BK channel properties correlate with neurobehavioral severity in three KCNMA1-linked channelopathy mouse models

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Abstract KCNMA1 forms the pore of BK K+ channels, which regulate neuronal and muscle excitability. Recently, genetic screening identified heterozygous KCNMA1 variants in a subset of patients with debilitating paroxysmal non-kinesigenic dyskinesia, presenting with or without epilepsy (PNKD3). However, the relevance of KCNMA1 mutations and the basis for clinical heterogeneity in PNKD3 has not been established. Here, we evaluate the relative severity of three KCNMA1 patient variants in BK channels, neurons, and mice. In heterologous cells, BK\textsuperscript{N999S} and BK\textsuperscript{D434G} channels displayed gain-of-function (GOF) properties, whereas BK\textsuperscript{H444Q} channels showed loss-of-function (LOF) properties. The relative degree of channel activity was BK\textsuperscript{N999S} > BK\textsuperscript{D434G} > WT > BK\textsuperscript{H444Q}. BK currents and action potential firing were increased, and seizure thresholds decreased, in Kcnma\textsuperscript{1N999S/WT} and Kcnma\textsuperscript{1D434G/WT} transgenic mice but not Kcnma\textsuperscript{1H444Q/WT} mice. In a novel behavioral test for paroxysmal dyskinesia, the more severely affected Kcnma\textsuperscript{1N999S/WT} mice became immobile after stress. This was abrogated by acute dextroamphetamine treatment, consistent with PNKD3-affected individuals. Homozygous Kcnma\textsuperscript{1D434G/D434G} mice showed similar immobility, but in contrast, homozygous Kcnma\textsuperscript{1H444Q/H444Q} mice displayed hyperkinetic behavior. These data establish the relative pathogenic potential of patient alleles as N999S>D434G>H444Q and validate Kcnma\textsuperscript{1N999S/WT} mice as a model for PNKD3 with increased seizure propensity.

Editor’s evaluation

This study is of broad interest to neuroscientists interested in membrane excitability and translational biologists and physicians eager for robust animal models for disorders involving mutations in the KCNMA gene, such as paroxysmal nonkinesigenic dyskinesia PNKD3. Here, phenotypes of mouse models of three of the more common patient disease-related mutations in KCNMA are evaluated for similarities to patient phenotypes. This work establishes that BK channel mutations linked to human neurological disease can, on their own, cause similar pathology in mice, and it also begins to provide neurological bases for the associated behavioral deficits. Importantly, one of the mutant alleles expressed in mice most closely phenocopies the patient phenotype, which is rescued with a new treatment for PNKD3 in KCNMA1-N999S patients, further validating it as an important animal model for studies seeking therapeutic treatments for the resulting debilitating disease moving forward.
eLife digest So far, only 70 patients around the world have been diagnosed with a newly identified rare syndrome known as KCNMA1-linked channelopathy. The condition is characterised by seizures and abnormal movements which include frequent ‘drop attacks’, a sudden and debilitating loss of muscle control that causes patients to fall without warning.

The disease is associated with mutations in the gene for KCNMA1, a member of a class of proteins important for controlling nerve cell activity and brain function. However, due to the limited number of people affected by the condition, it is difficult to link a particular mutation to the observed symptoms; the basis for the drop attacks therefore remains unknown. Park et al. set out to ‘model’ KCNMA1-linked channelopathy in the laboratory, in order to determine which mutations in the KCNMA1 gene caused these symptoms.

Three groups of mice were each genetically engineered to carry either one of the two most common mutations in the gene for KCNMA1, or a very rare mutation associated with the movement symptoms. Behavioural experiments and studies of nerve cell activity revealed that the mice carrying mutations that made the KCNMA1 protein more active developed seizures more easily and became immobilized, showing the mouse version of drop attacks. Giving these mice the drug dextroamphetamine, which works in some human patients, stopped the immobilizing attacks altogether.

These results show for the first time which specific genetic changes cause the main symptoms of KCNMA1-linked channelopathy. Park et al. hope that this knowledge will deepen our understanding of this disease and help develop better treatments.

Introduction

KCNMA1-linked channelopathy encompasses an array of neurological symptoms associated with clinical detection of a KCNMA1 variant. Affected individuals typically present with epilepsy and/or dyskinesia, but also have other disorders including ataxia, developmental delay, intellectual disability, and brain and structural abnormalities (Bailey et al., 2019; Liang et al., 2019; Miller et al., 2021). The basis for these symptoms is not mechanistically established but is likely similar to other neurological channelopathies involving direct or indirect changes in neuronal excitability leading to excitation-inhibition imbalance (Benatar, 2000; Menezes et al., 2020). KCNMA1 genotype-phenotype correlation is an active area of investigation with >40 variants identified in this patient population to date (Miller et al., 2021, and ALM unpublished data). Since most variants arise de novo in a single heterozygous proband, whether ‘KCNMA1 channelopathy’ is a bona fide monogenic disorder, or results from intergenic and developmental interactions, is not well understood. Animal models for the most common variants are needed to validate genotype-phenotype associations and to investigate disease mechanisms and manifestations over lifespan (MacArthur et al., 2014).

