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Review

# A selected review of recent advances in the study of neuronal circuits using fiber photometry

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ABSTRACT

To understand the correlation between animal behaviors and the underlying neuronal circuits, it is important to monitor and record neurotransmission in the brain of freely moving animals. With the development of fiber photometry, based on genetically encoded biosensors, and novel electrochemical biosensors, it is possible to measure some key neuronal transmission events specific to cell types or neurotransmitters of interest with high temporospatial resolution. This review discusses the recent advances and achievements of these two techniques in the study of neurotransmission in animal models and how they can be used to complement other techniques in the neuroscientist's toolbox.

## 1. Introduction

The visualization of neuronal interaction is an essential step in understanding overall brain organization. Both fiber photometry and electrochemical biosensors are used to examine neuron populations in regard to neurotransmission and can be used to associate said activity with behavior. In this review, we will discuss how fiber photometry has proven to be a valuable technique in studying the activity of neuronal subpopulations and how it has inspired derivations from the original technique in an effort to overcome some of its limitations. Additionally, we will examine a more established tool, electrochemical biosensors, and their contribution to the field. We will also illustrate how these technologies can either be used alone or in combination with other previously established methods to enhance our understanding of neuronal circuits. Finally, we will highlight recent advances achieved with both techniques, by contrasting both methods based on their applicability and efficiency.

## 2. Fiber photometry

Fiber photometry is a technique designed to measure the afferent activity of neurons projecting to specific downstream targets in vivo, a variable that was previously difficult to study (Gunaydin et al., 2014). By doing so, researchers now have the ability to investigate behaviorally-evoked patterns of neurotransmission. Having the ability to associate neurotransmission with behavior is key to understanding how the brain works (Martianova et al., 2019).

### 2.1. The mechanics of fiber photometry

Fiber photometry measures the light emitted from fluorescent molecules in the brain via time-correlated single-photon counting (TCSPC)-based fiber optics. This can be done using either a dual- or single-fiber optic design, in which a small fiber optic probe is surgically implanted directly above the brain region of interest (Fig. 1.A). In both designs, excitation pulses are delivered down the fiber probe to excite the fluorophore of interest, in which the fluorescence emissions are then collected, and the photons are delivered out of the probe, either by the same fiber (single-fiber optic) or an additional fiber (dual-fiber optic) (Gunaydin et al., 2014; Martianova et al., 2019). The collected emission signal is then spectrally separated by a dichroic mirror, passed through a band filter, and then focused onto a photodetector, as shown in Fig. 1.A (Gunaydin et al., 2014). Although the single fiber optic design does make a simpler system for recording in freely moving animals, it does not completely replace the dual-fiber optic design. By having an individual optical fiber assigned specifically for collecting fluorescence

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emission, the dual-fiber optic design offers greater sensitivity by providing enhanced detection of emission signals in deep brain tissue and being less susceptible noise due to wire movement.

The advantages of using fiber photometry vary depending on the approach. In general, the implanted fiber optic probe is small and flexible, resulting in minimal surrounding tissue damage and allows recording from multiple brain regions simultaneously. Due to its ability to measure single photons, TCSPC-based fiber optics is an ultrasensitive tool for detecting emitted light; enabling the detection of fluorescence with low intensity excitation light, thus reducing the chances of photobleaching, and extends recording time (Girven and Sparta, 2017).

While there are considerable advantages of the fiber photometry technique, it is also limited by many factors as well. The main limitation being the inability to record the activity of individual cells, as the probe detects fluorescence from the surrounding neuronal population that is expressing the biosensor. This issue has been addressed with the use of transgenic animal lines, as this enables the targeting of particular neuronal subtypes. Although this provides a bit more specificity, the entire neuronal subpopulation is still being monitored, thus providing very little information regarding individual projections. Fiber photometry is also limited by the inability to resolve cellular structures, as the emitted photons are "scrambled" and collected by the fiber as "bulk" fluorescence signals, thereby preventing spatial information being obtained (Siciliano and Tye, 2019). Finally, when recording from a functionally diverse set of neurons, it may be difficult to measure the fluorescence if the emission is at too great of a distance from the probe, making the distal proximity between the neurons of interest and the implanted probe an important consideration (Girven and Sparta, 2017).

### 2.2. Genetically encoded biosensors

In order to obtain activity-dependent fluorescence, biosensors have been designed to correspond with various molecular signatures of activated neurons. Genetically encoded biosensors are a class of biomolecular signaling agents that are incorporated into a cell's genome and are then produced by its molecular machinery. These protein structures can be designed to change conformation in the presence of a chemical substance, or change in cellular environment, allowing a fluorescent signal to be produced. To study neurotransmission, several genetically encoded biosensors have been developed to detect the presence of various molecular signatures corresponding to neural activation, as shown in Fig. 1.B.

(1) *Membrane depolarization*. Once an action potential (AP) is achieved, the AP will propagate down the axon, and depolarize the membrane corresponding to the presynaptic bouton (from −65 mV to +40 mV or greater) (Wang et al., 2019a). This membrane depolarization serves as a molecular signature of neuronal activation and can be detected via genetically-encoded voltage indicators (GEVIs) (Knöpfel and Song, 2019).

