in lobster nerve cord using the acoustoelectric effect. *Appl Phys Lett* **163902**, 10–13 (2013).
61. Berthon, B., Dansette, P. M., Tanter, M., Pernot, M. & Provost, J. An integrated and highly sensitive ultrafast acoustoelectric imaging system for biomedical applications. *Phys Med Biol* **62**, 5808–5822 (2017).
62. Olafsson, R., Jia, C., Huang, S. W., Witte, R. S. & O'Donnell, M. Detection of electrical current in a live rabbit heart using ultrasound. *Proc IEEE Ultrason Symp* 989–992 (2007) doi:10.1109/ULTSYM.2007.252.
63. Song, X., Su, X., Chen, X., Xu, M. & Ming, D. In Vivo Transcranial Acoustoelectric Brain Imaging of Different Steady-State Visual Stimulation Paradigms. *IEEE Transactions on Neural Systems and Rehabilitation Engineering* **30**, 2233–2241 (2022).
64. Zhou, Y. *et al.* In Vivo Transcranial Acoustoelectric Brain Imaging of Different Deep Brain Stimulation Currents. *IEEE Transactions on Neural Systems and Rehabilitation Engineering* 1–1 (2024) doi:10.1109/TNSRE.2024.3356440.
65. Zhou, Y. *et al.* Coding Biological Current Source with Pulsed Ultrasound for Acoustoelectric Brain Imaging: Application to Vivo Rat Brain. *IEEE Access* **8**, 29586–29594 (2020).
66. Song, X., Chen, X., Guo, J., Xu, M. & Ming, D. Living Rat SSVEP Mapping With Acoustoelectric Brain Imaging. *IEEE Trans Biomed Eng* **69**, 75–82 (2022).
67. An, C. Y., Syu, J. H., Tseng, C. S. & Chang, C. An Ultrasound Imaging-Guided Robotic HIFU Ablation Experimental System and Accuracy Evaluations. **2017**, (2017).
68. Esslinger, A. *et al.* Acoustoelectric study of localized states in the quantized Hall effect. *Solid State Commun* **84**, 939–942 (1992).
69. Shilton, J. M. *et al.* Experimental study of the acoustoelectric effects in GaAs-AlGaAs heterostructures. *Journal of Physics: Condensed Matter* **7**, 7675–7685 (1995).

70. Shilton, J. M. *et al.* On the acoustoelectric current in a one-dimensional channel. *Journal of Physics Condensed Matter* **8**, L337 (1996).
71. Hodgkin, A. L. & Huxley, A. F. Action potentials recorded from inside a nerve fibre [8]. *Nature* **144**, 710–712 (1939).
72. Häusser, M. The Hodgkin-Huxley theory of the action potential. *Nature Neuroscience* 2000 3:11 **3**, 1165–1165 (2000).
73. Lavandier, B., Jossinet, J. & Cathignol, D. Experimental measurement of the acousto-electric interaction signal in saline solution. *Ultrasonics* **38**, 929–936 (2000).
74. Lavandier, B., Jossinet, J. & Cathignol, D. Quantitative assessment of ultrasound-induced resistance change in saline solution. *Med Biol Eng Comput* **38**, 150–155 (2000).
75. Olafsson, R., Witte, R. S. & O'Donnell, M. Measurement of a 2D electric dipole field using the acousto-electric effect. *Medical Imaging 2007: Ultrasonic Imaging and Signal Processing* **6513**, 65130S (2007).
76. I. S. Grant, W. R. P. Electromagnetism, 2nd Edition. 122–123 (1991).
77. Jossinet, J., Lavandier, B. & Cathignol, D. Impedance modulation by pulsed ultrasound. *Annals of the New York Academy of Sciences* vol. 873 396–407 Preprint at <https://doi.org/10.1111/j.1749-6632.1999.tb09489.x> (1999).
78. Partington, J. R. *Effect of Pressure on the Viscosity of Liquids. In An Advanced Treatise on Physical Chemistry. Vol. 2, p. 89.* (Longmans, Green., New York, 1955).
