Impaired excitability of fast-spiking neurons in a novel mouse model of *KCNC1* epileptic encephalopathy

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Abstract The recurrent pathogenic variant *KCNC1*-p.Ala421Val (A421V) is a cause of developmental and epileptic encephalopathy characterized by moderate-to-severe developmental delay/intellectual disability, and infantile-onset treatment-resistant epilepsy with multiple seizure types including myoclonic seizures. Yet, the mechanistic basis of disease is unclear. *KCNC1* encodes Kv3.1, a voltage-gated potassium channel subunit that is highly and selectively expressed in neurons capable of generating action potentials at high frequency, including parvalbumin-positive fast-spiking GABAergic inhibitory interneurons in cerebral cortex (PV-INs) known to be important for cognitive function and plasticity as well as control of network excitation to prevent seizures. In this study, we generate a novel transgenic mouse model with conditional expression of the Ala421Val pathogenic missense variant (*Kcnc1*-A421V/+ mice) to explore the physiological mechanisms of *KCNC1* developmental and epileptic encephalopathy. Our results indicate that global heterozygous expression of the A421V variant leads to epilepsy and premature lethality. We observe decreased PV-IN cell surface expression of Kv3.1 via immunohistochemistry, decreased voltage-gated potassium current density in PV-INs using outside-out nucleated macropatch recordings in brain slice, and profound impairments in the intrinsic excitability of cerebral cortex PV-INs but not excitatory neurons in current-clamp electrophysiology. *In vivo* two-photon calcium imaging revealed hypersynchronous discharges correlated with brief paroxysmal movements, subsequently shown to be myoclonic seizures on electroencephalography. We found alterations in PV-IN-mediated inhibitory neurotransmission in young adult but not juvenile *Kcnc1*-A421V/+ mice relative to wild-type controls. Together, these results establish the impact of the recurrent Kv3.1-A421V variant on neuronal excitability and synaptic physiology across development to drive network dysfunction underlying *KCNC1* epileptic encephalopathy.

Introduction

Variants in *KCNC1*, which encodes the voltage-gated potassium (K^+) channel subunit Kv3.1, cause *KCNC1*-related neurological disorders, a spectrum of clinical phenotypes ranging from nonspecific intellectual disability to progressive myoclonus epilepsy and developmental and epileptic encephalopathy (***Oliver et al., 2017***; ***Cameron et al., 2019***; ***Park et al., 2019***; ***Li et al., 2021***; ***Clatot et al., 2023***; ***Feng et al., 2024***). Kv3.1 is one of four members (Kv3.1-Kv3.4) of the Kv3 subfamily of voltage-gated K^+ channels. Kv3 channels show unique biophysical properties relative to other voltage-gated K^+ channels including a depolarized voltage-dependence of activation, fast rates of activation and deactivation, and little/no inactivation, properties that are exquisitely tuned to generate brief spikes and limit inter-spike interval, and thereby support rapid cycling required for reliable fast-spiking in Kv3-expressing neurons (***Weiser et al., 1995***; ***Massengill et al., 1997***; ***Sekirnjak et al., 1997***; ***Gan and Kaczmarek, 1998***; ***Martina et al., 1998***; ***Wang et al., 1998***; ***Erisir et al., 1999***; ***Rudy and McBain, 2001***; ***Akemann and Knöpfel, 2006***; ***Sacco et al., 2006***; ***Martina et al., 2007***). Thus, Kv3 channels are highly and specifically expressed in cellular populations throughout the brain known to generate APs at high-frequency, including cerebellar granule and Purkinje cells, neurons of the reticular thalamus, as well as parvalbumin-positive fast-spiking GABAergic inhibitory interneurons (PV-INs) in the neocortex, hippocampus and basal ganglia (***Rudy et al., 1999***; ***Kaczmarek and Zhang, 2017***). Alterations in Kv3.1 function would be expected to have a profound impact on neuronal excitability of fast-spiking neurons with downstream effects on circuits containing Kv3-expressing cells.

Our previous study using a novel mouse model of Progressive Myoclonus Epilepsy Type 7 (PME7 or EPM7) harboring the recurrent missense variant *KCNC1*-p.Arg320His indicated that loss of Kv3.1 function alters excitability and synaptic neurotransmission in cerebral cortex PV-INs and cerebellar granule cells in adult heterozygous *Kcnc1*-p.R320H/+ mice (***Feng et al., 2024***). In contrast to EPM7, patients harboring de novo heterozygous *KCNC1*-p.Ala421Val variants exhibit developmental and epileptic encephalopathy (DEE), with moderate to severe developmental delay/intellectual disability without regression, variable but mild nonprogressive ataxia, and treatment-resistant epilepsy onset in infancy with multiple seizure types including myoclonic seizures (***Oliver et al., 2017***; ***Cameron et al., 2019***; ***Park et al., 2019***; ***Li et al., 2021***). Examination of the function of voltage-gated K^+ channels containing variant versus wild-type (WT) Kv3.1 in heterologous systems has indicated that the A421V variant is a near-complete loss-of-function at the level of the channel, generating K^+ currents that are significantly reduced in magnitude relative to WT (***Cameron et al., 2019***; ***Park et al., 2019***). Hence, while both the R320H and A421V variants are loss of function with a proposed dominant negative action on tetrameric Kv3 channels composed of WT and variant subunits in heterologous systems, the A421V variant is a markedly more severe loss of function, consistent with the associated clinical phenotype with earlier age of onset and treatment-resistant epilepsy. The A421 residue is localized between the selectivity filter and the PVP motif of Kv3.1. Molecular modeling shows that the A421V variant does not lead to obvious steric hindrance in the channel yet could possibly influence gating and selectivity through the addition of hydrophobic carbon atoms in the Kv3.1 pore (***Li et al., 2021***). Yet, the precise mechanisms underlying how the A421V variant impacts native neuronal Kv3 currents, neuronal physiology, and ultimately results in developmental and epileptic encephalopathy, is unclear.

In this study, we generated a novel mouse model of *KCNC1* DEE – *Kcnc1*-Flox(p.Ala421Val)/+ (i.e., *Kcnc1*-A421V/+) mice – to determine the impact of heterozygous expression of the *Kcnc1*-p.A421V variant on native voltage-gated K^+ channel currents, intrinsic excitability of Kv3.1-expressing neurons, inhibitory synaptic neurotransmission and function in cortical microcircuits, and epilepsy phenotype. Our results indicate that global expression of the *Kcnc1*-p.A421V allele results in developmental impairment, epilepsy including prominent myoclonic seizures, and premature lethality due to seizure-induced sudden death. Patch-clamp electrophysiological recordings demonstrate that Kv3-like voltage-gated K^+ current density is significantly reduced in PV-INs driven at least in part by

impaired trafficking and cell surface expression of Kv3.1, with resulting alterations in AP waveform and impaired intrinsic excitability. Excitatory cell physiology was unchanged in the *Kcnc1*-A421V/+, suggesting that the phenotype of *Kcnc1*-A421V/+ mice is related to inhibitory neuron dysfunction. Investigation of synaptic neurotransmission revealed no significant differences between WT and *Kcnc1*-A421V/+ PV-IN-mediated inhibitory neurotransmission at the early juvenile time window (P16-21), but significantly altered properties at the young adult time point (P32-42), consistent with the observed progressive worsening of epilepsy in the mouse model and suggesting that altered Kv3.1 function leads to impairments in PV-IN synaptic function in a developmentally-regulated manner. Overall, these results indicate that the *Kcnc1*-A421V variant is physiologically loss-of-function in native neurons with resulting impairment of intrinsic excitability and synaptic transmission of Kv3.1-expressing parvalbumin-positive fast-spiking cells to yield epilepsy and early mortality.

Results

Generation of the *Kcnc1*-A421V/+ mouse model of *KCNC1* Epilepsy

We generated a novel transgenic mouse (see Materials and Methods) that conditionally expresses the *Kcnc1*-p.A421V/+ variant (*Kcnc1*-p.A421V/+ mice) homologous to a recurrent *KCNC1* variant previously identified in human patients with developmental and epileptic encephalopathy (***Oliver et al., 2017***; ***Cameron et al., 2019***; ***Park et al., 2019***). Briefly, the *Kcnc1* c.1262C>T missense was introduced into an ES cell line via gene targeting, converting a GTC to GTT and leading to the Ala421Val amino acid change. A targeting vector containing part of intron 1 followed by the coding sequence of exons 2-4 flanked by loxP sites was then introduced upstream of the modified endogenous sequence (Figure 1A). Thus, in the absence of Cre recombinase, there is expression of the introduced 5' wild-type exons 2-4; in the presence of Cre recombinase, there is Cre-mediated excision of the floxed wild-type exons 2-4 coding sequence and expression of *Kcnc1* harboring the c.1262C>T substitution resulting in the single amino acid change p.Ala421Val (Figure 1A). Sanger sequencing confirmed the knock-in point mutation in exon 2 and subsequent PCR showed Cre-dependent genome recombination of the variant (Figure 1B-C). We utilized a breeding strategy which allowed us to examine the behavior and physiology of mice expressing the *Kcnc1* variant globally (via cross to ActB-Cre mice; JAX#: 003376), as is the case with human patients harboring the A421V as a de novo pathogenic variant (Figure 1D). We also used a transgenic mouse line (C57BL/6-Tg(Pvalb-tdTomato)15Gfng/J; JAX#: 027395) which fluorescently labels parvalbumin-positive inhibitory interneurons (PV-INs) with the red fluorescent protein tdTomato driven by the endogenous parvalbumin promotor (Figure 1D). Our triple transgenic breeding strategy therefore produced experimental *Kcnc1*-A421V/+ mice and WT littermates of both sexes containing Cre and with 50% harboring the tdTomato allele to guide physiological experiments targeting PV-INs. The overall survival curve demonstrated that *Kcnc1*-A421V/+ mice (N=33) exhibited premature death relative to their WT littermates (N=46) with no *Kcnc1*-A421V/+ mice surviving beyond 122 days (***P<0.001 by Mantel-Cox Test; Figure 1E).

Counts of PV cells per unit of cortical area were no different between WT and *Kcnc1*-A421V/+ mice indicating that expression of A421V did not alter the density of cortical PV-INs (Supplementary Figure 1A-B), consistent with the fact that interneuron migration is complete prior to expression of *Kcnc1* in mouse. We separately used immunohistochemistry to validate our genetic strategy for labeling PV-INs (Supplementary Figure 1C-D): The average sensitivity rates were greater than 0.8 in both groups as previously reported (***Kaiser et al., 2016***) and were not different by genotype (Supplementary Figure 1C), with the average false positive rate less than 0.1 for each group (Supplementary Figure 1D).

Kcnc1-A421V/+ mice underwent an assessment of developmental milestones at postnatal day five through 15 (P5-15), as done previously (***Feng et al., 2024***). Although *Kcnc1*-A421V/+ mice exhibited reduced body (Supplementary Figure 2A-B) and brain (Supplementary Figure 2C) weights relative to their WT littermates – as seen previously with *Kcnc1* knockout mice (***Ho et al., 1997***) – we did not detect other developmental abnormalities in the onset of fur appearance, eye opening,

ear canal opening, incisor eruption, head elevation, shoulder elevation, auditory startle, horizontal screen test, vertical screen test, cliff avoidance, quadruple walking, and negative geotaxis (Supplementary Figure 2D-E). These results suggest that while body and brain weights are reduced, the *Kcnc1*-A421V/+ mice show otherwise typical gross anatomical and functional development within the early developmental timepoint examined (P5-15).

Kcnc1-A421V/+ mice exhibit reduced voltage-gated potassium channel currents and altered membrane Kv3.1 expression

Previous studies in heterologous expression systems have reported that the A421V variant is physiologically loss-of-function and generates strongly attenuated voltage-gated K^+ channel currents in Xenopus laevis oocytes (***Cameron et al., 2019***; ***Park et al., 2019***), albeit with conflicting conclusions related to the presence of a dominant-negative action. Considering that Kv3.1 channels form heterotetramers with other Kv3 channel isoforms (likely Kv3.2) in PV-INs, we sought to clarify the impact of the A421V *Kcnc1* variant on native neuronal voltage-gated K^+ channel function in PV-INs from *Kcnc1*-A421V/+ mice. Outside-out nucleated macropatch recordings allowed for high-quality electrophysiological recordings of somatic voltage-gated K^+ currents (Figure 2A-C). Relative to WT controls (*n*=13 cells, *N*=3 mice), the current density of voltage-gated K^+ currents in the *Kcnc1*-A421V/+ mice (*n*=17 cells, *N*=3 mice) was markedly reduced across all voltages examined (***P<0.001; Repeated-measures Two-way ANOVA; Figure 2B-D). Peak current densities were significantly lower in PV-INs from *Kcnc1*-A421V/+ mice compared to WT controls (***P<0.001; t-test; Figure 2E). We did not observe differences in the voltage-dependence (Figure 2F) or kinetics of activation (Figure 2G) when comparing voltage-gated K^+ channel currents from WT and *Kcnc1*-A421V mice. Together, these results demonstrate that PV-IN voltage-gated K^+ channel function is strongly impaired in the context of the *Kcnc1*-p.A421V variant.

The markedly decreased K^+ current magnitude observed in PV-INs without alterations in gating properties is consistent with impaired function of the population of Kv3 channels, but could also be explained by impaired trafficking to the cell membrane. To investigate this possibility, we examined Kv3.1 expression in PV-INs via immunohistochemistry. Our results indicated that the amount of Kv3.1 at membrane relative to cytosol was significantly altered in the *Kcnc1*-A421V/+ mice (*n*=27 cells, 9 cells from each of 3 slices, *N*=3 mice) compared to WT controls (*n*=27 cells, 9 cells from each of 3 slices, *N*=3 mice; (Figure 2H-I). These findings support the conclusion that impaired trafficking to the cell surface contributes to the observed decrease in K^+ current in PV-INs in *Kcnc1*-A421V/+ mice.

Intrinsic excitability of PV-INs is altered in *Kcnc1*-A421V/+ mice

We next examined intrinsic neuronal excitability in PV-INs in somatosensory neocortex to determine the impact of the voltage-gated K^+ channel dysfunction across two age ranges, juvenile (P16-21; Figure 3A-E) and young adult mice (P32-42; Figure 3F-J). We performed whole-cell current-clamp recordings to generate a detailed comparison of passive membrane properties, properties of individual APs, and of repetitive firing, for PV-INs from primary somatosensory neocortex in WT vs. *Kcnc1*-A421V/+ mice at both time points (Figure 3,Table 1,Table 2). PV-INs from both genotypes generated trains of repetitive APs in response to depolarizing current injection; however, frequency was profoundly reduced in *Kcnc1*-A421V/+ mice relative to the WT control PV-INs at all current magnitudes examined (***P<0.001, Repeated-measures Two-way ANOVA; Figure 3B-D). When examining the properties of individual APs, *Kcnc1*-A421V/+ and WT PV-INs showed significant differences in downstroke velocity and half-maximal AP duration (APD50), properties that are determined by Kv3 channel function. AP amplitude was elevated in the *Kcnc1*-A421V/+ mice at juvenile (Figure 3E);Table 1) and adult (Figure 3J; Table 2) timepoints, but only reached significance at P32-42 (***P<0.001, unpaired t-test). Overall, PV-INs from *Kcnc1*-A421V/+ mice exhibited specific impairments consistent with reduction in Kv3 current including altered properties of individual

APs leading to a reduction in firing frequency, with a relative preservation of passive membrane properties not thought to be directly regulated by Kv3 channels.

We sought to further extend these results by examining another prominent Kv3.1-expressing cellular population linked to epilepsy pathogenesis, PV-positive neurons in the nucleus of the reticular thalamus (RTN) (***Porcello et al., 2002***) (Supplementary Figure 3). In response to hyperpolarizing current injections of various magnitudes, RTN neurons from *Kcnc1*-A421V/+ mice (*N*=19 cells, 5 mice) generated fewer rebound APs than their WT counterparts (*n*=16 cells, *N*=4 mice; Supplementary Figure 3B-C). As in neocortical PV-INs, the relationship between AP frequency and depolarizing current injection was attenuated in reticular thalamic cells from *Kcnc1*-A421V/+ mice relative to WT counterparts (*P=0.0109; Supplementary Figure 3B,D). And as in neocortical PV-INs, the magnitude of the downstroke velocity was significantly reduced in *Kcnc1*-A421V/+ RTN neurons, while other parameters did not reach significance at this timepoint (Table 1). Thus, impairments in intrinsic physiology extend beyond cortical PV-INs to Kv3.1-expressing cells in other brain regions.

