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# Only Hit the Bad Guys: A Gene Therapy Approach to Selectively Silence Highly Active Neurons Reduces Chronic Seizures in Epileptic Mice

**On-Demand Cell-Autonomous Gene Therapy for Brain Circuit Disorders**

Qiu Y, O'Neill N, Maffei B, Zourray C, Almacellas-Barbanoj A, Carpenter JC, Jones SP, Leite M, Turner TJ, Moreira FC, Snowball A, Shekh-Ahmad T, Magloire V, Barral S, Kurian MA, Walker MC, Schorge S, Kullmann DM, Lignani G. *Science*. 2022;378(6619):523-532. doi:10.1126/science.abq6656. PMID: 36378958; PMCID: PMC7613996

Several neurodevelopmental and neuropsychiatric disorders are characterized by intermittent episodes of pathological activity. Although genetic therapies offer the ability to modulate neuronal excitability, a limiting factor is that they do not discriminate between neurons involved in circuit pathologies and "healthy" surrounding or intermingled neurons. We describe a gene therapy strategy that down-regulates the excitability of overactive neurons in closed loop, which we tested in models of epilepsy. We used an immediate early gene promoter to drive the expression of Kv1.1 potassium channels specifically in hyperactive neurons, and only for as long as they exhibit abnormal activity. Neuronal excitability was reduced by seizure-related activity, leading to a persistent antiepileptic effect without interfering with normal behaviors. Activity-dependent gene therapy is a promising on-demand cell-autonomous treatment for brain circuit disorders.

## Commentary

Almost a third of people with epilepsy do not respond to anti-epileptic drugs and thus new treatments are needed to help these patients. Gene therapy, where a treatment induces new genetic material to control the disorder, has long been a source of hope for drug-resistant forms of epilepsy. One general approach to gene therapy in epilepsy has been to express an inhibitory protein in the brain that can rescue the hyperexcitability that causes seizures. Proof-of-principle of such approaches have been shown in rodent models of epilepsy, with generally positive results that suggest gene therapy is a viable approach to controlling seizures.[1] However, previous studies have focused on reducing excitability of many neurons simultaneously, which may be too broad for clinical use as they can induce off-target effects on cognition, anxiety, and other behaviors. Since seizures appear to be initiated by a subset of neurons that are hyperexcitable, a more precise manipulation that targets only the cells with abnormal excitability would hold great potential to control seizures without negative side effects.

In an exciting new study, Qiu and colleagues[2] developed a targeted gene therapy approach that is able to selectively silence only the neurons that are highly active, and found compelling evidence that this approach can reduce seizure burden with minimal off-target effects. This study is particularly novel because it used an "activity-dependent" approach, a method commonly used in rodents to study memories, to transiently reduce neural signaling only in highly active cells. This allows for a closed-loop system, where the therapeutic intervention is only directed toward cells that are highly active at any given time and thus responding directly to the pathological activity in the brain. This targeted approach can pinpoint the genetic manipulation to the specific cells that are driving seizures and leave other cells unperturbed, maintaining normal brain function.

The key innovation in this new approach is the use of an activity-dependent promoter that both detects which cells are highly active and then drives the production of an inhibitory protein in those cells. To develop this new system, the authors tested many different combinations of activity-dependent promotors and inhibitory proteins. They found that using the *cfos* promoter with a modified Kv1.1 potassium channel (referred to as EKC, engineered potassium [K+] channel) was the most ideal combination to reduce neural firing in active cells. The authors then performed several proof-of-principle *in vitro* experiments to demonstrate that their system can effectively inhibit recently active cells for up to 24 hours. They then tested whether this inhibition could reduce seizure susceptibility in control mice using acute injections of pentylenetetrazole (PTZ),

a drug that reliably produces generalized seizures in rodents. The experimenters first expressed the transgenic mechanism in the ventral hippocampus and triggered a large amount of neural activity by inducing a seizure with PTZ. One day later, the cells that were activated by the seizure (most of the cells in ventral hippocampus) then expressed the inhibitory EKC channel and when the animals were again given PTZ, they had less severe seizures, suggesting this transgenic therapy was able to reduce seizure susceptibility. To extend this finding to chronic epilepsy, the experimenters then expressed the transgenic mechanism in chronically epileptic mice and found that this treatment was able to suppress spontaneously occurring seizures for weeks after the treatment. Taken together, these results clearly demonstrated that the engineered *cfos*-EKC genetic therapy was able to reduce seizure susceptibility in both control and chronically epileptic mice.

By targeting only the highly active neurons that drive the *cfos* promoter, the authors hoped to avoid any off-target effects on cognition, anxiety, and locomotion. However, while the goal is to target only the pathologically hyperactive neurons, the *cfos* promoter is also engaged by normal learning processes and thus this system will also drive inhibition of learning-activated neurons, and could interfere with normal memory processes. The authors directly tested this possibility and found no effect on Pavlovian fear conditioning, anxiety-like behaviors, locomotion, and working memory. Yet, while these specific behaviors were not affected, it remains possible that more subtle behavioral effects may emerge with additional testing. In this study, the authors expressed the transgene bilaterally in ventral hippocampus, and there is strong evidence that the activity of *cfos*-expressing neurons in ventral hippocampus are critical for memory,[3] social behavior,[4] and observational fear.[5] The lack of off-target effects observed in this study may be due to the limited subset of neurons that expressed the activity-dependent inhibitory protein, or perhaps the specific behavioral assays performed. In addition, any off-target effects will be impacted by the brain region targeted and thus future studies are necessary to determine how targeting other brain regions may impact the results. Given that there is exceptionally large variability in the localization of the epileptogenic zone in humans across epilepsy disorders, it will be essential to consider how distinct brain regions mediate seizure susceptibility and how targeting various brain regions may impact behavioral effects. In fact, gene therapy may be most beneficial for patients without a clear seizure localization, who would not be candidates for surgical resection. These patients may require broad expression of the transgene across several brain regions and the selective nature of this activity-dependent transgene may allow for widespread inhibition with minimal side effects. Regardless, the suppression of seizures observed with this new gene therapy approach may outweigh any minor off-target effects.

The work by Qui and colleagues[2] is an exciting preclinical advancement that has the potential to greatly improve the treatment of chronic epilepsy. Yet there are several challenges that will need to be overcome before this gene therapy can be used in patients. Perhaps the greatest challenge is how to safely deliver the genetic material to the brain. The use of adeno-associated viruses (AAV) is generally considered to have an ideal safety profile,[6] and several AAV-based gene therapies have been approved for clinical use or are in clinical trials for neurological disorders.[7] However, no AAV-based therapies targeting the brain have been approved for clinical use, and there are still several safety and expression challenges to overcome with this technique.[6] A second important consideration is how human patients may differ in the expression of activity-dependent promoters, or in the response to inhibitory proteins. To this end, Qui and colleagues provide strong evidence that human-derived cells grown in a dish can express the *cfos*-EKC transgenic therapy and this can regulate neural excitability. This suggests that both the activity-dependent and inhibitory mechanisms are preserved across species and their combined use is likely to induce similar effects in humans. Finally, while expression of the c-Fos protein (driven by the *cfos* promotor) has traditionally been used to indicate when a cell has recently been active, the relationship between cellular activity and c-Fos expression is not entirely clear and is still actively being investigated.[8] Thus, continued refinement of the selective activity-dependent promoters and inhibitory payloads that will best control the symptoms of epilepsy will be critical to improving the clinical outcomes of this exciting technological development.

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