Passive cavitation detection-based feedback control for ultrasound-mediated blood-brain barrier opening in non-human primates

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***Abstract*—Despite the progress of focused ultrasound (FUS)-mediated blood-brain barrier (BBB) disruption, neuro-inflammatory responses and the high variability of the FUS transmission through the human skull make the control of the acoustic parameters challenging. In this study, we developed a high-field (7-T) magnetic resonance (MR)-guided FUS system with a feedback control based on passive cavitation detection (PCD) to explore BBB opening in non-human primates (NHP). The sonication parameters were: 2 min duration, 500-kHz frequency, pulse length of 10 ms, and pulse repetition frequency of 5 Hz. T1-weighted MR images acquired every 5 min revealed a maximum contrast enhancement of 67% ± 15% relative to muscle after 30 min of sonication. Safe sonications were achieved in the 3 sessions using real-time PCD-based feedback control of the acoustic pressure. The high resolution anatomical images and the high temporal/spatial resolution of contrast agent diffusion provide a unique tool for studying the mechanisms of BBB disruption and drug delivery in NHP. Furthermore, the PCD-based feedback control allows repeatable safe sonication regardless of the variation of skull attenuation, allowing comparisons across animals and experimental sessions.**

***Keywords*—blood-brain barrier; drug delivery; magnetic resonance-guided focus ultrasound; passive cavitation detection**

I. Introduction

Cavitating microbubbles driven by focused ultrasound (FUS) can temporarily and locally increase the permeability of the blood-brain barrier (BBB) [1]. The interaction of microbubbles with endothelial cells inhibits active transport proteins [2] and disrupts endothelial barrier function by mechanically loosening tight junctions and triggering transcytosis [3]. This non-invasive technique has the promising potential to enable clinically relevant substances for the treatment of Parkinson's disease, Alzheimer's disease, and brain tumors, among other brain diseases to reach the parenchyma [3].

Broadband acoustic emission of cavitating microbubbles indicates harmful regime of cavitation (inertial cavitation), in which collapsing microbubbles create brain tissue damage [4]. Passive cavitation detection (PCD) can be used to monitor the acoustic emission in real-time providing an endpoint criterion for the sonication. In addition, the real-time monitoring of harmonic, ultra-harmonic, and sub-harmonic emission components have been proposed for the feedback control of the acoustic pressure [5-7]. However, these techniques essentially become an open-loop at threshold level and give rise to false positive detections due to other sources of scattering such as inadequate acoustic coupling (i.e. bubbles trapped in the coupling gel or water in the coupling tank/balloon not adequately degassed) [5].

Safety is the main concern for the clinical translation of the FUS-mediated BBB disruption technique, due to its potential inflammatory responses [8]. To minimize the risks, it is crucial to use techniques to ensure correct targeting (i.e. magnetic resonance imaging-guided focused ultrasound – MRgFUS [9]) and a reliable feedback control of the acoustic pressure. Furthermore, experiments conducted in non-human primates (NHP) constitute a more adequate evaluation of the bio-effects of sonication due to their similarity to humans in physiology and neuroanatomy. Previous studies with NHP have shown the safety of repeated sonication sessions [10], the influence of anesthesia in the BBB opening volume, and the drug delivery efficiency associated with brain vasculature heterogeneity [11].

In this study, we report a NHP-dedicated MRgFUS system with PCD-based feedback control. We demonstrate the capability of the high signal-to-noise ratio provided by a 7-T MRI system that allows superior evaluation of the contrast permeation in the brain. Furthermore, the relative spectrum of the PCD signal [12] was used to extract microbubble emissions from background signals caused by other sources of scattering.

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II. Materials and Methods

A. Animal preparation

All procedures were approved by the ethics committee CEtEA n°44 and the Ministry of Research and Education (Authorization n° APAFIS#908-2015062410594279v2). Four male cynomolgus monkeys (*Macaca fascicularis,* supplied by Noveprim, Mauritius Island) aged 4-6 years, weighing 4.1 – 7.9 kg were used in this study. The animals were initially induced with a mixture of ketamine/xylazine (10:1 mg/kg) and maintained anesthetized with an intravenous infusion of propofol throughout the experiment at a rate of 1 ml/kg/h. A heating pad (Resp./2_CH IBP, SA Instruments Inc., NY, USA) maintained the animal's temperature at 37°C and the respiratory and heart rates were continuously monitored (SA Instruments Inc., NY, USA).