Normal physiological function in excitatory neurons from *Kcnc1*-A421V/+ mice

We next investigated the voltage-gated K^+ channel function and intrinsic excitability in excitatory cells from both WT and *Kcnc1*-A421V/+ mice (Figure 4). While voltage-gated K^+ channel currents in neocortical layer IV excitatory neurons were of significantly lower magnitude compared to that observed in PV-INs, there were no genotype differences in K^+ currents between excitatory cells from WT and *Kcnc1*-A421V/+ mice, consistent with a lack of Kv3.1 expression in excitatory cells (Figure 4B-D). Neither voltage-dependent current density (Figure 4D), peak current density (Figure 4E), voltage-dependent activation (Figure 4F), nor the voltage-dependent rate of activation (Figure 4G) were altered in excitatory neurons of *Kcnc1*-A421V/+ mice relative to WT controls. We also recorded these cells in current-clamp to characterize intrinsic excitability (Figure 4H-K). Across a range of depolarizing current injection magnitudes, we did not detect any differences in steady-state AP frequency in excitatory neurons between WT and *Kcnc1*-A421V/+ mice (Figure 4I-K). We also did not detect any statistically significant alterations in the passive membrane properties or properties or single APs between WT and *Kcnc1*-A421V/+ mice (Table 1). Overall, these data suggest that intrinsic excitability of neocortical excitatory neurons is not altered in juvenile *Kcnc1*-A421V/+ mice (either directly or via a secondary network effect), while impaired intrinsic excitability of PV-positive neurons in the neocortex and reticular thalamus is most likely a direct result of cell-autonomous reduction in Kv3.1 current density.

PV-IN and excitatory cell synaptic neurotransmission is functionally intact in juvenile *Kcnc1*-A421V/+ mice

Within fast-spiking cells, Kv3 channels are expressed in specific subcellular compartments and are functionally involved not only in AP generation, but also AP propagation in the axon and inhibitory neurotransmission at the synaptic terminal (***Goldberg et al., 2005***; ***Rowan et al., 2014***, ***2016***; ***Rowan and Christie, 2017***). Additionally, prior work has shown that block of presynaptic Kv3 current leads to an increase in the efficacy of synaptic transmission (***Ishikawa et al., 2003***; ***Brooke et al., 2004***; ***Goldberg et al., 2005***). For that reason, we sought to determine the impact of the A421V variant on PV-IN-mediated inhibitory synaptic neurotransmission in juvenile (P16-21) WT vs. *Kcnc1*-A421V/+ mice (Figure 5). We collected simultaneous whole-cell patch-clamp electrophysiology recordings from one PV-IN and one nearby (<100 μm inter-soma distance) excitatory neuron of which 21 of 64 (32.8%) WT neuron pairs and 15 of 43 (31.2%) *Kcnc1*-A421V/+ neuron pairs exhibited unitary inhibitory post-synaptic currents (uIPSCs) in the excitatory cell in response to AP generation in the PV-IN at 5, 10, 20, 40, 80, and 120Hz (Figure 5A-E). Rates of failure of the first five APs (AP is successfully initiated in the PV-IN, but no uIPSC is observed in the excitatory cell) were not different in WT vs. *Kcnc1*-A421V/+ mice (Figure 5F). The magnitudes of the first five uIPSCs at various stimulation frequencies were also not significantly differently between the two genotypes (Figure 5G-I). The paired-pulse ratios, either $uIPSC_2/uIPSC_1$ or uIPSCLast/$uIPSC_1$ were not different between WT

and *Kcnc1*-A421V/+ (Figure 5J-K). Finally, we failed to detect a significant difference in synaptic latency of the uIPSC between WT and *Kcnc1*-A421V/+ neuron pairs (Figure 5L). These data suggest that, despite expression of Kv3.1 in the axon and synaptic terminal in WT mice, neocortical PV-IN-mediated inhibitory synaptic neurotransmission remains intact in *Kcnc1*-A421V/+ mice at this juvenile developmental timepoint. Yet, inhibitory transmission will be impaired in *Kcnc1*-A421V/+ mice secondary to the abnormal excitability and impaired spike generation of PV-INs.

In nine of 61 neuron (14.8%) pairs for WT mice and six of 43 pairs (14.0%) for *Kcnc1*-A421V/+ mice, we observed unitary excitatory synaptic currents (uEPSCs) from the excitatory neuron onto the PV-IN which allowed us to investigate whether there were abnormalities in excitatory neurotransmission (Supplementary Figure 4A-D). The frequency-dependent rate of failure showed no significant effect for genotype (Supplementary Figure 4E). Lastly, the magnitude (Supplementary Figure 4F), paired-pulse ratio (Supplementary Figure 4G), and the latency (Supplementary Figure 4H) of the uEPSCs were not significantly different between WT and *Kcnc1*-A421V/+ neuron pairs indicating that excitatory neuron synaptic transmission onto PV-INs is not altered in juvenile *Kcnc1*-A421V/+ mice.

PV-IN synaptic neurotransmission is altered in adult *Kcnc1*-A421V/+ mice

As Kv3.1 exhibits developmentally-regulated expression, we next assessed PV-IN mediated inhibitory neurotransmission in young adult (P32-42) *Kcnc1*-A421V/+ mice relative to WT controls (Figure 6). As in the younger mice, we recorded pairs of cortical PV-IN and excitatory neurons that were synaptically connected—14 of 36 pairs (38.9%) in WT mice (*N*=8 mice) and 13 of 36 pairs (36.1%) in *Kcnc1*-A421V/+ mice (*N*=8 mice; Figure 6A-D). We did not detect differences in failure rates between WT and *Kcnc1*-A421V/+ mice over a range of stimulation frequencies (Figure 6E). Notably, the average magnitude of the first five uIPSCs was significantly increased in *Kcnc1*-A421V/+ relative to WT recordings (**P<0.01 at 20 Hz, *P<0.05 at 40 Hz, and *P<0.05 at 80 Hz; Figure 6F-H). Additionally, we observed a reduced paired-pulse ratio for the second uIPSC relative to the first uIPSC in *Kcnc1*-A421V/+ neuron pairs from young adult mice across a range of stimulation frequencies (*P<0.05; Figure 6I), but found no genotype effect in the average ratio between the last uIPSC to the first uIPSC (Figure 6J). The uIPSC latency measured from AP peak to onset of the synaptic event was not significantly different between WT and *Kcnc1*-A421V/+ neuron pairs (Figure 6K). Overall, these complex results align with prior work showing that Kv3.1 is an important regulator of synaptic neurotransmission in PV-INs and suggests that synaptic dysfunction may contribute to the pathogenesis of epilepsy in *Kcnc1*-A421V/+ mice in a developmentally determined manner.

2-Photon *in vivo* calcium imaging reveals paroxysmal hypersynchronous discharges in *Kcnc1*-A421V/+ mice

We then utilized two-photon (2P) calcium-imaging to investigate neural activity in neocortical circuits in both WT and *Kcnc1*-A421V/+ mice in layer II/III of primary somatosensory cortex *in vivo* (Figure 7A). We observed striking instances of paroxysmal hypersynchronous discharges in seven of seven *Kcnc1*-A421V/+ mice examined, but never in WT mice (Figure 7B-D). During each hypersynchronous discharge, the mouse was stationary and non-ambulatory, but displayed a brief diffuse twitch involving the facial musculature and bilateral limbs (Supplementary Video 1). Imaging of mice with pan-neuronal viral expression of GCaMP8m and tdTomato labeling of PV-INs driven by the S5E2 enhancer revealed that there was no significant recruitment of PV+ or PV- somata before or after the synchronous discharges (Figure 7E-G), but did show recruitment of layer II/III PV+ and PV- somata during the synchronous discharges (although it is possible that the small observed increase in somatic fluorescence may remain contaminated by the large magnitude increase in neuropil fluorescence during the discharge) (Figure 7F-G). Taken together, these *in vivo* imaging results indicate that *Kcnc1*-A421V/+ mice exhibit paroxysmal synchronous discharges that may correlate with seizures, potentially myoclonic seizures.

Spontaneous seizures and sudden death in *Kcnc1*-A421V/+ mice.

To further investigate the nature of the events observed in *Kcnc1*-A421V/+ mice during *in vivo* 2P calcium-imaging, we performed continuous video electroencephalogram (EEG) monitoring for periods of two to seven days (Figure 8). In seven of 11 mice, we observed clear convulsive seizures including tonic, clonic, and tonic-clonic limb movements with loss of consciousness and fall, associated with an electrographic correlate (seizure duration was 105.9 ± 84.2 seconds; Figure 8A-B; Supplementary Video 2). As in our 2P *in vivo* calcium imaging experiments, we also observed brief diffuse jerks involving the face and limbs associated with large-amplitude spikes on the EEG (Figure 8A-C; Supplementary Video 2. In seven of 11 mice, we observed brief runs of epileptiform spikes without apparent behavioral correlate (seizure duration was 14.5 ± 0.7 seconds; Figure 8C). Interestingly, we were also able to capture four *Kcnc1*-A421V/+ seizure-induced sudden death events on video-EEG with two additional occurrences with video only (Supplementary Video 3). In each case, sudden death was directly preceded by a generalized tonic-clonic seizure with hindlimb extension, whereas nonfatal seizures did not lead to the hindlimb extension associated with the tonic phase of a generalized seizures (Figure 8A-B). Overall, our *in vivo* monitoring reveals that the *Kcnc1*-A421V/+ mouse recapitulates core features of *Kcnc1* DEE including a range of seizure types including myoclonic seizures as well as seizure-induced sudden death.

Discussion

The recurrent pathogenic variant *Kcnc1* p.A421V leads to DEE characterized by treatment-resistant epilepsy with onset in the first year of life and with multiple seizure types including myoclonic seizures, moderate to severe global developmental delay/intellectual disability, and variably present but mild nonprogressive ataxia. Further elucidation of the mechanistic links between *Kcnc1* variants, Kv3.1 subunit-containing K^+ channel dysfunction, impairments in the intrinsic excitability of Kv3.1-expressing neurons, and synaptic and circuit neurophysiology is critical towards clarification of underlying disease pathomechanisms and development of potential targets for therapeutic intervention. Our study reports the generation of a mouse model of *Kcnc1* DEE and determine the physiological mechanisms of disease at the level of ion channels, single neuron intrinsic excitability, and synaptic neurotransmission, as well as in circuits *in vivo*, within epilepsy-related brain regions.

Kv3.1 expression and function

Kv3.1 is specifically expressed in high-frequency firing neurons throughout the nervous system, including PV-INs in the neocortex, hippocampus, amygdala, and basal ganglia, as well as cells of the reticular thalamus, and Purkinje, granule cells, and molecular layer interneurons of the cerebellum (***Chow et al., 1999***). Due to its rapid activation and deactivation kinetics and voltage-dependence (more positively shifted than any other K^+ channel), Kv3.1 and the other members of the Kv3 family (Kv3.2, Kv3.3) are associated with neurons that generate APs at particularly high frequencies >200 Hz (***Erisir et al., 1999***; ***Kaczmarek and Zhang, 2017***).

Kv3.1 knockout (Kv3.1-/-) animals have previously been used to investigate the functional contribution of Kv3.1 to neuronal spiking. These mice have reduced body weight and altered sensory/motor function, as we observed in the *Kcnc1*-A421V/+ mice, but do not display spontaneous seizures (***Ho et al., 1997***). The identified impairments in intrinsic neuronal excitability of Kv3.1-expressing neurons were relatively subtle in Kv3.1 knockout mice: RTN neurons from Kv3.1-/- mice exhibited slightly wider APs and a use-dependent spike broadening that produced a mild impairment in AP frequency; but, overall, there seemed to be functional and/or genetic compensatory upregulation of other Kv3 subfamily members in response to Kv3.1 deletion (***Porcello et al., 2002***). Interestingly, mice lacking Kv3.2 (Kv3.2-/-), the other Kv3 family member highly expressed in PV-INs and which has near-identical biophysical properties, exhibit a more similar phenotype to that identified in the *Kcnc1*-A421V/+ mice, with impaired excitability of neocortical PV-INs and spontaneous seizures observed in a subset of mice (***Lau et al., 2000***). Overall, for mechanistic reasons

yet unclear, it seems that the heterozygous *Kcnc1*-A421V/+ mice reported here have a more severe phenotype than either Kv3.1 or Kv3.2 null mice, perhaps because compensation occurring in knockout mice might not occur with heterozygous expression of a missense variant. Our results show that the A421V variant leads to decreased Kv3-like current in nucleated macropatches from neocortical PV-INs as well as impaired cell surface expression of Kv3.1 in neocortical PV-INs. Future studies should clarify the mechanism underlying precisely how this missense variant impacts trafficking to and incorporation into heteromultimeric Kv3 channels in various subcellular compartments of PV-INs and other Kv3.1-expressing cells. We found that Kv3.1-expressing neocortical PV-INs and cells of the RTN, but not excitatory cells (which do not express Kv3.1), were hypoexcitable in *Kcnc1*-A421V/+ relative to WT mice, generating fewer APs in response to depolarizing current injections. Hypofunction of PV-INs has been associated with various types of epilepsy including, most notably, Dravet syndrome, another DEE driven by loss of function variants in *SCN1A* encoding the voltage-gated sodium channel subunit NaV1.1. Hence, Dravet Syndrome and *Kcnc1* DEE converge on specific impairment of GABAergic inhibitory interneurons, and on PV-INs in particular. Yet, there are likely important differences between these syndromes which may explain the divergent clinical presentation in patients (***Clatot et al., 2024***). For one, deficits in Kv3.1 in *Kcnc1* DEE may be differentially compensated by other Kv3 isoforms when compared to possible compensation for reduced NaV1.1 in Dravet syndrome by other voltage-gated sodium channel α subunits. Secondly, there is a differential cell type-specific expression pattern between Kv3.1 and NaV1.1 in the cerebral cortex, with NaV1.1 being also expressed in non-fast-spiking interneurons such as somatostatin and VIP-expressing interneurons (***Tai et al., 2014***; ***Rubinstein et al., 2015***; ***Goff and Goldberg, 2019***), whereas Kv3.1 is largely specific for PV-INs. Yet, Kv3.1 is more prominently expressed in superficial layers of mouse neocortex with Kv3.2 more prominently expressed in deep layer PV-INs, while NaV1.1 appears to be expressed in PV-INs across layers of the neocortex. Our results here also indicate another point of divergence between mechanisms of Dravet Syndrome and *Kcnc1* DEE: In adult *Kcnc1*-A421V/+ mice we observed an increase in the magnitude and altered paired-pulse ratio of PV-IN-mediated inhibitory postsynaptic currents relative to WT mice accompanied by no change in failure rate, which contrasts with the increased rate of failure and prolonged synaptic latency that we previously observed in PV-IN-mediated neurotransmission in Dravet syndrome (*Scn1a*$^{+/-}$) mice. We interpret the augmentation in postsynaptic current magnitude alongside reduced paired-pulse ratio observed in young adult (P32-42, after epilepsy onset) *Kcnc1*-A421V/+ mice to be generally consistent with a role for Kv3.1 in regulating neurotransmitter release by controlling spike-evoked calcium release via presynaptic AP width, as shown previously (***Goldberg et al., 2005***), although there could also be roles for secondary dysregulation of or compensatory alterations in other determinants of synaptic transmission (such as GABA receptor expression). How these specific patterns of abnormalities in intrinsic excitability and synaptic transmission contribute to circuit dysfunction in *Kcnc1*-A421V/+ mice and how this differs vs. *Scn1a*$^{+/-}$ mice could be more thoroughly examined in future studies.

We were initially surprised that we did not find alterations in inhibitory synaptic neurotransmission at the P16-21 timepoint, as PV-INs already exhibited markedly impaired intrinsic excitability and reduced magnitude of somatic voltage-gated K^+ currents. These results may indicate that the physiological contribution of Kv3.1 in different subcellular regions (i.e. soma, dendrite, axon, terminal, etc.) and its corresponding role in regulating the associated physiological phenomena (AP generation, propagation, and neurotransmitter release) evolves over development. For example, the demonstrated impairment in trafficking of Kv3.1-A421V variant subunit containing Kv3 channels imply that distal synaptically-localized Kv3 channels may not contain variant subunits at early time points and hence local AP waveform at the synapse might remain largely intact via residual Kv3 channels containing WT Kv3.1 and/or Kv3.2 (or Kv3.3) subunits. A more detailed mechanistic explanation for this age-dependent effect would provide further insight into disease pathomechanisms and could explain why the epilepsy phenotype appears at/around the time of weaning and increases in severity in this mouse model (in stark contrast to *Scn1a*$^{+/-}$ mice, where epilepsy

severity decreases with age). However, this would require a detailed exploration of the specific composition of heterotetrameric Kv3 channels in WT vs. *Kcnc1*-A421V/+ mice in various subcellular compartments and across development, as suggested above.

In this study, we focused on PV-INs and excitatory neurons in somatosensory cortical layer II-IV as well as PV-positive neurons of the reticular thalamus – epilepsy-linked brain regions – due to the prominent epilepsy phenotype and seizure-related early mortality observed in *Kcnc1*-A421V/+ mice. However, there are many other cellular populations across various brain regions that express Kv3.1 which could also be examined in future studies. Given that our mouse model expresses the knock-in A421V variant under the control of Cre recombinase, we are well-positioned to explore how cell type and developmental timing of altered Kv3.1 function might contribute to overall behavior phenotype.

