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New imaging methods for monitoring dopaminergic neurotransmission

Yizhou Zhuo[1,2] & Yulong Li[1,2,3*]

[1]State Key Laboratory of Membrane Biology, Peking University School of Life Sciences, Beijing 100871, China;
[2]PKU-IDG/McGovern Institute for Brain Research, Beijing 100871, China;
[3]PKU-THU Center for Life Sciences, Academy for Advanced Interdisciplinary Studies, Peking University, Beijing 100871, China

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Dopamine is a crucial monoamine neuromodulator, which is primarily synthesized in the ventral midbrain in the central nervous system and activates G protein-coupled dopamine receptors to modulate the neuronal circuit dynamics. Five subtypes of mammalian dopamine receptors have been identified and are classified into D1-like (D1, D5) or D2-like (D2, D3, D4) groups with different functions (Beaulieu and Gainetdinov, 2011). The D1-like group primarily couples to the Gs family of G proteins, whereas the D2-like class mainly interacts with the Gi/o protein. Dopamine regulates multiple neural functions across human brain and body mainly through two well-described structures. The nigrostriatal circuit starting from substantia nigra to the dorsal striatum is important for motor control and action selection. The mesocorticolimbic circuit, which originates in the ventral tegmental area and projects to the limbic structure and prefrontal cortical region, is responsible for reward processing, emotion control and reinforcement learning. Conversely, imbalanced or malfunctioned dopaminergic signaling is implicated in the pathogenesis of many neurological disorders, including Parkinson's disease, schizophrenia, addiction and attention deficit disorders, which are commonly treated by drugs that regulate dopamine neurotransmission. Though many efforts have been made to decode the information underlying dopaminergic neurotransmission, how the specific dopaminergic signals might sculpt neuronal circuits in normal brain functions or diseases remains largely unknown due to the lack of tools to measure precise spatiotemporal dopamine dynamics *in vivo*.

Unlike classic neurotransmitters which are mainly released into the synaptic cleft, dopamine transmission is believed to occur on a broader spatial scale, with synaptic release driving both local communication between the pre- and post-synaptic neurons and volume transmission for widespread receptor activation of target cells (Marder, 2012). In addition to the coupling of the G protein-activated inward rectifier K^+ channel (GIRK) to D_2R for the DA release in the midbrain (Beaulieu and Gainetdinov, 2011), most dopamine transmissions are not associated with postsynaptic currents which are detectable using electrophysiology or genetically-encoded voltage sensors. Previous dopamine measurement relies on analytical chemistry techniques such as fast-scan cyclic voltammetry (FSCV) that are limited by micron-scale spatial resolution and relatively poor dopamine specificity over norepinephrine, or microdialysis suffering from spatiotemporal constraints. This dilemma has started to change with the development of optical methods.

Optical imaging methods address these challenges and enable direct, sensitive, and long-term measurement of dopamine dynamics with high spatiotemporal resolution. Genetically targeted optical sensors have been widely used *in vitro* and *in vivo* since the first appearance of GCaMP (Nakai et al., 2001), and have become increasingly versatile, effective, and popular over the past decades. The GCaMP

*Corresponding author (email: yulongli@pku.edu.cn)

construct was initially designed by fusing a circularly permuted GFP (cpGFP) in-between the calcium-binding motif of calmodulin (CaM) to the CaM-binding peptide M13/RS20. In GCaMP, the structural change of the CaM-M13/RS20 module that occurs in the presence of Ca^{2+} causes an increase in fluorescence of the cpGFP, thereby enabling optical imaging of calcium which serves as a universal proxy for neuronal activity. More recently, this strategy has been adapted for the direct detection of additional signaling molecules based on the coupling of other genetically encoded receptors to cpGFP.

