Final Report – FUSF Research Grant

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

Prepared by Seung-Schik Yoo and Wonhye Lee, November 21, 2014

Introduction

The *main technical focus* of the project was to develop a comprehensive FUS environment that provides (1) image-guidance of the sonication focus using functional MRI (fMRI) and diffusion tensor MRI (DTI) on eloquent functional areas of the brain, while enabling (2) on-site electrophysiological assessment of the neuromodulatory outcomes, including the electromyogram (EMG) and electroencephalogram (EEG) activities (*motor evoked potential; MEP* and visual evoked potential; *VEP*). These capabilities are crucial to evaluate the efficacy of the sonication parameters on-site, and to further optimize them through closed-loop control.

A. Study Outcome #1: Comprehensive Functional Neuroimaging in Sheep

Only a limited amount of neuroimaging information on healthy sheep was available. Therefore, we were motivated to examine the feasibility of applying a routine human neuro MRI protocol to image the sheep brain. The study was conducted under the approval of the Harvard Medical Area Standing Committee on Animals. Sheep (Dorset, all female, weight = 32.6 ± 4.4 kg; mean ± SD, 25–38 kg, n = 8) were anesthetized with Telazol (Tiletamine; N-methyl-D-aspartate; NMDA receptor antagonist + zolazepam, initial dose 2–4 mg/kg *i.v.* + additional doses as needed) for the MRI procedures. Anatomical MRI was conducted along with fMRI during photic (presented through the closed eyelid) and tactile stimulation (gentle pinching of the unilateral hind leg). Also, voxel-wise apparent diffusion coefficient (ADC), along with fractional anisotropy (FA; as a measure of the directionality of diffusion) values were obtained using DTI, and the spatial orientations of corticospinal tracts were visualized using a tractography technique (DTIStudio).

fMRI was performed to identify the primary sensorimotor (SM1) and visual area (V1) of the sheep brain, using T_2*-weighted blood-oxygenation-level-dependent (BOLD) sensitive gradient-echo echo-planar-imaging (EPI) sequence (TR/TE = 2000/40 ms; flip angle = 90 °; FOV = 18 × 18 cm^2; image matrix = 64 × 64; slice thickness = 3 mm; slices = 20, no gap; voxel size = 2.81 × 2.81 × 3.00 mm^3). The entire brain volume was imaged in the oblique axial orientation using the same scan location as the anatomical T_1- and T_2-weighted brain images. The activations of the SM1 and V1 areas were provoked using a passive tactile (*i.e.* gentle 2 Hz squeeze of the right hind leg muscle) and photic stimulation (*i.e.* 2 Hz white strobe lights to both eyes with the eyelid closed), respectively. Three blocks of the stimulation period (20 s, synchronized with the scanner operation) were interleaved by four resting periods of equal duration (20 s). The fMRI data was processed by the SPM8 software package.

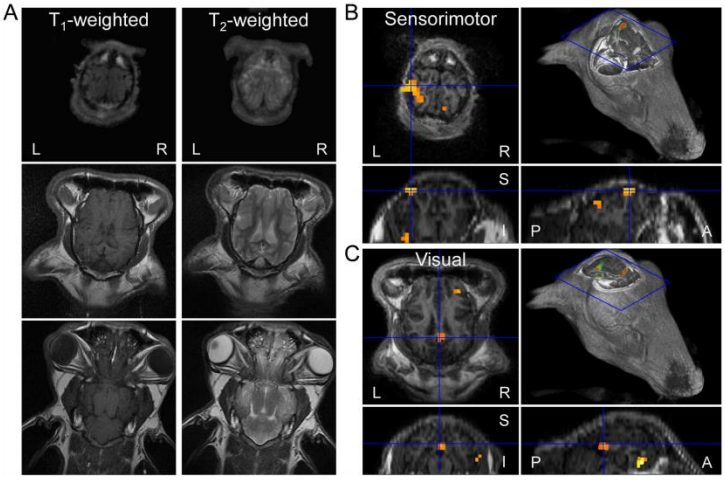

Figure 1. Example of MRI data from an individual sheep (A) T_1-weighted (left column) and T_2-weighted (right column) axial images from superior (top) to inferior (bottom). Individual fMRI activation maps from (B) the sensorimotor and (C) visual stimulations were overlaid on axial, coronal, and sagittal views as well as on a 3D MRI head rendering. Crosshairs (in blue) indicate the location of the local maxima on the fMRI activation map from the square frame (in blue) showed on an axial plane of the 3D rendering. L: left, R: right, S: superior, I: inferior, P: posterior, and A: anterior.