Kcnc1-A421V/+ mice recapitulate the core phenotype of *Kcnc1* epilepsy seen in human patients

Patients harboring the *Kcnc1*-p.A421V variant exhibit treatment-resistant epilepsy with various seizure types including myoclonic, focal, atypical absence, and generalized tonic-clonic seizures with onset in the first year of life (***Cameron et al., 2019***; ***Park et al., 2019***). The novel *Kcnc1*-A421V/+ mouse model well captured the range of seizure phenotypes observed: Spontaneous seizures with different behavioral manifestations were observed including myoclonic jerks, convulsive seizures, and tonic-clonic seizures (those associated with hindlimb extension leading to sudden death). We directly observed abnormal neocortical neural activity in the *Kcnc1*-A421V/+ mice in our *in vivo* 2P calcium-imaging experiments accompanied by behavioral correlates of myoclonic seizures, demonstrating this mouse represents a potentially useful model for study of mechanisms of spontaneous seizures – including myoclonic seizures – using 2P calcium imaging in awake, behaving mice. These experiments revealed that apparent myoclonic seizures were associated with hypersynchronous paroxysmal discharges seen across all neurons within the field of view. Although we separately labeled fast-spiking PV-INs and other cells in our *in vivo* imaging experiments, we did not observe a temporal separation in the activity between PV-INs and non-PV cells which might indicate a causal relationship between aberrant PV-IN activity and the hypersynchronous discharge. However, it is perhaps more likely that such seizures engaged diffuse brain networks and the observed hypersynchronous dischargers were driven by distal activity. Nevertheless, these otherwise brief and intermittent events were clearly identified via 2P imaging which led to subsequent EEG studies confirming such events to be seizures. Future studies should expand on the *in vivo* imaging completed here to more thoroughly investigate the cellular and network architecture of the neural activity underlying the spontaneously occurring myoclonic seizure events. Moreover, *Kcnc1*-A421V/+ mice may prove to be a particularly tractable model for the study of myoclonic seizures.

Beyond seizures, human patients harboring *Kcnc1* variants show moderate to severe developmental delay and intellectual disability (***Cameron et al., 2019***; ***Park et al., 2019***). *Kcnc1*-A421V/+ mice showed developmental differences in body/brain weights; however, we did not detect other gross impairments in developmental milestones between postnatal days 5 and 15, perhaps because of the sensitivity of such assays to early subtle differences. We again focused here on the epilepsy phenotype but future studies could further evaluate cognitive and motor function in *Kcnc1*-A421V/+ mice across development.

The A421V *Kcnc1* variant leads to a loss of voltage-gated potassium channel function in PV-INs.

Previous studies have reported that A421V is a loss-of-function variant when examined in heterologous cells (in this case, Xenopus oocytes) (***Cameron et al., 2019***; ***Park et al., 2019***). However, such work is conflicting as to the exact mechanism, with one paper showing evidence for a dominant-negative effect, with another paper finding no evidence for dominant-negative action of the A421V variant. Our results using outside-out nucleated macropatch recordings of somatic voltage-gated

K^+ currents in brain slice showed clear approximately 50% reduction in K^+ current density in PV-INs (but not excitatory cells) without changes in the biophysical properties of gating. In our examination of surface Kv3.1, we found a reduction in the amount of Kv3.1 that reaches the membrane in PV-INs from *Kcnc1*-A421V/+ mice. While we cannot rule out the possibility that some Kv3 tetramers at the cell surface contain Kv3.1-A421V subunits and act to decrease channel conductance, our data is consistent with the view that the variant acts at least in part via incorporation of Kv3.1-A421V subunits into heterotetrameric Kv3 channels (likely in the endoplasmic reticulum) with impaired trafficking to the membrane. Consistent with this, a previous study identified A421V as exerting only slight steric hindrance relative to other developmental encephalopathy-causing *Kcnc1* variants, supporting the conclusion that the profound impact of this variant on recorded currents may not be due to impact on gating (***Li et al., 2021***). Similar structural approaches may also help better determine the mechanism of trafficking deficiency and the extent to which A421V channels impair the trafficking of heteromultimeric Kv3 channels containing WT Kv3.1 and/or Kv3.2 subunits.

Kv3.1 as a drug target in epilepsy

Given the powerful influence of Kv3 channels on the excitability of neocortical PV-INs and neurons of the cerebellum, pharmacological modulators of Kv3.1 have been put forth as potential treatment for a range of neurological and psychiatric conditions including in a mechanistically targeted fashion for patients with *Kcnc1*-related disorders such as EPM7 (***Rosato-Siri et al., 2015***; ***Brown et al., 2016***; ***Boddum et al., 2017***; ***Chambers et al., 2017***; ***Munch et al., 2018***; ***Feng et al., 2024***). Previous reports showed that AUT-1 and related compounds facilitate greater firing frequency and spiking reliability of fast-spiking cells (***Rosato-Siri et al., 2015***; ***Brown et al., 2016***; ***Boddum et al., 2017***; ***Chambers et al., 2017***; ***Munch et al., 2018***; ***Feng et al., 2024***). Our study did not investigate the impact of Kv3.1 modulators in *Kcnc1*-A421V/+ mice, but future work could prioritize such studies. Yet, considering the decreased cell surface expression of Kv3.1 in PV-INs from *Kcnc1*-A421V/+ mice, one might predict limited efficacy of a small-molecule channel activator, unless such compounds could exert therapeutic effect via action on WT Kv3 channels not containing variant Kv3.1-A421V subunits. On the other hand, genetic approaches to either knock down expression of the A421V variant (perhaps using an antisense oligonucleotide) or boost expression levels of the WT Kv3.1 should be explored.

Limitations of the study

We focused our study on the global impact of the *Kcnc1*-A421V variant on mouse development, epilepsy, and neuronal physiology of selected neuron types in epilepsy-linked brain regions, using ACTB-Cre to drive global expression from the blastocyst stage so as to best model the human condition. However, future work using cell type-specific Cre-drivers or Cre delivery to restricted brain regions will enable greater mechanistic clarity in linking cell type and brain region to specific aspects of the mouse phenotype, including epilepsy and non-epilepsy comorbidities of cognitive and motor impairment. However, given the early onset of neurological dysfunction in our mice, specific expression of the variant using, for example, PV-Cre mice, might not yield greater mechanistic insight, as PV itself is not expressed at appreciable levels until at/beyond P10 in mice and hence efficient recombination and expression of the *Kcnc1*-p.A421V variant in PV-INs will likely not faithfully reproduce the appropriate developmental expression pattern.

Conclusion

In summary, we report a mouse model that recapitulates the core features of *Kcnc1* developmental and epileptic encephalopathy due to the recurrent K^+ channel variant *Kcnc1*-p.A421V. *Kcnc1*-A421V/+ mice exhibit epilepsy with multiple seizure types including myoclonic seizures as well as seizure-linked premature lethality. This was associated with a pattern of specific impairments in intrinsic excitability and synaptic transmission consistent with Kv3 dysfunction, observed in Kv3.1-expressing neurons linked to epilepsy including neocortical PV-INs and neurons of the reticular

thalamic nucleus, but not excitatory cells. Future studies and ongoing therapeutic development promise to expand this mechanistic understanding in pursuit of improved outcomes for patients with this severe yet currently incurable and untreatable disorder.

Methods and Materials

Experimental animals

All procedures were approved by the Animal Care and Use Committee at the Children's Hospital of Philadelphia and were conducted in accordance with the published ethical guidelines by the National Institutes of Health. Male and female mice were used in approximately equal numbers in each experiment throughout the study and, while our study was not powered to detect sex differences *a priori*, we observed no significant interaction between sex and genotype in our study. In experiments on pre-weanling mice ages postnatal day 16-21, mice were housed with the dam; in post-weaning experiments, mice were group-housed by sex (≤5 mice/cage) and had access to food/water ad libitum in a temperature- and humidity-controlled room with a 12:12 hour light:dark cycle.

Kcnc1-Flox(A421V)/+ mice were generated via a gene targeting strategy. The targeting vector contained part of intron 1 and exons 2-4 of the *Kcnc1* coding sequence flanked by LoxP sites. A point mutation C>T (Ala421Val) was introduced into exon 2 in the 3' homology arm. The linearized vector was electroporated into ES cells and homologous recombination was confirmed by Southern blot. The targeted ES cell clone was then injected into mouse blastocysts and founder animals were identified by coat color. Germline transmission was confirmed by breeding with C57BL/6 mice and subsequent genotyping of the offspring. Hence, in the absence of Cre recombinase, the inserted wild-type sequence is expressed; in the presence of Cre recombinase, there is Cre-mediated excision of the inserted wild-type sequence, leading to the expression of the modified *Kcnc1* allele. Genotyping was performed using primers designed to detect upstream and downstream LoxP sites. The presence of the knock-in point mutation site was confirmed via Sanger sequencing. The homozygous floxed *Kcnc1* males were crossed to hemizygous Pvalb-tdT BAC transgenic reporter females (JAX# 027395) for generation of double transgenic *Kcnc1*-Flox(A421V)/+:Pvalb-tdT/+ mice. The female offspring were then crossed to homozygous ActB-Cre males (JAX# 003376) for generation of experimental mice. This strategy produced roughly equal numbers of WT:ActB-Cre:Pvalb-tdT (WT) and *Kcnc1*-A421V/+:Pvalb-tdT mice of both sexes in which PV-INs were fluorescently labeled, all on a C57BL/6 genetic background. After generation and establishment of the line, routine genotyping was completed through Transnetyx automated genotyping services.

Immunohistochemistry

Mice were deeply anesthetized with isoflurane and underwent transcardial perfusion with 10 mL of 4% paraformaldehyde (PFA) in PBS. Whole brains were extracted and postfixed for 24 hours. Parasagittal brain sections were cut at 40 µm intervals using a vibratome (Leica VT1200S). After washing in PBS, the slices were incubated in blocking solution (3% normal goat serum (NGS), 2% bovine serum albumin (BSA), 0.3% Triton X-100 in PBS) for 1 h at room temperature and then washed in PBS. The sections were then transferred into primary antibody solution containing rabbit Kv3.1b antibody (1:500; Alomone Labs APC-014) and mouse IgG1 parvalbumin antibody (EMD Millipore MAB1572) in blocking solution (1% NGS, 0.2% BSA, 0.3% Triton X-100 in PBS) and incubated at 4°C overnight. Next, sections were washed with PBS and incubated for 1h at room temperature in secondary antibody solution containing goat anti-rabbit Alexa Fluor 488 (1:1000, Molecular Probes A11034) and goat anti-mouse IgG1 Alexa Fluor 568 (1:1000, Molecular Probes A21124) in blocking solution (2% BSA, 0.3% Triton X-100 in PBS). Finally, sections were washed 3 times with PBS, incubated with DAPI (1:1000, Thermo Fisher D3571) for 10 min, and washed again with PBS. Sections were mounted on glass slides using polyvinyl alcohol mounting medium with DABCO (Sigma 10981). Images were acquired from somatosensory cortex using a confocal microscope (Leica SP8)

equipped with 10X and 40X objectives and image processing was performed with ImageJ software (NIH, USA).

Image Analysis

Parvalbumin-positive cells from somatosensory cortex layers II-IV were imaged and manually traced using the parvalbumin signal to define the cell soma and the DAPI signal to define the cell nucleus. A membrane compartment was defined as the outermost 1 micron of the parvalbumin-defined cell soma. A cytosol compartment was defined as the region inside the membrane compartment and outside the DAPI-defined nucleus. The mean Kv3.1b signal in the membrane compartment of each cell was measured and compared to the mean signal in the cytosolic compartment. To analyze cell density, parvalbumin-positive cells in somatosensory cortex layers 2-4 were imaged using a 10X objective and identified using automatic thresholding based on the ImageJ Triangle threshold method. Manually identified cells were manually verified for quality control. All analysis was done blinded to genotype, and statistical comparisons were made using an unpaired t-test.

Developmental Milestone Assessments

WT and *Kcnc1*-A421V/+ mice were examined for developmental milestones from postnatal day 5 through 15 as previously described (***Armstrong et al., 2020***; ***Feng et al., 2024***). Mice were examined manually for onset of fur appearance, eye opening, ear canal opening, incisor eruption, and elevation of the head and shoulders as previously described. Auditory startle was determined by examining for flinching in response to presentation of a loud stimulus (hand clap near cage). Horizontal and vertical screen tests, cliff avoidance, quadruped walking, and negative geotaxis were completed as previously described (***Armstrong et al., 2020***; ***Feng et al., 2024***). Statistical comparison of age-dependent brain and body weights were determined using a Two-way ANOVA.

In vivo two-photon calcium imaging

For stereotaxic viral injection and cranial window implantation, WT and *Kcnc1*-A421V/+ mice age >P35 were anesthetized with isoflurane (induction at 3–4%; maintenance at 1–1.5%). A 3 mm craniotomy over primary somatosensory cortex (centered at 1 mm posterior, 3 mm lateral to the bregma) was made, and a Nanoject III (Drummond Scientific) was used to inject 60 nL of AAV9-syn-jGCaMP8m-WPRE (Addgene #162375) alone or mixed with PHP.eB-E6-S5E2-dTom-nlsdTom (Addgene #135630), each diluted to a titer of 2e12 in sterile PBS) at a depth of 300-500 μm and a rate of 1 nL/sec using a 50 μm diameter beveled tip glass pipette. A 3 mm circular coverslip glued to a 5 mm circular coverslip was affixed over the craniotomy and a custom stainless steel headbar was cemented to the skull. Mice were given buprenorphine-SR 0.5 mg/kg, cefazolin 500 mg/kg, and dexamethasone 5 mg/kg perioperatively and monitored for recovery and infection for 48 h following surgery. 2-3 weeks following the cranial window implantation, mice were habituated to head fixation on the Mobile Homecage Apparatus (Neurotar) in a custom chamber with transparent siding for 2 days or until the mouse showed spontaneous running bouts and the absence of escape or freezing behaviors. Airflow into the Mobile HomeCage stage provided ~40–45 db pink noise during the experiment. Locomotion speed was tracked by the Mobile HomeCage locomotion tracking software (Neurotar). An infrared (IR) 850 nm light source and IR CCD camera (Grasshopper 3, Teledyne FLIR) was used to record mouse behavior during imaging. 2P imaging was performed on an Ultima 2P-plus microscope (Bruker) equipped with a resonant scanner using a tunable femtosecond-pulsed IR InSight X3 laser (Spectra-Physics) with output controlled by a Pockels cell (Conoptics). Imaging was performed at a sampling rate of 30 Hz using an excitation wavelength of 950 nm through a 16X/0.8 NA water immersion objective (Nikon) with an additional 2X optical zoom. GCaMP8m and dTomato signal were collected with a gallium arsenide phosphide photodetector (H742240, Hamamatsu) and a multi-alkali detector (R3896, Hamamatsu) respectively. 10-minute recordings were performed across 3-5 distinct fields of view in each mouse.

In vivo 2-photon calcium imaging analysis

Cell detection and extraction of neuronal activity were performed as previously described (***Goff et al., 2023***). Briefly, we used the Suite2p package to perform a nonrigid registration, detection of cell ROIs, and extraction of fluorescence values from ROIs (***Pachitariu et al., 2017***). Manual quality control was performed on all potential ROIs by a blinded experimenter. Fluorescence values extracted from each ROI and the mean fluorescence of the whole FOV were transformed into $\frac{dF}{F_0}$ values, which was calculated as $\frac{dF}{F_0} = \frac{F(t)-F_0}{F_0}$, where F_0 is the 10th percentile of the fluorescence trace, adjusted by using a linear interpolation between the average F_0 values for each 1000 frames of the recording. Paroxysmal synchronous discharges were detected in the traces of the mean dF/F_0 for the whole field of view by high-pass filtering the trace at 1 Hz, then identifying peaks with a zscore greater than 5.

In vivo Seizure Monitoring

Chronic video-EEG recordings were collected as previously described (***Feng et al., 2024***) using a wireless EEG system (Data Sciences International, St. Paul, MN) and standard surgical approaches. Mice were anesthetized with isoflurane and four small burr holes were made in the skull above the mouse motor and barrel cortices. A telemetry device containing four electrode leads was implanted subcutaneously on the back of the mouse. The insulation on the positive and negative leads was removed and the exposed wire was manually bent to create a relatively flat terminal to place on the surface of the dura. The leads were stably secured in the head cap via adhesive cement (C&B-Metabond, Parkell Inc, Brentwood, NY). Once the incision was sutured, the mice were given local treatment with antibiotic ointment (OTC Generics, Patterson Veterinary, Houston, TX) and were singly housed in home-cages for recovery. Continuous video and EEG recordings were collected for two to seven days using the Ponemah Software System (DSI, St. Paul, MN) and the EEG signal was acquired at 500 Hz. The aligned video and EEG signals are accessed using Neuroscore (DSI) software. First, the EEG signals are preprocessed by filtering with a powerline filter (60 Hz notch filter) followed by 1Hz high pass filtering. Then, an analysis protocol is designed in the Neuroscore software for spike and spike train detection to determine periods of abnormal EEG. Spikes were detected when the EEG signal had a minimum amplitude of 200 muV and was greater than the root-mean-squared value of the activity within the preceding minute. A spike train was detected when at least five spikes were detected, the inter-spike interval was between 0.05 and 0.6 seconds, and the duration of the train was at least three seconds. Detected spikes and spike trains were then analyzed manually alongside the recorded video for behavior to classify events into runs of epileptiform spikes, spontaneous seizures, myoclonic seizures, or seizure-induced sudden death events. Runs of epileptiform spikes were detected spike-trains that lacked clear behavioral manifestation. Spontaneous seizures were detected as spike trains of at least three seconds coincident with mouse loss of balance and/or convulsive movements. Myoclonic seizures were identified by large and brief (<200 ms) single epileptiform spikes that were coincident with periodic whole-body spasm-like movements. All seizure-induced sudden death events were characterized as spontaneous seizures which culminated in hindlimb extension and suppressed EEG signal. All EEG data along with identified events are exported into MATLAB to produce raster plots.