(2) *Voltage-gated calcium* ($Ca^{2+}$) *channel activation and influx*. When membrane depolarization occurs in the presynaptic active zone, voltage-gated $Ca^{2+}$ channels open resulting in a rapid $Ca^{2+}$ influx. This elevated cytoplasmic $Ca^{2+}$ concentration (from ~100- ~ 400 nM) can last about 50–150 milliseconds and can be detected via genetically encoded $Ca^{2+}$ indicators (GECIs) (Wang et al., 2019a; Helmchen et al., 1997).

(3) *Vesicular fusion/exocytosis*. Elevated cytoplasmic $Ca^{2+}$ levels trigger the exocytosis of the readily-releasable pool of synaptic vesicles which are loaded with neurotransmitters (Südhof, 2013; Wang et al., 2019b). During exocytosis, when the synaptic vesicle fuses with the plasma membrane, the extracellular medium (pH ~ 7.4) neutralizes the acidic pH of the vesicle lumen (pH ~ 5.5), and this neutralization can be detected using pH indicators, such as pHluorin (Miesenböck et al., 1998).

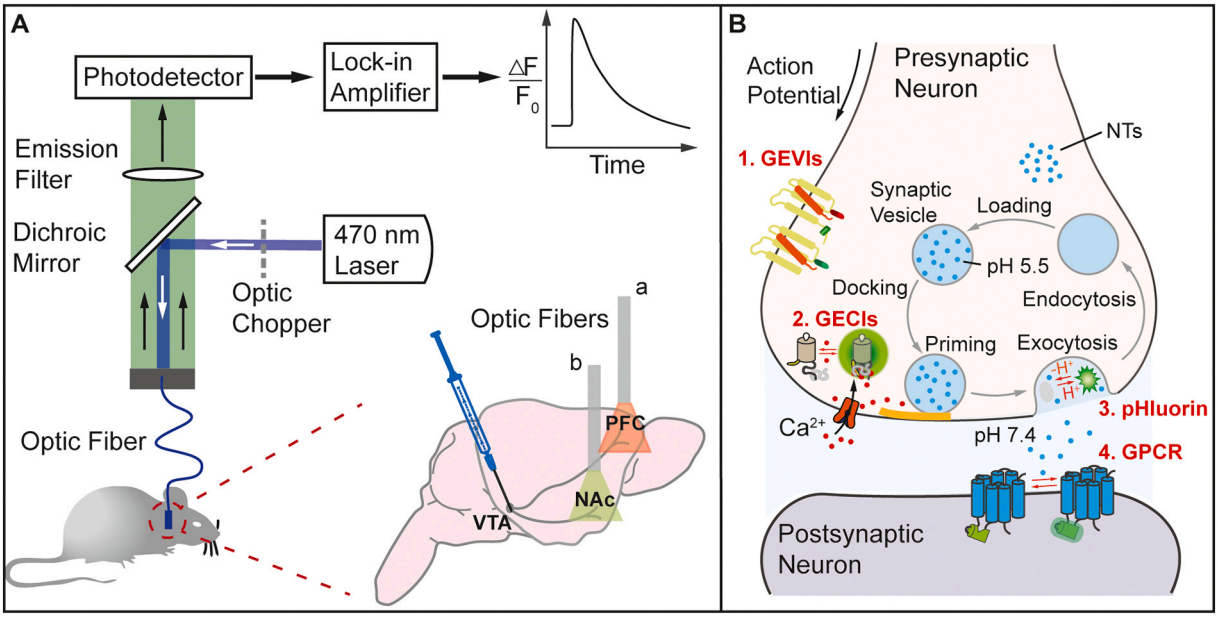


**Fig. 1.** Fiber photometry and genetically-encoded biosensors. A. Schematic representation of the fiber photometry setup and signal processing (Gunaydin et al., 2014). In this model, genetically-encoded biosensors are virally injected into the ventral tegmental area (VTA) and their signal is recorded from the Nucleus Accumbens (NAc) and prefrontal cortex (PFC). B. Schematic representation of four different genetically-encoded biosensors and their corresponding molecular signatures that underlie neurotransmission. Following action potential (AP) propagation, voltage gated calcium ($Ca^{2+}$) channels are triggered to open, causing a rapid $Ca^{2+}$ influx. This induces the exocytosis of synaptic vesicles, which releases neurotransmitters (NTs) into the synaptic cleft in which they can then bind to their corresponding receptors and activate the postsynaptic neuron (Wang et al., 2019a). These molecular signatures can be detected via genetically-encoded biosensors, such as genetically-encoded voltage indicators (GEVIs) (Knöpfel and Song, 2019), genetically-encoded $Ca^{2+}$ indicators (GECIs) (Helmchen et al., 1997), genetically-encoded pH indicators (e.g., pHluorin) (Miesenböck et al., 1998) and genetically-encoded NT indicators (G protein-coupled receptor, GCPR) (Jones-Tabah et al., 2020). Schematics are not drawn to scale.