KCNMA1 encodes the ‘Big K+’ (BK) channel, activated by voltage and intracellular Ca2+ (Figure 1). BK currents are prominent in the central nervous system and smooth muscle (Bailey et al., 2019; Contet et al., 2016; Latorre et al., 2017). Neuronal BK channels regulate action potential repolarization and fast afterhyperpolarizations (fAHP) to set firing rates (Gu et al., 2007; Montgomery and Meredith, 2012; Sah and Faber, 2002; Shao et al., 1999) and neurotransmission (Golding et al., 1999; Raffaelli et al., 2004; Sailer et al., 2006; Tazerart et al., 2022). KCNMA1 knockout mice (Kcnma1−/−) show prominent smooth muscle, neurobehavioral, and locomotor deficits, associated with widespread alterations in cellular excitability (MGI:99923; Bailey et al., 2019; Meredith et al., 2004; Sausbert et al., 2005; Sausbert et al., 2004). However, Kcnma1−/− mice do not overtly exhibit KCNMA1-linked channelopathy symptoms. Moreover, the largest cohort of clinically distinguishable patients harbor gain-of-function (GOF), rather than loss-of-function (LOF), alleles with respect to BK channel activity (Miller et al., 2021).

Two GOF KCNMA1 variants, D434G and N999S, account for half of the patient population (Bailey et al., 2019; Miller et al., 2021). Both variants cause BK channel activation at more negative membrane potentials, speed activation, and slow deactivation (Diez-Sampedro et al., 2006; Du et al., 2005; Li et al., 2018; Moldenhauer et al., 2020a; Wang et al., 2009; Yang et al., 2010). The majority of individuals harboring D434G and N999S variants present with paroxysmal non-kinesigenic dyskinesia (PNKD type 3; OMIM #609446), characterized by varying degrees of negative motor phenomena...
Figure 1. Location and consequence of KCNMA1 variants in the BK K⁺ channel. (A) KCNMA1 forms the homotetrameric BK channel. Each α subunit is comprised of seven transmembrane domains (S0–S6) and an intracellular gating ring with hydrophobic segments (S7–S10, black). Pore (+) opening and closing is regulated by voltage-sensitive residues in S2–S4 (VSD), the AC domain (βA to αC), and two Regulators of Conductance of Potassium (RCK) domains in the gating ring (gray), each containing a Ca²⁺ binding site (green) (Yang et al., 2015; Giraldez and Rothberg, 2017). (B) BK channel
structure showing two opposing subunits with Ca\textsuperscript{2+} bound in the gating ring (PDB 6V38). H444Q (purple) and D434G (blue) are located within the βαβ and αA and β of the AC domain, respectively, a region within RCK1 affecting Ca\textsuperscript{2+}-dependent gating (Du et al., 2005; Tao and MacKinnon, 2019). N999S (red) is located at the helix bend in the middle of the S10 domain within RCK2 (Tao and MacKinnon, 2019). (C) Representative inside-out patch-clamp recordings from BK\textsuperscript{WT}, BK\textsuperscript{N999S}, BK\textsuperscript{D434G}, BK\textsuperscript{H444Q} channels expressed in HEK293 cells. Macroscopic BK currents were recorded in symmetrical K\textsuperscript{+} and 1 μM intracellular Ca\textsuperscript{2+} by holding patches at −100 mV, stepping from −100 to 250 mV for 30 ms, followed by a tail step −100 mV for 15 ms. Scale bars: 1 nA, 5 ms. (D) Normalized conductance-voltage (G-V) relationships fit with Boltzmann functions (solid lines). There was no change in the slope factor (z) for any of the variants (p=0.06, one-way ANOVA). BK\textsuperscript{WT} (n=12), BK\textsuperscript{N999S} (n=9), BK\textsuperscript{D434G} (n=12), and BK\textsuperscript{H444Q} (n=12). (E) Voltage of half-maximal activation (V\textsubscript{1/2}) obtained from Boltzmann fits for individual patches. *p<0.0001. One-way ANOVA with Dunnett’s post hoc. (F) Activation time constants (τ\textsubscript{act}) BK\textsuperscript{N999S} and BK\textsuperscript{D434G} channels had decreased τ\textsubscript{act} compared to BK\textsuperscript{WT}, either across all voltage steps (mixed effects model for repeated measures with Bonferroni post hoc, p<0.01) or above 120 mV (p<0.05), respectively. At lower voltages, BK\textsuperscript{D434G} channels were more steeply voltage dependent, but did not exceed the fast activation time constants of BK\textsuperscript{N999S} channels. BK\textsuperscript{H444Q} channels had increased τ\textsubscript{act} compared to BK\textsuperscript{WT} between 160 and 250 mV (p<0.05). Inset: Representative current traces from 170 mV step, scaled to the maximal current to illustrate activation timecourse (x-axis scale bar: 10 ms). τ\textsubscript{act} for BK\textsuperscript{WT} currents was 3.8±0.3 ms, while BK\textsuperscript{N999S} and BK\textsuperscript{D434G} currents activated faster (0.9±9.1 and 1.8±0.1 ms, respectively) and BK\textsuperscript{H444Q} activated slower (6.5±0.5 ms). (G) Deactivation time constants (τ\textsubscript{deact}). BK\textsuperscript{N999S} and BK\textsuperscript{D434G} channels had increased τ\textsubscript{deact} compared to BK\textsuperscript{WT}, across all voltage steps (mixed effects model for repeated measures with Bonferroni post hoc, p<0.01), with the exception of −160 (p>0.05), respectively. BK\textsuperscript{H444Q} channels had decreased τ\textsubscript{deact} compared to BK\textsuperscript{WT} between −190 mV and between −140 and −20 mV (p<0.05). (E) Representative current traces from −20 mV step, scaled to the maximal current to illustrate deactivation timecourse (x-axis scale bar: 10 ms). τ\textsubscript{deact} for BK\textsuperscript{WT} currents was 0.7±0.01 ms, while BK\textsuperscript{N999S} and BK\textsuperscript{D434G} currents deactivated slower (4.5±0.7 and 1.5±0.1 ms, respectively) and BK\textsuperscript{H444Q} deactivated more quickly (0.4±0.01 ms). Data are presented as mean ± SEM. Additional data on the effects of stimulants on BK\textsuperscript{WT} and BK\textsuperscript{N999S} channels appears in Figure 1—figure supplement 1.

