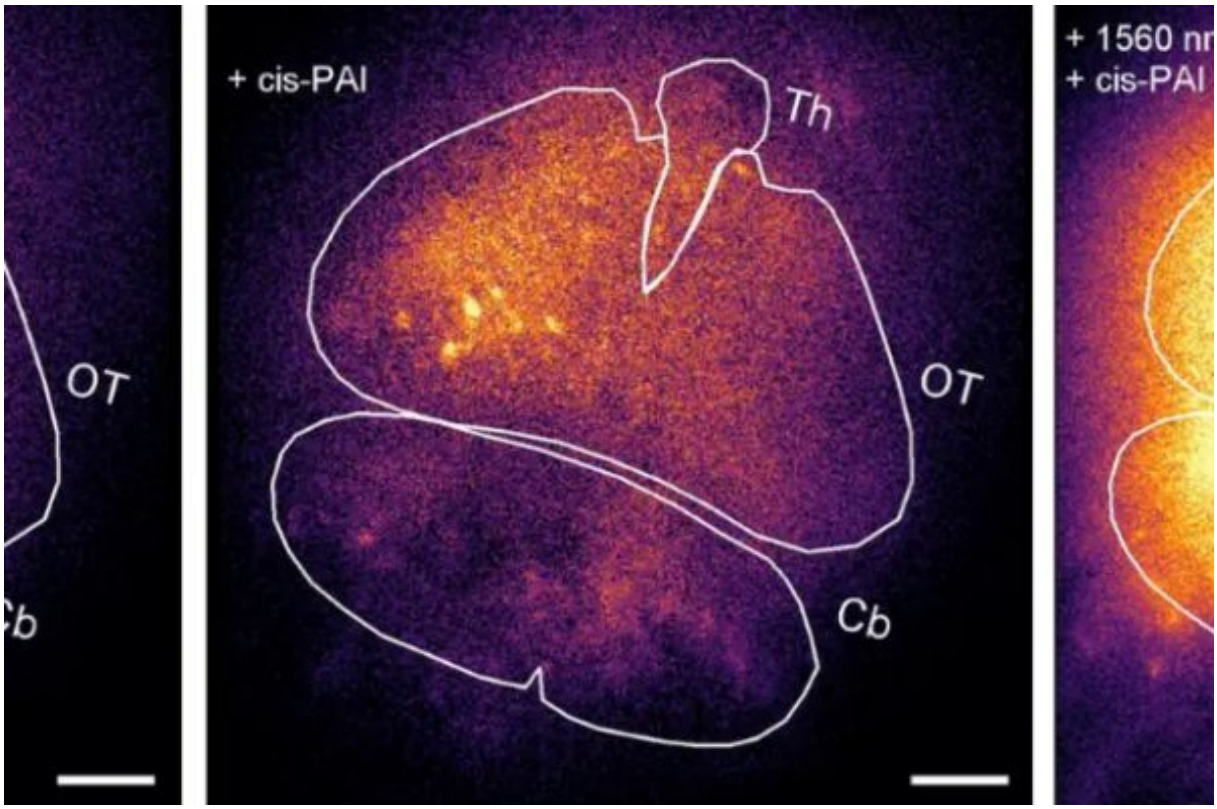

Researchers induce brain activation using infrared light-controlled drugs

A pioneering study led by IBEC in collaboration with ICFO has unveiled a method to control brain activity in living organisms using drugs activated by infrared light. This cutting-edge technique activates a specific neurotransmitter receptor using light that can penetrate deep into tissue and offers unparalleled pharmacological and spatiotemporal precision in three dimensions. The findings open new frontiers for neurobiology research and the development of light-based noninvasive neuromodulation therapies.

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Studying the brain remains one of the most challenging endeavors in neuroscience. Researchers have explored various methods for live imaging and stimulation of deep brain activity. One such method is multiphoton excitation using pulsed infrared (IR) light. This type of light is weakly absorbed by tissues and can penetrate through the bone and deep into organs like the brain. However, it has its limitations in producing focused images and

controlling cellular activity with precision. To overcome this, scientists have been exploring three-photon excitation with ultrafast pulsed IR light, to achieve whole cortex imaging and the observation of neuronal activity. Yet, until now, in-vivo neuronal stimulation using three-photon excitation has not been reported.

