A Prototype Stimulator System for Noninvasive Low Intensity Focused Ultrasound Delivery

Amit P. MULGAONKAR*,[1,2] Rahul SINGH,[1,2,3] Meghedi Babakhanian,[1,2] Martin O. CULJAT,[1,2,3] Warren GRUNDFEST,[1,2,3,4] Alexander BYSTRITSKY,[5] Alessandra GORGULHO,[6] Goran LACAN,[7] and William P. MELEGA[1,7]

[1]*Center for Advanced Surgical and Interventional Technology (CASIT),* [2]*Biomedical Engineering IDP,*
[3]*Department of Surgery,* [4]*Department of Electrical Engineering,* [5]*Department of Psychiatry and Biobehavioral Science,*
[6]*Department of Neurosurgery,* [7]*Department of Molecular and Medical Pharmacology*
University of California, Los Angeles, CA USA;
** Corresponding author contact at:amitm@ucla.edu*

Abstract. A prototype Low Intensity Focused Ultrasound (LIFU) stimulator system for non-invasive neuromodulation in a large animal model was developed and tested. We have conducted a feasibility study on a Göttingen minipig, demonstrating reversible, targeted transcranial neuromodulation. The hypothalamus of the minipig was repeatedly stimulated with LIFU which evoked temporally correlated increases in both heart rate and blood pressure.

Stud Health Technol Inform. 2012;173:297-303. PubMed PMID: 22357005.

Introduction

The use of high intensity focused ultrasound (HIFU) was first proposed over 40 years ago to reversibly modulate the function of the central nervous system (CNS) [1, 2]. Until 1990, only a few papers were published on the use of ultrasound for neuromodulation, with much of the work coming from the laboratory of Gavrilov in the former Soviet Union [3-7]. These studies demonstrated reversible neuromodulation with ultrasound without any observable damage to neuronal tissue. Several other groups also explored the effects of focused ultrasound energy on neuronal tissue and demonstrated similar results [8-12]. More recently, a permanent nerve block on a rabbit sciatic nerve was successfully shown *in-vivo,* using high intensity (>1.5 kW/cm^2) and 30s-long, continuous sonication [13]. However, these studies were confounded by temperature elevations that were accompanied by the delivery of HIFU.

Since the excessive heat and pressure associated with HIFU may damage brain tissue, low-intensity focused ultrasound sonication (LIFU) has been suggested as an alternative for brain neuromodulation. Studies with *ex-vivo* experimental set-ups have provided supporting evidence that LIFU may be used to modulate neuronal activity [14, 15], possibly through modulation of the mechanosensitive properties of voltage-gated ion channels and axonal conductance. A recent *in-vivo* study in a rodent model of acute seizures has demonstrated modulation of regional neural activity in brain by suppression of ictal activity [15, 16].

LIFU has only recently been advanced for consideration of clinical applications with the advent of superior ultrasound instrumentation and brain MR imaging that allows for accurate and safe targeting in humans. Demonstration of LIFU efficacy in a large animal model can bridge the gap between exploratory work in small animals and future applications in humans. Accordingly, we have used an experimental LIFU system and developed a coupling mechanism and associated surgical techniques for the targeted noninvasive stimulation of brain structures in the Göttingen minipig. The gyrencephalic minipig's brain resembles the human brain more in anatomy, growth and development than do the brains of commonly used small laboratory animals [17-20], while the relatively larger size of the minipig brain compared to rodents allows for targeting of cortical and subcortical structures by MRI imaging techniques [21, 22].

We have conducted a feasibility study in a minipig to demonstrate that our surgical procedures, LIFU instrumentation, and MR-brain targeting can be successfully used to achieve LIFU-evoked physiological responses in a large animal model. To achieve these goals, LIFU pulses were targeted to the dorsomedial hypothalamus using techniques described below. A range of pulse parameters were attempted and associated heart rate/blood pressure (HR/BP) changes were recorded in the anesthetized minipig.

Methods & Materials

Stimulator System: The study was performed using the Brainsonix BX1001 system, consisting of a stimulating unit and a transducer. Ultrasound pulse burst trains were generated on a console containing a digital function generator (Agilent 33220A) feeding a high-power RF power amplifier (Electronics + Innovation Model 240L) that drove a custom piezo-ceramic transducer. An RF coupler (Werlatone C5948-10) and power meter (Agilent E4419B) allowed for real-time monitoring of the electrical power delivered to the transducer.