B. Experimental setup

The animals (n= 4; 7 sessions) had the head shaved and were positioned in a stereotaxic frame in sphinx position inside a 7-T MR imaging system (Varian-Agilent Technologies Inc., California, USA). The FUS transducer was fixed to the stereotaxic frame and coupled to the animal's head with an expandable balloon filled with degassed water. A 14-element annular array transducer was driven by a RF-amplifier (LabFUS, Image Guided Therapy, Bordeaux, France) at 500 kHz during 2 min with pulse length of 10 ms and pulse repetition frequency of 5 Hz. The PCD was performed by a planar mono-element 1.5 MHz transducer positioned at the center of the FUS transducer. A bolus of MRI contrast agent (Gadolinium "Gd", dose: 0.20 mL/kg; DOTAREM®, Guerbet, Roissy, France) and of microbubbles (dose: 0.30 mL/kg; SonoVue, Bracco Imaging S.p.A., Italy) were injected intravenously before sonication.

C. Feedback control based on PCD

The signal detected by the PCD transducer was digitized at 31.25 MHz (PicoScope 5242B, Pico Technology, Cambridgeshire, UK) and processed in real-time in a software developed in Python (version 2.7.12, Python Software Foundation, Delaware, USA). The inertial and stable cavitation doses (ICD and SCD, respectively) were calculated as described by previous studies [13-14] with bandwidths of 50 kHz around the sub-harmonic (f_{sub} = f/2), 100 kHz around the harmonics (f_{harm} = n*f, with n=2, 3, and 4), and 50 kHz around the ultra-harmonics (f_{ultra} = m*f, with m=1.5, 2.5, 3.5, and 4.5). The relative power spectra [12] of the ICD and SCD obtained from the ratio of the spectra before and after microbubble injection were used to suppress scattering sources other than microbubble emission as a feedback control. Before sonication with injected microbubbles, averaged baseline signals were acquired at the acoustic pressure range (calibrated at focus in free water) from 90 to 1157 kPa with steps of 9 kPa (n=5 for each pressure level). During feedback controlled sessions, the acoustic pressure was increased gradually until CD threshold levels were reached. In addition, the verification of nucleation generation in sham acquisitions using PCD (same experimental conditions except for the absence of injected microbubbles) was performed to check for acoustic coupling quality.

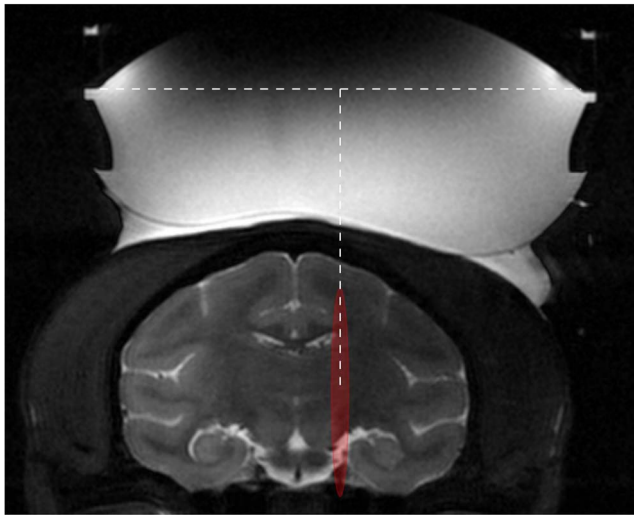

Fig. 1. Example of target planning and acoustic coupling verification on T_2-w MR images. The target planning was performed by tracing a normal incidence (white dashed line) of the focus (in red) based on the transducer geometry and calibrations in free water.

