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Dynamics of acetylcholine release in mouse visual cortex: from resting state to virtual environment sense.

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Aims: Acetylcholine (ACh) controls computation of visual inputs and attentional processes in the visual cortex [1]. Thanks to innovative imaging probes [2], the spatiotemporal release of ACh from basalocortical projections can now be mapped in the cortex during visually guided behaviors. In this study, spatial and temporal ACh release dynamics were measured in gACh 3.0 mice using mesoscopic imaging during resting state, gold-standard visual stimulation and presentation of a virtual reality (VR) dynamic environment.

Methods: 3-4 months old C57/BL6 mice received a 4 µl icv injection of AAV9-hSYN-ACh4.3 (gACh3.0) provided by Dr. Y. Li (Peking University). ACh release was measured in head-fixed awake mice (n=8) using mesoscopic imaging during resting state or in response to bilateral presentation of sinusoidal gratings of various contrast or moving dots (RDK) of various motion coherence or a Unity-generated VR sequence in motion. The VR sequence simulated a journey in the cage environment with different shelters and a salient sinusoidal grating appearing in the upper binocular visual field. Amplitude responses (dF/F) were evaluated at the level of the primary visual cortex (V1), and secondary areas (PM, AL, LM) using Matlab Umit Toolbox, and compared by 2-way ANOVA.

Results: During resting state, ACh signals were correlated between AL and LM or PM and V1 but there were no obvious correlations between hemispheres. During grating or RDK stimulation, most visual areas exhibited activation up to 60% in V1 for ACh release in a contrast-dependent manner. There was no adjustment of these signals for levels of motion coherence during RDK presentation. VR triggered significant ACh release across visual, motor, sensorimotor, and associative areas at different time points.

Conclusion: The research addressed in this study aimed to determine how the ACh release was adjusted to visual stimulus in the visual cortex including during resting state. ACh release was evoked throughout the cortical mantle but particularly in the visual cortex by the dynamic VR sequence, whereas it was more limited to the visual areas in response to standard visual stimuli. This observation indicates an important role of ACh in visual perception.

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Optogenetic Activation of Prefrontal Circuits via Upconversion Technology for Depression Treatment

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Depression involves dysregulated brain circuits [1.2], but identifying the most critical target regions remains a challenge. Through brain-wide screening, we found that c-fos expression in the prefrontal cortex (PFC) exhibited the most significant changes among various regions and that the PFC serves as the downstream hub in depression-related neural circuits [3]. We therefore targeted the PFC using a viral vector approach combined with upconversion-based optogenetics to investigate its therapeutic potential.

Using a chronic unpredictable mild stress (CUMS) mouse model [4], our findings revealed that optogenetic stimulation of the PFC significantly alleviated depressive-like behaviors, including increased sucrose preference and reduced immobility in the forced swim test. Laser speckle imaging demonstrated enhanced cerebral blood flow in the PFC, while immunohistochemistry confirmed restored neuronal activity as evidenced by elevated c-fos expression. This study highlights the potential of precise circuit modulation in the PFC as a novel intervention for depression and underscores the importance of upconversion technology in advancing optogenetic research.

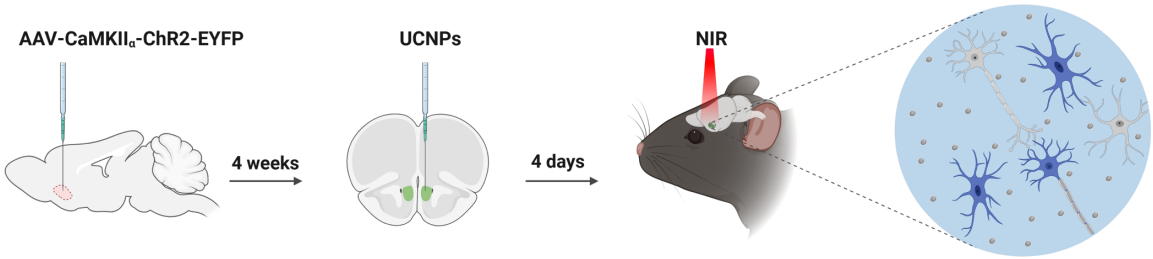

***Fig. 1**: Viral delivery and optogenetic stimulation setup targeting the PFC in a CUMS mouse model.*

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P3

Photopharmacological control of biased signaling by the serotonin 2A receptor: Novel tools to study hallucinogenesis and antidepressant properties of psychedelics

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Serotonin (5-hydroxytryptamine, 5-HT) is an important modulatory neurotransmitter involved in mood, sleep, cognition and other vital functions. Among the numerous different serotonin receptor subtypes, the serotonin 2A receptor (5HT2AR) is the main target for psychedelics, which can induce hallucinations but also in some instances show promise as rapid acting antidepressants. The underlying intracellular signaling pathways responsible for hallucinations and the antidepressant effects are poorly understood.

Photopharmacology allows optical control over pharmacological properties. It holds the promise to allow activation of specific serotonin receptors with high spatiotemporal control *in vivo* to enable highly controlled studies of where and how 5HT2AR must be activated to elicit hallucinations and/or antidepressant effects.

We here describe the development and detailed pharmacological characterization of a novel set of 5HT2AR agonists with the ability to be activated and to become biased agonists upon demand using light. Our studies also shed light onto aspects of how biased agonism is achieved on a molecular level.

Illuminating Insights – how well plate material influences light stimulation efficiency

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Investigations into photosensory receptors and other light-dependent research domains demand rigorous attention to the optical properties of experimental apparatuses, including multiwell plates.
Our study critically evaluates the transmittance characteristics of a variety of multiwell plates, which are pivotal in light-based experiments, mapping their combined absorption and scattering traits across the UV-VIS spectrum.
We demonstrate that although transmittance remains comparably high above 450 nm, considerable differences are observed at lower wavelengths. Some plates show exceptionally high transmittance, making them suitable for sensitive applications, whereas other plates show significant light loss at lower wavelengths. Importantly, micro-structured well plates can decrease transmittance across the whole UV-Vis spectrum by up to 50%.
In response to these findings, we have integrated the transmittance data into an innovative illumination platform that ensures consistent and even illumination across different well plate types. This platform, complemented by a computational tool, enables researchers to adapt light dosages to accommodate the unique optical properties of each plate. This advance is crucial for researchers across diverse disciplines where light plays a pivotal role, ensuring that experimental conditions are reproducible and results are reliable.
The study emphasizes the need for meticulous recalibration of lighting when altering well plate types and sets a foundation for further research on the impact of various experimental media on light transmittance, thereby enhancing the precision of light-based research methodologies.

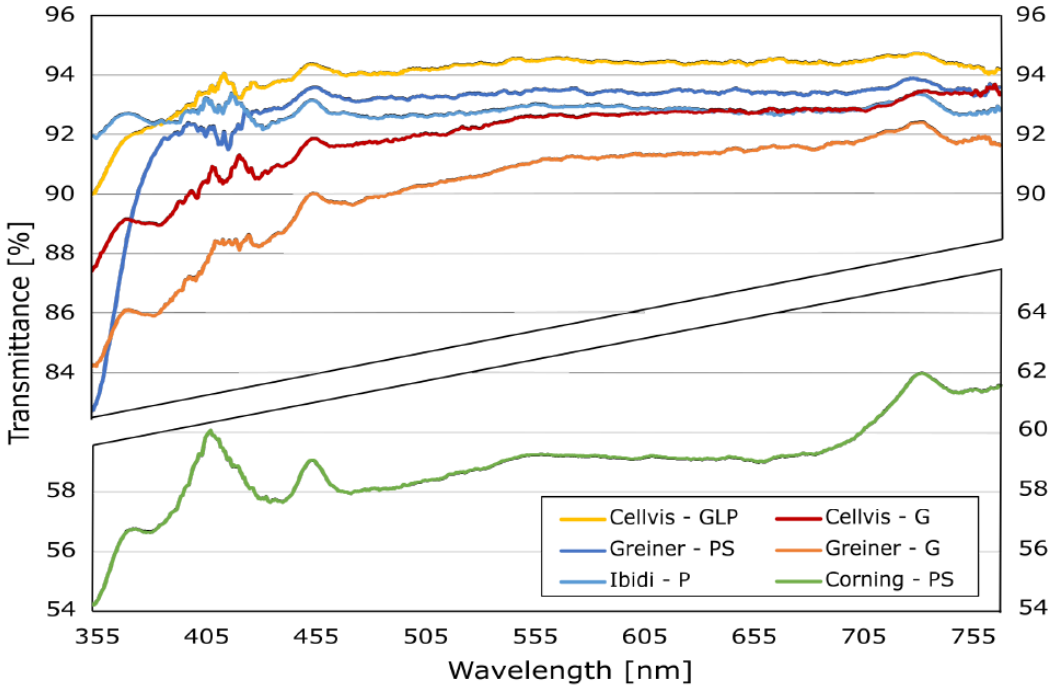

Figure 6. Transmittance through the well plate bottom from 350 nm to 770 nm for different well plate types, averaged from three independent experiments.

Mechanical analysis of microstructured polymer fiber neural interfaces

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Polymer optical fiber (POF)-based neural interfaces are widely used in central nervous system optogenetics due to their flexibility, which reduces inflammation compared to silica glass fibers [1–3]. However, their high flexibility also makes them susceptible to buckling failure during implantation [4]. Integrating functional microstructures like microfluidic channels (MC) and electrodes further complicates their mechanical properties [5–9]. This study employs the finite element method to analyze the critical load factor and bending stiffness of POFs, aiming to provide design guidelines for POF-based interfaces to prevent buckling while maintaining flexibility to reduce inflammation.

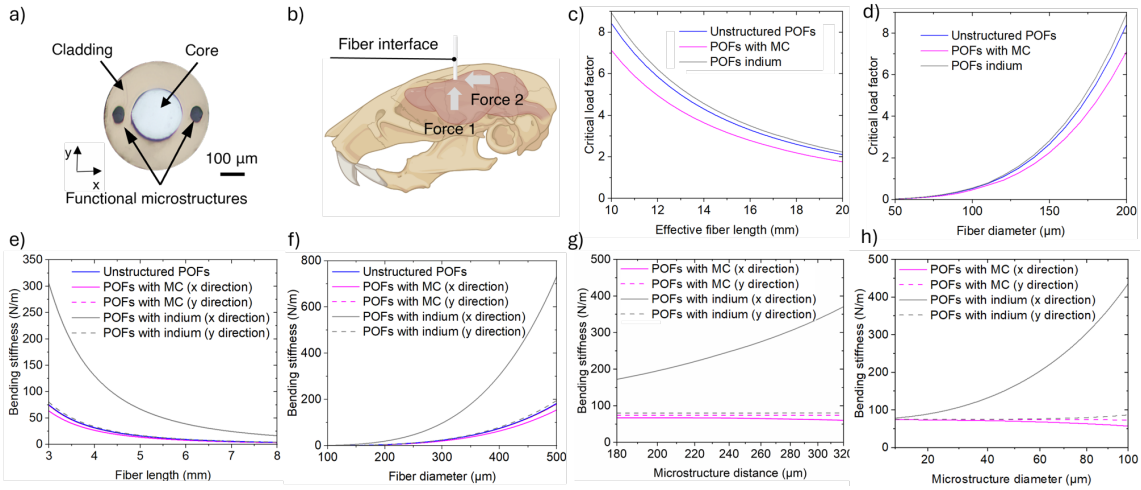

***Fig. 1**: a) Cross section of the microstructured POFs. b) Force applied to the POFs during (force 1) and after (force 2) implantation in mechanical analysis. Critical load factor as a function of effective fiber length (e), fiber diameter(f), microstructure distance (g), and microstructure diameter (h).*

Acknowledgments

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AMPA receptor targeted Au bipyramids immobilization on rat primary CTX neurons for photothermal neuromodulation

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Abstract. Plasmonic nanoparticles offer a promising avenue for neuronal modulation by converting light into localized heat. The rapid temperature changes at the nanoscale can alter neuronal excitability through mechanisms such as membrane capacitance shifts and ion channel modulation. In this study, we present experimental evidence on the immobilization of gold bipyramids (AuBPs)[1] onto AMPA receptors (AMPARs) and their application in neuromodulation via infrared (IR) laser stimulation. Selective targeting of AMPARs was achieved through a bioorthogonal two-step functionalization process,[2] enabling the stable immobilization of AuBPs on the neuronal membrane, as confirmed by transmission electron microscopy (TEM) analysis. This precise localization allowed for controlled photothermal stimulation using a 785 nm IR laser coupled to a multielectrode array system. We found that neuronal activity was modulated as a function of laser pulse duration and the delivered power. This approach enables precise and adjustable neuromodulation, paving the way for targeted neural interfaces and optical neurotechnologies.

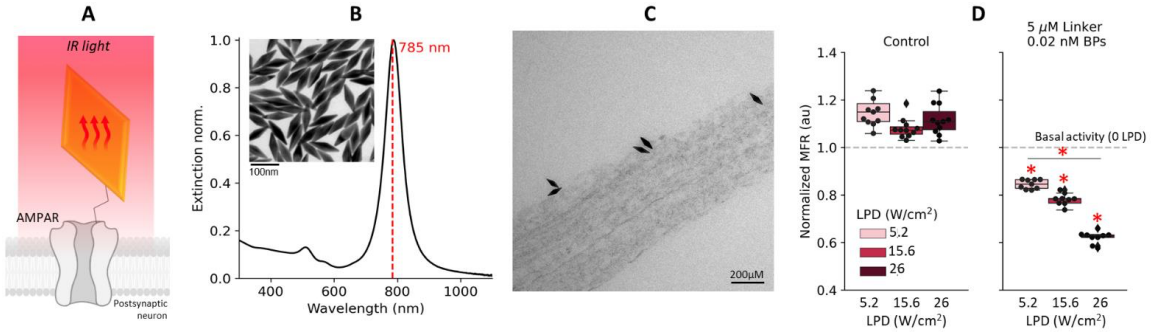

Figure 1. A) Immobilization of AuBPs to the AMPAR for photothermal modulation of the activity trough IR light irradiation. B) The AuBPs exhibit the peak of the localized surface plasmon resonance band at 790 nm, and are 100 ± 2 nm in length and 32 ± 1 nm in width, calculated by TEM imaging. C) TEM images of primary rat cortical neurons incubated with AMPAr linker for 1h and AuBPs for 30min. D) MFR response with increasing laser power density at 100ms pulses duration. Measurements are normalized to the initial activity of each electrode in the well where the linker/Au and laser treatment is applied.

