Focused-ultrasound-mediated delivery of virus-encoded red-shifted channelrhodopsin for fully non-invasive and remote neuronal activation *in vivo*

Antonios N. Pouliopoulos[1], Nancy Kwon[1], S. Abid Hussaini[2], Elisa E. Konofagou[1,3]

1. **Department of Biomedical Engineering,**
2. **Department of Pathology and Cell Biology, and**
3. **Department of Radiology**

Columbia University, New York, USA

OBJECTIVES

Channelrhodopsin (ChR) is a light-sensitive protein used in optogenetics to elicit neuronal responses *in vivo*. Typically, ChR is encoded by an adeno-associated virus (AAV) delivered via direct injection into the brain and activated via blue-light illumination through implanted optical fibers. Neuronal activity is usually recorded with implanted electrodes. However, these highly invasive procedures are sources of morbidity and damage to the surrounding tissues and alter physiological brain responses. Recently, red-shifted ChR variants have enabled non-invasive neuronal activation using red light, which can penetrate deeper through the skull and tissues. Here, we aimed at fully non-invasive and remote activation of deep structures in the murine brain, using focused-ultrasound(FUS)-assisted viral delivery and red light exposure.

METHODS

AAVs encoding a red-shifted ChR variant (tdTomato - Chrimson, peak absorption wavelength ~ 600 nm) were delivered into the brain of wild-type mice through FUS exposure (fc: 1.5 MHz, peak-negative pressure: 0.8 MPa, pulse duration: 10 ms, pulse repetition frequency: 5 Hz), in the presence of systemically circulating microbubbles. Following non-invasive blood-brain barrier opening and viral delivery, mice were allowed to survive for 2 weeks to allow for sufficient viral transduction. To remotely trigger neuronal activity, we illuminated the treated areas with red light through the intact skull using an LED source (635nm, beam size: 4 mm). Mice were then sacrificed and their brains were imaged with fluorescence microscopy to confirm the presence of Chrimson. Staining for Arc protein provided indirect evidence of neuronal activation in the areas expressing the light-sensitive channel. Measurements were conducted to detect differences between the ipsilateral (i.e., treated) and contralateral (i.e., control) hemispheres.

RESULTS

Fluorescence imaging confirmed that AAVs were successfully delivered in the sonicated region. Viral delivery (figure 1A, top) was prominent in the treated hemisphere, however we detected limited Chrimson expression in the contralateral hemisphere. Similar AAV delivery ($p > 0.05$,

figure 1B, left) was achieved both in illuminated (2.1 ± 0.4-fold increase) and control mice (1.8 ± 0.6-fold increase). Arc staining showed that neuronal activation (figure 1A, bottom) occurred at areas with the highest rate of AAV expression, suggesting that light-sensitive channels were activated due to red light exposure (figure 1B, right). Arc upregulation was primarily detected with the neuronal nuclei, but also diffused throughout the activated area (figure 2). Arc activation was on average 66 ± 37 % higher on the ipsilateral side compared to the contralateral side in illuminated brains. In contrast, the difference in Arc activation between the ipsilateral and contralateral side in control mice, which were not exposed to light, was 4.57 ± 4.25 %. Although viral delivery was equivalent between illuminated and control mice ($p > 0.05$), Arc activation was significantly higher ($p < 0.05$) in mice exposed to red light.

CONCLUSIONS

In this proof-of-principle study, we showed that AAVs encoding red-shifted ChR variants can be non-invasively delivered using FUS and microbubbles. By exposing the treated brains to deep-penetrating red light, we found evidence of neuronal activation in deep structures. Our initial findings indicate that neuronal activity can be triggered remotely *in vivo* using a combination of ultrasound and light exposure through the intact skull.

