



Algal Proteins Illuminate Epilepsy

Closed-Loop Optogenetic Control of Thalamus as a Tool for Interrupting Seizures after Cortical Injury.

Paz JT, Davidson TJ, Frechette ES, Delord B, Parada I, Peng K, Deisseroth K, Huguenard JR. *Nat Neurosci* 2013;16:64–70.

Cerebrocortical injuries such as stroke are a major source of disability. Maladaptive consequences can result from post-injury local reorganization of cortical circuits. For example, epilepsy is a common sequela of cortical stroke, but the mechanisms responsible for seizures following cortical injuries remain unknown. In addition to local reorganization, long-range, extra-cortical connections might be critical for seizure maintenance. In rats, we found that the thalamus, a structure that is remote from, but connected to, the injured cortex, was required to maintain cortical seizures. Thalamo-cortical neurons connected to the injured epileptic cortex underwent changes in HCN channel expression and became hyperexcitable. Targeting these neurons with a closed-loop optogenetic strategy revealed that reducing their activity in real-time was sufficient to immediately interrupt electrographic and behavioral seizures. This approach is of therapeutic interest for intractable epilepsy, as it spares cortical function between seizures, in contrast with existing treatments, such as surgical lesioning or drugs.

On-Demand Optogenetic Control of Spontaneous Seizures in temporal Temporal lobe Lobe Eepilepsy.

Krook-Magnusen E, Armstrong C, Oijala M, Soltesz I. *Nature Communications* 2013;4. doi: 10.1038/ncomms2376.

Temporal lobe epilepsy is the most common type of epilepsy in adults, is often medically refractory, and due to broad actions and long-time scales, current systemic treatments have major negative side-effects. However, temporal lobe seizures tend to arise from discrete regions before overt clinical behaviour, making temporally and spatially specific treatment theoretically possible. Here we report the arrest of spontaneous seizures using a real-time, closed-loop, response system and in vivo optogenetics in a mouse model of temporal lobe epilepsy. Either optogenetic inhibition of excitatory principal cells, or activation of a subpopulation of GABAergic cells representing < 5% of hippocampal neurons, stops seizures rapidly upon light application. These results demonstrate that spontaneous temporal lobe seizures can be detected and terminated by modulating specific cell populations in a spatially restricted manner. A clinical approach built on these principles may overcome many of the side-effects of currently available treatment options.

Commentary

The age of optogenetics is upon us, and as these two articles illustrate, this experimental approach is now illuminating our field of epilepsy, characterizing mechanisms of seizure initiation and propagation with a heretofore unprecedented level of specificity.

Optogenetics has exploded onto the neuroscience scene during the past ten years. This field began when several groups—notably, including Karl Deisseroth and Ed Boyden's laboratory at Stanford—realized the potential utility and importance of a set of microbial opsin proteins characterized primarily in algae and halobacteria. These opsins are light-activated membrane proteins that, upon illumination with appropriate wavelength light, can alter diverse cellular functions, including (for several important members of this

protein family) reversibly changing membrane potential by opening channels or activating ionic pumps. The genes encoding multiple opsins were cloned, allowing introduction of these proteins into neurons using viral strategies, and, more recently, transgenic mice (1). Coupling the ability of opsins to activate or silence neurons with the ability to express these proteins in specified subsets of neurons, using multiple targeting techniques, has facilitated investigation of the roles played by individual neuron populations in generation of circuit behaviors. These include disease states involving the nervous system, such as psychiatric disorders, neurodegenerative diseases, and epilepsy (2, 3).

The two studies from the Huguenard and Soltesz laboratories built on a recent exploration of the utility of optogenetic intervention in stopping seizure activity in vivo (3). This study introduced an opsin that hyperpolarizes neurons upon exposure to yellow light (halorhodopsin) into principal cells within the area of interest—a region of motor cortex previously injected with tetanus toxin—creating a seizure focus. Yellow light was then administered directly to neurons

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within the seizure generating focus in an open loop, 20 sec on/20 sec off duty cycle for a 1000 s period. The effects on EEG recorded seizure activity, spectral power, and line length were examined. Illumination significantly reduced high-frequency power and EEG coastline compared to the 1000 s baseline period, and the frequency of automatically detected epileptiform events was also significantly reduced. Optogenetic inhibition was therefore demonstrated by this study as having the potential to exert an anticonvulsant effect and the further potential to abort seizures on demand. However, although this was important to demonstrate, the end result was not hugely surprising, given the efficacy of these reagents in controlling neuronal excitability and the fact that optogenetic inhibition by halorhodopsin was previously shown to be efficacious in terminating evoked epileptiform activity *in vitro* (4).