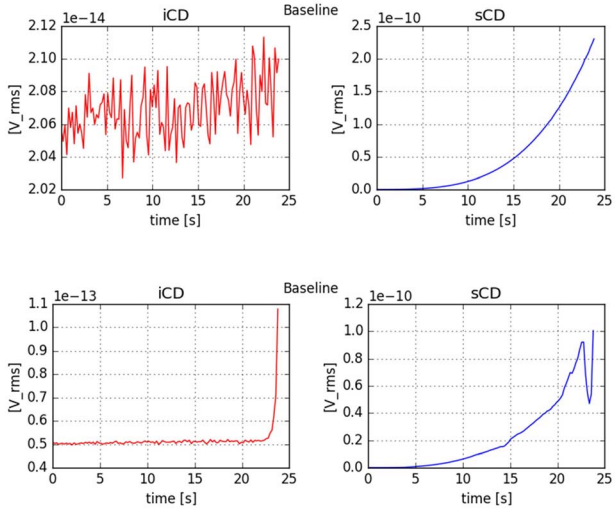

Fig. 2. Examples of acoustic coupling at baseline. (Top) adequate acoustic coupling produced constant and very low ICD, whereas SCD smoothly increased with the gradual increase of the acoustic pressure. (Bottom) examples of ICD and SCD baselines with nucleation in a session with inadequate acoustic coupling.

D. MRI

Targeting and acoustic coupling were checked in T_2-w (weighted) images (fast spin-echo sequence; TR=4750 ms, TE=20 ms, 450x450 μm^2 in-plane resolution, 40 coronal slices, slice thickness = 1 mm). BBB disruption was confirmed with contrast enhanced 3-D T_1-w images (TR=2000 ms, TE=3 ms, TI=680 ms, resolution=450x450x2000 mm^3, 64 coronal/sagittal slices) acquired every 5 min after sonication during 40 min. Absolute difference MR images were obtained from the subtraction of images before and after Gd

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injection/sonication. The contrast enhancement in the parenchyma was compared with the average enhancement found in the muscles adjacent to the target area on the coronal image.

III. Results and Discussion

The targeting and acoustic coupling quality were confirmed using T_2-w MR images (Fig. 1). The focus position was estimated based on the transducer geometry and the focus zone obtained from the transducer calibration in free water. T_2-w MR images were used to confirm the absence of bubbles trapped in the coupling gel or in the balloon. In addition to that, the SCD and ICD plots obtained during PCD baseline acquisition also helped identify whether water in the balloon was adequately degassed or small bubbles were trapped in the coupling gel (Fig. 2). Fig. 2 shows examples of cases in which adequate and inadequate acoustic coupling were detected by PCD at baseline.

The high resolution anatomical images and the high temporal/spatial resolution of contrast agent diffusion provide a unique tool for studying the mechanisms of BBB disruption and drug delivery in NHP. T_1-w dynamic MR images after contrast agent injection confirmed the BBB opening and allowed the measurement of its uptake kinetics (Fig. 3). T_2- and T_2*-w MR images acquired after sonication revealed potential hemorrhages. The amount of Gd in the sonicated area was maximal at 30 min post sonication (67% ± 15% relative to adjacent muscle). In sessions without feedback control, high variability of results were found across animals and even within the same animal when targeting different brain regions. Using 697 kPa (calibrated in free water) a mild BBB disruption was obtained in one animal (S1) and no BBB opening in another animal (S2) when targeting the same brain region. When pressure was increased to 1.16 MPa, a more lateral targeting (10.8 mm from midline) resulted in mild opening (S3), whereas targeting more central (5.6 mm from midline) resulted in a permanent brain lesion (S4). The skull heterogeneity caused high variability in the ultrasound transmission into the brain. Therefore, the ultrasound intensity has to be carefully adjusted for a safe and an effective BBB opening.

The high resolution anatomical images and the high temporal/spatial resolution of contrast agent diffusion provide a unique tool for studying the mechanisms of BBB disruption and drug delivery in NHP. Using data of the session resulting in a permanent brain lesion, the safe acoustic pressure range was estimated by intersecting the estimated lateral focus size (from transducer calibration) with the profiles (lateral size) of the BBB opening (from T_1-w MR image acquired within 5 min of sonication) and of the brain lesion (from T_2-w MR image acquired 14 days after sonication). Considering 71% of attenuation caused by the skull, the acoustic pressure range that resulted in BBB opening without bleeding was estimated to range from 185±22 kPa to 266±4 kPa.