The transducer consisted of a spherically curved air-backed lead-zirconate-titanate (PZT) element (Channel Industries, Santa Barbara, CA) with conductive epoxy electrodes operating at a resonant frequency of 650 KHz. The transducer had a 6 cm aperture and 7 cm radius of

curvature. Silicone was used to pot the element into a sealed acrylic housing. The acoustic performance of the transducer and system were evaluated in a precision acoustic measurement tank (AIMS, Sonora/Unisyn, Longmont, CO) using a calibrated membrane hydrophone (Sonora Medical Systems Model 804).

In Vivo Procedure: Targeting of the hypothalamic region was planned according to regional landmarks identified in a published stereotactic brain atlas of the Göttingen minipig species. T1 weighted coronal, axial, and sagittal MR sections were obtained throughout the hypothalamus region and a corresponding trajectory was planned using the iPlan 2.6 software (BrainLab, Feldkirchen, Germany).

A circular burr-hole was made in the outer layer of the trabecular porcine skull, with the lower skull wall (~2mm) remaining intact so as to maintain the integrity of the brain cavity and to provide a rough approximation of the challenges to be faced in delivering LIFU through the human skull. These techniques were validated in prior studies in the minipig [23, 24].

Transducer Positioning and Mounting: The transducer was mounted in a custom acoustic coupler attached to a commercially available stereotactic frame (Cosman-Roberts-Wells) that was affixed to the minipig's head. The stereotactic frame allowed the transducer to be positioned accurately at a predetermined location on the head. The frame-mounted ultrasonic transducer was coupled to the minipig skull through use of an acrylic water-filled conical cylindrical enclosure. The coupling assembly was placed into the burr hole and placed against the lamina, therefore ensuring that the transducer was acoustically coupled to the lamina of the skull. Acoustic coupling is necessary to avoid impedance mismatches in the ultrasonic path and to ensure correct beam propagation and focus.

The transducer was inserted through the acrylic cylindrical enclosure, with the rear of the transducer attached to a positioning shaft that allows the transducer to be translated within the enclosure (Fig. 1). This arrangement also allows for precise control over the targeting path, and depth of the ultrasound pulse. The water filling this enclosure was kept in a degassed state and monitored for rises in temperature. Acoustic modeling with PZFlex software (Weidlinger Associates) was used to verify that the coupler geometry did not impede acoustic propagation of the wave (Fig 2).

Results

The transducer was measured as having an in-water focus at 5.6 cm and a -3 dB focus diameter of 5.6 mm. These measurements represent the minimal focus volume that can be delivered by the transducer. The dispersion of the focus due to the scattering and geometric lensing effects of the skull in a transcranial application is currently being studied. The ex-vivo acoustic attenuation of the skull was measured as 28 dB.

The coupling technique and surgical procedure enabled transcranial delivery of ultrasound energy into the hypothalamus region of the brain. A set of pulse parameters (target depth 30 mm, input 275 mV_{p-p}, 25 cycles per pulse, 25 kHz PRF) were identified that appeared to cause reversible neuromodulation in the minipig. Upon LIFU delivery to the hypothalamus region for periods of 90s, sustained increases in BP and HR were observed that reversed upon termination of stimulation.

We also conducted experiments with fresh ex-vivo mini pig skulls and demonstrated significant alterations to the depth and width of the acoustic focus based on the morphology of the skull, and the location of stimulation. Further, the acoustic attenuation of the Water to Minipig Skull to Brain interface was measured as being significantly more than theoretical values of approximately 5 dB. However, observing a HR/BP change while targeting the hypothalamus region suggests that at least part of the acoustic energy reached the intended target.

Conclusions & Discussion

The work summarized above is the first known demonstration to date of LIFU-based neuromodulation in a large animal model and was intended to be a 'proof of concept' for the effective use of our stimulator system, targeting techniques, coupling mechanism, and surgical procedures to be applied to subsequent full-scale experimentation.

While we have demonstrated LIFU-evoked physiological responses in a porcine model, there remain significant challenges for accurate transcranial LIFU delivery. For example, the presence of the lower lamina of the skull alters the focus and intensity of the ultrasound beam relative to its unimpeded water tank ('best case') value. This makes it difficult to quantify the exact location and acoustic energy at the point of stimulation within the brain. This is a significant limitation of the present study. Future studies that include real-time MR guidance of the stimulation are planned to address this issue.