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Implantable neural interfaces for the central nervous system based on multifunctional optical fibers

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As optical methods for interrogating neurons such as optogenetics and infrared neural stimulation are gaining traction in the neuroscience community, multifunctional optical fibers have emerged as a powerful tool for a broad range of investigations within the central nervous system [1–3]. Polymer optical fibers (POFs), in particular, have gained traction as a promising platform for developing implants targeted at chronic *in vivo* experiments since the wide range of available polymers allow to precisely tailor the optical and mechanical properties of POFs for the intended application, while their low bending stiffness with respect to standard silica glass reduces the foreign body response from the surrounding tissue [4,5]. Here, we present our work on the fabrication and *in vivo* validation of multifunctional POFs for neuromodulation, drug delivery, electrical interrogation and sensing [6–11].

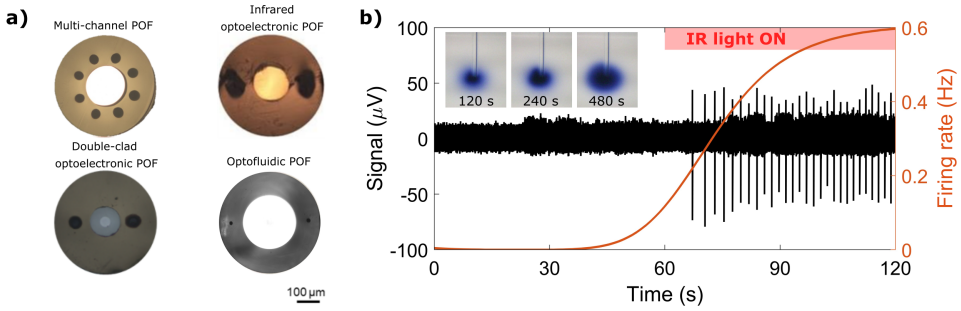

***Fig. 1**: a) Microscope images of different multifunctional POFs; b) Infrared neural stimulation and electrophysiology through an optoelectronic POF (inset: fluid delivery through an optofluidic POF).*

Acknowledgments

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Evaluation of AAV-Mediated Optogenetic Expression in Human Retinal Organoids

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Over the past two decades, multiple pre-clinical have been used to develop optogenetic therapies to treat retinal disease [1]. Many of these studies have shown promising proof-of-concepts for optogenetic vision restoration [2]. However, the models have limitations which have prohibited the successful translation of these outcomes in large animal models, and to date, none have been recapitulated in clinical trials. This study aimed to develop retinal organoids as a viable model to evaluate optogenetic tools in translation to human trials.

Human retinal organoids were treated with optogenes tagged with fluorescent markers, coupled to the CAG promoter and packaged into AAV vectors. Optimisation studies were performed regarding vector dose and timing of vector application. Live cell imaging was performed using an EVOS Cell Imaging System on organoid cultures. Organoids were embedded in gelatin blocks, and sections were obtained using a cryostat and mounted on slides. Immunofluorescence was observed using a confocal microscope. An ATP assay was performed using the Cell Viability Kit.

Gene expression steadily increased over four weeks. For each capsid, AAV2.2, AAV2.5 and AAV2.7M8, the expression was dose-dependent, with a higher dose (1E+11) resulting in greater transduction efficiency than the lower dose (1E+10), with AAV2.7M8 leading to the highest level of expression. Notably, strong expression was observed in retinal ganglion cells and bipolar cells, as indicated by co-staining with cell-specific markers. The ATP cell viability assay showed that the transgene expression did not make a significant contribution to the viability of retinal organoids (97%) compared to untreated organoids (100%) ($P = 0.87$, $n = 5$).

We recommend human-derived retinal organoids as a promising model to assess optogene expression patterns and their impact on cell viability. In organoid transduction, the efficacy depends upon the AAV capsid type and vector dose, with AAV2.7M8 leading to the strongest expression from the capsids we tested. AAV-mediated human rhodopsin expression in retinal organoids did not affect retinal organoids' viability, indicating good initial safety data for this optogene for further translational studies.

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POT: an optogenetics-based endogenous protein degradation system

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Precise regulation of cellular protein abundances is important for maintaining normal cell functions, and whose dysfunction is prone to diverse diseases in human. Optogenetics allows rapid and reversible control of precisely defined cellular processes, which has the potential to be utilized for regulation of protein dynamics at various scales. Here, we developed a novel optogenetics-based protein degradation system, namely Peptide-mediated OptoTrim-Away (POT) which employs expressed small peptides to effectively target endogenous and unmodified proteins. By engineering the light-induced oligomerization of the E3 ligase TRIM21[1], POT can rapidly trigger protein degradation via the proteasomal pathway. We chose phosphoinositide 3-kinase (PI3K) 110α and glutathione peroxidase 4 (GPX4) as targets for their functions in cell have been well characterized, and both of them have no specific nanobodies available. Our results showed that the developed POT-PI3K and POT-GPX4 modules, which used the iSH2 [2] and FUNDC1 domains [3] to specifically PI3K and GPX4 respectively, were able to potently induce the degradation of these endogenous proteins by light. Both live-cell imaging and biochemical experiments validated the potency of these tools in downregulating cancer cell migration, proliferation and even promotion of cell apoptosis. Therefore, we believe the POT offers an alternative and practical solution for rapid manipulation of endogenous protein levels, and it could potentially be employed to dissect complex signaling pathways in cell and for targeted cellular therapies.

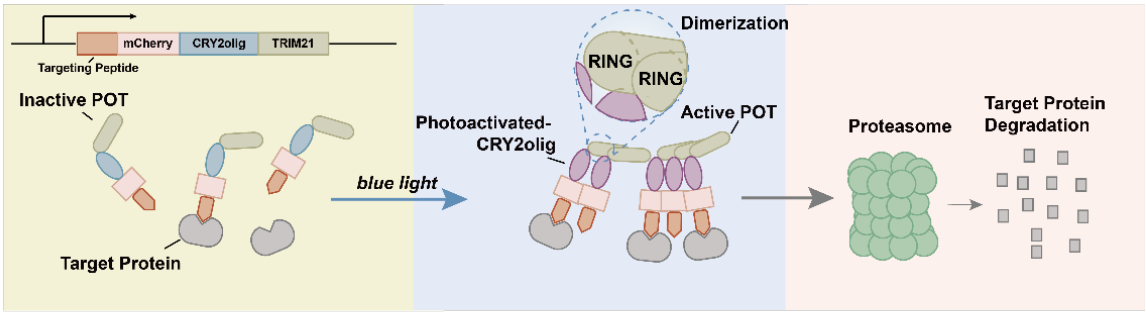

***Fig. 1**: **Development of an optogenetics-based tool for light-induced protein degradation.** Schematic of the POT system. An N-terminal peptide was used to target the specific protein of interests. Blue light illumination promotes the CRY2olig oligomerization, which induces the TRIM21-mediated protein degradation. Of note, the protein icons used in the figure are symbolic only and do not represent the actual size of the proteins.*

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Initial Insights into Direct Measurement of Dopamine Release in the Retina

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Dopamine is the most abundant catecholamine in the vertebrate retina, where it is released by dopaminergic amacrine cells located in the intermediate layers of the retina [1]. It acts both at synapses and through volumetric transmission, playing a crucial role in light-dark adaptation and circadian rhythm regulation [1]. Dopamine production and release are driven by illumination levels and follow a circadian pattern. The spiking activity of dopaminergic amacrine cells is also modulated by visual stimulation [2]. Moreover, dopamine has been implicated in the development of certain diseases, such as myopia. However, the precise light conditions that regulate its release remain unclear, largely due to the challenge of directly recording dopamine release. Recent advances in genetically encoded dopamine sensors, such as the GPCR-based GRABDA and dLight series, have enabled in vivo measurements of dopamine dynamics in mice[3,4].

In this study, we investigate dopamine dynamics in the retina under different light conditions using the genetically encoded sensors GRABDA, delivered via intravitreal injections to target retinal neurons. Dopamine responses were recorded in ganglion cell somas and dendrites within both the OFF and ON sublayers of the inner plexiform layer in the dorsal retina. Our preliminary results indicate that dopamine release occurs in response to light. Most responses were dominated by a sustained component, where even the lowest light intensity tested (2.68 $\mu W/cm^2$) was sufficient to trigger a response. Notably, these responses persisted despite the blockade of photoreceptor transmission, suggesting a potential contribution of melanopsin-expressing ganglion cells to dopamine release.

These findings confirm the successful expression of GRABDA in the retina, enabling real-time measurement of light-induced dopamine release. Future experiments will further investigate the mechanisms underlying this release by testing various visual stimuli and imaging the release on specific retinal cell types.

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The optogenetic potential of photocyclic rhodopsin

P11

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Vertebrate rhodopsins are photosensitive proteins which have been widely used in the development of optogenetic tools. One of the main limitations of opsins is the requirement of 11-*cis* retinal replenishment. Sakai and co-workers identified a single amino acid mutation from glycine to cysteine at position 188 (G188C) in bovine rhodopsin overcomes this limitation [1]. G188C mutation leads to photoconversion to the active state, which then thermally relaxes back to the dark state, revealing photocyclic behaviour in bovine rhodopsin and bypassing the need for retinal replenishment [1, 2]. To explore its potential as an optogenetic tool, we introduced the G188C mutation into human rhodopsin. Multiple G188C variants of human rhodopsin exhibited robust photocyclic activity, maintaining stable activation across repeated light stimulations in HEK-29 and Neuro2A cell lines [3, 4, 5]. Furthermore, human rhodopsin G188C based chimeras were developed, which retained the photocyclic behaviour of G188C mutation and the G-protein activation profile of the endogenous receptor. These findings highlight the G188C mutant of human rhodopsin as a promising optogenetic tool for answering physiologically relevant questions due to its advantages for *in vivo* application.

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OLEDs: The Cornerstone for Next-Generation Neural Interfaces

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Light-based neurostimulation aims to improve spatial resolution and specificity for targeted neuronal activation. However, precise delivery of light in tissue remains challenging. OLEDs offer a compelling solution due to their thin film nature, enabling direct integration onto a wide range of substrates, from CMOS backplanes to flexible neuroprobes. OLEDs can be fabricated with different form factors, one of them being µm-sized, individually addressable pixels, allowing high-density, multiplexed stimulation with minimal heat generation and low driving voltages. Their spectrally tunable emission enhances compatibility with various opsins, while their scalability and conformability make them an adaptable building block for neurotechnology. While achieving the brightness levels required for optogenetics (0.1 to several mW/mm²) remains challenging, recent advancements have allowed several breakthroughs.

We present the direct integration of both high brightness orange and blue top-emitting OLEDs onto CMOS-based neuroimplants, featuring four-shank architectures with 1024 addressable pixels (20×20 µm², 24.5 µm pitch), achieving stable brightness levels of 0.2 mW/mm² and a >90% pixel yield through optimized plasma-based surface treatments.[1] This advancement enables, for the first time, in vivo single-OLED/single-neuron stimulation with CMOS implants, addressing a critical technological gap for precise optical neuromodulation.[2] Furthermore, the OLED fabrication process is seamlessly adapted to alternative substrates, including flexible polyimide-based neuroprobes originally designed for electrophysiology. We also report magnetoelectric platforms for wireless neurostimulation.[3] These examples of modular integration of OLEDs demonstrate the value of this technology as a versatile and scalable toolset for next-generation optoelectronic neural interfaces.

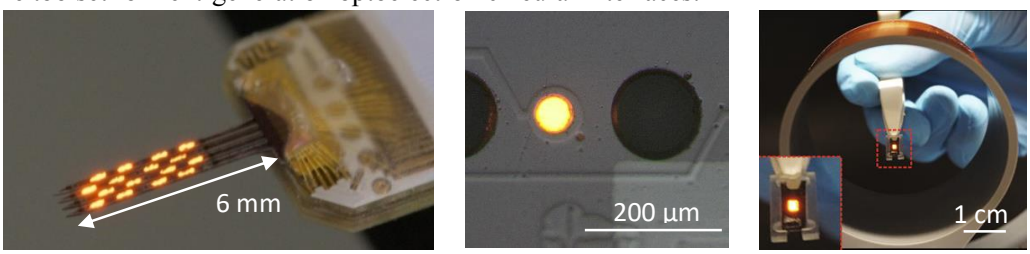

Figure 1 a) OLED-on-CMOS for optogenetics, photograph of neuroimplant with 512 out of the 1,024 OLEDs activated over the four needles in a chequerboard pattern. b) Commercial electrode array for electrophysiology with 32 electrodes at a pitch of 300 µm and with a diameter of 50 µm each, repurposed for optogenetics by integration of OLEDs. Microscopy image showing one active OLED pixel with orange emission. c) Photograph of a wirelessly operated magnetoelectric OLED.

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Potassium-selective channelrhodopsins can exert hyper- or depolarizing effects in excitable cells of *Caenorhabditis elegans*

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The collection of optogenetic tools used for the control of excitation and inhibition of muscles and neurons is ever-increasing, but their functionality and applicability can differ between different model organisms. Our work focused on the study and characterization of potassium-selective channelrhodopsins (KCRs) in excitable cells of the nematode *Caenorhabditis elegans*. We focused on HcKCR1 and WiChR, which were suggested in previous studies to inhibit excitation through hyperpolarization by inducing potassium efflux [1,2]. Through body length assays we found that, upon light-induced stimulation of channel opening, they induce only brief hyperpolarization followed by longer and persistent depolarization in body wall muscles of transgenic worms. These findings are also supported by the results of electrophysiological measurements. This might be caused by the high conductivity of the channels causing macroscopic changes in the ionic gradients over the membrane, causing an initial, intense potassium efflux, which, when ceased, is followed by sodium influx through the channels as a secondary effect. Consequently, we found that lower expression strength, decreased light intensities, wavelengths that differ from the absorption maximum and pulsed instead of continuous illumination, all of which would be expected to minimize the stimulation and thus the membrane conductance mediated by the light-gated channels, increase the hyperpolarizing ability and prevent or decrease the intensity of the subsequent depolarization [3].

In collaboration with Dr. Shiqiang Gao (University Würzburg, Germany), we are testing HcKCR1 mutants that were found to reduce the depolarizing tendency of the channel in *Drosophila*. These mutations also appear to improve the hyperpolarizing abilities in *C. elegans* in initial experiments.

