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BUSTA 1 (estratta)

1) il candidato descriva la sua esperienza nell'ambito dell'elettrofisiologia di cellule eccitabili cardiache o neuronali
2) il candidato faccia degli esempi, presi dalla letteratura, di utilizzo di tecniche di imaging per lo studio dell'elettrofisiologia di cellule eccitabili cardiache o neuronali
3) il candidato legga e traduca il brano estratto dal seguente articolo: Patriarchi T, Cho JR, Merten K, Howe MW, Marley A, Xiong WH, Folk RW, Broussard GJ, Liang R, Jang MJ, Zhong H, Dombeck D, von Zastrow M, Nimmerjahn A, Gradinaru V, Williams JT, Tian L. Ultrafast neuronal imaging of dopamine dynamics with designed genetically encoded sensors. Science. 2018 Jun 29;360(6396):eaat4422. doi: 10.1126/science.aat4422. Epub 2018 May 31. PMID: 29853555; PMCID: PMC6287765.

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Consiglio Nazionale delle Ricerche

RESEARCH ARTICLE

NEUROSCIENCE

Ultrafast neuronal imaging of dopamine dynamics with designed genetically encoded sensors

Tommaso Patriarchi[1]*, Jounhong Ryan Cho[2]*, Katharina Merten[3], Mark W. Howe[4]†, Aaron Marley[5], Wei-Hong Xiong[6], Robert W. Folk[3], Gerard Joey Broussard[1], Ruqiang Liang[1], Min Jee Jang[2], Haining Zhong[6], Daniel Dombeck[4], Mark von Zastrow[5], Axel Nimmerjahn[3], Viviana Gradinaru[2], John T. Williams[6], Lin Tian[1]‡

Neuromodulatory systems exert profound influences on brain function. Understanding how these systems modify the operating mode of target circuits requires spatiotemporally precise measurement of neuromodulator release. We developed dLight1, an intensity-based genetically encoded dopamine indicator, to enable optical recording of dopamine dynamics with high spatiotemporal resolution in behaving mice. We demonstrated the utility of dLight1 by imaging dopamine dynamics simultaneously with pharmacological manipulation, electrophysiological or optogenetic stimulation, and calcium imaging of local neuronal activity. dLight1 enabled chronic tracking of learning-induced changes in millisecond dopamine transients in mouse striatum. Further, we used dLight1 to image spatially distinct, functionally heterogeneous dopamine transients relevant to learning and motor control in mouse cortex. We also validated our sensor design platform for developing norepinephrine, serotonin, melatonin, and opioid neuropeptide indicators.

Animal behavior is influenced by the release of neuromodulators such as dopamine (DA), which signal behavioral variables that are relevant to the functioning of circuits brainwide. Projections from dopaminergic nuclei to the striatum and cortex, for example, play important roles in reinforcement learning, decision-making, and motor control. Loss of DA or dysfunction of its target circuits has been linked to disorders such as Parkinson's disease, schizophrenia, and addiction (*1*–*3*).

Much work has been devoted to determining how neural representations of behavioral states are encoded in the firing patterns of neuromodulatory neurons (*4*–*9*), but very little is known about how the precise release of neuromodulators alters the function of their target circuits (*10*, *11*). To address this problem, an essential step is to monitor the spatiotemporal dynamics of neuromodulatory signals in target circuits while also measuring and manipulating the elements of the circuit during behavior.

Analytical techniques such as microdialysis and electrochemical microsensors have provided useful insights about neuromodulator presence (*12*, *13*) but suffer from poor spatial and/or temporal resolution and cannot be targeted to cells of interest. Optical approaches such as injected cell-based systems (CNiFERs) (*14*) and reporter gene–based iTango (*15*) can reveal DA release with high molecular specificity. However, these systems are limited by poor temporal resolution (seconds to hours), preventing direct detection of DA release events that occur on a subsecond time scale (*16*, *17*).

High-quality single fluorescence protein (FP)–based sensors that report calcium or glutamate transients with subsecond temporal resolution have recently been developed and are widely used (*18*, *19*). Here, we report the development of a set of single FP–based DA sensors, named dLight1, that enables imaging of DA transients with high spatiotemporal resolution in behaving animals.

