Impaired Fast-Spiking Interneuron Physiology in a Mouse Model of *KCNC1* Epilepsy

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Abstract

Objective

To determine how the disease-associated A421V variant of the *KCNC1* gene affects the intrinsic physiology of fast-spiking GABAergic parvalbumin-expressing interneurons (PV-INs), and to create a schema for how $K_V3.1$ dysfunction results in neural circuit hyperexcitability.

Methodology

Electrophysiological recordings of PV-INs in cortical layers II/III and layer IV of WT (wildtype) mice and a novel mouse model of the A421V variant of *KCNC1* (*Kcnc1-A421V/+*) were performed to examine and compare their intrinsic physiological properties.

Conclusion

Compared to WT PV-INs, *Kcnc1* PV-INs have:

- significantly slower downstroke velocity
  → significantly higher AP amplitude
  → significantly higher APD50
- tendency to undergo depolarization block at higher current injections
  → significantly lower AP frequency

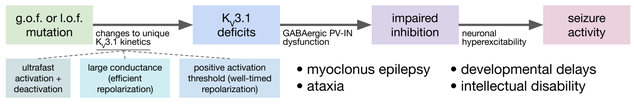
Interpretation

Kcnc1-A421V/+ mutation in $K_V3.1$ channels
↓
major reduction of K^+ current density
↓
impaired firing of fast-spiking GABAergic PV-INs
↓
insufficient levels of inhibition
↓
neural circuit hyperexcitability

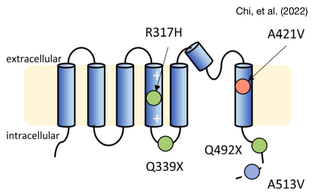
Background

The *KCNC1* gene encodes the $K_V3.1$ subunit of voltage-gated potassium channels, which are specifically expressed in fast-spiking GABAergic interneurons in the hippocampus and neocortex. The unique kinetic properties of the $K_V3.1$ channel render it a key contributor to membrane repolarization and the termination of action potentials. These properties also enable neurons to sustain high-frequency firing, crucial for learning and memory, sensory processing, and the regulation of sleep-wake cycles.

***KCNC1* Mutations**



Mutations affecting K_V3 channels lead to severe neurological conditions, including ataxias, movement disorders, and epilepsies. However, *KCNC1* had not been linked to human disease until the gene was recently implicated in MEAK (myoclonus epilepsy and ataxia due to K^+ channel mutation). Since then, the A421V (p.Ala421His, c.1262C > T) mutation has been identified as a recurrent *KCNC1* variant in patients who present with developmental and epileptic encephalopathy (DEE).

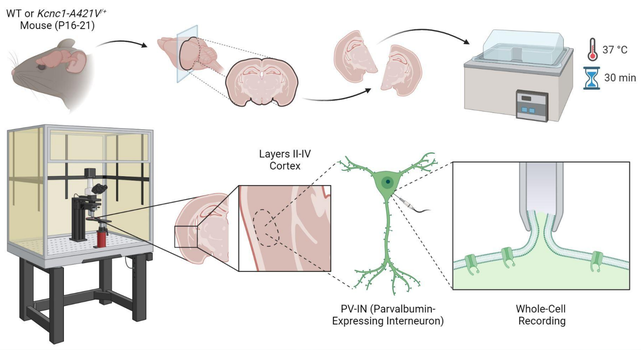


The A421V variant of the *KCNC1* gene is a loss-of-function missense mutation that occurs between SF (the selectivity filter) and the PVP (Pro-Val-Pro) hinge on transmembrane segment 6 of $K_V3.1$ (voltage-gated potassium channel 3.1).

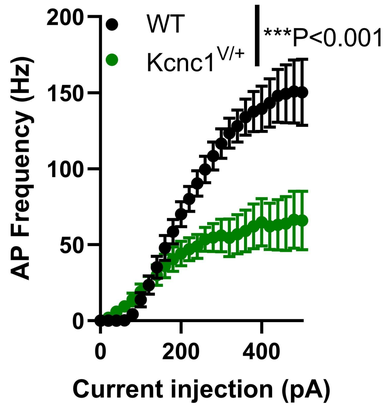
How does the Kcnc1-A421V/+ mutation affect the intrinsic physiology of PV-INs?

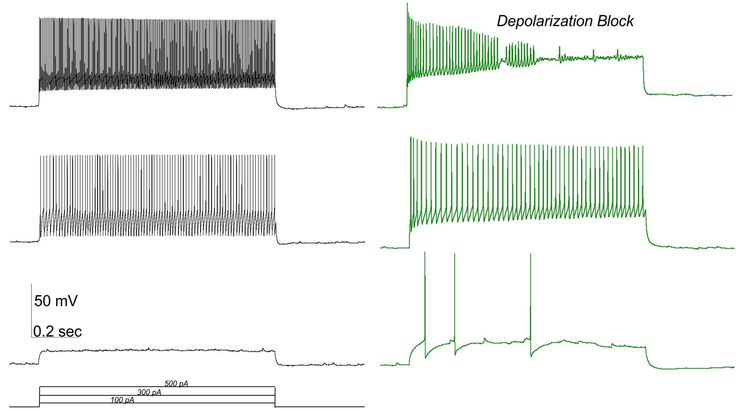
How can $K_V3.1$ dysfunction lead to neural circuit hyperexcitability and developmental and epileptic encephalopathy (DEE)?