The detailed data collection parameters were described in the submitted manuscript (**BMC Veterinary Research, Appendix 1**). All animals successfully underwent the MRI session. Exemplar T_1- and T_2-weighted axial images of the sheep brain are shown in **Fig. 1A**. The activated functional areas, from the sensorimotor stimulation (**Fig. 1B**) and visual stimulation (**Fig. 1C**) of one sheep, were visualized (P < 0.001 threshold) and overlaid on axial, coronal, and sagittal slices, along with a 3D-rendered head view. Also, the measurements taken from DTI (FA and ADC values) as well as the tractography showed the feasibility of examining the WM macrostructures.

B. Study Outcome #2: FUS Sonication and Electrophysiological Recordings

For the FUS sonication, we initially intended to use a MRI-guided ExAblate system (220 kHz; Insightec, Tirat Carmel, Israel); however, due to the clinical transition of the system for human use at BWH, the system became unavailable for

animal experiment. Therefore, a single-element FUS transducer was used, along with full image-guidance for sonication targeting.

B.1. Functional and Anatomical Neuroimage Data Acquisition and Processing for Sonication Planning

The acquired MRI and fMRI data were co-registered volumetrically using the Normalized Mutual Information technique (Maes, Collignon et al. 1997), whereby T_1-weighted volumetric MRI data were used as the registration target (*i.e.* a fixed volume). The co-registered imaging data were loaded in a custom-built software that was developed for image-guided FUS navigation (Kim, Chiu et al. 2012). The accuracy of spatial registration between the image data and the animal subjects were evaluated by examining the locations of the sheep's natural anatomical features (inferior helix of the posterior auricle of the ears, inner canthus of the eyes, and the nose tip) prior to the sonication.

B.2. Sonication Setup A single-element ceramic FUS transducer (Channel Industries, Santa Barbara, CA), having 250 kHz operating frequency *via* piezoelectric mechanism, was used for the transcranial sonication of the SM1 and V1 of the sheep brain. The transducer had a shape of a segmented-sphere, with an outer diameter (OD) of 6 cm and a radius-of-curvature (ROC) of 7 cm, which was housed in an air-backed water-proof plastic casing. The transducer unit was submerged in degassed water that was held in a cone-shape, thin film vessel (linear low-density polyethylene; LLDPE; ~75 μm in thickness). The film did not induce any measureable acoustic attenuation or distortion of the sonication path. The transducer and the vessel were installed on an articulated mechanical arm, which allowed operators to manually adjust and lock the position and orientation of the FUS transducer.

Neuroimaging-based image-guidance was used to assist the operators to align the acoustic focus on the target location of the sheep's specific brain structures. A rigid-body tracker (containing four infrared-reflective markers) was placed onto the FUS transducer as a physical landmark that provides its spatial coordinates and orientations in real-time, as detected by the optical tracking system (NDI, Ontario, Canada) (Kim, Chiu et al. 2012). The target sonication areas were guided for aiming the unilateral SM1 of left hemisphere and the V1 based on the fMRI activation map (local maxima of activation probability thresholded $p < 0.001$) and/or the anatomical MRI. The location of the focal point and the path of the FUS sonication with respect to the transducer was displayed and updated in real-time (Kim, Chiu et al. 2012) (**Figs. 2a and b**). The incident acoustic beam was manually aligned perpendicular to the scalp curvature around the entry region of the beam to minimize the travel distance as well as to reduce the refraction through the cranial structure.

