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RESEARCH REVIEW

# Hacking the Brain:
## Gene Therapy as a Tool to Combat Epilepsy

**MAXWELL NG, KRISH BILIMORIA, KARANBIR BRAR, NIKESH PANDEY**
Bachelor of Health Sciences (Honours) Program, Class of 2018
McMaster University
Correspondence: ngmc2@mcmaster.ca

## ABSTRACT

Since its inception in 1972, gene therapy has demonstrated remarkable progression and advancement. Initially through the use of engineered viral vectors and later through the use of complex RNA-based editing tools, the fundamental processes and techniques used in gene therapy have grounded themselves on a watershed of discovery. A potential application of gene therapy is in epilepsy, which is a prevalent set of neurological disorders characterized by epileptic seizures of numerous divergent pathologies. Though many epileptic disorders are idiopathic in nature, the genetic predisposition of generalized epilepsy with febrile seizures plus (GEFS+) has been well documented in scientific literature. GEFS+ is believed to be caused by a mutation in the SCN1A gene, coding for a subunit of a specific ion-gated sodium channel on bipolar and pyramidal neurons. In a rat model of GEFS+, we propose the use of herpes simplex virus type 1 as a vector to deliver zinc finger nucleases and a corrected SCN1A gene sequence to simultaneously knock out and replace the mutated SCN1A gene. This experimental design would demonstrate the prospects of epileptic gene therapy in the rodent brain and, by extension, validate the plausibility of genetic editing procedures in a clinical setting.

## INTRODUCTION

Epilepsy is a brain disorder predominantly characterized by recurrent and unpredictable seizures.[1] Generalized epilepsy with febrile seizures plus (GEFS+) is a subtype of autosomal dominant epileptic syndromes characterized by over 90 mutations of the sodium channel voltage gated type 1 alpha subunit (SCN1A) gene.[2,3] GEFS+ pathogenesis is mostly genetic. One form of GEFS+ is Dravet syndrome, a rare and lethal form of epilepsy also known as Severe Myoclonic Epilepsy of Infancy (SMEI). 80% of patients with Dravet Syndrome have mutations in the SCN1A gene.[4] These mutations confer either hypoexcitability, a repressed reaction to stimuli, or hyperexcitability, an excessive reaction to stimuli, in the sodium ion channel $Na_v1.1$. As a result, afflicted individuals have a greater susceptibility to seizures due to a widespread dysfunction of their neural network inhibition.[2,3]

Multiple causal factors are associated with epileptogenesis. Since the early 1990s, pharmacological approaches have had limited progress in treating epileptic symptoms, with no decrease in drug-resistant epilepsy cases.[5,6] This provides the impetus for the development of new, more effective treatments, namely in the form of gene therapy.[7]

Gene therapy is the process of delivering nucleic acids including adenine, thymine, guanine, and cytosine in a packaged vector to patient cells.[4] Replication-defective recombinant herpes simplex virus type 1 (HSV-1) is an effective and unique vector for neuronal gene therapy. It is a virus that preferentially infects the brain by spreading across synapses, establishing latent infection, and evading immune responses.[8,9] HSV-1 is able to disguise itself upon infection to allow the DNA vector to be conserved in neurons for extremely prolonged periods of time, thereby maintaining long-term neuronal infection. Lastly, HSV-1 has a large genome containing few spliced genes, allowing for the insertion of large foreign genes for gene therapy applications.[10,11]

Zinc finger nucleases (ZFNs) are a recent development in genetic engineering for the cleavage of DNA strands. ZFNs combine the nonspecific cleavage domain of FokI endonucleases, restriction enzymes that break phosphodiester bonds between nucleotide bases, with zinc finger proteins to achieve site specific gene targeting and editing. Subsequent non-homologous end joining occurs after cleavage activity.[12] When paired with a viral vector, ZFNs allow for a targeted combinatorial method of gene editing that has powerful potential for therapeutic applications. ZFNs have also been cleared through Phase II of clinical trials, lending towards their credibility as a safe and scalable system for genetic editing. If our proposed treatment method involving ZFNs is successful in a rat model, it can be further applied to humans and potentially serve as a therapy for this form of epilepsy.