79. Pethig, R. Dielectric properties of biological materials: Biophysical and medical applications. *IEEE Transactions on Electrical Insulation* **EI-19**, 453–474 (1984).
80. Li, Q., Olafsson, R., Ingram, P., Wang, Z. & Witte, R. Measuring the acoustoelectric interaction constant using ultrasound current source density imaging. *Phys Med Biol* **57**, 5929–5941 (2012).
81. Song, X., Qin, Y., Xu, Y., Ingram, P. & Witte, R. S. Tissue Acoustoelectric Effect Modeling From Solid Mechanics Theory. *IEEE Trans Ultrason Ferroelectr Freq Control* **64**, 1583–1590 (2017).
82. Olafsson, R., Witte, R. S., Huang, S. W. & O'Donnell, M. Ultrasound current source density imaging. *IEEE Trans Biomed Eng* **55**, 1840–1848 (2008).

83. Parker, K. J. Radiation pattern of a focused transducer: A numerically convergent solution. *Journal of the Acoustical Society of America* **94**, 2979–2991 (1993).
84. Langlands, T. A. M. & Henry, B. I. The accuracy and stability of an implicit solution method for the fractional diffusion equation. *J Comput Phys* **205**, 719–736 (2005).
85. Rintoul, J. L. Acoustoelectric k-space simulation and data viewing tools. Preprint at <https://doi.org/10.1038/s42005-023-01198-w> (2021).
86. Sim4Life Application and Support Team. Sim4Life light Reference Guide: Release 4.4. (2018).
87. Philip, N. M. Adiabatic and isothermal compressibilities of liquids. *Proceedings of the Indian Academy of Sciences - Section A* 1939 9:2 **9**, 109–120 (1939).
88. Dielectric Properties » IT'IS Foundation. <https://itis.swiss/virtual-population/tissue-properties/database/dielectric-properties/>.
89. Rintoul, J. L. Acoustoelectric heterogeneous simulation. Preprint at <https://doi.org/https://doi.org/10.6084/m9.figshare.26948188.v1> (2021).
90. Chen, X., Schwarz, K. Q. & Parker, K. J. Radiation pattern of a focused transducer: A numerically convergent solution. *J Acoust Soc Am* **94**, 2979–2991 (1993).
91. Chen, P. *et al.* Acoustic characterization of tissue-mimicking materials for ultrasound perfusion imaging research. *Ultrasound Med Biol* **48**, 124–142 (2022).
92. McGarry, C. K. *et al.* Tissue mimicking materials for imaging and therapy phantoms: a review. *Phys Med Biol* **65**, (2020).
93. Song, X., Qin, Y., Xu, Y., Ingram, P. & Witte, R. S. Tissue Acoustoelectric Effect Modeling From Solid Mechanics Theory. *IEEE Trans Ultrason Ferroelectr Freq Control* **64**, 1583–1590 (2017).
94. Yexian Qin, Zhaohui Wang, Pier Ingram, Qian Li, R. S. W. Optimizing Frequency and Pulse Shape for Ultrasound Current Source Density Imaging. *IEEE Trans Ultrasound Ferroelectrics and Frequency Control* **23**, 1–7 (2019).
95. Şen, T., Tüfekçioğlu, O. & Koza, Y. Mechanical index. *Anatol J Cardiol* **15**, 334 (2015).
96. Duck, F. A. The Meaning of Thermal Index (TI) and Mechanical Index (MI) Values. *Ultrasound* **5**, 36–40 (1997).

97. Yarmolenko, P. S. *et al.* Thresholds for thermal damage to normal tissues: an update. *Int J Hyperthermia* **27**, 320–343 (2011).
98. Treeby, B. E. & Cox, B. T. k-Wave: MATLAB toolbox for the simulation and reconstruction of photoacoustic wave fields. *J Biomed Opt* **15**, 021314 (2010).
99. Treeby, B. E. & Cox, B. T. k-Wave: MATLAB toolbox for the simulation and reconstruction of photoacoustic wave fields. *J Biomed Opt* **15**, 021314 (2010).