Sensor engineering

Sensitive optical readout of changes in DA concentration was achieved by directly coupling the DA binding–induced conformational changes in human DA receptors to changes in the fluorescence intensity of circularly permuted green fluorescent protein (cpGFP). We did this by replacing the third intracellular loop (IL3) of the human dopamine D1 receptor (DRD1), D2 receptor (DRD2), and D4 receptor (DRD4) with a cpGFP module from the genetically encoded calcium indicator GCaMP6 (Fig. 1A).

To determine the insertion site of cpGFP in IL3 that produces maximal coupling of ligand-induced conformational changes to cpGFP fluorescence, we aligned the sequences of DRD1 and DRD4 with that of the β_2 adrenergic receptor (B2AR) (Fig. 1B), for which both active and inactive structure are available (*20*). The initial variant, obtained by inserting a cpGFP module with original linker sequences (LSSLE-cpGFP-LPDQL) between Lys^{232} and Lys^{269} of DRD1, was well expressed at the plasma membrane of human embryonic kidney (HEK293) cells and showed a fluorescence decrease ($\Delta F/F_{max}$ = −19.4 ± 0.02%) in response to puffed DA (fig. S1A). To obtain a positive-response sensor, we screened a library of 585 variants in HEK cells (Fig. 1C and fig. S1B). The variant with the largest positive fluorescence response (max $\Delta F/F_{max}$ = 230 ± 9%) and excellent membrane localization was named dLight1.1 (Fig. 1D). In situ DA titration on HEK cells revealed submicromolar apparent affinity of dLight1.1 (affinity constant K_d = 330 ± 30 nM; Fig. 1E).

We next sought to further tune the dynamic range and affinity of the sensor. Mutation of Phe^{129}, a highly conserved residue among many G protein–coupled receptors (GPCRs) (*21*), into Ala (dLight1.2) slightly increased dynamic range (max $\Delta F/F_{max}$ = 340 ± 20%, K_d = 770 ± 10 nM; Fig. 1, D and E). Optimizing the cpGFP insertion site in dLight1.1 and dLight1.2 (fig. S1, C to G) greatly increased the dynamic range but also reduced the affinity to micromolar range (dLight1.3a: $\Delta F/F_{max}$ = 660 ± 30%, K_d = 2300 ± 20 nM, fig. S2, A and B; dLight1.3b: $\Delta F/F_{max}$ = 930 ± 30%, K_d = 1680 ± 10 nM; Fig. 1, D and E). Insertion of the cpGFP module into DRD4 and DRD2 produced dLight1.4 and dLight1.5, respectively, which exhibited nanomolar affinity with a relatively small dynamic range [dLight1.4: $\Delta F/F_{max}$ = 170 ± 10%, K_d = 4.1 ± 0.2 nM, Fig. 1, B, D, and E; dLight1.5: DA, $\Delta F/F_{max}$ = 180 ± 10%, K_d = 110 ± 10 nM; quinpirole (synthetic agonist of D2 dopamine receptors), $\Delta F/F_{max}$ = 124 ± 19%, fig. S2, A to C]. In addition, we engineered a control sensor by incorporating a D103A mutation in dLight1.1 to abolish DA binding (control sensor: $\Delta F/F$ = 0.4 ± 4%, Fig. 1E) (*22*). Because dLight1.1 and dLight1.2 produced large responses at low DA concentration (e.g., 100 nM) without approaching response saturation (Fig. 1E, inset) and had submicromolar affinity, we further characterized these two sensors.

Sensor characterization

These two sensors showed peak emissions at 516 nm and 920 nm for one- and two-photon illumination in HEK cells, respectively (fig. S3). In situ titration on dissociated hippocampal neurons and on HEK293 cells showed similar apparent affinities to DA (Fig. 1E and fig. S4, A to C). Single 5-ms pulses of uncaged DA were robustly detected on the dendrites of cultured neurons, and the fluorescence response tracked uncaging pulse duration (fig. S4, D to F). In cultured

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BUSTA 2

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RESEARCH ARTICLE

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