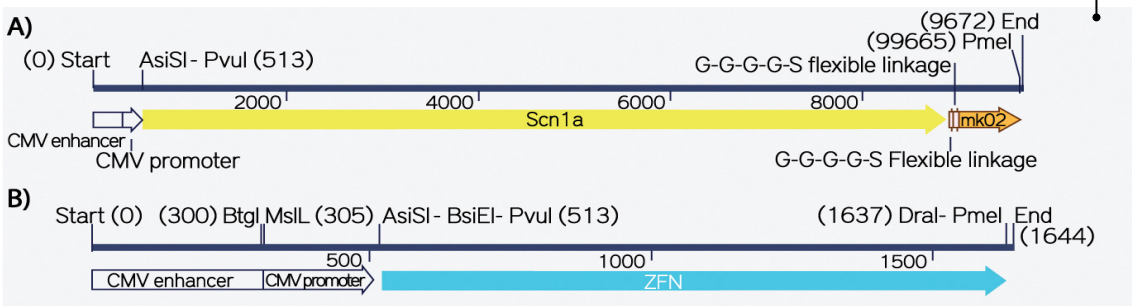


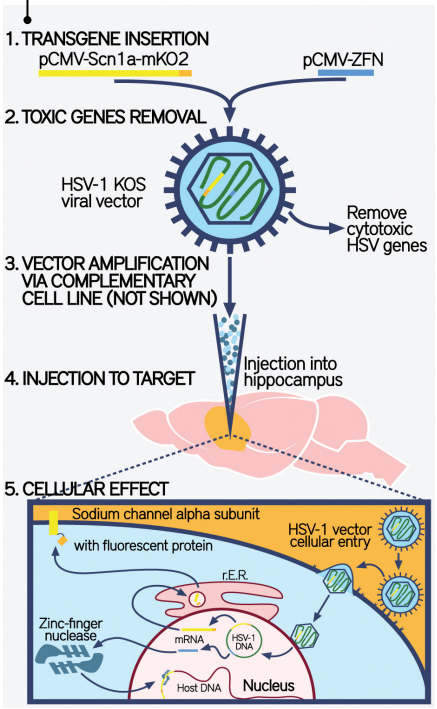

FIGURE 1: a) pCMV-SCN1A-mKO2 transgene. The CMV enhancer and promoter pair is upstream from the corrected SCN1A gene. The mKO2 orange fluorescent protein is connected to the SCN1A by a flexible linkage to minimize interference. b) pCMV-ZFN transgene. The CMV enhancer and promoter pair is upstream from the zinc finger nuclease (ZFN) encoding gene that will remove the mutated SCN1A gene present in the host DNA.

## RESEARCH DESIGN AND RESULTS

The following series of experiments outline a 'proof-of-concept' genetic treatment on a rat model for the specific form of epilepsy being studied. These animal subjects are characterized by GEFS+ type 2 symptoms, which are inducible via hyperthermia, due to a mutation in their Scn1a gene and are therefore known as hyperthermia-induced seizure-susceptible (Hiss) rats. The treatment is a solution of standard artificial cerebrospinal fluid (CSF) harboring HSV-1 vectors containing Scn1a-specific-ZFN DNA and the correct Scn1a gene. The expressed ZFNs will cleave the mutated Scn1a gene in the host DNA. The HSV-1 vector has a large DNA capacity of 150 kilobase pairs (kbp) that can allot for the rat Scn1a gene and the smaller ZFN.[10,11]

The experimental method involves an *ex vivo* study using rat brain slices and an in vivo study using rats. For *in vivo*, the treatment solution will be injected directly into the hippocampus of rats using stereotaxic surgery, a method of injection using stereotaxic coordinates that map to specific brain structures.[13] Direct injection circumvents processes in the bloodstream and the blood-brain barrier that could act as confounding variables in the experiment. An overview of the treatment design is shown in Fig. 2.

