Ultrasonic stimulation of peripheral nervous tissue: an investigation into mechanisms

C J Wright¹, J Rothwell² and N Saffari¹
1. Department of Mechanical Engineering, University College London, Gower Street, London, WC1E 6BT, UK
2. UCL Institute of Neuroscience, Queen Square, London, WC1N 3BG
E-mail: ucemcjw@live.ucl.ac.uk

Abstract. Neuro-stimulation has wide ranging clinical and research potential but this is currently limited either by low resolution, penetration or by highly invasive procedures. It has been reported in previous studies that ultrasound is able to elicit a neuro-stimulatory effect at a higher resolution than other non-invasive approaches but both the underlying mechanism that makes this possible and the practical details of how it can be implemented are still poorly understood. The current study has identified the main issues that need to be resolved in the field, proposing several different approaches to tackling these areas. An isolated in vitro peripheral nerve bundle was chosen as a simple model to demonstrate and investigate the neuro-stimulatory effect after preliminary results showed successful stimulation in a skin-nerve preparation. Early results from the nerve bundle show successful neurostimulation, indicating that structures in the peripheral nerve axon are sensitive to ultrasound. Further research using this model should reveal more precisely what structures are being affected and how to optimise the effect, helping to inform the design of future procedures and devices used in in vivo applications.

1. Introduction
Several effective medical techniques already exist for stimulating the Central Nervous System (CNS). The FDA has recently approved DBS (Deep Brain Stimulation) for various mental health and neurodegenerative disorders [25]. Transcranial Magnetic Stimulation (TMS) has been approved for treatment of depression and for VNS (Vegas Nerve Stimulation) and is being assessed for epilepsy and depression [10]. Transcranial ultrasound theoretically has the potential to replace or improve all of these treatments, solving many of their drawbacks. Low Intensity, Low Frequency Ultrasound (LILFU) is well placed to make rapid changes to clinical practice with the widespread use of ultrasound (US) in hospitals, High Intensity Focused Ultrasound (HIFU) devices especially can be adapted to produce the LILFU stimulation protocols allowing potentially rapid transition from the lab to the clinic. Indeed with the medical safety record of therapeutics and imaging ultrasound protocols LILFU is not expected to be dangerous and cutting edge researchers are already testing ultrasound neuromodulation protocols in humans [18].

The use of US for neuromodulation has been investigated sporadically for over 60 years but is only recently gaining significant momentum as a viable research area. Initial studies by Fry and others showed a variety of different US effects on neural tissue including potentiation and inhibition [7, 37]. The current research field can be divided into those studying peripheral nerve
stimulation and others targeting central nervous structures, with the focus in both being on in vivo work.

The first study to re-ignite interest in ultrasound neurostimulation of the CNS was in 2008 [34], shortly followed by another in vitro study in 2009 [24]. This sparked a series of in vivo studies demonstrating the efficacy of ultrasound neurostimulation by targeting the motor cortex of mice and rabbits [32, 39, 20, 14, 40, 23]. By contrast the studies on the peripheral nervous system only show much success when targeting specifically mechanosensitive structures such as the skin [18, 8, 24, 17, 4] or neuropathic tissue [33]. Attempts at inducing Action Potentials (APs) by specifically targeting nerve axon have been hitherto mostly unsuccessful, instead resulting in inhibition only [19, 11], although modulation of APs stimulated electrically is easily observed [29, 22, 31]. Only one study has reported successful US stimulation of a peripheral nerve trunk [13] though their stimulus was transmitted through brain tissue, which given reflections that inevitably occur within a rat’s skull, raises questions about their stimulation specificity.

In almost all of the literature, in both peripheral and central nervous studies, the theme follows that very little to no effort has been made to control or correct for reflections of ultrasound. This leads to mistrust of stated values of intensity and pressure and when combined with the variety of models and often conflicting results, studies become very difficult to compare. The mixed findings in the literature have even on occasion called into question existence of the stimulatory effect itself [2, 8]. The purpose of this research is to gain a better understanding of how ultrasound affects nervous tissue and create a hypothesis, backed by experimental evidence for how the mechanical forces of ultrasound are transduced into propagating electrical activity in the nerve fibres.

