MAE 207 Research Proposal

Epilepsy Treatment with Targeted Injection of ChR2 and Upconverting Nanoparticles

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CONTENTS

01
BACKGROUND

BACKGROUND

Epilepsy

- Epilepsy affects ~ 50 million people worldwide (Fisher)
- Characterized by recurrent seizures, impairing an individual's quality of life (Devinsky)
- Up to ⅓ epileptics do not achieve adequate seizure control w/ medication alone (Krook-Magnuson)

The New York Times

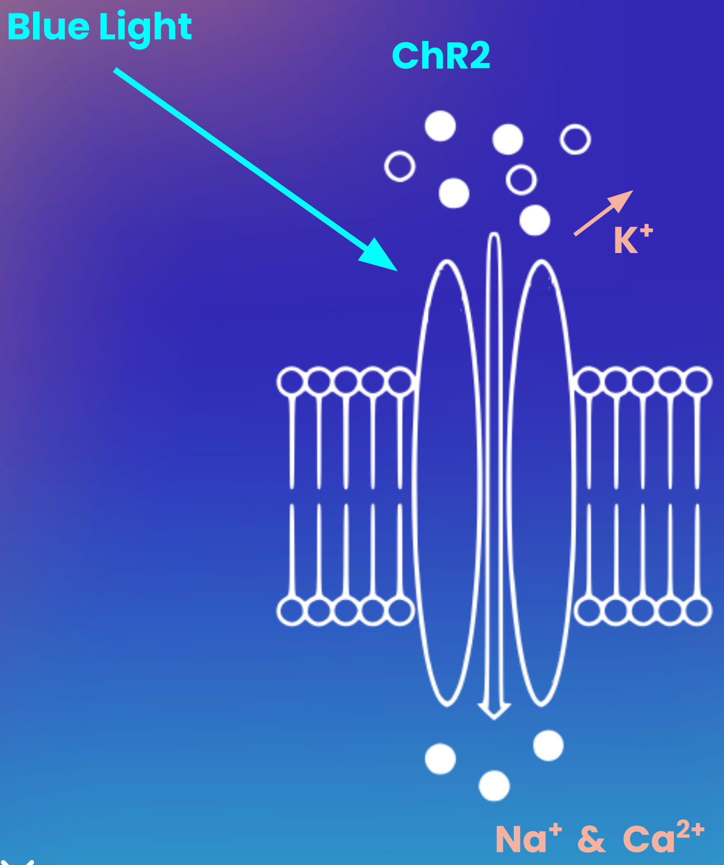
Optogenetics

- Holds great promise as an emerging field for treatment of epilepsy
- Light-sensitive proteins, opsins, selectively expressed in specific neurons using genetic techniques (Zhang)
- When activated by light, opsins modulate neuronal activity and control activity of specific neural circuits (Tønnesen)

ChR2 Opsin

- Non-selective cation protein channel
- Regulates the influx of cations across the cell membrane under blue light stimulation (Zhang)
- ChR2 has been explored as a promising tool in optogenetics for epileptic seizures and other central nervous system injuries (Zhang, Geng)

