Opto-Electronic Advances

Supplementary information

2022, Vol. 5, No. 12

DOI: 10.29026/oea.2022.210081

All-fiber-transmission photometry for simultaneous optogenetic stimulation and multi-color neuronal activity recording

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Supplementary information for this paper is available at https://doi.org/10.29026/oea.2022.210081

Section 1：The spectra of GEFIs and opsin-sensors

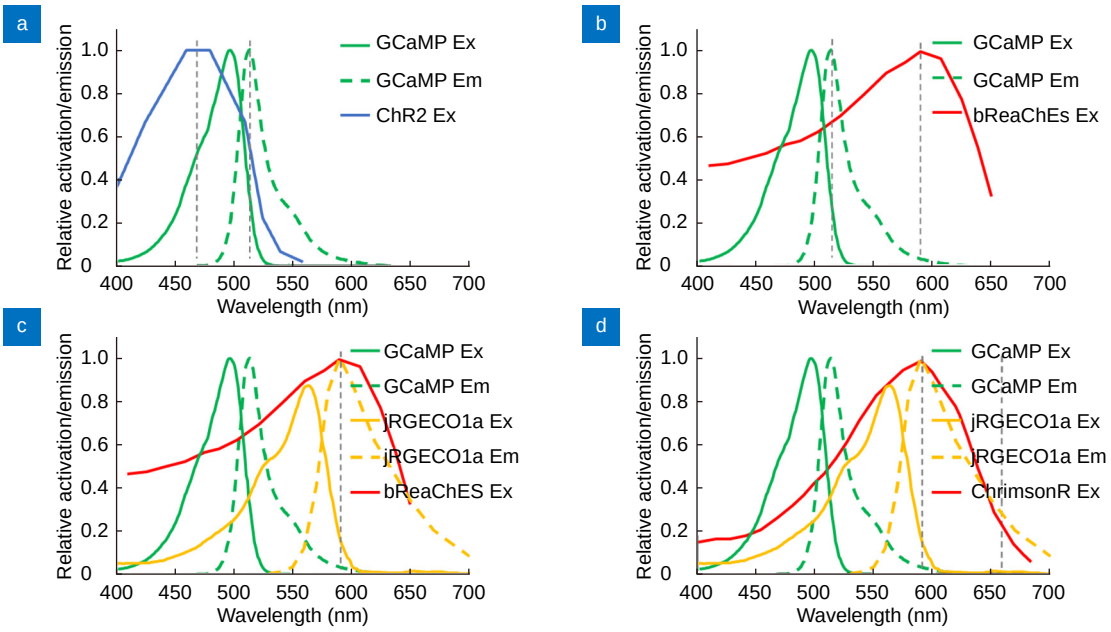

Fig. S1 | The spectra of GEFIs and opsin-sensors. (**a**) The excitation spectrum of ChR2 is close to the emission spectrum of GCaMP. Gray lines: GCaMP (510 nm) emission and ChR2 (473 nm) excitation wavelengths. (**b**) The separated excitation spectrum of bReaChES[61] and the emission spectrum of GCaMP allowed simultaneous recording and optogenetic manipulation without artifacts. Gray lines: GCaMP (510 nm) emission and bReaChES (593 nm) excitation wavelengths. (**c**) The excitation spectrum of bReaChES overlaps with the emission spectrum of GCaMP. Stimulation artifact from a 590-nm excitation light is still a problem when involving dual-color recording. Gray lines: jRGECO1a (593 nm) emission and bReaChES (593 nm) excitation wavelengths. (**d**) The effective excitation wavelength of ChrimsonR[43] can separate from the emission spectrum of GEFIs when involving dual-color recording and optogenetics. Gray lines: jRGECO1a (593 nm) emission and ChrimsonR (660 nm) effective excitation wavelengths. The ChrimsonR is a red-shift channelrhodopsin and is effectively more light-sensitive, which can be excited by the 470~720-nm light with milliwatt. The spectra of GEFIs and opsin-sensors are available for this paper at https://www.fpbase.org. Abbreviations: Ex, excitation; Em, emission; GEFIs, genetically encoded fluorescent indicators.

Section 2：Schematic of the traditional epi-fluorescence fiber photometry system

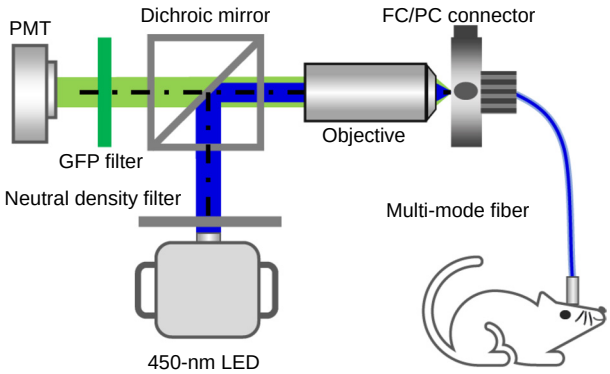

Fig. S2 | Schematic of the traditional epi-fluorescence fiber photometry system[14]. The excitation light from the 450-nm LED is reflected by the dichroic mirror and coupled into the multi-mode fiber by the objective lens. Fluorescence is collected by the same fiber and objective, then detected by the PMT after passing through the dichroic mirror and optical filter. Abbreviations: PMT, Photo Multiplier Tube; LED, Light Emitting Diode.