Establishing KCRs provides an important addition to the optogenetic toolbox, as it allows direct access to mediating fluxes of potassium, the main ion used for by cells for repolarization and for defining their resting membrane potential.

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Laminae specific optogenetic perturbation of frontotemporal circuits in macaques

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Perceptual inference is a fundamental function in humans and nonhuman animals, allowing the brain to construct meaningful representations of the environment. Predictive coding theories suggest that this process relies on internal models built by cortical microcircuits, the functional units of the neocortex. In these models, superficial layers in sensory cortex are thought to compute prediction errors and relay them to higher-order brain areas such as prefrontal cortex through gamma oscillations, while deeper layers in higher-order areas encode predictions that are feedback to sensory cortex through low-frequency oscillations. To probe these mechanisms, developing precise circuit-level manipulations with high spatial and temporal resolution is crucial, yet their application in macaque models remains challenging [1]. Here, we present preliminary findings from an awake macaque monkey, demonstrating optogenetic manipulation of single-neurons and local field potentials limited to specific cortical layers. The viral vector AAV9-CamKII-ChR2-eYFP was injected into the rostral part of the non-primary auditory cortex (rAC) and the ventrolateral prefrontal cortex (areas 44/45) to specifically target pyramidal neurons for channelrhodopsin (ChR2) light sensitive ion channel expression, activated by blue light. Single-unit activity and local field potentials (LFPs) were simultaneously recorded from the auditory and frontal cortices with 16-channel linear electrode arrays (Plexon S-probes) integrated with optical fibres. Stimulation with blue- (473 nm) or red- (635 nm) laser light was applied in one-second bursts at either theta (5 Hz) or gamma (40 Hz) frequencies, targeting either superficial or deeper cortical layers. In our preliminary results, we have identified single-units that exhibited modulation in response to optogenetic stimulation in the site of stimulation. These neurons fired action potentials at the same frequency as the applied stimulation and selectively for the blue, but not the red, laser light stimulation wavelength, providing direct evidence of targeted optogenetic activation at cellular resolution. Furthermore, similar wavelength-dependent changes were observed in the LFP power, specific to the frequency of stimulation. By regulating the laser light intensity, we were able to titrate the spatial specificity of these effects to certain sets of layers. Finally, we demonstrated that optogenetic manipulation of neurons in one region resulted in rapid changes in single-unit activity and LFP power within the interconnected region, providing novel neurophysiological evidence for excitatory connections between these two areas (2,3). These results highlight the ability to modulate activity with high spatiotemporal precision in single neurons across the cortical layers in a primate model, targeting either superficial or deeper layers of the cortex. This laminar resolution approach enables the dissection of cortical microcircuits, providing insights into fronto-temporal neuronal circuit motifs underlying perceptual inference in the primate brain.

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In vitro stability of polymer fibers for optical interfaces

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Introduction

In aiming towards chronic (>30 days) *in vivo* studies for neural implants, it is essential to not only evaluate biocompatibility, but also to understand degradation and failure mechanisms of implants and their modalities *in vitro*. This leads to a better understanding of what may occur *in vivo* and allows for evaluating reliability and performance of the device development process [1]. For polymer fiber-based optical neural interfaces, this entails evaluating the fiber's ability to deliver light over time.

Methods

Polymer fiber samples consisting of a polysulfone (PSU) core and a fluorinated ethylene propylene (FEP) cladding with four fluidic channels (Fig 1A) were stored in phosphate-buffered saline (PBS) at 37 °C (body temperature) and 60 °C (accelerated aging) and characterized over time. Chemical stability of the fibers was evaluated by acquiring scanning electron microscopy (SEM) images. Optical stability was then evaluated by transmission measurements at the fiber tip (Fig 1D).

Results

SEM images of fiber samples showed residue on the fiber surface, salt buildup in the interface between the core and cladding, and crystal formation within the fluidic channels (Fig 1B-C); thus, demonstrating a need for evaluating optical stability *in vitro*, since these changes can affect optical transmission.

Conclusion

Salt residue on the fiber surface and in the interface between the core and cladding contribute to optical losses, while crystal formation within fluidic channels would impede fluid delivery and need to be investigated further.

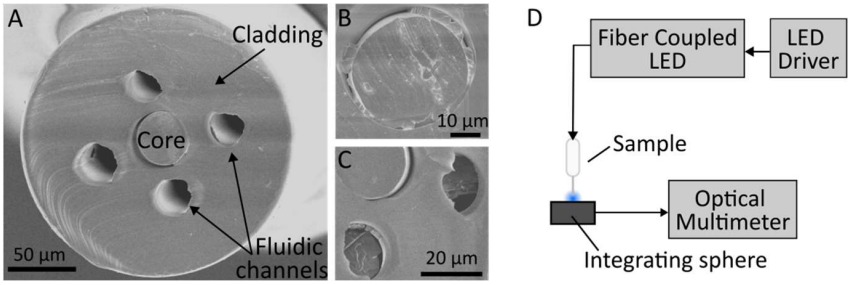

***Fig. 1**: (A-C) SEM images of fibers after storage in PBS, illustrated as A) reference after 1 day in PBS, B) buildup between the core and cladding observed after 34 days, C) salt crystal formation in channels after 42 days. D) Illustration of the optical measurement setup.*

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Funded by the European Union
Move2Treat

Evaluating the efficacy of nuclear receptor factors and optogenetics in axon regeneration

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Abstract

The spinal cord serves as critical pathway for transmitting signals between the brain and rest of the body. The spinal cord's complex networks regulate multidimensional motor functions in mammals but have limited regeneration capacity after injury that leads to permanent, irreversible functional impairment. Current therapeutic strategies often focus on isolated approaches, gene therapy to promote regenerative growth and behavioral modulation to leverage neuroplasticity for functional recovery [1,2]. Literature survey combined with our preliminary analyses suggest that nuclear receptor transcription factors (NRTFs) are critical for neural development, however their role in regulating regeneration in adults is unknown [3,4]. Therefore, we are now testing the hypothesis that combinations of NRTFs regulate axon growth across regeneration. To this end, we overexpressed selected NRTFs in in vivo model and assessed their impact in regeneration and sprouting. Though, NRTF-based gene therapy showed robust axon growth, behavioral functional recovery was limited. Possibly because of aberrant connections between pre-existing and newly formed axons. Therefore, we plan to employ Optogenetics based strategy to selectively stimulate regenerating axons and promote meaningful functional recovery. Ultimately, the outcome of our research holds promising implications for identifying innovative therapeutic targets, potentially enhancing regenerative outcomes and behavioral recovery post-neural injuries.

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OLED integration on electrode arrays for all optical stimulation and recording *in vivo*

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Optogenetics has achieved unprecedented precision through the genetic targeting of neurons, further enhanced by the recent advancements in opsins with significantly improved light sensitivity. Further advances in attaining even more precise and highly parallel stimulation of neuronal networks now require progress in the spatial resolution and versatility of the light-sources involved in optostimulation, especially when reaching deep into the brain.

Here, we present an innovative approach to high spatial resolution for optogenetic stimulation that is based on the monolithic integration of organic light-emitting diodes (OLEDs) on commercially available flexible electrophysiology probes. Due to their thin-film nature, OLEDs can be directly deposited onto many different substrates.[1,2] In the present work, we fabricate a total of 28 OLEDs, each with a diameter of 50 µm, on a 1.3 mm x 2.5 mm sized implantable flexible probe. The OLEDs can be driven in pulsed and burst mode at power densities sufficient for optogenetic stimulation. After OLED integration, each neuroprobe is encapsulated by an optimized chemical vapor based thin-film deposition process in order to render it biocompatible and stable for implantation.

The neuroprobe is implanted into the somatosensory cortex to stimulate red-shifted opsins like C1V1 and Crimson at different depth along the cortical column. Simultaneously, to achieve all optical stimulation and recording, GCaMP-based cellular Ca^{2+}-imaging [3] is carried out performing two-photon intravital microscopy in head-fixed mice. Our results demonstrate that OLED-based probes offer a versatile and scalable solution for next-generation optogenetic research, opening new avenues for spatiotemporally precise neuromodulation and readout in both fundamental and applied neuroscience.

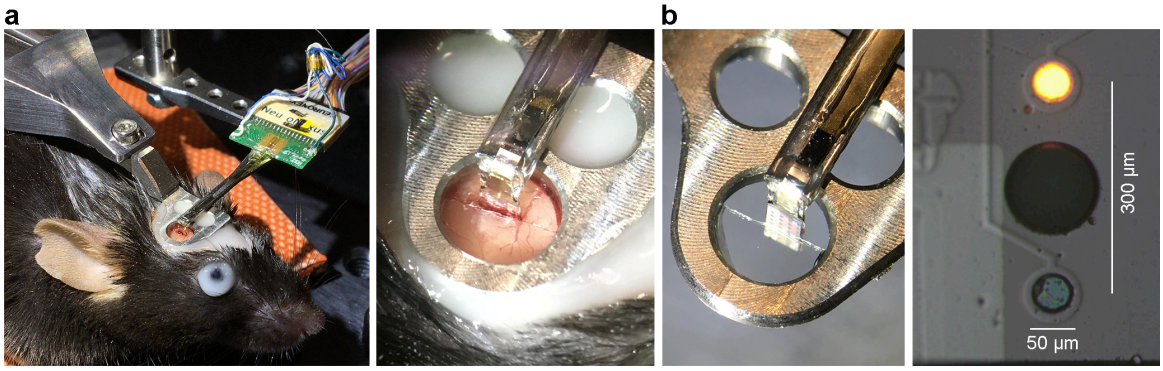

***Fig. 1**: a) Positioning of the probe in the cortex of the mouse (left) and zoom (right). b) Commercial electrode array for electrophysiology with 32 electrodes at a pitch of 300 µm and with a diameter of 50 µm each, repurposed for optogenetics by integration of OLEDs. Microscopy image showing one active OLED pixel with orange emission.*

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The dual role VIP+ inhibitory neurons in the retina

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A major challenge in the retina is to understand the role of the many types of amacrine cells (ACs), the main class of inhibitory interneurons, in shaping ganglion cell (GC) responses (Bae *et al*., 2018; Yan *et al*., 2020).

Recent studies have made significant strides in identifying and characterizing ACs expressing the vasoactive intestinal peptide (VIP+) (Zhu *et al*., 2014; Akrouh & Kerschensteiner, 2015; Park *et al*, 2015; Pérez de Sevilla Müller *et al*., 2019). Anatomical studies suggest these VIP+ ACs have both inhibitory GABAergic synapses and gap junctions (Park *et al*, 2015). However, we don't know how these two types of synapses can impact GC activity, and the role of VIP+ ACs in retinal processing remains unclear.

Here, we show that VIP+ ACs have a dual role in modulating GC activity: they activate specific types of GCs through gap junction to synchronize their activity and inhibit other types through their GABAergic synapses.

We expressed optogenetic proteins specifically in VIP+ ACs by injecting an AAV into a transgenic mouse (Zhu *et al*). We stimulated individual VIP+ ACs using 2-photon digital holography with high spatial resolution while recording the impact of this stimulation on GC activity with a multi-electrode array (MEA).

Our results show that VIP+ ACs have a double and opposite mode of action: they can individually suppress but also activate some types of ON and OFF GCs. We demonstrated that the inhibition of VIP ACs is mediated by GABAergic signalization through GABA-A receptors, and the excitation is mediated through direct gap junctions between VIP+ ACs and GCs. This particularity allows VIP+ cells to provide GCs with different types of signals to encode different visual features.

To determine how VIP+ ACs affect the visual response of GCs, we combined visual stimulation of the retina and 2-photon holographic stimulation. We demonstrated that VIP+ ACs synchronize some types of GC via their gap junctions, suggesting they play an important role in the processing of visual information in the retina. Finally, we also engineered a genetic mouse line where gap junctions were selectively knocked out in VIP+ cells. This reduced the level of synchronized activity between GCs of specific types. This shows that VIP+ interneurons do not only act through their GABAergic inhibition but also synchronize excitatory GCs through gap junctions. Our results uncover a new role for VIP+ cells beyond inhibiting excitatory neurons.

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Photoswitchable Carbamazepine Analogs for Non-Invasive Neuroinhibition *In Vivo*

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A problem of systemic pharmacotherapy is off-target activity, which causes adverse effects. Outstanding examples include neuroinhibitory medications like antiseizure drugs, which are used against epilepsy and neuropathic pain but cause systemic side effects. There is a need of drugs that inhibit nerve signals locally and on-demand without affecting other regions of the body. Photopharmacology aims to address this problem with light-activated drugs and localized illumination in the target organ. Here, we have developed photoswitchable derivatives of the widely prescribed antiseizure drug carbamazepine. For that purpose, we expanded our method of ortho azologization of tricyclic drugs to meta/para and to N-bridged diazocine. Our results validate the concept of ortho cryptoazologs (uniquely exemplified by Carbazopine-1) and bring to light Carbadiazocine (8), which can be photoswitched between 400-590 nm light (using violet LEDs and halogen lamps) and shows good drug-likeness and predicted safety. Both compounds display photoswitchable activity in vitro and in translucent zebrafish larvae. Carbadiazocine (8) also offers in vivo analgesic efficacy (mechanical and thermal stimuli) in a rat model of neuropathic pain and a simple and compelling treatment demonstration with non-invasive illumination.