- Light sources (lasers, LEDs) require invasive surgical procedures

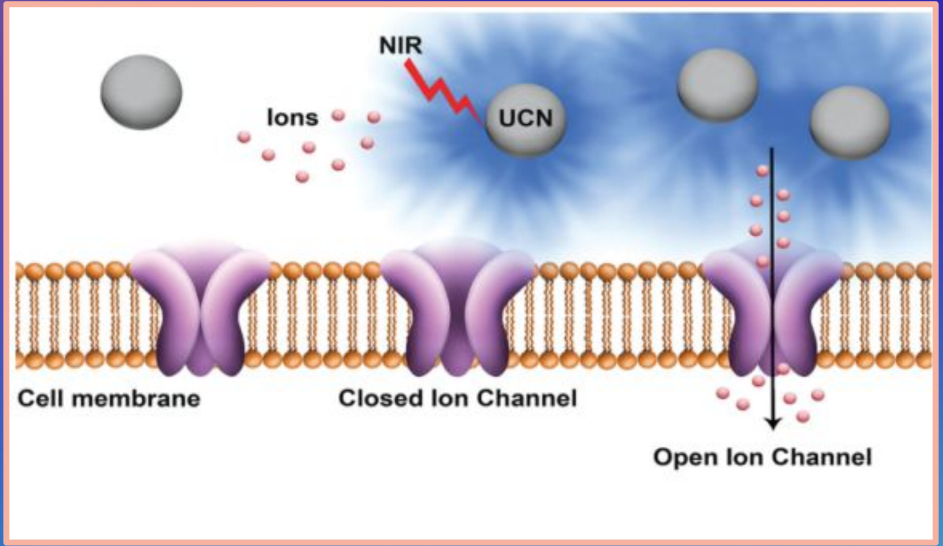


02
MOTIVATION & RATIONALE

UCNs

- Low energy NIR converted to high energy blue light
- Generally 3 to 5 photons absorbed
- UCN material: transition metal, lanthanide, or actinide ions doped into a solid-state host
- Generally requires a light source partially embedded in the skull

(J. C. Boyer et al. 2006)
(Chatterjee et al. 2010)

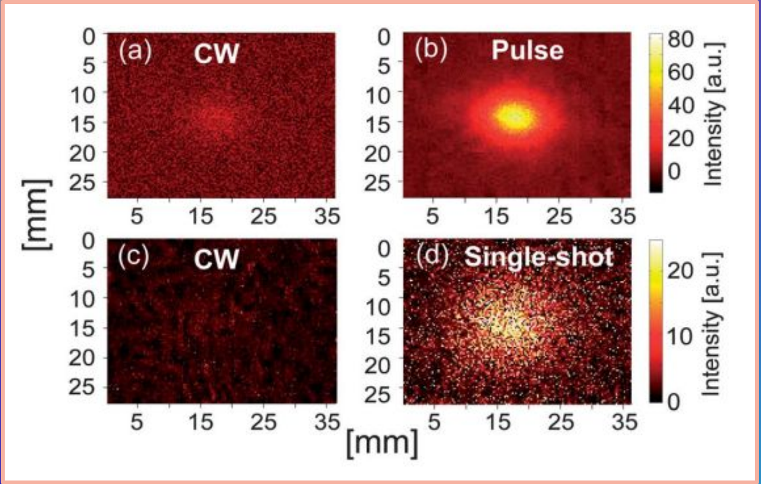


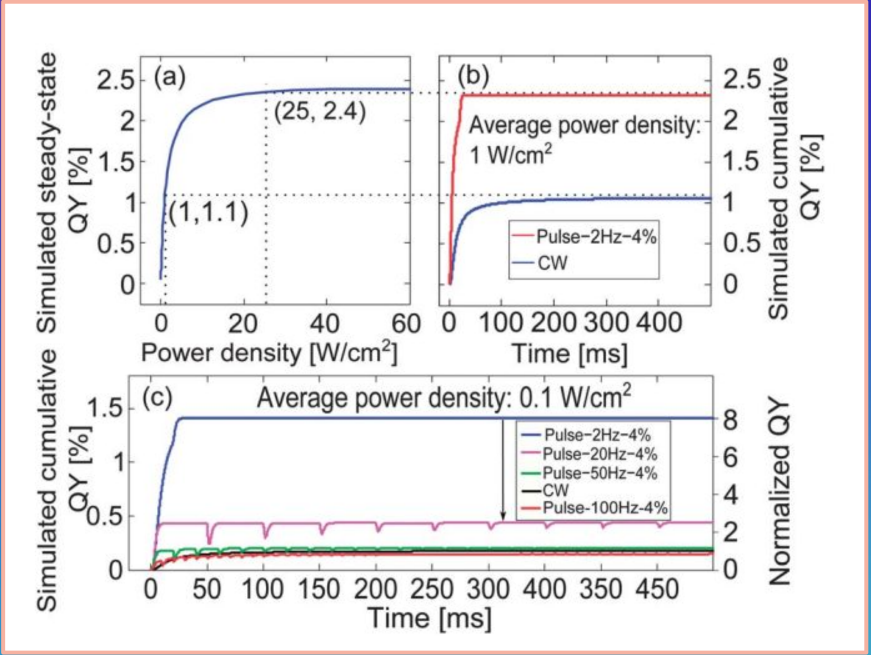
(Bansal et al. 2016)

QUASI-CW LIGHT

- Pulsed light
- Increases the quantum yield

(Liu et al. 2013)





03
SPECIFIC AIMS

Specific Aim 1: Transcranial UCN responsiveness

- Test UCN photostimulation with quasi-CW NIR light (980 nm)
- Use phantom skull and tissue (recreate characteristic extinction coefficient)
- Determine optical laser power and UCN concentration
- Imaging with confocal microscopy