Section 3: Representative trace of DA signals during the sucrose delivery experiment

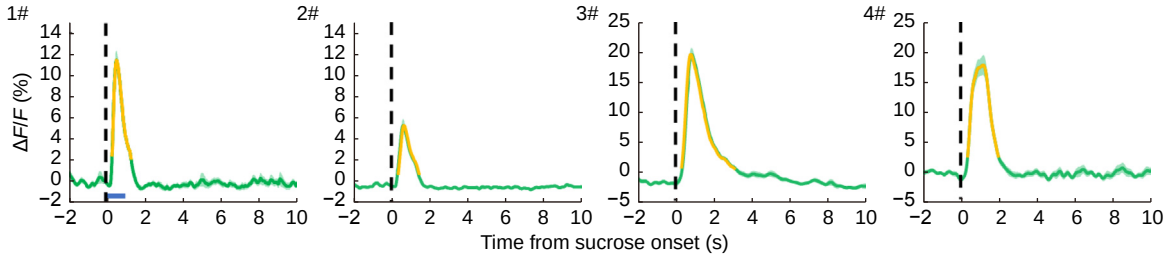

Fig. S3 | Representative trace of DA signals during the sucrose delivery experiment. The onset times of 5% sucrose solution delivery are indicated above the trace with blue bars.

Section 4: Averaged DA signal and neuronal Ca^{2+} signal transients in response to unexpected sucrose solution

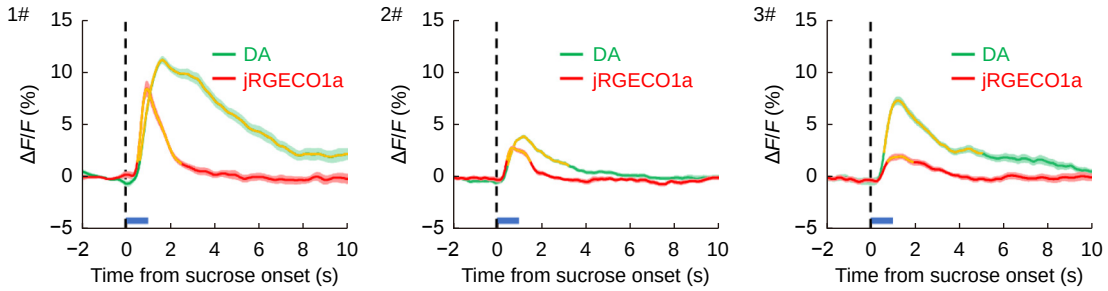

Fig. S4 | Averaged DA signal and neuronal Ca^{2+} signal transients in response to unexpected sucrose solution. The shaded area represents the SEM (±). The blue bar represents the 1 s sucrose solution. Orange segments indicate a statistically significant increase from the baseline (*P<0.05, Wilcoxon's signed-rank test; n = 20 pairs).

Section 5: Heatmap of DA signal and neuronal Ca^{2+} signal transients in response to phasic optogenetic stimulation

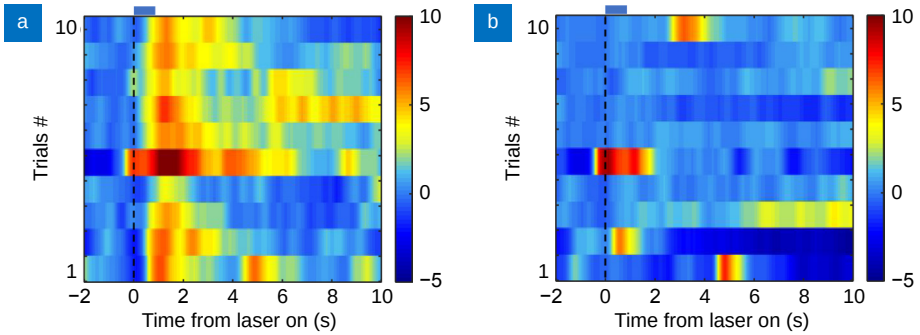

Fig. S5 | Heatmap of DA signal (**a**) and neuronal Ca^{2+} signal (**b**) transients in response to phasic optogenetic stimulation. The blue bar indicates the time of stimulation.