Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Behavioral Sciences

Review

Genetically encoded dopamine sensors: principles, applications, and future directions

Hui Dong[1,2], Zhenghua Wang[1,3] and Yulong Li[1,3,4,5]

Dopamine is a crucial neuromodulator involved in various physiological and pathological states. Detecting dopamine dynamics with high spatial and temporal resolution serves as a fundamental basis for understanding the multifaceted functions of dopamine. Recently, emerging genetically encoded dopamine fluorescent sensors enable high spatiotemporal resolution *in vivo* detection of dopamine dynamics within the living brain. Here, we summarize the principles and features of genetically encoded dopamine sensors. We then highlight the advantages of these dopamine sensors through studies that have utilized them. Finally, we present perspectives on future directions for the development of next-generation dopamine sensors.

Addresses
[1] State Key Laboratory of Membrane Biology, Peking University School of Life Sciences, Beijing 100871, China
[2] Department of General Practice, Songjiang Hospital and Songjiang Research Institute, Shanghai Key Laboratory of Emotions and Affective Disorders, Shanghai Jiao Tong University School of Medicine, Shanghai 201600, China
[3] Academy for Advanced Interdisciplinary Studies, Peking–Tsinghua Center for Life Sciences, Peking University, Beijing, China
[4] PKU-IDG/McGovern Institute for Brain Research, Beijing 100871, China
[5] National Biomedical Imaging Center, Peking University, Beijing 100871, China

Corresponding author: Li, Yulong (yulongli@pku.edu.cn)
Li, Yulong (𝕏 @yulonglilab)

Current Opinion in Behavioral Sciences 2025, **62**:101489

This review comes from a themed issue on **Dopamine**

Edited by **Benjamin Saunders** and **Camilla Bellone**

Available online xxxx

Received: 27 August 2024; Revised: 16 December 2024;
Accepted: 3 February 2025

https://doi.org/10.1016/j.cobeha.2025.101489

Introduction

Dopamine is a crucial monoamine neurotransmitter in the brain, playing a pivotal role in movement, learning, memory, reward, and motivation [1]. Disruptions in the dopamine system lead to a variety of psychiatric and neurological disorders, including Parkinson's disease, addiction, and schizophrenia. Pioneering studies using formaldehyde-induced fluorescence revealed multiple dopamine-rich neuronal nuclei in the brain, distributed from the mesencephalon to the olfactory bulb, designated as A8-A16 [2]. Among these, the substantia nigra (A9) and ventral tegmental area (VTA) (A10) in the mesencephalon have received the most extensive research attention. These dopaminergic neurons project to various brain regions, with the densest projections to the dorsal striatum and nucleus accumbens and sparser projections to the cortex and amygdala.

Unlike classical neurotransmitters, dopamine functions as a neuromodulator, lacking the formation of classical synaptic structures [3,4]. Dopamine terminals form varicosities and often fail to establish typical postsynaptic structures. Upon release from presynaptic terminals, dopamine acts on dopamine receptors located on nearby neuronal cell bodies, dendrites, or even axons through volume transmission. In vertebrates, dopamine exerts its effects through two major classes of dopamine receptors: the Gs/olf-coupled D1-like (D1 and D5) receptors and the Gi/o-coupled D2-like (D2, D3, and D4) receptors.

Nongenetically encoded methods for dopamine detection *in vivo*

Tools and methods for dopamine detection are the cornerstones of understanding dopamine signaling (Table 1). Classical approaches include microdialysis and electrochemical methods. Microdialysis has been widely used to measure the dopamine dynamics in the living brain [5]. It involves extracting soluble chemicals like dopamine from the brain's cerebrospinal fluid using a semipermeable membrane, followed by further analysis of its concentration using chromatographic and analytical techniques. By enhancing the sensitivity of analytical methods, the temporal resolution of microdialysis techniques has been improved to the order of seconds; however, the commonly used microdialysis methods still operate at a temporal resolution of minutes. [6] Microdialysis probes usually has 100–200 μm diameter. Microdialysis offers high sensitivity and molecular specificity but suffers from low temporal resolution and invasiveness, potentially causing brain tissue damage. Electrochemical methods, including amperometry and fast-scan cyclic voltammetry, are commonly employed

Table 1

Advantages and disadvantages of methods for dopamine detection.

Method	Advantages	Disadvantages
Microdialysis	1. High sensitivity 2. High molecular specificity 3. Simultaneous detection of various chemicals 4. Quantitative detection of dopamine level	1. Low temporal resolution (usually minute-level sampling rate) 2. Low spatial resolution (usually 100–200 μm probe diameter) 3. Invasiveness (probe insertion potentially causes tissue damage)
FSCV	1. High sensitivity 2. High temporal resolution (subseconds level) 3. Higher spatial resolution than microdialysis (usually 5–10 μm probe diameter)	1. Unable to distinguish between DA and NE 2. Unable to be extended to the detection of other nonelectrochemically molecules
Nanosensor	1. High temporal resolution (subseconds level) 2. High spatial resolution (micrometer level) 3. NIR emission provides good tissue penetrance for *in vivo* imaging	1. Unable to distinguish between DA and NE 2. The demand for external injection of sensors limits *in vivo* applications
Genetically encoded sensors ($GRAB_{DA}$ and dLight)	1. High sensitivity 2. High temporal resolution (subseconds level) 3. High spatial resolution (subcellular level) 4. High molecular and cell type specificity 5. Low invasiveness	1. Currently cannot achieve the emission wavelength of NIR 2. Currently cannot achieve quantitative detection of dopamine level

FSCV, fast - scan cyclic voltammetry; DA represents dopamine; NE, norepinephrine.

for transient dopamine detection, providing high temporal resolution under the order of subseconds [7]. Carbon fiber microelectrodes are among the most commonly used in electrochemical methods for recording dopamine, with a tip diameter of approximately 5–10 μm, which provides a reasonably good spatial resolution [7]. However, due to the similar oxidation–reduction potentials of dopamine and norepinephrine, these methods often face difficulties in distinguishing between the two.

