PROGRESS REPORT

Blood-brain barrier opening for facilitating drug delivery in neurodegenerative diseases in non-human primates (PI: Konofagou)

Below we report on the progress below each one of the two specific aims. We have so far acquired six animals, two with this grant and four from other funding. Methods and results are reported on two out six monkeys and experiments in the remaining four will be completed at the end of the period.

1. To test and demonstrate delivery of neurotrophic factors to the hippocampus and putamen of monkeys.

In this aim, the accuracy of this system was tested by targeting the hippocampus and caudate nucleus of the basal ganglia in two macaque monkeys. We have performed 24 sonications with a 96% success rate (23/24). The average lateral targeting error of the system was ~2 mm while the axial targeting error, i.e., along the ultrasound path, was ~0.2 mm (Figure 1). We have also developed a real-time treatment monitoring technique based on cavitation spectral analysis. This technique also allowed for delineation of a safe and reliable acoustic parameter window for blood-brain barrier (BBB) opening. In summary, the targeting accuracy of the system developed was deemed to be suitable for the selective delivery of drugs to specific sub-structures of the basal ganglia. This established the method and the system developed as a potentially highly useful tool not only for clinical use, but also for neuroscientists trying to dissect the role of role of basal ganglia in neuropsychiatric disorders.

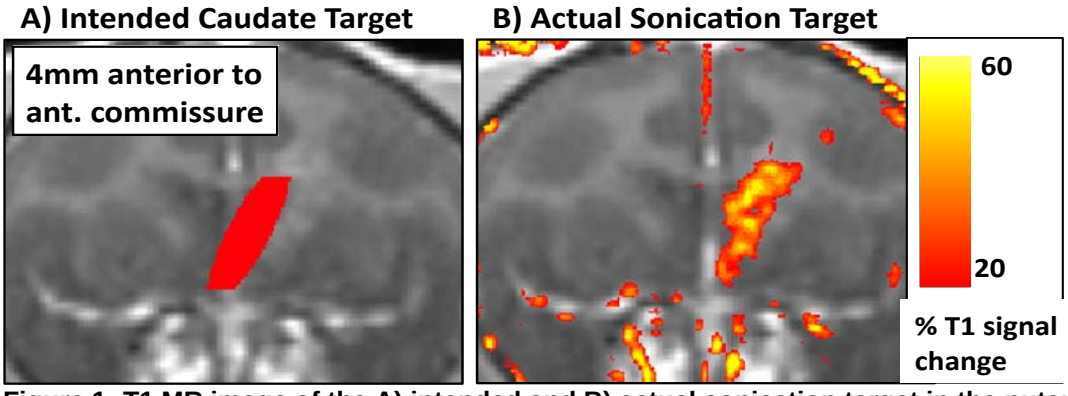

Figure 1: T1 MR image of the A) intended and B) actual sonication target in the putamen.

The neurotrophic factors have been prohibitively expensive to cover through the FUS funding. Based on our mouse studies, the approximate BDNF dose would be 30mg/kg and at $3500/mg, the cost would be $945,000 per monkey (~9-kg). This would require partnering with a pharmaceutical company in the future.

Instead, we have administered drugs intramuscularly (IM) that have been shown not to cross the blood-brain barrier and to inhibit motor control upon direct injection. Since the target is the putamen in this case, we expect that the motor control would be affected as a result of the BBB opening. The drugs tested were carbidopa and domperidone which are known to be D2 antagonists. The left putamen was sonicated and therefore the right arm was expected to experience slowness in motion. We have seen no significant effects when the monkeys were tested with a cognitive (touch panel) test (Figure 2).

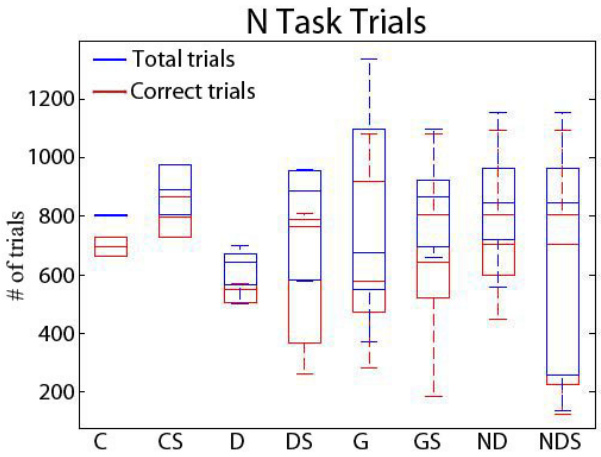

Figure 2: Motivation testing in monkey N for the following IM-injected drugs: C = carbidopa, D = domperidone, G = GABA, ND = No Drug. When a 'S' is affixed behind the previous notations, it signifies sonication.

We have hypothesized that the issue was not with the FUS method but with the drug delivery route, so we have now switched from IM to intravenous injection of those drugs, which has already some signs of our hypothesis being valid. The results are too preliminary to show in this report however, but we will show them at the 12-month report. Finally, we have started using PET imaging which just became available again on the Columbia University Medical Center (CUMC). The PET center had been closed for more than three years due to an incident. This will facilitate detection of binding to dopamine receptors in the brain and we have already completed our first baseline experiment by injecting raclopride which is not to highlight the receptors (Figure 3). We expect to see higher signal on the sonicated side and, more importantly, assess the delivered dose in vivo, over the remainder of the funding period.