100. Mirzakhaili, E., Barra, B., Capogrosso, M. & Lempka, S. F. Biophysics of Temporal Interference Stimulation. *Cell Syst* **11**, 557-572.e5 (2020).
101. King, R. L., Brown, J. R., Newsome, W. T. & Pauly, K. B. Effective Parameters for Ultrasound-Induced In Vivo Neurostimulation. *Ultrasound Med Biol* **39**, 312–331 (2013).
102. Rintoul, J. L., Neufeld, E., Butler, C., Cleveland, R. O. & Grossman, N. Remote focused encoding and decoding of electric fields through acoustoelectric heterodyning. *Communications Physics* 2023 6:1 **6**, 1–11 (2023).
103. Rintoul, J. L. Open 3D Phantom. Preprint at <https://doi.org/https://doi.org/10.6084/m9.figshare.26968963.v1> (2021).
104. Francis, M. J. Effect of Degassing on the Electrical Conductivity of Pure Water and Potassium Chloride Solutions. *Journal of Physical Chemistry C* **112**, 14563–14569 (2008).
105. Cogan, S. F., Garrett, D. J. & Green, R. A. Electrochemical Principles of Safe Charge Injection. *Neurobionics: The Biomedical Engineering of Neural Prostheses* 55–88 (2016) doi:10.1002/9781118816028.CH3.
106. Differential filter design Texas Instruments. (2010) doi:<https://doi.org/10.6084/m9.figshare.26968963.v1>.
107. Nasiri, H. *et al.* Electrode Configuration with Howland Current Source and Demodulator for Electrical Impedance Tomography. *IFAC-PapersOnLine* **54**, 79–84 (2021).
108. Ruiz-Vargas, A., Ivorra, A. & Arkwright, J. W. Design, Construction and Validation of an Electrical Impedance Probe with Contact Force and Temperature Sensors Suitable for in-vivo Measurements. *Scientific Reports* | **8**, (2018).

109. Xu, K., Kim, Y., Boctor, E. M. & Zhang, H. K. Enabling low-cost point-of-care ultrasound imaging system using single element transducer and delta configuration actuator. 31 (2019) doi:10.1117/12.2513169.
110. Chan, K. Application Report Design of Differential Filters for High-Speed Signal Chains. (2007).
111. Casas, Ó. & Pallás-Areny, R. Basics of analog differential filters. *IEEE Trans Instrum Meas* **45**, 275–279 (1996).
112. Cooley, J. W. & Tukey, J. W. An Algorithm for the Machine Calculation of Complex Fourier Series.
113. Harris, F. J. On the Use of Windows for Harmonic Analysis with the Discrete Fourier Transform.
114. Heinzl, G., Rüdiger, A. & Schilling, R. Spectrum and spectral density estimation by the Discrete Fourier transform (DFT), including a comprehensive list of window functions and some new flat-top windows. (2002).
115. Tai, C. T. & Collin, R. E. Radiation of a Hertzian dipole immersed in a dissipative medium. *IEEE Trans Antennas Propag* **48**, 1501–1506 (2000).
116. Dzialecka, P., Zabouri, N., Barnes, S. & Grossman, N. Cortical calcium dynamics evoked by temporal interference (TI) electrical stimulation in vivo. *Brain Stimul* **16**, 342 (2023).
117. King, R. L., Brown, J. R., Newsome, W. T. & Pauly, K. B. Effective Parameters for Ultrasound-Induced In Vivo Neurostimulation. *Ultrasound Med Biol* **39**, 312–331 (2013).
118. Yuan, Y., Wang, Z., Liu, M. & Shoham, S. Cortical hemodynamic responses induced by low-intensity transcranial ultrasound stimulation of mouse cortex. *Neuroimage* **211**, 116597 (2020).
119. Cameron, C. G. Electrochemical capacitors. *Springer Handbooks* **38**, 563–589 (2017).
120. Singh, M. B. & Kant, R. Debye-Falkenhagen dynamics of electric double layer in presence of electrode heterogeneities. *Journal of Electroanalytical Chemistry* **704**, 197–207 (2013).