In recent years, fluorescent nanosensors based on single-walled carbon nanotubes (SWNTs) have been developed for the detection of catecholamines. One such nanosensor with near-infrared (NIR) excitation is known as the NIR catecholamine nanosensor (nIRCat) [8]. This technology utilizes an SWNT noncovalently functionalized with single-strand (GT)6 oligonucleotides to form the nIR catecholamine nanosensor (nIRCat). The nIRCat is employed in the striatal nuclei, where dopamine projections are dense, to monitor the spatiotemporal dynamics of dopamine, offering subsecond temporal resolution and micrometer spatial resolution. However, these probes are not yet adept at distinguishing between dopamine and norepinephrine. These nanosensors exhibit fluorescence emission in the range of near-infrared, which is well-suited for *in vivo* imaging due to good tissue penetrance. However, there is currently a lack of in vivo application examples.

The design and features of genetically encoded dopamine sensors

In recent years, genetically encoded neuromodulator fluorescent indicators have been developed (Table 2), enabling high spatiotemporal resolution detection of dopamine dynamics *in vivo* [9–13]. Two prominent series include the $GRAB_{DA}$ and dLight sensors. Both series share a similar principle — they incorporate a dopamine sensing domain, typically a dopamine receptor, and a fluorescent reporter domain, such as circular-permutated enhanced green fluorescent protein (cpEGFP) or circular-permutated mApple (cpmApple) (Figure 1a). The sensing domain, dopamine receptor, has a highly conserved conformational change in fifth (TM5) and sixth (TM6) transmembrane domains upon dopamine binding. A conformation-sensitive fluorescent protein is inserted into the third intracellular loop (ICL3) between TM5 and TM6 of the dopamine receptor. Dopamine binding to the dopamine probe triggers a conformational change in the binding domain, which afterward change the micro-environment surrounding the fluorophore and in turn alters the fluorescence intensity of the fluorescent protein. This fluorescence intensity change therefore serves as an indicator of dopamine dynamics.

Both $GRAB_{DA}$ and dLight sensors offer several outperforming advantages over existing methods for monitoring extracellular dopamine dynamics *in vivo*. First, these sensors are genetically encoded, which making them feasible to express in specific cell type or brain region either through virus-based approaches or transgenic line. Second, these dopamine sensors provide exceptional spatiotemporal resolution for *in vivo* dopamine imaging. They have subsecond kinetics and high signal-to-noise ratio, which makes them enable to track dopamine transients in real time in behavioral animals. Combining with advanced microscope, these fluorescent sensors enable to detect dopamine dynamics in high temporal resolution. Finally, these sensors offer

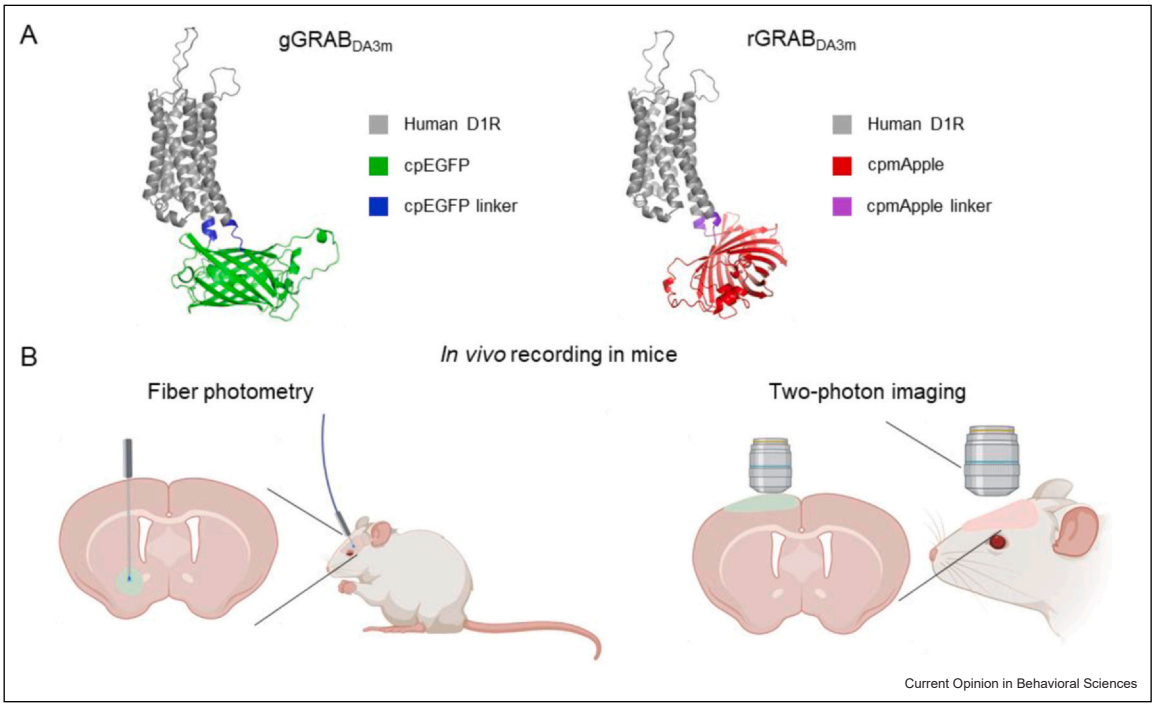
Table 2

List of genetically encoded dopamine sensors.