G-protein coupled receptors (GPCRs) are the largest family of membrane proteins and the targets for multiple extracellular signaling molecules, including most neurotransmitters and neuromodulators, providing an essential library for biosensor development. A key and common feature of GPCR architecture is the structural rearrangement of transmembrane (TM) helices upon ligand binding, especially the movement between TM5 and TM6. We and others have recently utilized these conserved conformational changes to develop a series of green genetically-encoded single-wavelength dopamine indicators, the $GRAB_{DA}$ family as well as the dLight sensors (Patriarchi et al., 2018; Sun et al., 2018). The $GRAB_{DA}$ family consists of D_2R with an inserted cpEGFP module in the third intracellular loop (ICL3) with close proximity between conformation-sensitive protein and reorganized transmembrane region (Figure 1), while the dLight sensors are engineered with a similar design but with different GPCR backbones D_1R, D_2R and D_4R. The GPCR based design confers the dopamine probes with ideally suited affinity within the physiologic range (nanomolar to micromolar), molecular specificity and sub-second kinetics, which are similar or even identical to their native receptors, to capture endogenous dopamine fluctuations. Both $GRAB_{DA}$ and dLight sensors are able to directly visualize the spatial and temporal release of dopamine with high resolution in cultured cells, brain slices and also behaving animals *in vivo* (Figure 1). Additionally, the same research groups have recently introduced red fluorescent variants for each ($rGRAB_{DA}$ and RdLight1) based on cpRFP module that has been iteratively evolved in red calcium indicators (Patriarchi et al., 2020; Sun et al., 2020), allowing multicolor imaging of complex neurochemical interaction.

These new imaging approaches have already enabled us to expand the knowledge of the spatiotemporal dynamics of dopamine transmission. For instance, combining widefield imaging to track dopamine dynamics over a large spatial scale of the dorsal striatum, the Moore lab reported spatiotemporally heterogeneous dopamine signals with wave-like patterns which are regionally tailored for reward learning (Hamid et al., 2021). By applying these fast genetically encoded dopamine probes, the Sabatini lab also explored the real-time relationship between dopamine and PKA in spiny projection neurons expressing either D_1R or D_2R in the nucleus accumbens (NAc) of mice during learning (Lee et al., 2021). They demonstrated the asynchronous modulation of positive or negative dopamine signals in PKA-dependent pathways in each neuron population during learning.

Moreover, it is worth noting that the value of these GPCR based sensors extends well beyond imaging dopamine dynamics. First, since most neurotransmitters and neuromodulators could be sensed by GPCRs, the GPCR based design provides many opportunities for engineering new sensors for other neuromodulators beyond the dopamine system. Second, since most of the ligand binding sites are maintained during sensor engineering, specific pharmacological properties of these sensors appear to be well preserved from the native receptors. This may be beneficial for dopamine pharmaceutics and represent a useful tool for drug screening for different dopamine receptor subtypes or even boosting GPCR drug discovery. Future efforts are expected to allow even more signals to be studied simultaneously by further expanding the color spectrum. To this aim, one feasible design might be the replacement of the existing fluorescent module with conformation-sensitive near-infrared (nIR) variants.

Apart from the above GPCR-cpFP strategy, carbon nanotubes present a promising alternative for detecting neurotransmitter release and fill the niche of sensors with near-infrared emission wavelengths. For example, the Landry lab recently designed a synthetic nanoscale nIR fluorescent probe that can report catecholamine dynamics, namely nIRCat (Beyene et al., 2019). The nIRCat sensor comprises semiconducting single-wall carbon nanotubes (SWNTs) which are noncovalently functionalized with single-strand $(GT)_6$ oligonucleotides. Unlike probes with GPCR scaffold, this nanosensor is specifically responsive to dopamine and norepinephrine, and compatible with dopamine pharmacology. Although nIRCat is not selective for dopamine over norepinephrine, the probe functions as a dopamine sensor in the dorsal striatum or NAc where dopaminergic innervation is dense and norepinephrinergic innervation is negligible. However, this lack of specificity may complicate the attempts to image specific dopamine signals outside these regions. SWNTs-based probes are also largely excluded from the synaptic cleft due to their nanoscale size. niRCat has been used to measure dopamine in the mouse brain slices but has not yet been validated *in vivo*, demonstrating an alternative to genetically introduced dopamine sensors with feasibility across species where gene delivery and protein expression are undesirable.

