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# Mom Controls the Thermostat: Mitochondria Influence the Neuronal Firing Set Point

**Mitochondrial Regulation of the Hippocampal Firing Rate Set Point and Seizure Susceptibility**

Styr B, Gonen N, Zarhin D, Ruggiero A, Atsmon R, Gazit N, Braun G, Frere S, Vertkin I, Shapira I, Harel M, Heim LR, Katsenelson M, Rechnitz O, Fadila S, Derdikman D, Rubinstein M, Geiger T, Ruppin E, Slutsky I. *Neuron*. 2019. pii: S0896-6273(19)30334-4. doi:10.1016/j.neuron.2019.03.045. [Epub ahead of print] PMID: 31047779.

Maintaining average activity within a set-point range constitutes a fundamental property of central neural circuits. However, whether and how activity set points are regulated remains unknown. Integrating genome-scale metabolic modeling and experimental study of neuronal homeostasis, we identified mitochondrial dihydroorotate dehydrogenase (DHODH) as a regulator of activity set points in hippocampal networks. The DHODH inhibitor teriflunomide stably suppressed mean firing rates via synaptic and intrinsic excitability mechanisms by modulating mitochondrial Ca2+ buffering and spare respiratory capacity. Bidirectional activity perturbations under DHODH blockade triggered firing rate compensation, while stabilizing firing to the lower level, indicating a change in the firing rate set point. In vivo, teriflunomide decreased CA3–CA1 synaptic transmission and CA1 mean firing rate and attenuated susceptibility to seizures, even in the intractable Dravet syndrome epilepsy model. Our results uncover mitochondria as a key regulator of activity set points, demonstrate the differential regulation of set points and compensatory mechanisms, and propose a new strategy to treat epilepsy.

Epileptologists sometimes explain that seizures result from an imbalance in the ratio of neuronal excitatory and inhibitory (E/I) mechanisms. They may invoke a simple analogy that describes homeostasis in the nonepileptic brain as a thermostat set at the "ideal temperature" (E/I balance) and that controls the "excitatory" furnace and "inhibitory" air conditioner to maintain the ideal state. Employing the same analogy, homeostasis in the epileptic brain uses the same thermostat but has an underperforming air conditioner. Sometimes during a hot summer day, the thermostat will not sufficiently drive inhibitory cooling to the ideal E/I "temperature" and a seizure will develop. Accordingly, much of current epilepsy research is focused on uncovering and modulating cellular processes that cause overactive furnaces or underperforming air conditioners. In contrast, Styr and colleagues tackled a different problem in this article and investigated mechanisms that control the thermostat's set point. Instead of an underperforming air conditioner, seizures could develop in a home with a thermostat set at an elevated temperature and could be prevented if the thermostat could be turned to a lower setting.

Styr and colleagues hypothesized that the neuronal excitatory set point could be modulated by cellular metabolism. Therefore, to search for possible metabolism genes that could affect E/I balance, they performed an in silico analysis of publicly accessible archived epileptic and control transcriptome data obtained from human postsurgical specimens and from hippocampi from pilocarpine and kainate rat epilepsy models. Epileptic and matching control transcriptome data were analyzed using a computational method (integrated Metabolic Analysis Tool [iMAT]) that predicts the flow of metabolic intermediates through the different known cellular metabolic pathways. Using the transcriptome data, iMAT maximizes the flux through pathways containing highly expressed enzymes and minimizes the use of low-expressed metabolic enzymes. The authors then used the iMAT-predicted metabolic differences between epileptic/nonepileptic tissues in a second in silico analysis, the metabolic transformation algorithm (MTA), that predicts enzymes that could be modulated to transform the epileptic metabolic state to a nonepileptic one. The top-predicted enzyme identified by the iMAT/MTA computations was dihydroorotate dehydrogenase (DHODH), a mitochondrial enzyme that catalyzes the oxidation of dihydroorotate to orotate in the pyrimidine biosynthetic pathway ("Mom" in this commentary's title refers to maternally derived mitochondria). Interestingly, separate iMAT/MTA analyses of hippocampi from nonepileptic rats fed a ketogenic diet and from Dravet syndrome mice also identified DHODH as a top enzyme.

Based on the results of the in silico analyses, the investigators determined whether DHODH inhibition altered E/I balance as measured by the neuronal spontaneous mean firing rate (MFR). With hippocampal neurons plated on multiple electrode arrays, they found that both teriflunomide (TERI), a DHODH inhibitor, and short hairpin RNA DHODH knockdown reduced MFR by 60% and 34%, respectively. Consistent with the ex vivo results, TERI also reduced hippocampal CA1 neuron MFR in vivo after an intracerebroventricular (ICV) infusion.

Dihydroorotate dehydrogenase inhibition modifies at least 2 physiological parameters that could decrease MFR. First, intracellular current clamp recordings demonstrated that TERI decreases the maximal action potential firing rate without altering other intrinsic physiological processes. Second, DHODH inhibition reduces excitatory synaptic transmission; in hippocampal brain slices, TERI reduced the amplitude of both electrically evoked field potentials and spontaneous miniature excitatory postsynaptic currents (mEPSC) and decreased mEPSC frequency. Therefore, compared to control neurons, TERI-inhibited neurons do not fire as many action potentials and, when action potentials are blocked, do not release as much excitatory glutamate. Their "furnace" is less active.