Dopamine sensors	GPCR scaffold	Reporter domain	Ex/ Em (nm)	Isosbestic point (nm)	Responses (maximum $\Delta F/F_0$)	Affinity (EC_{50}/nM)	Ref
$GRAB_{DA}$							
gDA3m	human D1R	cpEGFP	495/520	410	11.4	86	Zhuo, et al., *Nat. Methods*, 2023
gDA3h	bovine D1R	cpEGFP	500/520	-	12.5	22	
gDA2m	human D2R	cpEGFP	-	-	3.4	90	Sun, et al., *Nat. Methods*, 2020
gDA2h	human D2R	cpEGFP	500/520	440	2.8	7	
gDA1m	human D2R	cpEGFP	-	-	0.9	130	Sun, et al., *Cell*, 2018
gDA1h	human D2R	cpEGFP	-	-	0.9	10	
rDA3m	human D1R	cpmApple	560/595	-	10.3	130	Zhuo, et al., *Nat. Methods*, 2023
rDA3h	human D1R	cpmApple	565/585	-	10.2	20	
rDA2m	ant D2R	cpmApple	565/595	-	6.6	180	
rDA2h	ant D2R	cpmApple	565/595	-	4.1	9	
rDA1m	human D2R	cpmApple	565/595	-	1.5	95	Sun, et al., *Nat. Methods*, 2020
rDA1h	human D2R	cpmApple	565/595	-	1.2	4	
dLight							
dLight1.1	human D1R	cpEGFP	-/516	-	2.3	330	Patriarchi, et al., *Science*, 2018
dLight1.2	human D1R	cpEGFP	-/516	-	3.4	770	
dLight1.3a	human D1R	cpEGFP	-	-	6.6	2300	
dLight1.3b	human D1R	cpEGFP	-/513	-	9.3	1680	
dLight1.4	human D4R	cpEGFP	-	-	1.7	4.1	
dLight1.5	human D2R	cpEGFP	-	-	1.8	110	
Ydlight1	human D1R	cpEGFP	-/525	-	3.1	1630	Patriarchi, et al., *Nat. Methods*, 2020
RdLight1	human D1R	cpmApple	-/588	-	2.3	860	

excellent molecular specificity to dopamine. The latest dopamine sensor $gGRAB_{DA3m}$ could effectively distinguish dopamine from norepinephrine and minimally receives the interference of endogenous norepinephrine during the process of recording dopamine signals in the prefrontal cortex of mice [11].

Figure 1



Structural models and application genetically encoded dopamine sensors. **(a)** Structural models of $gGRAB_{DA3m}$ (left) and $rGRAB_{DA3m}$ sensors (right) generated using AlphaFold. **(b)** In vivo recording of dopamine dynamics combined with dopamine sensors and fiber photometry or two-photon imaging.

Instrument for recording of dopamine sensor signals

Since the fluorescent module of the novel dopamine sensors employs similar principle (single fluorophore-based strategy) to that of genetically encoded calcium indicators, the same modality used for recording calcium probes can be utilized without the need for new equipment. Two widely used approaches, fiber photometry and fluorescence microscopy (Figure 1b), have been employed in capturing fluorescent signals from dopamine sensors *in vivo*. Fiber photometry enables the acquisition of integrated bulk fluorescence signals from a targeted brain region via an optical fiber in behavior animals [14,15]. This method is particularly valued for its accessibility and compatibility with freely moving animals, although it inherently lacks precise spatial resolution. Conversely, one-photon and multiphoton microscopy provide high-resolution visualization of dopamine dynamics but require animals to be head-fixed in most cases. Recent advancements in miniaturized microscopy have expanded the application of this technique to freely moving animals. Fiber photometry and microscopy offer complementary advantages, and the optimal method should be based on the specific research objectives. Fiber photometry is widely used to record sensor signals from deep brain nuclei of freely moving rodents, while microscopy is employed in recording dopamine dynamics in the cortex of rodents [11], as well as in the brains of flies and zebrafish [9].

The application of dopamine sensors

We will proceed to review some of the applications that have been developed by previous researchers, leveraging the advantages of these sensors.

Dopamine sensors enable the detection of dopamine dynamics in high temporal resolution in the brains of freely moving mice. The mesolimbic dopaminergic pathway plays a crucial role in reinforcement learning, and dopamine signals are believed to represent the difference between reward prediction and actual reward, known as reward prediction error (RPE) [16]. Utilizing dopamine probes in conjunction with fiber photometry, researchers have recorded dopamine dynamics in the mouse nucleus accumbens during associative learning. During the initial learning phase, dopamine exhibits a rapid response to rewards. As learning progresses, dopamine primarily responds to nonconditioned stimulus cues, while its response to rewards diminishes [9,12]. These findings corroborate the role of dopamine in representing the difference between reward prediction and actual reward. Dopamine also contributes to sleep–wake regulation. Hasegawa et al. examined dopamine dynamics across the sleep–wake cycle in several brain regions. Intriguingly, they found a transient increase in dopamine levels in the basolateral amygdala (BLA) during the transition from nonrapid eye movement (NREM) sleep to rapid eye movement (REM) sleep initiation [17]. Furthermore, leveraging optogenetics and chemogenetics techniques, they have elucidated the transient increases in dopamine in the BLA promote REM sleep from NREM sleep in normal mice, while triggers cataplexy in narcoleptic mice.

