Epilepsy Treatment with Targeted Injection of ChR2 and Upconverting Nanoparticles

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Introduction & Background

Epilepsy affects approximately 50 million people worldwide.[1] It is characterized by recurrent seizures, impairing an individual's quality of life.[2] Up to one-third of epileptics do not achieve adequate seizure control with medication alone.[3] Thus, alternative therapies are urgently needed. Optogenetics holds great promise as an emerging field for treatment of epilepsy.[4] It involves the use of light-sensitive proteins, opsins, which are selectively expressed in specific neurons using genetic techniques.[5] When activated by light, these opsins can modulate neuronal activity, controlling the activity of specific neural circuits.[6] The opsin ChR2 is a non-selective cation protein channel that regulates the influx of cations across the cell membrane under blue light stimulation, causing the depolarization and subsequent activation of neurons (see figure 1, *right*).[7] ChR2 has been explored as a promising tool in optogenetics for epileptic seizures and other central nervous system injuries.[8,9] Generally, however, light sources such as lasers and LEDs require invasive surgical procedures to bypass the skull and activate opsins, especially in the case of blue-shifted light which falls outside the optical window.[9]

Motivation & Rationale

Transcranial penetration can be addressed using upconverting nanoparticles (UCNs). These are luminescent-material particles that can can convert NIR to blue light by absorbing multiple (three to five) low energy photons and releasing them at shorter wavelengths, also called *Anti-Stokes emission*.[10,11] This is achieved through the use of transition metal, lanthanide, or actinide ions doped into a solid-state host.[12] Transcortical photostimulation using UCNs is a promising technique in optogenetics for ChR2 activation (see figure 1 below).[9]

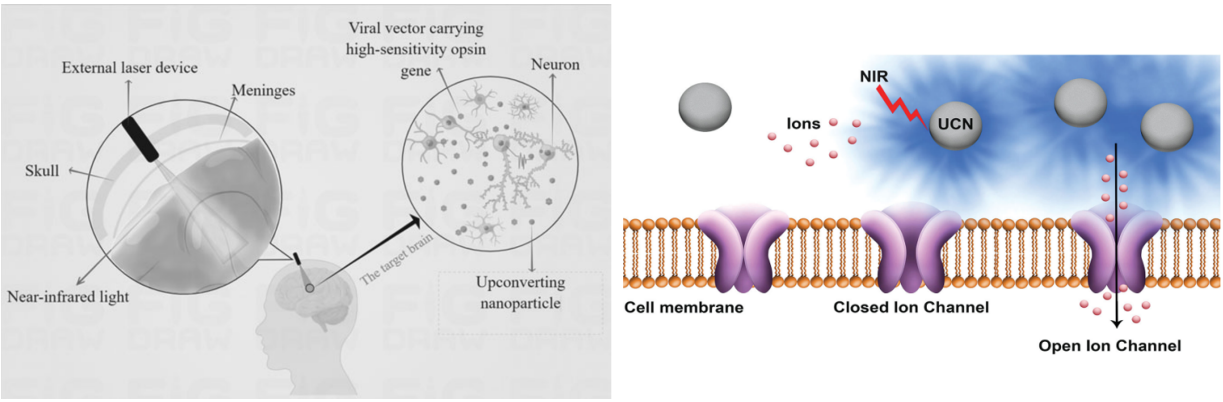

Figure 1. (Left) A schematic showing transcortical stimulation of opsins with NIR light[9] (Right) A schematic representation of ChR2 ion channel manipulation via blue light produced by UCNs upon NIR irradiation[11]

As seen in figure 1 (left), transcortical stimulation requires that an external laser device be embedded in the skull to achieve adequate penetration depth.[9] However, quasi-continuous wave (quasi-CW) light, which increases UNP transducer efficiency, can potentially remove the need for implants in optogenetics.[11] **This proposal aims to create a method for treating epilepsy by injecting ChR2 opsins and UCNs into the hippocampus, and achieving photoactivation through the use of UCNs with quasi-CW NIR light.**