121. Anderson, J. E. The Debye-Falkenhagen effect: experimental fact or friction? *J Non Cryst Solids* **172–174**, 1190–1194 (1994).
122. Gabriel, C. Compilation of the Dielectric Properties of Body Tissues at RF and Microwave Frequencies. (1996) doi:10.21236/ADA303903.
123. Heddle, D. W. O. *Electrostatic Lens Systems*. vol. 59 (CRC Press, 2000).
124. Blewett, J. P. Radial Focusing in the Linear Accelerator. *Physical Review* **88**, 1197–1199 (1952).
125. Fine, R. A. & Millero, F. J. Compressibility of water as a function of temperature and pressure. *J Chem Phys* **59**, 5529 (2003).
126. Ward, L. M. Synchronous neural oscillations and cognitive processes. *Trends Cogn Sci* **7**, 553–559 (2003).
127. Grossman, N. *et al.* Noninvasive Deep Brain Stimulation via Temporally Interfering Electric Fields. *Cell* **169**, 1029-1041.e16 (2017).
128. Hutcheon, B. & Yarom, Y. Resonance, oscillation and the intrinsic frequency preferences of neurons. *Trends Neurosci* **23**, 216–222 (2000).
129. Babcock, W. C. Intermodulation Interference in Radio Systems: Frequency of Occurrence and Control by Channel Selection. *Bell System Technical Journal* **32**, 63–73 (1953).
130. Sacko, D. & Kéïta, A. A. Techniques of Modulation: Pulse Amplitude Modulation, Pulse Width Modulation, Pulse Position Modulation. *International Journal of Engineering and Advanced Technology (IJEAT)* 2249–8958 (2017).
131. Räisänen, Antti V.; Lehto, A. *Radio Engineering for Wireless Communication and Sensor Applications*. (Artech House, 2003).
132. Stanford Research Systems. Lock-In Amplifier Basics. (2020).
133. Blaunstein, N., Christodoulou, C. & Sergeev, M. *Introduction to Radio Engineering. Introduction to Radio Engineering* (2016). doi:10.1201/9781315367194.
134. Weisstein, E. W. Correlation Coefficient--Bivariate Normal Distribution.
135. Hotelling, H. New Light on the Correlation Coefficient and its Transforms. *Journal of the Royal Statistical Society: Series B (Methodological)* **15**, 193–225 (1953).
136. Dayan, A., Huang, Y. & Schuchinsky, A. Passive Intermodulation at Contacts of Rough Conductors. *Electronic Materials* 2022, Vol. 3, Pages 65-81 **3**, 65–81 (2022).

137. Jossinet, J., Lavandier, B. & Cathignol, D. Impedance modulation by pulsed ultrasound. *Annals of the New York Academy of Sciences* vol. 873 396–407 Preprint at <https://doi.org/10.1111/j.1749-6632.1999.tb09489.x> (1999).
138. Ragnar Olafsson, Russell S. Witte, Sheng-Wen Huang, M. O. Ultrasound Current Source Density Imaging. **55**, 1840–1848 (2008).
139. Yang, R., Li, X., Song, A., He, B. & Yan, R. A 3-D reconstruction solution to current density imaging based on acoustoelectric effect by deconvolution: A simulation study. *IEEE Trans Biomed Eng* **60**, 1181–1190 (2013).
140. Berthon, B., Dansette, P. M., Tanter, M., Pernot, M. & Provost, J. An integrated and highly sensitive ultrafast acoustoelectric imaging system for biomedical applications. *Phys Med Biol* **62**, 5808–5822 (2017).
141. Courant, E. D. & Snyder, H. S. Theory of the alternating-gradient synchrotron. *Ann Phys (N Y)* **3**, 360–408 (1958).
142. Brain, M. E. R., Hutcheon, B. & Yarom, Y. Resonance , oscillation and the intrinsic frequency preferences of neurons. *Trends in Neuroscience* **23**, 216–222 (2000).