Dopamine fluorescent probes can achieve high spatial resolution for detecting the spatial dynamic characteristics of dopamine. Dopamine fulfills a crucial teaching signal in olfactory associative learning paradigms in the fly brain. To synchronously monitor the dynamics of dopamine across multiple regions within the fly mushroom body, transgenic dopamine sensor fly was generated. Subsequently, two-photon microscopy was employed to detect signals emanating from the dopamine probes during various stimuli. Odor stimuli and electric shocks induce compartmentally heterogeneous dopamine release in the mushroom bodies of Drosophila [9]. Under the fiber photometry recording, we know that dopamine encodes RPEs in the mammalian brain; however, it is unknown whether dopamine release spatial heterogeneous in striatum in mice just like mushroom body in fly. Using widefield imaging with dLight sensors, Hamid et al. reported wave-like spatiotemporal dopamine dynamics across the dorsal striatum [18]. Intriguingly, the wave trajectory propagating directions depend on behavior tasks. Rewards after an instrumental trial triggered medially sourced, laterally propagating waves, whereas rewards in the Pavlovian task promoted laterally initiated, medially propagating waves. The probe diameter in microdialysis is relatively large, which poses a significant challenge when attempting to apply it to small animals like fruit flies, where precise targeting of brain regions is crucial. Moreover, both microdialysis and voltammetry are characterized by a lower throughput, which hampers their ability to capture the intricate, wave-like dynamics of dopamine release observed in the brains of mice. The compartmentally heterogeneous dopamine release in the fly brain and the wave-like spatiotemporal dopamine dynamics in the mouse brain exemplify the unique advantages of dopamine fluorescent sensors.

Red-shift spectrum dopamine sensors have distinct and well-separated spectra from GFP-based sensors, thus enabling orthogonally combine with commonly used optical sensors to simultaneous monitoring of other neurotransmitter, neuromodulator, or intracellular chemicals. Striatal dopamine release is considered to be regulated by local neuromodulators, especially acetylcholine (ACh) [19,20]. Using dual-color imaging of dopamine and ACh *in vivo*, dopamine and ACh exhibit anticorrelated transients and fluctuate spontaneously and periodically across behavioral states more than just reward [21–23]. The β2-containing nAChR blocker attenuated striatal dopamine dynamics [24]; however, genetic deleting β2 nAChR subunit in dopamine neurons or inhibition of ACh release

minimally perturb striatal dopamine dynamics [21,22]. In addition to studying the regulation between dopamine and other neurotransmitters, the downstream cell signaling regulated by dopamine is also important. Dopamine D1-like receptors are coupled to Gαs to activate adenylate cyclase then to increase intracellular cAMP. Assessed using red $GRAB_{DA}$ and green fluorescent cAMP indicator G-Flamp1 [25], intracellular cAMP accompanied with dopamine dynamics in mating behavior in the NAc [11]. When using mApple-based red dopamine sensors, it is important to be mindful of photoactivation, also known as 'photoswitching' or 'photoconversion'. Upon exposure to blue light, these fluorescent probes may exhibit a rapid, artificial increase in fluorescence signals, which is not due to an increase in dopamine concentration but rather a photoactivation phenomenon. For instance, the red dopamine sensor dLight signals in the NAc were readily increased upon blue light activation [26,27]. Therefore, control experiments should be designed carefully to account for or mitigate these potential artifacts.

Previous generation of dopamine sensors are widely and limitedly used in the striatum and nucleus accumbens, which receive dense dopaminergic innervation. Low sensitivity limits the application of these sensors in brain regions where dopamine projections are sparse. The new generation of GRAB dopamine sensor is super sensitive to dopamine and enables to monitor sparse dopamine release in cortex and amygdala. Using fiber photometry recording, the super sensitive dopamine sensor, $gGRAB_{DA3h,}$ enables to monitor prefrontal cortex dopamine release by one-pulse optogenetic stimulation of VTA dopaminergic neurons in awake behavior mice [11]. Using two-photon imaging, $gGRAB_{DA3h}$ sensor allows the mapping of spatially and functionally diverse patterns of dopamine release in the motor cortex under forced running and tail shock conditions [11]. In the future, we can harness these supersensitive dopamine sensors in conjunction with microscopy to investigate the dynamic patterns of dopamine in the cortex under various physiological and pathological behavioral states.

Dopamine sensors can be introduced into specific cells or brain regions either by employing viruses as delivery vehicles or by generating transgenic mice that harbor the fluorescent sensor. Beyond these approaches, establishing stable cell lines that constitutively express the dopamine sensor — referred to as 'dopamine sniffer cells' —allows for the direct monitoring of dopamine release by seeding sniffer cells on top of cultured dopaminergic neurons [28]. Furthermore, the development of fiber-optic probes incorporating engineered cells offers a convenient, real-time tracking of dopamine levels in animals that are freely moving. These techniques can expedite the process of capturing dopamine fluctuations within tissues, bypassing the need for lengthy plasmid transfections or viral expression. They also hold the potential to be applied in species where genetic manipulation and protein synthesis are either impractical or labor intensive.

Future directions

The development and application of genetically encoded sensors have already led the dopamine detection into a new era. Current dopamine sensors allow sensitive detection for *in vivo* dopamine dynamics during physiological and pathological conditions, even in regions with sparse dopaminergic innervation. However, current dopamine sensors still face challenges in multiplex imaging and quantitative measurements. Besides, although the selectivity of current dopamine sensors could already fulfill the requirements for the discrimination between dopamine and norepinephrine under certain circumstances, there is still space for improvement. Optimizations for these limitations will be a focus for future research in this field.

Multiplex imaging of dopamine and other neurotransmitter dynamics is crucial for research on the functions of neurotransmitters. Many physiological processes and pathological conditions in brain are related to dopamine and several other neurotransmitters. The interplay between dopamine and other neurotransmitter systems is highly universal and physiologically relevant in the nervous system [29]. Green and red neurotransmitter sensors have been successfully developed and applied to enable dual-color imaging of two different neurotransmitters [10,11,13]. On this basis, multiplex imaging using three and even more colors for the simultaneous detection of different neurotransmitters should be further developed so as to answer more questions. For example, various neurotransmitters such as dopamine, norepinephrine, serotonin, histamine, and adenosine are involved in the regulation of the sleep–wake cycle [30,31]. Expanded multicolor imaging would elucidate the coordination and regulation among these neurotransmitters. To realize the goal, it is necessary to expand the sensor's spectrum further, particularly extend the wavelength to a range of far-red (650–700 nm) and NIR (> 700 nm). Far-red/NIR sensors also have other advantages, including increased imaging depth, reduced background, and phototoxicity [32]. Several far-red/NIR FPs in circularly permutated form or split form have been utilized to develop single-FP-based calcium and zinc ion sensors [33–36], providing possible choices for fluorescent modules of dopamine sensors. However, the relatively low brightness of these far-red/NIR FPs could bring challenges for the sensitivity of dopamine sensors. Introducing the chemigenetic strategy into the development of dopamine sensors can be an alternative approach. Fluorescent chemical dyes can covalently bind with self-labeling proteins such as HaloTag through specific ligands and provide higher brightness and

