Case study

High throughput optogenetic stimulation for auditory neuroscience

Tools:
SyncroPatch 384, Optogenetic Stimulation Tool

Dr. Thomas Mager
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Dr Thomas Mager is a group leader in the Institute for Auditory Neurosciences at the University Medical Center Göttingen. His research focuses on the development of novel optogenetic tools for the clinical restoration of hearing and vision. Using the SyncroPatch 384 coupled with the optogenetic stimulation tool, he screens large libraries of light sensitive ion channels to assess their function and clinical utility

Optogenetics is a fascinating technology which aims to control neuronal function using pulses of light. This is possible through the insertion of light-sensitive ion channels into the membranes of excitable cells. These proteins, known as opsins, are derived from microbial organisms that usually originate in the aquatic microbiome such as unicellular algae. Opsins can be either excitatory (e.g., Channelrhodopsin 2 [1]) or inhibitory (e.g., Kalium channelrhodopsins [2]), enabling the activation or suppression of neuronal firing, respectively.

Before being used in a physiological system, opsin function must be tested and validated using the patch-clamp technique. This approach is difficult and requires extensive user expertise. It is, by definition, a low-throughput method. New developments in automated patch-clamp (APC) instrumentation have removed this limitation, with high throughput devices such as the new SyncroPatch 384 which can measure up to 384 cells simultaneously in a single experiment. Importantly, this device is also compatible with a modular optogenetic stimulation tool (OST) that can guide 96 separate LED sources directly onto each cell as it is being patched. In combination with the SyncroPatch 32-well mode for partial plate use, the OST offers flexibility with 10 different excitation wavelengths to choose from and can be easily implemented into a variety of existing voltage protocols.

The field of optogenetics is a key focus of the group of Thomas Mager at the institute for auditory neurosciences in Göttingen. Led by Tobias Moser, this institute aims to develop optical cochlear implants for hearing restoration. In approximately 1 million patients, standard cochlear implants improve speech perception by bypassing the dysfunctional sensory organ and electrically stimulating the spiral ganglion neurons (SGNs) of the auditory nerve. However, cochlear implant users have problems understanding speech in the noisy environment of daily life. In the saline-filled cochlea, large spread of electric currents from the electrode contacts of the implant leads to unspecific SGN activation, therefore the transfer of frequency-specific information is limited and imprecise. Using light pulses instead of electrical activation

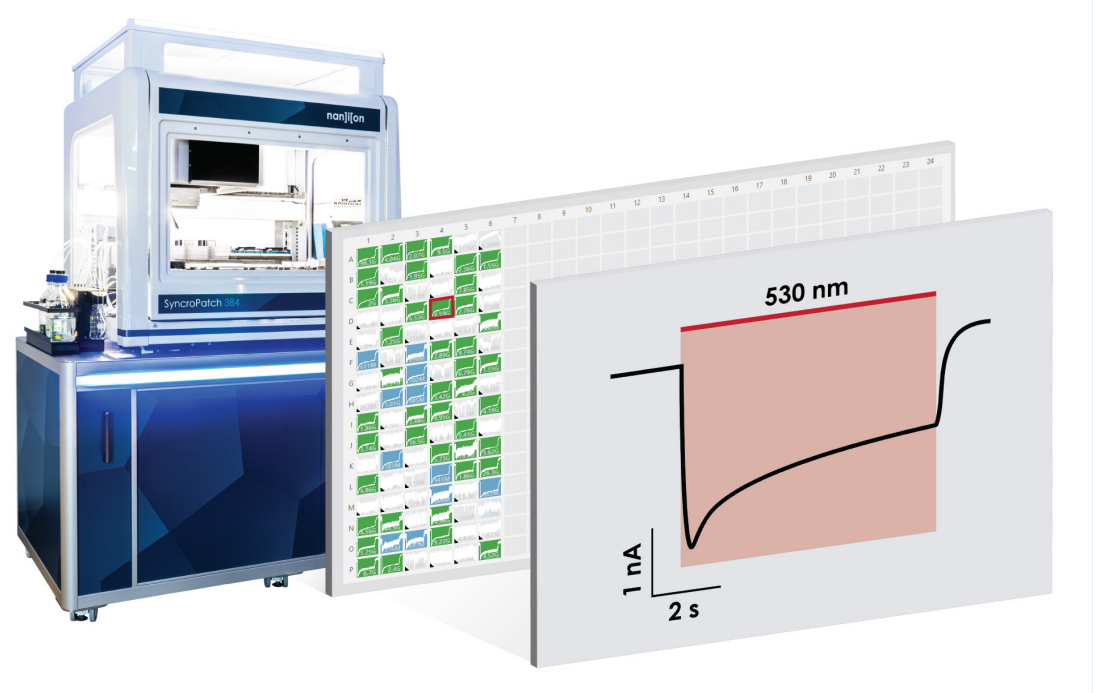
The SyncroPatch 384 with Optogenetic Stimulation Tool ensures efficient combination of high throughput patch clamp electrophysiology and optical activation. The Optogenetic Stimulation tool is seamlessly integrated into the SyncroPatch 384 for light activation of up to 96 wells simultaneously.

