Light-induced action potentials and stem cell-derived sensory neurons: an automated patch clamp investigation

Kefan Yang[1], Irene Lu[2], Tim Strassmaier[2], Patrick Mumm[1], Nadine Becker[1], Tom A Götze[1], Alison Obergrussberger[1], Michael George[1], Niels Fertig[1]

[1]Nanion Technologies GmbH, Munich, Germany; [2]Nanion Technologies Inc., Livingston, NJ, USA

Contact: ali@nanion.de

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1 Introduction

Channelrhodopsins (ChRs) are light-gated proteins that control phototaxis in protists and are commonly used in optogenetics to manipulate neuron activity. ChR2-$Na_V1.5$, a fusion of ChR2 with cardiac-specific Na^+ channel, modulates sodium channel kinetics and produces photosensitive inward currents[1]. Using automated low and high throughput patch clamp (APC), we found that blue-light intensity directly correlates with ChR2 channel activity, with higher intensities resulting in larger peak amplitudes under constant light exposure durations. We also found that light could be used to activate ChR2, depolarizing the membrane sufficiently in current clamp mode to elicit action potentials. This is demonstrated on both the Port-a-Patch, a semi-automated patch clamp device recording from a single cell at a time, and the high throughput fully automated device, the SyncroPatch 384. The SyncroPatch 384 could also be used to investigate different ChR2 variants. Several different constructs could be activated using blue light on a single NPC-384 chip. In this study we used HEK cells stably expressed ChR2-$Na_V1.5$ (kindly provided by Axxam SpA) but the technology could be extended to hiPSC-neurons or other stem cells or primary cells which express light activated ion channels.

2 Voltage and current clamp recordings of ChR2-$Na_V1.5$ on the Port-a-Patch

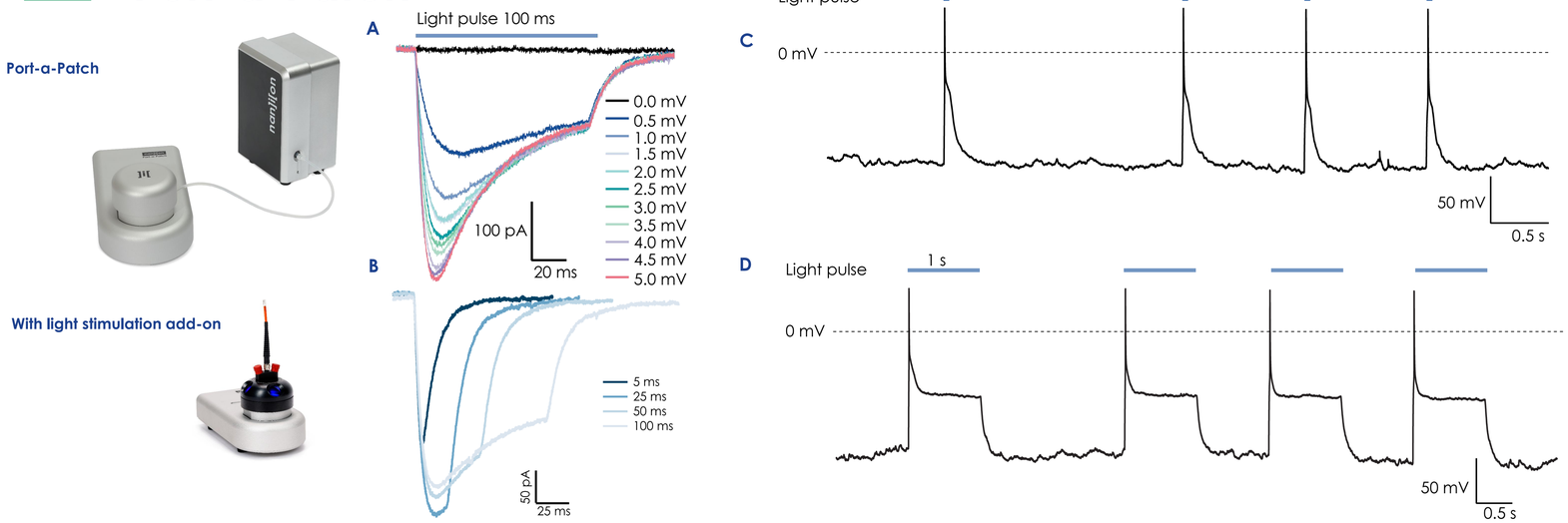

Figure 1: **ChR2-$Na_V1.5$ expressed in HEK cells recorded on the Port-a-Patch with light stimulation add-on. A** The whole-cell ChR2 currents were elicited using increasing light intensities under constant 100 ms light exposure. **B** Elicited ChR2 currents using increasing light exposure durations under constant 5 mV light intensity. **C** Action potentials recorded with 50 ms blue light pulse durations and **D** 1 s blue light pulses. Activation of ChR2 depolarized the membrane potential which in turn activated $Na_V1.5$ causing further membrane depolarization. HEK cells expressing ChR-$Na_V1.5$ were kindly provided by Axxam SpA.

