Assessment of Lexiscan for Blood Brain Barrier disruption to facilitate Fluorescence brain imaging

Rebecca W. Pak[1], Hanh N. D. Le[2], Heather Valentine[3], Daniel Thorek[3], Arman Rahmim[3], Dean Wong[3] and Jin U. Kang[2]

[1]Department of Biomedical Engineering, Johns Hopkins University, 720 Rutland Avenue, Baltimore, MD 21205, USA,
[2]Department of Electrical and Computer Engineering, Johns Hopkins University, 3400 North Charles Street, Baltimore, MD 21218, USA,
[3]Department of Radiology and Radiological Science, Johns Hopkins University, 601 N. Caroline St., Baltimore, MD 21287, USA
rpak2@jhmi.edu

Abstract: Mouse brain fluorescence was imaged after the tail vein injections of indocyanine green (ICG) dye and Lexiscan. The through-skull images showed dye in the vasculatures and permeating through the blood brain barrier into the tissue.
OCIS codes: (110.0110) Imaging systems; (170.0170) Medical optics and biotechnology.

1. Introduction

Many current methods for interrogating brain activity involve craniotomy, cranial windows, and other invasive preparations. On the less invasive side, techniques such as functional magnetic resonance imaging (fMRI) and calcium channel signaling have also been employed. However, both these signals are indirect measures. fMRI assesses the blood-oxygen-level dependent (BOLD) signal and relies on the coupling of blood flow with neural activity [1], which is not always present, particularly in diseased neurological models [2]. On the other hand, calcium signals indirectly measure dendritic membrane potential and have several confounding factors [3]. However, one of the main limitations of such methods is the lack of ways to effectively deliver the dye to the brain tissue. With this in mind, in this study we investigated a pharmacological method using Lexiscan to deliver voltage-sensitive dye to the brain through minimally invasive methods and performed fluorescence imaging to evaluate the feasibility of such procedures.

2. Methods

The fluorescence imaging system setup is shown in Fig. 1. To facilitate minimally invasive procedures, dyes with longer wavelength excitations and emissions, in the near infrared (NIR) region, were chosen and continue to be synthesized to achieve greater depth penetration. Thus, the system includes a commercial 780 nm laser diode for excitation of these dyes. For high resolution imaging, we used laser scanning microscopy, implemented using a pair of galvo mirrors (Thorlabs) to scan the region of interest plane on the mouse head. A dichroic mirror in conjunction with an 800 nm longpass filter were used to minimize intensity loss while also filtering out any excitation light from the camera. An Orca-Flash 4.0 sCMOS digital camera (Hamamatsu, Japan) was used for detection to minimize noise and maximize quantum efficiency.

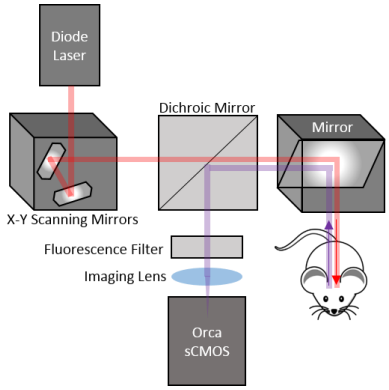

Fig. 1: Fluorescence imaging system

3. Results and Discussion

White mice, chosen for their minimal melanin, were injected with indocyanine green (ICG), a fluorescent dye (spectrum in Fig. 2). The injection was done intravenously (IV) through the mice tail veins and monitored with the

above fluorescence imaging system. More detail can be seen without the skin, but clear fluorescence signal is evident through the skin also (Fig. 3). Reflectance images are provided for comparison.

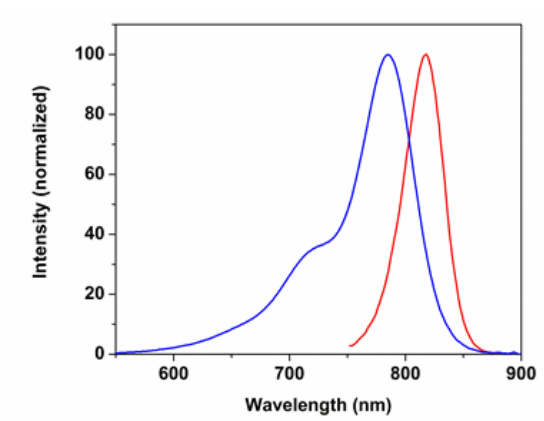

Fig. 2: ICG absorption (blue) and emission (red) spectrum [4].