The online version of this article includes the following source data and figure supplement(s) for figure 1:

Source data 1. Data file for Figure 1D-G.

Figure supplement 1. Sequences for CRISPR/Cas9 editing of the mouse Kcnma1 gene.

Figure supplement 2. Effects of lisdexamfetamine (lis) and dextroamphetamine (d-amp) on BK\textsuperscript{WT} and BK\textsuperscript{N999S} channels expressed in HEK293 cells.

Figure supplement 2—source data 1. Data file for Figure 1—figure supplement 2.

described as dystonia, hypotonia, non-narcoleptic cataplexy, and akinesia. PNKD3 episodes manifest as postural lapses with immobility (‘drop attacks’) that occur with short duration and high frequency, often hundreds of times per day (Du et al., 2005; Heim et al., 2020; Keros et al., 2022; Li et al., 2018; Wang et al., 2017; Zhang et al., 2015). Just under half of patients experience seizure of varying types, including absence, atonic, myoclonic, and generalized tonic-clonic (GTC). However, epilepsy and PNKD are not consistently co-morbid (Du et al., 2005; Miller et al., 2021). Individuals with putative LOF variants report additional movement disorders including dyskinesia, axial hypotonia, tremor, or ataxia, in addition to various seizure types (Du et al., 2020; Liang et al., 2019; Rodrigues Bento et al., 2021; Tabarki et al., 2016; Yeşil et al., 2018). It is not yet clear whether variations in symptomatic presentation result from incomplete or inconsistent clinical evaluations, or genuine genotype-phenotype differences within either GOF or LOF cohorts.

We address these questions through heterologous, neuronal, and neurobehavioral validation for three patient-associated KCNMA1 variants in mouse models. The GOF BK\textsuperscript{N999S} and BK\textsuperscript{D434G} channels produced increased neuronal BK currents and firing as heterozygous alleles in transgenic mice, while heterozygous LOF BK\textsuperscript{H444Q} channels were insufficient to alter neuronal properties. Mice were evaluated in a series of spontaneous and evoked seizure and locomotor assays. N999S propagated the largest symptomatic burden with chemoconvulsant challenge and stress-triggered dyskinesia, supporting the conclusion that this variant has the greatest monogenic pathogenicity, followed by D434G, Kcnma1\textsuperscript{−/−}, and H444Q. The results identify Kcnma1\textsuperscript{N999S/WT} mice as a PNKD3 model with the highest phenotypic similarity to patients harboring KCNMA1 GOF variants, including symptomatic resolution with acute dextroamphetamine treatment. Our findings further establish the stress-induced PNKD assay to delineate distinct symptomatic manifestations between GOF and LOF alleles, supporting its utility in a battery of neurobehavioral evaluations to define KCNMA1-linked channelopathy models.
Results

Patient variants confer GOF (N999S and D434G) and LOF (H444Q) properties on BK channel activity

A comparative assessment for three dyskinesia-associated patient variants (N999S, D434G, and H444Q) was performed within the human BK channel (Figure 1). BK channel function was assessed using inside-out patch-clamp recordings in HEK293 cells. Patches from cells expressing BKWT, BKN999S, BKD434G, and BKH444Q channels were activated with depolarizing voltage steps, and the voltage dependence of activation and kinetics were assessed from macroscopic currents (Figure 1C). Conductance versus voltage (G-V) relationships (Figure 1D) were assessed by the voltage of half-maximal activation (V1/2; Figure 1E).

BKWT currents had a V1/2 of 125±2 mV. Introduction of N999S and D434G mutations shifted the G-V relationships to more negative membrane potentials (V1/2: BKN999S 41±3 mV and BKD434G 88±2 mV), confirming their GOF effect at all voltages. The decrease in V1/2 for BKN999S channels compared to BKWT was 20–30 mV larger than observed in prior studies with different splice variants and intracellular Ca2+ (Li et al., 2018; Moldenhauer et al., 2020a). Here, under equivalent conditions, N999S produced a larger hyperpolarizing shift from WT (ΔV1/2 = 84 mV) versus D434G (ΔV1/2 = 37 mV). In addition, N999S and D434G produced faster BK channel activation and slower deactivation compared to WT (Figure 1F–G). Altogether, BKN999S channels showed greater GOF properties than BKD434G in all parameters under these conditions, corroborating the relative severity predicted from prior work.

In contrast, introduction of the H444Q variant shifted the G-V relationship to more positive potentials (V1/2: BKH444Q 155±4 mV), consistent with LOF effects. H444Q produced changes in channel opening and closing further consistent with LOF effects, slowing activation and speeding deactivation (Figure 1D–G). H444Q produced a smaller difference from WT than either GOF variant (ΔV1/2 = 30 mV), identifying H444Q as a comparatively mild variant. The results indicate that N999S produces the strongest effect on BK channel activation in the GOF direction, followed by D434G (GOF) and H444Q (LOF).