A groundbreaking study, led by the Institute for Bioengineering of Catalonia (IBEC) in collaboration with the ICFO - Institute of Photonic Sciences, both CERCA centers and members of BIST, has introduced the first method for controlling brain activity in living organisms using drugs activated by three-photon excitation and mid-infrared light. This method is based on activating a specific receptor for acetylcholine, a vital neurotransmitter involved in various brain processes such as learning, attention, and memory.

To achieve this, researchers utilized PAI, a light-responsive molecule previously developed at IBEC, employing the lowest drug concentration and the longest photoactivation wavelength ever recorded.

The study, published in Angewandte Chemie journal, was led by **Dr. Pau Gorostiza,** ICREA Research Professor and Leader of the Nanoprobes and nano switches group at IBEC and by **Dr. Pablo Loza-Alvarez**, head of the Super-resolution microscopy and Nanoscopy (SNL) lab at ICFO.

i¿½The novelty of these results lies in the demonstration that the activity of drugs can e controlled with IR light and that most of the photoswitchable ligands previously used n photopharmacology with ultraviolet and visible light are amenable to three-photon excitati n using mid-infrared light, which is gentler on tissues‰Explains Gorostiza.

i¿½Plus, this technique, being IR illumination, provides the opportunity to penetrate de p within the tissue with submicrometric resolutions in all three dimensions. In simple terms, t means we can pinpoint the activation right at the laser beam's focal point, illuminating t externally through the skull.i¿½Adds Loza-Alvarez.

These results showcase the potential of three-photon pharmacology and open up new horizons for fundamental research in neurobiology and the development of light-based neuromodulation therapies.

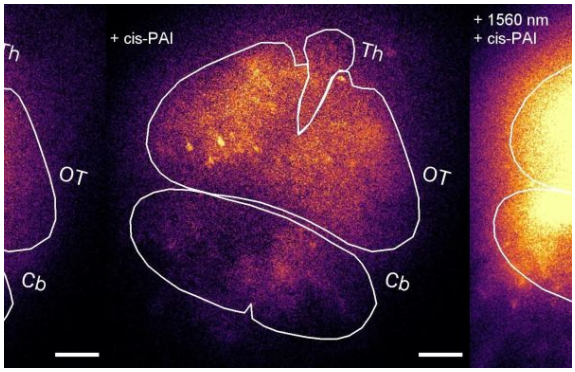
The power of light

Light can be harnessed to enable targeted drug action within specific areas of the body through photopharmacology. This innovative approach involves modifying the chemical structure of a drug by adding a light-activated molecular switch, ensuring the drug activates only when exposed to a particular color of light.

In previous research, IBEC scientists tried to activate PAI using one-photon and two-photon excitation with limited reversibility and control in vivo. In their recently published paper, researchers turned to longer wavelength IR light and theoretical calculations to enhance the activation of PAI through multi-photon excitations. These calculations, performed by the laboratory of Josep Maria Lluch and Jordi Hernando at the Autonomous University of

Barcelona suggested that three-photon excitation could be more efficient than the two-photon alternatives and that the principle could be widely applicable.
Subsequently, the researchers conducted in vivo experiments to validate these theoretical predictions. They used zebrafish larvae genetically engineered to express a fluorescent calcium indicator. Neural activity leads to rapid fluctuations in intracellular free calcium, which is why calcium indicators are commonly employed to visualize neural activity.
This experimental setup allowed them to observe and analyze alterations in calcium levels when the brain was exposed to light stimulation through three-photon excitation: i¿½The fir t time we saw the brain responses we were really impressed. We administered the P I compound in its inactive form, which was overtaken by the zebrafish brain, and when e exited it with the three-photon flashes, the entire brain lit up. Using our setup, we cou d clearly see the PAI activation as changes in brain activity,i¿½highlights **Rosalba Sortino**, first author of the study and recently doctorated researcher from Gorostiza's group at IBEC.
In their future work, researchers plan to apply even shorter and more focused stimulation to study these endogenous responses in detail.

Zebra fish larvae



Zebrafish larvae brain activation with PAI after light stimulation