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Novel all-fiber-optic technology for control and multi-color probing of neural circuits in freely-moving animals

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All-fiber-optic photometry system based on a multi-branch fiber bundle has achieved, for the first time, simultaneous optogenetic manipulation and dual-color recording of neuronal Ca^{2+} or neurotransmitter signals in freely moving animals, providing a powerful tool for comprehensive analysis of neural circuit function and the study of neurological diseases.

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The human brain has about 100 billion neurons, connected through trillions of synapses, that form complex neural circuits. These circuits are regulated by various types of neurons and neurotransmitters that exhibit different activity patterns and underlie the sophisticated functions of the brain. Abnormal neuronal activity can lead to (or indicate) behavioral disorders and mental illnesses. Therefore, technology capable of real-time recording and manipulating cell-type-specific neuronal activity in behaving animals with high spatiotemporal resolution would be fundamentally valuable in neuroscience research for decoding neural circuit structure and function as well as for mechanistic studies of brain diseases.

Various techniques, such as electrophysiology[1] and fluorescence imaging[2–4], have been developed for elucidating the intricate functions of neural networks. Similar to electric circuits, one way to probe the neural networks is to assess the responses of a given network after providing inputs. This can be achieved using an electrode or an array of electrodes; however, it is invasive. Optogenetics offers a new avenue of generating an input to a neural network (e.g., by controlling the ion channel's opening or closing using light), which can be highly cell type, spatially, and temporally selective[5]. The combination of optical microscopy, genetically encoded calcium-sensitive fluorescent proteins, and optogenetic stimulation makes it possible to explore dynamic neural activities in live animals with high spatial resolution. Recently, a novel compact optical imaging platform termed fiber photometry[3,4,6] was developed. Distinct from a bench-top microscope, this exciting new platform is simple, stable, and minimally invasive, and, most importantly, it enables exploration of the dynamic interconnections among neurons in freely-moving animals. Fiber photometry, along with optogenetic stimulation, offers an exciting opportunity to unveil the causal relationship between neuron activities and functions and assess neural circuits under near-nature physiological and social conditions.

Advances have been made in optogenetics technology to avoid the spectral overlap between optogenetic

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stimulation light and excitation/emission light from genetically encoded calcium sensing fluorescein proteins (such as GCaMP) and other cell-typing/labelling proteins (such as RFP or YFP)[7,8], thus avoiding or minimizing unwanted artifacts in neural activity signals or in optogenetic stimulation. Both optogenetic stimulation and multi-color cell-type-specific neural activity imaging can be realized in a bench-top microscopy system by using properly chosen optical filters/dichroic mirrors, but not in the currently available fiber photometry systems —which only cover the spectrum of light between 405–600 nm[8]. This precludes the possibility of optogenetic stimulation and multi-color neural activity imaging in freely behaving animals.

An innovative work recently reported by Ling Fu et al in *Opto-Electronic Advances* has overcome the above challenge, successfully unleashing the potential of fiber photometry and optogenetics technology for assessing neural networks[9]. Their elegant approach leverages a multi-branch optical fiber bundle in a photometry system, which is for the first time capable of simultaneous optogenetic stimulation and dual-color recording of neural activities in freely-moving animals. Their novel design entails a four-branch optical fiber bundle (see Fig. 1(a)), which can deliver three different excitation wavelengths (450 nm and 561 nm for excitation of fluorescent indicators, and 660 nm for optogenetic stimulation) through three branches (i – iii) to the brain and collect fluorescence from the brain through the fourth branch (iv). A lock-in amplifier (LIA) is used to enhance the detection sensitivity of the fluorescence signals from the two types of neural sensors simultaneously. The intensities of the two excitation lights are sinusoidally modulated at different frequencies (211 Hz for 450 nm and 531 Hz for 561 nm) while the intensity of the 660 nm light (for optogenetic stimulation) is modulated with a low frequency (0–50 Hz) square wave. The detected fluorescence signals from the PMT are then demodulated and filtered by the LIA. This approach enables simultaneous real-time acquisition of the two fluorescence signals with minimal crosstalk between the two channels, and it also reduces the system cost and eliminates the risk of potential instability compared to an alternative detection scheme involving multiple PMTs and dichroic mirrors. In order to suppress potential artifacts caused by the optogenetic stimulation, a short-pass filter is placed in front of the PMT to remove the backscattered optogenetic stimulation laser light at 660nm (in addition to a narrow line-width bandpass filter for the 660 nm laser source). This innovative design affords an all-fiber-optic transmission for both excitation and emission light, making the photometry system simpler, more flexible, and more robust, critical features for enabling neural imaging in freely-moving animals. Notably, this all-fiber-

