29th PhotoIUPAC
IUPAC Symposium on Photochemistry
14-19 july Valencia 2024

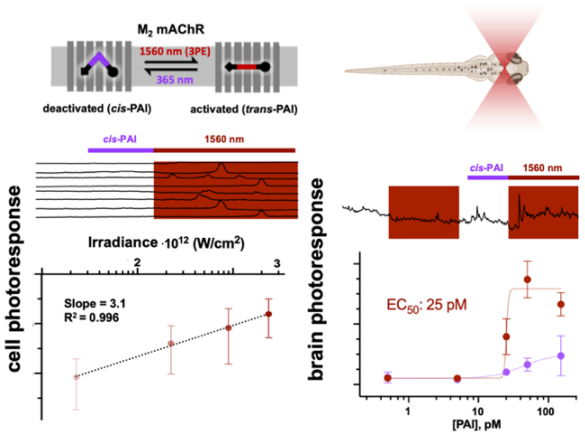
Three-Photon Infrared Stimulation of Endogenous Neuroreceptors in Vivo

Rosalba Sortino[1,2]; Marina Cunquero[3]; Gustavo Castro-Olvera[3]; Ricard Gelabert[4]; Miquel Moreno[4]; Fabio Riefolo[1,2,#]; Carlo Matera[1,2,%]; Noèlia Fernàndez-Castillo[2,5,6,7]; Luca Agnetta[8]; Michael Decker[8]; José M. Lluch[4,9]; Jordi Hernando[4]; Pablo Loza-Alvarez[3,*]; Pau Gorostiza[1,2,10,*]

*[1]Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute for Science and Technology, Barcelona, 08028, Spain. [2]CIBER-BBN, ISCIII, Madrid, 28029, Spain. [3]ICFO - Institut de Ciencies Fotoniques, The Barcelona Institute of Science and Technology, Castelldefels (Barcelona), 08860 Spain. [4]Departament de Química, Universitat Autònoma de Barcelona (UAB), Bellaterra, 08193, Spain. [5]Departament de Genètica, Microbiologia i Estadística, Facultat de Biologia, Universitat de Barcelona, Barcelona, 08028, Spain. [6]Institut de Biomedicina de la Universitat de Barcelona (IBUB), Barcelona, 08028, Spain. [7]Institut de Recerca Sant Joan de Déu (IRSJD), Esplugues de Llobregat, 08950, Spain. [8]Pharmaceutical and Medicinal Chemistry, Institute of Pharmacy and Food Chemistry, Ludwig Maximilian University of Würzburg, Würzburg, 97074, Germany. [9]Institut de Biotecnologia i de Biomedicina (IBB), UAB, Bellaterra, 08193, Spain. [10]Catalan Institution of Research and Advanced Studies (ICREA), Barcelona, 08010, Spain. [#]Current address: Teamit Institute, Partnerships, Barcelona Health Hub, Barcelona, 08025, Spain. [%]Current address: Department of Pharmaceutical Sciences, University of Milan, Milan, 20133, Italy. *Corresponding authors. E-mail: pablo.loza@icfo.eu (P. L. A.), pau@icrea.cat (P. G.).*

Abstract:

To interrogate neural circuits and crack their codes, *in vivo* brain activity imaging must be combined with spatiotemporally precise stimulation in three dimensions using genetic or pharmacological specificity. This challenge requires deep penetration and focusing as provided by infrared light and multiphoton excitation, and has promoted two-photon photopharmacology[1] and optogenetics[2]. However, three-photon brain stimulation *in vivo* remains to be demonstrated. We report the regulation of neuronal activity in zebrafish larvae by three-photon excitation of a photoswitchable muscarinic agonist at 50 pM, a billion-fold lower concentration than used for uncaging, and with mid-infrared light of 1560 nm, the longest reported photoswitch wavelength. Robust, physiologically relevant photoresponses allow modulating brain activity in wild-type animals with spatiotemporal and pharmacological precision. Computational calculations predict that azobenzene-based ligands have high three-photon absorption cross-section and can be used directly with pulsed infrared light. The expansion of three-photon pharmacology will deeply impact basic neurobiology and neuromodulation phototherapies.



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