Generation of N999S, D434G, and H444Q mouse models

Correlation between patient genotype and phenotype has only been established for a single KCNMA1 variant so far, D434G, an autosomal dominant that co-segregates with PNKD and epilepsy in a multi-generation pedigree (Du et al., 2005). D434G pathogenicity is further corroborated by mouse and fly models, which show alterations in neuronal excitability, brain and motor function (Dong et al., 2021; Kratschmer et al., 2021). In contrast, N999S and H444Q lack this direct evidence due to the absence of familial transmission among the children that carry these variants (Miller et al., 2021). N999S is the most common de novo KCNMA1 variant (~17% of all patients), found as heterozygous in every case. About half of individuals harboring N999S alleles are diagnosed with seizure, PNKD, or both (Keros et al., 2022; Li et al., 2018; Wang et al., 2017; Zhang et al., 2015), suggesting a strong potential to be causative in channelopathy symptoms. H444Q is found in a single case and is one of several putative LOF variants where affected individuals have dyskinesia-like paroxysms (Miller et al., 2021). This proband had a history of abnormal EEG, unresolved with respect to the diagnosis of epilepsy, but also harbors three additional genetic findings (ALM unpublished data).

To establish genotype-phenotype correlations, heterozygous mice replicating the patient genotypes were first evaluated. Each variant was introduced as a single nucleotide mutation into the mouse Kcnma1 gene using CRISPR base-editing (Figure 1—figure supplement 1). In all animal experiments, investigators were blinded to genotype during data collection and analysis, and WT controls were compared to transgenic littermates within individual transgenic lines. Kcnma1N999S/WT, Kcnma1D434G/WT, and Kcnma1H444Q/WT mice were grossly behaviorally and morphologically normal with no notable spontaneous paroxysms, gait abnormalities, or visually detectable seizures during home cage observation. Kcnma1D434G/WT and Kcnma1H444Q/WT intercrosses produced homozygous progeny that were also visually normal. However, Kcnma1N999S/WT intercrosses produced no homozygous pups (see Materials and methods). The absence of homozygous N999S progeny is similar to Tg-BKΔ507Q mice harboring another strong GOF mutation that showed lethality in the homozygous allele configuration (Montgomery and Meredith, 2012). Given the inability to generate homozygous N999S mice, gene expression was analyzed from hippocampus and cerebellum of Kcnma1N999S/WT and WT littermates (n=3 mice each genotype and tissue). No significant differences were found in the levels of Kcnma1 (1.07-fold change,
Figure 2. Increased BK current in Kcnma1\textsuperscript{WT/WT} and Kcnma1\textsuperscript{D434G/D434G} granule neurons. Whole-cell macroscopic BK currents were recorded in 1 μM tetrodotoxin (TTX) and 2 mM 4-aminopyridine (4-AP), isolated with 10 μM paxilline, and normalized to cell capacitance. Activating voltage steps were applied from V, of −90 mV, stepping from −100 to +30 mV for 150 ms, and back to −90 mV for 130 ms. (A–C) Peak BK current density versus voltage relationships. Data are presented as mean ± SEM. * and †, p<0.05, two-way repeated measures ANOVA with Bonferroni post hoc. Insets: Representative BK current traces at 30 mV. Scale bars: 500 pA, 5 ms. (A) BK current density was larger in Kcnma1\textsuperscript{N999S/WT} neurons (n=16 neurons, 5 mice) compared to Kcnma1\textsuperscript{WT/WT} (n=14 neurons, 4 mice) at −30 mV (p=0.0114), −20 (p=0.0210), −10 (p=0.0426) voltage steps (indicated with *). (B) BK current density was larger in Kcnma1\textsuperscript{D434G/WT} neurons (n=12 neurons, 3 mice) compared to Kcnma1\textsuperscript{WT/WT} (n=10 neurons, 4 mice) at density at −40 mV (p=0.0112), −30 (p=0.0026), −20 (p=0.0031), −10 (p=0.0038), 0 (p=0.0078), 10 (p=0.0068), 20 (p=0.0071), 30 (p=0.0088) voltage steps (*). Kcnma1\textsuperscript{D434G/WT} mice (n=9 neurons, 3 mice) had higher BK current density compared to Kcnma1\textsuperscript{WT/WT} at −30 mV only (p=0.0321). (C) BK current density was not different in Kcnma1\textsuperscript{N999S/WT} neurons (n=7 neurons, 2 mice) compared to Kcnma1\textsuperscript{WT/WT} (n=6 neurons, 3 mice).

The online version of this article includes the following source data for figure 2:

Source data 1. Data file for Figure 2A–C.

p=0.79, FDR = 0.99, ANOVA with eBayes test), or BK\(\beta\) subunits expressed in brain: Kcnmb1 (−1.07, p=0.14), Kcnmb2 (1.00, p=0.84), and Kcnmb4 (−1.03, p=0.46). The results show no gross up- or down-regulation of BK channel components in either the hippocampus or cerebellum of Kcnma1\textsuperscript{N999S/WT} neurons. However, since the microarray-based probe set does not distinguish between the WT and N999S Kcnma1 transcripts, no further conclusion can be made regarding the specific expression ratio of each allele.