The Huguenard and Soltesz studies under discussion take these findings of the Kullmann laboratory (3) several steps further, with significant translational and mechanistic implications. The first additional aspect explored by both studies was to examine the utility of closed-loop optogenetic silencing of proscribed brain areas in aborting already initiated seizures before behavioral manifestations of seizures emerge. Seizures were detected on-line, and this detection event was then utilized to activate the laser administering silencing illumination to halorhodopsin-expressing neurons in various locations. Although the seizure detection algorithms, brain areas, and experimental epilepsy models utilized in these two studies differed, the end results were the same: Both studies were able to significantly truncate EEG seizures and also reduce the incidence and duration of behavioral seizures using closed-loop optogenetic intervention.

However, the findings in these two studies extend much beyond this important translational milestone of closed-loop seizure control. The cellular specificity of optogenetics allowed these investigators to explore mechanistic aspects of seizure initiation and propagation in a manner never before examined.

In the study from the Huguenard laboratory, Paz and colleagues studied spontaneous seizure activation in an experimental photo-thrombotic model of stroke-induced epilepsy in rats. Setting the stage for subsequent intervention strategies, specific alterations in the intrinsic properties of thalamic neurons interconnected with affected cortical regions were identified, secondary to the cortical injury. These affected thalamic neurons were then targeted for viral transfection of halorhodopsin driven by an alpha calmodulin kinase-2 promoter (restricting halorhodopsin to excitatory relay neurons). A combined unit recording and light-emitting electrode was targeted to this transfected thalamic region, accompanied by EEG recordings ipsi- and contralateral to the experimental infarct. Optogenetically silencing the thalamus by illumination during seizure activation immediately terminated both the ipsi- and contralaterally recorded spontaneous electrographic cortical seizure, and also terminated the behavioral manifestations of the seizure. This demonstrated for the first time that activity in thalamocortical relay neurons is required for seizure generation in a model of post-stroke epilepsy. Thalamic neuronal alterations, therefore, are not

secondary, downstream echoes of a damaged cortex; rather, they constitute an important contributor to the emergence of the epileptic state.

The Krook-Magnusen et al. study from the Soltesz laboratory also had significant mechanistic implications for seizure initiation and propagation—over and above the anticonvulsant findings discussed above. They examined the effects of several different optogenetic manipulations on seizures originating from an epileptic focus created by localized, unilateral administration of kainic acid into the hippocampus of a mouse. Rather than using viral targeting strategies, these investigators took advantage of the availability of transgenic mice expressing the inhibitory opsin halorhodopsin, or the excitatory opsin channelrhodopsin 2 under control of Cre recombinase. These opsin mice were then crossed to alpha calmodulin kinase Cre or parvalbumin Cre mouse strains to restrict expression of opsins to excitatory forebrain neurons or parvalbumin-expressing GABAergic interneurons, respectively. Direct illumination of the hippocampal epileptic focus during a seizure was able to terminate the event in mice expressing halorhodopsin in principal excitatory neurons—proof of principle for closed-loop seizure control. However, these investigators extended this to show that blue light illumination-mediated *excitation* of parvalbumin-expressing channelrhodopsin-positive interneurons was also sufficient to terminate ongoing seizures. These cells constitute less than 5% of the neuronal population but still have such a powerful role in regulating circuit excitability that their activation is sufficient to rein in the runaway excitation underlying seizure discharges. Also of interest, this effect of exciting PV-positive interneurons on seizures was evident when stimulating either the ipsilateral (focal) or contralateral hippocampus, implicating a bilateral network involved in seizure generation in temporal lobe epilepsy.

What overarching conclusions can be drawn from these two studies using distinct experimental models of epilepsy and divergent optogenetic strategies to control seizures? First, and most obviously, both studies demonstrate that closed-loop seizure detection–intervention strategies are possible and have the potential for significant efficacy in controlling epilepsy. Second, and perhaps more interestingly, both studies support the hypothesis that—instead of being just followers passively driven in seizure generalization—interconnected structures remote from the site of an epileptogenic injury can be critically involved in the initiation and/or generalization of seizure activity. This supports the emerging concept that distributed, anatomically widespread networks of neurons are intimately involved in seizure generation. This has significant implications for our concept of a seizure focus (is there such a thing?) and also for the development of intervention strategies, which now may conceivably be implemented in structures remote from the site of injury in focal epilepsies.

by Douglas A. Coulter, PhD

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