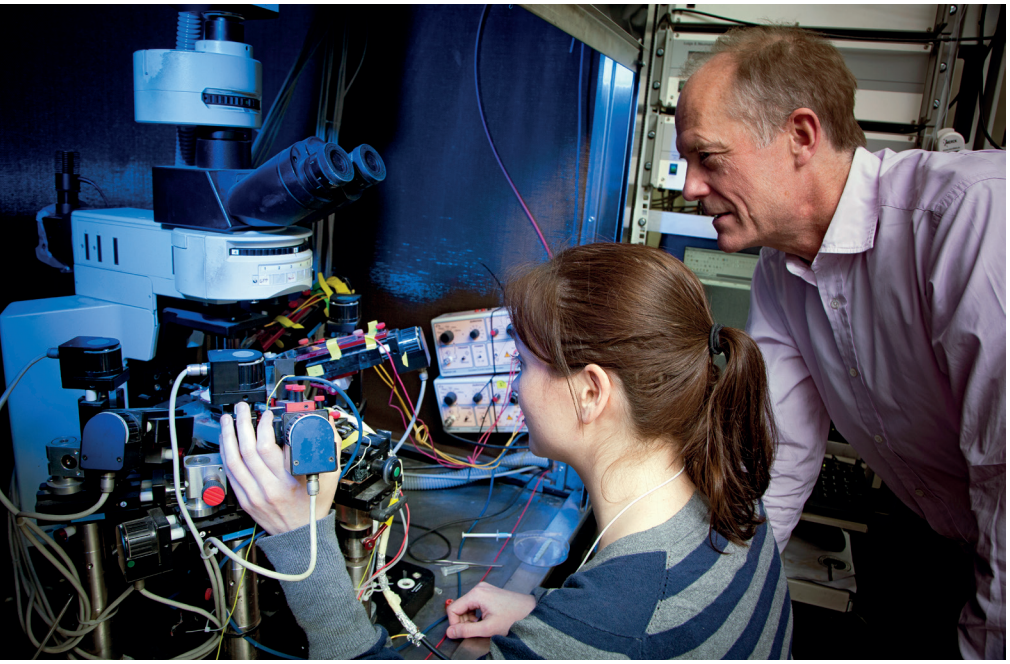


DAVID BISHOP/UCL HEALTH CREATIVES

Elizabeth Nicholson and Dimitri Kullmann at University College London.

NEUROLOGY

Repairs for a runaway brain

Gene therapy could damp down epilepsy seizures in people for whom current drugs are ineffective.

BY LIAM DREW

The seizures of around one-third of people with epilepsy are resistant to available medicines — a statistic that haunts neurology. It has been this way for decades. The medicines have got better by becoming safer and causing fewer side effects. But still there are people for whom the drugs simply don't work — and for them, epilepsy can be ruinous.

"There's stigma; they can't drive; they have difficulty holding down jobs; they have difficulty maintaining relationships," says Dimitri Kullmann, a neurologist and neuroscientist at University College London (UCL).

Currently, the main hope for people with severe drug-resistant epilepsy is surgery. Someone whose seizures arise from a well-defined region of the brain might be offered an operation to remove that region. This is a drastic procedure, but not especially rare; it is carried out about 500 times every year in the United States.

Kullmann is hoping that gene therapy can make such surgery unnecessary. His group and others are investigating the potential benefits of introducing different genes into the brains of people with epilepsy, each one selected to quell the rampage of electrical activity that causes epileptic seizures. The most advanced projects are now being readied for clinical trials.

EXCITATION AND INHIBITION

Epilepsy comes in many forms. It is defined by the repeated occurrence of seizures — but these seizures can vary in their nature, intensity and frequency. And the disorder can arise from numerous causes, progress in different ways and affect distinct parts of the brain.

Crucially, epilepsy can either be focal, with seizures beginning in a specific brain region, or generalized, with seizures developing across wide spans of the brain. Focal epilepsy is more common, and it can be further subcategorized according to whether the seizures remain focal or spread to become generalized. There is also variation in the size of the seizure-generating focus and whether it is discrete, and therefore potentially removable, or enmeshed with vital brain tissue, and thus inoperable.

Brains essentially work by relaying electrical signals from neuron to neuron through the release of chemical neurotransmitters. Excitatory neurons release neurotransmitters that electrically stimulate neighbouring cells, whereas inhibitory neurons release neurotransmitters that suppress electrical activity. A seizure is a period of runaway electrical activity during which the normal balance between excitation and inhibition is lost. Current anti-seizure drugs either dampen excitatory mechanisms or boost inhibitory ones. But they do so indiscriminately, producing wide-ranging side effects by affecting neural circuits throughout the nervous system.