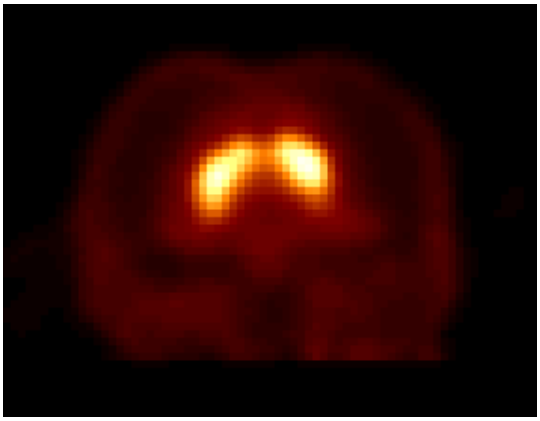

Figure 3: PET image of raclopride binding in the putamen (baseline only, no FUS).

2. To assess the safety of the FUS method in monkeys.

All monkeys have been survived so far and continuously monitored over the course of the entire study before, after and during sonication. Briefly, there have been no changes in neither the cognitive behavior (basic touch panel, Figure 2), vital (heart rate,blood pressure, etc.) or

physiological signs (food consumption, water consumption, etc.) of the monkeys after sonication (Figure 4). The drop in heart rate has been attributed to the isofluorane anesthesia that is required for sonication but will be verified over the remainder of the study. We will continue to monitor the animals in a similar fashion and perform sacrifice of the two monkeys dedicated to the study for histological confirmation at the end of the 12 months.

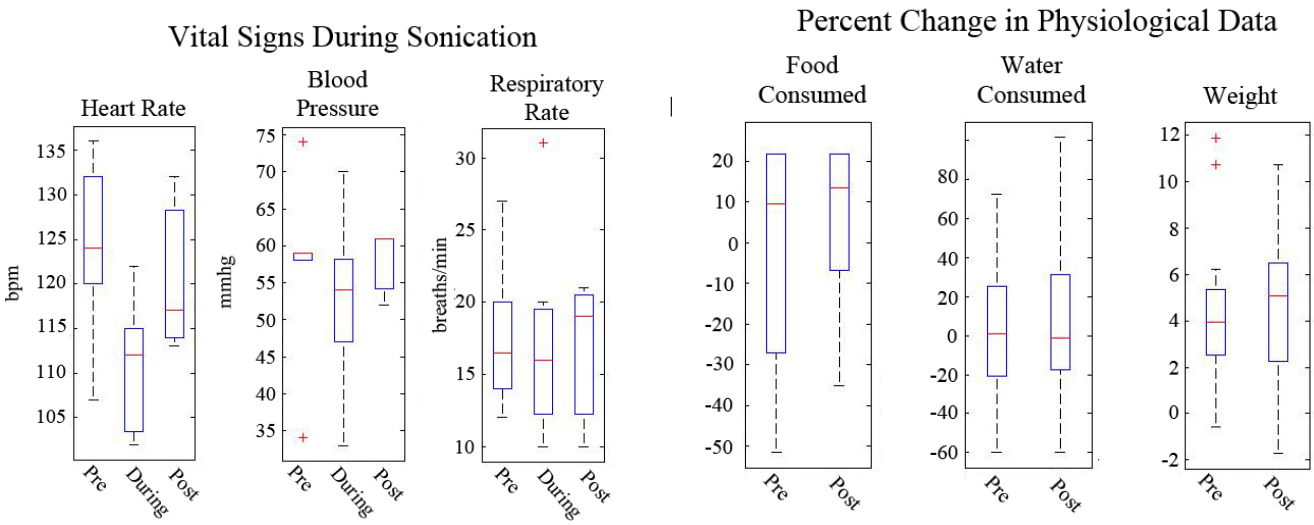

Figure 4: Vital and physiological signs averaged across the two monkeys studied and over all BBB openings.

Publications:

- Yao-Sheng Tung, Shih-Ying Wu, Fabrice Marquet, Konofagou E.E., Quantification of stable cavitation dose during FUS-induced blood-brain barrier opening in mice and in non-human primates, Proceedings of IEEE International Ultrasonics Symposium (Dresden, Germany), October 7 to 10, 2012 (in press).
- Shih-Ying Wu, Yao-Sheng Tung, Fabrice Marquet, Cherry C. Chen, Konofagou E.E., Non-human primate skull effects on the FUS-induced blood-brain barrier opening, Proceedings of IEEE International Ultrasonics Symposium (Dresden, Germany), October 7 to 10, 2012 (in press).
- Fabrice Marquet, Tobias Teichert, Shih-Ying Wu, Yao-Sheng Tung, Matthew Downs, Shutao Wang, Cherry Chen, Vincent Ferrera and Elisa Konofagou, Real-Time Monitoring of Opening of the Blood-Brain Barrier in Non-Human Primates, Proceedings of IEEE International Ultrasonics Symposium (Dresden, Germany), October 7 to 10, 2012 (in press).
- Fabrice Marquet, Tobias Teichert, Shih-Ying Wu, Yao-Sheng Tung, Matthew Downs, Shutao Wang, Cherry Chen, Vincent Ferrera and Elisa Konofagou, Real-time, transcranial monitoring of safe blood-brain barrier opening in non-human primates, PloS One (in preparation).