(Bansal et al. 2016)
(Ni et al. 2014)

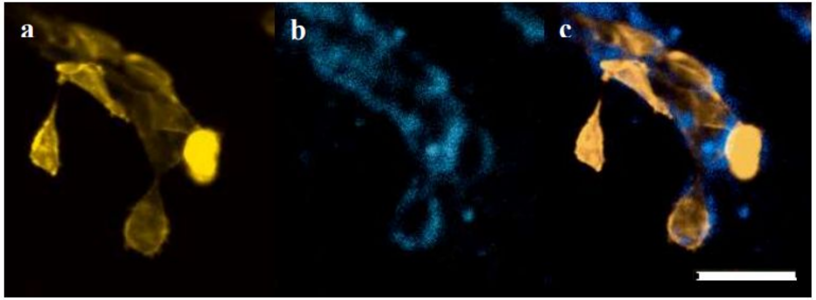
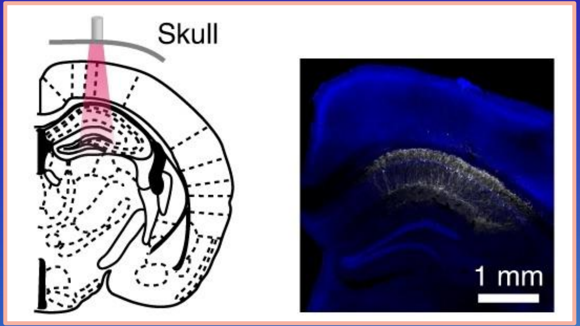


Figure S1 Hek293t cells transfected with ChR2-EYFP and incubated with UCNs showing **(a)** EYFP expression **(b)** Upconverted blue fluorescence from UCNs and **(c)** overlap of the two. Scale bar= 50 µm.

(Bansal et al. 2016) Supporting Information

Specific Aim 2: Determining conditions in which blue light stimulates ChR2 effectively

- Inject ChR2 expression AAV vector with PEG-UCNs into the hippocampal CA1
- Fix fiber optic cable over skull adjacent to injection site
- Test range of pulse durations, irradiance, and frequencies
- Place electrodes in hippocampal CA1 region and collect EEG signal
    - Record conditions that produce highest responsivity



Chen et. al. 2021

Specific Aim 3: In Vivo Anti-Epileptic Effect

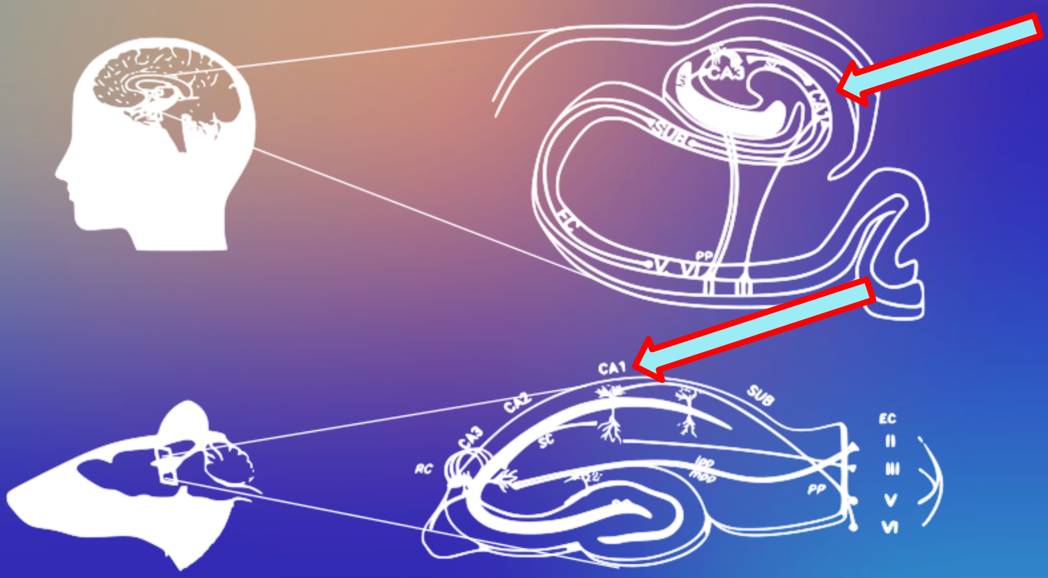


Figure Adapted from [Roux 2020]

Triggering Seizures

Electrical stimulation can trigger synchronized activity in a large number of neurons in the hippocampal CA1 region, leading to seizures.

