# Same Channel, Different Tune

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Reduced GABAergic Neuron Excitability, Altered Synaptic Connectivity, and Seizures in a KCNT1 Gain-of-Function Mouse Model of Childhood Epilepsy

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Gain-of-function (GOF) variants in K+ channels cause severe childhood epilepsies, but there are no mechanisms to explain how increased K+ currents lead to network hyperexcitability. Here, we introduce a human Na+-activated K+ (KNa) channel variant (KCNT1-Y796H) into mice, and using a multiplatform approach, find motor cortex hyperexcitability and early-onset seizures, phenotypes strikingly similar to those of human patients. Although the variant increases KNa currents in cortical excitatory and inhibitory neurons, there is an increase in the KNa current across subthreshold voltages only in inhibitory neurons, particularly in those with non-fast-spiking properties, resulting in inhibitory neuron-specific impairments in excitability and action potential generation. We further observe evidence of synaptic rewiring, including increases in homotypic synaptic connectivity, accompanied by network hyperexcitability and hypersynchronicity. These findings support inhibitory neuron-specific mechanisms in mediating the epileptogenic effects of KCNT1 channel GOF, offering cell type–specific currents and effects as promising targets for therapeutic intervention.

## Commentary

Accurately modeling human diseases in preclinical models is essential for the development of effective treatments. The manuscript by Shore et al is an excellent example of the necessity of modeling human mutations in preclinical models, demonstrating the ability to recapitulate the human condition and elucidate the complex mechanisms contributing to the phenotype.[1] The study underscores the fact that it is difficult to predict the consequences of a known human mutation without assessing the impact in an intact network, which may not produce the anticipated results via the presumed mechanism when examined in vivo. Thus, this study highlights the utility of animal models for studying human diseases, including epilepsy—a topic which was controversially debated at the 73rd Annual American Epilepsy Society meeting held in 2019[2] (https://www.pathlms.com/aes/courses/17742). As the authors state these data are critical for "emphasizing that an understanding of disease mechanisms beyond channel biophysics is necessary to advance clinical treatment" and demonstrate clearly that preclinical models have utility in understanding human disease.[1]

The article featured in this Commentary utilizes preclinical models to understand the mechanisms whereby gain-of-function (GOF) mutations in the $Na^+$-activated $K^+$ ($K_{Na}$) channel *KCNT1* lead to hyperexcitability and a range of epilepsies, including early infantile epileptic encephalopathies, malignant migrating partial seizures, and autosomal dominant nocturnal frontal lobe epilepsy[3] (https://www.ncbi.nlm.nih.gov/books/NBK525917/). Gain-of-function mutations in *KCNT1* have been proposed to increase excitability and seizure susceptibility by (1) increasing the excitability of excitatory neurons by accelerating action potential (AP) repolarization, (2) causing disinhibition by reducing the excitability of interneurons, or (3) changing neuronal development resulting in altered synaptic connectivity. Shore and colleagues introduced a GOF mutation in *KCNT1* (Y777H) into mice. The Y777H mutation corresponds to a human mutation associated with intractable childhood epilepsy (Y796H). The phenotype in mice with the Y777H mutation was strikingly similar to human patients. Consistent with this mutation recapitulating the phenotype of intractable childhood epilepsy, spontaneous seizures were observed in mice homozygous for the Y777H mutation. Seizures were 2× more common during the light phase, corresponding to a period of increased sleep in mice, and 90% of seizures arose during NREM sleep.[1] A limitation of the current study is the minimal assessment of seizure frequency, making seizure onset, progression, clustering, and so on, unable to be resolved. However, it is clear that the Y777H homozygous mutation in mice recapitulates the seizure phenotype of the human disease caused by this GOF *KCNT1* mutation in humans

(Y796H) and has utility for exploring the mechanisms contributing to the hyperexcitability and epileptic phenotype. However, it is important to note one interesting difference—patients are heterozygous for this mutation; whereas, in the mouse model, the phenotype recapitulating the human condition was observed only in animals homozygous for the mutation, not in heterozygous animals. This is commonly observed when attempting to model human genetic heterozygous mutations in mouse models, and while the causes are not fully understood, it may involve the expression of covariants in the human population which are not expressed in inbred mouse strains.