(4) *Neurotransmission.* Finally, once the neurotransmitters are released into the synapse, their functions vary depending on the transmitter type and can be detected via neurotransmitter (NT) indicators, such as iAChSnFR (intensity-based acetylcholine-sensing fluorescent reporter) and dLight. The genetically encoded acetylcholine (ACh) sensor iAChSnFR, was recently invented for detecting ACh transients in different biological samples (Borden et al., 2020). It has been designed to fluoresce upon choline-binding and has been used to measure hippocampal ACh transmission in response to reward activities in mice in vivo. dLight is a receptor-based genetically encoded dopamine (DA) indicator. It is constructed by replacing the third intracellular loop of the DA receptor (DAR) with a circularly permuted green fluorescent protein (cpGFP) (Patriarchi et al., 2018). dLight generates different fluorescent intensities corresponding to the amount of DA binding to DARs.

Although there are many different biosensors that are used to detect the various molecular signatures in neuronal activation, the most commonly used biosensors in fiber photometry are GECIs. Following $Ca^{2+}$ influx, GECIs fluoresce upon $Ca^{2+}$ binding, and the change in fluorescence corresponds proportionally to the changes in $Ca^{2+}$ concentration (Wang et al., 2019a). The most commonly used GECI for fiber photometry experiments (and in vivo imaging in general) is currently GCaMP6. Chen et al. first reported GCaMP6 in 2013 as an indicator that is very sensitive to changes in $Ca^{2+}$ concentration and its fast dynamics allow for the detection of individual APs (Chen et al., 2013). When created, GCaMP6 unveiled neuronal dynamics that were previously unachievable with protein sensors. To consider its structure, GCaMP contains both calmodulin and M13 domains that are located within a cpGFP. In the presence of high calcium concentration, GCaMP undergoes a conformational change, which induces a further change in the fluorescent proteins conformation around the chromophore thus enhancing fluorescence emission intensity (Wang et al., 2019a; Chen et al., 2013). By recording $Ca^{2+}$ signals in activated neurons via fiber photometry, researchers are able to make inferences about neurotransmission in response to behavior, such as been done for glutamate (Glu) (Montardy et al., 2019; Li et al., 2020). Aside from fiber photometry, GECIs are often utilized and coupled with other techniques (Girven and Sparta, 2017).

Genetically encoded biosensors have also been developed to measure fluorescence resonance energy transfer (FRET) between two intracellular proteins, allowing them to investigate second-messengers (e.g., activity levels), protein kinases, GTPases, post-translational modifications and protein-protein interactions (Jones-Tabah et al., 2020). In 2020 Jones-Tabah et al. used ratiometric fiber photometry in combination with FRET-based biosensors to examine GPCR (G protein-coupled receptor) signaling by measuring the activity levels of the enzymes PKA (protein kinase A) and ERK1/2 (extracellular signal-regulated kinase 1/2) (Jones-Tabah et al., 2020). These experiments showed how acute D1-like DA receptor (D1R)-induced activation of PKA and ERK1/2 is suppressed by repeated Levodopa (L-DOPA) treatment, thus D1R-driven dyskinesia increases although D1R signaling appears to be desensitized by chronic L-DOPA treatment. In addition to FP, similar experiments using FRET-based biosensors can be used in conjunction with other optical and imaging techniques (Jones-Tabah et al., 2020).

### 2.3. Applications of FP and brain mapping

Brain mapping is defined as the process of studying the anatomy and corresponding function of the brain. Due to the sensitivity of the brain tissue to mechanical force and the physical properties of fiber photometry, this method is sometimes limited to the outer surface of the brain (<2 mm depth). This allows recording with the highest resolution and the lowest possible amount of tissue damage or compression (Ohayon et al., 2018). Nonetheless, modifications have been made to allow for larger or deeper brain areas to be examined (Sych et al., 2019; Qin et al., 2019), while maintaining a good resolution (Pisano et al., 2019; Meng et al., 2018). The application of fiber photometry in brain mapping differs depending on the device used. While flat-cleaved optical fibers (FFs) are widely employed to assess neurotransmission, they lead to more substantial brain tissue damage, possess a limited collection depth due to light scattering and cannot examine multiple regions simultaneously. Less invasive probes utilize tapered optical fibers (TFs), which cause comparably less tissue damage, and possess a larger interface surface, allowing for a larger and deeper volume of collection on average. DA in concentrated in both the ventral and the dorsal striatum and can be measured during fiber photometry using dLight1.1 - a DA biosensor. Using TFs to detect DA transients, in free behaving operant conditioned mice, showed an association between movement and both striatal regions, but only the ventral striatum generated dopaminergic (DAergic) transients during rewards (Pisano et al., 2019).

In comparison to the previously described method, i.e., single-color photometry, Meng et al. developed a multi-color photometry method based on spectrometry to assess different fluorescent signals from deep brain tissue in 2018 (Meng et al., 2018). Meng et al. was able to use this method to measure the differences in neurotransmission between the direct- and indirect pathways within the spiny projection neurons (SPNs) of the basal ganglia in vivo. They quantified the pathways corresponding neurotransmission by using two different GECIs that emit fluorophores at two different wavelengths, GCaMP6f (green) and jRGECO1a (red). While observing the different magnitudes of activation between the direct- and indirect pathways, the neurotransmission within each hemisphere was synchronized, but desynchronized when comparing the two hemispheres. Strong direct-pathway activation led to a completed contraversive turn and forward movement, while strong indirect pathway activation led to an abortion of the turn which either stopped the movement or reversed the direction (ipsiversive direction) (Meng et al., 2018).