Current gene-therapy strategies, by contrast, use harmless viruses to introduce one or two therapeutic genes into the defined volume of tissue from which focal epilepsy emanates. "It is more personal, more targeted, and probably has fewer side effects because we treat the tissue that needs to be treated, instead of treating the whole body," says Merab Kokaia, a neuroscientist working on this approach at Lund University in Sweden. The strategies in development target focal epilepsy, but treating generalized epilepsy is a longer-term possibility.

RESTORING BALANCE

The brains of people with epilepsy contain increased amounts of neuropeptide Y (NPY), a chemical that certain neurons release when they are especially active. NPY acts on five receptors, Y1 to Y5, some of which are excitatory and some inhibitory. The levels of some of these receptors are also altered in epilepsy: notably, levels of Y2, which strongly inhibits neurotransmitter release, are higher. Overall, the accumulation of NPY and the altered levels of its receptors seem to represent an adaptive response — an intrinsic bid to hold back runaway brain activity.

In 2004, investigators used a viral vector to deliver the *NPY* gene into the brains of rats that had been manipulated to display a form of epileptic activity[1]. The resulting overexpression of NPY caused a reduction in seizure frequency. Other animal experiments also showed that overexpressing the neuropeptide galanin likewise suppressed seizures.

Kokaia, who was already working on NPY and epilepsy at the time, became interested in the therapeutic potential of this approach and started experimenting with introducing genes for neuropeptides, their receptors or both. He found that overexpressing NPY alone decreased seizure frequency, but simultaneously overexpressing it with the inhibitory Y2 receptor dramatically heightened the anti-seizure effect[2]. "What we are trying to do is boost the natural response of the brain by gene therapy," says Kokaia.

In 2015, Kokaia co-founded CombiGene in Lund, Sweden, to commercially develop this technique. In the past two years, CombiGene has confirmed the anti-seizure effects of the NPY–Y2 combination therapy, now called CG01, in further rodent models of epilepsy. And the company has successfully introduced the NPY and Y2 genes into brain tissue that was surgically removed from people with epilepsy.

Experiments using such tissue also ended

ESTHER KROOK-MAGNUSON

interest in the second neuropeptide, galanin. Whereas NPY suppressed neurotransmission in human tissue, galanin did nothing — human neurons lack functional receptors for it.

Kullmann's move into the epilepsy field was serendipitous. His group was investigating the voltage-gated potassium channel Kv1.1 — a type of ion channel that electrically quiets neurons — as part of work on an entirely different neurological condition, episodic ataxia. The group made a virus that transferred the Kv1.1 gene into neurons. Because a neighbouring laboratory was routinely using rodent models of epilepsy, Kullmann and colleagues thought it might be worth testing Kv1.1 in these animals. The effect, published in 2012, was a dramatic reduction in seizure frequency[3]. After seeing this effect in three separate animal models, Kullmann and UCL colleague Stephanie Schorge developed a viral vector that introduces a modified Kv1.1 gene specifically into excitatory neurons, and does not integrate the gene into the cell's genome.

In principle, CG01 or Kv1.1 could provide long-term suppression of epileptic seizures following a single injection, with the genes continually generating products that calm the neurons in which they are expressed.

TRIGGERED ACTIVATION

Several alternative approaches are mainly based on converting widely used basic-research technologies into clinical tools. These approaches are more complicated, but hold potential advantages over CG01 or Kv1.1.

Opsins, for example, are membrane proteins that are activated by light, and the genes encoding them have been isolated from microorganisms. When illuminated, some types excite neurons, whereas others inhibit them. The big appeal of opsins is that they could remain inert in neurons when brain function is normal and only be called into action when needed.

Esther Krook-Magnuson, a neuroscientist at the University of Minnesota in Minneapolis, has shown that opsins can control seizures in rats[4]. Her team introduced inhibitory opsins into the rats' epileptic foci, then implanted seizure-detecting electrodes into their brains, along with fibre optics that light up to activate the opsins. An algorithm switched on the light when it detected the first signs of epileptic activity, quashing seizures early. Krook-Magnuson notes that implanting electrodes and light sources into humans would be less invasive than the current option of removing an area of brain.

However, this system requires a reliable seizure-detection method, an effective light-delivery technique and a way to get the right amount of virus into the right neurons. All three components will have to be optimized before the system has a chance of reaching the clinic.