photostability compared with FPs [37–39]. Some chemical dyes are able to cross the blood–brain barrier, thus enable applications in brain [40,41]. The system combining circularly permutated HaloTag and context-sensitive chemical dyes has been applied in developing calcium and voltage sensors [42,43], indicating a possibility to develop far-red/NIR dopamine sensors based on the chemigenetic strategy. In addition to HaloTag, several other self-labeling protein tag systems, including TMP-tag [44] and SNAP-tag [45], offer the ability to label proteins with various chemical dyes simultaneously and orthogonally. Optimized versions such as TMP-tag3 [46] and SNAP-tag2 [47] exhibit enhanced brightness and labeling speed, making them valuable tools for live-cell imaging. These tags have the potential to be used in the development of neurotransmitter sensors, including those for dopamine and other molecules. This would enable orthogonal multiplex imaging of multiple neurotransmitters in the far-red/NIR spectrum.

Quantitative measurement is also urgently required for the detection of dopamine. Quantifying dopamine dynamic could help distinguish the phasic and tonic release [48], thus provide further insights into their physiological roles. Dopamine receptors such as D1R and D2R have significantly different affinities for dopamine and play opposite regulatory effects on downstream signals [49]. Quantification of dopamine concentration could also help explore whether different dopamine levels have different functions by acting on different receptors. Current dopamine sensors are facing challenges in quantitative measurement due to their dependence on fluorescence intensity detection [50], which can be influenced by various factors such as fluorophore concentration, excitation power fluctuation, and photobleaching. Ratiometric sensors are one of the solutions since they have intrinsic references to reduce intensity-related artifacts. They can be categorized into emission and excitation ratiometric sensors. Emission ratiometric sensors use two distinct emission wavelengths, one can respond to ligand binding and the other acts as a reference [51,52]. However, the occupation of two channels limits the potential for multiplex imaging. Excitation ratiometric sensors are based on the shift of the sensors' excitation curve when ligand binding. By exciting the sensor using two distinct wavelengths and measure a fixed emission wavelength, the emission ratio between two excitation wavelengths can be calculated and thus determine the sensor's state, which is corresponding to the ligand concentration [53]. Despite the advantages mentioned above, the readout of ratiometric sensors is still influenced by the wavelength-dependent scattering. Fluorescence lifetime offers another promising option. It is defined as the average time that an excited fluorophore remains in its excited state, which is an intrinsic property of the fluorophore independent of intensity-related factors, thus can be measured quantitatively using fluorescence lifetime imaging microscopy [54–56]. Fluorescence lifetime has been utilized to quantitatively measure the concentration of intracellular molecules such as calcium [57–59] and cAMP [60]. One of the published lifetime-based calcium sensor Tq-Ca-FLITS, utilizing the cyan fluorescent protein mTurquoise2, exhibits a significant change in fluorescence lifetime upon calcium binding. However, the relatively short excitation and emission wavelengths of Cyan fluorescent protein limit its usefulness for *in vivo* neuronal imaging. A pressing need exists for more red-shifted lifetime-based sensors that can quantitatively measure the dynamics of neurotransmitters, including dopamine, in living animals.

Selectivity between dopamine and norepinephrine is always an essential property of dopamine sensors due to the structural similarity and the importance in nervous system of these two neurotransmitters. As is mentioned above, the latest $gGRAB_{DA3m}$ sensor already has good sensitivity and selectivity that could enable dopamine recording in the mouse motor cortex without the interference from norepinephrine [11]. In some other typical brain regions with projections of adrenergic neurons such as the visual cortex, however, evidence shows that there are also sparse dopaminergic projections in studied species [61–63]. In order to enable dopamine recording and learn more about functions of dopamine in such regions, further optimization of selectivity for dopamine sensors might be required. To achieve better selectivity without reducing sensitivity, optimization of current sensors' ligand binding pocket could be performed under the guidance of protein structure [64]. Besides, screening of orthologous receptors derived from different species can provide better backbones for sensors with better selectivity [65]. This experience might also be used to screen highly selective dopamine sensors.

The development of dopamine sensors has significantly advanced our understanding of dopamine dynamics *in vivo*. In conclusion, future research should focus on improving multiplexing capabilities, quantitative measurements, and selectivity to further elucidate the complex role of dopamine in the brain. By addressing these key areas, we can anticipate further advancements in our understanding of dopamine's role in various physiological and pathological processes.

Declaration of Competing Interest

The authors declare no competing interests.