One limitation to single fiber photometry, and several other techniques, is the difficulty in studying larger brain circuits, especially beyond the surface of the brain. High-density multi-fiber photometry allows for the examination of multiple brain regions simultaneously in vivo (free behaving and head-fixed). In 2019, Sych et al. was able to examine 12 independent brain regions simultaneously using GCaMP6 to observe $Ca^{2+}$ dynamics (Sych et al., 2019). This method also carries the potential of combining different modifications, such as tapered (Pisano et al., 2019) or thinner optical fibers (Pisano et al., 2019).

Although recording neurotransmission within axon terminals is challenging due to size limitations, it is also important for understanding the conductance of signals within the brain. Previous techniques were mostly restricted to the brain surface in head-fixed animals or were too invasive. While fiber photometry offers low spatial resolution, in 2019 Qin et al. was able to produce a multichannel fiber photometry device that allows the recording of multiple fibers simultaneously, each within different brain regions (Qin et al., 2019). Although a reduction in fiber diameter lowered the recording range, this allowed the animals to be freely moving, whilst still maintaining the necessary sensitivity to detect $Ca^{2+}$ signals (Qin et al., 2019).

This reduction in fiber diameter has allowed researchers to adapt optical fibers to work in conjunction with other techniques. In 2018, Ohayon et al. designed a microendoscope using an ultra-thin multimode optical fiber (MMF) probe, and it operates with a high resolution that allows for the recording of neural dynamics (Ohayon et al., 2018). Compared to *endo*-microscopes with a gradient refractive index lens (GRIN), the MMF probe within microendoscopes can be inserted deeper into the tissue without risking tissue displacement or damage. The high resolution over a small area of neurons would allow for this technique to be used while examining specific pathways within a heterogeneous neuron population. The limitation of this device is the loss of resolution in freely moving animals, therefore restricting it to observations in head-fixed animals (Ohayon et al., 2018).

### 2.4. Frame-projected independent fiber photometry

In 2016 Kim et al. developed frame-projected independent-fiber photometry (FIP), in which cell population activity is projected onto a single sCMOS sensor, instead of the previously used photodetectors (Kim et al., 2016). An FIP microscope provides the opportunity to record GCaMP6f $Ca^{2+}$ signals from multiple fibers simultaneously. FIP utilizes a seven-fiber patch cord, in which the seven fibers are split into seven branches on one end, where a fiber optic interface can be implanted into seven different, widely dispersed brain regions of an adult mouse at a given time. Those same seven wires are then tightly bundled at the other end of the patchcord, where the sCMOS camera is imaging and measuring the fluorescence emission from all seven fibers simultaneously (Kim et al., 2016). FIP also allows simultaneous dual-color imaging and/or optogenetic stimulation through a single fiber. Although FIP enables recording from multiple fibers simultaneously through its projection onto a single sCMOS camera, it is still more effective to use multiple photosensors (the original version of the method). Multiple photosensors will obtain a more accurate measurement of the emission source as it detects photons from a broader range of the spectrum (Girven and Sparta, 2017).

### 2.5. FP & Optical Techniques

Complementing the behaviorally-evoked activity patterns measured via fiber photometry, Gunaydin et al. simultaneously applied optogenetic tools in order to define the fundamental roles of the same neuronal projections (Gunaydin et al., 2014). Optogenetics is a method which allows you to specifically activate or inhibit neurotransmission in vivo. Researchers often use optogenetics to investigate the effects of defined neuronal subpopulations on specific behaviors with great temporal precision (Wang et al., 2019a). However, optogenetic tools alone are not able to mimic or block the precise pattern of firing that is present during the naturally occurring behavior, this may cause an unrealistic effect. Therefore, optogenetic manipulations should be supported by evidence showing that the circuit is in fact naturally involved in the behavior, and this can be done using fiber photometry.

In the work of Gunaydin et al. (2014), the researchers designed a single multimode optical fiber that is sensitive enough to record from deep brain structures and detect signals so small that they correspond to changes in neurotransmission occurring in both axons and cell bodies during behavior. This single fiber has the ability to both deliver excitation and detect the activity-dependent fluorescence emitted, allowing for optogenetics and fiber photometry to be applied simultaneously. By using these techniques together, Gunaydin et al. found the DAergic projection from the Ventral Tegmental Area (VTA) to the Nucleus Accumbens (NAc) to potentially modulate social interaction (Gunaydin et al., 2014). The application of optogenetics enhanced the fiber photometric findings by not only showing how increased phasic activity in VTA-DA somata increased social behavior, but how it is the specific projection from the VTA to the NAc that predicts/mediates this effect. Using optogenetics in combination with fiber photometry enables researchers to investigate the functional significance of specific cells, their projections and their targets, rather than individual neurotransmitters (Gunaydin et al., 2014).