**N999S and D434G increase BK current in hippocampal neurons**

From heterologous cells, we predicted that the variants would have a strong (N999S), intermediate (D434G), or weak (H444Q) potential to alter neuronal BK current levels in transgenic mice. However, heterozygous patient genotypes create the possibility for hetero-tetramer channel formation (Geng et al., 2021), necessitating understanding the relative GOF and LOF effects in vivo from BK current levels in heterozygous transgenics compared to WT littermates. Recordings were made in the dentate gyrus of the hippocampus, where BK channels are highly expressed, regulate neuronal excitability, and where changes in BK channel properties are associated with seizure (Kaufmann et al., 2010; N999S/WT neurons had a 69% increase in BK current compared to WT littermates (Kcnma1\textsuperscript{WT/WT} 13.0±2.0 pA/pF and Kcnma1\textsuperscript{N999S/WT} 22.0±1.8 pA/pF at −10 mV; Figure 2A). The increased BK current likely results from alterations in BK channel activity, since KCNMA1 expression was not changed in Kcnma1\textsuperscript{N999S/WT} neurons. Kcnma1\textsuperscript{D434G/WT} BK currents were 73% larger (Kcnma1\textsuperscript{WT/WT} 12.1±2.6 pA/pF and Kcnma1\textsuperscript{D434G/WT} 20.9±3.2 pA/pF at −10 mV; Figure 2B), although not statistically different at most voltages due to variability. However, two copies of the D434G variant (Kcnma1\textsuperscript{D434G/D434G}) resulted in the largest increase in BK current across voltages from −40 mV to the maximum (203%; 36.7±5.9 pA/pF −10 mV). Interestingly, by direct
comparison Kcnma1<sup>D434G/WT</sup> BK current levels were similar to Kcnma1<sup>N999S/WT</sup>, despite the more severe phenotype for BK<sup>N999S</sup> channels in heterologous cells (Figure 1).

In contrast, BK currents in Kcnma1<sup>H444Q/WT</sup> neurons were not significantly different compared to WT littersmates at any voltage (Kcnma1<sup>WT/WT</sup> 10.9±1.0 pA/pF and Kcnma1<sup>H444Q/WT</sup> 9.5±1.9 pA/pF at −10 mV; -13% change; Figure 2C). This establishes an allelic series of Kcnma1<sup>D434G/D434G ≫ Kcnma1<sup>H444Q/WT</sup> ≫ Kcnma1<sup>N999S/WT</sup></sup> with respect to BK current magnitude and supports the potential for N999S and D434G to cause neurobehavioral changes. The detrimental potential for Kcnma1<sup>H444Q/WT</sup> is less clear and may require additional factors or mechanisms to support pathogenicity (i.e., other Ca<sup>2+</sup> conditions, cell types, or gene interactions).

These dentate granule whole-cell recordings represent an initial evaluation of the potential for each variant to affect BK currents under physiological conditions. For Kcnma1<sup>N999S/WT</sup>, the increased BK current is not associated with higher transcript expression. Although the ratio of WT and N999S transcripts could not be individually determined from the microarray probe set, the single nucleotide mutations introduced into coding exons provide no obvious mechanism to alter the allelic expression ratios. Assuming both alleles are expressed normally, it would suggest functional changes underlie the BK current increase. However, how the properties identified from homotetrameric channels (Figure 1C–G) contribute to the increased current in neurons is unknown. The apparent voltage dependence of activation was not different between N999S, D434G, H444Q, and their respective WT control currents in neurons (data not shown). Several factors that could mitigate differences in 

**N999S and D434G increase intrinsic neuronal excitability**

Intrinsic excitability was next assessed in dentate granule neurons as an independent validation for neuronal pathogenicity. Both GOF and LOF BK channel mutations have the ability to alter neuronal activity in either direction, depending on the context (Bailey et al., 2019; Brenner et al., 2005; Montgomery and Meredith, 2012; Gu et al., 2007; Sausbier et al., 2004). Dentate granule cell input-output firing relationships were assessed in current-clamp mode (Figure 3). Firing rates increased with current injection in each Kcnma1<sup>WT/WT</sup> littersmate control dataset, reaching a peak of ~40 Hz between 240 and 260 pA and then decreasing with higher current injections (Figure 3A–B). Kcnma1<sup>N999S/WT</sup> firing was greater than Kcnma1<sup>WT/WT</sup> littersmate neurons in several key places. First, across the whole current injection range, firing was significantly increased in the middle portion (160–240 pA), ranging from 25% to 30% higher than WT (Figure 3Ai, Bi). After reaching the maximum, the firing still decreased instead of remaining higher through the full range of current injections. In addition, the initial slope of firing (0–160 pA) was greater in Kcnma1<sup>N999S/WT</sup> neurons (0.22±0.01 Hz/pA) compared to Kcnma1<sup>WT/WT</sup> (0.18±0.01 Hz/pA, Figure 3Ci). Lastly, the maximal firing was 9.6±1.8 Hz (125%) higher in Kcnma1<sup>N999S/WT</sup> neurons versus Kcnma1<sup>WT/WT</sup> (Figure 3Di). Taken together, Kcnma1<sup>N999S/WT</sup> neurons respond to stimulation with higher firing and a shift in the input-output relationship.

Increased firing was also observed in Kcnma1<sup>D434G/WT</sup> neurons, but the shape of the input-output alteration was different than that observed in Kcnma1<sup>N999S/WT</sup>. Firing was 18–67% greater than WT controls at higher current injections only, from 260 to 400 pA (Figure 3Aii,Bii). Despite the increase at the higher end of the range, Kcnma1<sup>D434G/WT</sup> firing still decreased after reaching a maximum, while remaining higher than Kcnma1<sup>WT/WT</sup>. The initial firing rate slope was not different from Kcnma1<sup>WT/WT</sup> (Figure 3Cii). However, the maximal firing rate was 5.5±2.2 Hz greater (113%) for Kcnma1<sup>D434G/WT</sup> compared to Kcnma1<sup>WT/WT</sup> (Figure 3Dii). This increase was shifted to higher current injections and occurred over a wider range of voltages than that observed for Kcnma1<sup>N999S/WT</sup>.
Figure 3. Increased intrinsic excitability in Kcnma1<sup>N999S/WT</sup>, Kcnma1<sup>D434G/WT</sup>, and Kcnma1<sup>D434G/D434G</sup> granule neurons. In current-clamp mode, step currents from 0 to 400 pA were applied to dentate granule neurons under the same ionic conditions used to record BK currents. (Ai–Aiii) Representative AP trains elicited from the 200 pA current injection step in WT and transgenic neurons. Scale bar: 20 mV, 100 ms. (Bi–Biii) Input-output relationship for firing frequency versus step current injection. Data are presented as mean ± SEM. *p<0.05, two-way repeated measures ANOVA with Bonferroni post hoc test.