Further work will be required for quantifying any distortions to the ultrasound beam caused by the intact lamina of the minipig skull. Such work in the minipig will be necessary to advance LIFU neuromodulation to humans. Significantly, the use of this large animal model will allow for LIFU targeting of discrete brain structures, at depths of 30-40 mm from the brain surface, which is not realizable in studies using small rodents. In future studies, we plan to model potential clinical applications in humans by designing and evaluating corresponding methods for accurate targeting and precise LIFU delivery.

References

[1] Fry, W., and Fry, F., 1958, "Fundamental Neurological Research and Human Neurosurgery Using Intense Ultrasound," Phys. Med, 37(pp. 148.
[2] Fry, W. J., 1958, "Intense Ultrasound in Investigations of the Central Nervous System," Advances in biological and medical physics, 6(pp. 281.
[3] Gavrilov, L. R., Tsirul'nikov, E. M., and Shchekanov, E. E., 1975, "[Responses of the Auditory Centers of the Frog Midbrain to Labyrinth Stimulation by Focused Ultrasound]," Fiziol Zh SSSR Im I M Sechenova, 61(2), pp. 213-21.
[4] Gavrilov, L. R., Gersuni, G. V., Ilyinsky, O. B., Sirotyuk, M. G., Tsirulnikov, E. M., and Shchekanov, E. E., 1976, "The Effect of Focused Ultrasound on the Skin and Deep Nerve Structures of Man and Animal," Prog Brain Res, 43(pp. 279-92.
[5] Gavrilov, L. R., 1984, "Use of Focused Ultrasound for Stimulation of Nerve Structures," Ultrasonics, 22(3), pp. 132-8.
[6] Adrianov, O. S., Vykhodtseva, N. I., and Gavrilov, L. R., 1984, "[Use of Focused Ultrasound for Local Effects on Deep Brain Structures]," Fiziol Zh SSSR Im I M Sechenova, 70(8), pp. 1157-66.
[7] Adrianov, O. S., Vykhodtseva, N. I., Fokin, V. F., Uranova, N. A., and Avirom, V. M., 1984, "[Reversible Functional Shutdown of the Optic Tract on Exposure to Focused Ultrasound]," Biull Eksp Biol Med, 97(6), pp. 760-2.
[8] Foster, K. R., and Wiederhold, M. L., 1978, "Auditory Responses in Cats Produced by Pulsed Ultrasound," J Acoust Soc Am, 63(4), pp. 1199-205.
[9] Pp, L., 1963, "Effects of Focused Ultrasonic Radiation on Peripheral Nerve, with Observations on Local Heating. Experimental Neurology 8, 47–83.," Experimental Neurology, 8(pp. 47-83.
[10] Lele, 1963, "Effects of Focused Ultrasonic Radiation on Peripheral Nerve, with Observations on Local Heating. ," Experimental Neurology, 8(pp. 47-83.
[11] Rinaldi, P. C., Jones, J. P., Reines, F., and Price, L. R., 1991, "Modification by Focused Ultrasound Pulses of Electrically Evoked Responses from an in Vitro Hippocampal Preparation," Brain Res, 558(1), pp. 36-42.
[12] Velling, V. A., and Shklyaruk, S. P., 1988, "Modulation of the Functional State of the Brain with the Aid of Focused Ultrasonic Action," Neurosci Behav Physiol, 18(5), pp. 369-75.
[13] Foley, J. L., Little, J. W., Starr, F. L., 3rd, Frantz, C., and Vaezy, S., 2004, "Image-Guided Hifu Neurolysis of Peripheral Nerves to Treat Spasticity and Pain," Ultrasound Med Biol, 30(9), pp. 1199-207.
[14] Bachtold, M. R., Rinaldi, P. C., Jones, J. P., Reines, F., and Price, L. R., 1998, "Focused Ultrasound Modifications of Neural Circuit Activity in a Mammalian Brain," Ultrasound Med Biol, 24(4), pp. 557-65.
[15] Tyler, W. J., Tufail, Y., Finsterwald, M., Tauchmann, M. L., Olson, E. J., and Majestic, C., 2008, "Remote Excitation of Neuronal Circuits Using Low-Intensity, Low-Frequency Ultrasound," PLoS One, 3(10), pp. e3511.
[16] Min, B.-K., Bystritsky, A., Jung, K.-I., Fischer, K., Zhang, Y., Maeng, L.-S., In Park, S., Chung, Y.-A., Jolesz, F., and Yoo, S.-S., 2011, "Focused Ultrasound-Mediated Suppression of Chemically-Induced Acute Epileptic Eeg Activity," BMC Neuroscience, 12(1), pp. 23.
[17] Lind, N. M., Moustgaard, A., Jelsing, J., Vajta, G., Cumming, P., and Hansen, A. K., 2007, "The Use of Pigs in Neuroscience: Modeling Brain Disorders," Neurosci Biobehav Rev, 31(5), pp. 728-51.
[18] Larsen, M., Bjarkam, C. R., Ostergaard, K., West, M. J., and Sorensen, J. C., 2004, "The Anatomy of the Porcine Subthalamic Nucleus Evaluated with Immunohistochemistry and Design-Based Stereology," Anat Embryol (Berl), 208(3), pp. 239-47.
[19] Jelsing, J., Hay-Schmidt, A., Dyrby, T., Hemmingsen, R., Uylings, H. B., and Pakkenberg, B., 2006, "The Prefrontal Cortex in the Gottingen Minipig Brain Defined by Neural Projection Criteria and Cytoarchitecture," Brain Res Bull, 70(4-6), pp. 322-36.
[20] Nielsen, M. S., Sorensen, J. C., and Bjarkam, C. R., 2009, "The Substantia Nigra Pars Compacta of the Gottingen Minipig: An Anatomical and Stereological Study," Brain Struct Funct, 213(4-5), pp. 481-8.
[21] Sakoh, M., Rohl, L., Gyldensted, C., Gjedde, A., and Ostergaard, L., 2000, "Cerebral Blood Flow and Blood Volume Measured by Magnetic Resonance Imaging Bolus Tracking after Acute Stroke in Pigs: Comparison with [(15)O]H(2)O Positron Emission Tomography," Stroke, 31(8), pp. 1958-64.
[22] Lacan, G., De Salles, A. A., Gorgulho, A. A., Krahl, S. E., Frighetto, L., Behnke, E. J., and Melega, W. P., 2008, "Modulation of Food Intake Following Deep Brain Stimulation of the Ventromedial Hypothalamus in the Vervet Monkey. Laboratory Investigation," J Neurosurg, 108(2), pp. 336-42.
[23] Lathers, C. M., Jim, K. F., and Spivey, W. H., 1989, "A Comparison of Intraosseous and Intravenous Routes of Administration for Antiseizure Agents," Epilepsia, 30(4), pp. 472-9.
[24] Jim, K. F., Lathers, C. M., Farris, V. L., Pratt, L. F., and Spivey, W. H., 1989, "Suppression of Pentylenetetrazol-Elicited Seizure Activity by Intraosseous Lorazepam in Pigs," Epilepsia, 30(4), pp. 480-6.