143. Ozen, S. *et al.* Transcranial electric stimulation entrains cortical neuronal populations in rats. *Journal of Neuroscience* **30**, 11476–11485 (2010).
144. Rattay, F. The basic mechanism for the electrical stimulation of the nervous system. *Neuroscience* **89**, 335–346 (1999).
145. Berkelmann, L. *et al.* Tumour-treating fields (TTFields): Investigations on the mechanism of action by electromagnetic exposure of cells in telophase/cytokinesis. *Sci Rep* **9**, 1–11 (2019).
146. Demi, L. Practical guide to ultrasound beam forming: Beam pattern and image reconstruction analysis. *Applied Sciences (Switzerland)* **8**, (2018).
147. Kuzmin, A. *et al.* Fast low-cost single element ultrasound reflectivity tomography using angular distribution analysis. *Proceedings - International Symposium on Biomedical Imaging* **2016-June**, 1021–1024 (2016).
148. Wang, Z. & Witte, R. Simulation-based validation for four-dimensional multi-channel ultrasound current source density imaging. *IEEE Trans Ultrason Ferroelectr Freq Control* **61**, 420–427 (2014).

149. Preston, C., Kasoff, W. S. & Witte, R. S. Selective Mapping of Deep Brain Stimulation Lead Currents Using Acoustoelectric Imaging. *Ultrasound Med Biol* **44**, 2345–2357 (2018).
150. Witte, R. S., Olafsson, R. & Donnell, M. O. Acoustoelectric Detection of Current Flow in a Neural Recording Chamber. in *2006 IEEE Ultrasonics Symposium, Vancouver, BC, Canada*, 5–8 (2006). doi:doi:10.1109/ULTSYM.2006.16.
151. Qin, Y., Li, Q., Ingram, P. & Witte, R. S. Mapping the ECG in the live rabbit heart using Ultrasound current source density imaging with coded excitation. *IEEE International Ultrasonics Symposium, IUS* 910–913 (2012) doi:10.1109/ULTSYM.2012.0227.
152. Qin, Y., Ingram, P., Burton, A. & Witte, R. S. 4D acoustoelectric imaging of current sources in a human head phantom. *IEEE International Ultrasonics Symposium, IUS 2016-Novem*, 3–6 (2016).
153. Zhou, Y. *et al.* Acoustoelectric brain imaging with different conductivities and acoustic distributions. *Front Physiol* **14**, 1241640 (2023).
154. Zhang, H. *et al.* Experimental and simulation studies of localization and decoding of single and double dipoles. *J Neural Eng* **19**, (2022).
155. Olafsson, R. *et al.* Cardiac activation mapping using ultrasound current source density imaging (UCSDI). *IEEE Trans Ultrason Ferroelectr Freq Control* **56**, 565–574 (2009).
156. Yexian Qin, Pier Ingram, Qian Li, Russell S. Witte & Zhaohui Wang. Optimizing Frequency and Pulse Shape for Ultrasound Current Source Density Imaging. *IEEE Trans Ultrasound Ferroelectrics and Frequency Control* **23**, 1–7 (2019).
157. Zhou, Y. *et al.* Coding Biological Current Source with Pulsed Ultrasound for Acoustoelectric Brain Imaging: Application to Vivo Rat Brain. *IEEE Access* **8**, 29586–29594 (2020).
158. Song, X. *et al.* An adaptive acoustoelectric signal decoding algorithm based on Fourier fitting for brain function imaging. *Front Physiol* **13**, 1054103 (2022).
159. Su, M. *et al.* Acoustoelectric Signal Decoding Based on Fourier Approximation. *Proceedings of the Annual International Conference of the IEEE Engineering in Medicine and Biology Society, EMBS 2020-July*, 988–991 (2020).

160. Zhou, Y. *et al.* Acoustoelectric brain imaging with different conductivities and acoustic distributions. *Front Physiol* **14**, (2023).
161. Song, X., Su, X., Chen, X., Xu, M. & Ming, D. In Vivo Transcranial Acoustoelectric Brain Imaging of Different Steady-State Visual Stimulation Paradigms. *IEEE Transactions on Neural Systems and Rehabilitation Engineering* **30**, 2233–2241 (2022).