FIGURE 2: Treatment concept. Cytotoxic herpes simplex virus (HSV) genes are removed from the HSV-1 KOS viral vector. The pCMV-SCN1A-mKO2 and the pCMV-ZFN transgenes are cloned into the the HSV dsDNA. The viral vector is amplified via a complementary cell line. These viral vectors are then injected into the hippocampal region of the host. The viral vector inserts its DNA, including the transgenes, into the cell's nucleus where it circularizes and expresses the transgene proteins. The ZFN removes the mutated SCN1A host DNA and the new SCN1A channel protein with linked fluorescent protein, eventually replacing the mutated SCN1A channel proteins.




### Vector

The transgenes for the correct donor Scn1a (pCMV-Scn1a-mKO2) (see Figure 2.a) and the ZFN pair (pCMV-ZFN) (see Figure 2.b) will be enclosed in a replication defective HSV-1 vector. To minimize possible adverse effects, the less pathogenic KOS HSV-1 strain is chosen, while cytotoxic and replication-essential genes are removed.[14] The expressed ZFN will knock out mutant Scn1a in targeted hippocampal cells, while the new fluorescent correct sodium channel subunit will serve as a replacement. The mKO2 fluorescent protein is used as a reporter to verify the gene's successful transfection and expression. This novel use of HSV-1 and ZFNs offers the ability to correct the Scn1a gene and alleviate GEFS+ type 2 symptoms in Hiss rats, a specific rat model for this epilepsy.

### Ex Vivo

Our ex vivo experiments are divided into two broad categories: electrophysiology and expression testing. The electrophysiology testing will first use hippocampal slices in artificial CSF prepared from 12 day-old Hiss rats and wild-type F344/NSIc rats. Slices are chosen and allocated to obtain one F344/NSIc set (1) and five Hiss sets. The five Hiss brain slice sample sets are: (2) Mutant Control - Untreated cell culture; (3) Vehicle Control - "Empty" HSV-1 vector; (4) ZFN Test - HSV-1 carrying Scn1a specific ZFNs; (5) Reporter Control - HSV-1 with a gene for the mKO2 fluorescent protein; and (6) Treatment - HSV-1 carrying the ZFN, mK02 reporter, and the correct Scn1a cDNA. Whole-cell patch clamp electrophysiology measurements for the F344/NSIc slices and various Hiss slices will act as controls to determine baseline electrical recordings for comparison with the test groups.[15]

Next, our expression measurements will measure RNA transcription. Quantitative reverse transcriptase PCR (qRT-PCR) will be utilized to determine the difference in transcription of Scn1a RNA between the control F344/NSIc and untreated Hiss slices and the four test groups, the treated Hiss slices. Western blots for the alpha subunit of $Na_v1.1$ channels will then measure the level of the protein expression in each group. Fluorescent microscopy should detect the mKO2 reporter in cultures (5) and (6) to determine the quantity and location of successfully transfected cell populations in the brain slices. Expected results, per group, are shown in Table 1.

### In Vivo

Our in vivo testing will use experimentally naïve Hiss rats and experimentally naïve wild-type F344/NSlc rats (line from which the mutated Hiss line was derived), all starting at an age of four weeks.[16] Stereotaxic surgery will inject the viral vector precisely into the hippocampal region.[13,17] Weekly tests will involve inducing epileptic seizures by situating the rats in a hot water bath at 45˚C and scoring the intensity of seizures on the 0-8 stage Moshé Racine scale.[18] Unlike some methods, hot water baths can induce seizures in rats of up to 10 weeks of age.[16] Rat behaviour will be videotaped at all times to monitor the presence and intensity of spontaneous seizure activity over the length of the experiment. At 10 weeks of age, rats will be euthanized with $CO_2$ and their brains will be removed and flash frozen prior to sectioning. Expected results are shown in Table 1. Animals are to be housed and tested in compliance with guidelines provided by the Guide to the Care and Use of Experimental Animals.[19]