Inhibiting Seizures

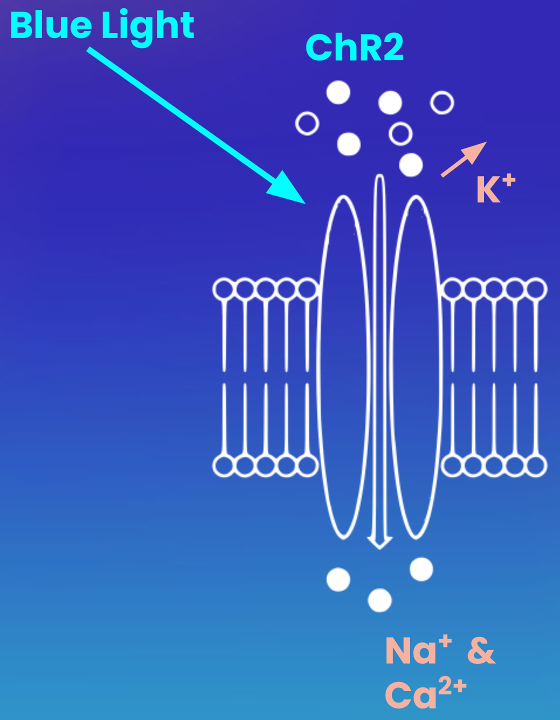
Utilizing the optimal parameters ascertained in Specific Aims 1 and 2, KCNQ2/3 channels will be targeted in the Hippocampal CA1 area to pursue an anti-epileptic effect.

Specific Aim 3: In Vivo Anti-Epileptic Effect

Raimondo et al. (2012) showed activation of ChR2 in hippocampal neurons led to the activation of KCNQ2/3 channels.

Active targeting of KCNQ2/3 will be accomplished using a promoter for the KCNQ2/3 gene.

Allows controlled expression of ChR2 to the cells that also express KCNQ2/3 channels, yielding control of response through light stimulation



Specific Aim 3: In Vivo Anti-Epileptic Effect

Quantification of Seizure Response

Monitor and record electrophysiological recordings from CA1 of mice before/after ChR2 activation

Compare frequency and duration of activity.

Electrophysiology: physiology pertaining to flow of ions in biological tissues

Electrical recording: place electrodes into biological tissue (Scanziani)

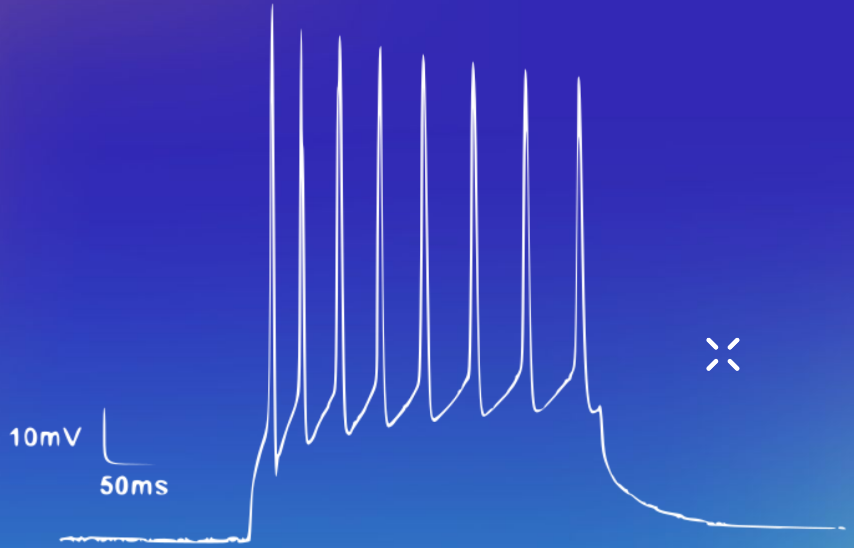


Figure: Example of electrophysiological recordings from a current clamp type device

Adapted from [Creative Commons]

04

ANTICIPATED IMPACT & DELIVERABLES

- Potential for noninvasive & precise suppression of epileptic seizures
- Contribution to development of real-time optogenetic treatments of serious diseases and disorders

Thank You!

Questions?

RESEARCH RESOURCES

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