1.1. Biological Target Structure

Neurons are cells that have highly electrically polarised membranes; at rest their interior is highly negative with respect to the positive outside and they fire by depolarising the membrane in a wave that travels along the nerve. This electrical gradient is maintained by the cell by pumping charged ions in antiport against their concentration gradient. The major ions involved along the nerve axon are sodium and potassium. Studies reporting US neurostimulation by targeting the motor cortex of mammals [39, 34] have no way of knowing which part of the neuron is responding to ultrasound if indeed the effect is region specific. The wide variety of structures in different regions of a neuron makes it difficult to predict exactly how the ultrasound energy is being transduced into a cellular response. However, as one of the most essential parts of a nerve depolarisation response, ion channels themselves are a likely candidate.

Opening or closing of ion channels will alter their radius or hydrophobic thickness through conformational change, meaning that all of them will be to some extent sensitive to mechanical forces through the surrounding membrane or other sources, known as mechanosensitivity [35]. The simplest theory for US neurostimulation therefore is that ion channels are both the direct receiver of the US force and the only biological effectors involved in the AP generation. Ion channels shown to exhibit particular sensitivity to mechanical stimulation include non-selective cation superfamily, two pore domain potassium channel superfamily, amiloride sensitive sodium channel superfamily and voltage gated ion channels [12]. The proposed leg nerve model used in this study will contain two pore potassium leak channels that help maintain resting potential and are constitutively active. It will also contain the voltage gated sodium and potassium channels which depolarise the membrane when activated. It is hoped that future work using the current model will be able to determine the extent to which each of these channel types are affected by US.
1.2. Energy Transduction Mechanism
The 5 influences of US propagation through living tissue are outlined by Wulff et al. in 1951 [38] as: "(1) heating caused by absorption of acoustic energy; (2) periodic pressure changes; (3) radiation pressure; (4) streaming or flow in viscous media; and, (5) high temperatures and pressures associated with cavitation, defined as the formation of holes in liquid media”. Numbers 1 and 5 are usually discounted in recent studies due to the low average intensities making the predicted heating negligible and the chance of inertial cavitation low. It is possible however that stable cavitation (oscillation and growth/shrinkage without collapse) of pre-existing bubbles may be occurring.

Despite the passage of more than 60 years it has still not been determined which of the remaining mechanisms are the primary source of bio-effects in nerves. Of course all of them will be occurring to some extent in the complex environment of tissues but the importance of each will change based upon the parameters of the US stimulus being applied and the structure of the tissue. Hence the community must focus on a tight set of parameters which are optimum for eliciting certain desired forces so that its effect in different tissues can be determined. In the case of neurostimulation no optimum or desirable characteristics of the US stimulus have been arrived at by any consensus in the past literature which leaves the conjecture about the mode of action and method of energy transduction very open.

1.3. In Vitro Nerve Model
In the current study the effect of US on the excitability of peripheral crab leg nerves is tested. This model was chosen for its simple, well characterised nerve bundle [9] that is easy to extract and take recordings from. The relative biological simplicity of this nerve bundle together with a well characterised US field that is not distorted by obstructions or overlaying tissue is intended to give the current study clear and repeatable results where independent variables can be isolated and definite conclusions reached with confidence. High accuracy of US field characterisation is made possible by the lack of any solid tissue depth, allowing accurate modelling of forces on the micro and macro scale which can be compared between different studies and biological models.

Along with the benefits of an in vitro model, the nerve bundle will be exposed to stresses and temperature changes not expected in vivo which may affect sensitivity to US bio-effects. Also, the differences between central and peripheral nerves such as channel subtypes, supporting tissues and morphology mean that this model will not be used in direct comparison to recent CNS stimulation studies. However, as the model only involves axonal tissue, the Hodgkin and Huxley model AP, axon morphology and broad ion channel types are all well conserved and consistent with unmyelinated axons in humans [29, 26].