Acknowledgements

This work was supported by the National Natural Science Foundation of China, China, (32471031), the National Key R&D Program of China, China (2022YFC3300905 to H.D.); the National Key R&D Program of China, China (2022YFE0108700), the National Natural Science Foundation of China, China (31925017), the Beijing Municipal Science & Technology Commission, China (Z220009), and the NIH BRAIN Initiative, USA

(1U01NS113358 and 1U01NS120824) and grants from the Feng Foundation of Biomedical Research, China, the Clement and Xinxin Foundation, China, and the New Cornerstone Science Foundation, China through the New Cornerstone Investigator Program and the XPLORER PRIZE (to Y.L.).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Garritsen O, van Battum EY, Grossouw LM, Pasterkamp RJ: **Development, wiring and function of dopamine neuron subtypes**. *Nat Rev Neurosci* 2023, **24**:134-152, https://doi.org/10.1038/s41583-022-00669-3

2. Bjorklund A, Dunnett SB: **Dopamine neuron systems in the brain: an update**. *Trends Neurosci* 2007, **30**:194-202, https://doi.org/10.1016/j.tins.2007.03.006

3. Liu C, Goel P, Kaeser PS: **Spatial and temporal scales of dopamine transmission**. *Nat Rev Neurosci* 2021, **22**:345-358, https://doi.org/10.1038/s41583-021-00455-7

4. Özçete ÖD, Banerjee A, Kaeser PS: **Mechanisms of neuromodulatory volume transmission**. *Mol Psychiatry* 2024, **29**:3680-3693, https://doi.org/10.1038/s41380-024-02608-3

5. Lee GJ, Park JH, Park HK: **Microdialysis applications in neuroscience**. *Neurol Res* 2013, **30**:661-668, https://doi.org/10.1179/174313208×289570

6. Wang M, Slaney T, Mabrouk O, Kennedy RT: **Collection of nanoliter microdialysate fractions in plugs for off-line in vivo chemical monitoring with up to 2s temporal resolution**. *J Neurosci Methods* 2010, **190**:39-48, https://doi.org/10.1016/j.jneumeth.2010.04.023

7. Jaquins-Gerstl A, Michael AC: **A review of the effects of FSCV and microdialysis measurements on dopamine release in the surrounding tissue**. *Analyst* 2015, **140**:3696-3708, https://doi.org/10.1039/c4an02065k

8. Beyene AG, *et al*.: **Imaging striatal dopamine release using a nongenetically encoded near infrared fluorescent catecholamine nanosensor**. *Sci Adv* 2019, **5**:eaaw310810.1126/sciadv.aaw3108.

9. Sun F, *et al*.: **A genetically encoded fluorescent sensor enables rapid and specific detection of dopamine in flies, fish, and mice**, e419, *Cell* 2018, **174**:481-496, https://doi.org/10.1016/j.cell.2018.06.042

10. Sun F, *et al*.: **Next-generation GRAB sensors for monitoring dopaminergic activity in vivo**. *Nat Methods* 2020, **17**:1156-1166, https://doi.org/10.1038/s41592-020-00981-9

11. •• Zhuo Y, *et al*.: **Improved green and red GRAB sensors for monitoring dopaminergic activity in vivo**. *Nat Methods* 2024, **21**:680-691, https://doi.org/10.1038/s41592-023-02100-w.
The authors developed new generation of GPCR-activation based green and red $GRAB_{DA}$ sensors enabling *in vivo* dopamine detection with high sensitivity, selectivity, and spatiotemporal resolution. Newly optimized $gGRAB_{DA3h}$ sensor is sensitive enough to achieve dopamine detection in cortex and amygdala. Newly optimized red-shifted spectrum $GRAB_{DA}$ sensors provide tools for combining with other green sensors in order to monitor dopamine together with other neurotransmitters or intercellular chemicals simultaneously.

12. Patriarchi T, *et al*.: **Ultrafast neuronal imaging of dopamine dynamics with designed genetically encoded sensors**. *Science* 2018, **360**:eaat4422.

13. Patriarchi T, *et al*.: **An expanded palette of dopamine sensors for multiplex imaging in vivo**. *Nat Methods* 2020, **17**:1147-1155, https://doi.org/10.1038/s41592-020-0936-3

14. Simpson EH, *et al*.: **Lights, fiber, action! A primer on in vivo fiber photometry**. *Neuron* 2024, **112**:718-739, https://doi.org/10.1016/j.neuron.2023.11.016

15. Gunaydin LA, *et al*.: **Natural neural projection dynamics underlying social behavior**. *Cell* 2014, **157**:1535-1551, https://doi.org/10.1016/j.cell.2014.05.017

16. Schultz W, Dayan P, Montague PR: **A neural substrate of prediction and reward**. *Science* 1997, **275**:1593-1599, https://doi.org/10.1126/science.275.5306.1593

17. • Hasegawa E, *et al*.: **Rapid eye movement sleep is initiated by basolateral amygdala dopamine signaling in mice** (-+), *Science* 2022, **375**:994, https://doi.org/10.1126/science.abl6618.
The authors recorded dopamine dynamics utilizing $GRAB_{DA}$ sensors during REM and NREM sleep of mice. They found that the transient increase of dopamine in the BLA during NREM terminates NREM sleep and initiates REM sleep, revealing the critical role of dopamine signaling in the BLA in initiating REM sleep and provide a neuronal basis for REM sleep cycle generation.

18. Hamid AA, Frank MJ, Moore CI: **Wave-like dopamine dynamics as a mechanism for spatiotemporal credit assignment**, e2716, *Cell* 2021, **184**:2733-2749, https://doi.org/10.1016/j.cell.2021.03.046

19. Zhou FM, Liang Y, Dani JA: **Endogenous nicotinic cholinergic activity regulates dopamine release in the striatum**. *Nat Neurosci* 2001, **4**:1224-1229, https://doi.org/10.1038/nn769

20. Cragg SJ: **Meaningful silences: how dopamine listens to the ACh pause**. *Trends Neurosci* 2006, **29**:125-131, https://doi.org/10.1016/j.tins.2006.01.003

21. • Chantranupong L, *et al*.: **Dopamine and glutamate regulate striatal acetylcholine in decision-making**. *Nature* 2023, **621**:577-585, https://doi.org/10.1038/s41586-023-06492-9.
The authors studied the regulation of dopamine and acetylcholine release by monitoring dopamine and acetylcholine signals in the ventrolateral striatum of mice. They found that dopamine dynamics and reward encoding do not require the acetylcholine release by cholinergic interneurons, but dopamine inhibits acetylcholine transients in a D2R-dependent manner, revealing a dynamic relationship between dopamine and acetylcholine in striatum during reward-based decision-making.