Methods

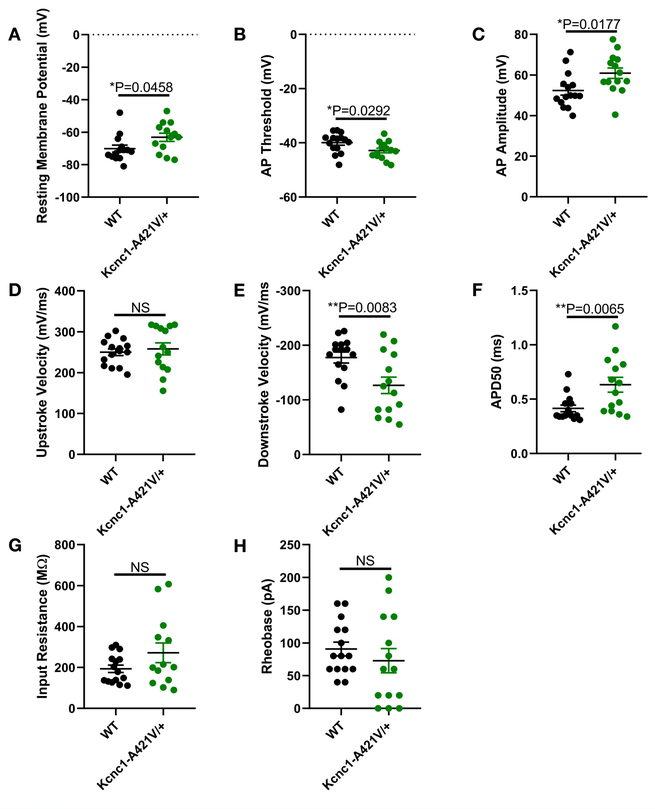


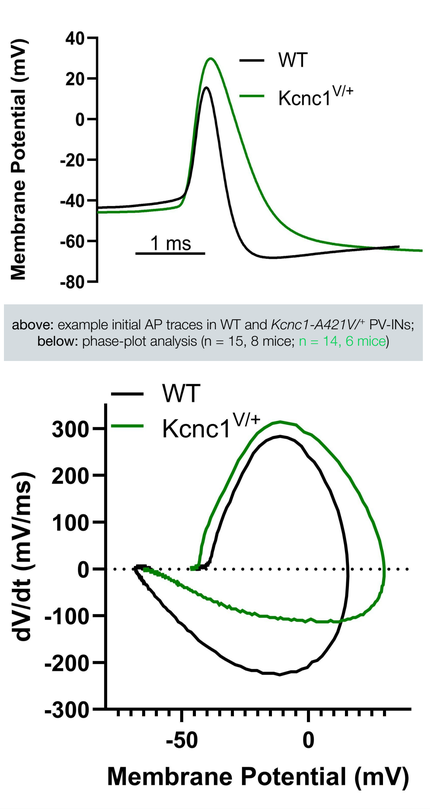
Results





depolarization block - increasing current injections cause sustained depolarizations of the membrane, leading to the inactivation of voltage-gated sodium channels; the reduced availability of voltage-gated sodium channels prevent ('block') neurons from firing APs

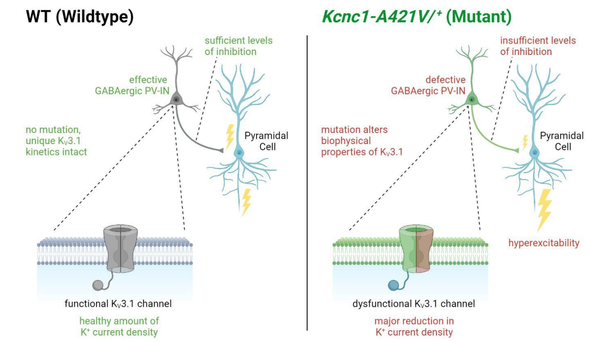




above: example initial AP traces in WT and *Kcnc1-A421V/+* PV-INs;
below: phase-plot analysis (n = 15, 8 mice; n = 14, 6 mice)

Conclusion

- AP frequency of *Kcnc1* PV-INs is significantly lower than WT PV-INs. *Kcnc1* PV-INs undergo depolarization block at higher current injections, whereas WT PV-INs do not.
- No significant difference in upstroke velocity for either genotype. However, *Kcnc1* PV-INs have a significantly slower downstroke velocity than WT PV-Ins. AP amplitude and APD50 of *Kcnc1* PV-INs are greater than WT PV-INs.
- V_{rest} of *Kcnc1* PV-INs is significantly more depolarized than WT PV-INs, while $V_{threshold}$ of *Kcnc1* PV-INs is significantly more hyperpolarized.