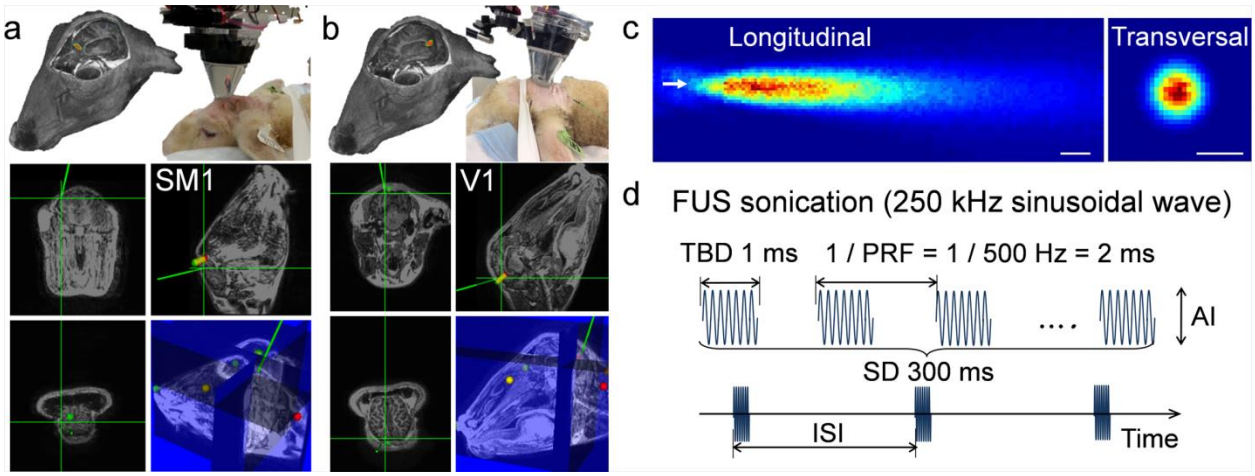

Figure 2. Schematics of the image-guided transcranial FUS sonication setup and parameters. Example of the FUS experimental setup for the sonication on the primary (a) sensorimotor (SM1) and (b) visual (V1) areas, based on the image-guided planning by anatomical and functional neuroimage data. 3D rendered views in the upper row of panels (a) and (b) show the localized eloquent functional areas of the SM1 and V1. (c) Acoustic intensity mapping in longitudinal and transversal planes of the acoustic focus of the 250 kHz FUS transducers. Scale bar, 10 mm. (d) Schematics of the pulsed FUS sonication with fundamental frequencies of 250 kHz, tone-burst-duration (TBD) of 1 ms, pulse-repetition-frequency (PRF) of 500 Hz, sonication duration (SD) of 300 ms, and the inter-stimulation-interval (ISI) of 5 for MEP and 1 s for VEP measurements.

B.3. Elicitation of Physiological Responses The acoustic intensity was varied up to a spatial peak pulse-average intensity (I_{sppa}) of 15.7 W/cm^2 at the target. The electromyogram (EMG) was measured from both hind legs for the SM1 stimulation (**Fig. 3**) while electroencephalography (EEG) visual activity was measured upon the stimulatory sonication on the V1 (**Fig. 4**). The motor evoked potentials (MEP) were elicited only from the hind leg contralateral to the site of the SM1 while the

visual-evoked potentials (VEP) were detected in the absence of external tactile/visual stimuli. The stimulation-related physiological responses were only detected over a certain acoustic intensity threshold, and also exhibited individual variability among the sheep. The detailed information was described in the attached drafted manuscript (journal target **TBD, Appendix 2**).