22. • Krok AC, *et al*.: **Intrinsic dopamine and acetylcholine dynamics in the striatum of mice**. *Nature* 2023, **621**:543-549, https://doi.org/10.1038/s41586-023-05995-9.
The authors studied the dynamics of dopamine and acetylcholine using dual-color imaging in the dorsal striatum of mice. They found that dopamine and acetylcholine exhibit anticorrelated transients and fluctuate spontaneously and periodically across behavioral states more than just reward.

23. Jing M, *et al*.: **An optimized acetylcholine sensor for monitoring in vivo cholinergic activity**. *Nat Methods* 2020, **17**:1139-1146, https://doi.org/10.1038/s41592-020-0953-2

24. • Liu C, *et al*.: **An action potential initiation mechanism in distal axons for the control of dopamine release**. *Science* 2022, **375**:1378-1385, https://doi.org/10.1126/science.abn0532.
The authors found that acetylcholine-releasing neurons induce action potential firing in distal dopamine axons in the striatum, deeply examined the release mechanisms of dopamine, and studied the functional relevance of ACh-induced dopamine release in freely moving mice using dual-color fiber photometry. The study reveals an endogenous mechanism for action potential initiation independent of somatodendritic integration and establishes that this mechanism segregates the control of dopamine signaling between axons and somata.

25. Wang L, *et al*.: **A high-performance genetically encoded fluorescent indicator for in vivo cAMP imaging**. *Nat Commun* 2022, **13**:5363, https://doi.org/10.1038/s41467-022-32994-7

26. Mohebi A, Collins VL, Berke JD: **Accumbens cholinergic interneurons dynamically promote dopamine release and enable motivation**. *eLife* 2023, **12**:e85011, https://doi.org/10.7554/eLife.85011

27. Melani R, *et al*.: **Comment on 'Accumbens cholinergic interneurons dynamically promote dopamine release and enable motivation**. *eLife* 2024, **13**:e95694, https://doi.org/10.7554/eLife.95694

28. Klein Herenbrink C, Støier JF, Reith WD, Dagra A, Gregorek MAC, Cola RB, Patriarchi T, Li Y, Tian L, Gether U, Herborg F: **Multimodal detection of dopamine by sniffer cells expressing**

genetically encoded fluorescent sensors. *Commun Biol* 2022, **5**:578, https://doi.org/10.1038/s42003-022-03488-5

29. Lovinger DM, *et al*.: **Local modulation by presynaptic receptors controls neuronal communication and behaviour**. *Nat Rev Neurosci* 2022, **23**:191-203, https://doi.org/10.1038/s41583-022-00561-0

30. Liu D, Dan Y: **A motor theory of sleep-wake control: arousal-action circuit**. *Annu Rev Neurosci* 2019, **42**:27-46, https://doi.org/10.1146/annurev-neuro-080317-061813

31. Peng W, *et al*.: **Regulation of sleep homeostasis mediator adenosine by basal forebrain glutamatergic neurons**. *Science* 2020, **369**:eabb0556, https://doi.org/10.1126/science.abb0556

32. Karasev MM, Stepanenko OV, Rumyantsev KA, Turoverov KK, Verkhusha VV: **Near-infrared fluorescent proteins and their applications**. *Biochemistry* 2019, **84**:32-50, https://doi.org/10.1134/S0006297919140037

33. Dalangin R, *et al*.: **Far-red fluorescent genetically encoded calcium ion indicators**, 2020.11.12.380089, *bioRxiv* 2020, https://doi.org/10.1101/2020.11.12.380089 2020.11.12.380089.

34. Wu, T *et al.* : A Genetically Encoded Far-Red Fluorescent Indicator for Imaging Synaptically Released Zn2+; *Science Advances* **9**, eadd2058, doi:⟨10.1126/sciadv.add2058⟩.

35. Qian Y, *et al*.: **A genetically encoded near-infrared fluorescent calcium ion indicator**. *Nat Methods* 2019, **16**:171-174, https://doi.org/10.1038/s41592-018-0294-6

36. Qian Y, *et al*.: **Improved genetically encoded near-infrared fluorescent calcium ion indicators for in vivo imaging**. *PLoS Biol* 2020, **18**:e3000965, https://doi.org/10.1371/journal.pbio.3000965

37. Los GV, *et al*.: **HaloTag: a novel protein labeling technology for cell imaging and protein analysis**. *ACS Chem Biol* 2008, **3**:373-382, https://doi.org/10.1021/cb800025k

38. Gautier A, Tebo AG: **Sensing cellular biochemistry with fluorescent chemical–genetic hybrids**. *Curr Opin Chem Biol* 2020, **57**:58-64, https://doi.org/10.1016/j.cbpa.2020.04.005

39. Cook A, Walterspiel F, Deo C: **HaloTag-based reporters for fluorescence imaging and biosensing**. *ChemBioChem* 2023, **24**:e202300022, https://doi.org/10.1002/cbic.202300022

40. Abdelfattah AS, *et al*.: **Bright and photostable chemigenetic indicators for extended in vivo voltage imaging**. *Science* 2019, **365**:699-704, https://doi.org/10.1126/science.aav6416

41. Grimm JB, *et al*.: **A general method to optimize and functionalize red-shifted rhodamine dyes**. *Nat Methods* 2020, **17**:815-821, https://doi.org/10.1038/s41592-020-0909-6

42. Wang L, Hiblot J, Popp C, Xue L, Johnsson K: **Environmentally sensitive color-shifting fluorophores for bioimaging**. *Angew Chem Int Ed* 2020, **59**:21880-21884, https://doi.org/10.1002/anie.202008357