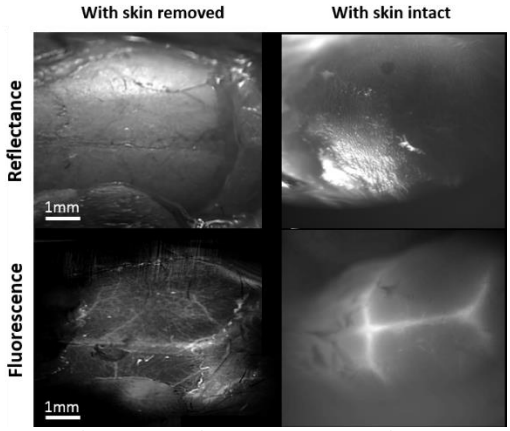

Fig. 3: Reflectance and fluorescence images of mouse brain with and without skin (skull intact).

A second set of experiments was conducted to test pharmacological treatment as a method to bypass the blood brain barrier (BBB). Baseline images with only ICG injected were taken. Vasculatures were visible in these images taken through the skull (Fig. 4a). With the additional injection of Lexiscan (adenosine receptor agonist) to open the BBB, dye fluorescence was observed to permeate through vessels and spread into the surrounding tissues (Fig. 4b). This demonstrated successful delivery of dye to the tissue through the BBB. This delivery method paves the way for the use of voltage-sensitive dyes in the brain in the future.

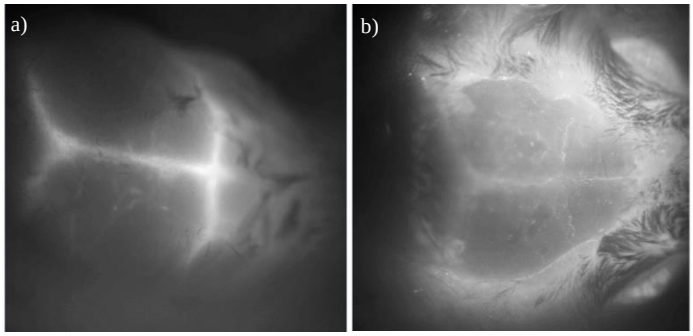

Fig. 4: Fluorescence imaging of mouse brain through intact skull a) with the injection of ICG and b) with injections of ICG and Lexiscan.

4. Conclusion

Pharmacological treatment with Lexiscan through tail vein injections successfully increased permeability of the blood brain barrier and allowed for tissue exposure to the ICG dye. This was verified by minimally-invasive fluorescence imaging through the skull. This confirmed the ability to deliver the dye to the brain tissue through IV injection. Continued development includes the injection of novel voltage-sensitive dyes synthesized by the Loew lab to facilitate real-time monitoring of neuron firing for neuroscience applications. For this application, micro-dialysis will also be used to correlate neurotransmitter concentrations with neuron firing and behavior.

5. References

[1] E. M. Hillman, “Coupling mechanism and significance of the BOLD signal: a status report,” *Annu Rev Neurosci*, vol. 37, pp. 161–181, 2014.

[2] S. Zacchigna, D. Lambrechts, and P. Carmeliet, “Neurovascular signalling defects in neurodegeneration,” *Nat Rev Neurosci*, vol. 9, no. 3, pp. 169–181, 2008.

[3] C. D. Acker, M. B. Singh, and S. D. Antic, “Intracellular Voltage-Sensitive Dyes for Studying Dendritic Excitability and Synaptic Integration,” 2016, pp. 247–265.

[4] AAT Bioquest, “ICG amine,” 2016. [Online]. Available: https://www.aatbio.com/products/188.