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Appendix E1

Ultrasound Pressure Measurements and Pressure Threshold

The acoustic field generated by the focused ultrasound transducer (f_c: 1 MHz, focal depth: 60.5 mm, diameter: 90 mm; Sonic Concepts) was calibrated using a needle hydrophone (diameter: 0.2 mm, Precision Acoustics Ltd) in a degassed water tank. The ultrasound beam had a lateral diameter of 2 mm, an elevational diameter of 1 mm and an axial length of 20 mm as defined by the peak-rarefactional pressure full width at half maximum (FWHM). The acoustic pressures reported in this study were de-rated using an 11% attenuation that we measured experimentally. In brief, the top layer of the mouse skull ($n = 4$) was excised postmortem and the attenuation value was calculated as the percent decrease in peak-rarefactional pressure at the focal point before and after the skull was placed between the transducer and focal point.

The pressure threshold at which dextran was delivered across the BBB was determined by assessing dextran delivery at 0.18 ($n = 3$) and 0.35 ($n = 5$) $MPa_{pk\text{-}neg}$ (Fig E1) using the long pulse sequence. We detected no dextran delivery at 0.18 MPa using the NOD ($P > .01$).

Ultrasound Targeting

The focus of the therapeutic transducer was positioned over the left hippocampus using the therapeutic transducer in pulse-echo mode (2). In this arrangement, the focused ultrasound transducer was connected to a pulser-receiver (DPR300; JSR) and moved using the computer-controlled 3D positioning system (Velmex). A 1 mm thick metal cross, placed at the bottom of the water container, aligned with the lambdoid and sagittal suture of the skull. We generated a 2D raster scan of the cross and used it to position the transducer 3 mm laterally from the sagittal suture and 0.5 mm anterior from the lambdoid suture. We adjusted the depth of the focus using the pulse-echo distance from the skull. We placed the focal point approximately 3 mm beneath the top of the skull. We chose the hippocampus as a target due to the low attenuation of the parietal bone and its potential use as a therapeutic target. The right hippocampus was not sonicated and used as a control in every experiment.

Brain Preparation and Histology

Within five minutes after the end of sonication, the mice were transcardially perfused with 20 mL PBS and 20 mL 10% formalin fixative (PFA; Sigma Aldrich). For frozen sections, 1.5 mm was trimmed from the bottom of the OCT embedded brain and sixty 30 µm slices were taken to cover the entire hippocampus using a cryostat (CryoStar NX70; Thermo Fisher). Immunohistochemistry (IHC) was performed on three frozen sections per brain to detect the presence of albumin. For paraffin-embedded brains, 1.2 mm was trimmed from the top of the brain to reach the hippocampus and eight sections were acquired per level (12 in total), spanning over 48 µm of brain tissue in each level. In between levels, 80 µm of tissue was discarded. The first two sections of each level were stained with H&E, leaving the rest for future staining of cells and proteins.

Fluorescence Microscopy Analysis

To measure the normalized drug delivery dose and distribution, each brain had five sections analyzed. Regions of interest around the left and right hippocampus were selected using Matlab, manually removing any artifacts from these regions. The extent of albumin extravasation was quantified by calculating the NOD for three slices for each brain treated with the RaSP (n = 5) and long pulse (n = 5) sequence.

Acoustic Emissions Analysis

The energy and spectral content of the focused passive cavitation detector (PCD, center frequency: 7.5 MHz, focal length: 76.2 mm; Olympus Industrial) signals were analyzed to obtain information regarding the type, magnitude and duration of microbubble-seeded cavitation activity. To obtain the energy, the time-varying voltage signal was squared, integrated over time and corrected for electronic and experimental noise. Each energy value in the 10 msec pulse length was subtracted by the mean noise energy value in a region where no acoustic cavitation was present to correct for electronic noise. The mean energy of the control was also subtracted from each energy point and this difference was plotted over time. The duration of emissions was determined by calculating the t_{80} constant. To determine the mode of cavitation activity, we analyzed the spectral content of the acoustic emissions using the FFT to observe how the frequency content of the signal changes over time.