162. Ridder, W. H. & Nusinowitz, S. The visual evoked potential in the mouse—Origins and response characteristics. *Vision Res* **46**, 902–913 (2006).
163. Buzsáki, G., Anastassiou, C. A. & Koch, C. The origin of extracellular fields and currents — EEG, ECoG, LFP and spikes. *Nature Reviews Neuroscience* **2012** 13:6 **13**, 407–420 (2012).
164. Sulistyaningsih *et al.* Performance comparison of blackman, bartlett, hanning, and kaiser window for radar digital signal processing. *2019 4th International Conference on Information Technology, Information Systems and Electrical Engineering, ICITISEE 2019* 391–394 (2019) doi:10.1109/ICITISEE48480.2019.9003791.
165. Nakayama, K. The relationship of visual evoked potentials to cortical physiology. *Ann N Y Acad Sci* **388**, 21–36 (1982).
166. Ridder, W. H. & Nusinowitz, S. The visual evoked potential in the mouse - Origins and response characteristics. *Vision Res* **46**, 902–913 (2006).
167. Miura, T. & Davis, L. E. Evaluation of intermodulation distortion of a ferrite element by the two-tone method. *IEEE Trans Microw Theory Tech* **57**, 1500–1507 (2009).
168. Guasch, L., Calderón Agudo, O., Tang, M. X., Nachev, P. & Warner, M. Full-waveform inversion imaging of the human brain. *npj Digital Medicine* **2020** 3:1 **3**, 1–12 (2020).
169. Riis, T., Feldman, D., Mickey, B. & Kubanek, J. Controlled noninvasive modulation of deep brain regions in humans. *Communications Engineering* **2024** 3:1 **3**, 1–12 (2024).
170. Gilden, D. L., Thornton, T. & Mallon, M. W. 1/f Noise in Human Cognition. *Science* (1979) **67**, 1837–1839 (1995).
171. Francois Beguin, E. F. *Carbons for Electrochemical Energy Storage and Conversion Systems (Advanced Materials and Technologies)*. (2009).

172. Brockbank, R. A. & Wass, C. A. A. Non-linear distortion in transmission systems. *Journal of the Institution of Electrical Engineers - Part III: Radio and Communication Engineering* **92**, 45–56 (1945).
173. Tennant, K. A. *et al.* The organization of the forelimb representation of the C57BL/6 mouse motor cortex as defined by intracortical microstimulation and cytoarchitecture. *Cerebral Cortex* **21**, 865–876 (2011).
174. King, R. L., Brown, J. R. & Pauly, K. B. Localization of Ultrasound-Induced In Vivo Neurostimulation in the Mouse Model. *Ultrasound Med Biol* **40**, 1512–1522 (2014).
175. Brunner, C., Montaldo, G. & Urban, A. Functional ultrasound imaging of stroke in awake rats. *Elife* **12**, (2023).
176. Murphy, K. R. *et al.* Optimized ultrasound neuromodulation for non-invasive control of behavior and physiology. *Neuron* (2024) doi:10.1016/J.NEURON.2024.07.002.
177. Green, R. & Abidian, M. R. Conducting Polymers for Neural Prosthetic and Neural Interface Applications. *Advanced Materials* **27**, 7620–7637 (2015).
178. Babcock, W. C. Intermodulation Interference in Radio Systems: Frequency of Occurrence and Control by Channel Selection. *Bell System Technical Journal* **32**, 63–73 (1953).
179. Dalecki, D. Mechanical bioeffects of ultrasound. *Annu Rev Biomed Eng* **6**, 229–248 (2004).
180. Fenlon, F. H. An Extension of the Bessel-Fubini Series for a Multiple-Frequency CW Acoustic Source of Finite Amplitude. *J Acoust Soc Am* **51**, 284–289 (1972).
181. Buzsáki, G. & Draguhn, A. Neuronal oscillations in cortical networks. *Science* **304**, 1926–1929 (2004).
182. Åström, M., Diczfalusy, E., Martens, H. & Wårdell, K. Relationship between neural activation and electric field distribution during deep brain stimulation. *IEEE Trans Biomed Eng* **62**, 664–672 (2015).
