

A novel technique for infusion of optogenetics viral vectors in non-human primates (NHPs) cortex using MR-guided convection enhanced delivery (CED)

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Optogenetics is an ideal tool for manipulating neural activity artificially with high temporal and spatial precision [1]. We hope to use this technology for studying complex behaviors in NHPs in order to dissect functional neural circuits and to develop clinically relevant stimulation-based approaches to treating human neurological diseases [2]. However there are several challenges that remain for conducting long-term optogenetic experiments in these animals. One of the challenges is delivering a large volume of virus into a specific region of the cortex. Conventional injection techniques use multiple small injections and rely on diffusion of the viral vector from the injection site, typically over a range of 1-3 mm. Due to the large size of cortical regions in these animals, this approach is time consuming and delivers highly variable density of viral vector over the region. Therefore, we see a need for a technique to deliver these viruses more efficiently and more evenly across the target tissue. In addition with conventional techniques, one cannot precisely localize the injection site within the tissue to monitor the spread of virus during infusion. We have addressed these issues using a Convection Enhanced Delivery (CED) technique that has been developed for other gene-therapy methods [3]. In this technique relatively large volumes are injected with sufficient pressure to drive bulk fluid flow (convection), and hence achieve a wider and more even distribution of the vector. In addition, we have performed these injections in the MRI scanner in order to obtain real-time visualization of the site of injection and the spread of viral vector.

We have used a reflux-resistant cannula [3] to infuse an AAV9-CamKIIa-ChR2-EYFP viral vector into the primary somatosensory cortex of a rhesus macaque monkey. The cannula was inserted 2 mm below the surface of the brain. To monitor the infusion via real-time MR images, the viral vector was co-infused with a gadolinium MR contrast agent (See Figure 1). The initial infusion rate started with 1 uL/min and was increased to 5 uL/min by 1 uL/min steps as the spread of the virus was monitored in the cortical region. The virus was infused at two sites and 47 uL was injected through each site (Figure 1b and 1c). After eight weeks recovery, and under pentobarbital anesthesia, surface illumination and imaging was used to confirm the presence of the EYFP fluorescent reporter [4]. Later histological analysis, using immunostaining against the reporter, was performed. We observed widespread and dense ChR2 expression in areas 1, 2, 3a and 3b of cortex. The highest expression was observed in areas 1 and 3b, which were closer to the site of injection.

This technique enables us to obtain expression across larger areas of cortex in a shorter period of time with minimal damage compared to the current techniques [4] and can be an optimal approach for optogenetics viral delivery in NHPs.

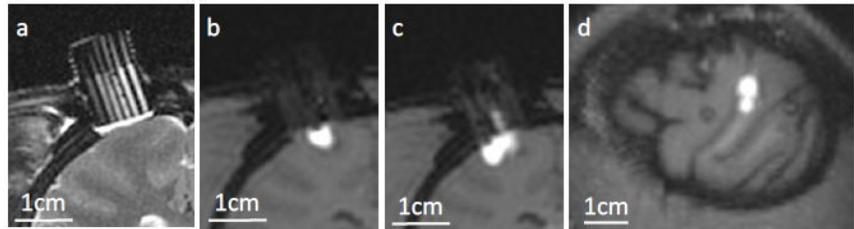


Figure 1. a) Coronal section of S1 before injection showing injection cylinder with cannula guides., b,c) viral vector spread after the first and second injections respectively. d) Surface rendering showing extent of viral vector spread between

REFERENCES

- [1] O. Yizhar, L. E. Fenno, T. J. Davidson, M. Mogri, and K. Deisseroth, "Optogenetics in neural systems," *NEURON*, vol. 71, pp. 9-34, Jul 14 2011.
- [2] X. Han, "Optogenetics in the nonhuman primate," *Prog Brain Res*, vol. 196, pp. 215-33, 2012.
- [3] A. P. Kells, P. Hadaczek, D. Yin, J. Bringas, V. Varenika, J. Forsayeth, and K. S. Bankiewicz, "Efficient gene therapy-based method for the delivery of therapeutics to primate cortex," *Proc Natl Acad Sci U S A*, vol. 106, pp. 2407-11, Feb 17 2009.
- [4] I. Diester, M. T. Kaufman, M. Mogri, R. Pashaie, W. Goo, O. Yizhar, C. Ramakrishnan, K. Deisseroth, and K. V. Shenoy, "An optogenetic toolbox designed for primates," *Nat Neurosci*, vol. 14, pp. 387-97, Mar 2011.

This work was supported by the Defense Advanced Research Projects Agency (DARPA) Reorganization and Plasticity to Accelerate Injury Recovery (REPAIR; N66001-10-C-2010).

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