The need to develop more than one technology can put off potential investors, says Kullmann. He has first-hand experience of this from trying to transform another research tool — DREADDs (designer receptors exclusively activated by designer drugs) — into a therapy. DREADDs are genetically engineered receptors that, like opsins, sit silently in neurons unless they are activated by a stimulus, but in this case, the stimulus is a drug rather than light.

Both Kullmann and Kokaia have found that inhibitory DREADDs can suppress seizures when the genes encoding them are inserted into the seizure foci of epileptic animals using viral vectors. If the therapy were translated to humans, people might take the activating drug regularly in a similar way to current epilepsy medicines — but with the advantage that the DREADDs would not inhibit brain tissue outside the region where the DREADD is situated. Alternatively, people might receive the drug automatically through an implanted, seizure-activated drug-delivery system, or simply take the drug when they feel the first indications of a seizure.

Kullmann is also exploring an ion channel that was originally identified in nematode worms. In nematodes, the glutamate-gated chloride (GluCl) channel is inhibitory and is activated by the neurotransmitter glutamate. But in mammals, glutamate is the main excitatory neurotransmitter that is responsible for driving excess activity during seizures, and none of its receptors is inhibitory.

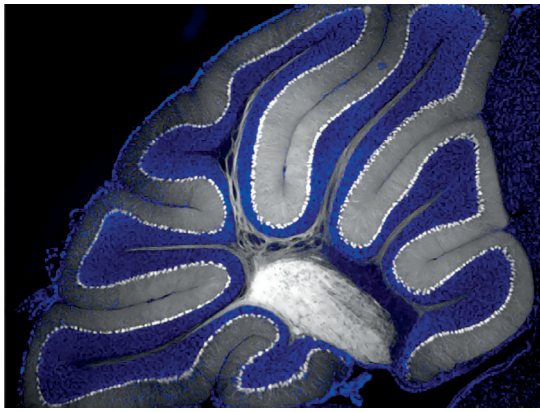
Kullmann and his colleague Andreas Lieb were interested in using an engineered version of the GluCl channel that is activated by a drug, but then they learnt that mutations in GluCl can change its glutamate sensitivity. If they picked a mutated channel that was insensitive to normal levels of glutamate, but activated by the high levels of glutamate that occur during seizures, they might have an appealing gene-therapy agent: an inhibitory ion channel that is ordinarily inactive but called into action during seizures. Early findings are encouraging: in two rat models, GluCl decreases seizure frequency[5].

PRIMED FOR CLINICAL TRIALS

In January, CombiGene partnered with the London-based incubator Cell and Gene Therapy Catapult to develop manufacturing processes for CG01 in preparation for clinical trials. And in April, Kullmann and Schorge received nearly £2 million (US$2.5 million) from the UK Medical Research Council to move the modified Kv1.1 virus towards the clinic.

Several technical hurdles remain, including scaling up the drug-delivery system: a human brain is around 700 times larger than the rat brains in which the viral vectors have been tested. But a major advantage of using NPY, Y2 and Kv1.1 is that they are derived from human genes — and therefore unlikely to evoke an immune response. By contrast, microbial opsins and GluCl from nematodes carry the risk of rejection by the immune system.

The hope is that gene-therapy treatments will be applicable to all drug-resistant focal epilepsies, including in people whose larger or awkwardly located foci make them ineligible for surgery, says CombiGene chief executive Jan Nilsson. And, more speculatively, if it is successful, gene therapy could potentially be adopted by some people instead of conventional drugs.



Brain cells could be manipulated using light.

But for the time being, CombiGene and Kullmann's team are planning safety and tolerability trials that will involve only people with drug-resistant epilepsy who are awaiting surgery. This is not because people in this group are the sole intended recipients of gene therapy — rather, they present a unique opportunity.

> ***"What we are trying to do is boost the natural response of the brain by gene therapy"***

The virus is likely to be given during presurgical investigations of the seizure locus, then allowed to enter neurons and deposit its genetic cargo while the patient spends weeks to months awaiting surgery. In phase I trials, surgeons will then almost certainly remove the focus. This procedure will allow researchers to carefully examine whether the gene delivery worked, and will also provide a fail-safe mechanism for excising genetically modified tissue should any safety issues arise.

The alternative is that people could opt out of surgery. If gene therapy is to be approved for epilepsy, numerous larger, more stringently controlled trials specifically designed to look at anti-seizure effects will be needed. But Kullmann allows himself to imagine a best-case scenario with the first exploratory trial. Someone who has stopped having seizures after the gene transfer, he says, might simply elect not to have surgery — entering a realm where their seizures are quelled not by conventional medication, but by DNA. ■

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