Utilizing the Y777H GOF *KCNT1* mutant mouse model, the authors perform widefield $Ca^{2+}$ imaging, in vivo electrocorticography, and video-electroencephalogram to characterize the increased network excitability, the spontaneous seizure phenotype, and to localize the hyperexcitability to specific brain regions, such as the somatosensory cortex.[1] To explore the mechanisms contributing to this hyperexcitable phenotype, the authors examine changes in $K_{Na}$ currents at different voltages. Interestingly, they observe cell type–specific differences in the impact of the mutation on $K_{Na}$ currents. Although the mutation is expressed in both excitatory and inhibitory neurons, the mutation results in different emergent properties in different cell types. In inhibitory neurons—particularly non-fast-spiking neurons—the mutation affected $K_{Na}$ currents across subthreshold voltages, impacting excitability to a greater extent in these neurons compared to excitatory neurons.[1] In glutamatergic neurons, there was no difference in resting membrane potential, input resistance, AP threshold, or rheobase firing (minimum amount of current needed to reach AP threshold) in *KCNT1* mutants compared to controls.[1] Further, the AP repolarization rate, AP half-width, and afterhyperpolarization (AHP) were not altered in excitatory neurons in *KCNT1* mutants compared to controls.[1] In contrast, fast-spiking interneurons in *KCNT1* mutant mice demonstrate an increase in rheobase firing and non-fast-spiking neurons from *KCNT1* mutants show even more profound changes in excitability, including a decrease in input resistance, increased capacitance, increased rheobase firing, narrower AP half-width, faster AP repolarization rate, and a larger AHP.[1] Further probing the impact of the KCNT1 mutation demonstrates cell type differences on $K_{NA}$ currents. Glutamatergic neurons only showed increased $K_{Na}$ currents at very depolarized voltages; whereas, fast-spiking neurons showed increased $K_{Na}$ currents only at more negative voltages ($-50$ to $-10$ mV) and non-fast-spiking interneurons exhibited the broadest increase in $K_{Na}$ currents,[1] consistent with the observation that the excitability of these neurons is the most affected by the *KCNT1* mutation.

The GOF *KCNT1* mutation, Y777H, is also associated with altered synaptic connectivity in *KCNT1* mutants, resulting in increased homotypic connections—excitatory to excitatory (E–E) connections and inhibitory to inhibitory (I–I) connections[1]—which collectivity would result in increased excitability and increased disinhibition, respectively. Interestingly, cultured neurons also show changes in synaptic connectivity resulting in hyperexcitability.[1] However, it remains unclear how the GOF mutation in *KCNT1* alters synaptic connectivity. Given that this mutation is associated with a developmental epilepsy, it would also be interesting to know whether this mutation is associated with altered network properties during development, such as altered presence of giant depolarizing potentials, known to play a critical role in neuronal maturation and network development.[4,5]

The currently highlighted study demonstrates that this translationally relevant GOF mutation in *KCNT1* (Y777H) alters $K_{Na}$ currents in inhibitory neurons across subthreshold voltages, reducing their intrinsic excitability, resulting in disinhibition and a hyperexcitable network, and altering synaptic connectivity.[1] The cell type–specific effects of the mutation emphasize the limitations of relying on heterologous systems to examine the effects on channel function. These data reinforce the importance of studying mutations in an intact network rather than just in heterologous systems since epilepsy is a network disorder, and therefore, must be assessed as such.[6]

A critical question remains regarding how the same mutation results in different channel properties in different cell types. This is a fundamental question with relevance that extends beyond just this GOF mutation in *KCNT1*. Some potential mechanisms mediating the cell type–specific differences in channel function include differential expression of alternative spliced forms of *KCNT1*, which have been shown to have different activation kinetics, or coexpression of other channels which could form heteromers and alter biophysical properties of the channel. Additional potential mechanisms may include transcriptional, spliceosomal, epigenetic, and proteasomal regulation of ion channel expression.[7] A deeper understanding of the mechanisms mediating the differential impact of channel mutations in different cell types will further our understanding of network dysfunction leading to network hyperexcitability and epilepsy.

In conclusion, the highlighted manuscript beautifully dissects the complexity of cell type–specific changes in excitability resulting from the same mutation in the same channel, contributing to network hyperexcitability and epilepsy. This study also reinforces that preclinical studies are required to appreciate the developmental, cellular, and synaptic complexity resulting from human mutations which is necessary for effective translation to the clinic.

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## References


1. Shore AN, Colombo S, Tobin WF, et al. Reduced GABAergic neuron excitability, altered synaptic connectivity, and seizures in a KCNT1 gain-of-function mouse model of childhood epilepsy. *Cell Rep*. 2020;33(4):108303.

2. TOBASL. Moshe. Translational research symposium: advances in translation for traumatic brain injury (TBI) and antiepileptogenesis. 2019. *73rd Annual American Epilepsy Society Meeting*. National Institute of Neurological Disorders and Stroke. https://www.pathlms.com/aes/courses/17742
3. Gertler T, Bearden D, Bhattacharjee A, Carvill G. *KCNT1-Related Epilepsy*. University of Washington; 2018. https://www.ncbi.nlm.nih.gov/books/NBK525917/
4. Mohajerani MH, Cherubini E. Role of giant depolarizing potentials in shaping synaptic currents in the developing hippocampus. *Crit Rev Neurobiol*. 2006;18(1-2):13-23.
5. Kasyanov AM, Safiulina VF, Voronin LL, Cherubini E. GABA-mediated giant depolarizing potentials as coincidence detectors for enhancing synaptic efficacy in the developing hippocampus. *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences; 2004;101:3967-3972.
6. Kramer MA, Cash SS. Epilepsy as a disorder of cortical network organization. *Neuroscientist*. 2012;18(4):360-372.
7. Ohya S, Kito H, Hatano N, Muraki K. Recent advances in therapeutic strategies that focus on the regulation of ion channel expression. *Pharmacol Ther*. 2016;160:11-43.