2. Experimental set up
In order to target an ex-vivo nerve with a controlled and known ultrasonic intensity the organ bath was floated on a 20cm deep water bath with a 2cm thick, high density foam US absorbing layer at the bottom of the tank to reduce the effect of reflections on focal amplitude, measured to be <5% of the peak focal amplitude. The water was kept cold (0-4°C) using ice to slow the nerve AP and to maintain the viability of the nerve bundle for longer. The ice was kept from the US propagation path by 2 sheets of stainless steel mesh (figure 1 and 2a & b).

The organ bath had to be designed and built to allow for simultaneous use of various modes of stimulation. A slot was cut into the acrylic wider than the beam profile of the PA409 transducer at the focal point (figure 3a) to allow ultrasound to pass through, preventing reflection or absorption by the chamber that might amplify or alter the effects on the nerve.
Figure 1. Schematic diagram of the experimental setup used to generate the alternate electrical and US stimuli recordings seen in figure 5 and 6. Equipment details: **function generator** - Agilent 33220A. **Power amplifier** - E&I 1020L 200W class AB linear amplifier with 55dBm gain. **Transducer** - Precision Acoustics 409 PZT curved ceramic 0.67MHz HIFU. **Signal Amplifier** - WPI DAM50 differential amplifier at 100X DC gain. **Electrical stimulator** - Digitimer DS3 constant current isolated stimulator. **Oscilloscope** - Lecroy HDO6054 500MHz. The water bath and US coupling cone were filled with degassed and deionized water. The organ bath was filled with white mineral oil.

Thin mylar film (12μm) was used to cover the slot as it has an acoustic impedance closely matched to water, providing a trench to hold the nerve bundle and isolate it from the water bath. Silver foil (25μm thick) strips were placed at intervals over the mylar film and fixed into the acrylic to be used as stimulation and recording electrodes. These electrodes were chosen to be orders of magnitude thinner than the wavelength of ultrasound in water (2.3mm at 0.67MHz) to avoid unwanted absorption. Silver was necessary for a low electrical resistance through the requisite thin foil and so the metal could be silver-chloride coated to provide high resistance to current flow through the saline around the nerve.

To further increase electrical resistance between the different electrodes white mineral oil was used to fill the organ bath, acting as US transmission medium that was electrically insulating, only a thin sheath of saline was maintained around the nerve to keep it alive for the duration of the experiment. A second chilled bath filled with saline was kept next to the main test tank where the nerve was kept if not being stimulated for prolonged periods. The organ bath and all implements were cleaned and sterilised with ethanol before every use.

A water-containing coupling cone was built to provide an US propagation path from the transducer to the oil in the organ bath. The aperture of this cone was covered with mylar film and placed over the nerve between electrode 4 and 5 using a spacer attachment to ensure accurate
alignment between the nerve bundle and the focal region of the US. Electrical stimulation was applied via electrode 3 using a Digitimer Ltd. DS3 model constant current isolated stimulator delivering a 0.2ms 2-20mA electrical stimulus pulse (figure 2a) at the start of the procedure and between each US stimulus. This was done to keep track of the health of the saturated Compound Action Potentials (CAPs) and therefore the state of the nerve bundle. Each stimulus event (of either modality) was separated by 3 seconds.

Ultrasound parameters were chosen based on precedent in the literature for successful neurostimulation protocols. A higher power was used here than in previously published studies due to predictions made from intensities used in peripheral nerve studies [18, 22, 7]. US parameters used are described in figure 5. Orders of parameters being tested were randomised to prevent systematic error from nerve inhibition or other effects.

To check the metrology of the pressure estimates at the focal point the US field was modelled
using a modified Khokhlov-Zabolotskaya-Kuznetzov (KZK) equation:

$$\partial^2_{T,z} - \frac{1}{2} \partial^2_T U^2 \beta \partial^3_T U - \gamma \Delta_y U = 0$$

where $\beta$ and $\gamma$ are positive constants, $T$ is time, $y$ and $z$ are two linear dimension variables and $U$ is velocity [41, 16]. This method was used to predict any non-linear propagation that may be occurring (figure 3) by modelling 50 harmonics around the fundamental frequency. At the maximum driving power used in the current study (20W) the peak positive pressure was indeed found to be higher than the peak negative (figure 3a) by a small amount along with minor harmonics generated (figure 3b). Both these effects are not seen at lower amplitude driving powers (figure 3c&d). These non-linearities should be considered in any estimation of biological forces, especially when considering medical applications with higher tissue depths. The predicted pressure field profile was found to be in good agreement with previously predicted values and hydrophone measurements (figure 3e).

