PREDICTING SUCCESSFUL GENERATION AND INHIBITION OF SEIZURE-LIKE AFTERDISCHARGES AND MAPPING THEIR SEIZURE NETWORKS USING FMRI

CROSS REFERENCE

[0001] This application claims priority to U.S. Provisional Application No. 62/980,964, filed February 24, 2020, which is incorporated herein in its entirety for all purpose.

INTRODUCTION

[0002] Targeted therapies such as deep brain stimulation (DBS) or more recently optogenetics promise to revolutionize the treatment of refractory epilepsy by interfering with specific neuronal circuits involved in the generation and propagation of seizures. However, while considered a goal of many experimental epilepsy therapies, it is unknown whether local inhibition at the seizure origin is sufficient to curtail seizure activity or whether rapid seizure propagation across a wider network necessitates a multi-site approach. Furthermore, because of our poor understanding of seizure networks in animal models, there has been limited development toward such approaches. A greater understanding of seizure propagation pathways is a necessary step toward enabling targeted therapies for treating refractory patients.

[0003] Optogenetics employs genetic targeting, which can be used to disentangle the roles of different cell types and networks in seizure maintenance. Most investigations into using optogenetics for seizure control have focused on spontaneous seizure models, which on the one hand afford an opportunity to study ictogenesis and how different brain states relate to intervention efficacy. On the other hand, without temporal control of seizure onset, it is challenging to define the exact origin of the seizures or employ advanced imaging methods such as optogenetic functional MRI (of MRI) or calcium imaging.

[0004] Development of methods to inhibit seizures and to map seizure networks are of great clinical interest. The present disclosure addresses this issue.

SUMMARY

[0005] Methods are provided herein for mapping and reducing seizures. In one aspect, the present disclosure provides a method of analyzing a seizure network, the method comprising: a) delivering a first polynucleotide that encodes a depolarizing light-reactive protein to a target neuron, to express the light-reactive protein in the target neuron; b) administering to the subject an effective amount of light that specifically activates the light-reactive protein thereby leading to optogenetic stimulation; and c) generating an activation map with fMRI. In some ~~embodiment~~examples seizures are inhibited locally, for example with local optogenetic inhibition. In some ~~embodiment~~examples the seizures result from optogenetically induced afterdischarges (AD). Methods are also provided for generating a seizure network map, which can find use in identification of targets for intervention. ADs that can be suppressed locally show similar but less widespread seizure networks compared with those that could not be inhibited. It is shown that success in generating or curtailing seizures is related to their onset time, with inhibition proving less effective on earlier-

onset afterdischarges. To identify downstream targets for intervention, simultaneous fMRI and LFP can be used to map the seizure network of afterdischarges initiated from the dorsal and ventral hippocampus.

[0006] A seizure induction and inhibition model is provided that can be analyzed with simultaneous electrophysiology, including local field potential (LFP) and functional MRI (fMRI). In some ~~embodiment~~examples, specific neurons or overlapping sets of neurons comprise both an inhibitory and excitatory light-reactive polypeptides. The model provides a means mapping seizure induction and inhibition. For example, whole-brain fMRI imaging can be used to determine which regions are involved in ADs that could not be curtailed using local inhibition. Imaging of induced seizures events can be analyzed in fine detail. For example, distinct networks were identified for ADs originating from the VH compared with those generated from the DH. Models include an animal brain, e.g. a live animal, which may be a mammal, e.g. a rodent such as a rat, mouse, etc., non-human primate, and the like. In some ~~embodiment~~examples, to image induced seizures with simultaneous LFP-fMRI in an animal model, the animal may be sedated and treated with a short-acting neuromuscular blocker to abolish motion during imaging of seizures, exemplary agents for this purpose include, without limitation, dexmedetomidine sedation and vecuronium.

