

***In vivo* Three Photon Imaging of Neuronal Activities from Hippocampus in Intact Mouse Brain**

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Optical imaging has enabled chronic observation of living mouse brain structure and function with single cell resolution [1]. Multiphoton microscopy (MPM) has significantly extended imaging depth to subcortical layers in highly scattering mouse brain [2-3]. Combined with genetically encoded calcium indicator GCaMP6s, we demonstrated that 3PM with 1350 nm excitation is capable of simultaneous recording of calcium transients in a neuron population in *stratum pyramidale* (SP) layer of *cornu ammonis* (CA1) region of hippocampus in intact mouse brain.

The excitation source for the custom-built multiphoton microscope was an optical parametric amplifier (OPA) operated at 1350 nm and a repetition rate of 250 kHz. After dispersion compensation, laser pulse width was ~ 65 fs after the objective. We imaged brain of mouse under anesthesia through cranial window two weeks after injection of AAV2/1 encapsulated GCaMP6s. The imaging site was approximately 2 mm posterior and 2.5 mm lateral to the bregma point.

With all overlaying cortical tissue intact, we imaged tissue structure in a stack from 700 μm to 1250 μm below brain surface. Fluorescence and third harmonic generation (THG) were simultaneously recorded. The THG signal delineates boundaries between deep cortical neurons, external capsule (EC), and hippocampus (Figure 1a). The maximum average power used to image SP layer in CA1 region of hippocampus was ~ 36 mW.

We further recorded calcium activities from 25 SP layer neurons simultaneously for 8 minutes (Figure 1b). Neuronal activities were continuously recorded with 128x128 pixel frames at a frame rate of 6.1 Hz. The time resolution was 164 ms, which is adequate for GCaMP6s with a half decay time of 1.8 s for 10 action potentials (AP) [4]. Observed spike duration varies from 1 s to ~20 s. Relative fluorescence change ($\Delta F/F_0$) of the spikes was as high as 700% (Figure 1c).

The method demonstrated here enables *in vivo* functional imaging of subcortical neuron population with single cell resolution within intact mouse brain. We anticipate that this technology will have an impact on minimally invasive investigation of neuronal circuit deep within a mouse brain.

References

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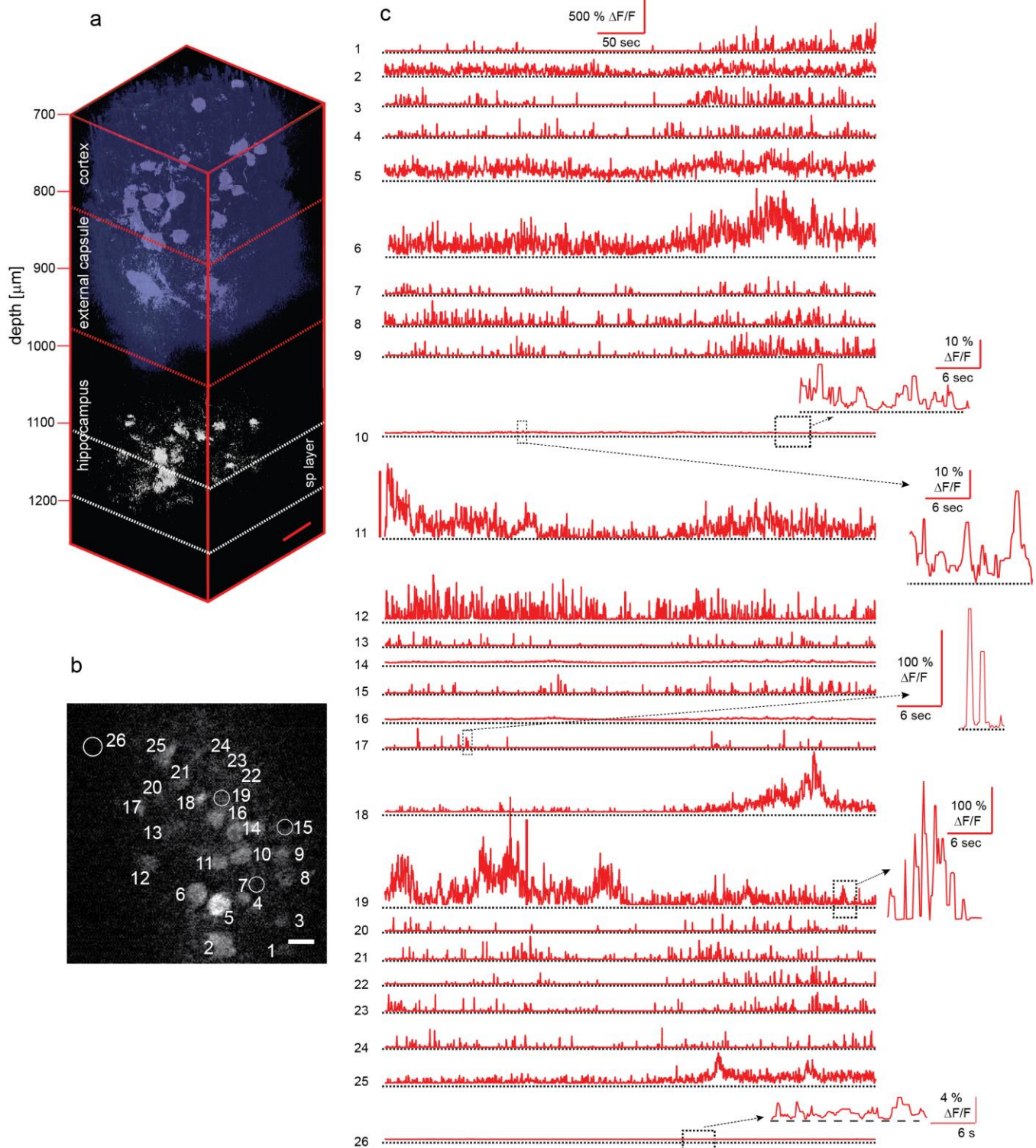


Figure 1. (a) Image stack of GCaMP6s labeled neurons spanning from cortex to hippocampus (fluorescence colored in bright grey, THG in dark purple, 175 μm x 175 μm field-of-view (FOV), taken with increments of 2 μm in depth, scale bar 50 μm) (b) Neuronal population in the CA1 region of 1121-μm depth (0.21 frames/s, 20 averages, 512x512 pixels, scale bar 20 μm) (c) Spontaneous Ca²⁺ transients recorded from the somata indicated in (b)