"The optogenetic stimulation tool for the SyncroPatch384 enables the precise characterization of multiple optogenetic actuators in a short period of time, which brings optogenetic tool development to the next level."

Dr. Thomas Mager, University Medical Center Göttingen

has the potential to improve spectral information transfer as light can be much better confined in space. Following the viral insertion of a suitable channelrhodopsin into the SGNs of the inner ear, a thin, multi microLED-implant inserted into the cochlea would allow for highly localized stimulation of SGNs at a level far more specific than currently available electrical cochlea implants.[3]

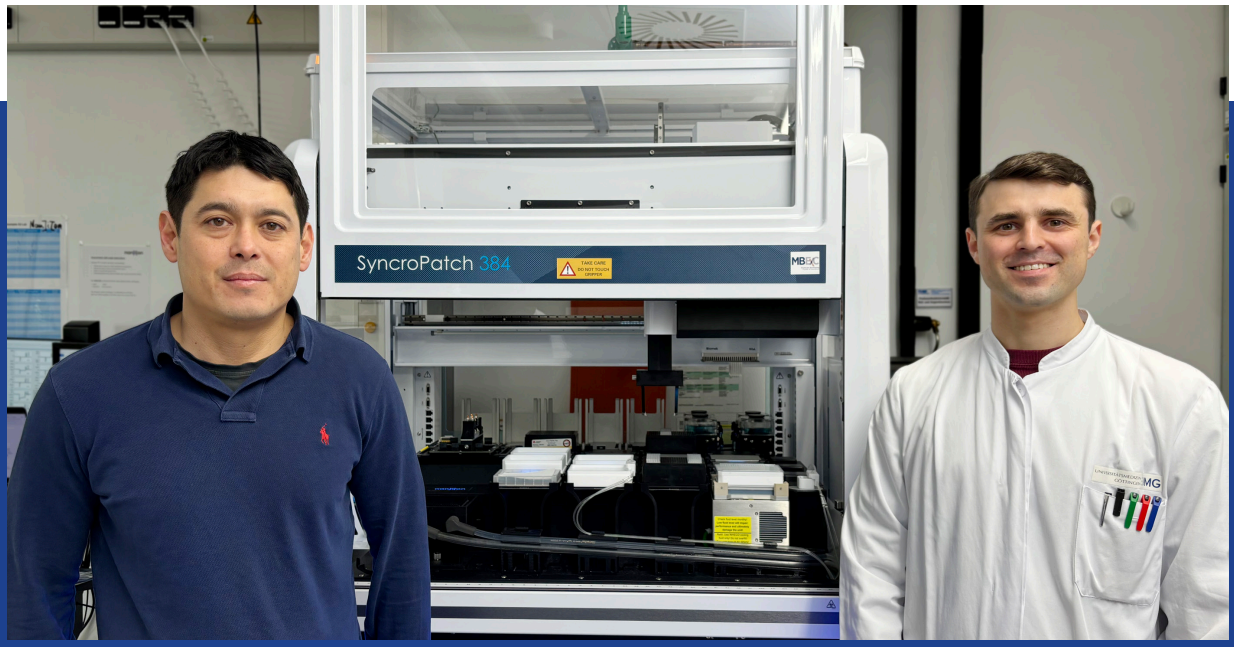
In order to achieve this goal, the Mager group must first identify and optimize channelrhodopsins that are suitable for sensitive *in vivo* systems like the inner ear. The efficiency of optogenetic control of excitable cell activity is limited by the low single-channel conductance of ChRs. This is highly relevant for future clinical applications like the optical cochlear implant, as proteostatic stress upon strong ChR expression as well as high light doses are potentially harmful. The Mager group optimizes ChRs by structure-guided mutagenesis to provide suitable action spectra, kinetics, and ion selectivity for future optogenetic therapies. In 2018, Thomas Mager and colleagues pioneered the development of red-shifted opsins with very fast closing times through a mutation of the "Chrimson" channelrhodopsin, originally derived from the alga Chlamydomonas noctigama in the lab of Ernst Bamberg at the Max-Planck-Institute of Biophysics in Frankfurt. [4,5] The improved opsin, labeled f-chrimson, enabled SGN activation at close to physiological rates using pulses of red light, which strongly reduces the risk of blue light induced phototoxicity. [4]



