Laser and Viral Optogenetic Neuromodulation of Peripheral Nerve Pain in a Rodent Model

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RESEARCH QUESTION

Optogenetics is an attractive mechanism for experimental neuromodulation, primarily due to the ease of manipulating light in frequency, intensity, and specificity. The delivery of these genes can be accomplished by multiple mechanisms, all of which have their respective pros and cons. In this retrospective analysis, we will compare the pros, cons, and overall effectiveness of neuromodulation for pain reduction resulting from both viral and laser gene delivery systems in a mouse model. The analysis will also examine potential behavioral differences arising from differing gene delivery systems.

BACKGROUND

Pain management has been a significant target for debate in medicine, economics, and politics for the past few years. Opioid medications are the mainstay of treatment; however, their utilization has severe, potentially life-threatening consequences. There is a need for alternative treatments for patients who present with severe, debilitating pain. Pain sensation and perception involve multiple nervous system structures that play a role in sensing, transmitting, processing, modulating, and perceiving pain. These processes all occur by integrating several cells at multiple biochemical signaling levels. Certain areas of the central nervous system, such as the Anterior Cingulate Cortex (ACC), the Dorsal Root Ganglion (DRG), and the Spinal Cord, are all intricately involved in pain perception (Figure 1). Here we are investigating the use of optogenetic neuromodulation of multiple regions of the central nervous system via two common gene delivery mechanisms in hopes of finding an alternative therapy to opioid medications for pain relief (Figure 2). Neuromodulation can potentially ameliorate pain without the severe side effects of contemporary pain therapy.

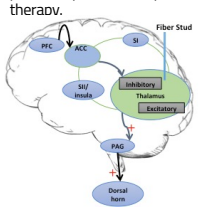

Figure 1: A schematic of pain sensation and modulation in the Central Nervous System (CNS). The Prefrontal Cortex (PFC), Anterior Cingulate Cortex (ACC), Primary Somatosensory Cortex (SI), Secondary Somatosensory Cortex (SII), Periaqueductal Gray (PAG), Thalamus, and Dorsal Horn are just some of the many structures thought to be involved.

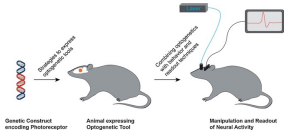

Figure 2: A diagram of optogenetic use for changes in neural activity in a rodent.[1]

METHODS

Using a glutamic acid decarboxylase (GAD) promoter, our group delivered our highly sensitive optogenetic modulator Multi-Characteristic Opsin (MCO) to target inhibitory GABAergic Anterior Cingulate Cortex (ACC), Dorsal Root Ganglion (DRG), and spinal neurons in a rodent model. Opsin delivery was accomplished by both adeno-associated viral (AAV) vectors for optogenetic stimulation and functional gold nanorods (fGNR) for optical stimulation. Optogenetic and optical stimulation was manipulated by a light source that delivered 630 nm red light, which our MCO was specific and sensitive to (Figure 3). We also controlled light delivery with a wireless phone application we created (Figures 4-6). The light's frequency and intensity were handled through the wireless application. Acute pain responses were assessed via a formalin pain model. Neuropathic pain responses were evaluated via a sciatic nerve ligation model, and Von Frey assays.

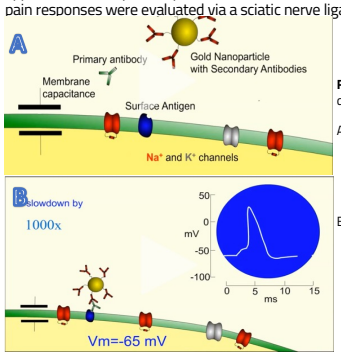

Figure 3: Optical stimulation by fGNR diagram.[2]

A) Primary antibody developed for specificity to GAD67 (GABAergic) neuron in the pain pathway. A secondary antibody with fGNRs is delivered to bind to the primary antibody.

B) The binding and delivery of laser allow for the heating of gold nanoparticles. A laser is delivered to the gold nanoparticles, which transmit heat to the target cell's membrane, thereby increasing the target cell membrane capacitance and allowing an action potential to occur.

- Overall, both laser and viral opsin gene delivery are equal in terms of efficacy of pain relief.
- However, laser gene delivery is preferential to viral delivery in several ways.
  - A. Avoidance of immunologic response and subsequent degeneration of involved tissues.
  - B. Increased specificity and control of delivery to intended targets.

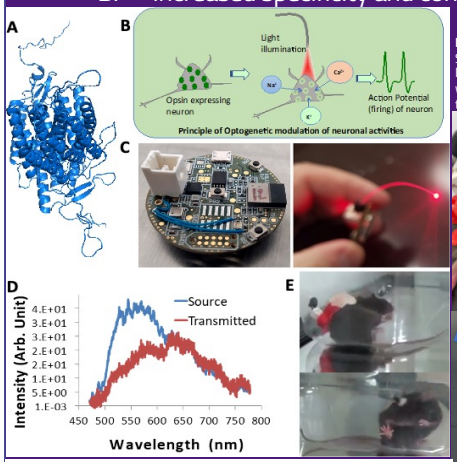

Figure 4: A) The protein structure of the Multi-Characteristic Opsin (MCO). B) Schematic of opsin-mediated neuromodulation. C) Wireless Optogenetic Pain Modulator (OPM) Device with attached fiber and light source D) Intensity and wavelength of the light source and transmitted light. E) The OPM was sutured to the backs of the rodents with the light source entering a defect in their skulls.