After propagation through water to the focal point a single pulse was measured using a fibre optic hydrophone to demonstrate the amplitude profile that the nerve bundle would encounter (figure 4). The ramping up and trailing off of the pulse is due to the transducer’s damping properties.

3. Results
Delivering a rapid pulse train of focused ultrasound directly into axonal tissue was able to produce significant excitation (figure 5) that only occurred in the first of 5 repeat stimuli. This data was collected over 5 separate crabs (all large male *Cancer pagurus*), the large CAP response was seen in 6 out of 9 (67%) nerve extractions that responded to electrical stimulation. Of these responsive nerves only 23% of US stimulus pulse trains resulted in observable CAPs. Significant CAP events were primarily seen in response to the first US stimulus, the application of subsequent pulse trains failing to result in recorded CAP events despite the electrical stimulation showing the nerve to be functional. The electric stimulation did reveal a successive reduction in CAP amplitude between each dose of US, especially visible in figure 5a where the response to the US was also highest, although this inhibition during the 5 stimulus protocol was not always observed(figure 5c and 6f). The lower or upper thresholds for producing the neurostimulatory effect were not found in these preliminary experiments.
Figure 3. Objects a to d are predicted plots of the US field using the KZK acoustic model at the top and bottom end of input powers experimentally used (a&b - 20 Watt, c&d 0.17 Watt) a&c) Pressure oscillation at the focal point of the PA409 transducer. b&d) Transverse scan of the pressure amplitude profile at the modelled focal point. Second and third harmonics are displayed in green and red respectively. e) Pressure amplitude profile measured from the PA409 HIFU transducer using a Precision Acoustics plane tipped fibre-optic hydrophone in degassed and de-ionised water at the geometric focal point.

Figure 4. Acoustic pressure amplitude trace of a single pulse in the stimulation protocol. Recorded using a plane tipped fibre optic hydrophone placed in the focal region of a 0.67MHz PA407 HIFU transducer in degassed and deionised water.
Figure 5. Recorded voltage amplitude profiles involving CAP activity from a crab leg nerve bundle using alternating electrical (200μs, 2mA) and ultrasonic stimulation (10kHz PRF, 50% duty cycle, 0.67MHz sinusoidal wave, b) 1MPa d) 1.3MPa peak focal pressure (66 W/cm² and 118.1 W/cm² $I_{SPT}$P respectively), total stimulus duration of 8ms (80 pulses)) of a crab leg nerve bundle with 2 seconds between each recording. Figures a and c are overlaid CAP recordings where the nerve was stimulated electrically, a and c correspond to US stimulation recordings in b and d respectively.

Recovery times of up to 20 minutes after observation of a successful US induced CAP were investigated (1,5,10 and 20 minute recovery periods) where no electrical or US stimulus was given and the nerve was not moved, the stimulus amplitude and pulse parameters were kept the same (figure 6). Recovery time was found to have no effect on susceptibility to US as no US induced CAPs were observed in 3 trials (12 total US stimuli events).

Movement of the nerve by 5mm so that a new section was under the US focus for each stimulus session was able to improve the US response reliability from 23% to 71% (total of 14 exposures at 260 W/cm² $I_{SPT}$P). This success did not appear to depend on which direction the nerve was moved.
Figure 6. Recorded voltage amplitude profiles from a crab leg nerve bundle using 2MPa (260W/cm²) peak focal pressure, otherwise identical protocol to those in figure 5. Objects a to e are nerve responses from US stimuli with longer recovery times left between each stimulus event (0, 1, 5, 10 and 20 minutes respectively). f represents an example electrical stimulus profile taken alternately with the recordings in a. Electrical stimulus recordings were also made during US stimuli b to e but are not displayed here.
4. Discussion