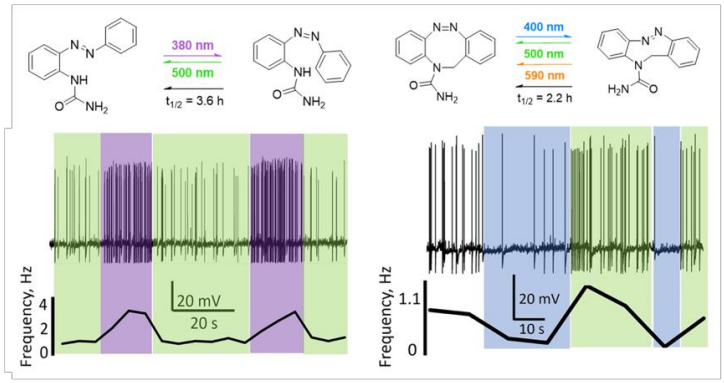

***Fig. 1**: Photoswitchable derivatives of the antiseizure drug carbamazepine have been developed to inhibit nerve signals locally and on-demand using light. Two compounds allow controlling hippocampal neuron firing and zebrafish larvae locomotion with light, and Carbadiazocine (right) also offers in vivo analgesic efficacy in a rat model of neuropathic pain and a simple and compelling treatment demonstration with non-invasive illumination.*

Engineered kalium channelrhodopsins for efficient and targeted neuronal inhibition

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Optogenetics has been extensively used to control genetically targeted cells with high spatial and temporal precision. However, reliable manipulation of synaptic transmission remains challenging due to unsatisfactory functional properties and/or expression level of the available opsins. To tackle this issue, we engineered high-performance optogenetic tools for robust inhibition of neurotransmission at the pre- and post-synaptic sites. Our tools are based on HcKCR1, a recently discovered kalium channelrhodopsin (KCR) from *Hyphochytrium catenoides* [1]. KCRs are potentially advantageous over pump-based opsins (e.g., NpHR and ArchT) as they are devoid of unwanted chloride accumulation or pH change after prolonged stimulation. We first improved the expression and axonal localization of HcKCR1 for presynaptic inhibition. The resulting axon-targeted HcKCR1 (**HcKCR1.AT**), when expressed in the CA3 pyramidal neurons, traveled efficiently to both ipsilateral and contralateral CA1 regions of the mouse hippocampus. In this paradigm, electrically evoked excitatory postsynaptic potentials (EPSPs) in the CA1 pyramidal cells could be strongly suppressed by a brief flash of green light, demonstrating robust presynaptic silencing by the ionic mechanism of **HcKCR1.AT**. To achieve more sustained inhibition in behaving animals, we further engineered a performance-improved kalium channelrhodopsin (**piKCR**) by optimizing the gating kinetics and potassium selectivity of HcKCR1. When expressing the axon-targeted version of piKCR (**piKCR.AT**) in the cerebellar cortex, illumination in the deep cerebellar nuclei (DCN; innervated by Purkinje cells) can robustly induce ataxia-like behaviors, indicating successful suppression of the Purkinje cell → DCN projection. Aside from presynaptic silencers, we are also engineering synapse-targeted opsins for manipulating neurotransmission on the postsynaptic sites. Through these efforts, we hope to provide new and better tools to enable neurobiology studies that were previously hindered by the limited power of inhibitory opsins.

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Optogenetic Silent Substitution: 50 Shades of Colors to Decouple Optogenetic and Photoreceptor Activation in the Retina

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Optogenetics is a powerful tool for manipulating neural activity, but its use in the retina remains challenging due to the tissue's intrinsic photosensitivity. The light used for optogenetic activation overlaps with the absorption spectrum of endogenous opsins expressed in photoreceptors. Retinal responses to light stimulation can thus be due to optogenetic activation, or to the undesired stimulation of photoreceptors. To address this issue, we developed an optogenetic equivalent of the Silent Substitution technique [1,2], the Optogenetic Silent Substitution (OSS). We switched between two spectral configurations, one including a red LED capable of activating a red-shifted transfected opsin (such as ChRmine [3]), and another without red illumination. We aim to maintain a constant photoreceptor isomerization rate between configurations, while only the red-inclusive configuration activates the optogenetic protein. We achieved this by adjusting green and yellow LED intensities to compensate for photoreceptor activation induced by the red LED.

To calibrate light intensity levels, we used multi-electrode arrays to measure the responses of optogenetic-free wild-type retinas to various spectral configurations. Our goal was to identify a pair of spectral configurations—one including red illumination and one without—that photoreceptors perceived as equivalent, ensuring that alternating between them does not evoke a change in ganglion cell response. Preliminary results suggest that there is a narrow range of LED intensity values that achieves an effective “silent substitution”: they are indistinguishable by photoreceptors, but only one configuration has the red light, at a strong enough level to activate optogenetic proteins.

Our findings demonstrate that OSS is a viable technique to decouple optogenetic and photoreceptor activation. This new tool should thus allow to use optogenetics in the retina without blocking photoreceptors nor having photoreceptor crosstalk.

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Heterodimerization and Interaction of the Serotonin-Receptors 5-HT1A and 5-HT2C

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G-Protein coupled receptors (GPCRs) are among the most prominent receptors in the central nervous system. Their malfunction is implicated in various neurological and neuropsychiatric disorders, making them a common target for medical treatment. Thus, it is crucial to gain a comprehensive understanding of GPCR functions and mechanisms to develop more effective and targeted medical treatments with fewer side effects.
This study examines the potential interplay between the serotonin receptors 5-HT1A and 5-HT2C, which play a significant role in the pathology of depression. Experiments were performed in transfected HEK-293 cells expressing both receptors.
Our findings provide initial evidence of heterodimerization between the 5-HT1A and 5-HT2C receptors, as indicated by FRET in acceptor photobleaching measurements. A significant increase in fluorescence intensity of the CFP-tagged 5-HT2C receptor of approximately 10% was observed after bleaching of the YFP-tagged 5-HT1A receptor. Further analysis of potential heterodimerization involves FLIM and Co-Immunoprecipitation/Western-Blot techniques.
Additionally, calcium imaging experiments demonstrated significant signals in HEK-293 cells transfected with both receptors and GCaMP8 when exposed to serotonin.
The potential interaction between the 5-HT1A and 5-HT2C receptors is further investigated by Patch-Clamp experiments.

Serotonin1A-Receptor-mediated signaling in Astrocytes and its influence on Major Depressive Disorder

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Major depressive disorder (MDD) is one of the most common psychiatric disorders worldwide, affecting more than 200 million people [1]. Despite the long-standing hypothesis that serotonin (5-HT) plays a role in the development and manifestation of depression, the precise mechanisms underlying this condition remain poorly understood. Glial cells, such as astrocytes, are well known for their passive role in the nervous system, protecting neurons and maintaining homeostasis. Recent studies suggest that these cells play a larger role than previously thought. There is considerable evidence for bidirectional communication between astrocytes and neurons, particularly at the synapse [2]. Astrocytes are able to respond to neurotransmitter release from nearby neurons by increasing intracellular calcium and subsequently releasing a neuroactive transmitter. Since astrocytes express different 5-HT receptors, such as the 5-HT1A receptor, which has been implicated in depression, our study focuses on the influence of 5-HT1A receptor-mediated signaling in astrocytes within the medial prefrontal cortex on depressive-like symptoms in mice.

We hypothesize that 5-HT1A-mediated calcium elevation in medial prefrontal cortex (mPFC) astrocytes may mediate antidepressant effects. To test this hypothesis, we used our previously published light-activatable 5-HT1A receptor chimera [3] to perform optogenetically manipulated experiments including calcium imaging, behavioral experiments, and brain slice electrophysiology.

Our findings suggest that astrocytic 5-HT1A-R signaling regulates neuronal activity and exerts antidepressant effects, highlighting a potential astrocyte-mediated mechanism in MDD pathophysiology.

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Brain-wide neural ensembles associated with pontine waves across sleep stages

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Sleep plays a crucial role in various brain homeostatic functions, including memory consolidation. Different sleep states exhibit distinct neural events: non-rapid eye movement (NREM) sleep features slow oscillations, delta waves, thalamocortical spindles, and hippocampal sharp wave-ripples, while REM sleep is characterized by hippocampal theta oscillations and ponto-geniculo-occipital waves ("P-waves" in rodents). Although P-waves originate from mesopontine cholinergic and glutamatergic nuclei, their interactions with broader neural events remain poorly understood.

Our previous research demonstrated that P-waves functionally couple with hippocampal sharp wave-ripples during NREM sleep and hippocampal theta oscillations during REM sleep [1], [2]. In our current study, we investigate how P-waves influence neural ensembles throughout the brain in a sleep state-dependent manner.

Using Neuropixels probes in mice, we simultaneously recorded P-waves and neuronal activity across multiple brain regions throughout sleep-wake cycles. We observed P-wave-related changes in neuronal activity across cortical regions, hippocampal formation, and striatal, thalamic, and midbrain nuclei. Notably, during NREM sleep, neuronal activity across most recorded areas decreased with P-waves onset, while during REM sleep, peak neuronal activity often aligned with P-waves.

To further examine the role of pontine cholinergic neurons in P-waves generation and memory consolidation, we conducted sleep state-specific closed-loop optogenetic manipulations. We present findings in behavioural memory testing, effects on P-waves frequency, sleep architecture, and hippocampal oscillatory power.

Overall, our findings reveal a sleep state-specific difference in how P-waves functionally couple with brain-wide neuronal ensembles, highlighting the significance of P-waves in brain function and suggesting distinct roles and mechanisms during NREM versus REM sleep.

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Optical Characterization of a Phase-Only Light Modulator (PLM) for Optogenetics Applications

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Light-targeting optogenetics enables the control of selected subsets of neurons with high spatiotemporal precision [1]. This is typically achieved using wavefront shaping approaches such as computer-generated holography (CGH), which shapes light onto the sample by modulating the phase of an illuminating beam. In practice, phase modulation is commonly achieved by using pixelated liquid crystal spatial light modulators (LC-SLM). In this case, the phase of the incident beam is dynamically modulated by adjusting the voltage at each pixel, allowing local reorientation of the LC molecules and control of the phase shifts of the incident beam. While LC-SLMs offer high efficiency (>80%), high bit depth (typically 8-bit), and high resolution (millions of pixels), they suffer from a relatively low refresh rate (typically between 60 Hz and 300 Hz), which may partially compromise the ability to temporally tune the activity of neural networks.
In this work, we explore the use of a new type of spatial light modulator called phase-only light modulator (PLM) [2]. PLM integrates a high-speed megapixel micromirror array, where each pixel can switch between 16 distinct vertical positions, enabling dynamic 4-bit phase modulation. Recent reports show high speed wavefront shaping up to 1.4 kHz. Here, we characterize the optical performances of PLM to generate light patterns typically used for light-targeting optogenetic control of neuronal circuits and compare these results with standard LC-SLM.

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A bistable inhibitory OptoGPCR for multiplexed optogenetic control of neural circuits

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Information is transmitted between brain regions through the release of neurotransmitters from long-range projecting axons. Understanding how the activity of such long-range connections contributes to behavior requires efficient methods for reversibly manipulating their function. Chemogenetic and optogenetic tools, acting through endogenous G-protein coupled receptor (GPCRs) pathways (Fig. 1), can be used to modulate synaptic transmission, but existing tools are limited in sensitivity, spatiotemporal precision, or spectral multiplexing capabilities. Here we systematically evaluated multiple bistable opsins for optogenetic applications and found that the *Platynereis dumerilii* ciliary opsin (*Pd*CO) is an efficient, versatile, light-activated bistable GPCR that can suppress synaptic transmission in mammalian neurons with high temporal precision *in vivo*. *Pd*CO has superior ideal biophysical properties that enable spectral multiplexing with other optogenetic actuators and reporters. We demonstrate that *Pd*CO can be used to conduct reversible loss-of-function experiments in long-range projections of behaving animals, thereby enabling detailed synapse-specific functional circuit mapping.

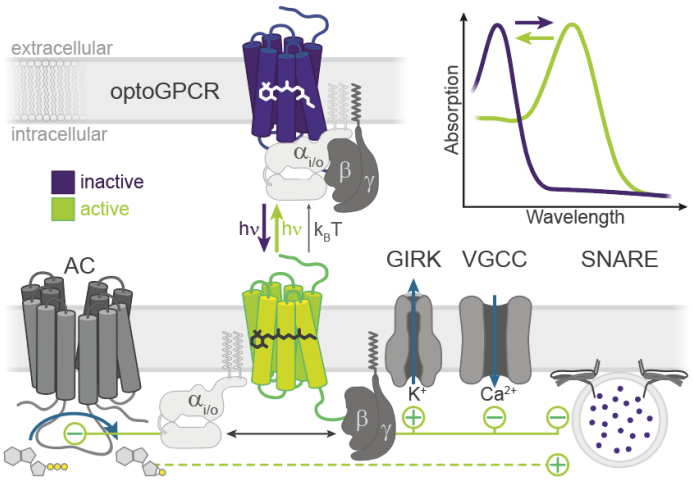

***Fig. 1**: Scheme of inhibitory optoGPCRs that couple via the Gi/o-pathway. A dark, inactive optoGPCR bound to the heterotrimeric Gαβγ protein is shown (top). Once the optoGPCR is activated by light, the heterotrimeric G-protein separates into the active Gα and Gβγ subunits (bottom). Gβγ activates G-protein coupled inward rectifying potassium channels (GIRK), inhibits voltage gated calcium channels (VGCCs) and may interfere with the SNARE (N-ethyl-maleimide sensitive factor attachment receptor) vesicle fusion apparatus. The Gα subunit inhibits adenylyl cyclases (ACs), thus reducing production of cAMP. OptoGPCRs can relax thermally (kBT) to the non-signaling ground state or, if their active state is spectrally separated from the ground state (inset absorption), absorption of a second photon with longer wavelength (hv) can terminate the signaling activity.*

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pHRoG: pH Regulating optoGenes for all-optical control of subcellular pH

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Life is organized in cells and even smaller compartments that create functional environments for signal processing and enzymatic reactions. In most subcellular organelles, such as mitochondria and lysosomes, proton concentrations are tightly regulated, and prolonged changes in lysosomal pH are associated with neurodegenerative diseases such as Alzheimer's or Parkinson's, cellular aging, and the adaptation of different types of cancer to their increased metabolic activity [1]. Despite the importance of subcellular pH for cell homeostasis and its role in various diseases, molecular tools for organelle-specific, time-resolved, and quantitative manipulation of subcellular pH remain limited. Chemical drugs like bafilomycin A1 or hydroxychloroquine, used to manipulate endolysosomal pH, affect all organelles along the pathway simultaneously and are slowly taken up and cleared by the cell.

By comparing the targeting and performance of different ion-transporting opsins in subcellular organelles, we developed a suite of pH-Regulating optoGenes, called pHRoG, that, in combination with spectrally complementary sensors for voltage and pH, allow spatially and temporally precise manipulation of organelle-specific physiology. In lysosomes, we show how these tools can be used to manipulate subcellular enzyme activity and study the pH and buffering capacity of individual lysosomes under physiological conditions. We demonstrate bidirectional control of local pH levels in different cell lines and neurons, and quantify both the potential and limitations of subcellular optogenetic pH manipulation using state-of-the-art opsins.