Patch-clamp Electrophysiology Recordings

Acute brain slices were prepared as previously described (***Wengert et al., 2021***, ***2019***, ***2022***; ***Kaneko et al., 2022***). Mice were deeply anesthetized with 5% isoflurane and decapitated so that the brain could be quickly removed and chilled in ice-cold artificial cerebrospinal fluid (ACSF) containing in mM: 125 NaCl, 2.5 KCl, 1.25 NaH_2PO4, 2 $CaCl_2$, 1 $MgCl_2$, 2 Na-pyruvate, 0.5 L-ascorbic acid, 10 D-glucose, and 25 $NaHCO_3$ (osmolarity = 305 mOsm). Horizontal slices (300 uM thick) were gently collected, bisected, and transferred to 37oC ACSF for 30 minutes. The slices were then kept at room temperature for up to 5 hours. Throughout all procedures the ACSF was constantly bubbled

with 95/5 O2/CO2 carbogen gas. For recording, acute brain slices were gently transferred to a recording chamber on the stage of an upright light microscope (Olympus) and were superfused at 3mL/min (SmoothFlow Model Q100-TT-ULP-ES TACMINA Corporation, Japan) with ACSF warmed to 32oC (TC-324C Warner Instruments).

Recordings of intrinsic excitability and synaptic neurotransmission Cortical PV-INs in layers II-IV of the somatosensory cortex were targeted for patch-clamp electrophysiology recordings due to their red fluorescence and non-pyramidal morphology while excitatory cells (spiny stellate and pyramidal neurons) were reliably targeted based on cellular morphology and orientation and the identity of cells was confirmed by examining electrophysiological properties (i.e. fast-spiking for PV-INs, and regular spiking for excitatory cells). Red-fluorescent neurons were also easily identifiable in the reticular nucleus of the thalamus. Whole-cell patch-clamp electrophysiology recordings were collected using borosilicate patch pipettes (Sutter Instruments OD=1.5 mm; ID=0.86 mm) pulled using either a P-97 or P-1000 Flaming-Brown micropipette puller (Sutter Instruments) to have resistance values of 2.5-4.5 MΩ when filled and placed in the bath solution. Voltage-gated K^+ channel function was assessed through recordings of outside-out nucleated macropatches as done previously (***Bekkers, 2000***; ***Korngreen and Sakmann, 2000***). After achieving the whole-cell recording configuration in PV-INs, light negative pressure was applied to bring the nucleus to the pipette. Slow retraction of the pipette enabled a large piece of the somatic membrane to be pulled off while reestablishing the gigaseal. Upon formation of the macropatch, the membrane capacitance was offset and the series resistance was confirmed to be below 12 MΩ. Voltage-gated K^+ channel currents were generated by 100-ms voltage steps from -80 to 40 mV in increments of 5 mV. The macropatch recordings used an internal solution containing in mM: 130 K-gluconate, 6.3 KCl, 1 MgCl2, 10 HEPES, 0.5 EGTA, 10 phosphocreatine-Tris, 4-ATP (magnesium salt), 0.3-GTP (disodium salt) and was adjusted with KOH to have a final pH of 7.3

The pipette internal solution for recordings of intrinsic excitability and synaptic neurotransmission contained in mM: 65 K-gluconate, 65 KCl, 2 MgCl2, 10 HEPES, 0.5 EGTA, 10 phosphocreatine-Tris, 4-ATP (magnesium salt), 0.3-GTP (disodium salt) and was adjusted with KOH to have a final pH of 7.3 and an osmolarity of 290 mOsm. This internal had a calculated reversal potential for chloride at ECl = -17 mV to enable larger signal to noise ratio for recordings of unitary inhibitory post-synaptic currents (uIPSCs). Membrane potential was sampled at 100 or 33 kHz with a Multiclamp 700B amplifier (Molecular Devices), filtered at 5 kHz or in some cases 2 kHz, and digitized using a Digidata 1550B digitizer (Molecular Devices), and acquired using the pClamp 11 software suite (Molecular Devices). Cells were discarded if the resting membrane potential was visibly unstable or more depolarized than -50 mV, or if the access resistance changed by >20% over the duration of the experiment. Throughout the study, we left our liquid-junction potentials uncorrected.

Intrinsic excitability of individual neurons was assessed similarly to previous reports (***Wengert et al., 2019***, ***2021***). For AP properties, analysis was completed on the first AP generated in response to a ramp of depolarizing current (100 pA/sec). Resting membrane potential and/or spontaneous excitability were determined as the median value of a Gapfree recording collected 2 minutes after achieving the whole-cell configuration. Upstroke and downstroke velocity were determined as the maximum and minimum of the first-derivative of the first AP. Threshold was determined as the membrane potential in which the first-derivative exceeded 5% of the upstroke velocity. Amplitude was calculated as the difference between the threshold and the peak of the AP. Input resistance was calculated using the change of voltage in response to the negative -20 pA current injection. Rheobase was approximated by taking the largest current injection step that did not evoke APs. The relationship between current injection and AP frequency was assessed by 1-second current injections of magnitudes ranging from -100 pA to 500 pA with a 1.5 second intersweep interval. APs were counted only if they reached a peak value of at least -10 mV. Neurons which had resting membrane potentials more depolarized than -65 mV (but less than -50 mV) were injected with DC current to bring to -65 mV to compare excitability across all cells. To examine synaptic neurotransmission between PV-INs and excitatory cells, pairs of nearby cells (<100 μ m inter-soma dis-

tance) were simultaneously recorded in the whole-cell configuration similar to our previous studies (***Kaneko et al., 2022***; ***Feng et al., 2024***). Monosynaptic connections were determined by stimulating APs in one neuron via depolarizing current injections and looking for unitary post-synaptic potentials in the other cell. Potential connections were confirmed by switching the postsynaptic cell to voltage-clamp (holding potential, -70 mV). Recordings of unitary synaptic currents were obtained by stimulating the presynaptic cell with trains of 1-ms square wave pulses of 2 nA at frequencies of 5,10, 20, 40, 80, and 120 Hz (for PV-INs only). Unitary synaptic currents were detected if their amplitude exceeded 10 pA and were otherwise considered a failure event. Connection probabilities were compared using Fisher's Exact test. Synaptic latency was calculated by taking the time difference between the peak of the AP and the onset of the evoked uIPSC.

All electrophysiology analysis was completed using custom-written MATLAB analysis scripts and/or confirmed manually using ClampFit (Molecular Devices). Statistical comparisons for physiological measures were completed using either unpaired t-tests or repeated-measures Two-way ANOVA followed by Sidak's multiple comparisons test. Significance was determined and denoted as *P<0.05, **P<0.01, and ***P<0.001.

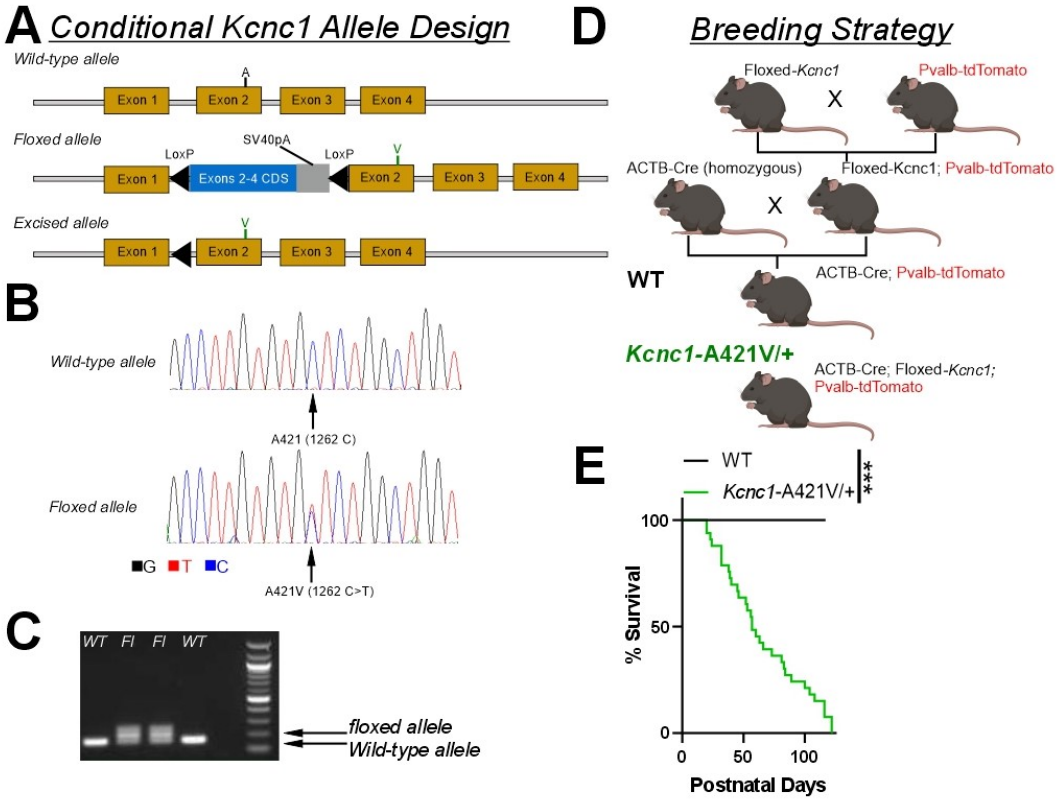

Figure 1. Design of a novel mouse model of *Kcnc1* developmental and epileptic encephalopathy. A. Design and structure of the conditional *Kcnc1*-A421V allele. Upon Cre-mediated recombination, the inserted WT coding sequence (CDS) flanked by LoxP sites is removed and the A421V variant inserted into exon 2 is expressed. **B.** Sequencing results indicate successful targeting of c.1262C>T to introduce the heterozygous A421V variant. **C.** PCR confirmation of two HET founders (*Kcnc1*-A421V/+) and two WT littermates. 167 bp, WT allele fragment; 207 bp, floxed allele fragment. **D.** Breeding strategy to generate control and experimental mice in which the *Kcnc1*-A421V variant is expressed globally and PV cells are fluorescently-labeled for targeted recording. **E.** Survival plot of WT (*N*=46; black) and *Kcnc1*-A421V/+ (*N*=33; green) mice. ***P<0.0001 by log-rank Mantel-Cox curve comparison.

Figure 1—figure supplement 1. Pvalb-TdTomato reporter effectively labels PV-INs in WT and *Kcnc1*-A421V/+ mice.

Figure 1—figure supplement 2. Early postnatal development of *Kcnc1*-A421V/+ mice.

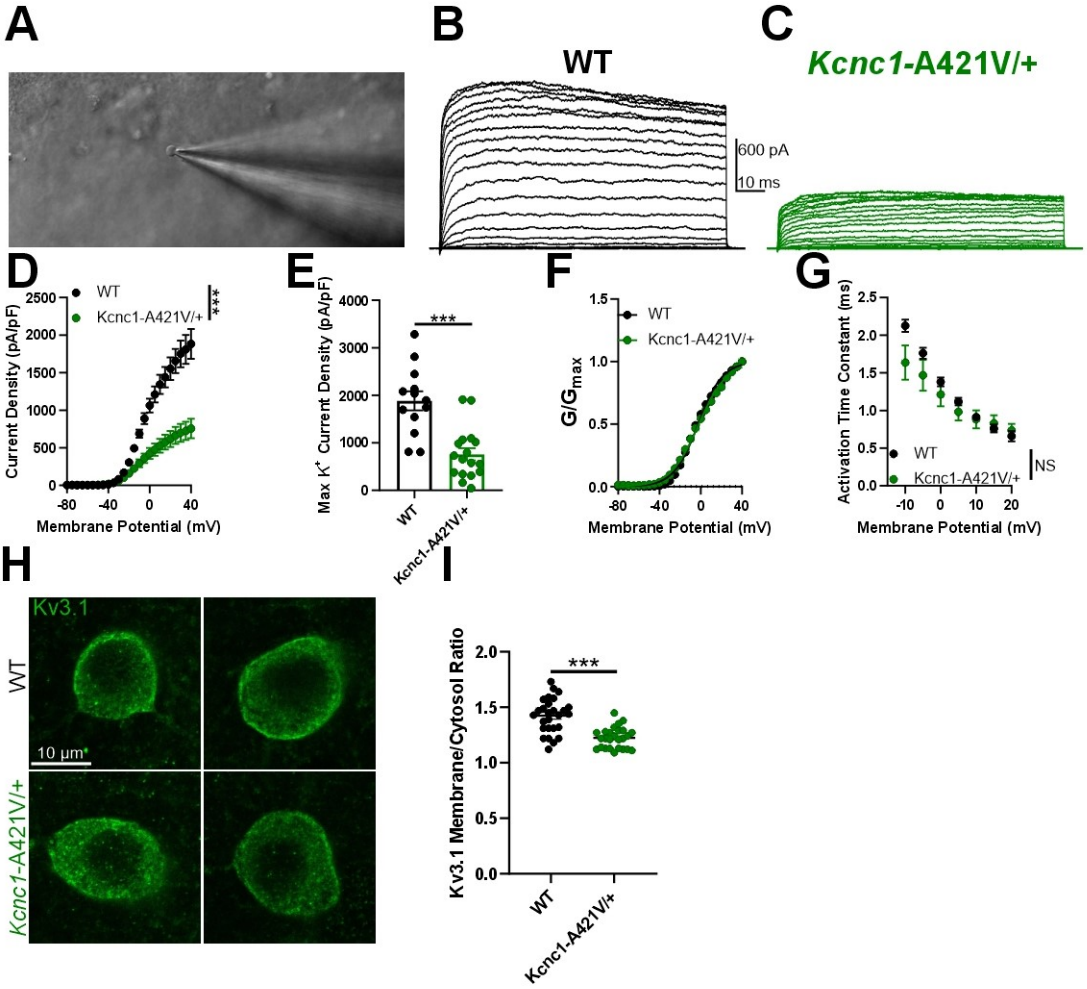

Figure 2. PV-INs from *Kcnc1*-A421V/+ mice exhibit attenuated voltage-gated potassium channel currents and impaired membrane Kv3.1 expression. A. Representative image of a cell being recorded in the outside-out nucleated macropatch configuration. **B-C.** Example family of traces of voltage-gated K^+ channel currents from a PV-IN from WT (B, black) and *Kcnc1*-A421V/+ (C, green) mice. **D.** Average voltage-gated K^+ channel current density for WT (*n*=13 macropatches, *N*=3 mice) and *Kcnc1*-A421V/+ (*n*=17, *N*=3 mice). **E.** Maximum K^+ channel current density per PV-IN macropatch in WT and *Kcnc1*-A421V/+ mice. **F.** Averaged normalized plots of K^+ conductance relative to voltage command indicating voltage-dependence of activation curves for WT and *Kcnc1*-A421V/+ mice. **G.** Average activation time constant for the voltage-gated K^+ channel currents relative to voltage command potential in both WT and *Kcnc1*-A421V/+ mice. **H.** Representative images of individual cortical PV-INs from WT and *Kcnc1*-A421V/+ mice stained for Kv3.1 (green). **I.** Group quantification of ratio of membrane to cytosolic Kv3.1 for WT (*n*=27 cells, *N*=3 mice) and *Kcnc1*-A421V/+ (*n*=27 cells, *N*=3 mice). Data are shown as mean ± SEM or individual data points and significance was determined using either Repeated-measures Two-way ANOVA or unpaired t-test where ***P<0.001.