## DISCUSSION

The Hiss rat model used in this experiment has several limitations. Firstly, it focuses on a single missense mutation - a change in one nucleotide base resulting in a single substituted amino a in the

| Group | Rat Strain | Measuring Expression (Expected to be the same *ex vivo* and *in vivo*) | | *ex vivo*: Electrophysiological Recordings | *in vivo*: Behavioural Analysis |
|---|---|---|---|---|---|
| | | Scn1a RNA Levels | $Na_v1.1$ Protein Levels | | |
| 1 | F344/NSlc | Wild-type Control | Wild-type Control | Wild-type Control: Normal recordings | Wild-type Control: Relatively low incidence and intensity of hyperthemia induced seizues (HIS) |
| 2 | Hiss | Mutant Control | Mutant Control | Mutant Control: Hyperpolarizing shift in voltage dependence of inactivation and higher persistent sodium current in bipolar neurons | Mutant Control: Relatively high incidence and intensity of HIS |
| 3 | Hiss | Equivalent to Mutant Control | Equivalent to Mutant Control | Equivalent to Mutant Control | Equivalent to Mutant Control |
| 4 | Hiss | ↓Mutated | ↓Mutated | Marked changes in overall electrical activity | ↑Incidence and intensity of HIS ↑Incidence of spontaneous seizures |
| 5 | Hiss | Equivalent to Mutant Control | Equivalent to Mutant Control | Equivalent to Mutant Control | Equivalent to Mutant Control |
| 6 | Hiss | ↑Correct ↓Mutated | Resemble Wild-type control | Resemble Wild-type Control | ↓Incidence and intensity of HIS, resemble Wild-type Control |

TABLE 1: Expected results

protein's peptide sequence. This is not an accurate model of human GEFS+, which is characterized by numerous mutations in the SCN1A gene. Further testing of the therapy on different mouse models would be required to improve its validity as a treatment. In addition, other species may have varied immune responses to HSV, as well as different neuronal cell populations that will have different transfection rates; thus, the treatment will need to be altered before any potential application in humans. Furthermore, the seizures in this model are only hyperthermia-induced and are therefore not representative of other causes of seizures, such as light-induced or drug-induced. Moreover, seizure activity is only tested for the first 10 weeks of life, which does not provide information about long-term efficacy of the treatment. Lastly, the vector is injected in the hippocampus in this model. Although this site has been implicated as the origin of epileptic activity GEFS+, it may not be the sole locus of seizures in the disorder.[20]

Moving forward, it is important to develop a global delivery system that would enable expression of the therapeutic SCN1A gene in the entire mouse brain. To avoid activation in other cells aside from neurons, as measured using fluorescence, the currently designed CMV promoter can be replaced with a synapsin promoter.[21] The experiment should also be repeated over a longer time frame using different SCN1A mutations and methods of inducing seizure to establish the long-term effects of the therapy in various scenarios. Finally, if this step is successful, we would subsequently evaluate non-invasive methods of delivery (e.g. exosomes, sonoporation) and techniques to deliver the therapy across the blood-brain barrier, which would be required for any human applications in the future.

## CONCLUSION

Gene therapy is one of the most rapidly developing fields in science, and this proposal presents the foray of this technique into the field of epilepsy, a combination that has not been heavily explored in previous literature. Given the novelty of delivering ZFNs in conjunction with the therapeutic gene, our study would also represent a major advancement for gene therapy techniques. If successful, this therapy would have major implications in the treatment of debilitating epilepsies caused by mutations in the SCN1A gene, including Dravet Syndrome, It would set a precedent for future investigation into the use of gene therapy as a treatment for genetic brain diseases, and would be a hallmark in genetic editing science.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**REVIEWED BY DR. ERIC SEIDLITZ** EDITED BY ELLEN HE



Dr. Eric Seidlitz is a distinguished researcher in the department of Pathology and Molecular Medicine and is a member of the Singh Lab at McMaster University. His research focuses extensively on the mechanisms surrounding pain in cancer and explores novel techniques for its management. In addition to his academic endeavours, Dr. Seidlitz is also known for teaching within the Bachelor of Health Sciences program.