Progress report

Project Title: "FUS-mediated Functional Neuromodulation for Neurophysiologic Assessment in a Large Animal Model"

Prepared by Seung-Schik Yoo December 9, 2013

We would like to submit a brief report on our experience and progress.

A. Neuroimaging of sheep for image-guidance

Method Two sheep (Dorset), ~20 kg weight on their arrivals, were acclimated for more than 1 week, and imaged using a 3 Tesla clinical MRI scanner (GE). A quadrature head coil was used for imaging. The animals were anesthetized with Tiletamine (NMDA receptor antagonist). Animals underwent standard sequences of T_1-weighted anatomical MRI, diffusion tensor MRI (DTI), and fMRI (sensorimotor and visual area).

For fMRI, we used two different stimulation paradigms, i.e. somatomotor and visual. For sensorimotor stimulation, the hind leg muscle was gently squeezed at a rate of ~ 1Hz. For the visual stimulation, white stroboscopic light (LED) was shown to both eyes at a frequency of 4 Hz. The stimulation was delivered as three 20 sec-long blocks, interleaved with four non-stimulation periods of equal duration. The fMRI data was processed using SPM platform and the co-registered with anatomical MR images for the image-guided FUS application (Figure 1).

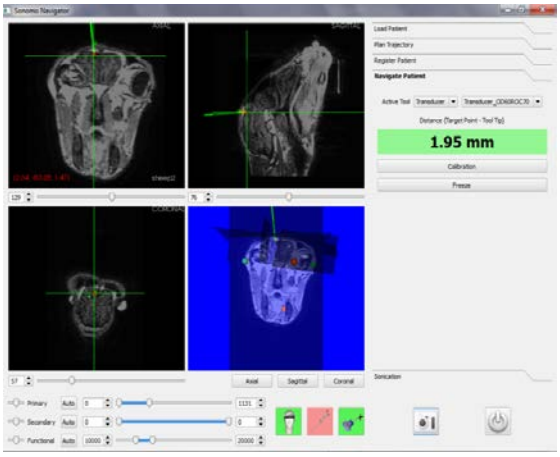

Figure 1. An example of the sonication guidance procedures for the sonication on the left sensorimotor areas of the sheep brain.

B. Stimulatory FUS Sonication to sensorimotor cortex

The animal was anesthetized using I.V. Tiletamine. Re-dose of the agent was given as monitored by the veterinarian staff. A single-element FUS transducer (operating at 250 kHz fundamental frequency), segmented sphere in shape, was used for stimulation, with image-guidance for positioning of the transducer. For stimulatory parameters, we used the one based on our previous experience in optimizing the pulse parameters for stimulation of the rat brain, i.e. 1 ms tone-burst-duration, 500Hz pulse-repetition frequency, sonication duration 300 ms. The incident acoustic intensity, between 3.4 to 8.4 W/cm^2 I_{sppa} (we assumed that 67% of the acoustic pressure will be transmitted), was given, although the actual measurement of the acoustic attenuation by the skull is pending. We plan to conduct an *ex vivo* measurement of the acoustic attenuation from the extracted sheep skull.

The subdermal EEG electrodes were inserted near the surface of gastocnemius muscle to detect the presence of aan evoked potential from stimulation of the sensorimotor area, while a motion sensor was wrapped around the hind leg to detect any muscle movement. For the detection of the visual evoked potential, the same subdermal EEG electrodes (Ives Solutions, Ontario, Canada) were applied to F_z- and O_z-equivalent montage in sheep, and subsequently recorded using data acquisition system (AD instruments).

C. FUS-mediated MEP detection

We detected the EMG signal from the hind leg muscle contralateral to the sonication from both sheep. This phenomenon was not accompanied by the actual muscle movement (Figure 2).

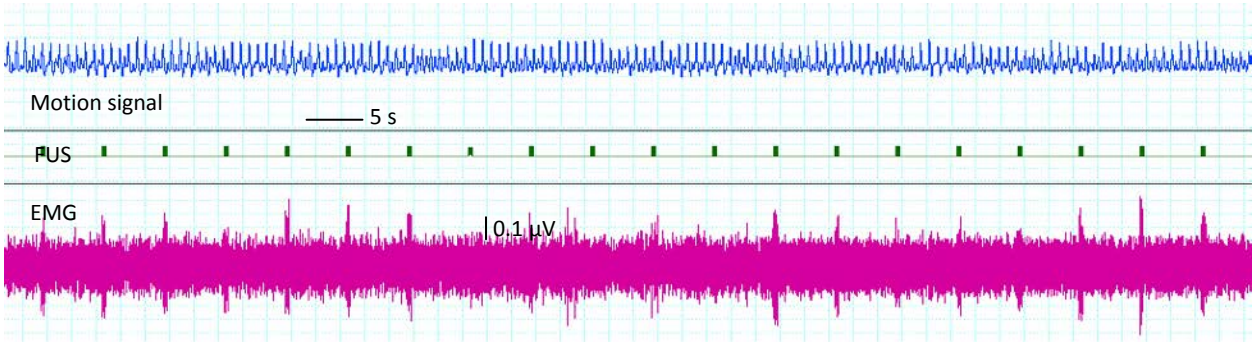

Figure 2. Demonstration of motor-evoked potential (MEP) by the administration of FUS. Top panel shows the motion signal from the hind leg which does not show any sonication-mediated movement (heart-related signals were detected). The middle section shows the FUS sonication timing, and the bottom panel shows the FUS-mediated EMG signal obtained from the contra-lateral hind leg (20-50 Hz bandpass filter).