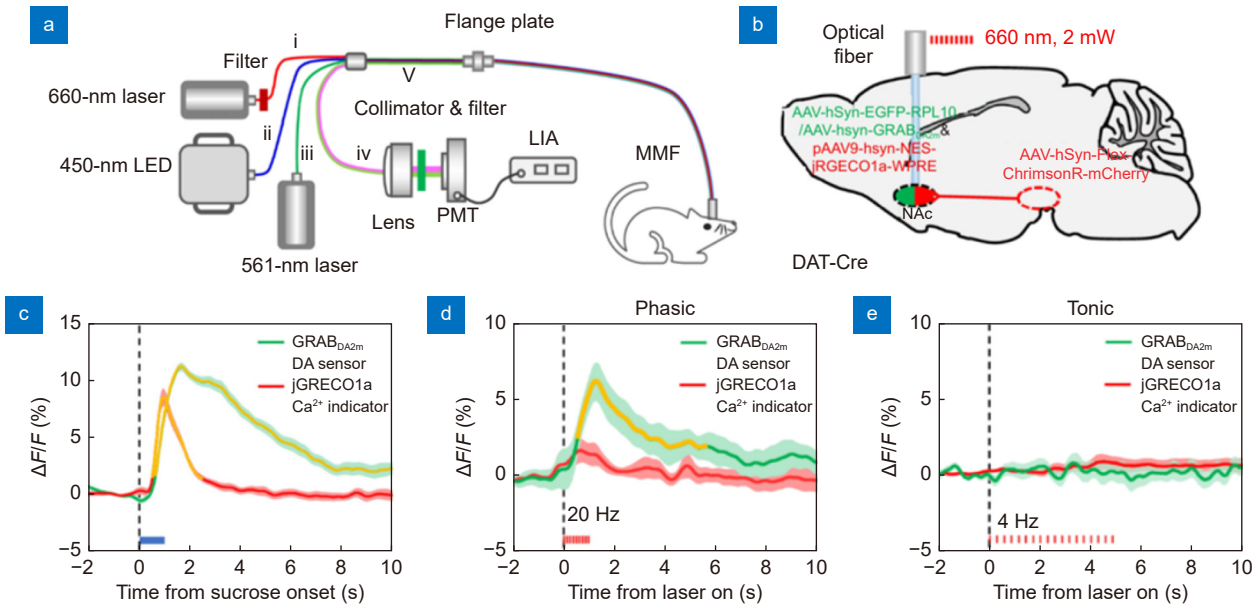

Fig. 1 | All-fiber-transmission photometry system based on a multi-branch fiber bundle[9]. (**a**) Schematic diagram of the system; (**b**) Schematic diagram of animal surgery and virus injection; (**c**) Averaged DA signal and neuronal Ca^{2+} signal transients in response to unexpected sucrose solution; (**d**) Averaged DA signal and neuronal Ca^{2+} signal transients in response to phasic and tonic optogenetic stimulation. (Credit: this figure was provided by the authors of Ref.9.)

optic photometry system can be conveniently modified for accommodating different fluorescent probes and optogenetic sensors by choosing appropriate light sources and optical filters while keeping the optical path unchanged.