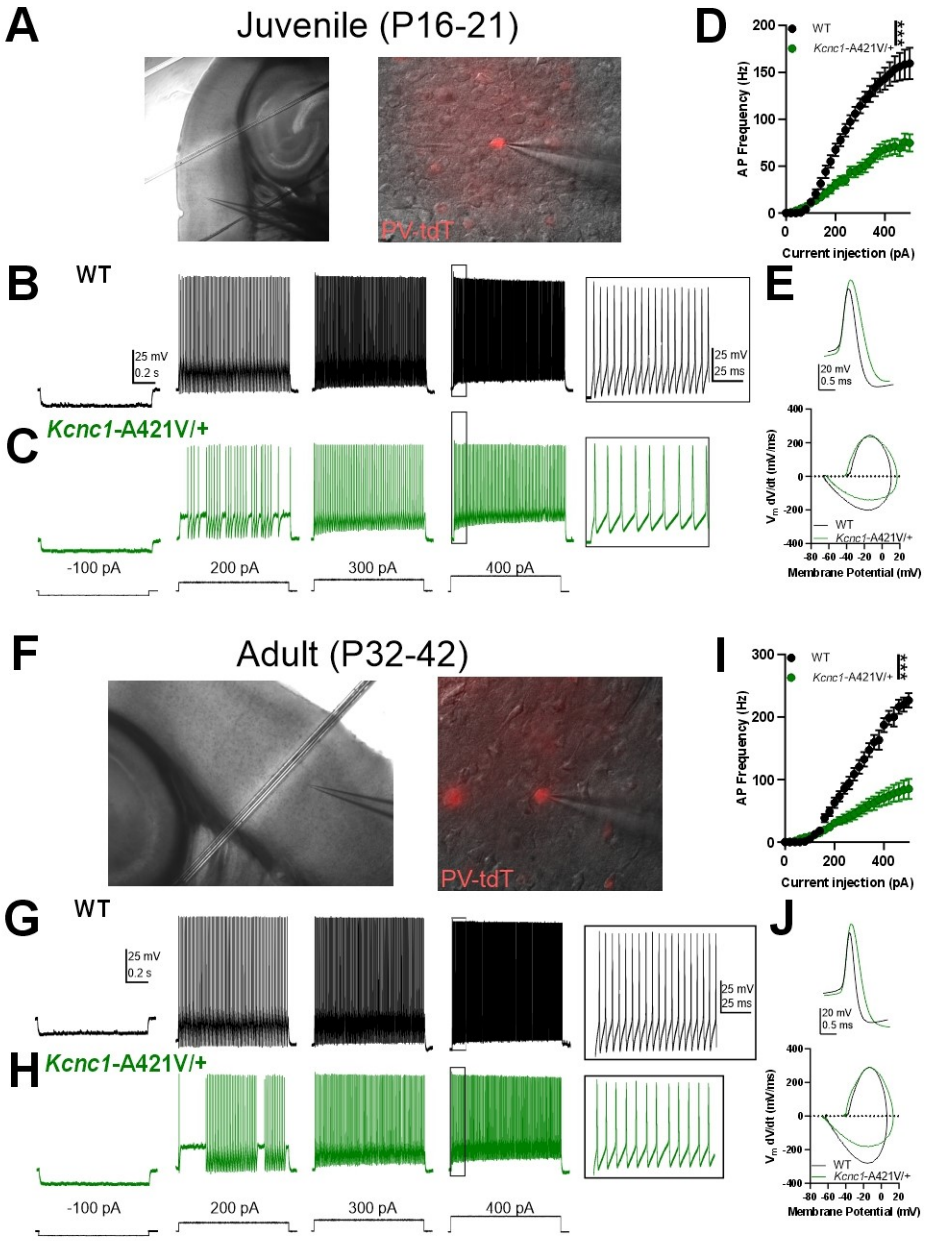

Figure 3. Impaired PV-IN intrinsic Excitability in Juvenile and Adult *Kcnc1*-A421V/+ mice. A. Representative images taken at 10X (left) and 40X (right) magnification of a layer IV neocortical PV-IN recorded in the whole-cell configuration to characterize intrinsic excitability. **B-C.** Representative example traces for juvenile (P16-21) WT (B, black) and *Kcnc1*-A421V/+ (C, green) PV-INs generating APs at current injections of -100, 200, 300, and 400 pA. The inset shows an expanded view of APs generated in response to the 400-pA current injection in both genotypes. **D.** Average relationship between PV-IN AP frequency in response to a range of current injections for juvenile WT (n=20 cells, N=9 mice) and *Kcnc1*-A421V/+ (n=36 cells, N=12 mice). **E.** Representative overlaid examples of single APs and the corresponding phase plots for juvenile WT (black) and *Kcnc1*-A421V/+ (green) PV-INs. **F.** Representative images for a layer IV neocortical PV-IN from an adult mouse. **G-H.** Representative example traces displaying intrinsic excitability in adult (P32-42) WT and *Kcnc1*-A421V/+ PV-INs. Inset show an expanded view of APs induced by the 400-pA current step. **I.** Average relationship between PV-IN AP frequency and current injection for adult WT (n=14 cells, N=3 mice) and *Kcnc1*-A421V/+ mice (n=17 cells, N=5 mice). **J.** Representative overlaid examples of single APs and the corresponding phase plots for adult WT (black) and *Kcnc1*-A421V/+ (green) PV-INs. Data are shown as mean ± SEM and significance was determined using either Repeated-measures Two-way ANOVA.

Figure 3—figure supplement 1. Abnormal Intrinsic Physiology in PV-INs of the Reticular Thalamus.

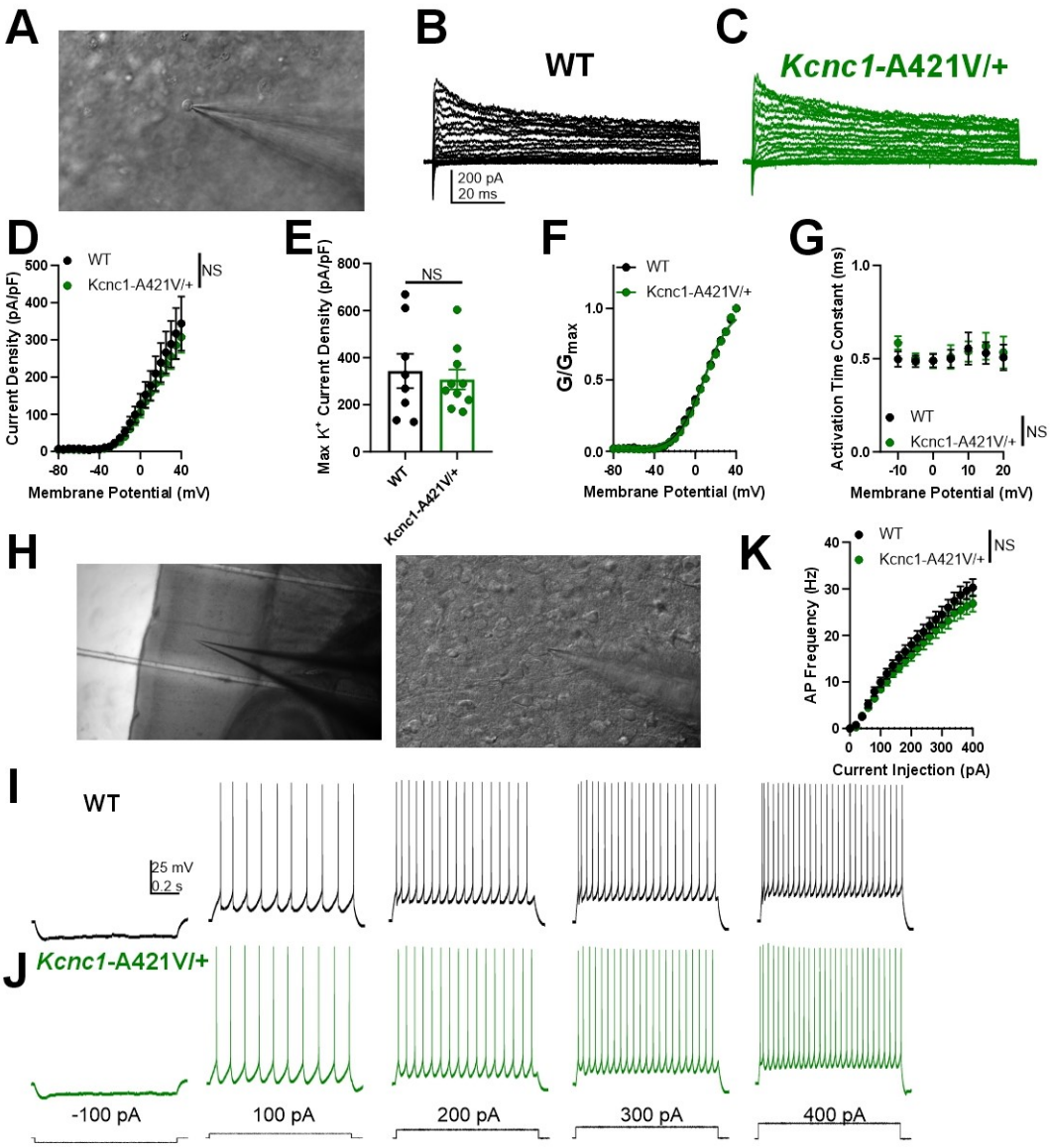

Figure 4. Unaltered physiological function of excitatory neurons in *Kcnc1*-A421V/+ mice. A. Representative image of a neocortical excitatory cell being recorded in the outside-out nucleated macropatch configuration. **B-C.** Example family of traces of voltage-gated K^+ channel currents from a excitatory cell from WT (B, black) and *Kcnc1*-A421V/+ (C, green) mice. **D.** Average voltage-gated K^+ channel current density for WT (*n*=8 macropatches, *N*=3 mice) and *Kcnc1*-A421V/+ (*n*=10, *N*=3 mice) relative to membrane potential. **E.** Peak voltage-gated K^+ channel current density in WT and *Kcnc1*-A421V/+ mice. **F.** Normalized voltage-dependent activation curves for WT and *Kcnc1*-A421V/+ mice. **G.** Average voltage-dependent activation time constant for the voltage-gated K^+ channel currents. **H.** Representative images taken at 10X (left) and 40X (right) magnification of a layer IV neocortical excitatory cell recorded in the whole-cell configuration to characterize intrinsic excitability. **I-J.** Representative example traces showing excitatory cell AP generation in WT (I, black) and *Kcnc1*-A421V/+ (J, green) in response to depolarizing current injections. **K.** Average relationship between excitatory cell AP frequency in response to a range of current injections for WT (*n*=23 cells, *N*=3 mice) and *Kcnc1*-A421V/+ (*n*=22, *N*=3 mice). Data are shown as mean ± SEM and all results failed to reach significance determined via Repeated-measures Two-way ANOVA or unpaired t-test.

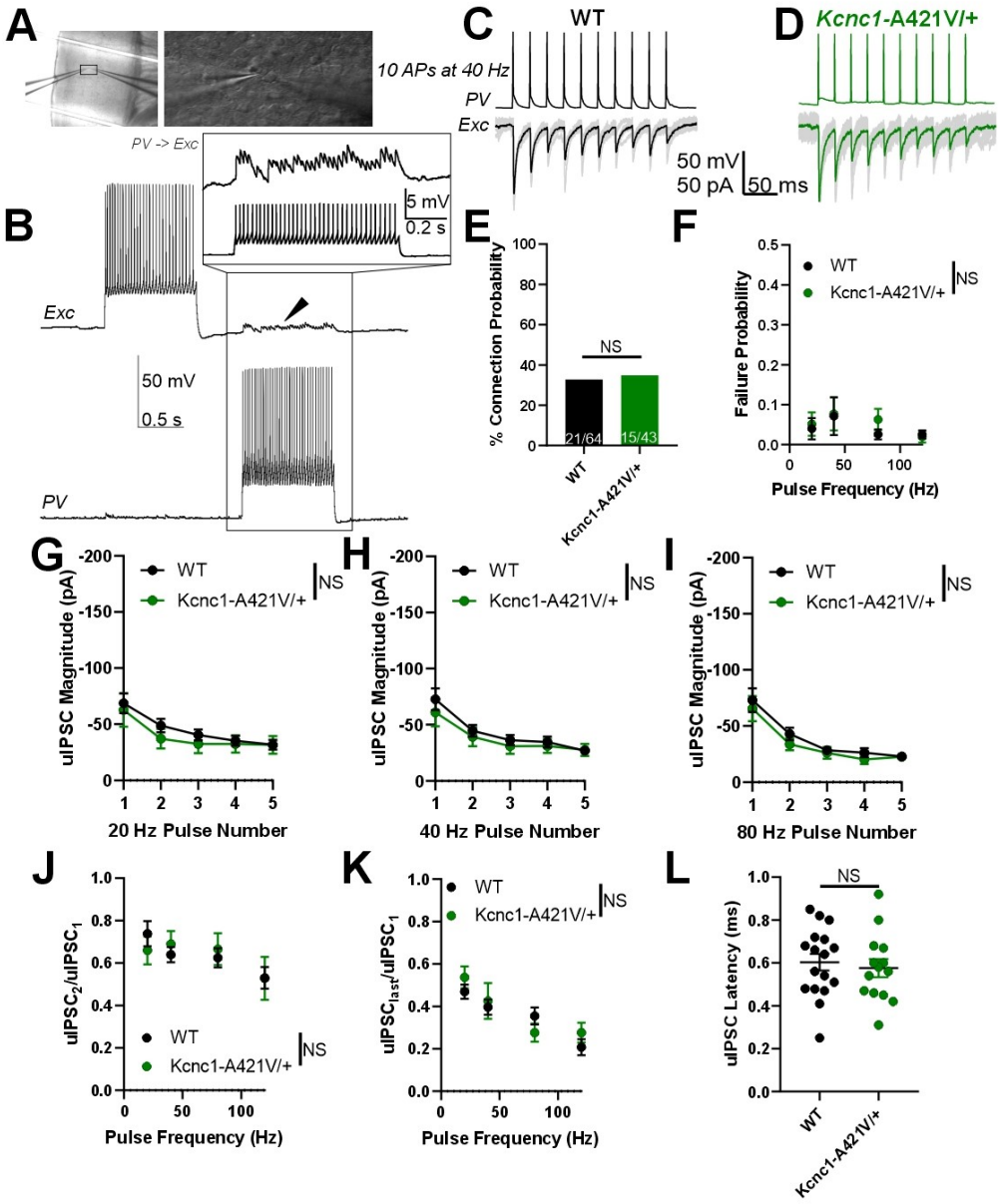

Figure 5. Juvenile *Kcnc1*-A421V/+ mice exhibit normal PV-IN mediated inhibitory synaptic neurotransmission. A. Representative images showing simultaneous whole-cell patch-clamp recordings of cortical PV-IN and nearby excitatory cell (left, 10x magnification; right 40x magnification). **B.** Example traces of a PV-IN and excitatory cell pair in which generation of APs in the PV-IN (bottom trace) is sufficient to induce clear uIPSPs in the excitatory cell (arrowhead, top trace). The inset shows an example view of the individual uIPSPs corresponding to each AP in the PV-IN. **C-D.** Example traces of uIPSCs in both WT (C) and *Kcnc1*-A421V/+ pairs of synaptically-connected neurons. 10 APs were generated in the PV-IN (top trace) at 40 Hz and the uIPSCs are shown below. The black and green traces are the averages of numerous individual sweeps shown in gray. **E.** Probability of synaptic connection between PV-IN and excitatory cell in WT (n=21 64 pairs from N=7 mice) and *Kcnc1*-A421V/+ (n= 5 of 43 pairs from N=11 mice). **F.** Average failure probability relative to presynaptic stimulation frequency in WT and *Kcnc1*-A421V/+ neuron pairs. **G-I.** Average uIPSC magnitude for the first five APs in WT and *Kcnc1*-A421V/+ at 20 Hz (G), 40 Hz (H), and 80 Hz(I) stimulus frequencies. **J-K.** Paired-pulse ratios for both WT and *Kcnc1*-A421V/+ mice relative to stimulus frequency. Ratio of second uIPSC to the first is provided in J, while K displays the average ratio of the last uIPSC to the first. **L.** Average latency from peak of presynaptic AP to peak of the uIPSC in WT and *Kcnc1*-A421V/+ mice. Data are shown as mean ± SEM and all results failed to reach significance determined via Repeated-measures Two-way ANOVA or unpaired t-test.

Figure 5—figure supplement 1. Excitatory neuron to PV-IN unitary excitatory synaptic neurotransmission is unaltered in juvenile *Kcnc1*-A421V/+ mice.