How are these findings different from the numerous other studies of neuronal E/I homeostasis? Is DHODH simply a switch for the cellular furnace? Styr et al demonstrated that DHODH inhibition differed from other manipulations of E/I processes because it did not evoke homeostatic compensation. Previous studies found that other techniques that reduced MFR (eg, overexpression of a recombinant potassium channel[1] and incubation with a $GABA_B$ agonist[2]) cause acute alterations of MFR, but then evoke slow compensation mechanisms (days) that gradually restore MFR to its baseline value. In contrast, continuous TERI application for 2 days produced sustained decreases in MFR and mEPSC frequency and amplitude without compensation. Did DHODH inhibition break the thermostat to prevent compensation? The authors showed that homeostatic mechanisms in TERI-treated neurons are still functional. After DHODH inhibition establishes a new MFR set point ($\sim$50% baseline), further sustained manipulations of other pharmacological pathways (treatment with the $GABA_B$ agonist, baclofen to reduce MFR to 10% baseline, or inhibition of the glutamate transporter, DL-threo-β-benzyloxyaspartic to increase MFR to 70% baseline) are followed by a gradual 1 to 2 day returns to the 50% set point. Therefore, in contrast to many of the known pathways that affect E/I balance by acting on the furnace or air conditioner, DHODH inhibition works by dialing down the thermostat.

How does a mitochondrial enzyme control the E/I thermostat? Styr and colleagues found that although DHODH inhibition did not affect presynaptic adenosine triphosphate stores, it did reduce oxygen consumption and spare respiratory capacity (SRC). Because SRC is linked to cytosolic and mitochondrial calcium concentration, the investigators tested whether TERI altered calcium flux. In cultured neurons, TERI increased the action potential calcium concentration in the mitochondria and decreased the action potential calcium in the cytoplasm. Because cytosolic calcium concentration is critical for presynaptic neurotransmitter release,[3,4] DHODH's effect on mitochondrial calcium buffering may mediate its control on the MFR set point.

Despite the in silico analyses being performed using transcriptome data from epilepsy specimens, all experiments discussed so far have been performed in nonepileptic samples. Does the DHODH's influence on the MFR thermostat apply to seizures? Styr et al tested the effects of DHODH inhibition in 2 seizure models. First, in a genetic mouse model of Dravet syndrome, a 2 to 4-hour ICV TERI infusion reduced hippocampal epileptiform spikes by 1.8-fold and a 3-day ICV TERI pretreatment increased the seizure induction temperature threshold by 1.54°C. Second, wild-type mice that received a 3-day ICV TERI pretreatment experienced substantially less severe pentylenetetrazol (PTZ)-induced seizures than vehicle-treated animals. In cultured hippocampal neurons, TERI-treated samples exhibited smaller increases in cytosolic calcium than untreated samples, a result that could explain TERI's effect on PTZ-evoked seizure severity.

This article established a unique direct link between mitochondrial respiration and epilepsy. There has been increased interest in the association of altered metabolism and seizures.[5] However, most previously-studied conditions that affect mitochondrial function and produce seizures, such as mutations of nuclear or mitochondrial DNA, are associated with reduced mitochondrial function.[6] Surprisingly, this study found that the diminished neuronal excitability and the associated anticonvulsant effect conferred by DHODH inhibition was also associated with reduced mitochondrial oxygen consumption. This finding highlights the need for additional studies to uncover the molecular mechanisms by which DHODH inhibition reduces the MFR set point. Possibly, DHODH inhibition directly reduces the MFR set point (eg, via increased mitochondrial calcium buffering) and the resulting lowered metabolic demand decreases oxygen consumption. Alternatively, TERI may directly (but partially) reduce mitochondrial function which would thereby increase mitochondrial calcium buffering and lower the set point.

Styr and colleagues also made use of a novel in silico analysis of transcriptome data to identify the DHODH enzyme as a key regulator of the metabolic state and this approach may be useful to epileptologists studying other epilepsy syndromes. However, after the in silico analysis, the authors then elucidated the effects of DHODH inhibition on neuronal firing, synaptic signaling, and calcium homeostasis in nonepileptic neurons. Is the MFR thermostat really turned to a "higher temperature" in epileptic conditions or did TERI simply reduce seizure severity in the Dravet and PTZ models by turning down the temperature from a normal set point. The latter interpretation is almost certainly the case for the acute PTZ model since this was performed in wild-type mice that would be expected to have a normal set point. Empirical data are now needed to determine if enhanced DHODH-sensitive metabolic signaling

is present in epilepsy and whether different epilepsy syndromes exhibit distinct metabolic profiles.

A final highlight of this research is its focus on modulation of the MFR set point—the thermostat. Whether or not DHODH modulation plays a future role in the treatment of seizures, it is important for epileptologists to consider homeostatic control as well as basic E/I mechanisms. It is well-established that some patients with epilepsy have an initial beneficial response (honeymoon) to an antiseizure drug (ASD) followed by the gradual worsening of their seizures after sustained drug exposure.[7] Slow homeostatic mechanisms may be working to counteract the effects of the ASD and result in drug tolerance. Possibly, instead of targeting the furnace or air conditioner, new treatments that "dial down the thermostat" may produce longer effects on E/I balance and seizure freedom.

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