Using the SyncroPatch 384 to activate Channelrhodopsins. *The OST add-on for the SyncroPatch 384 can be used to elicit light-activated currents expressed in cells. Different wavelengths can be used, in this case, currents activated by light at a wavelength of 530 nm are shown.*

Using the SyncroPatch 384 coupled with the OST for recordings of ChR photocurrents, the Mager group recently demonstrated that ChRmine, a ChR variant derived from the microalgae Rhodomonas lens [6,7] exhibits a considerably elevated single channel conductance. This was the first statistically confirmed description of a higher-single channel conductance ChR under standardized conditions. [8] The utility of ChRmine however, was compromised by a decrease of the photocurrent (desensitization) upon sustained optogenetic stimulation. The Mager group minimized photocurrent desensitization in ChRmine by introducing mutations in a key domain for channel function, unleashing its potential for life science and medical applications. The advanced ChRmine variant was nicknamed ChReef (ChR that excites efficiently). The authors showed that in the living mouse, the auditory pathway was stimulated successfully under low energy conditions. This is particularly exciting from a clinical standpoint, as lowering the energy threshold represents a realistic step closer to wearable optogenetic devices for effective hearing restoration. Such technology is not only restricted to the auditory system. The authors have also shown the applicability of opsins like ChReef in the visual pathway through restoration of retinal ganglion cell function, recording clear responses in the primary visual cortex following optogenetic stimulation. [8]

The development of efficient optogenetic actuators is not only important for the clinical restoration of hearing or vision, but also has useful applications across the cardiac field for heart rhythm control and cardiac disease modelling. Red shifted opsins developed by Mager and colleagues have been recently used for the development of an *in vitro* model of atrial fibrillation, a serious and complex heart condition experienced by millions of patients worldwide.[9] By uniquely allowing long-term optical pacing of induced pluripotent stem cell-derived atrial cardiomyocytes, this scalable model will provide a deeper mechanistic understanding of how the disease progresses. In the future, these models will facilitate the development of effective and much needed treatments for atrial fibrillation and other cardiac arrhythmias.



Dr. Thomas Mager and Dr. Alexey Alekseev use the SyncroPatch 384 and OST to perform patch-clamp experiments. *Their research focuses on optimization and validation of opsins for use in medical research and devices.*

"The OST expands our ability to screen natural variants and follow previously impossible protein engineering approaches for the purpose of advancing optogenetic tools for basic sciences and future medical therapies."

Dr. Thomas Mager, University Medical Center Göttingen

The work of the Mager group highlights the vital importance of screening power for the discovery and development of new opsins. The high throughput nature of the SyncroPatch 384 coupled with the OST is invaluable for the systematic screening of thousands upon thousands of possible optogenetic actuators. Identification of the few that offer enhanced functionality and versatility is paramount to pushing the field of optogenetics into new frontiers. It is clear that high throughput optogenetic screening not only accelerates basic science research, but also holds profound implications for the development of clinical therapies.

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