

# Non-Invasive Delivery of Adeno-Associated Viral Vectors to the Brain in Non-Human Primates with Theranostic Ultrasound

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## Objectives

To elucidate the feasibility of transcranial gene delivery with a 500 kHz theranostic ultrasound (ThUS) linear array and ultra-short pulses in the non-human primate brain.

## Methods

A male rhesus macaque (~30 y.o., 13 kg) was dosed intravenously with adeno-associated virus (AAV9-CAG-GFP,  $2.0 \times 10^{13}$  gc/kg) and proprietarily-formulated polydisperse microbubbles immediately prior to synchronous BBB opening (BBBO) and power cavitation imaging (PCI) with a linear array in three brain regions: right caudate/putamen, left substantia nigra, and midbrain. Contrast-enhanced T<sub>1</sub>-weighted MRI confirmed BBBO 30 min post-ThUS. The NHP was euthanized 4 weeks later for brain and peripheral tissue collection, histopathological examination, and AAV genome quantification.

## Results

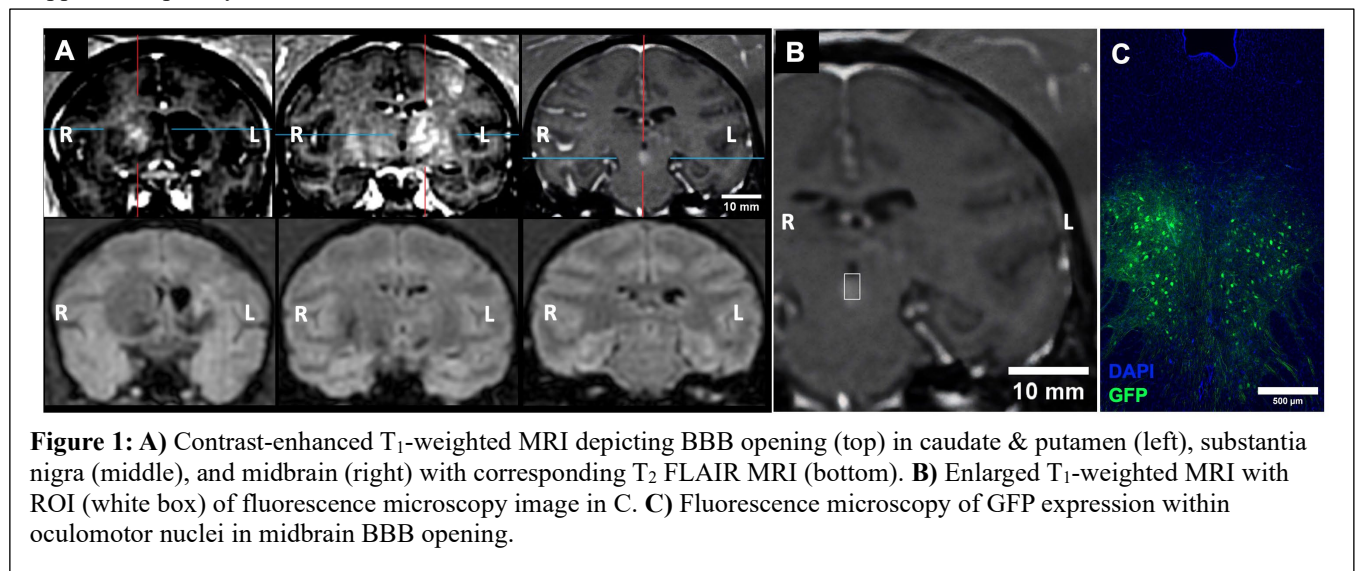
After AAV injection, PCI acquired during sonication demonstrated a substantial increase in cavitation activity along all target trajectories after each bolus injection of microbubbles, accompanied by confirmed BBB opening in all target brain regions (Fig. 1A). No radiological damage was detected on T<sub>2</sub>-FLAIR MRI acquired immediately prior to gadodiamide contrast injection (Fig. 1A). While histology for transgene expression visualization and AAV DNA quantification is ongoing for the caudate, putamen, and substantia nigra targets, fluorescence microscopy in the midbrain trajectory revealed a ~200-fold increase in GFP+ cells (oculomotor nuclei) within the contrast-enhanced region on MRI relative to unsonicated midbrain (Fig. 1B-C).

## Conclusions

In the study presented herein, we demonstrated transcranial gene delivery to the rhesus macaque brain with short, 3-cycle pulses operated by a portable, low-cost, ThUS linear array with similar focusing and gene delivery capabilities as other recently reported studies investigating FUS-mediated AAV delivery in non-human primates.

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**Figure 1:** **A)** Contrast-enhanced T<sub>1</sub>-weighted MRI depicting BBB opening (top) in caudate & putamen (left), substantia nigra (middle), and midbrain (right) with corresponding T<sub>2</sub> FLAIR MRI (bottom). **B)** Enlarged T<sub>1</sub>-weighted MRI with ROI (white box) of fluorescence microscopy image in C. **C)** Fluorescence microscopy of GFP expression within oculomotor nuclei in midbrain BBB opening.