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Two-photon voltage imaging with rhodopsin-based GEVIs

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The optical recording of neural membrane potential overcomes limitations of electrical approaches and promises minimally-invasive observation of population activity with single-cell and single-spike resolution. While early efforts used voltage sensitive dyes, protein engineering allowed transitioning to genetically-encoded voltage indicators (GEVIs). GEVIs, used for action potential detection, are divided into two families: ASAP-type sensors are coupling a phosphatase domain to a fluorescent protein and the other group employs microbial rhodopsins as voltage sensors, either using the weak rhodopsin fluorescence as output or fusing a fluorophore (FRET-opsin). While ASAP-type sensors have been used for two-photon voltage imaging, rhodopsin-based sensors reportedly loose voltage sensitivity under two-photon illumination.

We report the rational design of a FRET-opsin voltage sensor based on *Acetabularia* rhodopsin fused to the brightest known fluorescent protein *Aa*FP1. We found the transfer of the pAce mutations and a combination of targeting sequences yielded a highly expressing, bright sensor with comparable ΔF/F to pAce and named it Jarvis. We demonstrate that Jarvis and pAce are highly compatible with scanless two-photon illumination and reported fast changes of membrane potential with high fidelity (SNR > 15) at a 0.5 kHz acquisition rate; SNR was significantly reduced for 2P scanning illumination at comparable conditions. We used Jarvis under scanless two-photon illumination *in vitro* (hippocampal slices) and *in vivo*, where it reliably reported single action potentials at high SNR (> 5). To the best of our knowledge this is the first report of 2-photon voltage imaging with a fully genetically-encoded rhodopsin-based GEVI for the detection of action potentials at high contrast.

Fiber Bundle-Based Microscope for All-optical Investigation over Enlarged Field of View or in Deep-brain Region in Freely Moving Mice

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A key question in neuroscience is to unravel causal relations between neuronal circuits and behavior. The precise study of neuronal circuits requires to measure and manipulate neuronal activity with high spatial (single cell) and temporal resolution within large ensembles. All-optical experiments using two-photon (2P) calcium imaging and holographic optogenetic photostimulation offer a promising approach to studying neuronal circuits in vivo in mice [1]. However, they have so far focused on experiments in head restrained mice. We recently developed a flexible two-photon microendoscope 2P-FENDO capable of all-optical brain investigation at near cellular resolution in the L2/3 of the barrel and visual cortex of freely moving mice [2]. The system performs fast two-photon (2P) functional imaging and 2P holographic photostimulation of single and multiple cells using axially confined extended spots [2]. Here, we present a significantly optimized 2P-FENDO-II system that achieves a four times larger field of view, a more homogeneous light distribution across the field of view, both for imaging and photostimulation, while achieving better flexibility and thus optimal adaptation to the study of freely moving mice. We have demonstrated the performance and versatility of 2P-FENDO-II in experiments targeting the somatosensory cortex, the visual cortex or the cerebellar cortex, in which we showed concomitant calcium imaging with jGCaMP7s and optogenetic control with ChRmine. We also developed another variation of the system, 2P-FENDO-III capable of imaging and photostimulating at fast acquisition rate in deep-brain regions. Proof-of-principle experiments were performed in freely moving mice co-expressing jGCaMP8s and the opsin ChRimson in the CA1 region of the hippocampus. In a field of view of 200 µm in diameter, we demonstrate functional imaging at a frame rate of up to 100 Hz and precise photostimulation of single and multiple pyramidal neurons. These enhancements establish 2P-FENDO-II and 2P-FENDO-III as groundbreaking tools for investigating complex neuronal dynamics and behavior with unprecedented detail in naturalistic situations.

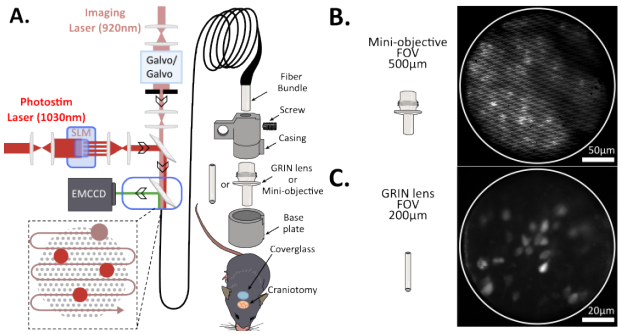

***Fig. 1**: A. Schematic of the system. B. Example Mini-objective FOV over the wS1 cortex (~110 µm deep) in mouse co-expressing jGCaMP7s and ChRmine. C. Example GRIN lens FOV over the hippocampus CA1 in mouse co-expressing jGCaMP8s and ChRimsonR*

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Sleep-dependent microglial calcium dynamics in Alzheimer's disease mouse models

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Sleep is becoming increasingly recognised to be associated with Alzheimer's disease (AD), yet their relationship remains to be determined [1]. It is known that microglia play a role in both sleep and AD. For example, microglia alter their intracellular calcium levels across the sleep-wake cycle and are involved in neuroinflammatory events throughout AD [2, 3]. However, little is understood about the influence of sleep loss and AD pathology on microglial calcium activity. To this end, we report an optical strategy to monitor microglial calcium signals across multiple brain states and regions in two AD mouse models *in vivo*. We used a triple transgenic approach to express a genetically encoded calcium indicator, jGCaMP8s [4], in microglia. We crossed either 5xFAD [5] or $App^{NL\text{-}G\text{-}F}$ [6] AD mouse models with Tmem119-CreER2 [7] and Cre-dependent jGCaMP8s mice, where tamoxifen was administered to induce recombination. Next, we chronically implanted electrophysiological electrodes and a tapered optical fibre [8, 9] into brain regions with varied amyloid pathology. First, we confirmed that this transgenic approach can target microglia. In 5xFAD;Tmem119-CreER2;jGCaMP8s and $App^{NL\text{-}G\text{-}F}$;Tmem119-CreER2;jGCaMP8s mice, we observed brain-wide jGCaMP8s expression in Iba1-positive microglia. To this end, we established a novel optical setup with an electrophysiological recording capability to adopt state-of-the-art tapered fibre photometry across sleep-wake cycles. This approach revealed fluctuations of microglial calcium levels across brain states and regions for the first time. The use of two AD models allowed cross-validation of our findings across mouse models with differing Aβ pathology. Further characterisation of brain-region-specific and state-dependent calcium dynamics in microglia will provide insight into how sleep and AD pathogenesis are related to homeostatic microglial calcium activity. Specifically, determining if sleep loss and AD pathology can contribute to dysregulation of microglial calcium dynamics and their neuroprotective functions is crucial. Consequently, this could provide opportunities to develop a novel intervention strategy for AD.

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Potent Photoswitch for Expression of Biotherapeutics in Mammalian Cells by Light

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Precise temporal and spatial control of gene expression greatly benefits the study of specific cellular circuits and activities. Compared to chemical inducers, light-dependent control of gene expression by optogenetics achieves a higher spatial and temporal resolution. This could also prove decisive beyond basic research for manufacturing difficult-to-express proteins in pharmaceutical bioproduction. However, current optogenetic gene-expression systems limit this application in mammalian cells as expression levels and fold induction upon light stimulation are not sufficient. To overcome this limitation, we designed a photoswitch by fusing the blue light-activated light-oxygen-voltage receptor EL222 from *Erythrobacter litoralis* to the three tandem transcriptional activator domains VP64, p65, and Rta. The resultant photoswitch, dubbed DEL-VPR, allows an up to 400-fold induction of target gene expression by blue light, achieving expression levels that surpass those for strong constitutive promoters. Here, we utilized DEL-VPR to enable light-induced expression of complex monoclonal and bispecific antibodies with reduced byproduct expression, increasing the yield of functional protein complexes. Our approach offers temporally controlled yet strong gene expression and applies to both academic and industrial settings.

Molecular engineering of a far-red light activated ion channel

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Bestrhodopsins are a newly identified family of light-modulated ion channels composed of a pentameric bestrophin channel surrounded by a ring of five rhodopsin pseudodimers [1]. By studying the absorption spectra of new members of this group, we demonstrate the remarkable spectral diversity of this opsin family, with absorbances ranging from UV to far-red light. However, when characterizing them electrophysiologically in HEK293 or HeLa cells, most bestrhodopsins do not exhibit light-activated bestrophin currents, with *Karlodinium veneficum* Rhodopsin-Rhodopsin-Bestrophin (*Kv*RRB) being the only known exception.

Using single-turnover electrophysiology, we show that green-light-induced channel opening and UV-triggered channel closure in *Kv*RRB are delayed by tens of milliseconds following single laser flashes, indicating a loose functional coupling between light absorption in the opsin domain and final gating of the bestrophin channel. Site-directed mutagenesis of residues in the bestrophin channel pore further allowed us to identify key amino acids essential for light-induced channel opening in *Kv*RRB. Most importantly, we identified a single glycine that, when transferred to the previously non-functional, far-red-absorbing *Pa*RRB from *Paraphysomonas antarctica*, enabled light modulation of bestrophin currents with far-red light.

Although photocurrents of *Pa*RRB V958G remain small, they demonstrate the feasibility of engineering a rhodopsin-based, far-red-light-activated ion channel and provide a promising foundation for future optogenetic engineering of far-red-shifted actuators for deep-tissue optogenetics.

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In Vivo Investigation of Synaptic Mechanisms Regulating Interneuron Function in the Mouse Primary Visual Cortex During Neurodevelopment

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The developmental dysregulation of GABAergic cells, such parvalbumin interneurons (PV-INs) is linked to the pathophysiological mechanisms that underline neurodevelopmental disorders such as autism and schizophrenia [1]. Reductions in the number and activity of PV-INs, are proposed to drive symptom progression in these conditions [2]. Hypothesis suggests that altered PV-IN function arises from disrupted NMDA receptor (NMDAR) signaling [3]. However, various subtypes of interneurons express NMDARs, and the role of NMDAR signaling in regulating normal brain function within these other inhibitory populations is still not well understood. The main aim of this work is to investigate the synaptic mechanisms, and particularly the relevance of synaptic NMDARs, in the operation of cortical interneurons *in vivo* in adult animals and during development. ASD patients display atypical sensory perception of visual stimuli which is suggested to be related to an excitation/inhibition imbalance [4]. Thus, this work study the function of cortical interneurons in the mouse primary visual cortex. Three different developmental time points were chosen: Adult mice (P>60) and two time points early in development (P=15/18 and P=20/23). In the adult group, mice were injected with AAV9-syn-FLEX-jGCaMP8s-WPE (1:1) in the L2/3 of V1, followed by window implantation on PV-cre, PV-cre::NR1-flox and SST-cre mice. After 30-40 days, the somas of interneurons were visualized using fluorescence two-photon imaging, and the animals were subjected to a visual stimulation protocol. For young animals, the same AAV construct was injected in the ventricle at P0 and the window implantation performed at P12-14 followed by two-photon imaging sessions between P15-23. The results in adult mice indicate that contrast sensitivity increases sublinearly with the contrast level in PV-INs and SST-INs, and that NMDARs do not alter contrast sensitivity in PV-INs. Orientation selectivity in PV-INs is independent of contrast level, also removal of NMDARs do not seem to alter this pattern. In SST-INs orientation selectivity decreases with contrast level. Upon inspecting the results throughout development, preliminary data suggest that orientation tuning and contrast response in PV-INs are developmentally regulated, whereas no such regulation is observed in SST-INs. Surprisingly, across all ages tested data analysis shows a strong response to stimulus offset selectively in PV cells after termination of visual stimulus. The analyses in PV cells without NMDARs showed the same pattern, probability representing the non-involving of NMDA in this cell behavior. These results suggest that synaptic mechanisms regulating contrast and orientation selectively of cortical INs are developmentally controlled. We are planning to use optogenetic tools to understand the mechanisms underlying the off responses in PV-INs.

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CandOR – For strong light-induced Calcium-Influx without Depolarisation

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Here we present CandOR (Calcium-permeable-non-depolarizing Optogenetic Regulator) as a synergetic multicomponent construct that allows for high calcium influx without depolarizing cell membranes. Calcium plays a nearly ubiquitous role in cellular processes. In neurons Ca^{2+} is essential for synaptic transmission and plasticity. In astrocytes intracellular waves of Ca^{2+} are used for communication as well as neurovascular coupling and modulation. And in myocytes Ca^{2+} release is the main driving force for contraction. CapChR2 is a channelrhodopsin (ChR) that under physiological conditions shows increased Ca^{2+} permeability at negative membrane voltages but still predominantly conducts protons and other cations [1]. Through further mutation, aiming to create additional Ca^{2+} binding sites along the channel-pore, we tried to further enhance its Ca2+ conductance and selectivity. In a parallel improvement strategy, we combined CapChR2 with the potassium-selective WiChR [2] and created a blue-light activated optogenetic antiporter, that we called CandOR.
The simultaneous outflux of K^+-ions upon activation inhibits the depolarization of excitable cells, maintaining a constant driving force for Ca2+ influx and allowing for Ca^{2+} -Sensor responses higher than with CapChR2 alone. In excitable cells CandOR will enable the study of Ca^{2+} effects without the interference of action potentials possibly representing one of the most effective optogenetic tools for extracellular Calcium influx up to now.

