Focused Ultrasound Facilitated Adenoviral Delivery for Optogenetic Stimulation

Shutao Wang¹, Amanda Buch¹, Syed Abid Hussaini², Camilo Acosta¹, and Elisa Konofagou¹,³
¹Department of Biomedical Engineering, ²Department of Neurobiology, ³Department of Radiology
Columbia University, New York, USA

Abstract—Optogenetics is a recently developed technique that has been widely implemented in the field of neuroscience. Channelrhodopsins (ChR), i.e., proteins that function as optically activated ion channels, are introduced into neurons either by viral transduction or transgenic manipulations. Recent advances in focused ultrasound (FUS) offer an alternative, non-invasive approach to carrying out viral transduction in the brain. In this study, we propose a FUS-based, non-invasive adenoviral delivery scheme that is suitable for optogenetic applications. Wild-type mice were used in this study, and each animal was sonicated using a single-element FUS transducer (center frequency 1.5 MHz). The acoustic parameters used for each sonication are: peak-rarefractional pressure 0.55 MPa, pulse length 10 ms, pulse repetition frequency 5 Hz, and a duration of 120 s. Viral vectors (both AAV1 and AAV9) were mixed with in-house manufactured polydisperse microbubbles prior to intravenous injections. Animals were survived for 12 days after sonication and electrode (with optic fiber) was implanted for electrophysiology recording. Upon optical stimulation, neuronal depolarization was observed in mice that received FUS-facilitated AAV transduction. In addition, fluorescence imaging revealed abundant ChR expression in the FUS sonicated brain regions. In conclusion, the successful non-invasive AAV delivery can provide an alternate and safer route for Channelrhodopsin neuronal transduction for optogenetic stimulation.

Keywords—focused ultrasound; optogenetics; brain; AAV

I. INTRODUCTION

Optogenetics is a recently developed technique that has revolutionized the field of neuroscience. Utilizing the unique properties of light sensitive protein channels such as Channelrhodopsin (ChR), neuronal activities can be artificially manipulated [1]. By selecting a specific group of neurons, the corresponding circuit or behavior can be controlled with millisecond precision. Among the light-sensitive protein channels, channelrhodopsins and halorhodopsins were found to be capable of transporting various ions across the cell membrane when activated by light, leading to action potentials [2]. However, these protein channels are non-endogenous proteins in mammals and needed to be introduced artificially. Currently, there are two major approaches to introduce light-sensitive protein channels to mammalian brains, namely viral transduction and transgenesis. Nonetheless, each technique suffers from some limitations: viral transduction requires invasive procedures to infuse viral vectors directly into the desired brain region, while transgenesis loses all region specificity. Therefore, to achieve selective neuronal transduction for optogenetic stimulation, a non-invasive viral delivery technique is desirable.

Recent advances in the field of Focused ultrasound (FUS) offers an exciting opportunity for non-invasive viral delivery to the brain [3], [4]. Numerous groups have reported disruption of the blood-brain barrier (BBB) with FUS and ultrasound contrast agents (i.e. microbubbles) for the delivery of therapeutic compounds [5]–[7]. Systemically administered adeno-associated virus (AAV, approximately 4MDa), has also been shown to cross the BBB, resulting in abundant expression in the targeted brain regions [8]. Non-invasive neuron-specific AAV transduction was also reported using synapsin promoters [3]. These studies have paved the way for non-invasively introduction light sensitive protein channels to mammalian brains.

In this study, we propose to take advantage of FUS and microbubbles to non-invasively and selectively induce ChR protein channels to the mouse brain. Our hypothesis is that FUS-facilitated viral delivery is sufficient to achieve neuronal activation with optogenetic stimulations.

II. METHODS

A. Animal Preparation

Wild-type mice (Strain: C57BL/6, Harlan, Indianapolis, IN) were used in this study. For both FUS sonication and electrode implantation procedures, each mouse was anesthetized with a mixture of oxygen and 1-3% isoflurane (SurgiVet, Smiths Medical PM, Inc., WI) and placed prone with its head immobilized by a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). The hair on the mouse scalp was removed using an electrical trimmer and depilatory cream to minimize impedance mismatch for ultrasound exposure. All procedures involving animals were approved by the Columbia University Institutional Animal Care and Use Committee. The viral vectors used in this study are AAV1-CAG-mcherry-ChR2 and AAV9-CAG-mcherry-ChR2 (UPenn Vector Core, Philadelphia, PA).
B. Microbubbles and FUS

Polydisperse microbubbles were manufactured in-house according to previously published protocol [9]. Briefly, 1, 2-distearyl-sn-glycero-3-phosphocholine and polyethylene glycol 2000 were mixed at a 9:1 ratio. Ten milligrams of the mixture was dissolved in a 10-mL solution consisting of filtered phosphate-buffered saline/glycerol (10% volume) / propylene glycol (10% volume) using a sonicator (Model 1510, Branson Ultrasonics, Danbury, CT, USA). Two milliliters of the solution were transferred to a 10 mL vial, which was filled with decafluorobutane (C4F10) gas. Each vial was activated using a mechanical shaker (VialMix®, N. Billerica, MA) for 45 seconds at room temperature. The microbubbles (approximately 2.5×10⁷ per animal) were diluted with 100 μl AAV9 vectors (titer 2.48×10¹³ GC/ml) immediately prior to intravenous injections.

