

## A 'Forward Optogenetics' approach to discover general and neural pathway-specific nociception genes

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Nociception is a conserved process associated with the detection and transmission of noxious stimuli by nociceptor neurons into the nervous system. The nociceptors are essential to prevent damage to the organism. Understanding the molecular substrates or properties of the nociceptors and the nociception neural circuits may help developing better therapeutic approaches for pain management.

My main goal was to use the powerful *C. elegans* genetic model in order to obtain new insights into the molecular mechanisms modulating nociception circuit function.

Classical genetic screens on nociception phenotypes are limited by several factors, among which the difficulty to implement reproducible stimuli and the existence of functionally redundant genetic/neural pathways. To circumvent these limitations, I implemented an optogenetic pipeline to create a 'synthetic behavior paradigm' targeting a narrow neural pathway. I used animals expressing channelrhodopsin (CoChR) specifically in FLP nociceptors neurons, enabling the generation of light-induced escape behaviors, mimicking the natural response to noxious heat and harsh touch. Then I performed an Ethyl methanesulfonate (EMS) mutagenesis screen using FLP::CoChR animals and looked for animal mutants with defective light-induced escape behavior. I recovered new mutant lines, which were mapped with Whole Genome Sequencing (WGS) and confirmed with rescue approaches. The genes identified are implicated in diverse functions such as transcriptional regulation, synaptic transmission or calcium signaling and most of them had not previously been associated with nociception.

Among those genes, *unc-68* is an orthologue of the human Ryanodine receptors. Despite a broad expression in muscles and neurons and being involved in numerous functions, my work further reveals a cell autonomous role for UNC-68 in controlling FLP heat-evoked intracellular calcium ( $Ca^{2+}$ ) activity and noxious-heat avoidance. In addition, this study shows the requirement of UNC-68 channel to sustain animals speed through the maintenance of  $Ca^{2+}$  tonic signaling after prolonged noxious-heat stimulation. Finally, I show evidence of *unc-68* tissue-specific transcript isoforms controlling specific functions.

Overall my work provides a proof-of-concept for a novel 'forward optogenetics' approach that could be applied to other neural pathways or to address more elaborated phenotypes. Furthermore, it provides new entry points for further investigations on nociception, as well as a refined view on the diverse functions of the Ryanodine receptor channel and on the role of alternative transcription start selection and alternative splicing.

Jury:

Prof. Dr. Dominique Glauser (thesis supervisor)

Prof. Dr. Attila Stetak (external co-examiner)

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