Open-source wearable miniature microscopes to study astrocytes and astrocyte-neuron interactions in freely behaving mice

Tsai-Yi Lu[1], Daniel Aharoni[1,2], Ben S. Huang[2], Peyman Golshani[2], and Baljit S. Khakh[1]
[1]Department of Physiology, [2]Department of Neurology, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, California, U.S.A.

Astrocytes display complex intracellular calcium dynamics. In the past two decades, it has been shown that the intracellular calcium concentration of astrocytes increases in response to local and/or general synaptic activity, which may modulate nearby neurons via a variety of proposed mechanisms. A critical open task, however, is to study astrocyte calcium signaling and astrocyte-neuron interactions *in vivo*, especially in relation to animal behavior. Based on recent developments with genetically-encoded calcium indicators (GECIs, e.g. GCaMP6f) and head-fixed *in vivo* two-photon laser scanning microscopy (2PLSM), it has been shown that when mice receive robust sensory or behavioral stimuli, such as whisker stimulation and air puff startle, the intracellular calcium concentration of cortical astrocytes is robustly increased (Paukert et al., 2014; Srinivasan et al., 2015; Takata et al., 2011; Wang et al., 2006). Although the use of GECIs and 2PLSM have significantly advanced our knowledge of astrocyte calcium signaling, unavoidable head-fixation with 2PLSM largely prevents researchers from exploring astrocyte calcium signaling in multiple behavior-related contexts. New approaches are thus needed. In this presentation, we will describe how to make and use facile miniature wearable microscopes. We will describe the design, engineering and use of low-cost (< $500 per scope), open-source, miniaturized (< 3g) wide-field fluorescence microscopes (henceforth referred to as Miniscopes) that can record both neuronal and astrocyte calcium signals in the visual cortex of freely behaving mice. Miniscopes successfully captured the activation of astrocyte calcium waves in the visual cortex when mice were freely moving on a spherical treadmill and when they were startled with an air-puff. Our results demonstrate the utility of Miniscopes for studying astrocyte calcium dynamics for the first time in freely behaving mice and foreshadow heretofore unappreciated opportunities to explore astrocyte-neuron interactions *in vivo*. This presentation will provide a detailed primer on Miniscopes and report data gathered with their use.

References:

Paukert, M., Agarwal, A., Cha, J., Doze, V.A., Kang, J.U., and Bergles, D.E. (2014). Norepinephrine controls astroglial responsiveness to local circuit activity. *Neuron 82*, 1263–1270.

Srinivasan, R., Huang, B.S., Venugopal, S., Johnston, A.D., Chai, H., Zeng, H., Golshani, P., and Khakh, B.S. (2015). Ca^{2+} signaling in astrocytes from $Ip3r2^{-/-}$ mice in brain slices and during startle responses in vivo. *Nat. Neurosci. 18*, 708–717.

Takata, N., Mishima, T., Hisatsune, C., Nagai, T., Ebisui, E., Mikoshiba, K., and Hirase, H. (2011). Astrocyte calcium signaling transforms cholinergic modulation to cortical plasticity in vivo. *J. Neurosci. 31*, 18155–18165.

Wang, X., Lou, N., Xu, Q., Tian, G.-F., Peng, W.G., Han, X., Kang, J., Takano, T., and Nedergaard, M. (2006). Astrocytic Ca^{2+} signaling evoked by sensory stimulation in vivo. *Nat. Neurosci. 9*, 816–823.