The popularity and utility of genetically encoded indicators is further increased by the ability to target their expression to defined cell types or subcellular compartments, which enables the visualization of neurochemical signals

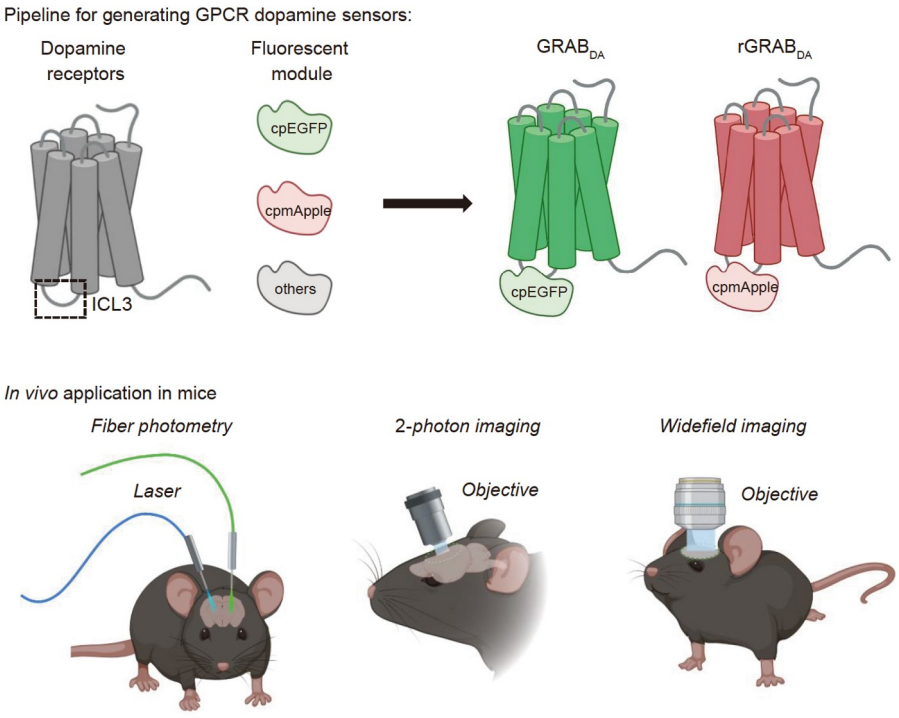

Figure 1 Design (top) and application (bottom) of GRAB dopamine sensors. Schematic illustration depicts the strategy for developing GRAB$_{DA}$ sensors. The general design requires a binding domain of the GPCR and a fluorescence reporting domain, e.g., cpEGFP and cpmApple. Combining a range of imaging modalities that possess different spatiotemporal resolutions, including fiber photometry, 2-photon microscope or mesoscope, these probes enable the ability to visualize dopamine signals while integrating neural circuits, systems and behaviors.

with cell-type specificity and/or subcellular resolution. Improved rhodamine dyes, such as the Janelia Fluor (JF) dyes, exhibit exceptional photophysical properties and flexible spectra range, which would be advantageous when combined with the self-labeling protein tags as well as protein sensor domains (Grimm et al., 2017). Recent work originating in the Schreiter lab and Lavis lab on chemigenetic voltage indicator (Voltron) serves as an elegant example (Abdelfattah et al., 2019). This approach involved the replacement of fluorescent proteins in the previously reported voltage indicators with the self-labeling HaloTag, followed by labeling with fluorogenic rhodamine dye. In a follow-up report, they re-engineered the HaloTag protein through circular permutation, converting the labeling system into a tunable scaffold for fluorescent imaging (Deo et al., 2021). This allows the genetic encoding of protein portion, utilization of cell-permeable small-molecule HaloTag ligands, and spectral flexibility of fluorogenic dyes. By simply swapping out the modulable fluorescent protein in established sensors with cpHaloTag, this hybrid genetic/small-molecule design offers an opportunity to develop new indicators to generate brighter and far-red probes for detecting dopamine and other neurochemical events.