Proposed Research

Specific Aim 1: Visualize UCN responsiveness to quasi-CW NIR light and conversion to blue light in the presence of phantom skull and tissue using confocal microscopy. Studies have shown that red-shifted light can enable transcranial photoactivation

of opsins with high light sensitivity, which can bypass transcranial surgery.[9,13] Recent advances in UCN technology enabled successful excitation *in vivo* with C. elegans using low power NIR quasi-CW light, with reduced photo-toxicity and heat damage to tissue.[11] NIR light allows for deep tissue penetration[14] and the high transducer efficiency achieved by quasi-CW NIR can potentially mimic the high-sensitivity absorber required to bypass surgery.[11] UCN photoactivation can be implemented via the method used by Chen et al., replacing the red-shifted excitation light with quasi-CW excitation[16] 980 nm (NIR) light[14] and using a phantom skull and tissue instead of mice. Blue light emission is dependent on the UCN concentration and laser power.[14] Confocal microscopy imaging used by Chen et al. can determine the optimal UCN concentration and laser power needed to achieve blue light emission in the presence of the phantom skull and tissue.

Specific Aim 2: Determining conditions in which blue light stimulates ChR2 opsins effectively. Adeno-associated virus (AAV) can be used as a vector for gene therapy.[15] The optogeneic control of ChR2 opsins for neuronal stimulation within the mouse brain can be achieved by injecting a ChR2-expressing AAV vector into a region of interest.[7,15,16] To transcranially stimulate neurons in the hippocampal CA1, these vectors can be injected with ANG/PEG-UCNs that have been shown to emit strong blue light at 980 nm excitation under deep penetration depths.[14,15,16] A fiber optic cable can be fixed over the intact skull next to the site of injection. This cable could deliver short pulses of 980 nm light at varying pulse durations, irradiance, and frequencies.[14,16] A trial range of pulse durations (1-100ms), irradiance (40–1,600mWmm−2 (5–200mW)), and frequencies (5-40 Hz) can be derived from the optogenetic study conducted by Chen et. al.[16] Electrodes can be placed in the hippocampal CA1 region following a craniotomy. The EEG signal can be collected by a data acquisition system and the resulting data would be analyzed with MATLAB.[16] The conditions that induce the highest neuronal responsivity— and thus the greatest ChR2 opsin excitation — can be recorded.

Specific Aim 3: Applying methodology to mice model to quantify effect on seizure frequency and duration. Electrical stimulation can trigger synchronized activity in a large number of neurons in the hippocampal CA1 region, leading to seizures.[17] Utilizing the optimal parameters ascertained in Specific Aims 1 and 2, KCNQ2/3 channels will be targeted in the Hippocampal CA1 area to pursue an anti-epileptic effect.[18,19] ChR2 activation leads to influx of positively charged ions (Na+ and Ca2+) into the cell, resulting in membrane depolarization and triggering an opening of voltage-gated potassium channels, including KCNQ2/3.[20] A study by Raimondo et al. (2012) showed activation of ChR2 in hippocampal neurons led to the activation of KCNQ2/3 channels. Active targeting of KCNQ2/3-expressing cells in hippocampal CA1 can be achieved with use of a promoter for the Kcnq2 gene. This promoter allows controlled expression of ChR2 to the cells that also express KCNQ2/3 channels, yielding control of response through light stimulation.[21,22] Quantification of seizure response can be monitored and recorded through in vivo electrophysiological recordings from the hippocampal CA1 region of mice before and after ChR2 activation, and the frequency and duration of seizure activity will be compared.[13]

Anticipated Impact & Deliverables

In exploring the NIR excitation of blue light emitting UCNs for the optogenetic treatment of epilepsy, this proposed study introduces the beginnings of a noninvasive yet precise suppression of epileptic seizures that can be implemented in surgical settings. This form of treatment circumvents the abnormal neuronal activity epilepsy inflicts upon the brain while minimizing the tissue damage seen with surgical intervention. On a larger scale, this methodology will pave the way towards the development of real-time optogenetic treatments of serious diseases and disorders.

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