### 2.6. FP with fMRI

In general, Blood Oxygen Level Detection (BOLD) fMRI (functional magnetic resonance imaging) is thought to be spatiotemporally linked to neurotransmission through the mechanism of vascular coupling. To get a closer look at the contributions of smaller populations of neurons to the BOLD signal electrophysiology techniques have been incorporated into fMRI studies. While these studies give rise to more specific information on neuronal circuitry, they are limited by the magnetic field required for fMRI which can generate artifacts in electrophysiological signals.

An alternative method to look at contributions of specific populations of neurons to the BOLD signal is to monitor neurotransmission through $Ca^{2+}$ spikes. Fiber optic (Schulz et al., 2012) and optogenetic (Schmid et al., 2016) studies have proven a link between the changes in $Ca^{2+}$ and BOLD signals in response to stimuli in vivo.

The recent advances in this field have led to the use of fiber photometry in conjunction with fMRI. This combination of techniques allows for a more spatially detailed experiment that can focus on a single cell type due to the nature of fiber photometry experiments. The first reported example of fiber photometry with fMRI was published in 2017 (Liang et al., 2017). Here, Liang et al. simultaneously monitored a GCaMP6 signal while conducting BOLD fMRI experiments in rats. Their study in the superior colliculus using visual stimuli found that $Ca^{2+}$ spikes reliably occurred just before the BOLD signal response, a difference that may be attributable to hemodynamic delay. While the study did lack a control in the form of a genetically encoded GFP in place of GCaMP6 expression, the technique proves to be inexpensive, repeatable within a subject, reproducible between subjects, and specific to the cell type of interest (Liang et al., 2017).

Subsequent studies have reinforced the efficacy of this combination of techniques. Providing more evidence of the link between $Ca^{2+}$ spikes and BOLD signals in different brain areas, with different cell types, animal models, both with and without stimulus and cognitive state (Schlegel et al., 2018; Wang et al., 2018; Tong et al., 2019; Ma et al., 2020). In fact, changes in astrocytic $Ca^{2+}$ levels have been linked to both positive and negative BOLD signals, giving insight into the controversial role of astrocytes in brain function, which may also be relevant to thalamic regulation. These findings may be relevant to brain disorder and injury models relevant to gliovascular disruption (Wang et al., 2018). These signals can now be monitored in multiple brain regions simultaneously by incorporating camera-based detection, instead of the more standard photodetector, and this method has been used to demonstrate a difference between resting and evoked state signals in rats (Tong et al., 2019).

The recent literature including fiber photometry techniques in fMRI experiments demonstrates a novel methodology that brings cellular resolution to fMRI scans. This combination of cell-type specific and global neuroscience research techniques has the potential to elucidate the contributions of individual neuronal subpopulations to larger brain circuits. Furthermore, the potential to monitor multiple subpopulations independently and simultaneously while also examining the brain as a whole.

### 2.7. FP with electrophysiology

Currently, electrophysiology is the gold-standard analytical technique to investigate the correlation between behavior and in vivo neuronal dynamics in freely behaving animals. Electrophysiology allows simultaneous recording from hundreds or thousands of cells, ion channels and neuronal activities (Viventi et al., 2011). It measures electric signals (i.e., current or potential) derived from ion flux with high temporal resolution on the order of millisecond. The electrode could land on a single cell and record intracellular signal changes, or land in the extracellular space to monitor electrophysiological signals across brain regions. Intracellular recordings are commonly applied on cultured cells or slices. When performing electrophysiological measurements, it is much more difficult to perform in vivo intracellular recordings than extracellular recordings in a complex brain environment (Robinson et al., 2008). Although cell identification is difficult to achieve, electrophysiological recordings allow identification through several combinatorial approaches like antidromic stimulation as shown in 1960s (Dreifuss and Kelly, 1972; Mantz et al., 1989), Juxtacellular labeling (Pinault, 1996; Schreihofer and Guyenet, 1997) and phototagging (Cohen et al., 2012; Lima et al., 2009; Nieh et al., 2015; Senn et al., 2014). Multi-electrode arrays allow for the monitoring of entire

neuronal circuits have also been developed (Deadwyler and Hampson, 2006). Similar to fiber photometry, microarrays record signals from all cells within proximity to the electrode tip. However, while fiber photometry requires the expression of fluorescent molecules through genetically-encoded indicators, electrophysiology indiscriminately records all electrical signals, thus not requiring any indicator, both providing simplicity to the method while removing the cell-type specificity provided through cell-targeted indicator expression (Patel et al., 2020). Due to the lack of temporal resolution in fiber photometry and the lack of cell-type specificity with in vivo electrophysiology, Sakata's team constructed a hybrid implant of an optic fiber cannula and a micropipette electrode that simultaneously monitors $Ca^{2+}$ transients and mesopontine field potentials in freely behaving mice (Patel et al., 2020). This novel technique enables the exploration of various brain regions and behavioral states.