The electrical responses to ultrasound stimulation reported here represent a significant new finding and demonstrate the usefulness of the *ex-vivo* peripheral nerve model as a vehicle for studying the nature of the US-nerve interaction. The data found in our experiments contradict previous studies reporting that *ex-vivo* peripheral nerves cannot be stimulated by US, only modulated or inhibited [19, 22, 31, 11] and provides several novel insights into the mechanism of action, proving that purely axonal tissue and its constituents are sufficient for neurostimulation and that the reliability of the effect is high enough to simultaneously stimulate many axons in the nerve bundle. The next step using the current model is to test several of the hypotheses proposed by different groups working in the field such as ultrasound induced intra-membrane bubble cavitation [15, 28, 27] or radiation force inducing membrane tension [8, 21]. Further research using this model should reveal more precisely what structures are being affected and how to optimise the effect, helping to inform the design of future procedures and devices.

To ensure temperature was a negligible factor HIFU simulation modelling was used to determine the temperature rise at the focal maximum at the highest intensities used in this study. The predicted rise was less than 0.1°C.

In figure 5a and c the rate of degradation of electrically induced CAPs is faster than a time series control experiment with no US stimulation predicted (30% and 26% reduction over 50 seconds vs a maximum of 8% over the same time period using the control time course) especially visible in figure 5a between the 2nd and 3rd stimulus. This warrants further investigation through microscopic or histological examination of the nerve tissue after exposure to US stimulus which will be carried out in any future experiments. If no signs of damage are found then this effect is likely due to a long lasting inhibitory effect of US on nerve conduction past the point of sonication.

A location-specific self-inhibitory effect of the US mechanism appears to be at play here given the results from figure 6 where very little inhibition of the electrical stimulation was seen (36% reduced maximum CAP amplitude over 36 minutes) despite total inhibition of the US response after the first stimulation. This implies that the lack of further US response is not due to mechanical damage, as this would be expected to similarly block or slow action potentials from electric stimulation. Without mechanical damage as a source of this inhibition the authors propose that the mechanism of the observed stimulation in this experiment involves bubbles or bubble nuclei which are somehow moved or depleted during a single pulse train stimulus.

This theory is supported further by the improvement of response reliability as new sections of the nerve are targeted and by well established bubble nucleation theory that predicts US parameters very similar to the ones here and in previous studies are ideal for generating cavitational activity [6].

Although the nature and location of cavitation nuclei in tissue is debated [1], their existence has long been known [3, 5, 36], meaning the same stable cavitation stimulation mechanism predicted here may be acting *in vivo*. However, the nature of an *in vitro* model makes the presence of micro-scale bubbles more likely than *in vivo*. The presence of micro-bubbles will be quantified at a later stage and if they are found, their behaviour and forces exerted on surrounding tissues can be modelled and reproduced in their absence. Alternately their existence can be induced *in vivo* by low power continuous wave ultrasound [30].

An important attribute of the crab leg nerve model is that all the fibres in the bundle are unmyelinated which may make them more susceptible to US forces than equivalent myelinated nerve fibres that have been tested previously in mammals and amphibians [19, 22, 31, 2]. They share this attribute with the majority CNS neurones (grey matter) where the stimulatory effect has been shown to be most reliable though further study is needed to test whether the myelination itself is a significant factor.
5. Conclusions

Reported here is a novel approach to studying US neurostimulation, expressly designed to determine the nature of the US tissue interaction, not just its effects. Already the methods described above have demonstrated the US sensitivity of the ex-vivo peripheral nerve model, confirming that US neurostimulation mechanisms are not restricted to the structures of the CNS. Furthermore, structures within purely axonal tissue are sufficient to receive US stimulation and produce action potentials as a direct result, vastly reducing the options for the critical biological US force receivers.

Using the equipment and experiments described above it is predicted that future work will quickly be able to determine the key parameters of US that are responsible for eliciting neuromodulation. This information would have immediate value in optimising stimulation parameters used in research, beginning to provide some theoretical consensus in the field and potentially start to unify the diverse range of observed US bioeffects. Answering the two questions at the core of this study: what is the key US force mechanism? and What is the key biological structure being affected? will certainly shed much needed light on this poorly understood phenomenon.