Figure 3 continued on next page.
Kcnma<sup>D434G/D434G</sup> neurons, which had the highest BK current levels, showed further differences from Kcnma<sup>D434G/WT</sup>. Firing was increased 22–47% in the early and middle of the current injection range, from 40 to 220 pA (Figure 3Aii, Bii). Both the initial slope (Kcnma<sup>D434G/D434G</sup> 0.25±0.01 Hz/pA versus 0.19±0.01 Hz/pA for Kcnma<sup>WT/WT</sup>) and the maximal firing rate were greater (117%, Figure 3Cii, Dii). Yet Kcnma<sup>D434G/D434G</sup> firing was qualitatively more similar to Kcnma<sup>N999S/WT</sup>, despite the finding that Kcnma<sup>D434G/D434G</sup> BK current levels were almost twice as much as those recorded from Kcnma<sup>N999S/WT</sup>. No significant differences in firing frequency, slope for the initial firing rate gain, or maximal firing rate were observed in Kcnma<sup>WT/WT</sup> or Kcnma<sup>D434G/D434G</sup> neurons compared to WT littermates (Figure 3Aiii–Diii). The lack of significant alteration in excitability was congruent with the absence of change in BK current levels in Kcnma<sup>N999S/WT</sup> neurons.

We conclude that both the N999S and D434G GOF variants have pathogenic potential through their ability to increase BK currents and action potential firing. The LOF H444Q variant does not substantiate the same pathogenic potential under these conditions. Mechanistically, despite the grossly similar BK current levels between Kcnma<sup>D434G/WT</sup> and Kcnma<sup>N999S/WT</sup> neurons, the non-identical input-output curves suggest a more complex relationship between BK channel properties and neuronal excitability in dentate granule neurons. Hints about the basis for these differences may be revealed by comparison of additional membrane parameters such as passive membrane properties and action potential waveforms. However, no differences in resting membrane potential or input resistance were observed between Kcnma<sup>N999S/WT</sup>, Kcnma<sup>D434G/WT</sup>, or Kcnma<sup>D434G/D434G</sup> and WT controls (Figure 3—figure supplement 1). With respect to action potentials, modulation of repolarization and the afterhyperpolarization (AHP) in repetitive firing occurs with both BK channel inhibition (slower repolarization and reduced AHP amplitude) and activation (faster repolarization and increased AHP amplitude) (Dong et al., 2021; Gu et al., 2007; Montgomery and Meredith, 2012; Shao et al., 1999). Analysis of waveforms from the 200 pA step corroborate BK channels regulate multiple phases of the action potential, but suggested that the basis for increased firing in Kcnma<sup>N999S/WT</sup> and Kcnma<sup>D434G/D434G</sup> neurons was a faster AHP decay rate (Figure 3—figure supplement 2), which would facilitate more rapid initiation of the next action potential. Since Kcnma<sup>D434G/WT</sup> neurons do not show increased firing at 200 pA, no differences would be expected in parameters related to setting firing frequency. Accordingly, no significant difference in AHP decay was found in Kcnma<sup>D434G/WT</sup> versus control waveforms.

The mechanism by which GOF BK channels facilitate AHP decay is not revealed in this study. Though the N999S and D434G variants both slow deactivation in heterologous cells, the deactivation rate remains to be defined in Kcnma<sup>N999S/WT</sup> and Kcnma<sup>D434G/D434G</sup> neurons under repetitive firing.
conditions with dynamic Ca$^{2+}$. In dentate gyrus, the GOF variants share some similarity to BK currents and action potential waveforms recorded from neurons lacking the β4 subunit (Brenner et al., 2005; Jaffe and Brenner, 2018; Wang et al., 2016). Kcnmb4$^{-/-}$ neurons have increased BK current and increased firing associated with accelerated an AHP decay. Like Kcnmb4$^{-/-}$, GOF BK currents may speed AHP decay rate indirectly by affecting another current, most likely SK current due to the lack of change in the AHP amplitudes. GOF variants in the context of β4 deletion would be predicted more severe than either alone, potentially speeding repolarization and further shortening the AHP.

**N999S and D434G reduce seizure thresholds in mice**

Neuronal hyperexcitability is coincident with establishment of an epileptic network, and about half of all individuals with KCNMA1 channelopathy, including those with N999S, D434G, and H444Q variants, report a history of seizures or epilepsy (Bailey et al., 2019; Miller et al., 2021). Individuals harboring the D434G variant primarily have absence seizures, if present (Du et al., 2005). Dentate gyrus hyperexcitability can both contribute to, and result from, epileptiform activity (Dengler et al., 2017; Krook-Magnuson et al., 2015; Mehranfar et al., 2015; Scharfman, 2019). In β4$^{-/-}$ mice, increased granule neuron firing is found in the setting of hippocampal epileptiform discharges, non-convulsive seizures, and lower chemoconvulsant-induced seizure thresholds (Brenner et al., 2005; Whitmire et al., 2017). We hypothesized that Kcnma$^{N999S/WT}$ and Kcnma$^{D434G/WT}$ mice would show increased number, duration, or severity of seizure events compared to WT controls. However, since half of those harboring LOF variants also report seizures (Liang et al., 2019; Miller et al., 2021), including the H444Q and individuals with putative truncation alleles, Kcnma$^{H444Q/WT}$ and Kcnma1$^{-/-}$ mice were assessed in parallel. No seizures have been previously reported in two established Kcnma1$^{-/-}$ mouse models (Bailey et al., 2019; ALM unpublished data), but spontaneous epilepsy was reported in a Kcnma1-exon4 frameshift mouse line (Yao et al., 2021).

