only intramembrane cavitation can explain all the observed aspects of ultrasonic neuromodulation.

Methods

We analyzed the relevant experimental literature using modified Rayleigh–Plesset intramembrane cavitation BLS biomechanics and acoustic radiation pressure gradients (RPG) - induced membrane dynamics. By coupling these biomechanical models to biophysical membrane models we predict dynamical biophysical responses of artificial bilayer membranes, and of three common neocortical single cell Hodgkin-Huxley type models: i) Regular Spiking (RS) cortical pyramidal neuron, ii) Fast Spiking (FS) cortical inhibitory neuron and iii) Low Threshold Spiking (LTS) cortical inhibitory neuron, RS-FS-LTS Hodgkin-Huxley based network model and CNS axon model. In addition, live brain tissue RPG subjected areal strains were evaluated in a viscoelastic brain model.

Results

Only the Neuronal Intramembrane Cavitation Excitation (NICE) models were able to explain US-induced action potential generation through BLS-type pulsating nano-bubbles inside the bilayer plasma membrane: the leaflets' periodic vibrations induce US-frequency membrane capacitance and potential oscillations, leading to slow charge accumulation across the membrane (on a time scale of tens of milliseconds), until action potentials are generated. In contrast, the analysis of RPG-induced membrane capacitance variations associated with membrane area changes explain artificial membrane results, but were found to be highly unlikely sources for neural excitation, when considering the areal strains expected to form in brain tissue during normal sonication. Further, the NICE-LTS inhibitory neurons show a much higher relative sensitivity to sparse ultrasonic stimulation compared to the other neurons, resulting from their T-type voltage gated calcium channels. This model-based prediction was found to explain the results of a significant body of suppression and excitation experimental studies, including in humans.

Conclusions

These results provide a unified theoretical framework for a large body of experiments in multiple preparations across the field of US neuromodulation, lending further support to the hypothesis that intramembrane cavitation is responsible for ultrasonic neuromodulation. They could thus pave the way towards new CNS therapeutic protocols, using the only method that currently allows targeted non-invasive neuromodulation with millimetre spatial resolution essentially anywhere in the brain.

O12

Ultrasonic stimulation of mammalian retina *in-vitro*

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Objectives

Following previous in vivo stimulation of the retina, we aimed to achieve a first direct measurement of the response of mammalian retinal neurons to ultrasonic (US) stimuli, and to study and characterize this response.

Methods

We coupled a high-density phased array (986 elements on a 25x35 mm^2 area) to a system for multi-electrode-array (MEA) recording with 256 contacts. Mouse retinas were dissected and placed on the MEA, and sonicated at 2.3 MHz, applying varying durations and intensities, as well as stimulated by light. The acquired data were processed to detect action potentials (spikes) elicited by retinal ganglion cells, and analysed to reveal the relations between the stimuli and the responses.

Results

We found prominent spike responses for stimuli in the range of 4.3-7.3 W/cm^2 and 0.5-1 s, which disappeared when the focus was steered 1.5 mm away. Furthermore, we found that the relation between the response strength and the stimulation intensity, or duration, followed a logistic sigmoid curve, while the response latency was described by a decreasing exponent. Lastly, we found indications that the observed responses to US stimuli are related to the "2^{nd} OFF" component in the responses to light stimuli.

Conclusions

These findings are the first direct demonstration of the response of the mammalian retina to US stimulation. The properties of the US transducer and the stimulation frequency indicate that non-invasive US stimulation of human retina is feasible, and may potentially evolve as an important tool for diagnosis and treatment of retinal diseases.