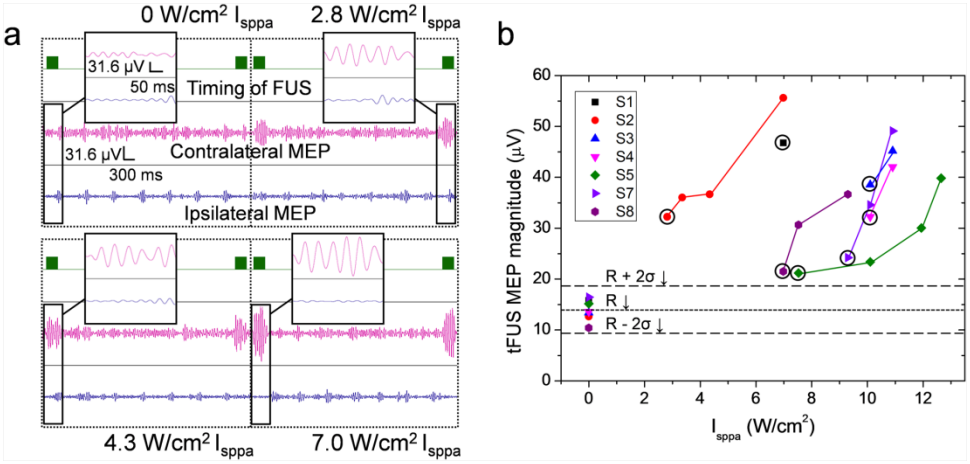

Figure 3. The individual and group data of the MEP elicited by FUS sonication on the SM1 area in the sheep brain. (a) Green squares in the top row shows the timing of sonication and the time-course MEP signals (bandpass filter 15–30 Hz), (b) Scatter plot of the FUS-mediated MEP magnitudes with varying acoustic intensity ranged from 0 to 12.7 W/cm^2 I_{sppa}. The horizontal dotted line at the level of 13.92 μV represents the mean (R) of the resting signals measured when no FUS sonications were given, and the horizontal dashed lines represent the level of +/- 2σ μV derived from the second standard deviation (2σ) of the R. The black circles indicate the acoustic intensity threshold level and the corresponding MEP magnitude of each sheep.

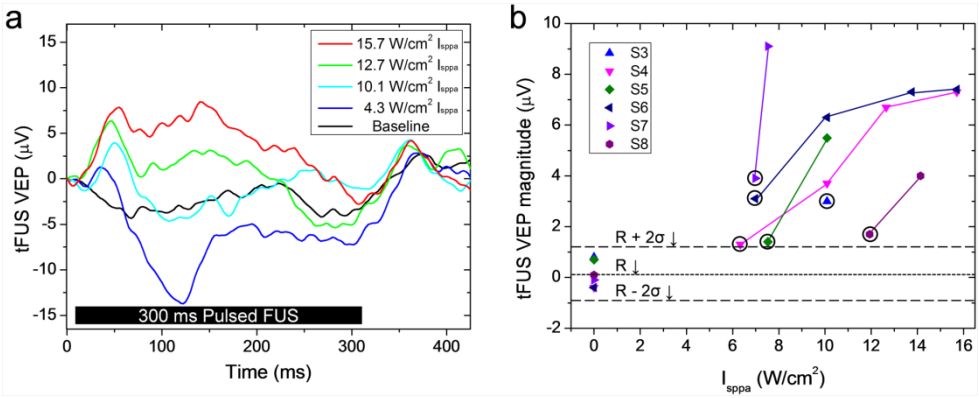

Figure 4. (a) Example of the elicited VEP signals, in the absence of external visual stimuli. (b) Scatter plot of the FUS-mediated VEP magnitudes measured at the F_z and O_z-equivalent EEG sites of the sheep (n = 6) with varying acoustic intensity ranging from 0 to 15.7 W/cm^2 I_{sppa}. The horizontal dotted line at the level of 0.12 μV represents the mean (R) of the peak VEP measured when no FUS sonications were given, and the horizontal dashed lines represents the level of +/- 2σ μV derived from the second standard deviation (2σ) of the R. The black circles indicate the acoustic intensity threshold level and the corresponding elicited VEP magnitude of each sheep.