43. Deo C, *et al*.: **The HaloTag as a general scaffold for far-red tunable chemigenetic indicators**. *Nat Chem Biol* 2021, **17**:718-723, https://doi.org/10.1038/s41589-021-00775-w

44. Miller LW, Cai Y, Sheetz MP, Cornish VW: **In vivo protein labeling with trimethoprim conjugates: a flexible chemical tag**. *Nat Methods* 2005, **2**:255-257, https://doi.org/10.1038/nmeth749

45. Keppler A, *et al*.: **A general method for the covalent labeling of fusion proteins with small molecules in vivo**. *Nat Biotechnol* 2003, **21**:86-89, https://doi.org/10.1038/nbt765

46. Mo J, *et al*.: **Third-generation covalent TMP-Tag for fast labeling and multiplexed imaging of cellular proteins**. *Angew Chem Int Ed Engl* 2022, **61**:e202207905, https://doi.org/10.1002/anie.202207905

47. Kühn S, *et al*.: **SNAP-tag2: faster and brighter protein labeling**, 2024.08.28.610127, *bioRxiv* 2024, https://doi.org/10.1101/2024.08.28.610127 2024.08.28.610127.

48. Gonon FG: **Nonlinear relationship between impulse flow and dopamine released by rat midbrain dopaminergic neurons as studied by in vivo electrochemistry**. *Neuroscience* 1988, **24**:19-28, https://doi.org/10.1016/0306-4522(88)90307-7

49. Calabresi P, Picconi B, Tozzi A, Ghiglieri V, Di Filippo M: **Direct and indirect pathways of basal ganglia: a critical reappraisal**. *Nat Neurosci* 2014, **17**:1022-1030, https://doi.org/10.1038/nn.3743

50. Bowman AJ, Huang C, Schnitzer MJ, Kasevich MA: **Wide-field fluorescence lifetime imaging of neuron spiking and subthreshold activity in vivo**. *Science* 2023, **380**:1270-1275, https://doi.org/10.1126/science.adf9725

51. Ast C, *et al*.: **Ratiometric Matryoshka biosensors from a nested cassette of green- and orange-emitting fluorescent proteins**. *Nat Commun* 2017, **8**:431, https://doi.org/10.1038/s41467-017-00400-2

52. Kim BB, *et al*.: **A red fluorescent protein with improved monomericity enables ratiometric voltage imaging with ASAP3**. *Sci Rep* 2022, **12**:3678, https://doi.org/10.1038/s41598-022-07313-1

53. Grynkiewicz G, Poenie M, Tsien RY: **A new generation of Ca2+ indicators with greatly improved fluorescence properties**. *J Biol Chem* 1985, **260**:3440-3450, https://doi.org/10.1016/S0021-9258(19)83641-4

54. Yasuda R: **Imaging spatiotemporal dynamics of neuronal signaling using fluorescence resonance energy transfer and fluorescence lifetime imaging microscopy**. *Curr Opin Neurobiol* 2006, **16**:551-561, https://doi.org/10.1016/j.conb.2006.08.012

55. Becker W: **Fluorescence lifetime imaging — techniques and applications**. *J Microsc* 2012, **247**:119-136, https://doi.org/10.1111/j.1365-2818.2012.03618.x

56. Datta R, Heaster TM, Sharick JT, Gillette AA, Skala MC: **Fluorescence lifetime imaging microscopy: fundamentals and advances in instrumentation, analysis, and applications**. *J Biomed Opt* 2020, **25**:1-43, https://doi.org/10.1117/1.Jbo.25.7.071203

57. Zheng K, *et al*.: **Time-resolved imaging reveals heterogeneous landscapes of nanomolar Ca2+ in neurons and astroglia**. *Neuron* 2015, **88**:277-288, https://doi.org/10.1016/j.neuron.2015.09.043

58. van der Linden FH, *et al*.: **A turquoise fluorescence lifetime-based biosensor for quantitative imaging of intracellular calcium**. *Nat Commun* 2021, **12**:7159, https://doi.org/10.1038/s41467-021-27249-w

59. Farrants H, Shuai Y, Lemon WC, *et al*.: **A modular chemigenetic calcium indicator for multiplexed in vivo functional imaging**. *Nat Methods* 2024, **21**:1916-1925, https://doi.org/10.1038/s41592-024-02411-6

60. Massengill CI, *et al*.: **Sensitive genetically encoded sensors for population and subcellular imaging of cAMP in vivo**. *Nat Methods* 2022, **19**:1461-1471, https://doi.org/10.1038/s41592-022-01646-5

61. Descarries L, Lemay B, Doucet G, Berger B: **Regional and laminar density of the dopamine innervation in adult rat cerebral cortex**. *Neuroscience* 1987, **21**:807-824, https://doi.org/10.1016/0306-4522(87)90038-8

62. Phillipson OT, Kilpatrick IC, Jones MW: **Dopaminergic innervation of the primary visual cortex in the rat, and some correlations with human cortex**. *Brain Res Bull* 1987, **18**:621-633, https://doi.org/10.1016/0361-9230(87)90132-8

63. Berger B, Trottier S, Verney C, Gaspar P, Alvarez C: **Regional and laminar distribution of the dopamine and serotonin innervation in the macaque cerebral cortex: a radioautographic study**. *J Comp Neurol* 1988, **273**:99-119, https://doi.org/10.1002/cne.902730109

64. Zhuang Y, *et al*.: **Structural insights into the human D1 and D2 dopamine receptor signaling complexes**, e918, *Cell* 2021, **184**:931-942, https://doi.org/10.1016/j.cell.2021.01.027

65. Kagiampaki Z, *et al*.: **Sensitive multicolor indicators for monitoring norepinephrine in vivo**. *Nat Methods* 2023, **20**:1426-1436, https://doi.org/10.1038/s41592-023-01959-z