O13

Motor response elicitation and pupil dilation using megahertz-range focused ultrasound neuromodulation

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Objectives

Using transcranial focused ultrasound for the modulation of brain activity has been identified as a possible non-invasive means of treating neurological disorders. Most studies involving sedate rodents use frequencies in the kilohertz range, which allow for optimal transmission of acoustic power through the skull. The trade-off of using lower frequencies involves a lack of target specificity. Higher frequencies must be used in order to modulate activity in a more highly-specified manner. This study demonstrates that focused ultrasound in the megahertz range can be used to evoke motor- and cognitive-related responses in mice under deep anaesthesia by targeting specific brain structures. Contralateral-paired hind limb movements were observed when stimulating cortical regions, demonstrating the ability of MHz-range FUS to stimulate activity in highly-localized brain regions. Additionally, pupil dilation was observed when deep-seated anxiety-related structures were targeted, demonstrating the ability of FUS to modulate cognitive activity in a highly-specified manner.

Methods

For this study, wild-type adult male mice were anesthetized with intraperitoneal injections of sodium pentobarbital (65 mg/kg) and fixed in a stereotaxic frame. A single-element FUS transducer with fundamental frequency of 1.94 MHz was fixed to a 3D positioning system for accurate navigation through the brain. A 6x6 mm grid centred +2 mm rostral of the lambda skull suture was sonicated in a random order using a centre frequency of 1.9 MHz, pulse repetition frequency of 1 kHz, 50% duty cycle, 1 second pulse duration, 1 second inter-pulse interval for a total of 10 pulse repetitions. The acoustic pressure applied was varied in order to evaluate thresholds for eliciting physiological responses like motor movement, eye movement, or pupil dilation. Motor movements were validated using video recordings and electromyography via needle electrodes implanted into the biceps femoris of both hind limbs. Videos were recorded using a high-resolution camera focused at the right eye and processed to measure eye movements or changes in pupil size.

Results

The minimum acoustic pressure required to elicit motor movements was 1.45 MPa when targeting the somatosensory cortex, calibrated using an excised mouse skull. Higher pressures increased the success rate from 20% (at the 1.45 MPa threshold) to 70% (1.79 MPa). Targeting eye-motor and anxiety related regions of the brain elicited eye movements and pupil dilations up to 20%. Sonicating the superior colliculus resulted in both eye movement and pupil dilation at a lower threshold pressure (1.20 MPa) than the hippocampus and locus coeruleus which required pressures greater than 1.80 MPa.

Conclusions

This study successfully demonstrated that MHz-range transcranial focused ultrasound can be used to elicit motor- and cognitive-related

physiological responses with high specificity in mice in vivo. It was also shown that the success rate of stimulation increased with acoustic pressure for motor movements associated with cortical activity modulation but highly depends on the region of the brain targeted. These findings emphasize the complex and yet to be determined mechanism of action involved in ultrasonic neuromodulation.

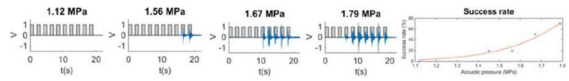

Fig. 4 (abstract O13). Evaluation of the pressure threshold and success rate associated with applying FUS to location within the somatosensory cortex. This location resulted in contralateral hind-limb movement relative to the sonication site. Moving the transducer symmetrically about the midline resulted again in contralateral movement relative to the new sonication site

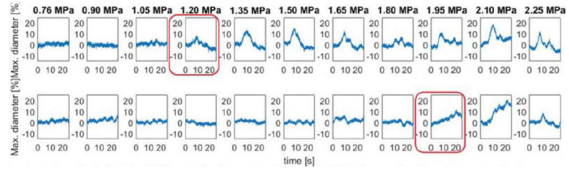

Fig. 5 (abstract O13). Superior colliculus (*top*) threshold determined to be approximately 1.2 MPa while the locus coeruleus (*bottom*) was evaluated to be greater than 1.8 MPa

O14

Thermal dose effects by MR-guided focused ultrasound on the pig brain tissue - preliminary results