B.4. Post-sonication Behavior Monitoring and Histological Analysis The animal was subject to sacrifice (1 week and 2 months after the sonication) and the brain was extracted and fixated using the immersion fixation technique. A maximum I_{sppa} of 12.7 W/cm^2 for the SM1 and 15.7 W/cm^2 for the V1 were applied, and there was no loss of life during the study period and the sheep's health status was normal throughout the whole duration of the study period before sacrifice. Based on the visual inspection of the EEG signals, the baseline resting-state EEG before and after the sonication did not show any changes. Post-sonication animal behaviors of all sheep were normal across all survival durations. In histological analysis, there was no apparent tissue damages from the SM1 sections, but several localized regions of the sonicated V1 regions showed micro hemorrhages from a few sheep (n = 4, S3, S5, S6, and S7). The common feature of sonication trials among these sheep was that five or more FUS-VEP sessions were performed consecutively (with only a brief pause between the sessions), resulting in 500 or more FUS sonication events given with an interval of 1 s, at an acoustic intensity ranging from 7.0–13.8 W/cm^2. On the other hand, the sheep S4 and S8, although exposed to an acoustic intensity ranging from 4.3 and 14.1 W/cm^2, did not show any histological abnormalities. These animals were only exposed to a maximum of 3 and 4 consecutive FUS sessions, respectively, which resulted in 400 or fewer FUS sonication events (also given in 1 s interval). Among the sheep showing the micro hemorrhages (extravasated erythrocytes in perivascular space without the presence of edema) in the V1 sections, sheep S3 showed the most severe tissue damages (**Fig. 5**) and was exposed to a total of 6 consecutive FUS-VEP sessions (ranging from 10.1 to 12.7 W/cm^2 I_{sppa}). In terms of the maximum acoustic intensity exposure, both S4 and S6 were exposed to sonication given at 15.7 W/cm^2 I_{sppa}, but only S6 showed signs of micro hemorrhages. Since there were no histological damages from the M1 area, we believe 12.7 W/cm^2 I_{sppa} can be applied as long as it is given in moderate numbers (< 400 times) with enough intervals (> 5 sec). We also attempted to examine the immunohistological responses, but staining was not possible due to the use of the immersion fixation technique.

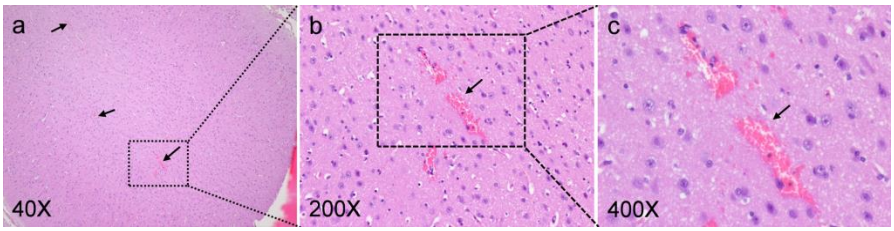

Figure 5. Example of the histology results from the left visual cortex of sheep S3 with different magnifications of (a) 40× (b) 200× and (c) 400× after the sonication. Black arrows indicate the regions showing the micro hemorrhages, and dashed square boxes shows the magnified region-of-interests (ROI) in panels (b) and (c). This is the most severe case of micro hemorrhaging among all sheep brain areas across.

C. *Study Outcome #3: Continuous vs. Pulsed FUS Sonication*

We were also motivated to test stimulatory efficiency of a continuous versus pulsing scheme having the same overall energy deposition on the same brain locations listed above. Continuous sonication seems to generate a greater magnitude of MEP compared to the pulsed counterpart, but this trend was opposite in the case of the visual cortex stimulation (**Fig. 6**), which merits further investigation. Along with the data from previous rodent models (King et al. 2013 UMB) and the Bilayer Sonophore Model (Plaskin et al. 2014), these findings confirmed that both pulsed and continuous sonication can stimulate the brain. These findings are interesting since the neuromodulatory effects may be expected from the short, but continuous application of FUS to the brain.

