

All-optical Electrophysiology in DRG Neurons: Applications in Drug Discovery

Christopher A. Werley, Pin W. Liu, Hongkang Zhang, Graham T. Dempsey, Owen B. McManus

Background: More than 100M people in the US suffer from chronic pain. Although several therapeutic classes are available, including NSAIDs, opioids, and gabapentinoids, a substantial fraction of patients suffer from poorly controlled pain and/or suffer significant side effects. Long-term NSAID use causes problems with the stomach, kidney, and liver, and opioids have adverse effects on respiratory, gastrointestinal and CNS function. Furthermore, patients often develop opioid tolerance, and abuse of the highly addictive drugs is a current public health crisis. Recent efforts to develop improved, non-opioid analgesics have floundered, and chronic pain remains a major area of unmet medical need, highlighting the importance of new tools and approaches.

Platform technology: To help develop new pain therapeutics, Q-State brings its proprietary platform for high-throughput all-optical electrophysiology in neurons. Optopatch recordings¹, which combine blue light-driven optogenetic stimulation with red light-excited voltage recordings using fluorescent proteins, have the rich information content of manual patch clamp data but with >10,000-fold higher throughput. Our custom *Firefly* microscope² enables electrophysiology measurements from 100s of sensory neurons in each well of a 96-well plate at a rate of 800 wells/day, a throughput sufficient to support phenotypic drug screening or medicinal chemistry. We routinely make Optopatch measurements on primary rat sensory neurons from the dorsal root ganglion (DRG), as well as human induced pluripotent stem cell (hiPSC)-derived sensory neurons.

An additional advantage of the platform technology is that the optical tools enable identification of neuronal sub-types using morphological characteristics and fluorescent labels. DRG neurons are highly diverse: they sense touch, temperature, pain, and itch. Using the TrpV1 promoter, we can label pain-sensing nociceptors, and evaluate compound effects specifically on this key cell type.

Pain-in-a-dish models: For many chronic pain conditions, pain *in vivo* is mediated by secreted soluble factors. For example, in osteoarthritis (OA), physical joint damage causes the accumulation of danger-associated molecular patterns (DAMPs), such as broken-down cartilage components and alarmins from damaged cells. DAMPs are detected by local immune and repair cells, which secrete inflammatory mediators that accumulate in the synovial fluid and modulate neuronal behavior. We can model this pain in cultured rat DRG neurons by applying a cocktail of these mediators, a Sensitizing PAin Reagent Composition (SPARC), which induces a nearly 3-fold increase in neuronal firing rate and causes neurons to fire action potentials in response to much more mild optogenetic stimuli. We have developed SPARC formulations for OA pain, tumor-induced pain, and inflammatory pain.

Discussion: We will discuss pharmacological testing of this assay system and future applications in phenotypic screening.

References:

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2. Werley, C. A., Chien, M.-P. & Cohen, A. E. An ultrawidefield microscope for high-speed fluorescence imaging and targeted optogenetic stimulation. *Biomed. Opt. Express* **8**, 5794 (2017).

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