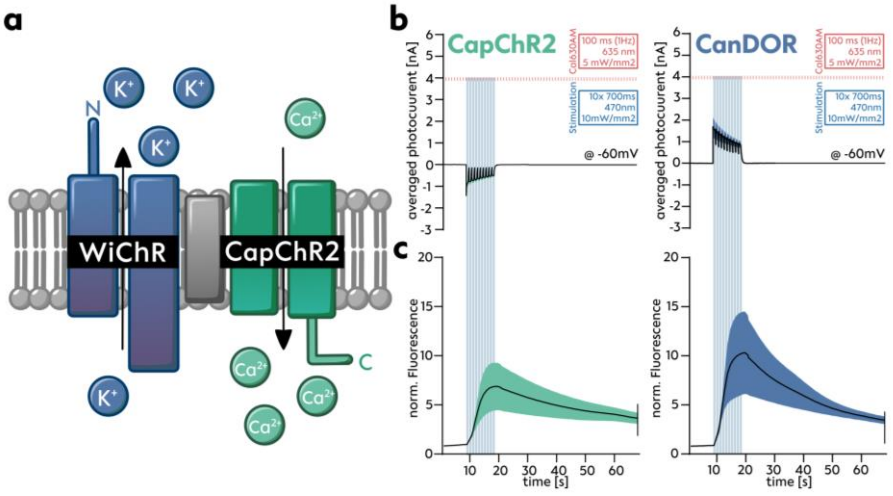

***Fig. 1**: **a**: Conceptual scheme of CandOR, in which the KCR WiChR and the Ca^{2+}-permeable channelrhodopsin CapChR2 are linked for simultaneous blue light-activated outflux of K^+-ions and influx of Ca2+-ions. **b**: averaged photocurrents during whole-cell patch clamp recordings in Voltage Clamp mode for CapChR2 and CandOR. **c**: Simultaneously recorded averaged fluorescence measurements of the Calcium-sensor CalBryte 635 for CapChR2 and CandOR.*

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Miniature 2P microscopes coupled with aberration corrected GRIN lenses enable extended field-of-view deep brain imaging in freely moving mice

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Miniaturized two-photon microscopes (MINI2Ps) in combination with genetically encoded fluorescent calcium indicators paved the way to the investigation of the activity of mammalian brain circuits with high spatial resolution in freely moving animals [1]. To image deep (> 1 mm) brain areas, MINI2Ps can be coupled to gradient index (GRIN) lenses, which relay laser light to the target imaging plane. However, GRIN lenses are affected by optical aberrations, which degrade the point-spread-function especially in marginal portions of the field-of-view (FOV) decreasing the area within which imaging can be performed with high spatial resolution. We recently showed that GRIN aberrations can be significantly reduced using specifically designed 3D-printed corrective microlenses positioned on the back end of GRIN lenses (aberration corrected GRINs [2,3]). This correction method enabled 2P functional imaging with improved spatial resolution over an enlarged FOV in deep brain regions of head-fixed mice [2,3] and it is particularly well suited for miniaturized 2P microscopy in freely moving animals, as the corrective lens is lightweight and tens to hundreds of micrometers in size. Here, we tested the hypothesis that MINI2Ps combined with long (length > 4 mm) aberration corrected GRIN lenses display enhances optical performance compared to MINI2Ps combined with uncorrected GRIN lenses. We characterized MINI2Ps coupled with two types of aberration corrected GRIN lenses (GRIN length, 4.1 mm and 6.4 mm; GRIN diameter, 500 μm; GRIN NA, 0.5). Measurements of subresolved fluorescent beads revealed that the corrected system (MINI2P + aberration corrected GRIN lens) had higher and more homogeneous spatial resolution across an enlarged FOV for both types of GRIN lens compared to the uncorrected system (MINI2P + uncorrected GRIN lens). The on-axis z-resolution of the corrected system was also improved compared to the uncorrected system. Moreover, the on-axis z-resolution of the corrected system was improved compared to that of the MINI2P alone for the 4.1 mm-long GRIN lens and it was comparable to that of the MINI2P alone for the 6.4 mm-long GRIN lens. The enlargement of the FOV was confirmed by imaging subresolved fluorescent films and mouse fixed brain slices expressing a fluorescent dye in neurons. We finally tested the corrected MINI2P system *in vivo* by longitudinally imaging large populations of jGCaMP7f-expressing neurons in the thalamic ventral posteromedial nucleus (VPM) of mice performing open field navigation and novel object recognition (NOR) tasks. Taken together, these results demonstrate that coupling of MINI2Ps with aberration corrected GRIN lenses is a promising approach to enable large-scale high-resolution recordings of neuronal population activity in deep regions of the mouse brain during naturalistic behavior.

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Optogenetic manipulation of dendritic signal integration

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Layer 5 pyramidal neurons (L5PN) in the somatosensory cortex are essential for cortical processing and have been linked to higher order cognitive functions, including perception and consciousness [1, 2]. L5PNs integrate information across all cortical layers as their dendritic tree spans the entire cortical column. Key active properties of L5PNs arise from two main spiking zones: Na^+ spikes initiated at the perisomatic region and Ca^{2+} plateau potentials in the distal dendrites. Ca^{2+} signals travel from apical dendrites to the soma and shape the neuronal output [3]. These signals are strongly modulated by dendritic G protein-coupled receptors (GPCRs) [4, 5]. Here, we investigate the interplay of GPCR signals and Ca^{2+} on signal integration in L5PNs of mice somatosensory cortex using optoGPCRs. Using a combination of electrophysiology and optogenetics we examine the effect of photocontrolled GPCR signals on the supralinear dendritic events in L5PNs.

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Overcoming the limitations of fluorescent protein-based biosensor development

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Fluorescent protein (FP)-based biosensors are genetically encodable imaging tools that can visualize the dynamics of target molecules/ions in cells and tissues. The current methodology of development of FP-based biosensors can be divided into three steps; 1. Identification of appropriate binding proteins that bind to the target showing conformational change, 2. Design of the prototype, and 3. Further optimization of biosensors. Here, I introduce a new method that enables the development of multiple FP-based biosensors targeting various molecules/ions. We focused on a histidine kinase (HK) binding protein, which is the component of two-component system (TCS) (**Fig. A**). HK binding proteins are exposed to the outer environment and bacteria sense the various surrounding molecules/ions through this system. This TCS and our single FP-based intensiometric biosensors share the same mechanism, which is a conformational change of the binding protein upon the binding to the target. In our previous work, we developed a GFP-based citrate biosensor, Citron1, using the CitAP, a histidine kinase citrate binding protein[1]. Convinced by the high performance of Citron1, we decided to create a large library of FP-based biosensor prototypes of the combination of GFPs and HK binding proteins. As shown in **Fig. B**, we screened this library by the target molecules/ion to find potential prototypes.

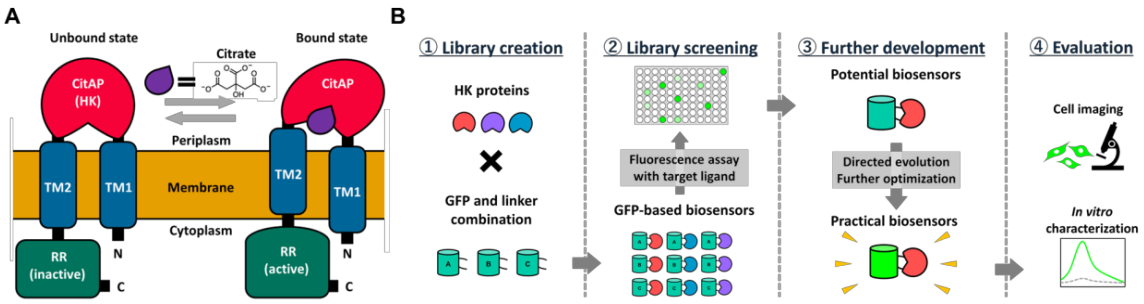

Figure: A. *Schematic of histidine kinase protein,* B. *General workflow of this project.*

I would like to share the latest results of this project including the development of four different biosensors targeting L-serine, nitrate, succinate, and boric acid with $\Delta F/F = 5 - 20$ throughout directed evolution without a time-consuming process of linker optimization. This unprecedented method would accelerate the development of FP-based biosensors and expand the target, which might expand the range of application of FP-based biosensors.

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Physics-Informed Deep Learning for Digital Twin of Turbid Media

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Abstract: This work presents a novel methodology for characterizing and creating a digital twin of turbid media using only intensity measurements. Our approach leverages a unique physics-informed neural network that not only integrates known physical laws with deep learning but also uncovers the polarization-dependent transmission matrix of the medium. Unlike previous methods that function as black boxes, our technique emphasizes transparency and interpretability, providing a deeper understanding of how light propagates and interacts with complex scattering environments. By capturing these nonlinear modulations, our model reduces the need for extensive calibration while offering a more accurate representation of the medium's optical properties.

A key strength of our approach lies in its ability to facilitate a data-driven discovery of the complex, nonlinear modulations that occur during light transmission. This capability is demonstrated by successfully employing gradient-based calculations to recover the original wavefront, as showcased in the image transmission problem. he higher accuracy of our method, compared to models optimized solely for this task [1,2], highlights its potential to enhance applications in neuromorphic computing, deep learning, and various photonic and optical systems. Overall, our work represents a significant advancement in digital twin technology, offering both improved performance and deeper physical insights.

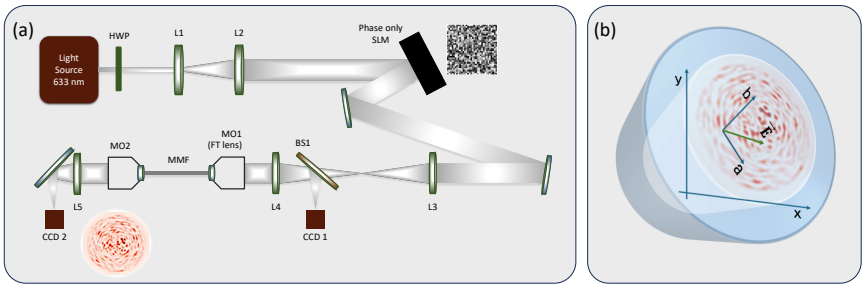

***Fig. 1**: a) The utilized optical setup, and (b) a schematic illustrating the tangential electric field vector on the distal facet of the fiber, along with the selected Cartesian coordinate systems for the speckle patterns and the polarization of the electric field, denoted by the axes pairs (x, y) and (a, b), respectively.*

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Toward the realization of chronically implantable neural probes using two-photon lithography

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Deciphering the complexity of the human brain requires the use of bidirectional neural probes able to monitor and modulate neural activities simultaneously at the cellular level [1]. Among them, implantable multimode optical fibers and particularly tapered fibers (TFs) have recently emerged as a promising tool for delivering light and collecting fluorescence to and from the brain tissue, allowing spatially resolved bidirectional optical neural interfaces [2]. Compared to their flat-cleaved counterparts, TFs are less invasive for the brain tissue and offer controlled light emission along the taper by exploiting the modal properties of the gradually narrowing waveguide [3]. Despite their highly non-planar surface, TFs can be patterned to control the modal content and host electric elements for simultaneous performance of optogenetic stimulation and electrophysiological recordings [4]. However, establishing a stable interface between them and the brain tissue is challenging due to the mechanical mismatch in place. The high bending stiffness of glass silica fibers induces inflammation and consequently neuronal death and tissue encapsulation in the implant surroundings, when applied in chronic settings [5]. This adverse immune response could be minimized by replacing silica with a material that matches more closely with the mechanical properties and micro-scale movements of the brain. As a result, over the last years, there has been an increased interest in developing polymeric optical fibers [6]. In this work, we demonstrate a method for the single-step fabrication of soft and flexible polymeric air-clad tapers using two-photon polymerization (2PP) and investigate their prospective use in chronic neural interfaces. 2PP is a versatile additive manufacturing technique that enables the fabrication of 3D structures of arbitrary shape with sub-diffraction limit resolution and tuneable mechanical properties and has been successfully employed for the fabrication of optical fiber integrated photonic elements [7]. To fabricate soft polymeric tapers, we used the commercially available IP-PDMS photoresist, which is a photocurable type of polydimethylsiloxane (PDMS). 2PP was performed using Nanoscribe's Photonic Professional system equipped with a femtosecond laser (λ=780 nm). 3D structures were printed in a layer-by-layer sequence using the Dip-in Liquid Lithography (DiLL) mode on top of polished multimode fiber facets. Following the exposure, not cross-linked material was removed and solid tapers were encapsulated with a thin conformal film of Parylene-C. Polymeric tapers were then characterized in terms of light delivery for prospective use in optogenetics. Moreover, the impact of the tapers on mechanically induced trauma was assesed *in vitro*, by performing insertion force measurements using phantom brains. Our results suggest that IP-PDMS tapers hold great promise as chonically implantable neural probes.

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Activation of TLR signaling with light in mammalian cells

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Toll-like receptors (TLRs) are single-spanning transmembrane receptors, which recognize pathogen-associated patterns (PAMPs) such as LPS, flagellin, bacterial and viral nucleic acids. Mammalian TLRs are expressed by immune and non-immune cells and trigger expression of cytokines through the activation of MyD88 pathway and NFkB signaling. To engineer TLRs, activated with far-red light we changed extracellular TLR domain in several homodimeric TLRs (4, 5, 7, 8 and 9) to the photosensory domain of *Deinococcus radiodurance* (eDr). Previously we demonstrated, that such strategy allows to engineer light-activated RTKs. Same engineering strategy allowed us to develop eDr-TLRs which activated NFkB pathway in the light-dependent manner in mammalian cells. Additionally, we reprogrammed the MyD88 pathway to induce apoptosis, rather than cytokine production, providing a new tool to control immune and cell death signaling with light.

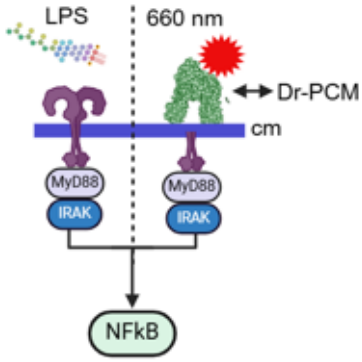

***Fig. 1**: Design of light-activated TLRs. On the left: TLR4 is activated with LPS. Activation of TLR triggers downstream NFkB signaling. On the right: LPS-recognizing extracellular domain of TLR4 is changed to the photosensory domain of Deinococcus radiodurans. Illumination with the red (660 nm) light leads to the reorganization of the intracellular TLR domains and activation of NFkB signaling.*

Toward the integration of active recording pads on optical neural implants by two-photon lithography

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The use of multifunctional neural interfaces for sensing and actuating neural activity in the living mouse brain is driving the development of a wide range of innovative experimental paradigms for neuroscientists. This progress is made possible by the integration of multiple functionalities into minimally invasive neural implants, capable of probing deep brain regions with high temporal and spatial resolution [1]. A widely used stimulation technique involves visible light radiation to induce action potentials in genetically modified neurons that express light-sensitive ion channels, leveraging optogenetics to activate specific neuronal populations [2]. Depth-resolved light delivery is achieved through implantable optical devices such as micro light-emitting diode (μLED) arrays, semiconductor waveguides, and tapered optical fibers (TFs) [3, 4]. Neural activity readout is usually conducted using extracellular recording electrodes, which measure electrical signals via capacitive coupling [5]. However, neuronal function is not only governed by electrical signals. Neurochemical signals including neurotransmitters, ions and reactive oxygen species also play a significant role in physiological and pathological processes in the living brain, fact that renders their sensing equally important [6]. In this context, the perspective of adding the capability of electrochemical detection onto an optical neural interface would allow for a better understanding of the multifaceted nature of neural signalling and related functions deep in the brain. Active devices such as Organic Electrochemical Transistors (OECTs) have recently emerged as a promising tool for neurochemical sensing due to their high sensitivity, amplification capability and high signal-to-noise ratio (SNR) [7]. In this work, we demonstrate a method for the integration of OECT sensing on TF-based neural interfaces. To this aim, we employed two-photon lithography (TPL) – exploiting its high versatility and high spatial resolution – enabling precise fabrication of custom, non-planar metallic and/or dielectric patterns [8]. Therefore, using an original TPL approach, source and drain electrodes with respective tracks and connection pads were realized as a protective resist mask onto a metallized (Cr/Au) TF, which was then subjected to metal lift-off and wet etching for resist removal. Following the deposition of protective Pt pads on the Au electrodes *via* focused ion beam (FIB), devices were encapsulated with a conformal film of Parylene-C. This was followed by a recess on the electrodes and channel between, to allow for the subsequent electrodeposition of PEDOT: PSS. The electrical characterization of our preliminary OECT device on TF exhibited a transconductance of 300 nS, validating its functionality. The versatility of TPL patterning approach allows for further enhancement of the performance of the OECT device via tuning of the geometrical characteristics of the channel, thereby advancing the development of multifunctional optoelectronic neural interfaces.