PCD harmonic emissions correlated with safe BBB opening, whereas broadband and sub-harmonic emissions were present in the sonication S4 that resulted in permanent lesion (Fig. 4). Safe sonications were achieved in all 3 sessions using

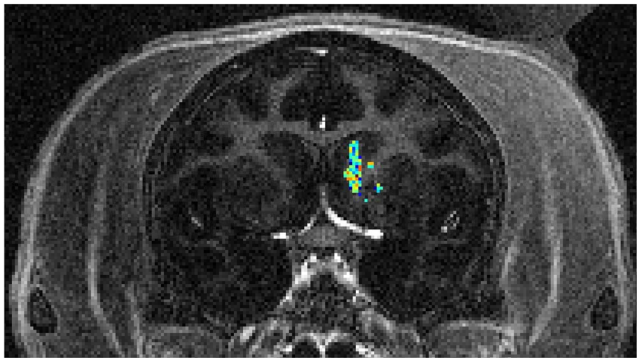

Fig. 3. Contrast enhanced T_1-w MR image obtained after 30 min of sonication (background image, grayscale) overlapped by the segmented image (BBB opening, color) resulting from the absolute difference between the baseline image and the background image.

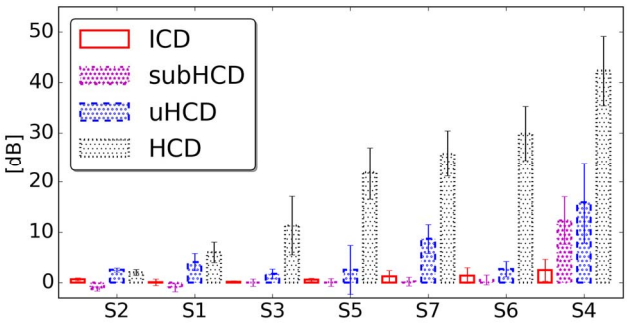

Fig. 4. Relative cavitation doses (CD) in all 7 sessions presented in ascending order. S1 to S4 were performed without feedback control. S5 to S7 were performed with feedback control. S4 resulted in a permanent lesion and presented higher ICD (inertial CD) and subHCD (sub-harmonic CD). HCD (harmonic CD) indicated the presence of harmonic components in all sessions with similar mean values in sessions with feedback control. uHCD (ultra-harmonic CD) indicated the presence of ultra-harmonic components in all sessions, but varied significantly across

real-time PCD-based feedback control of the acoustic pressure (S5 to S7). The PCD revealed that harmonic components were always present in successful sonication that resulted in BBB opening (Fig. 4). Broadband and sub-harmonic emissions were suppressed in sessions with safe sonication. The average sub-harmonic cavitation dose was more than 12 dB higher in the case that resulted in permanent lesion showing that this component could serve as an endpoint criteria for the sonication. The ultra-harmonic components varied across sessions and they were more prominent in sessions S7 (safe sonication) and S4 (harmful sonication). As expected [15], ultra-harmonic components may be present in inertial and stable cavitation events. In our study, this component could not be used as a threshold to indicate harmful sonication. The PCD-based feedback control allowed for repeated safe sonication (no edema or any macroscopic damage detected on MRI) regardless of the variation of skull attenuation.

IV. Conclusion

We have demonstrated that the skull heterogeneity makes the acoustic pressure control challenging without feedback

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control. The use of relative spectra of inertial and stable cavitation with PCD served as a robust readout for the feedback control of the acoustic pressure. Furthermore, baseline acquisitions prior to microbubble injection provide an evaluation of the acoustic coupling that could reveal the presence of trapped bubbles or inadequate degassing of the coupling gel or water in the coupling balloon. The feedback control based on the relative PCD spectrum is system-independent, allowing other systems to benefit from the real-time control of the acoustic pressure to ensure safe sonications.

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