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Objectives

The objective of this research is to investigate the effects of thermal dose (TD) delivered by magnetic resonance-guided focused ultrasound (MRgFUS) on in vivo pig brain tissue. In current clinical applications of transcranial MRgFUS systems, continuous acoustic wave emission is used to heat brain tissue to peak temperatures over 58°C. However, there are some situations where it has proven difficult to reach the desired peak temperature due to high absorption of acoustic energy by skull bone. There are reports that thermal effects on tissue are well correlated with thermal dose, which suggest that treatment delivery could be prescribed in terms of thermal dose rather than peak temperature or electric/acoustic power. It is also been demonstrated that the thermal dose threshold for permanent tissue damage is about 240 cumulative equivalent minutes (CEM) at 43°C for most of tissue. Currently available transcranial MRgFUS systems only allow the prescription of acoustic power and duration. In order to investigate the effects of thermal dose on in vivo brain tissue, we have developed a closed-loop control system to allow prescription thermal dose. This system monitors tissue heating via MR thermometry and provides pulse width modulation of output acoustic power in order to hold target tissue at a fixed temperature, and hence receives a nearly constant dose rate.

Methods

A FUS system (ExAblate 4000 Neuro 650 kHz system, InSightec) was used for sonication and an MRI system (Discovery MR75-3.0T, GE Medical systems) was used for thermometry and pre- and post-imaging. A closed-loop control system was implemented on a personal computer to control pulse width modulation of the FUS system acoustic power in order to maintain a specified temperature based on the MR thermometry. Accumulated thermal dose was calculated in real time and used to stop the sonication so that a prescribed thermal dose was delivered to the targeted tissue. Phantom studies were performed to test the control system to prepare for animal experiments. One acute and six chronic experiments (with three day survival) were conducted to observe the effects of TD on pig brain by behaviour observation and post MR imaging of the brain (1 hour and 70 hours post procedure). Craniotomy was performed to create an acoustic access window, and sonication was applied on 4 spots in the thalamus of each pig. Histology was also performed to compare it with MR imagery. Temperature in the pig brain tissue was estimated by rectal temperature for the MR thermometry baseline. TD was varied from 7 to 200 CEM while the target temperature was changed from 46 to 52 °C with appropriate acoustic power depending on target position and individual pig. This study was approved by the University of Virginia Institutional Animal Care and Use Committee.

Results

From the acute experiment, we could observe the lesions on MR images after 1 hour of sonication and histology subsequently confirmed the lesions. For the chronic experiments, no obvious problem was observed in the behavior of any of the six animals. Eighteen sonication spots in 5 pigs were analyzed through MR images. One pig experiment failed to control temperature due to introduction of air bubbles between the brain and scalp during surgery procedure, and 2 sonication spots were excluded due to technical problems. Large tissue changes were observed in MR images in all 6 spots over 100 CEM.

The diameter of those tissue changes in MR T2-weighted axial images were measured and averaged to 2.9 ± 0. 4 mm. There is inconsistency in generating lesions for TD below 100 CEM. No lesion was shown in some lower TD from 7 CEM and 61 CEM, while some smaller lesions (<2 mm in lesion diameter) were shown in TD from 18 CEM to 85 CEM except one large tissue change of 3.5 mm in diameter at 31 CEM. Some tissue changes were shown in both post MR images after 1 hour and 70 hours of sonication, while some were visible only at the 70 hour time point. Histology of 3 pig experiments is now available and the histology reports support the tissue changes and lesions in MR images. Lesion diameters in MR T2-weighted axial images versus TD in CEM are shown in Fig. 6 for all the results from the chronic pig study.

Conclusions

These preliminary results from pig brain tissue generally confirmed the previous results from rabbit brain tissue in generating tissue changes over a certain TD, even though there are some differences in the FUS systems and the experimental procedures and analysis. For lower thermal dose below 61 CEM, there is significant variability in generating of tissue changes, while large tissue changes whose average diameter is 2.9 mm were observed in MR T2-weighted axial images for higher TD over 100 CEM, which were reported with similar tendency but a little difference in TD from the rabbit brain study. These results may contribute to open the way to prescribe the thermal dose rather than peak temperature or acoustic power for brain treatments, and expand the treatment envelope beyond the current limitations in selecting targets and patients. This project is ongoing and will be further pursued with additional experiments for consolidation of the results and analysis.