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Label-free molecular analysis of brain thrombi by means of Raman spectroscopy

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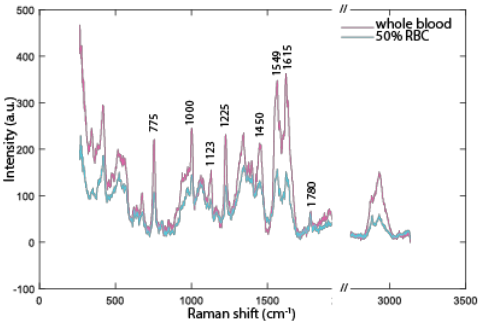
Strokes are among the leading causes of death worldwide, with brain strokes being the primary cause of disability and the third leading cause of mortality. Ischemic stroke occurs due to the occlusion of cerebral vessels by a clot with subsequent brain tissue necrosis caused by the lack of nutrients and oxygen. Depending on the etiopathogenic origin of the clot (atherosclerotic or cardiac-related), its cellular and molecular composition can vary. Fibrin (involved in coagulation mechanisms), red blood cells (RBCs), and platelets are its major constituents, while white blood cells are the secondary components [1]. Recanalization is achieved by clot lysis (use of an intravenous thrombolytic agent) or by direct mechanical removal of the thrombus via an endovascular catheter delivered intracranially or by means of a metallic device (i.e. stent retrievers)[2]. Importantly, a deep understanding of clot composition is crucial for selecting the most appropriate endovascular tools. Aiming at developing a label free technique for real-time molecular characterization of blood clots, we implemented a Raman spectroscopy-based approach to analyze synthetic clots derived from human blood. Clots were obtained from human whole blood treated with $CaCl_2$ and incubated in a warm bath at 37°C for two hours. A 785nm custom-made Raman setup equipped with a 4X air objective was used for acquiring signals in the fingerprint (FP, 800-1750 cm^{-1}) and in the high wavenumber (HW, 2800-3100 cm^{-1}) spectral ranges. Preliminary results highlight the presence of the heme prostetic group as it displays strong Raman scattering because of its high symmetry, with bands in the 1650-1400cm^{-1} assigned to the methin bridges and pyrrole rings stretching vibrations, and the 1549 cm^{-1} peak typically identifying RBC because of its absence in any other blood cell type [3,4]. Thanks to the high sensitivity and molecular specificity of Raman spectroscopy in identifying signals associated to the presence of the heme group, we are approaching a systematic analysis of synthetic blood clots characterized by different concentrations of RBC and fibrin. Leveraging on the possibility of using optical fibers for reliable Raman spectroscopy analysis [5], we foreseen the possibility of accessing thrombi via the surgical catheter, for *in vivo* real-time etiopathologic diagnosis, and prompt secondary prevention in order to reduce the risk of relapse.



Peak pos (cm^{-1})	Assignment
755	Pyr ring breathing
1000	Phenylalanine
1123	Cβ-methyl
1225	Oxy-hemoglobin
1450	σ(CH_2/CH_3)
1549	ν($C_β$-$C_β$)
1615	ν($C_α$-C_m)
1780	C-O, C=O bonds

Fig. 1: *Raman spectra of artificial blood clot acquired for clot originated from whole blood (pink) and for a clot with half the amount of RBC (cyan) and relative peak assignement*

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Deep learning-based Robust Raman Spectral Analysis: Overcoming Probe-Induced Artifacts with a Cascaded U-Net

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Raman spectroscopy using silica-based tapered optical fibers (TF) enables the elucidation of mouse brain cytoarchitecture, the monitoring of molecular alterations due to traumatic brain injury, and the detection of brain metastasis markers with high accuracy [1]. While these fibers enhance the signal-to-noise ratio (SNR), the Raman signal from the TF's silica in the fingerprint region can be orders of magnitude stronger than that of the target sample, complicating spectral data extraction. A cascaded U-Net model was proposed in [2] to address this challenge, ensuring noise removal and baseline correction. However, it lacked resilience to the signal generated by different implantable probes, which can generate different background based on different shapes and different processing temperatures when the taper is generated. This work overcomes that limitation by developing a deep learning model robust to the silica probe. A base training dataset to take into account TF's background variability was built by collecting Raman spectra from 739.6 cm-1 to 2399.8 cm-1 (excitation 785nm) generated by the probe itself under several different conditions. These included different ferrule materials (ceramic vs. metallic), taper lengths, laser power levels, exposure times, laser focus adjustments, and Raman system architectures. This base dataset was then expanded by data augmentation to obtain the final training dataset [3]. Each spectrum in the base dataset was augmented using linear combinations, Raman band modifications (expansion/contraction), random noise addition, and polynomial baselines of varying orders, generating a total of 120,000 spectra. Each synthetic spectrum contained 0–8 peaks of Moffat, Lorentz, Gaussian, and Voigt types, with peak heights ranging from 70 to 1,000 pixel counts and widths from 8 to 80 units. Additionally, spectra incorporated up to two cosmic rays and a polynomial baseline (maximum degree: 10). Gaussian noise, with a mean of 0 and variance between 10 and 70 counts, was also added. This augmented dataset trained the cascaded U-Net model (fig. 1), where each module was tasked with progressively refining the signal: the first stage removed the augmented silica background, the second eliminated the polynomial baseline, and the final stage suppressed noise, yielding a pure Raman spectrum. We aim to validate the model on spectra from different brain regions. These results pave the way for a universal model resilient to probe-induced variations, ensuring robust Raman spectral analysis across diverse experimental conditions.

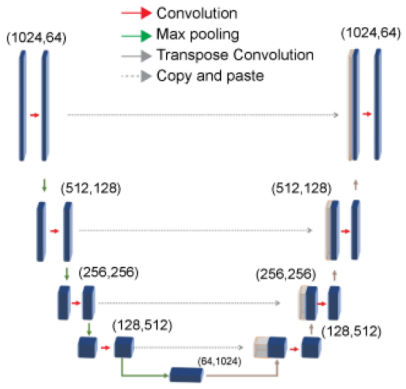

***Fig. 1**: Diagram of the network architecture with cascaded levels*

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Metalens-assisted wavefront shaping for augmented optical control in the near and far field of multimode optical fibers

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Endoscopic imaging to detect functional fluorescence at high spatiotemporal resolution emerged as a promising approach for investigating local microcircuits[1], mapping neural activity[2], and studying physiological and pathological states[3] in deep brain regions in a minimally invasive fashion. In this framework, multimode optical fibers (MMFs) represent an excellent platform, thanks to their small implant cross-section, their inherently light-couple nature, and the wealth of information carried out in the thousand modes sustained. However, MMFs have limited numerical aperture (NA), limiting spatial resolution and collection effciency. Wavefront shaping techniques[4], at the input of the fiber, allow the control of the properties of light at the fiber output, both in the near and far fields[5] and the improvement of the resolution of imaging systems, even allowing the observation of sub-cellular structures[6]. In this work, we tested the hypothesis that the intrinsic NA limitation of a MMF can be overcome by enriching the optical waveguide with a metalens designed to improve the angular capability of the optical system. Metalenses, which consist of meta-atoms designed to impart specific phase shifts to radiation, can modulate light both in amplitude and phase[7], compensate for aberrations[8], and focus light for optical trapping [9]. We designed a diverging lens (Fig. 1A) to increase the NA of a MMF (core diameter of 50µm) from NA=0.22 to NA=0.6. The metalens was then fabricated by two-photon additive manufacturing (Fig. 1B), employing a photoresist with refractive index n =1.53. The resulting photonic system was combined with a wavefront shaping setup to obtain scanning-focused spots at the fiber output, either in the near or the far field planes. In the far field, we extended optical control over k-vectors beyond the numerical aperture of the bare fiber (Fig. 1C), while in the near field, we obtained a sub-portion of the field of view with smaller focal waists, compatible with the higher NA (Fig. 1D). These results suggest that metalens-MMFs enable the generation of a scanning, focused spot at 25 µm from the fiber facet beyond the diffraction limit and the NA of the fiber itself, simultaneously improving the collection volume and likely enhancing the imaging capabilities of the system.

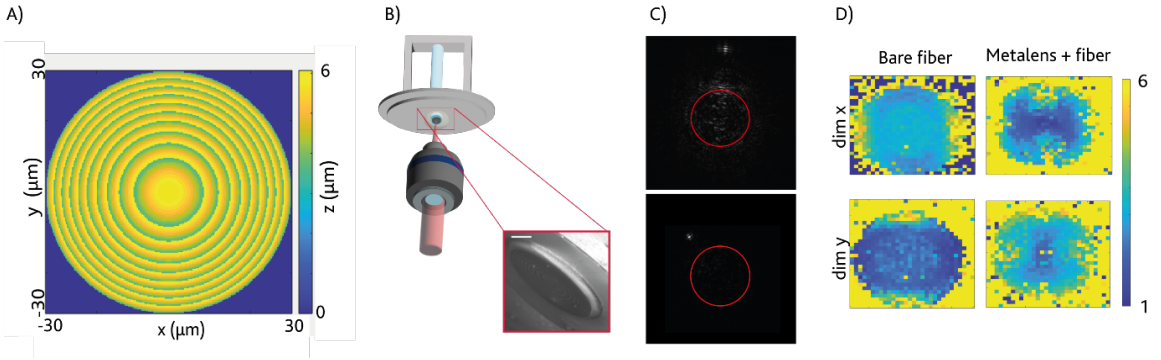

Fig. 1: A) Design of a diverging metalens with NA 0.6, including a base to promote adhesion. B) Schematization of the fabrication process. In the insert: SEM image of a metalens fabricated on a MMF with a scalebar 10 µm. C) Foci obtained in the far field of the metalens decorated fiber at 30° and 23°. The red circles indicate the 0.22 NA. D) Pseudocolor maps showing the size of the waists of the focal spots obtained both for a bare fiber and a metalens-decorated fiber.

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Plasmonic Tapered Fiber Sensors for Ultra-Sensitive Neurotransmitter Detection via Surface-Enhanced Raman Spectroscopy

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Surface-enhanced Raman spectroscopy (SERS) is a powerful label-free sensing technique with applications in biomedical diagnostics[1], chemical identification[2], food safety assessment[3], and environmental monitoring[4]. Integrating SERS-active substrates with endoscopic probes, such as multimode optical fibers, enables molecular sensing in previously inaccessible environments, including deep brain regions[5], hazardous sites[6], and live-cell interfaces[7].

Tapered optical fibers (TFs) offer unique advantages for SERS-based sensing, including enhanced coupling with plasmonic structures[8], reduced specimen damage due to their conical geometry, and a large active area that amplifies the SERS signal[9]. Here, we introduce a high-throughput, room-temperature method for fabricating SERS-active TFs by leveraging physical vapor deposition to nucleate ultrasmall gold nanoparticles. The non-planar TF surface drives nanoparticle self-organization, transitioning from a rough interconnected gold film to uniformly distributed spherical particles with sub-5 nm interparticle gaps—ideal for plasmonic enhancement.

These implantable sensors demonstrate remarkable sensitivity, detecting serotonin at its physiological resting concentration (10^{-9} M) even in a through-fiber measurement scheme. Additionally, SERS spectra obtained at varying concentrations reveal sensitivity to different stereoisomeric forms of serotonin. This novel approach enables highly sensitive, minimally invasive neurotransmitter detection, opening new avenues for biochemical sensing.

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Studying Photo-Thermoelastic Deformations of Thin Films using Laser Doppler Vibrometry for Optoacoustic Applications

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Understanding photo-thermoelastic deformations in thin films is crucial in photoacoustic experiments, as they directly affect the generation and propagation of acoustic waves. Photo-thermoelastic properties such as the coefficient of thermal expansion are key parameters in this context. Traditional measurement techniques such as mechanical dilatometry and X-ray diffraction have limitations in precision, sensitivity to environmental factors and applicability to composite materials [1]. Thus, non-invasive methods capable of simultaneously probing both temperature variations and mechanical expansion in situ, with high spatial and temporal resolution are needed.

In this study, we present a method to investigate photo-thermoelastic deformations in thin films for optoacoustic applications. It is based on a ns-pulsed laser exciting the material under investigation, whose temperature and mechanical expansion properties are simultaneously monitored by thermographic imaging and laser doppler vibrometry, respectively. A PDMS-based nanocomposite with silicone black pigments was used as a proof-of-concept material [2]. The pigment absorbs light, produces heat, and causes thermal expansion of the surrounding PDMS. The material was spin-coated on a glass coverslip and analyzed with the multimodal setup shown in Fig. 1(a) to measure the local thermoelastic response.

The film was locally excited by a 10ns-pulsed 640 nm laser, triggered by pulse trains: each single pulse with a period of 200ns and a duty cycle of 50%, while the train duration was 500µs and the train pulse repetition frequency was 1 kHz. Thus exciting the film for 500µs every 1ms.

Simultaneously, the laser Doppler vibrometer performed a frequency-domain scan, enabling to sense in situ local deformation. A representative graph of the recorded expansion of the polymer is presented in Fig. 1(b). Furthermore, Fourier analysis of the displacement response revealed a narrow peak at the main frequency of 1 kHz, where mechanical response was expected, along with secondary odd harmonics with decreasing amplitude, consistently with the repetition frequency of the pulse train.

These results confirm the effectiveness of this approach in characterizing local thermal expansion dynamics in thin films, paving the way for further advancements in photoacoustic material analysis.