3 ChR2-$Na_V1.5$ recorded on the SyncroPatch 384

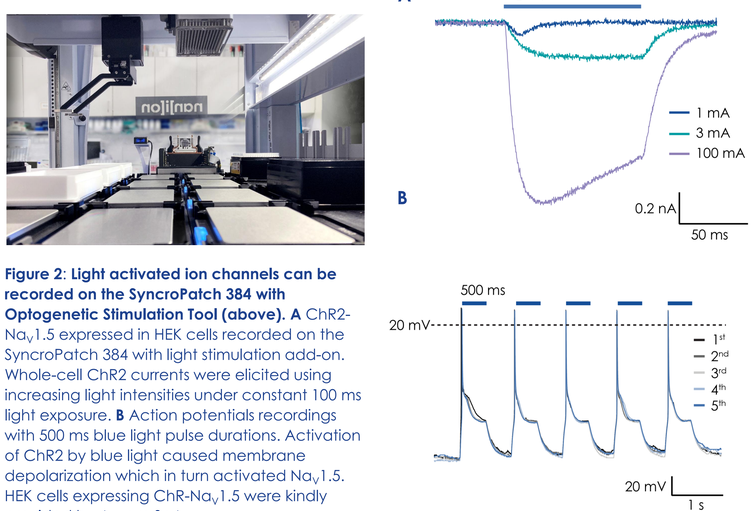

Figure 2: **Light activated ion channels can be recorded on the SyncroPatch 384 with Optogenetic Stimulation Tool (above). A** ChR2-$Na_V1.5$ expressed in HEK cells recorded on the SyncroPatch 384 with light stimulation add-on. Whole-cell ChR2 currents were elicited using increasing light intensities under constant 100 ms light exposure. **B** Action potentials recordings with 500 ms blue light pulse durations. Activation of ChR2 by blue light caused membrane depolarization which in turn activated $Na_V1.5$. HEK cells expressing ChR-$Na_V1.5$ were kindly provided by Axxam SpA.

4 Activation of different ChR2 variants

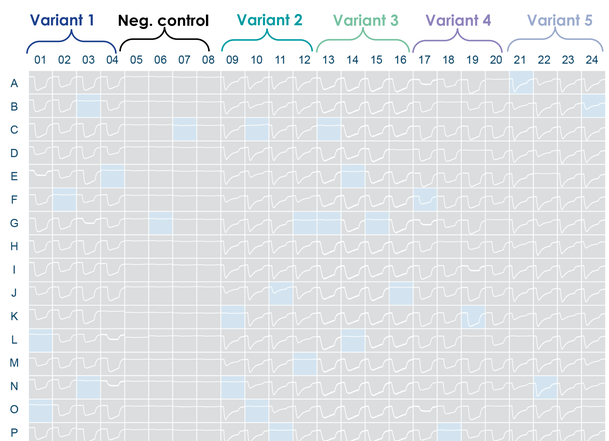

Figure 3: Investigating ChR using the SyncroPatch 384. Shown is an example of how the SyncroPatch 384 could be used to screen cells expressing different variants of ChR and activation during a 100 ms light pulse. In this theoretical example, 4 columns of the plate received one variant and 4 columns (05 – 08) received a negative control (untransfected cells).

5 Conclusions

- Recordings of ChR2-$Na_V1.5$-mediated currents and action potentials demonstrated the effectiveness of APC in light stimulation studies.
- Experiments could be scaled up to high throughput on the SyncroPatch 384 using light as the stimulus.
- Recordings could be made in voltage- and current clamp mode on the Port-a-Patch and SyncroPatch 384.
- The SyncroPatch 384 and Optogenetic Stimulation Tool could be used to screen channelrhodopsin variants.

References

1. Walther, F., et al. 2020. J. Gen. Physiol. 152(5):e201912489