References

[1] R. Arieli and A. Marmur. Decompression sickness bubbles: are gas micronuclei formed on a flat hydrophobic surface? *Respir Physiol Neurobiol*, 177:1923, 2011.
[2] V. Calucci. Focused ultrasound effects on nerve action potential in vitro. *Ultrasound in Medicine & Biology*, 35(10):1737–1747, 2009.
[3] V.P.J. Craig. Formation of micronuclei responsible for decompression sickness. *Journal of Colloid and Interface Science*, 183:260, 1996.
[4] D. Dalecki, S. Z. Child, C. H. Raeman, and E. L. Carstensen. Tactile perception of ultrasound. *The Journal of the Acoustical Society of America*, 97(5 Pt 1):3165–3170, May 1995.
[5] A. Evans and D. N. Walder. Significance of gas micronuclei in the aetiology of decompression sickness. *Nature*, 222(5190):251–252, April 1969.
[6] H.G. Flynn and C.C. Church. A mechanism for the generation of cavitation maxima by pulsed ultrasound. *The Journal of the Acoustical Society of America*, 76(2):505–512, 1984.
[7] W.J. Fry, V.J. Wulff, D. Tucker, and F.J. Fry. Physical factors involved in ultrasonically induced changes in living systems. Identification of non-temperature effects. *Acoust Sot Am*, 22:868, 1950.
[8] L.R. Gavrilov and E.M. Tsirulnikov. Focused ultrasound as a tool to input sensory information to humans. *Acoustical Physics*, 177:121, 58.
[9] G.A. Horridge and R.A. Chapman. Sheaths of the motor axons of the crab carcinus. *Quarterly Journal of Microscopical Science*, 3-105:175181, 1964.
[10] PG Janicak, Z Nahas, SH Lisanby, HB Solvason, and Sampson SM. Durability of clinical benefit with transcranial magnetic stimulation (tms) in the treatment of pharmacoresistant major depression: assessment of relapse during a 6-month, multisite, open-label study. *Brain Stimul.*, 2010.
[11] E.J. Juan, R. Gonzalez, G. Albers, M.P. Ward, and P Irazoqui. Vagus nerve modulation using focused pulsed ultrasound: Potential applications and preliminary observations in a rat. *International Journal of Imaging Systems and Technology*, 24(1):67–71, 2014.
[12] A. Kamkin and I. Kiseleva. *Mechanosensitivity of the Nervous System*, 2009.
[13] H. Kim, S.J. Taghados, K. Fischer, L. Maeng, S Park, and S.S. Yoo. Noninvasive transcranial stimulation of rat aducens nerve by focused ultrasound. *Ultrasound in Medicine & Biology*, 38(9):1568 – 1575, 2012.
[14] R.L. King, J.R. Brown, W.T. Newsome, and K.B. Pauly. Effective parameters for ultrasound-induced in vivo neurostimulation. *Ultrasound in Medicine & Biology*, 39:312331, 2013.
[15] B. Krasovitski, V. Frenkel, S. Shoham, and E. Kimmel. Intramembrane cavitation as a unifying mechanism for ultrasound-induced bioeffects. *PNAS*, 2011.
[16] V.P. Kuznetsov. Equations of nonlinear acoustics. *Soviet Physical Acoustics*, 1971.
[17] W. Lee, H. Kim, S. Lee, S. Yoo, and Y.A. Chung. Creation of various skin sensations using pulsed focused ultrasound: Evidence for functional neuromodulation. *International Journal of Imaging Systems and Technology*, 24(2):167–174, June 2014.
[18] W. Legon, A. Rowlands, A. Opitz, T.F. Sato, and W.J. Tyler. Pulsed ultrasound differentially stimulates somatosensory circuits in humans as indicated by eeg and fmr. *PLoS ONE*, 7:e51177., 2012.
[19] P.P. Lele. Effects of focused ultrasonic radiation on peripheral nerve, with observations on local heating. *Experimental Neurology*, 8(1):47 – 83, 1963.

[20] E. Mehic, J.M. Xu, C.J. Caler, N.K. Coulson, C.T. Moritz, and P.D. Mourad. Increased anatomical specificity of neuromodulation via modulated focused ultrasound. *Plos One*, 9(2):e86939, February 2014. WOS:000330631800016.