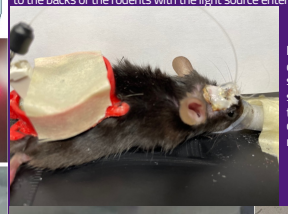

Figure 5: An LED device coupled with our fiber stub was glued to the skulls of our mice after they received a craniotomy for CNS modulation.

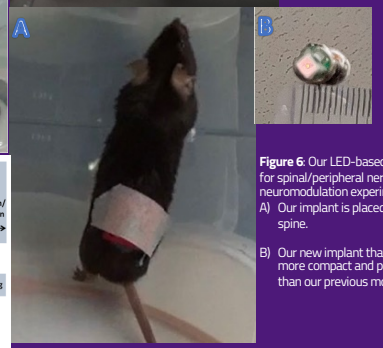

Figure 6: Our LED-based implant for spinal/peripheral nerve neuromodulation experiments.

A) Our implant is placed over the spine.

B) Our new implant that is much more compact and powerful than our previous model.

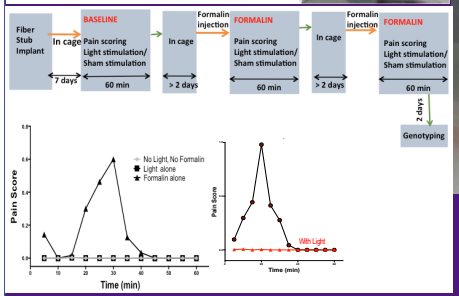

Figure 7: A schematic of our formalin assay showed a significant decrease in pain scoring in the presence of light, confirming the effects of optogenetic modulation.

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RESULTS

Optogenetic modulation of the ACC and DRG decreased acute pain responses in all experimental assays. During the (inflammatory) phase of responses to the presence of formalin, there were significant reductions in pain scoring. This reduction was only specific to this phase and not seen in the nociceptive phase of the formalin assay (Figure 7). Therefore, with the preservation of the acute (nociceptive) pain response and the significant reduction of the inflammatory phase, our results confirm that optogenetic stimulation effectively reduces prolonged pain due to inflammation. We also saw a statistically significant decrease in pain scores with our Von Frey assays as well (Figures 8 and 9). When comparing the pain reduction of laser gene delivery to AAV gene delivery, initial reductions in pain responses were not statistically different. There were also no statistically significant differences when comparing ACC versus DRG pain scores. Laser gene delivery was more consistently effective when comparing overall gene delivery between the two methods. Additionally, there was a single experiment where AAV gene delivery, unfortunately, resulted in the deaths of an entire cohort of mice.

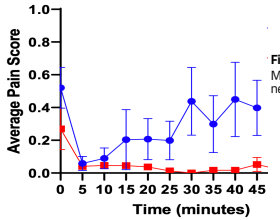

Figure 8: Von Frey Assay after sciatic nerve ligation. MCO spinal therapy was delivered to target peripheral nerve pain.

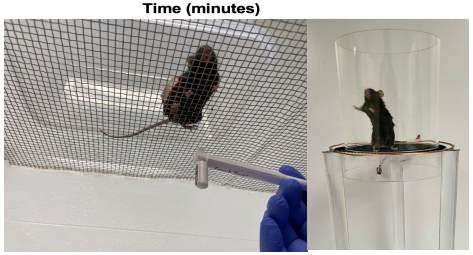

Figure 9: Von Frey assays are utilized to analyze mechanical allodynia and pain sensation in mice. The soles of the hind paw of the rodents were exposed to both pinprick and monofilament as corollaries for pain and control, respectively.

DISCUSSION AND CONCLUSION

Overall, our MCO was effective via AAV and laser particle delivery for reducing pain. This effect was present when the MCO was targeted to the ACC and the DRG. Our results were statistically significant from controls; however, experimental groups had no statistically significant differences. Overall, both laser and viral gene delivery are equal in terms of efficacy. However, it appears that laser gene delivery is preferential to viral delivery in two distinct manners – avoidance of immunologic response and subsequent degeneration of involved tissues and increased specificity and control of delivery to intended targets.

FUTURE DIRECTIONS

Previous studies have demonstrated that following the primary introduction of the viral vector, subsequent exposure induces a maladaptive immunologic response that may be responsible for tissue degeneration in a dose-dependent manner.[3] In our experience, we have additionally seen greater control of gene delivery in a manner that is superior to that seen in our viral vector studies. In conclusion, we advocate for laser gene delivery systems to become the new standard method for gene delivery – particularly in rodent models for neuromodulation.

ACKNOWLEDGEMENTS

The author Robert Benkowski has an equity interest in Designplex Biomedical, LLC. The author Samarendra Mohanty has an equity interest in Nanoscope Technologies, LLC.

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