Behavioral assessments and EEGs were made from transgenic and WT littermates for indications of seizure. No spontaneous twitching/jumping/convulsions, rigidity/immobility, anorexia/dehydration, or premature mortality were observed from transgenic (or control) mice in the home cage environment. After dural electrode implantation, 24 hr baseline EEGs were recorded. No interictal epileptiform discharges, spontaneous seizures, or other abnormalities (e.g., slowing) were observed in transgenic or control mice during baselines. The absence of spontaneous events was not surprising given that half of affected individuals do not report epilepsy, and among those that do, there is a wide range in frequency (isolated to daily), semiology, and age of onset (Bailey et al., 2019; Miller et al., 2021). However, this presents challenges to evaluating spontaneous EEG events in mouse models, especially those that could be occurring in deeper brain regions similar to β4$^{-/-}$ mice. The presence of EEG abnormalities could be more comprehensively assessed with longer monitoring, depth electrodes, or interrogation of additional ages and strain backgrounds (Lösch et al., 2017), which were beyond the capability of the present study.

Human epilepsy variants in rodent models without spontaneous abnormalities often exhibit decreased thresholds to triggered seizures (Feliciano et al., 2011; Watanabe et al., 2000; Yuskaitis et al., 2018), although this is not entirely predictive of epilepsy risk (Noebels, 2003). We hypothesized that Kcnma$^{N999S/WT}$ and Kcnma$^{D434G/WT}$ mice would show either decreased threshold or increased severity with 40 mg/kg pentylenetetrazol (PTZ) chemoconvulsant challenge. Kcnma$^{WT/WT}$ controls for each line developed seizures consistent with those observed with PTZ in other studies (Van Erum et al., 2019) ranging from abnormal posturing and myoclonic twitching (10/18 mice; modified Racine score 1 or 2) to tonic-clonic activity (7/18 mice; modified Racine 3 or 4) within minutes after PTZ injection (Figure 4—video 4–1).

Kcnma$^{N999S/WT}$ mice developed PTZ-induced seizures that were distinguishable from Kcnma$^{WT/WT}$ littermates in several parameters. Behaviorally, most Kcnma$^{N999S/WT}$ mice displayed tonic-clonic activity (9/13 mice modified Racine 3 or 4), with two reaching status epilepticus (2/13 mice; modified Racine 5). The latency to first seizure after PTZ injection was reduced to 75±15 s, compared to WT littermates (294±99 s; Figure 4Ai,C-D). EEG power, an estimation of seizure severity, showed a broader range with Kcnma$^{N999S/WT}$ mice, although the differences were not significant (Figure 4Bi,C-D). Interestingly despite these observations, mice exhibiting electrographic seizures did not look strikingly behaviorally different from control mice. One reason may be the movement suppression that developed in Kcnma$^{N999S/WT}$ mice after PTZ injection, quantified by EMG. After PTZ, Kcnma$^{WT/WT}$
Figure 4. Pentylenetetrazol (PTZ)-induced seizures in mice. (Ai–Aiv) Latency to initial seizure after PTZ injection. Data are individual mice with median and inter-quartile range. (Ai) Latency was decreased in Kcnma1<sup>N999S</sup>/WT mice (n=13) compared to Kcnma1<sup>WT/WT</sup> (n=18, *p=0.0006; Mann-Whitney test). (Aii) Latency was decreased in Kcnma1<sup>D434G</sup>/WT mice (n=7) compared to Kcnma1<sup>WT/WT</sup> (n=11, *p=0.0041; Mann-Whitney test). (Aiii) Seizure latency was comparable between Kcnma1<sup>H444Q</sup>/WT (n=7) and Kcnma1<sup>WT/WT</sup> (n=4, p=0.5273; Mann-Whitney test). (Aiv) No differences were found in seizure latency between Kcnma1<sup>−/−</sup> (n=9) and Kcnma1<sup>+/+</sup> mice (n=13, p=0.2282; Mann-Whitney test). (Bi–iv) Total EEG power after PTZ injection (y-axis in µV²/Hz × 10<sup>2</sup>). Data are individual mice with median and inter-quartile range. (Bi) EEG power was not different between Kcnma1<sup>N999S</sup>/WT (n=7) and Kcnma1<sup>WT/WT</sup> (n=11, p=0.0619; t-test). (Bii) Kcnma1<sup>D434G</sup>/WT (n=4) was not different from Kcnma1<sup>WT/WT</sup> (n=6, p=0.7563; t-test). (Biii) Kcnma1<sup>H444Q</sup>/WT (n=6) was not different from Kcnma1<sup>WT/WT</sup> (n=4, p=0.9641; t-test). (Biv) Kcnma1<sup>−/−</sup> (n=10) was not different from Kcnma1<sup>+/+</sup> (n=9, p=0.2134; t-test). (C) Representative EEG traces over 45 min at baseline and after PTZ injection (red line). (D) Expanded EEG traces for the first seizure indicated with the red boxes in (C). Representative videos for this dataset appear in Figure 4—videos 1–4.

The online version of this article includes the following video and source data for figure 4:

Source data 1. Data file for Figure 4A, B.