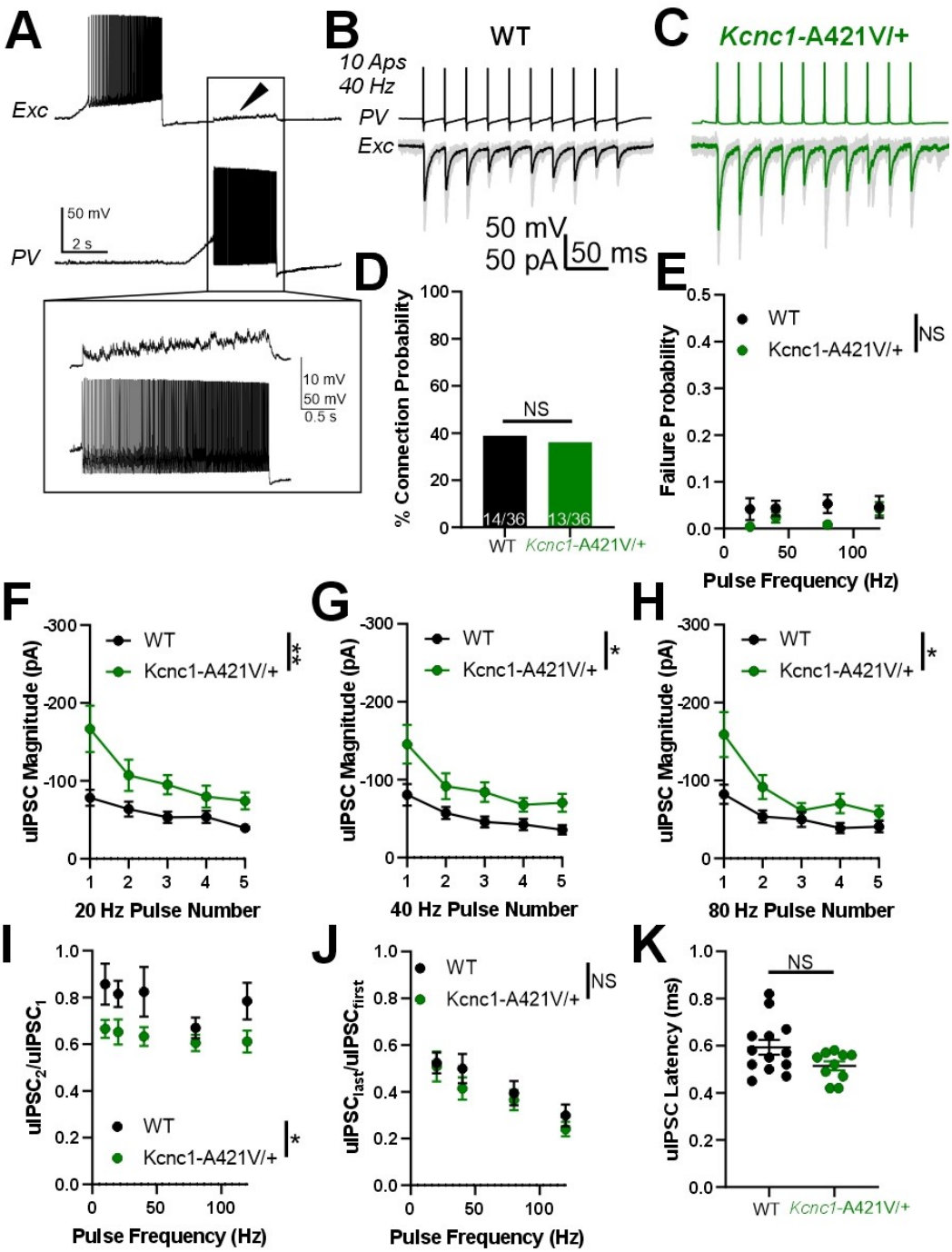

Figure 6. Adult *Kcnc1*-A421V/+ mice exhibit altered PV-IN mediated synaptic neurotransmission. A. Example presynaptic cortical PV-IN and post-synaptic excitatory neuron. Arrowhead and inset display the uIPSPs induced in the postsynaptic cell when the PV-IN generates APs. **B-C.** Example traces of uIPSCs in both adult WT (B) and *Kcnc1*-A421V/+ (C) pairs of synaptically-connected neurons. 10 APs were generated in the PV-IN at 40 Hz and the evoked uIPSCs are displayed in the trace below where the black and green traces are the averages of numerous individual sweeps shown in gray. **D.** Connection probability between WT (14 of 36, *N*=8 mice) and *Kcnc1*-A421V/+ (13 of 36, *N*=8 mice) pairs PV-INs and nearby excitatory cells. **E.** Average frequency-dependent rate of failure for the first five APs in adult WT and *Kcnc1*-A421V/+ neuron pairs. **F-H.** Average uIPSC magnitude of the first five APs for adult WT and *Kcnc1*-A421V/+ at 20 Hz (F), 40 Hz (G), and 80 Hz (H). **I-J.** Paired-pulse ratios for WT and *Kcnc1*-A421V/+ neuron pairs ($uIPSC_2/uIPSC_1$ provided in I, uIPSClast/uIPSCfirst provided in J) relative to stimulus frequency. **K.** Average latency from AP peak to onset of the uIPSC in WT and *Kcnc1*-A421V/+ mice. Data are shown as mean ± SEM or individual values and significance (*P<0.05, **P<0.01) was determined using unpaired t-test or Repeated-measures Two-way ANOVA.

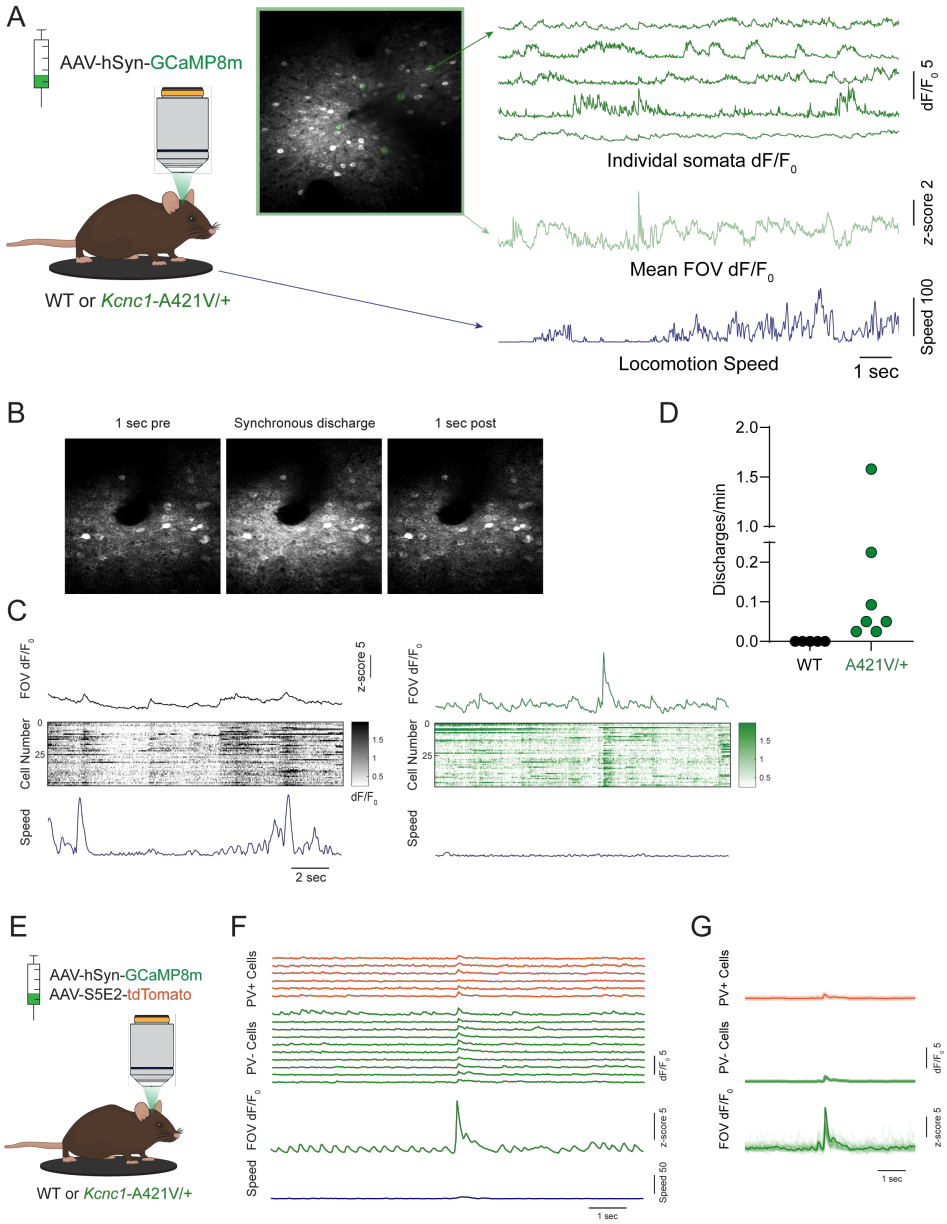

Figure 7. *In vivo* 2P calcium imaging reveals paroxysmal hypersynchronous discharges in *Kcnc1*-A421V/+ mice. A. Experimental setup for *in vivo* 2P calcium imaging with representative calcium transients from cells expressing AAV-hSyn-GCaMP8m and mean dF/F_0 of the whole field of view (FOV) aligned to locomotion speed. **B.** Representative 2P field of view during a hypersynchronous discharge in a *Kcnc1*-A421V/+ mouse. **D.** Mean dF/F of the field of view (top), calcium transients of individual somata (middle), and locomotion speed (bottom) during a paroxysmal discharge in the *Kcnc1*-A421V/+ mouse relative to typical baseline activity shown in a WT mouse. Note that, in contrast to epochs of low-amplitude synchronization in WT associated with transition from quiet wakefulness to locomotion, there is no locomotion during the larger-amplitude hypersynchronous discharges identified in *Kcnc1*-A421V/+ mice. **D.** Frequency of paroxysmal discharges in each mouse (N = 5 WT, 7 *Kcnc1*-A421V/+ mice). F. E. Experimental design for *in vivo* 2P calcium imaging of cells positive (PV+) and negative (PV-) for parvalbumin. **F.** Calcium transients of PV+ and PV- cells during a representative hypersynchronous discharge. Mean FOV dF/F_0 and locomotion speed shown below. **G.** Mean calcium transients for PV+ and PV- cells during hypersynchronous discharges (N = 3 mice, n = 43 PV+ cells, = 277 PV- cells; shading indicates bootstrapped 95% confidence intervals). Mean FOV dF/F_0 shown below, individual recordings in light green with mean trace overlaid in dark green.

Figure 7—video 1. Example *in vivo* 2P calcium imaging of synchronous discharge in a *Kcnc1*-A421V/+ mouse.

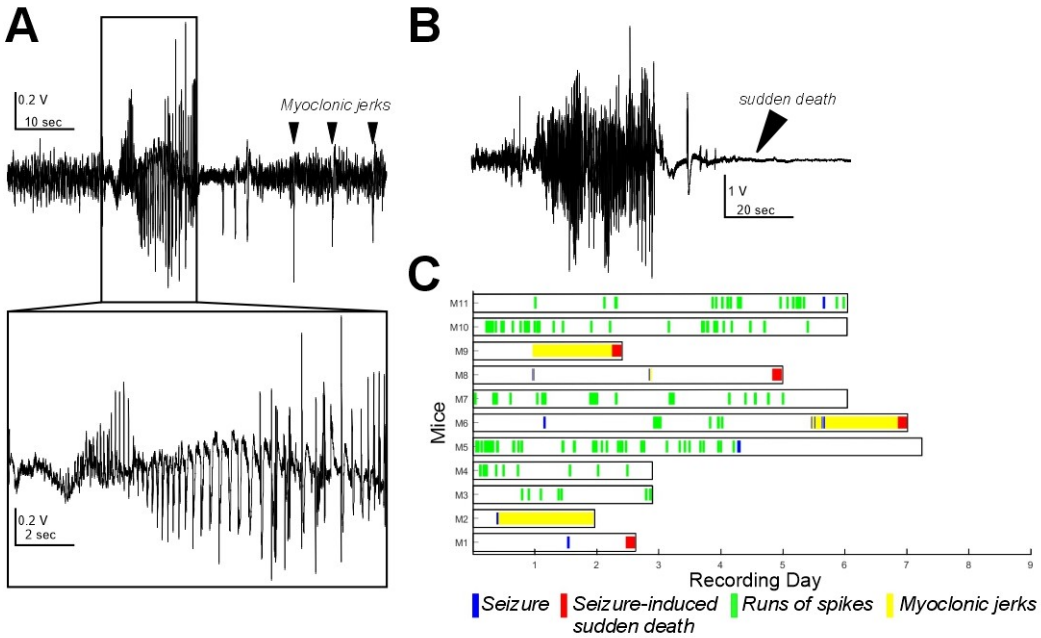

Figure 8. *Kcnc1*-A421V/+ mice exhibit spontaneous seizures and seizure-induced death. A. Representative example trace of the EEG collected from an adult *Kcnc1*-A421V/+ mouse during a nonfatal seizure. After the seizure-related spike-wave discharges, there are large spikes that are associated with diffuse whole-body jerks. **B.** Representative generalized tonic-clonic seizure resulting in seizure-induced sudden death in a *Kcnc1*-A421V/+ mouse. **C.** Raster plot indicating nonfatal seizures (blue bars), seizure-induced sudden death (red bars), interictal runs of spikes (green bars) without clear behavior manifestation, and periods of repetitive myoclonic seizures (yellow shading) for each mouse examined (N=11).

Figure 8—video 1. Example seizure with myoclonic jerks.

Figure 8—video 2. Example seizure-induced sudden death.

Cell Type	Group	V_m (mV)	AP Threshold (mV)	Upstroke Velocity (mV/ms)	Downstroke Velocity (mV/ms)	AP Amplitude (mV)	APD50 (ms)	Input Resistance (MΩ)	Rheobase (pA)
Layer II-IV PV-INs	WT (N=20,9)	-72.3 ± 1.3	-39.8 ± 0.8	253 ± 8	-183 ± 9	52.1 ± 1.8	0.40 ± 0.02	142 ± 13	93 ± 9
	Kcnc1-A421V/+ (N=36, 12)	-67.4 ± 1.3	-41.6 ± 0.5	252 ± 9	-138 ± 8	57.1 ± 1.6	0.54 ± 0.03	170 ± 21	145 ± 19
	Statistical Comparison	***P=0.017**	P=0.065	P=0.96	****P=0.0012**	P=0.055	****P=0.0053**	P=0.29	P=0.19
Layer IV Exc. Cells	WT (N=23, 3)	-66.7 ± 0.7	-43.4 ± 0.7	303 ± 13	-68.6 ± 3.9	79.8 ± 1.3	1.06 ± 0.05	155 ± 15	34 ± 6
	Kcnc1-A421V/+ (N=22,3)	-67.6 ± 1.0	-41.8 ± 0.7	286 ± 15	-64.5 ± 3.7	77.8 ± 1.6	1.10 ± 0.04	159 ± 14	37 ± 6
	Statistical Comparison	P=0.49	P=0.11	P=0.38	P=0.45	P=0.35	P=0.46	P=0.86	0.69
RTN	WT (N=18, 4)	-55.1 ± 1.4	-39.2 ± 0.8	227 ± 15	-189 ± 11	49.2 ± 1.8	0.38 ± 0.02	247 ± 39	24 ± 7
	Kcnc1-A421V/+ (N=19, 5)	-57.5 ± 2.3	-38.1 ± 1.0	195 ± 12	-160 ± 7	45.6 ± 1.7	0.42 ± 0.02	236 ± 30	48 ± 10
	Statistical Comparison	P=0.39	P=0.41	P=0.10	***P=0.034**	P=0.15	P=0.092	P=0.82	P=0.0805

Table 1. Membrane and Action Potential Properties of WT and *Kcnc1*-A421V/+ Neurons at P16-21.

Cell Type	Group	V_m (mV)	AP Threshold (mV)	Upstroke Velocity (mV/ms)	Downstroke Velocity (mV/ms)	AP Amplitude (mV)	APD50 (ms)	Input Resistance (MΩ)	Rheobase (pA)
Layer II-IV PV-INs	WT (N=14,3)	-66.1 ± 1.4	-41.5 ± 0.9	294 ± 25	-248 ± 21	51.1 ± 2.0	0.31 ± 0.02	141 ± 11	91 ± 10
	Kcnc1-A421V/+ (N=17, 5)	-68.3 ± 2.3	-43.1 ± 0.6	324 ± 16	-164 ± 18	67.8 ± 2.3	0.58 ± 0.07	173 ± 25	106 ± 23
	Statistical Comparison	P=0.45	P=0.16	P=0.29	**P=0.0051	***P<0.001	**P=0.0026	P=0.28	P=0.87

Table 2. Membrane and AP Properties of Adult (P32-42) WT and *Kcnc1*-A421V/+ PV-INs.

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References

Akemann W, Knöpfel T. Interaction of Kv3 Potassium Channels and Resurgent Sodium Current Influences the Rate of Spontaneous Firing of Purkinje Neurons. Journal of Neuroscience. 2006 Apr; 26(17):4602–4612. https://www.jneurosci.org/content/26/17/4602, doi: 10.1523/JNEUROSCI.5204-05.2006, publisher: Society for Neuroscience Section: Articles.

Armstrong EC, Caruso A, Servadio M, Andreae LC, Trezza V, Scattoni ML, Fernandes C. Assessing the developmental trajectory of mouse models of neurodevelopmental disorders: Social and communication deficits in mice with Neurexin 1 deletion. Genes, Brain and Behavior. 2020; 19(4). doi: 10.1111/gbb.12630.

Bekkers JM. Properties of voltage-gated potassium currents in nucleated patches from large layer 5 cortical pyramidal neurons of the rat. The Journal of Physiology. 2000; 525(3):593–609. https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1469-7793.2000.t01-1-00593.x, doi: 10.1111/j.1469-7793.2000.t01-1-00593.x, _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1469-7793.2000.t01-1-00593.x.