Here we outlined the existing optical methods for directly measuring dopamine dynamics concerning their properties, advantages as well as limitations, and discussed directions of future developments. There is no doubt that the development of new biosensor systems is critical for pushing the frontier of real-time biological imaging. And most importantly, with the rapid advances in *in vivo* imaging techniques, including multiplexed fiber photometry, head-mounted two-photon microscope, miniscope and mesoscope, and also expected future developments in the field, the optical sensors have the potential to renovate the field of dopamine sensing and achieve *in vivo* dopamine imaging with unprecedented spatiotemporal resolution.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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References

Abdelfattah, A.S., Kawashima, T., Singh, A., Novak, O., Liu, H., Shuai, Y., Huang, Y.C., Campagnola, L., Seeman, S.C., Yu, J., et al. (2019). Bright and photostable chemigenetic indicators for extended *in vivo* voltage imaging. Science 365, 699–704.

Beaulieu, J.M., and Gainetdinov, R.R. (2011). The physiology, signaling,

and pharmacology of dopamine receptors. Pharmacol Rev 63, 182–217.
Beyene, A.G., Delevich, K., Del Bonis-O'Donnell, J.T., Piekarski, D.J., Lin, W.C., Thomas, A.W., Yang, S.J., Kosillo, P., Yang, D., Prounis, G. S., et al. (2019). Imaging striatal dopamine release using a nongenetically encoded near infrared fluorescent catecholamine nanosensor. Sci Adv 5, eaaw3108.
Deo, C., Abdelfattah, A.S., Bhargava, H.K., Berro, A.J., Falco, N., Farrants, H., Moeyaert, B., Chupanova, M., Lavis, L.D., and Schreiter, E.R. (2021). The HaloTag as a general scaffold for far-red tunable chemigenetic indicators. Nat Chem Biol 17, 718–723.
Grimm, J.B., Muthusamy, A.K., Liang, Y., Brown, T.A., Lemon, W.C., Patel, R., Lu, R., Macklin, J.J., Keller, P.J., Ji, N., et al. (2017). A general method to fine-tune fluorophores for live-cell and *in vivo* imaging. Nat Methods 14, 987–994.
Hamid, A.A., Frank, M.J., and Moore, C.I. (2021). Wave-like dopamine dynamics as a mechanism for spatiotemporal credit assignment. Cell 184, 2733–2749.e16.
Lee, S.J., Lodder, B., Chen, Y., Patriarchi, T., Tian, L., and Sabatini, B.L. (2021). Cell-type-specific asynchronous modulation of PKA by dopamine in learning. Nature 590, 451–456.
Marder, E. (2012). Neuromodulation of neuronal circuits: Back to the future. Neuron 76, 1–11.
Nakai, J., Ohkura, M., and Imoto, K. (2001). A high signal-to-noise Ca^{2+} probe composed of a single green fluorescent protein. Nat Biotechnol 19, 137–141.
Patriarchi, T., Cho, J.R., Merten, K., Howe, M.W., Marley, A., Xiong, W. H., Folk, R.W., Broussard, G.J., Liang, R., Jang, M.J., et al. (2018). Ultrafast neuronal imaging of dopamine dynamics with designed genetically encoded sensors. Science 360.
Patriarchi, T., Mohebi, A., Sun, J., Marley, A., Liang, R., Dong, C., Puhger, K., Mizuno, G.O., Davis, C.M., Wiltgen, B., et al. (2020). An expanded palette of dopamine sensors for multiplex imaging *in vivo*. Nat Methods 17, 1147–1155.
Sun, F., Zeng, J., Jing, M., Zhou, J., Feng, J., Owen, S.F., Luo, Y., Li, F., Wang, H., Yamaguchi, T., et al. (2018). A genetically encoded fluorescent sensor enables rapid and specific detection of dopamine in flies, fish, and mice. Cell 174, 481–496.e19.
Sun, F., Zhou, J., Dai, B., Qian, T., Zeng, J., Li, X., Zhuo, Y., Zhang, Y., Wang, Y., Qian, C., et al. (2020). Next-generation GRAB sensors for monitoring dopaminergic activity *in vivo*. Nat Methods 17, 1156–1166.