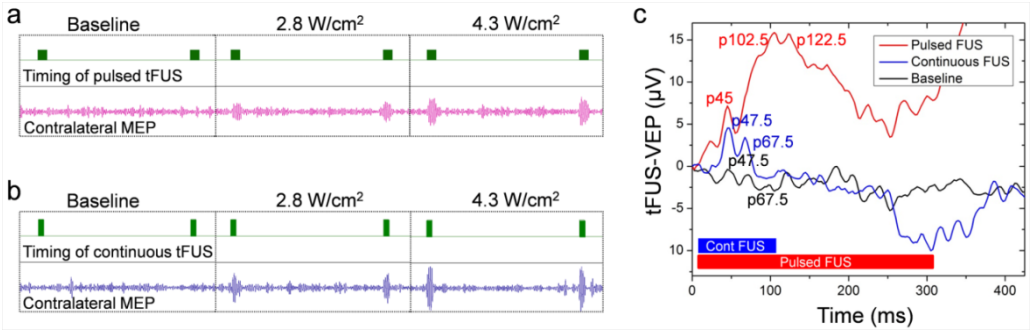

Figure 6. The elicited physiological responses elicited by the pulsed and continuous sonication on SM1 and V1 areas. (a and b) The elicited MEP data via (a) pulsed and (b) continuous sonication on the SM1, having the same overall energy deposition. (c) The elicited VEP data via pulsed (red) and continuous (blue) FUS sonication on the V1 without external visual stimulation. Baseline resting-state signal profile is also shown (black).

D. *Study Outcome #4: FUS Sonication on the White Matter (WM) Tract*

We also provided the pulsed FUS to the WM tract (the right optic radiation), based on the DTI data of the sheep, to examine whether the sonication can initiate the signal transduction from the WM. Various sonication parameters were tested, at varying intensities ranging from 7.5–15.7 W/cm^2, however, only two out of four sheep showed VEP responses from the unilateral sonication on the optic radiation (**Fig 7a**). As a comparison, VEP elicited by a photic stimulation was shown in **Fig. 7b** (not in the same time/voltage scale, see the region inside a circle). Therefore, the neuromodulatory effect on the WM tracts is deemed inconclusive in the present study. It is also possible that the sonication was actually given to the lateral geniculate nucleus (LGN) of the thalamus and triggered the stimulatory responses, without involving the WM tracts. Further study is needed to elucidate the possibility of eliciting stimulation from the white matter tract as it can be used as a completely new way of probing functional connectivity of the brain.

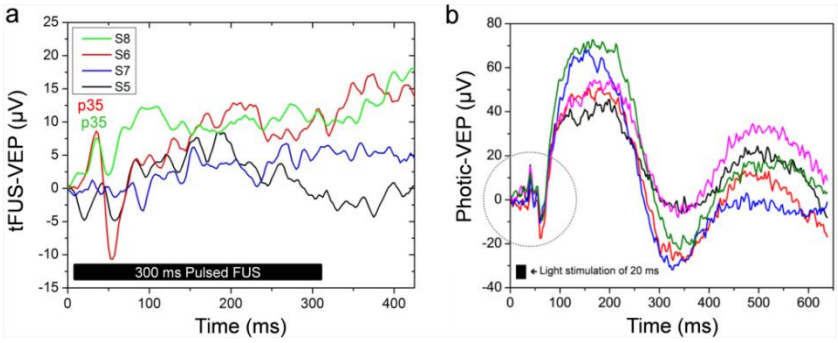

Figure 7. Example of visual evoked potential (VEP) elicited by transcranial FUS and by photic stimulation. (a) The VEP signal elicited by transcranial pulsed FUS without external visual stimulation. Sheep S6 and S8 showed the VEP peak at p35, but S5 and S7 did not show the elicited VEP peak. (b) The VEP signal elicited by a 20-ms long photic stimulation. The VEP signal profile inside a circled region can be compared to the FUS-mediated VEP profile in the panel (a).

Summary of Performance Indices

1. Planned publications: **(Appendix 1)** Functional and Diffusion Tensor Magnetic Resonance Imaging of the Sheep Brain, in review, BMC Veterinary Research, and **(Appendix 2)** Image-guided FUS-mediated Regional Brain Stimulation in Sheep, to be submitted to Neuroimage or Ultrasound in Med. Biol. (TBD)
2. Planned Research Activity: NIH R01 to be applied in January 2015.