Electrophysiology can also be used to complement other analytical methods, such as electrochemistry and imaging, to study neurotransmission and validate neuronal features (Borden et al., 2020; Bucher and Wightman, 2015; Feng et al., 2019). The efficiency of genetic knockdown in supramammillary nucleus (SuM)-dentate gyrus (DG) terminals was firstly validated by electrophysiology together with immunohistology in the work of Li et al. (2020). Using these methods in conjunction allowed the investigation of the role of SuM-DG circuits in regulating spatial memory retrieval via Glu release. Further, in vivo fiber photometry was performed by recording $Ca^{2+}$ dynamics in these genetic knockdown animal models (Feng et al., 2019). In the work of Feng et al. and Borden et al., they both performed whole-cell patch-clamp electrophysiology simultaneously with two-photon fluorescent microscopy to validate the expression of genetically-encoded norepinephrine (NE) and ACh biosensors in cultured cells, with brain slices and awake animals. Therefore, like fiber photometry, electrophysiology can be used by itself or in conjunction with other techniques to monitor neurotransmission both in vivo and ex vivo (Borden et al., 2020; Feng et al., 2019).

## 3. Electrochemical biosensors

Electrochemical techniques have significantly contributed to the neurochemistry field on a fundamental level. However, to encompass all components of neural circuitry, different analytical tools can be used in combination to enhance our understanding of neurochemistry.

### 3.1. The mechanics of fast-scan cyclic voltammetry and amperometry

To study the impact of NT release on behavior, disease states and disorders, electrochemical methods are often used to monitor NT fluctuations derived from neurotransmission. Fast-scan cyclic voltammetry (FSCV) and amperometry are the two most common approaches among all electrochemical methods (Cahill et al., 1996; Bruns, 2004; Park et al., 2009) (Fig. 2.A). Both methods can be used to detect electroactive NTs, such as DA, serotonin or NE, and does so by measuring the redox current at the electrode surface. The redox current is obtained by applying a sufficient potential to the carbon-fiber microelectrode, in which electroactive NTs will be easily oxidized or reduced upon interacting with the fibers surface (Keighron et al., 2020). FSCV measures the current generated by altering the potential between low and high in cycles at the electrode surface over a period of time. FSCV can provide chemical information that enables analyte identification by plotting the applied potential by the current output. This is useful when studying complex microenvironments, such as live brains, in which there are many interfering electroactive compounds (Bucher and Wightman, 2015; Cahill et al., 1996). Alternatively, amperometry records the redox current of analytes generated by applying a constant potential at the electrode surface, providing a measurement of concentration, but not chemical identity.

### 3.2. Enzyme based amperometric biosensors

While amperometry and FSCV easily measure electroactive NTs, non-electroactive NTs such as Glu and ACh are of high interest to the study of behavior, cognition and neurological diseases and disorders (Nadim and Bucher, 2014; Avery and Krichmar, 2017; Meldrum, 2000; Hasselmo, 2006). As previously discussed, fiber photometry can be used to monitor the release of nonelectroactive NTs by measuring the fluorescence change of genetically-encoded reporter molecules that correspond to transmission of these NTs (Montardy et al., 2019; Li et al., 2020). Electrochemical biosensors take a different approach to study neurotransmission, in which NT concentrations are measured directly.

As shown in Fig. 2.B, by immobilizing enzymes onto an electrode surface, non-electroactive NT release can be electrochemically monitored. These immobilized enzymes selectively catalyze non-electroactive NTs and produce a reporting molecule (e.g., hydrogen