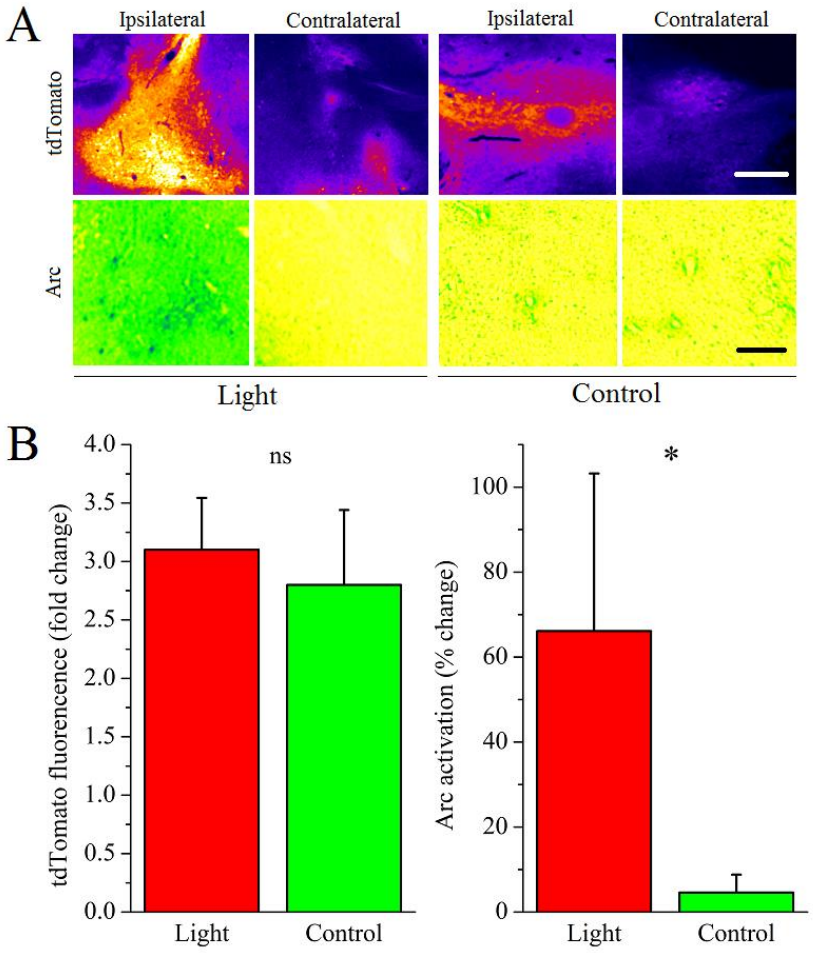

Figure 1: Remote neuronal activation following red-light exposure. A) tdTomato fluorescence indicated successful viral delivery predominantly in the ipsilateral side, in both light-exposed and control mice. Arc staining revealed areas of increased neuronal activation only in the light-exposed group. B) tdTomato fluorescence was enhanced in the ipsilateral side compared to the contralateral side in both light-exposed and control mice, without significant differences (ns: $p>0.05$). In contrast, Arc activation was significantly higher in light-exposed mice (: $p<0.05$). Data presented as mean ± s.d.. Bar: 100 μm.*

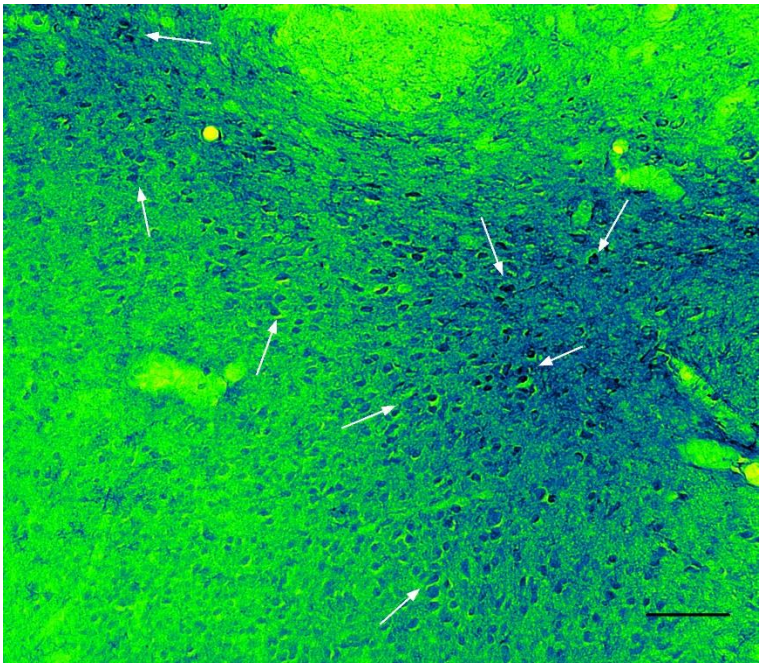

Figure 2: Arc distribution. Arc protein was primarily detected within the cell nuclei (in blue, annotated with white arrows), but also diffused throughout the entire activated area (in green). Bar: 50 μm.