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Commentary

Brain delivery of a virus to block seizures helps mice get a silent NACHT

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For people with epilepsy, frontline treatment remains with anti-seizure drugs (ASDs), a group of over 20 different medicines available for this common, disabling brain disease. ASDs are effective in controlling seizures in approximately two-thirds of patients, but this leaves a very large number of people for whom seizures continue. This has devastating effects on quality of life and increases the risk for serious adverse events, including sudden unexplained death in epilepsy (SUDEP). In fact, the treatment gap is greater still. Since most ASDs work by dampening brain excitability, either by boosting inhibition or reducing excitability, serious side effects such as sedation are common, further reducing the quality of life. Finally, ASDs are not disease-modifying, producing no long-term improvements to the underlying pathophysiology. As a result, there continues to be a major unmet need for more effective, safer ways to control seizures and modify the underlying hyperexcitable brain networks [1,2].

In an article in *EBioMedicine*, Yang and colleagues identify a promising new target for the treatment of epilepsy [3]. NACHT and WD repeat domain-containing protein 1, Nwd1, is a large (~175 kDa) cytoplasmic protein whose unusual name derives from structural features which are shared by various other molecules, some involved in the control of apoptosis [4]. One of these, Apaf-1, has previously been linked to the signaling pathways which result in cell death after prolonged seizures [5]. Some NACHT-containing proteins also serve in innate and adaptive immunity, including activating inflammatory signals [6]. This combination makes Nwd1 a very interesting protein, given that both excitatory communication and neuroinflammation are integral to the pathogenesis and maintenance of the epileptic state [7].

On this basis, and having noted the distribution of Nwd1 in the rodent brain [8], the authors hypothesize that the protein might be a component of the post-synaptic machinery linking surface ligand-gated ion channels to downstream intracellular molecules necessary for signal transduction. In an impressive study that neatly combines *in vitro* and *in vivo* animal studies with human data from patients with drug-resistant epilepsy, they uncover a role for Nwd1 in the control of brain excitability. They first show that seizures induced by kainic acid in mice increase levels of Nwd1 and that Nwd1 levels are also higher in resected brain tissue from patients with temporal lobe epilepsy. They demonstrate the protein is expressed only by neurons, consistent with the earlier work, and show evidence for co-localisation with a post-, but not pre-synaptic marker. To explore the function of Nwd1 they use a viral approach, delivering short hairpin RNAs (shRNA) against *Nwd1* enclosed in an AAV vector. The use of AAV as a delivery platform is sensible since similar vectors have been successful in gene therapy trials [9]. They show their AAV vector lowered but does not eliminate Nwd1 protein. Using electrophysiology, they reported that mouse neurons infected with the AAV-Nwd1-shRNA vector have selectively reduced excitatory post-synaptic potentials. Other measures of more complex network behaviour, carried out in brain slices, were also reduced, including polyspiking epileptiform activity. The vector did not interfere with inhibitory neurotransmission. Insights into the mechanism then came from biochemical studies where they demonstrated that reducing Nwd1 selectively lowered levels and phosphorylation of GluN2B, a part of the NMDA receptor that guides trafficking and synaptic localization. So, does this help the fight against seizures? To answer this, the authors infected mice with the AAV-Nwd1-shRNA vector *in vivo* and tested responses to kainic acid. Acute seizures were delayed in the mice, their severity reduced and brain sections from the animals had more surviving neurons.

Altogether, the study sheds important light on a little-understood protein and presents a promising new target for seizure control. From a translational point of view, however, several further studies will be needed to build on the discoveries here. First, the *in vivo* seizure tests were performed in an acute model of status epilepticus rather than a model of spontaneous recurrent seizures. Testing the vector in an *in vivo* model of genetic or acquired epilepsy will be an important next step. There are risks with manipulating NMDA signaling and many clinical trials have failed for reasons of safety and efficacy [10] and, notably, the authors found that knockdown of Nwd1 reduced dendritic spine density. Preclinical tests of cognition will be important in the future. The gene therapy approach taken here is elegant but getting this to patients would meet substantial challenges so a small molecule-based inhibitor might be a way to reach the clinic sooner. Finally, does Nwd1 serve pro-inflammatory roles in epilepsy? If Nwd1 bridges both excitatory neurotransmission and neuroinflammation, the authors may well have hit upon an optimal target for seizure control with disease modification that could one day benefit patients with epilepsy.

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