[0007] In some ~~embodiment~~examples, a seizure is generated by depolarizing neurons of interest in an individual. Neurons that find use in the ~~invention~~disclosure, include but is not limited to, neurons in the ventral hippocampus, dorsal hippocampus, medial pre-frontal cortex, medial temporal lobe, etc. including, for example, CaMKII-positive excitatory neurons in the dorsal and ventral hippocampus. Neurons may be depolarized using a variety of methods, which include but are not limited to, optogenetic stimulation, chemical treatment, electrical stimulation, etc. When optogenetic stimulation is used to induce seizure networks, a number of light-reactive polypeptides may be used. The light-activated polypeptide for stimulation may be, for example, a channelrhodopsin, including without limitation ChR2, ChR1, VChR1, etc. The light-reactive polypeptide may be operably linked to a promoter expressed in excitatory hippocampal neurons. Promoters that find use in this method, include without limitation, a hSyn promoter, CAMKII promoter, a thy1 promoter, etc.

[0008] In some ~~embodiment~~examples, a second light-reactive polypeptide is used to hyperpolarize neurons of interest after they have been depolarized, in order inhibit seizures, i.e. inhibit ADs. In some ~~embodiment~~examples, optogenetics is used to hyperpolarize neurons. When optogenetics is used to hyperpolarize (inhibit) neurons of interest, a number of light-reactive proteins may be used. Light-reactive polypeptides of interest include but are not limited to, NpHr, eNpHr2.0, eNpHr3.0, eNpHr3.1, GtR3, etc. The second light-reactive polypeptide may be operably linked to a promoter

expressed in excitatory hippocampal neurons. Promoters that find use in this method, include without limitation, a hSyn promoter, CaMKII promoter, a thy1 promoter, etc. In some ~~embodiment~~examples, the first light-reactive polynucleotide is operably linked to a promoter that is different from the second light-reactive polynucleotide.

[0009] In some ~~embodiment~~examples both excitatory light-reactive polypeptides and inhibitory light-reactive polypeptides are co-expressed in cells, or in overlapping cell populations. For example, ChR2 and eNpHR3.0 can be expressed in a cell from the same or different promoters. In some ~~embodiment~~examples the promoter is hSyn.

[0010] In some ~~embodiment~~examples, inhibiting ADs generated by optogenetic stimulation requires a minimum light intensity. For instance, a minimum of 10mW may be required in order to inhibit ADs generated by optogenetic stimulation. In some ~~embodiment~~examples, the light intensity may be, including without limitation, 1000-1500mW/mm^2, 1500-2000mW/mm^2, 2000-2500mW/mm^2, 2500-3000mW/mm^2, or greater than 3000mW/mm^2.

[0011] When using optogenetics for stimulation and/or inhibition, light is applied to neurons in an individual. Any suitable method may be used to deliver light to a region of interest, including without limitation, transdermally, through an implanted device, etc.

[0012] Early spiking is demonstrated to be predictive of successful inhibition of seizures. Onset time is predictive in seizures from both the dorsal and the ventral hippocampus. Short dorsal and ventral seizures are bilateral and unilateral, respectively fMRI networks are similar but broader when optogenetic inhibition is ineffective.

[0013] The data provided herein demonstrate that more progressed ADs with earlier ictal onset time are more likely to be sustained. Local optogenetic inhibition could curtail seizure-like ADs, although more progressed ADs cannot be interrupted using optogenetic inhibition. Voxel-wise activation time maps can be generated, comparing the spread of seizure activity between ADs originating in the dorsal and ventral hippocampus. fMRI activation maps for ADs originating from the ventral hippocampus indicate that ADs that did not progress were limited to the hypothalamus, amygdala, and prefrontal cortex. fMRI activation maps with dorsal hippocampus stimulation and inhibition show that ADs did not progress and were limited to the anterior dorsal hippocampus. Optogenetic inhibition applied during optogenetic stimulation dramatically attenuates the fMRI response, while applied by itself, optogenetic inhibition does not alter the cbv-weighted fMRI signal.

[0014] During after discharge induction using optogenetics, LFP recordings show that stimulations with earlier ictal onset times are more likely to result in afterdischarges and are more difficult to curtail with optogenetic inhibition. These results are generalizable across two