When there was no sonication given, the MEP was not elicited while the amplitude of the MEP appeared to have intensity dependency. As shown in Fig 3, the amplitude of MEP (when 8.4W/cm^2 I_{sppa} was applied) was greater than the one from the lower acoustic intensity (5.2 W/cm^2 I_{sppa}). Therefore, we determined that 5.2 W/cm^2 I_{sppa} was the minimum threshold for the elicitation of the MEP. Considering the 50% duty cycle, the effective acoustic intensity would be 2.6 W/cm^2 I_{spta}.

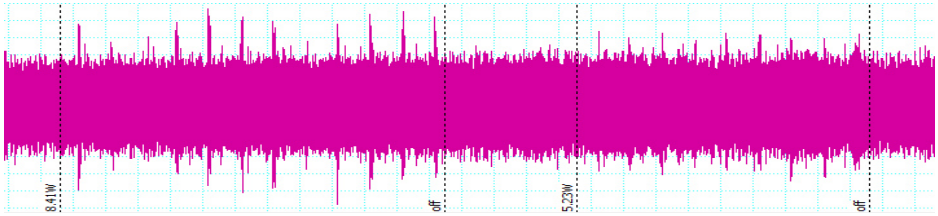

Figure 3. Demonstration of the time course of the MEP for the use of different acoustic intensities (left, 8.4W/cm^2 I_{sppa}) and at the intensity just above the threshold (right, 5.3W/cm^2 I_{sppa}), interleaved by the non-sonication period (in the middle).

The use of continuous FUS

We were also motivated to use the continuous pulse of FUS (sonication duration 160 ms) to examine the possibility of using the continuous (i.e. 100% duty cycle) FUS for the stimulation. The use of continuous FUS also resulted in the elicitation of MEP at 3.4 W/cm^2 I_{sppa} (Figure 4). In terms of energy deposition, 2.6 W/cm^2 I_{spta}× 300ms = 0.78J/cm^2 was given in a pulsed mode while the continuous acoustic wave seemed to elicit responses at 3.4 W/cm^2 I_{spta}× 160 ms= 0.54J/cm^2.

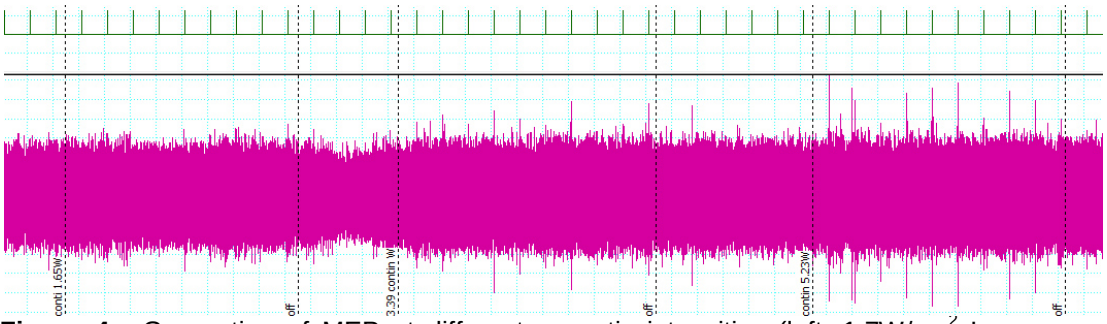

Figure 4. Generation of MEP at different acoustic intensities (left, 1.7W/cm^2 I_{sppa}; no response), at an intermediate intensity just above the threshold (middle, 3.4W/cm^2 I_{sppa}), and at higher intensity (left, 5.3W/cm^2 I_{sppa}) using continuous 160ms-long FUS. Timing of the sonication is given at upper panel in green lines (5 sec inter-sonication interval).

D. Acquisition of visual evoked potential (NOT FUS-mediated)

As the studies on measuring VEP from the sheep are rare, the method as well as the features of VEP was important to be established, prior to the FUS-induced suppression of the function of the sheep visual cortex. After a few trials and errors, robust VEP were measured from both sheep, as shown in the Figure 5. It is notable that VEP amplitude and shape are affected by anesthetic conditions and electrode montage setting, therefore, care should be given in order to establish the baseline conditions for the measurement.

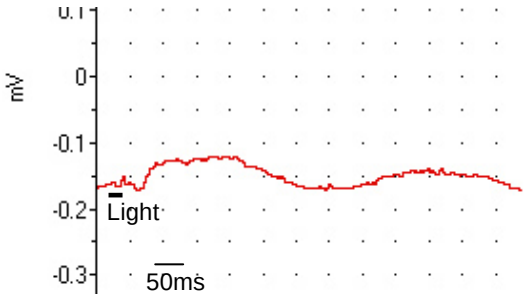

Figure 5. VEP from the sheep (averaged n=100 stimuli). The VEP amplitude at 90ms post stimulus onset was 43±5 μV (n=3 trials, session #1) and 42±7 μV (n=5 trials, in session #2). The results will be used to observe/detect any changes in suppression of visual activities by the FUS.

E. Post-sonication brain extraction

Both sheep underwent four sets of sonication experiments (separated by 8, 34, and 8 days). Throughout the entire course of the experiment, the sheep's status was normal. At day 4 post-sonication, the sheep were sacrificed and their brains were extracted. Histological analysis is pending.

F. Future directions

Here is the summary statement of the project's heading.

1. Continue to build more data on stimulatory effects of the FUS, examination on the intensity-dependency as well as the presence of threshold for successful stimulation events.
2. *Ex vivo* estimation of transcranial attenuation of the acoustic energy
3. Demonstration of suppressive effects on FUS on visual cortex, examined via suppression of VEP.
4. Accumulation of neuroimaging data as no fMRI work has been done in sheep (potential new publication)
5. Start transition to using the Insightec system, which is needed for stimulating the deeper and smaller structures.
6. Continue to examine the safety profile of the sonication in more sheep.