183. Ye, H. & Steiger, A. Neuron matters: electric activation of neuronal tissue is dependent on the interaction between the neuron and the electric field. *J Neuroeng Rehabil* **12**, 1–9 (2015).

184. Serrano-muñoz, D., Avendaño-coy, J., Simón-martínez, C., Taylor, J. & Gómez-soriano, J. 20-kHz alternating current stimulation: effects on motor and somatosensory thresholds. 1–7 (2020).
185. Brocker, D. T. & Grill, W. M. Principles of electrical stimulation of neural tissue. *Handb Clin Neurol* **116**, 3–18 (2013).
186. Histed, M. H., Bonin, V. & Reid, R. C. Direct Activation of Sparse, Distributed Populations of Cortical Neurons by Electrical Microstimulation. *Neuron* **63**, 508–522 (2009).
187. Xing, L., Huang, Y., Alja'afreh, S. S. & Boyes, S. J. A monopole water antenna. *LAPC 2012 - 2012 Loughborough Antennas and Propagation Conference* (2012) doi:10.1109/LAPC.2012.6402985.
188. Motovilova, E. & Huang, S. Y. A review on reconfigurable liquid dielectric antennas. *Materials* **13**, (2020).
189. Darvas, F., Mehić, E., Caler, C. J., Ojemann, J. G. & Mourad, P. D. Toward Deep Brain Monitoring with Superficial EEG Sensors Plus Neuromodulatory Focused Ultrasound. *Ultrasound Med Biol* **42**, 1834–1847 (2016).
190. Gao, R. Interpreting the electrophysiological power spectrum. *J Neurophysiol* **115**, 628 (2016).
191. Schober, P. & Schwarte, L. A. Correlation coefficients: Appropriate use and interpretation. *Anesth Analg* **126**, 1763–1768 (2018).
192. Schober, P. & Vetter, T. R. Correlation Analysis in Medical Research. *Anesth Analg* **130**, 332 (2020).
193. Feng, C. *et al.* Log-transformation and its implications for data analysis. *Shanghai Arch Psychiatry* **26**, 105–109 (2014).
194. Dalecki, D., Raeman, C. H., Child, S. Z. & Carstensen, E. L. Effects of pulsed ultrasound on the frog heart: III. The radiation force mechanism. *Ultrasound Med Biol* **23**, 275–285 (1997).
195. Yoo, S., Mittelstein, D. R., Hurt, R. C., Lacroix, J. & Shapiro, M. G. Focused ultrasound excites cortical neurons via mechanosensitive calcium accumulation and ion channel amplification. *Nature Communications* 2022 13:1 **13**, 1–13 (2022).

196. Yoo, S., Mittelstein, D., Hurt, R., Lacroix, J. & Shapiro, M. Focused ultrasound excites neurons via mechanosensitive calcium accumulation and ion channel amplification. *Nature Communications* 2022 13:1 1–15 (2022) doi:10.1101/2020.05.19.101196.
197. Mosayebi-Samani, M., Melo, L., Agboada, D., Nitsche, M. A. & Kuo, M. F. Ca²⁺ channel dynamics explain the nonlinear neuroplasticity induction by cathodal transcranial direct current stimulation over the primary motor cortex. *European Neuropsychopharmacology* **38**, 63–72 (2020).
198. Rattay, F. Analysis of models for extracellular fiber stimulation. *IEEE Trans Biomed Eng* **36**, 676–682 (1989).
199. Ma, X. *et al.* Study on the Role of Physical Fields in TMAS to Modulate Synaptic Plasticity in Mice. *IEEE Trans Biomed Eng* **71**, 1531–1541 (2024).
200. Li, X., Yu, K. & He, B. Magnetoacoustic tomography with magnetic induction (MAT-MI) for imaging electrical conductivity of biological tissue: A tutorial review. *Phys Med Biol* **61**, R249–R270 (2016).

194