The FUS sonications were carried out with a single-element focused transducer (focal length: 60 mm and radius: 30 mm, Imasonic, France), which has a center frequency of 1.5 MHz. The -6dB pressure focal zone of the FUS transducer was measured by a needle hydrophone (Precision Acoustics Ltd., Dorchester, UK) in degassed water to be 7.5 × 1 × 1 mm³. A confocally mounted pulse-echo transducer (radius: 11.2 mm, focal length: 60 mm, and center frequency: 10 MHz, Olympus NDT, Waltham, MA) was used for targeting. The transducer cone was filled with de-ionized and degassed water and sealed with a piece of polyurethane membrane (Trojan; Church & Dwight Co., Inc., Princeton, NJ). The FUS transducer was connected to a matching circuit and driven by a computer controlled function generator (Agilent, Palo Alto, CA) and a 50 dB power amplifier (ENI Inc., Rochester, NY). The transducer system was then mounted onto a computer controlled three-dimensional positioner (Velmex Inc., Lachine, QC, Canada). The hippocampus was selected to be the targeted brain structure in this study. The FUS transducer was moved according to the following coordinates from lambda: AP +2.5 mm and ML -2 mm. The center of the FUS focus was placed 2.5 mm below the skull and 30% attenuation was accounted for acoustic pressure loss through the skull. An estimated in situ peak rarefactional pressure (PRP) of 0.45 MPa was used for all sonications with a pulsing sequence of pulse repetition frequency (PRF) at 5 Hz, pulse length of 6.7 ms, and a total duration of 60 s.

C. Electrode implantation and optical stimulation

Mice received FUS sonications were allowed to survive 12 days before electrode implantation procedures. The implantation procedures were performed on a stereotaxic frame (David KOPF Instruments, Tujunga, CA). The selected target in this study was the hippocampus. The insertion site was located using the following coordinates derived from a mouse brain atlas: AP -2.5 mm, ML -2.0 mm, DV +2.0 mm (from bregma). A sixteen channelled tetrode bundle (Axona, St.Albans, UK) with an attached optrode was inserted through an opening in the skull. The tetrode bundle was secured on the mouse skull with dental cement upon the completion of the implantation. The optical stimulation was performed by connecting a LED source (blue light) to the optrode. Both burst stimulation (2s on and 2s off) and constant current stimulation (10s on) were performed.

D. MRI and Fluorescence Imaging

Upon the completion of FUS sonication, MR contrast agent gadolinium (200 μl) was administrated intraperitoneally. MRI scans were performed to confirm the BBB opening with a 9.4 T MRI system (Bruker Medical, Boston, MA). All acquisitions were carried out using a T1-weighted 2D FLASH sequence (TR/TE 230/3.3 ms, flip angle 70, resolution 100 μm × 100 μm × 400 μm).

Approximately one hour after the optical stimulation, mice were sacrificed and transcardially perfused with 30 mL phosphate-buffered saline and 60 mL 4% paraformaldehyde. The brains were collected and sectioned at 30 μm. The sections were mounted with ProLong Gold with DAPI (Life Technologies, Grand Island, NY). All fluorescence images were taken with a confocal microscopy (Nikon Instruments Inc., Melville, NY).

III. RESULTS

A. FUS Facilitated AAV-ChR2 Delivery
From contrast-enhanced MRI images, it was evident that the BBB was successfully opened with FUS and microbubbles. As shown in Figure 2 (top), the left hippocampus was successfully targeted with FUS. The viral gene contained both ChR2 and mcherry, which is used as a marker for ChR2 expression. The bottom image in Figure 2 revealed the ChR2 expression in mouse brains treated with unilateral FUS sonication. We observed abundant ChR2 expression (red) on the sonicated side of the brain, while minimal transduction was seen on the contralateral side. Comparing AAV1 and AAV9, serotype 9 AAV offered significantly better gene expression in vivo. As a result, AAV9 was used in all subsequent studies.

B. Optical Stimulation

Once we confirmed the feasibility of FUS facilitated AAV delivery and the abundance of ChR2 expression, optical stimulation was performed. The simulation sessions were carried out while the animals were fully awake. As shown in Figure 3a, neuronal activation was observed when the stimulation light was turned on every other two seconds. The amplitude of recorded signals was much higher upon stimulation than that in the resting state, indicating enhanced neuronal activity. Similarly, increased neuronal activity was observed at the beginning of the 10s stimulation. The gradual decrease of amplitude signifies the depletion of ionic gradients, where neurons transitioned to the fatigue state.

IV. DISCUSSION AND CONCLUSIONS

Optogenetics is a technology that has significantly advanced the field of neuroscience with widespread applications by numerous research laboratories around the world over the past decade. For the purpose of investigating neuronal circuitry and functionality, the intact nature of brain structures is essential. Current approaches introducing light-sensitive protein channels to mammalian brains suffer from either invasive procedure or lack of selectivity. FUS in combination with microbubbles provides an alternative method for non-invasive and selective introduction of light sensitive channels to the brain. In this study, we demonstrated, for the first time, the feasibility of FUS facilitated AAV-ChR2 delivery for optogenetic applications.

The BBB opening was first confirmed with contrast-enhanced MRI scans, which serves as a guide for later on electrode implantation. Abundant ChR2 expression was desirable since the optical stimulation has very limited effective range (on the order of millimeter). By comparing AAV1 and AAV9 vectors, we concluded that AAV9 which offers significantly higher transduction rate is more suitable for the purpose of the current study. Fluorescence imaging revealed selective AAV transduction on the sonicated side of the brain, while almost no expression was observed on the contralateral side. The response of neurons expressing ChR2 to light was further examined using optical stimulations. As
shown in Figure 3, neurons expressing ChR2, which was introduced non-invasively, respond well to both burst and constant-current optical stimulations. These results indicate that FUS facilitated non-invasive AAV delivery is a viable approach to introduce light sensitive protein channels to mammalian brains for optogenetic applications.

ACKNOWLEDGMENT

This study was supported in part by NIH R01EB009041, and NIH R01AG038961.

REFERENCES