As shown in Fig. 1(b), neural imaging in freely-moving mice with the all-fiber-optic photometry system has confirmed its excellent capability for simultaneous dual-color imaging of neuronal calcium activity and neurotransmitter dopamine (DA) dynamics, and optogenetically activating the terminals of the dopaminergic neurons in the nucleus accumbens core (NAc). DIO-ChrimsonR-mCherry virus was used to label the ventral tegmental area (VTA) DA neurons in a DAT-Cre mouse and some of these neurons project to the NAc (termed VTA-NAc DA neurons). These projected dopaminergic neurons can be optogenetically stimulated by light at 660 nm. The genetically encoded DA sensor $GRAB_{DA2m}$ (emitting green fluorescence) and the Ca^{2+} indicator jGRECO1a (emitting red fluorescence) were co-expressed in the NAc neurons. For imaging and stimulation, the fiber probe was placed over the NAc region. As a control test, they measured the DA fluorescence signals of the DA sensors and the neuronal Ca^{2+} indicators upon the delivery of unexpected sucrose solutions (for the mouse to lick) and observed a significant increase in the fluorescence signals, suggesting a positive correlation between NAc neurons and VTA-NAc DA neurons with the reward (Fig. 1(c)). The authors also noticed a clear difference in the time course of the DA sensors and calcium indicators. More specifically, the DA signals correlated with, but lagged behind, the neuronal Ca^{2+} signals. In addition, the DA signals exhibited an overall prolonged activation.

It is well-known that the firing patterns of VTA DA neurons can be either phasic (transient) or tonic (sustained), resulting in different downstream neuronal activities and physiological functions[10,11]. The above all-fiber-optic photometry system and the mouse model offer a perfect opportunity to explore neuronal responses to different types of stimulation. As a proof-of-concept experiment, the authors delivered two different patterns of optogenetic stimulation to induce tonic or phasic firing patterns at the VTA-NAc dopaminergic terminals. The results demonstrated that the DA signal displayed a significant, time-locked upward trend when a phasic stimulation was applied, whereas the neuronal Ca^{2+} signal only exhibited a slight upward trend (Fig. 1(d)). There was no significant change in the DA signal or neuronal Ca^{2+} signal when using a tonic stimulation. These findings suggest that dopamine transmitters in the NAc are predominantly released rapidly by the DA neurons through phasic stimulation, and the Ca^{2+} signals from population activity are not sensitive to dopamine released by VTA neurons in the NAc.

This proof-of-concept study using the fill-fiber-optic photometry sensing/stimulation system represents a significant advance in the field of neuroscience. It is the first one to achieve precise optogenetic stimulation of a specific type of neuron in freely-moving mice while simultaneously monitoring neuronal calcium activities and neurotransmitter dynamics in real time, without channel crosstalk and potential artifacts from optogenetic activation. The reported technology can be an effective tool for mechanistic studies of neural circuits. Moreover, this versatile technology also enables a closed-loop control for guiding optogenetic stimulation while monitoring the resulted dynamic neuronal activity in real time, which can advance our understanding of the pathogenesis and potential treatment of brain diseases.

Several technical improvements may further enhance the technology's efficacy. First, design improvements and fabrication of smaller, more flexible, and different types of fiber-optic probes[12] may enable researchers to target specific brain regions with higher resolution, higher sensitivity, a deeper imaging range, and a larger imaging volume. Second, new optical methods can be developed to improve resolution, particularly along depth, which can minimize unwanted signal contamination from neurons below or above the target ones. Third, new fluorescent biosensors and optogenetic tools[13–15] will expand the versatility and applicability of the all-fiber-optic photometry technique, which will facilitate investigations of more complex neuronal dynamics, and interactions between different types of neurons, neurotransmitters, and other molecules in the nervous system. Fourth, the fiber-optic photometry probe is passive and can readily be integrated with other imaging modalities. Researchers have already used fiber photometry techniques in fMRI experiments[16,17] to investigate the contribution of neurons to the BOLD signal. Fifth, advances in data analytics (e.g., using machine or deep learning algorithms) may help researchers better understand the data generated by fiber photometry recordings, potentially enabling systematic analysis of large amounts of time-course imaging data and providing unprecedented

insights about dynamic neural circuits. Finally, the development of new tools such as wireless fiber-optic systems[18] may help minimize the limitations and impacts on animal behavior and associated neuron activity recordings. In the future, when combined with implantable devices, wearable sensors, and other new technologies, fiber-optic photometry techniques may be applicable to human neuroscience research, offering unique opportunities to better understand the neural basis of human behavior and cognition and develop new methods of therapy for neurological diseases.

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