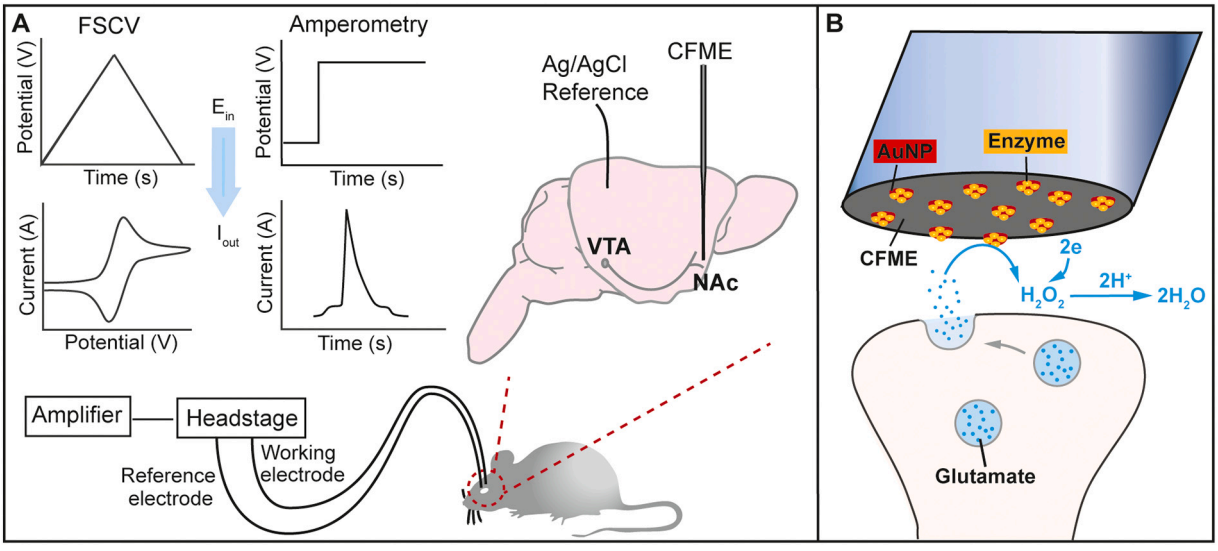


**Fig. 2.** Electrochemical Methods. A. Schematic diagram of the electrochemical approach for detecting neurotransmission in the mouse brain. Typically, a carbon fiber microelectrode (CFME) is implanted into a target brain region, the applied potential through the CFME drives redox reactions of the released neurotransmitters that can be detected as current over a period of time. B. The schematic of enzyme-based CFME with gold nanoparticle (AuNP) surface modification for detecting non-electroactive neurotransmitters (e.g., glutamate), which are released through exocytotic events. Schematics are not drawn to scale.

peroxide) that is electroactive and can be measured electrochemically. However, these analytical methods suffered from a low temporal resolution as they are recorded on a second or subsecond timescale (Vasylieva et al., 2013; Chatard et al., 2018). Recently, a novel ultrafast electrochemical biosensor with a thin layer or monolayer of enzyme coating was invented by Cans group. This advancement was made by carefully studying the interaction of enzymes with nanoparticle colloids in bulk solutions (Wang et al., 2020). The limited thickness of the enzyme coating at the sensor's surface (1) decreases diffusion rates of NTs to the enzyme active site and (2) shortens the diffusion distance of reporting molecules to the electrode surface. This new sensor design allows for the detection of vesicular ACh release events on the millisecond timescale (Keighron et al., 2015) as well as real-time monitoring on the submillisecond timescale and characterization the Glu exocytotic events in rodent brain slices (Wang et al., 2019b). Together with a newly established methodology using liposome models filled with known concentrations of NTs, the number of Glu molecules encapsulated inside of single synaptic vesicles and the fraction of Glu released during exocytotic events could be quantified (Wang et al., 2019c). This novel enzymatic biosensor allows researchers to monitor and characterize the kinetics of Glu exocytotic events in rodent brain slices (Wang et al., 2019b). Together with a newly established methodology, the number of Glu molecules encapsulated inside of single synaptic vesicles could be quantified (Wang et al., 2019c).

## 4. Comparison of fiber photometry and electrochemistry

As two powerful analytical techniques in neuroscience, the advantages, limitations and optimal conditions for fiber photometry and electrochemistry are summarized in Table 1 with detailed explanations:

### 4.1. Spatial resolution

Fiber photometry was designed to measure a cumulative response from total neuron populations instead of single cells or synaptic vesicles and does not provide high spatial resolution due to the scrambling of photons collected by the fiber (Siciliano and Tye, 2019; Patel et al., 2020). The most common size of an optical fiber used in neuroscience with core and cladding is usually on the order of 100 μm in diameter (Aravanis et al., 2007; Wan et al., 2020). These optical fibers are typically chronically-implanted and have a typical size range of 300–400 μm in diameter (Siciliano and Tye, 2019; Ung and Arenkiel, 2012), thus posing the chance of causing significant tissue damage (Patel et al., 2020). Indeed, optical fibers can be made smaller with a tapered tip by using the heat-and-pull method with a puller. In the work of Pisanello and his colleagues, they were able to taper the tip of optical fiber on the order of 10 μm (Pisanello et al., 2014) and even down to a submicrometer diameter (Pisano et al., 2019; Pisanello et al., 2014; Pisanello et al., 2017), which might be the smallest sized tapered optical fiber used for in vivo brain measurements. In comparison to the optical fibers used in fiber photometry, the electrodes typically used for electrochemical measurements are much smaller and can be limited to order of 10 μm and down to 10 nm in diameter (Cahill et al., 1996; Fathali et al., 2017; Li et al., 2015) contributing to a high spatial resolution. Therefore, electrochemical approaches enable the targeting of single exocytotic events at individual secretory cells while causing negligible tissue or cellular damage (Cahill et al., 1996; Robinson et al., 2003; Cover et al., 2019).

### 4.2. Temporal resolution and quantification

Fiber photometry is a cutting-edge technique that allows for the real-time detection of neurotransmission in freely moving animals (Montardy et al., 2019; Feng et al., 2019; Sun et al., 2018) with a temporal resolution at subsecond to second timescale (Patel et al., 2020; Feng et al., 2019). It is slower than ultrafast single neuronal events like exocytosis, which is important to neuronal communication and occurs at millisecond to submillisecond timescale. Although the sample rate of FSCV measurements can reach millisecond order (Roberts and Sombers, 2018), it is still limited to the scan rate and waiting times between scan cycles. Constant-potential amperometry measures diffusion-limited current (without background current generated by changing voltage at electrode surface with time in FSCV), which offers outstanding temporal resolution on a submillisecond timescale with a kHz sampling rate. Therefore, the constant-potential amperometry is often used for studying single exocytotic events and single vesicles as the main electrochemical approach (Robinson et al., 2008; Bruns, 2004; Zhou and Misler, 1995). Amperometry allows quantifying NTs released from single vesicles and individual exocytotic events by using Faradays' law (Keighron et al., 2020).