Boddum K, Hougaard C, Xiao-Ying Lin J, von Schoubye NL, Jensen HS, Grunnet M, Jespersen T. Kv3.1/Kv3.2 channel positive modulators enable faster activating kinetics and increase firing frequency in fast-spiking GABAergic interneurons. Neuropharmacology. 2017; 118. doi: 10.1016/j.neuropharm.2017.02.024.

Brooke RE, Moores TS, Morris NP, Parson SH, Deuchars J. Kv3 voltage-gated potassium channels regulate neurotransmitter release from mouse motor nerve terminals. European Journal of Neuroscience. 2004; 20(12):3313–3321. https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1460-9568.2004.03730.x, doi: 10.1111/j.1460-9568.2004.03730.x, _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1460-9568.2004.03730.x.

Brown MR, El-Hassar L, Zhang Y, Alvaro G, Large CH, Kaczmarek LK. Physiological modulators of Kv3.1 channels adjust firing patterns of auditory brain stem neurons. Journal of Neurophysiology. 2016; 116(1). doi: 10.1152/jn.00174.2016.

Cameron JM, Maljevic S, Nair U, Aung YH, Cogné B, Bézieau S, Blair E, Isidor B, Zweier C, Reis A, Koenig MK, Maarup T, Sarco D, Afenjar A, Huq AHMM, Kukolich M, Billette de Villemeur T, Nava C, Héron B, Petrou S, et al. Encephalopathies with KCNC1 variants: genotype-phenotype-functional correlations. Annals of Clinical and Translational Neurology. 2019; 6(7):1263–1272. doi: 10.1002/acn3.50822.

Chambers AR, Pilati N, Balaram P, Large CH, Kaczmarek LK, Polley DB. Pharmacological modulation of Kv3.1 mitigates auditory midbrain temporal processing deficits following auditory nerve damage. Scientific Reports. 2017 Dec; 7(1):17496. https://www.nature.com/articles/s41598-017-17406-x, doi: 10.1038/s41598-017-17406-x, publisher: Nature Publishing Group.

Chow A, Erisir A, Farb C, Nadal MS, Ozaita A, Lau D, Welker E, Rudy B. K+ channel expression distinguishes subpopulations of parvalbumin- and somatostatin-containing neocortical interneurons. Journal of Neuroscience. 1999; 19(21). doi: 10.1523/jneurosci.19-21-09332.1999.

Clatot J, Currin CB, Liang Q, Pipatpolkai T, Massey SL, Helbig I, Delemotte L, Vogels TP, Covarrubias M, Goldberg EM. A structurally precise mechanism links an epilepsy-associated KCNC2 potassium channel mutation to interneuron dysfunction. Proceedings of the National Academy of Sciences. 2024 Jan; 121(3):e2307776121. https://www.pnas.org/doi/abs/10.1073/pnas.2307776121, doi: 10.1073/pnas.2307776121, publisher: Proceedings of the National Academy of Sciences.

Clatot J, Ginn N, Costain G, Goldberg EM. A KCNC1-related neurological disorder due to gain of Kv3.1 function. Annals of Clinical and Translational Neurology. 2023 Jan; 10(1):111–117. doi: 10.1002/acn3.51707.

Erisir A, Lau D, Rudy B, Leonard CS. Function of Specific K+ Channels in Sustained High-Frequency Firing of Fast-Spiking Neocortical Interneurons. Journal of Neurophysiology. 1999 Nov; 82(5):2476–2489. https://journals.physiology.org/doi/full/10.1152/jn.1999.82.5.2476, doi: 10.1152/jn.1999.82.5.2476, publisher: American Physiological Society.

Feng H, Clatot J, Kaneko K, Flores-Mendez M, Wengert ER, Koutcher C, Hoddeson E, Lopez E, Lee D, Arias L, Liang Q, Zhang X, Somarowthu A, Covarrubias M, Gunthorpe MJ, Large CH, Akizu N, Goldberg EM. Targeted therapy improves cellular dysfunction, ataxia, and seizure susceptibility in a model of a progressive myoclonus epilepsy. Cell Reports Medicine. 2024; p. 101389. https://www.sciencedirect.com/science/article/pii/S2666379123006183, doi: https://doi.org/10.1016/j.xcrm.2023.101389.

Gan L, Kaczmarek LK. When, where, and how much? Expression of the Kv3.1 potassium channel in high-frequency firing neurons. Journal of Neurobiology. 1998; 37(1):69–79. https://onlinelibrary.wiley.com/doi/abs/10.1002/%28SICI%291097-4695%28199810%2937%3A1%3C69%3A%3AAID-NEU6%3E3.0.CO%3B2-6, doi: 10.1002/(SICI)1097-4695(199810)37:1<69::AID-NEU6>3.0.CO;2-6, _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/%28SICI%291097-4695%28199810%2937%3A1%3C69%3A%3AAID-NEU6%3E3.0.CO%3B2-6.

Goff KM, Goldberg EM. Vasoactive intestinal peptide-expressing interneurons are impaired in a mouse model of Dravet syndrome. eLife. 2019 Jul; 8:e46846. https://doi.org/10.7554/eLife.46846, doi: 10.7554/eLife.46846, publisher: eLife Sciences Publications, Ltd.

Goff KM, Liebergall SR, Jiang E, Somarowthu A, Goldberg EM. VIP interneuron impairment promotes in vivo circuit dysfunction and autism-related behaviors in Dravet syndrome. Cell Reports. 2023 Jun; 42(6). https://www.cell.com/cell-reports/abstract/S2211-1247(23)00639-3, doi: 10.1016/j.celrep.2023.112628, publisher: Elsevier.

Goldberg EM, Watanabe S, Chang SY, Joho RH, Huang ZJ, Leonard CS, Rudy B. Specific functions of synaptically localized potassium channels in synaptic transmission at the neocortical GABAergic fast-spiking cell synapse. Journal of Neuroscience. 2005; 25(21). doi: 10.1523/JNEUROSCI.0722-05.2005.

Ho CS, Grange RW, Joho RH. Pleiotropic effects of a disrupted K+ channel gene: Reduced body weight, impaired motor skill and muscle contraction, but no seizures. Proceedings of the National Academy of Sciences of the United States of America. 1997; 94(4). doi: 10.1073/pnas.94.4.1533.

Ishikawa T, Nakamura Y, Saitoh N, Li WB, Iwasaki S, Takahashi T. Distinct Roles of Kv1 and Kv3 Potassium Channels at the Calyx of Held Presynaptic Terminal. The Journal of Neuroscience. 2003 Nov; 23(32):10445–10453. https://www.jneurosci.org/lookup/doi/10.1523/JNEUROSCI.23-32-10445.2003, doi: 10.1523/JNEUROSCI.23-32-10445.2003.

Kaczmarek LK, Zhang Y. Kv3 channels: Enablers of rapid firing, neurotransmitter release, and neuronal endurance. Physiological Reviews. 2017; 97(4). doi: 10.1152/physrev.00002.2017.

Kaiser T, Ting JT, Monteiro P, Feng G. Transgenic labeling of parvalbumin-expressing neurons with tdTomato. Neuroscience. 2016 May; 321:236–245. https://www.sciencedirect.com/science/article/pii/S030645221500768X, doi: 10.1016/j.neuroscience.2015.08.036.

Kaneko K, Currin CB, Goff KM, Wengert ER, Somarowthu A, Vogels TP, Goldberg EM. Developmentally regulated impairment of parvalbumin interneuron synaptic transmission in an experimental model of Dravet syndrome. Cell Reports. 2022; 38(13). doi: 10.1016/j.celrep.2022.110580.

Korngreen A, Sakmann B. Voltage-gated K+ channels in layer 5 neocortical pyramidal neurones from young rats: subtypes and gradients. The Journal of Physiology. 2000; 525(3):621–639. https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1469-7793.2000.00621.x, doi: 10.1111/j.1469-7793.2000.00621.x, _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1469-7793.2000.00621.x.

Lau D, Miera EVSd, Contreras D, Ozaita A, Harvey M, Chow A, Noebels JL, Paylor R, Morgan JI, Leonard CS, Rudy B. Impaired Fast-Spiking, Suppressed Cortical Inhibition, and Increased Susceptibility to Seizures in Mice Lacking Kv3.2 K+ Channel Proteins. Journal of Neuroscience. 2000 Dec; 20(24):9071–9085. https://www.jneurosci.org/content/20/24/9071, doi: 10.1523/JNEUROSCI.20-24-09071.2000, publisher: Society for Neuroscience Section: ARTICLE.

Li X, Zheng Y, Li S, Nair U, Sun C, Zhao C, Lu J, Zhang VW, Maljevic S, Petrou S, Lin J. Kv3.1 channelopathy: a novel loss-of-function variant and the mechanistic basis of its clinical phenotypes. Annals of Translational Medicine. 2021 Sep; 9(18):1397. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8506712/, doi: 10.21037/atm-21-1885.

Martina M, Metz AE, Bean BP. Voltage-Dependent Potassium Currents During Fast Spikes of Rat Cerebellar Purkinje Neurons: Inhibition by BDS-I Toxin. Journal of Neurophysiology. 2007 Jan; 97(1):563–571. https://journals.physiology.org/doi/full/10.1152/jn.00269.2006, doi: 10.1152/jn.00269.2006, publisher: American Physiological Society.

Martina M, Schultz JH, Ehmke H, Monyer H, Jonas P. Functional and Molecular Differences between Voltage-Gated K+ Channels of Fast-Spiking Interneurons and Pyramidal Neurons of Rat Hippocampus. Journal of Neuroscience. 1998 Oct; 18(20):8111–8125. https://www.jneurosci.org/content/18/20/8111, doi: 10.1523/JNEUROSCI.18-20-08111.1998, publisher: Society for Neuroscience Section: ARTICLE.

Massengill JL, Smith MA, Son DI, O'Dowd DK. Differential Expression of K4-AP Currents and Kv3.1 Potassium Channel Transcripts in Cortical Neurons that Develop Distinct Firing Phenotypes. Journal of Neuroscience. 1997 May; 17(9):3136–3147. https://www.jneurosci.org/content/17/9/3136, doi: 10.1523/JNEUROSCI.17-09-03136.1997, publisher: Society for Neuroscience Section: Articles.

Munch AS, Saljic A, Boddum K, Grunnet M, Jespersen T, Munch AS, Hougaard C, Grunnet M. Pharmacological rescue of mutated Kv3.1 ion-channel linked to progressive myoclonus epilepsies. European Journal of Pharmacology. 2018; 833. doi: 10.1016/j.ejphar.2018.06.015.

Oliver KL, Franceschetti S, Milligan CJ, Muona M, Mandelstam SA, Canafoglia L, Boguszewska-Chachulska AM, Korczyn AD, Bisulli F, Di Bonaventura C, Ragona F, Michelucci R, Ben-Zeev B, Straussberg R, Panzica F, Massano J, Friedman D, Crespel A, Engelsen BA, Andermann F, et al. Myoclonus epilepsy and ataxia due to KCNC1 mutation: Analysis of 20 cases and K+ channel properties. Annals of Neurology. 2017; 81(5). doi: 10.1002/ana.24929.

Pachitariu M, Stringer C, Dipoppa M, Schröder S, Rossi LF, Dalgleish H, Carandini M, Harris KD, Suite2p: beyond 10,000 neurons with standard two-photon microscopy. bioRxiv; 2017. https://www.biorxiv.org/content/10.1101/061507v2, doi: 10.1101/061507, pages: 061507 Section: New Results.

Park J, Koko M, Hedrich UBS, Hermann A, Cremer K, Haberlandt E, Grimmel M, Alhaddad B, Beck-Woedl S, Harrer M, Karall D, Kingelhoefer L, Tzschach A, Matthies LC, Strom TM, Ringelstein EB, Sturm M, Engels H, Wolff M, Lerche H, et al. KCNC1-related disorders: new de novo variants expand the phenotypic spectrum. Annals of Clinical and Translational Neurology. 2019; 6(7):1319–1326. doi: 10.1002/acn3.50799.

Porcello DM, Ho CS, Joho RH, Huguenard JR. Resilient RTN fast spiking in Kv3.1 Null mice suggests redundancy in the action potential repolarization mechanism. Journal of Neurophysiology. 2002; 87(3). doi: 10.1152/jn.00556.2001.

Rosato-Siri MD, Zambello E, Mutinelli C, Garbati N, Benedetti R, Aldegheri L, Graziani F, Virginio C, Alvaro G, Large CH. A Novel Modulator of Kv3 Potassium Channels Regulates the Firing of Parvalbumin-Positive Cortical Interneurons. Journal of Pharmacology and Experimental Therapeutics. 2015 Sep; 354(3):251–260. https://jpet.aspetjournals.org/content/354/3/251, doi: 10.1124/jpet.115.225748, publisher: American Society for Pharmacology and Experimental Therapeutics Section: Drug Discovery and Translational Medicine.

Rowan MJM, Christie JM. Rapid State-Dependent Alteration in Kv3 Channel Availability Drives Flexible Synaptic Signaling Dependent on Somatic Subthreshold Depolarization. Cell Reports. 2017 Feb; 18(8):2018–2029. https://www.cell.com/cell-reports/abstract/S2211-1247(17)30138-9, doi: 10.1016/j.celrep.2017.01.068, publisher: Elsevier.

Rowan MJM, DelCanto G, Yu JJ, Kamasawa N, Christie JM. Synapse-Level Determination of Action Potential Duration by K+ Channel Clustering in Axons. Neuron. 2016 Jul; 91(2):370–383. https://www.cell.com/neuron/abstract/S0896-6273(16)30246-X, doi: 10.1016/j.neuron.2016.05.035, publisher: Elsevier.

Rowan MJM, Tranquil E, Christie JM. Distinct Kv Channel Subtypes Contribute to Differences in Spike Signaling Properties in the Axon Initial Segment and Presynaptic Boutons of Cerebellar Interneurons. Journal of Neuroscience. 2014 May; 34(19):6611–6623. https://www.jneurosci.org/content/34/19/6611, doi: 10.1523/JNEUROSCI.4208-13.2014, publisher: Society for Neuroscience Section: Articles.

Rubinstein M, Han S, Tai C, Westenbroek RE, Hunker A, Scheuer T, Catterall WA. Dissecting the phenotypes of Dravet syndrome by gene deletion. Brain. 2015 Aug; 138(8):2219–2233. https://doi.org/10.1093/brain/awv142, doi: 10.1093/brain/awv142.

Rudy B, Chow A, Lau D, Amarillo Y, Ozaita A, Saganich M, Moreno H, Nadal MS, Hernandez-Pineda R, Hernandez-Cruz A, Erisir A, Leonard C, DE Miera EVS. Contributions of Kv3 Channels to Neuronal Excitability. Annals of the New York Academy of Sciences. 1999; 868(1):304–343. https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1749-6632.1999.tb11295.x, doi: 10.1111/j.1749-6632.1999.tb11295.x, _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1749-6632.1999.tb11295.x.

Rudy B, McBain CJ. Kv3 channels: Voltage-gated K+ channels designed for high-frequency repetitive firing. Trends in Neurosciences. 2001; 24(9). doi: 10.1016/S0166-2236(00)01892-0.

Sacco T, De Luca A, Tempia F. Properties and expression of Kv3 channels in cerebellar Purkinje cells. Molecular and Cellular Neuroscience. 2006 Oct; 33(2):170–179. https://www.sciencedirect.com/science/article/pii/S104474310600159X, doi: 10.1016/j.mcn.2006.07.006.

Sekirnjak C, Martone ME, Weiser M, Deerinck T, Bueno E, Rudy B, Ellisman M. Subcellular localization of the K+ channel subunit Kv3.1b in selected rat CNS neurons. Brain Research. 1997; 766(1-2). doi: 10.1016/S0006-8993(97)00527-1.

Tai C, Abe Y, Westenbroek RE, Scheuer T, Catterall WA. Impaired excitability of somatostatin- and parvalbumin-expressing cortical interneurons in a mouse model of Dravet syndrome. Proceedings of the National Academy of Sciences of the United States of America. 2014; 111(30). doi: 10.1073/pnas.1411131111, iSBN: 1091-6490 (Electronic)\r0027-8424 (Linking).

Wang LY, Gan L, Forsythe ID, Kaczmarek LK. Contribution of the Kv3.1 potassium channel to high-frequency firing in mouse auditory neurones. The Journal of Physiology. 1998; 509(1):183–194. https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1469-7793.1998.183bo.x, doi: 10.1111/j.1469-7793.1998.183bo.x, _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1469-7793.1998.183bo.x.

Weiser M, Bueno E, Sekirnjak C, Martone ME, Baker H, Hillman D, Chen S, Thornhill W, Ellisman M, Rudy B. The potassium channel subunit KV3.1b is localized to somatic and axonal membranes of specific populations of CNS neurons. Journal of Neuroscience. 1995 Jun; 15(6):4298–4314. https://www.jneurosci.org/content/15/6/4298, doi: 10.1523/JNEUROSCI.15-06-04298.1995, publisher: Society for Neuroscience Section: Articles.