[21] M.D. Menz, . Oralkan, P.T. Khuri-Yakub, and S.A. Baccus. Precise neural stimulation in the retina using focused ultrasound. *J. Neurosci.*, 33:45504560, 2013.

[22] R.T. Mihran, F.S. Barnes, and H. Wachtel. Temporally-specific modification of myelinated axon excitability in vitro following a single ultrasound pulse. *Ultrasound in Medicine & Biology*, 16:297309, 1990.

[23] B. Min et al. Focused ultrasound modulates the level of cortical neurotransmitters: Potential as a new functional brain mapping technique. *International Journal of Imaging Systems and Technology*, 21(2):232–240, 2011.

[24] R. Muratore, J. LaManna, E. Szulman, M.S.A. Kalisz, M.S.M. Lamprecht, M. abd Simon, M.S.Z. Yu, N. Xu, B. Morrison, and E.S. Ebbini. Bioeffective ultrasound at very low doses: Reversible manipulation of neuronal cell morphology and function in vitro. *Respir Physiol Neurobiol*, page 2529., 2014.

[25] CW Olanow, MB Stern, and Sethi K. The scientific and clinical basis for the treatment of parkinson disease. *Neurology*, 2009.

[26] F.C. Page. A comparative study of five fresh-water and marine species of thecamoebidae. *Transactions of the American Microscopical Society*, 90:157173, 1971.

[27] M. Plaksin, S. Shoham, and E. Kimmel. Intramembrane cavitation as a predictive bio-piezoelectric mechanism for ultrasonic brain stimulation. *Phys. Rev. X*, 4:011004, Jan 2014.

[28] S.M. Rappaport, A.M. Berezhkovskii, J. Zimmerberg, and S.M. Bezrukov. Thermodynamics of interleaflet cavitation in lipid bilayer membranes. *Phys. Rev.*, E:87, 2013.

[29] A. Sheltawy and R.M. Dawson. The polyphosphoinositides and other lipids of peripheral nerves. *Biochem. J*, 100:1218, 1966.

[30] G.R. Ter Harr, S. Daniels, and K. Morton. Evidence for acoustic cavitation in vivo: Thresholds for bubble formation with 0.75-MHz continuous wave and pulsed beams. *IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control*, 33(2):162–164, March 1986.

[31] P. Tsui, S. Wang, and C. Huang. In vitro effects of ultrasound with different energies on the conduction properties of neural tissue. *Ultrasonics*, 43(7):560 – 565, 2005.

[32] Y. Tufail et al. Transcranial pulsed ultrasound stimulates intact brain circuits. *Neuron*, 66:681–694, 2010.

[33] R.E. Tych, M. Gofeld, J.G. Jarvik, M. Kliot, J.D. Loeser, A.M. McClintic, R.J. Ollos, K.D. Pederson, R.E. Sparks, G.W. Terman, and P.D. Mourad. Neuropathic tissue responds preferentially to stimulation by intense focused ultrasound. *Ultrasound Med Biol*, 39:111116, 2013.

[34] W.J. Tyler et al. Remote excitation of neuronal circuits using low-intensity, low-frequency ultrasound. *PloS One*, 3(10):3511, 2008.

[35] R. D. Vann, J. Grimstad, and C. H. Nielsen. Evidence for gas nuclei in decompressed rats. *Undersea Biomedical Research*, 7(2):107–112, June 1980.

[36] V.J. Wulff, W.J. Fry, D. Tucker, F.J. Fry, and C. Melton. Effects of ultrasonic vibrations on nerve tissues. *Exp Biol Med*, 76:361366, 1951.

[37] Y. Younan, T. Deffieux, B. Larrat, M. Fink, M. Tanter, and J.-F. Aubry. Influence of the pressure field distribution in transcranial ultrasonic neurostimulation. *Medical Physics*, 40:082902, 2013.

[38] E.A. Zabolotskaya and R.V. Khokhlov. Quasi-plane waves in the nonlinear acoustics of confined beams. *Soviet Physical Acoustics*, 1969.