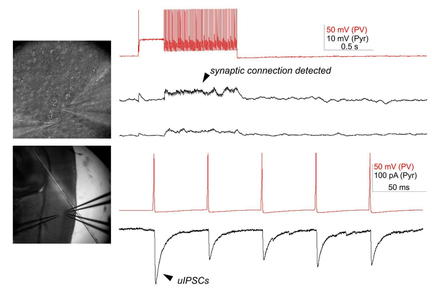
Interpretation

- The *Kcnc1-A421V/+* mutation may result in a reduction of K^+ current density, leading to dysfunctional $K_V3.1$ channels that impair the firing of fast-spiking GABAergic PV-INs.
- With a significantly slower downstroke velocity, *Kcnc1* PV-INs will take a longer period of time to repolarize, which impairs the recovery from inactivation of Na_V channels required for high-frequency repetitive firing typical of PV-INs.
- As current injection strength increases, *Kcnc1* PV-IN repolarization may be too slow to completely offset membrane depolarization. Subsequent APs could fire with fewer and fewer Na_V channels until *Kcnc1* PV-INs fail to fire APs at suprathreshold potentials.

A novel mouse model of *KCNC1* encephalopathy was generated due to the recurrent variant *KCNC1*-p.A421V. Results indicate that PV-INs - which are known to powerfully control inhibition in the cerebral cortex and specifically express *Kcnc1* - are prominently impaired in *Kcnc1*-p.A421V mice. Analysis of electrophysiological recordings identified a range of cellular defects consistent with potassium channel dysfunction.

Next Steps

Since $K_V3.1$ is also prominently expressed at PV-IN synapses, the intrinsic physiology of the *Kcnc1-A421V/+* mutant may affect synaptic excitability. The synaptic connectivity of WT and A421V mice can be examined and compared using a technique called 'multipatching.' Furthermore, the effects of K_V3-specific modulators on PV-IN physiology can be tested.



References

Ranjan, R., Logette, E., Marani, M., Herzog, M., Tâche, V., Scantamburlo, E., Buchillier, V., & Markram, H. (2019). A Kinetic Map of the Homomeric Voltage-Gated Potassium Channel (Kv) Family. *Frontiers in cellular neuroscience*, 13, 358. https://doi.org/10.3389/fncel.2019.00358

Chi, G., Liang, Q., Sridhar, A., Cowgill, J. B., Sader, K., Radjainia, M., Qian, P., Castro-Hartmann, P., Venkaya, S., Singh, N. K., McKinley, G., Fernandez-Cid, A., Mukhopadhyay, S., Burgess-Brown, N. A., Delemotte, L., Covarrubias, M., & Dürr, K. L. (2022). Cryo-EM structure of the human Kv3.1 channel reveals gating control by the cytoplasmic T1 domain. *Nature communications*, 13(1), 4087. https://doi.org/10.1038/s41467-022-29594-w

Muona, M., Berkovic, S. F., Dibbens, L. M., Oliver, K. L., Maljevic, S., Bayly, M. A., Joensuu, T., Canafoglia, L., Franceschetti, S., Michelucci, R., Markkinen, S., Heron, S. E., Hildebrand, M. S., Andermann, E., Andermann, F., Gambardella, A., Tinuper, P., Licchetta, L., Scheffer, I. E., Criscuolo, C., … Lehesjoki, A. E. (2015). A recurrent de novo mutation in KCNC1 causes progressive myoclonus epilepsy. *Nature genetics*, 47(1), 39–46. https://doi.org/10.1038/ng.3144

Cameron, J. M., Maljevic, S., Nair, U., Aung, Y. H., Cogné, B., Bézieau, S., Blair, E., Isidor, B., Zweier, C., Reis, A., Koenig, M. K., Maarup, T., Sarco, D., Afenjar, A., Huq, A., Kukolich, M., Billette de Villemeur, T., Nava, C., Héron, B., Petrou, S., … Berkovic, S. F. (2019). Encephalopathies with KCNC1 variants: genotype-phenotype-functional correlations. *Annals of clinical and translational neurology*, 6(7), 1263–1272. https://doi.org/10.1002/acn3.50822

Park, J., Koko, M., Hedrich, U., Hermann, A., Cremer, K., Haberlandt, E., Grimmel, M., Alhaddad, B., Beck-Woedl, S., Harrer, M., Karall, D., Kingelhoefer, L., Tzschach, A., Matthies, L. C., Strom, T. M., Ringelstein, E. B., Sturm, M., Engels, H., Wolff, M., Lerche, H., … Haack, T. B. (2019). KCNC1-related disorders: new de novo variants expand the phenotypic spectrum. *Annals of clinical and translational neurology*, 6(7), 1319–1326. https://doi.org/10.1002/acn3.50799

Carpenter, J. C., Männikkö, R., Heffner, C., Heneine, J., Sampedro-Castañeda, M., Lignani, G., & Schorge, S. (2021). Progressive myoclonus epilepsy KCNC1 variant causes a developmental dendritopathy. *Epilepsia*, 62(5), 1256–1267. https://doi.org/10.1111/epi.16867

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