Wengert ER, Miralles RM, Wedgwood KCA, Wagley PK, Strohm SM, Panchal PS, Idrissi AM, Wenker IC, Thompson JA, Gaykema RP, Patel MK. Somatostatin-Positive Interneurons Contribute to Seizures in SCN8A Epileptic Encephalopathy. The Journal of Neuroscience. 2021; 41(44). doi: 10.1523/jneurosci.0718-21.2021.

Wengert ER, Saga AU, Panchal PS, Barker BS, Patel MK. Prax330 reduces persistent and resurgent sodium channel currents and neuronal hyperexcitability of subiculum neurons in a mouse model of SCN8A epileptic encephalopathy. Neuropharmacology. 2019 Nov; 158:107699. https://www.sciencedirect.com/science/article/pii/S0028390819302576?via%3Dihub, doi: 10.1016/j.neuropharm.2019.107699, publisher: Pergamon.

Wengert ER, Wagley PK, Strohm SM, Reza N, Wenker IC, Gaykema RP, Christiansen A, Liau G, Patel MK. Targeted Augmentation of Nuclear Gene Output (TANGO) of Scn1a rescues parvalbumin interneuron excitability and reduces seizures in a mouse model of Dravet Syndrome. Brain Research. 2022; 1775:147743. https://www.sciencedirect.com/science/article/pii/S0006899321006028, doi: https://doi.org/10.1016/j.brainres.2021.147743.

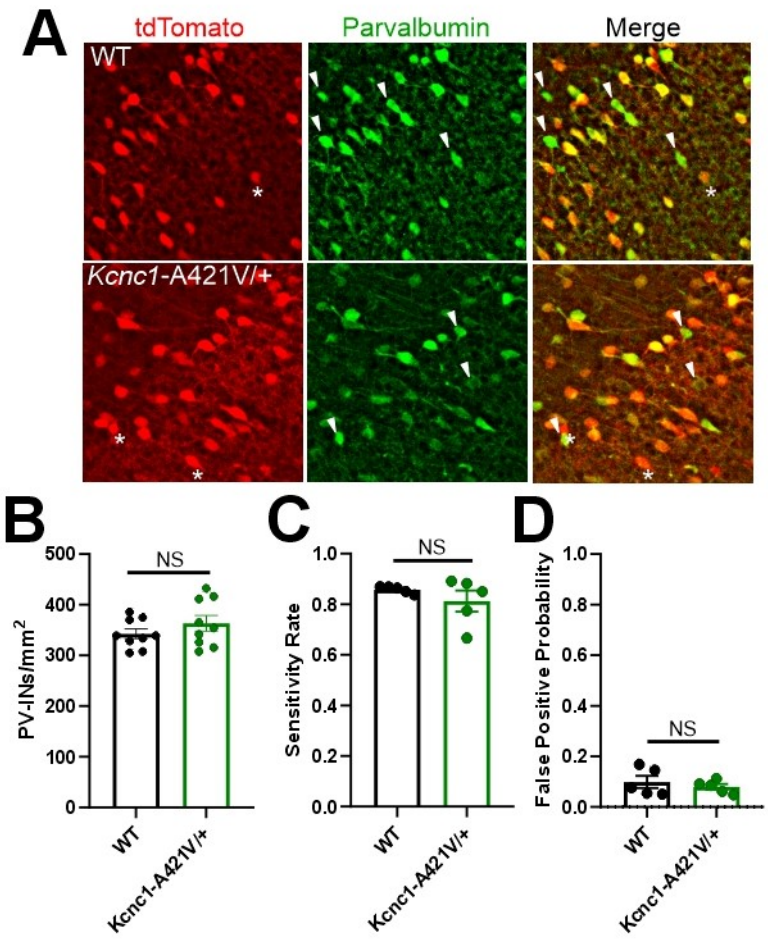

Figure 1—figure supplement 1. Pvalb-TdTomato reporter effectively labels PV-INs in WT and *Kcnc1*-A421V/+ mice. A. Representative immunohistochemistry image for parvalbumin in WT (top row) and *Kcnc1*-A421V/+ (bottom row) mice showing a high degree of overlap between the td-Tomato reporter and parvalbumin expression. The asterisk indicates cells that are tdTomato+, but parvalbumin-. The arrowhead indicates cells that are parvalbumin+ but tdTomato-. **B.** Counts of PV cells per unit area (mm^2) is not different between WT and *Kcnc1*-A421V/+ mice (N=5 mice/genotype). **C.** Sensitivity rate (probability of a cell being tdTomato+ if it is parvalbumin+). **D.** False-positive probability (proportion of all tdTomato+ cells that appear in parvalbumin- cells).

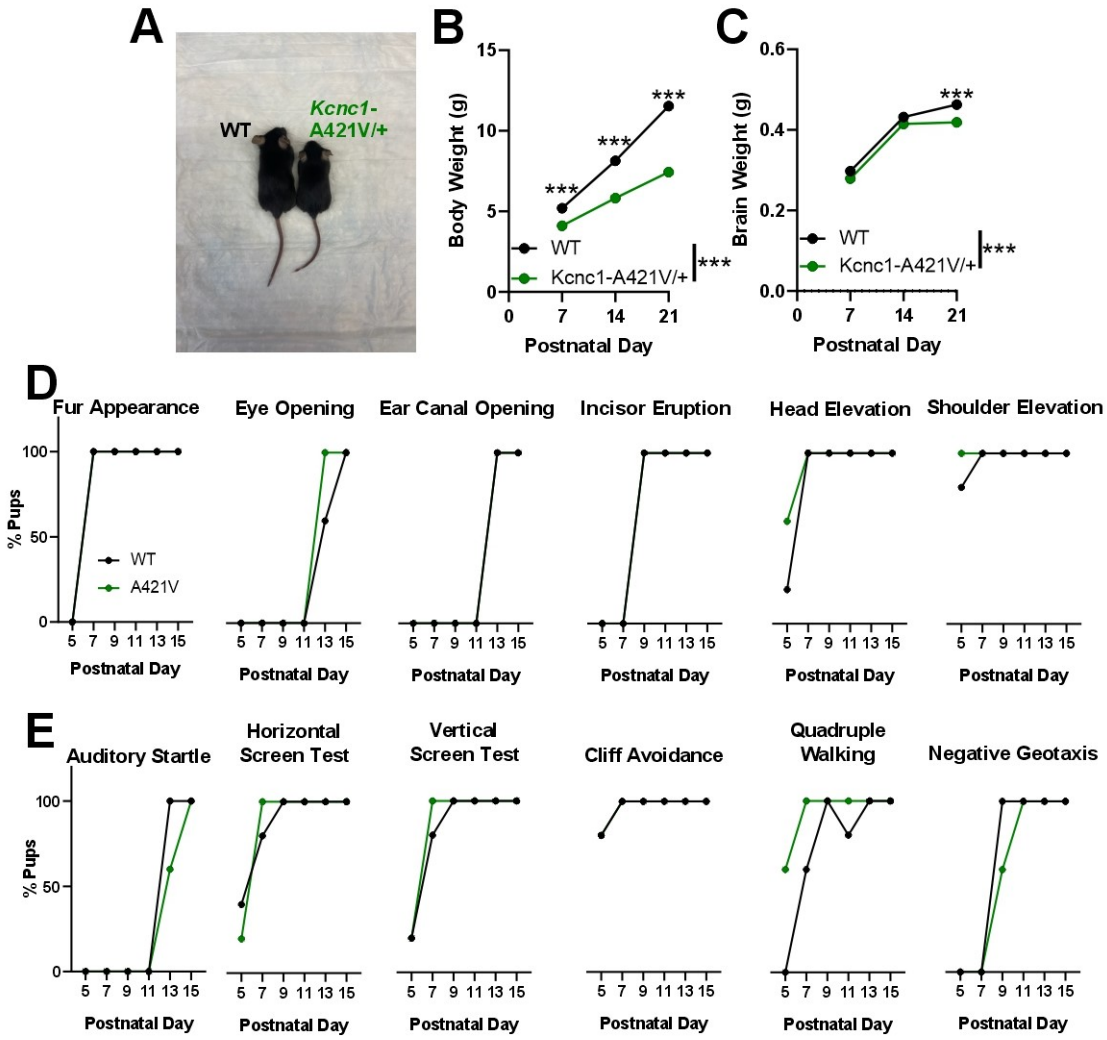

Figure 1—figure supplement 2. Early postnatal development of *Kcnc1*-A421V/+ mice. A. Representative example image showing littermate WT and *Kcnc1*-A421V/+ mice at postnatal day 21. **B.** Average body weights for WT (*N*=10; black) and *Kcnc1*-A421V/+ (*N*=11; green) at postnatal days 7, 14, and 21. **C.** Average brain weights for WT and *Kcnc1*-A421V/+ mice at postnatal day 7 (WT, N = 2; *Kcnc1*-A421V/+, N =6), 14 (WT, *N*=3; *Kcnc1*-A421V/+, *N*=3), and 21 (WT, *N*=4; *Kcnc1*-A421V/+, *N*=6). **D.** Onset of developmental and motor benchmarks for WT (*N*=7) and *Kcnc1*-A421V/+ (*N*=10) mice. **E.** Onset of startle reflex and other typical behavioral/motor milestones in WT (*N*=7) and *Kcnc1*-A421V/+ (*N*=10) mice. ***P<0.001 by Mixed-Effects Analysis followed by Sidak's Multiple comparisons post-hoc test.

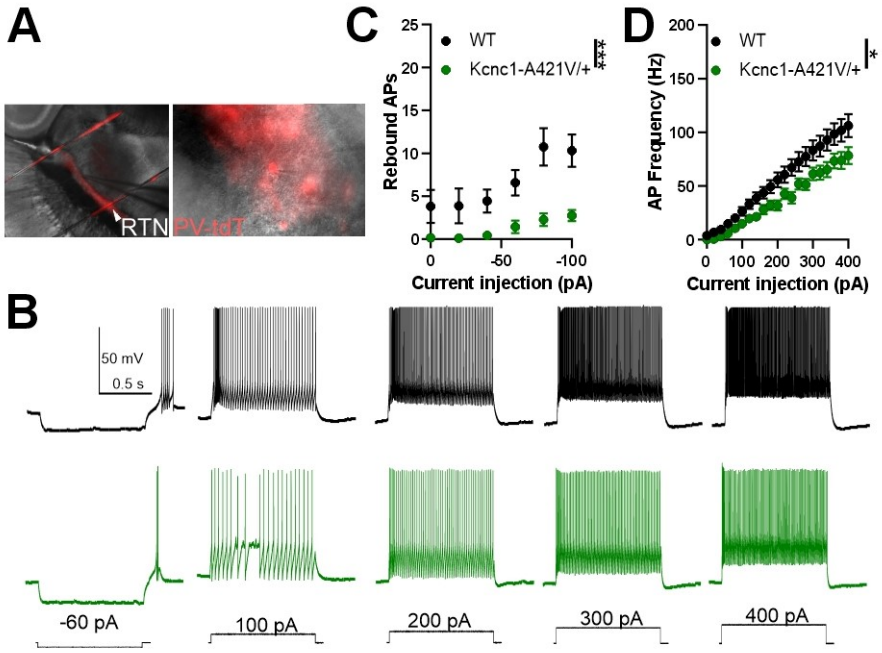

Figure 3—figure supplement 1. Abnormal Intrinsic Physiology in PV-INs of the Reticular Thalamus. Neurons of the reticular thalamus exhibit altered intrinsic excitability in *Kcnc1*-A421V/+ mice. **A.** Representative images taken at 10X (left) and 40X (right) magnification of a PV-positive cell in the reticular thalamus recorded in the whole-cell configuration to characterize intrinsic excitability. **B.** Representative example traces of APs generated in response to current injections of varying magnitudes. **C.** Average hyperpolarization-induced rebound APs in WT (*n*=16 cells, *N*=4 mice) and *Kcnc1*-A421V/+ (*n*=19 cells, *N*=5 mice) in response to various current injections from 0 to -100 pA. **D.** Frequency-current relationship shows impaired intrinsic excitability in the RT PV cells relative to depolarizing current injections (0-400 pA). Data are shown as mean ± SEM and significance is denoted as *P<0.05 or ***P<0.001 by Repeated-measures Two-way ANOVA.

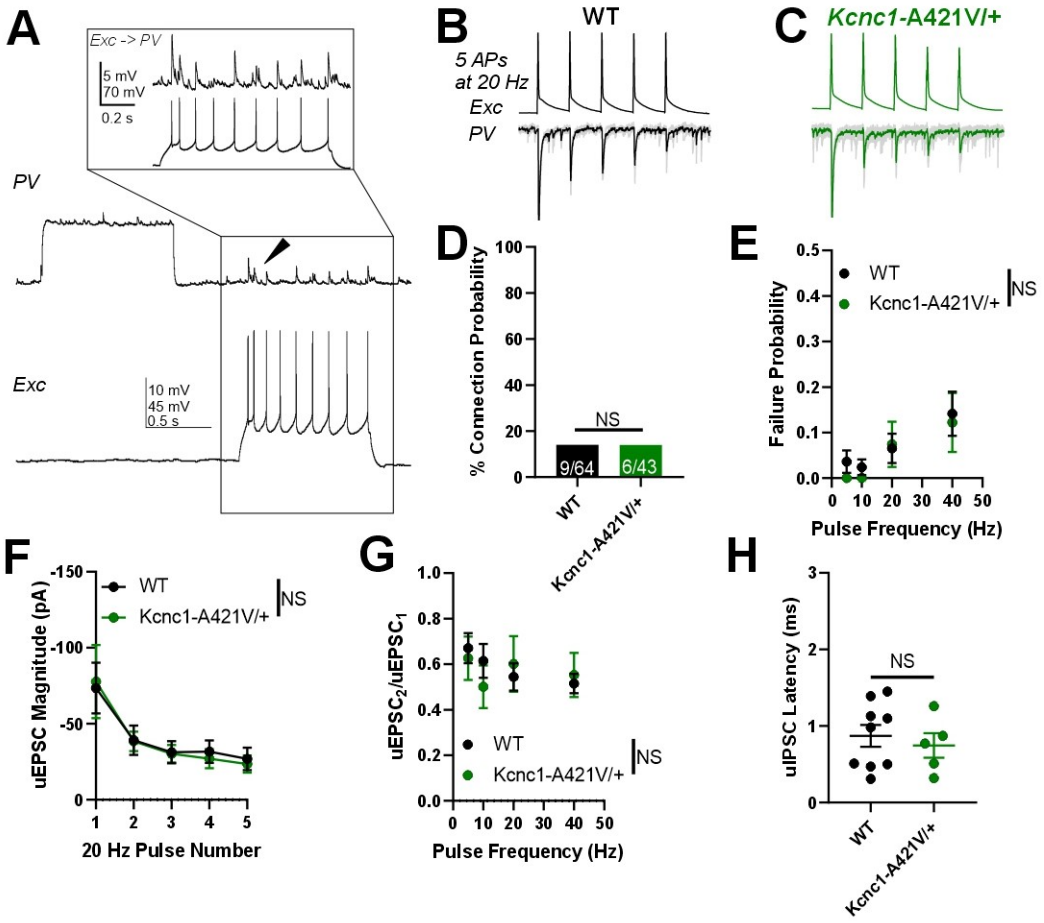

Figure 5—figure supplement 1. Excitatory neuron to PV-IN unitary excitatory synaptic neurotransmission is unaltered in juvenile *Kcnc1*-A421V/+ mice. Excitatory neuron to PV-IN excitatory synaptic neurotransmission is unaltered in *Kcnc1*-A421V/+ mice. **A.** Example traces of simultaneous recordings of a synaptically-connected PV-IN and excitatory cell neuron pair in which generation of APs in the excitatory cell leads to uEPSPs in the PV-IN. The inset shows an expanded view of the uEPSPs in the PV-IN generated from each AP in the excitatory cell. **B-C.** Representative example traces of uEPSCs generated in WT (B) and *Kcnc1*-A421V/+ pairs of excitatory cells (top trace) and nearby PV-INs (bottom trace). In response to a 20-Hz train of five APs generated in the excitatory cells, uIPSCs are recorded in the PV-INs (shown in gray) with the average of numerous sweeps shown for WT (black) or for *Kcnc1*-A421V/+ (green). **D.** Connection probability between the excitatory cell and the PV-IN for WT (*n*=9 of 64 pairs from *N*=7 mice) and *Kcnc1*-A421V/+ (*n*=6 of 43 pairs from *N*=5 mice). **E.** Average failure rate for WT and *Kcnc1*-A421V/+ neuron pairs relative to presynaptic stimulation frequency. **F.** Average uEPSC magnitude in response to a train of five APs generated at 20 Hz in WT and *Kcnc1*-A421V/+ neuron pairs. **G.** Paired-pulse ratio of the second uEPSC to the first uEPSC in WT and *Kcnc1*-A421V/+ mice. **H.** Average synaptic latency between peak of AP to peak of uEPSC in both WT and *Kcnc1*-A421V/+ mice.