Illustrations

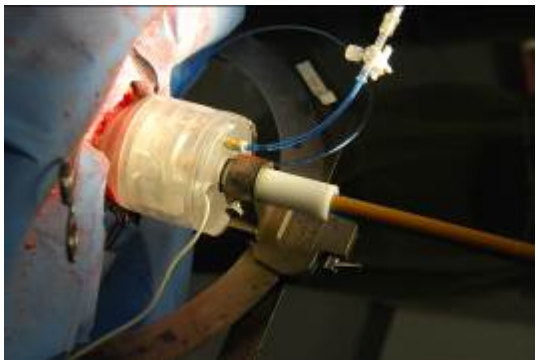

Fig. 1: Transducer embedded within cylindrical enclosure, with positioning shaft allowing the transducer to translate in the axial direction while maintaining acoustic coupling.

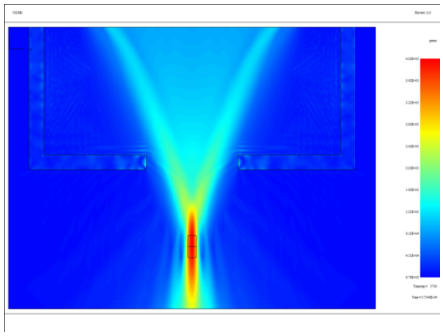

Fig 2: PZFlex acoustic finite element simulation of focused ultrasound beam propagation in water, with vertical axis depth (into brain) and horizontal axis the lateral axis. The model guided design of the coupling mechanism.