### 4.3. Selectivity and biocompatibility in pharmacology

FSCV is typically used as a complementary tool to amperometry and

**Table 1**
The comparison of fiber photometry and electrochemistry.

| | Fiber Photometry | FSCV | Amperometry |
|---|---|---|---|
| Spatial Resolution | Low (the most common size of optical fiber is on the order of 100 μm (Aravanis et al., 2007; Wan et al., 2020), but can be tapered down to submicrometer diameters) (Pisano et al., 2019; Pisanello et al., 2014; Pisanello et al., 2017) | High (the common sensor size is on the order of 10 μm; it is capable to taper the sensor tip down to the order of 10 nm) (Cahill et al., 1996; Fathali et al., 2017; Li et al., 2015) | High (the common sensor size on the order of 10 μm; it is capable to taper sensor tip to the order of 10 nm) (Cahill et al., 1996; Fathali et al., 2017; Li et al., 2015) |
| Tissue Damage | Larger (with the most common fiber size on 100 μm in diameter) (Siciliano and Tye, 2019) | Smaller (Robinson et al., 2003) | Smaller (Robinson et al., 2003) |
| Temporal Resolution | Low (low as subsecond) | Medium (usually on subsecond timescale, can be down to millisecond) | High (low as submillisecond to millisecond) |
| Vesicular & Exocytosis Quantification | No | No | Yes |
| Chemical Specificity | Yes | Yes | No (but it can be improved by immobilizing enzymes in sensor fabrication for detecting non-electroactive metabolites) |
| Cell Specificity | Yes | No | No |
| Compatibility of sensor with pharmacology | No (receptor-based genetically-encoded fluorescent biosensor can be interfered by drugs) | Yes | Yes |
| Optimal conditions | Investigate the correlation of neuronal circuits to behaviors; brain mapping; dissect presynaptic events from postsynaptic events; dissect cell type to cell type events, etc. | Investigate the correlation of neuronal activity to behaviors; identify chemical compounds (e.g., dopamine); measure chemical concentrations in the range of nM to μM, etc. (Robinson et al., 2003) | Monitor single exocytotic events; vesicular and exocytotic quantification; characterize exocytosis dynamics, etc. |

fiber photometry to identify the electroactive chemicals among interfering compounds. Nevertheless, FSCV does lack accuracy in distinguishing some NTs like DA from NE, due to their very similar molecular structures (Feng et al., 2019; Park et al., 2009; Robinson et al., 2003). DA and NE offer a nearly identical current response in FSCV measurements at the same concentration of analytes and experimental conditions (Feng et al., 2019; Robinson et al., 2003). To overcome this limitation, Li's group developed a genetically encoded GPCR activation-based NE sensor, to make NE more distinguishable from DA (Feng et al., 2019). This NE sensor can be measured with fiber photometry and can detect evoked NE release and dynamics in behaving mice and zebrafish with extraordinary high specificity. Compared to other analytical methods, amperometry measurements are lacking chemical selectivity and are limited to a simple environment without many interfering compounds. However, by introducing enzymes into the fabrication of electrochemical biosensors, the selectivity of amperometric measurement can be significantly improved (Wang et al., 2019b; Vasylieva et al., 2013).

As we previously mentioned, the sensors used for monitoring NTs in fiber photometry are usually genetically-encoded protein-based indicators (Wang et al., 2019a; Südhof, 2013; Wang et al., 2019b; Miesenböck et al., 1998; Borden et al., 2020; Patriarchi et al., 2018), and many of them are based on receptors to report NT transients (e.g., iAChSnFR and dLight1.1) (Borden et al., 2020; Patriarchi et al., 2018). Speculation in terms of the specificity of receptor-based sensors exists in pharmacology study when drugs are added. In this case, extensive validation is required to ensure the drug has no affinity to receptor-based sensors, or to ensure the emitted fluorescence seen in response to drug treatment is due to NTs binding to sensors. Otherwise, spurious results could be produced since even moderate affinity of drug binding to receptor-based sensors may dramatically alter the fluorescent intensity. However, electrochemical methods, such as FSCV and amperometry, can overcome these obstacles. They are commonly used for detecting electroactive NTs and have high compatibility with drugs that are not electroactive in pharmacology research.

## 5. Conclusions

Monitoring neurotransmission in the brain of freely moving animals is a way to investigate the role of neural circuits in behavior. The measurement of neurotransmission in behaving animals requires sufficient sensitivity, selectivity and high temporal and spatial resolution. In this review, fiber photometry based on genetically-encoded biosensors and electrochemical biosensors were comprehensively discussed with their latest achievements. The strength and weakness of these two techniques were also well compared to each other and to other techniques, such as optogenetics and electrophysiology. Each technique has its advantages, and both are quite useful in the correct setting. However, the summary of many research studies in this review shows that more details underlying neurological behaviors can be revealed by complementing or combining multiple techniques.

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