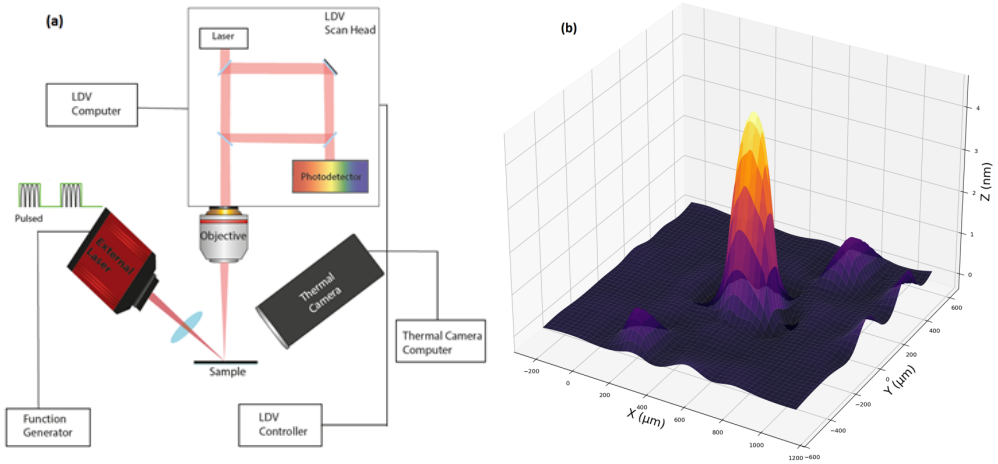

***Fig. 1**: (a) Experimental setup for investigating photo-thermoelastic deformations. (b) Reconstructed 3D deformation map of the film at the focal point under external laser stimulation.*

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A scalable approach for integrating microelectronics on tapered optical fiber-based neural interfaces

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The development of advanced neural interfaces requires technologies capable of reliably recording and stimulating neural activity with high spatial and temporal resolution. Multifunctional optical fibers have recently emerged as powerful tools in this context, enabling simultaneous optical stimulation and electrical recording through integrated bioelectronic components [1,2]. Among these, Organic Electrochemical Transistors (OECTs) stand out for their exceptional sensitivity, biocompatibility, and stable performance in physiological environments [3]. These features make them particularly suitable for integration with optogenetics, where precise monitoring of neuronal responses to light is essential [4]. Despite their promise, the scalable and reliable fabrication of OECTs remains a key bottleneck. Conventional techniques such as photolithography and screen printing face limitations in resolution, material compatibility, and process efficiency—hindering their use in flexible, fiber-based platforms. Photolithography, although widely adopted, involves multiple processing steps, costly infrastructure, and restrictions on material deposition. Screen printing, while more scalable, suffers from low resolution and significant material wastage. In contrast, high-precision capillary printing (HPCP) has emerged as a powerful additive manufacturing technique for bioelectronic devices, offering fine spatial control, material efficiency, and compatibility with soft and flexible substrates [5]. A critical aspect of HPCP is the role of capillary forces, which govern droplet formation, spreading, and substrate adhesion—directly impacting pattern uniformity, resolution, and deposition precision.

In this study, we explore the use of the Hummink system, a high-precision capillary printing technology inspired by Atomic Force Microscopy (AFM), for microscale patterning of PEDOT:PSS—a widely used conductive polymer. The focus is on optimizing the ink formulation to meet the stringent requirements of HPCP. PEDOT:PSS ink was systematically modified to enhance viscosity, surface tension, and wettability, enabling controlled deposition and improved electrical performance. These adjustments led to improved film homogeneity, stronger substrate adhesion, and optimized conductivity. Such enhancements are particularly impactful in applications requiring stable, high-fidelity signal acquisition, such as optogenetics-based neural interfaces [6].

Our results highlight the significance of capillary-driven effects in high-precision printing and demonstrate how tailored PEDOT:PSS formulations can improve fabrication processes—paving the way for scalable, next-generation bioelectronic devices compatible with optogenetic applications.

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Raman Spectroscopy for the Spatial Characterization of Neurospheroids

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Raman spectroscopy is a technique based on inelastic scattering of light, used for the non-invasive analysis of the chemical composition of biological samples. Its application in biological tissue studies has gained significant interest due to its ability to detect molecular biomarkers without the use of fluorescent dyes or contrast agents [1]. For instance, recent studies have demonstrated the effectiveness of Raman spectroscopy in analyzing neurodegenerative diseases and brain tumors, highlighting its potential for both diagnostics and neuroscience research [2]. On the other hand, spheroids and organs on chip systems also have emerged as complementary fundamental tools to evaluate physiological phenomena in three dimensions, also greatly impacting the studies on neurodegenerative diseases and neural networks [3]. The application of Raman spectroscopy on those *in vitro* models has opened the possibility to track their evolution over time, such as maturation and aging [4].

With the aim of implementing a method able to track biomarkers of neurodegenerative diseases with spatial resolution on neural spheroids, in this study we investigate the dependance of the Raman signal on the number of probed cells within the spheroid. Neurospheroids composed of human iPSC-derived dopaminergic neurons co-cultured with human astrocytes in a 9:1 ratio, were formed in ultra-low attachment 96-well plates, where cells aggregated to form compact three-dimensional structures. To perform spatially resolved Raman measurements, we implemented the experimental setup depicted in **Figure 1A**. A 785 nm CW narrow-band laser was chosen to minimize autofluorescence while keeping a good signal-to-noise ratio. A representative Raman spectrum collected with the system at 10 mW excitation power and beam waist of 70 µm is shown in Figure 1B. We were able to identify main peaks in both the fingerprint (800–1800 cm^{-1}) and high wavenumber (1800–3200 cm^{-1}) spectral ranges (**Figure 1B**). We characterized the dependence of the generated Raman signal on the number of contributing cells, by tuning the beam waist, thus identifying the right trade-off between spatial resolution and volumetric information. The here described approach will be exploited for the tracking of biomarkers with spatial resolution in neurospheroids, aiming at a better understanding of degenerative process in *in vitro* 3D models of neural diseases.

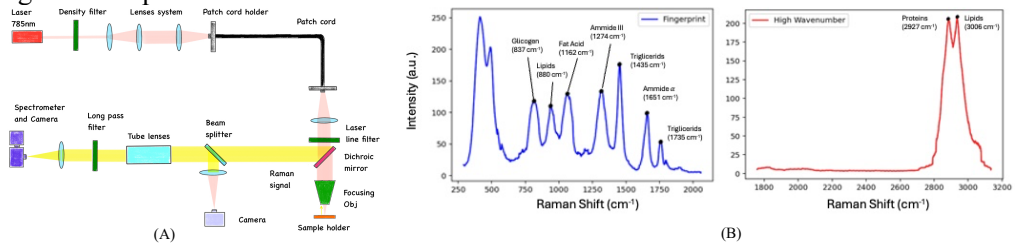

Figure 1. (A) Schematic representation of the experimental setup used for Raman spectroscopy. The laser beam, after passing through a series of filters and lenses, is coupled into an optical fiber and focused onto the sample via a 25× objective. The Raman signal is collected in backscattered configuration by the same objective and directed to the spectrometer for analysis. (B) Raman spectrum acquired with a laser spot size of approximately 70 µm. The main identified Raman peaks are shown in the graph.

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Development of a non-planar temporal focused holographic two-photon lithography (npTF-HoloTPL) system for high-resolution tapered optical fibers patterning

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In order to explore deep brain regions, the scientific community is focusing on developing implantable devices able to establish a bi-directional channel to optically trigger and electrically record neural activity. In this context, tapered optical fibers have emerged as a complementary tool to reduce photoelectric artifacts and tissue damage compared to other implantable systems [1]. However, microfabrication on their non-planar surfaces poses a significant challenge. While focus ion beam (FIB) milling and deposition enable sub-50 nm patterning resolution [2-4], the high atomic weight ions locally increase the waveguide's refractive index, reducing the photon transmission efficiency. On the other hand, laser ablation or two-photon photopolymerization have proven effective solutions to this issue, although they require extended fabrication times.

To overcome this issue and to realize complex patterns on non-planar surfaces, we propose to implement a non-planar temporal focused holographic two-photon lithography (npTF-HoloTPL) system. The concept is developed by starting from the temporal focusing used in two-photon imaging proposed in [5, 6] but applied to two-photon lithography. The system incorporates two spatial light modulators (SLMs) on the optical path: the first SLM for the generation of the hologram, the second SLM for spatial-resolved temporal focusing, after dispersion of the spectral frequencies on a grating [5]. We estimate to attain a voxel size of approximately 0.25 µm × 1 µm (lateral × axial size), which is beneficial for the realization of complex structures. We expect our system will allow us to project the hologram with a lateral field of view up to about 350 × 350 µm. As already demonstrated in [6], an axial displacement of the holographic light pattern up to 130 µm will enable multibeam and holographic patterning. A scan system comprising three galvanometric mirrors will be implemented to translate the projected pattern in the xy plane, and a nanometric roto-translational stage will also be adopted to achieve a complete fiber patterning [7]. This will allow a significant reduction in patterning times and improvement in throughput.

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Raman Spectroscopy with tapered optical fibers in the high wavenumbers discriminates different regions at depth in the mouse brain

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Raman spectroscopy is a label-free technique providing chemical information about the sample through vibrational fingerprints and has garnered significant attention for detecting and identifying complex molecular samples, such as tissues [1]. Here we present a low invasive approach to target and study deep brain regions, we exploited 785 nm Raman spectroscopy to collect signals from fixed mice brains by implanting a single tapered optical fiber (1 µm at its tip) at different depths, addressing Cortex (LPtA), Hippocampus, VL-thalamic nucleus, LH-Hypothalamic area and Striatum. Indeed, these brain areas display different cytoarchitectures and are characterized by different molecular profiles [2]. Spectra with a high signal-to-noise ratio were acquired in the high wavenumber spectra range (2750 cm^{-1} to 3100 cm^{-1}). We employed both supervised and unsupervised machine learning models for discriminating Raman spectral data from these five regions, including principal component analysis (PCA), Uniform Manifold Approximation and projection (UMAP), decision tree (Accuracy 72%), Linear discrimination analysis (Accuracy 56%), support vector machine (Accuracy 76%) and k-nearest neighbor (Accuracy 76%) [3]. Figure 1 shows a representative result in the range 2750 cm^{-1} to 3100 cm^{-1} .

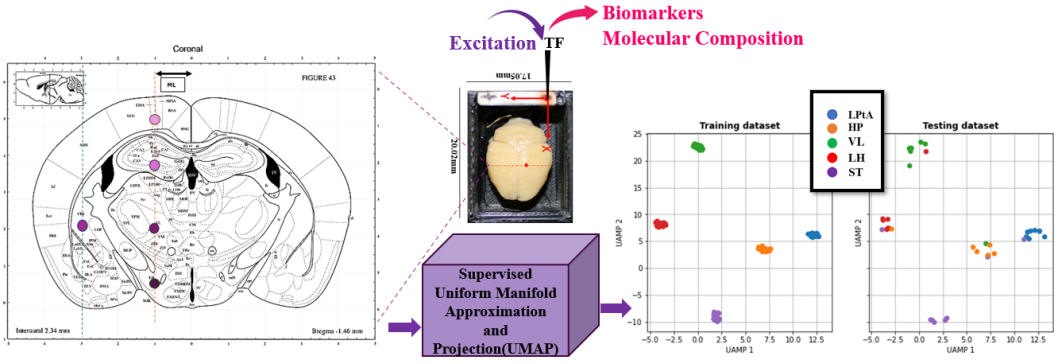

***Fig. 1**: Graphical depiction of workflow leading to discrimination of brain regions using Raman spectroscopy.*

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Numerical Simulation of Photoacoustic Ultrasound Pulses for Neural Applications

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In recent years, the study of neurological diseases has driven the demand for innovative devices capable of techniques such as blood brain barrier disruption or sonogenetics [1][2]. One possible way to address this demand is the use of ultrasound (US) waves. Photoacoustically (PA) generated US offers attractive properties of high penetration depth and high spatial resolution suitable for neurological applications [3]. In PA generation, powerful pulsed lasers are utilized for the generation of acoustic waves modulated by the thermal expansion of an absorbing elastomer [4]. To explore this phenomenon, we report a model based on Finite Element Method (FEM) approach using the software COMSOL Multiphysics. Specifically, since PA generation is based on the thermal expansion, our model used a Multiphysics approach to simulate the interactions between a radiative beam in an absorbing media, heat propagation in solids, mechanical deformation, and generation and propagation of acoustic waves.

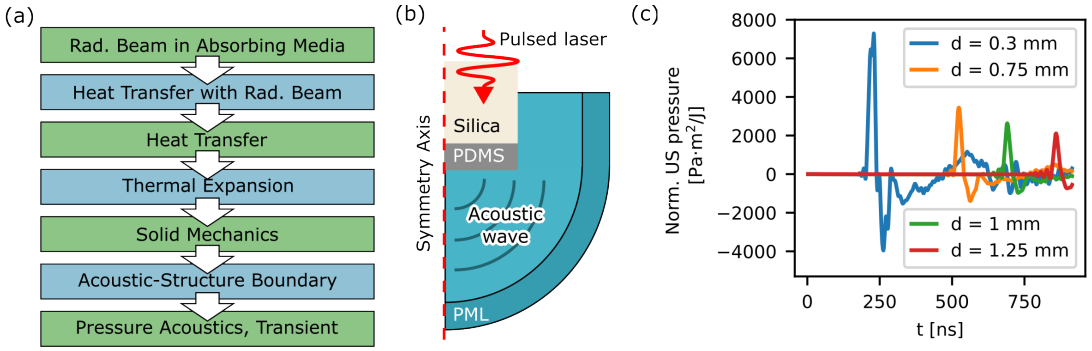

***Fig. 1**: (a): Flowchart of COMSOL Multiphysics modules utilized for the simulation. (b): Schematic of the simulated geometry. (c): Photoacoustically generated wave at different locations.*

Figure 1(a) shows a flowchart of the utilized physics and multiphysics interfaces, while figure 1(b) shows a schematic of the simulated geometry. A 6 ns laser pulse (100 W) is incident on a silica optical fiber (r = 0.100 mm) immersed in a water bath and terminated with an absorbing (α = 4.5e-2μm^{-1}) carbon/PDMS composite. As the fiber expands in response to the thermal absorption, an acoustic wave is generated in the water. The resulting US pressure, at a selection of distances, normalized with respect to the laser fluence is shown in figure 1(c).

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