Figure 4—video 1. Kcnma<sup>WT/WT</sup>, https://elifesciences.org/articles/77953/figures#fig4video1

Figure 4—video 2. Kcnma<sup>N999S</sup>/WT, https://elifesciences.org/articles/77953/figures#fig4video2

Figure 4 continued on next page
WT mice had infrequent bouts of sustained quiescent EMG activity, with average lengths of 45±7 s (n=16). However, the inactive bouts were longer for Kcnma1<sup>N999S/WT</sup> mice (311±126, n=10, p<0.0001, Mann-Whitney test) and were visually apparent (Figure 4—video 4–2). The movement suppression exhibited by Kcnma1<sup>N999S/WT</sup> mice under PTZ does not have a correlate in individuals harboring N999S variants, although a few report absence seizures among other types (Miller et al., 2021). Since no spontaneous EEG+/EMG− events were observed in the baseline EEG recording period of these mice, it remains to be determined whether the PTZ-elicited movement suppression is related to an absence-like seizure manifestation.

Within the D434G family, there is an intermediate penetrance for epilepsy (56%), the most frequent diagnosis being absence (Du et al., 2005; Miller et al., 2021). Like N999S, Kcnma1<sup>D434G/WT</sup> mice also showed a reduced latency to first seizure (101±27 s) compared to Kcnma1<sup>WT/WT</sup> mice (209±35 s; Figure 4Aii,C-D). However, this reduction was not as large as the difference between Kcnma1<sup>N999S/WT</sup> mice and their respective controls. Total EEG power from Kcnma1<sup>D434G/WT</sup> mice was not different from WT controls (Figure 4Bii). Therefore, the D434G variant also increased the propensity for seizure in the transgenic model, consistent with its ability to alter neuronal excitability, but was less severe than the N999S variant. The phenotype assessed here for Kcnma1<sup>D434G/WT</sup> is also less severe than reported in a knock-in mouse model with the D434G mutation introduced in the context of a Cre/lox cassette. Those mice showed spontaneous spike-wave discharges in both the heterozygous and homozygous configuration with complete penetrance (Dong et al., 2021), a phenotype that appears more severe than reported in the D434G pedigree, in which only half experience seizures (Du et al., 2005).

Although no patients have a homozygous D434G genotype, a limited number of Kcnma1<sup>D434G/D434G</sup> mice were available for EEG analysis. We tested whether Kcnma1<sup>D434G/D434G</sup> mice, producing only mutant BK channel homotetramers, had a more severe phenotype. These mice showed a trend toward the shortest latencies to seizure, with thresholds comparable to the lowest among the Kcnma1<sup>D434G/WT</sup> cohort (48, 58, and 69 s). Kcnma1<sup>D434G/D434G</sup> mice had EEG power overlapping with WT and heterozygous littermates (2172, 4237, and 3627). While not conclusive and requiring substantiation in a larger cohort, the Kcnma1<sup>D434G/D434G</sup> seizure data raise the possibility of a gene dosage effect with D434G Kcnma1 to first seizure or total EEG power in doxically, acute inhibition of BK channels has anti-seizure effects in other rodent models (Du et al., 2005). However, no differences were observed in latency to first seizure or total EEG power in Kcnma1<sup>H444Q/WT</sup> or Kcnma1<sup>+/−</sup> mice (Figure 4Aii-Aiv,Biii-Biv; Figure 4—video 4). For H444Q, this data suggests the lack of change in dentate granule neuron BK currents and excitability may be consistent with other areas of the brain, producing no change in seizure propensity indicative of widespread hyperexcitability. Overall, alterations in BK current and firing detected concurrently with lowered seizure threshold suggests that the GOF variants N999S and D434G have the potential to contribute to seizure risk by changing neuronal activity in a mouse model. However, partial (H444Q) or total (Kcnma1<sup>+/−</sup>) loss of BK channel function does not support the same potential in seizure etiology under equivalent conditions.

**N999S and D434G cause paroxysmal dyskinesia in mice**

One of the most recognizable symptoms in KCNMA1 channelopathy is a distinctive type of dyskinesia manifesting as sudden, brief paroxysms of axial hypotonia (PNKD3). These episodes sometimes resemble the immobility in non-narcoleptic cataplexy, but have preservation of some muscle tone that varies among individuals (Du et al., 2005; Heim et al., 2020; Keros et al., 2022; Miller et al., 2021; Wang et al., 2017; Zhang et al., 2015). Patients may slump or fall over but can often maintain position if appropriately supported, and consciousness is maintained. Normal activity is recovered...
relatively quickly without persistent impairment (see patient videos in Braverman, 2019; Sanders, 2018). PNKD3 episodes are not initiated by movement or exertion (non-kinesigenic), but rather by negative and positive triggers such as strong emotion (stress and excitement), cold, fatigue, or alcohol. The events are not associated with epileptiform activity on EEG and are generally unresponsive to anti-seizure medications (Keros et al., 2022; Miller et al., 2021). PNKD3 is associated with substantial morbidity due to its high frequency, with hundreds of episodes per day. All three variants tested in this study are associated with PNKD, with 75% of individuals harboring N999S and D434G carrying the diagnosis but also observed at lower incidence with LOF variants or VUS (variant of uncertain significance) (Miller et al., 2021).

There are currently no standardized behavioral assays for either PNKD3 or non-KCNMA1-associated PNKD. In other paroxysmal dyskinesia animal models, the phenotype is usually hyperkinetic, not the hypokinetic events observed in PNKD3. For example, Ca\(^{2+}\) channelopathy, Prrt2-deficient, and Pnkd mutant mice are characterized by dystonia, chorea, and tonic-clonic episodes (Fureman et al., 2002; Lee et al., 2012; Michetti et al., 2017; Tan et al., 2018; Pan et al., 2020). No spontaneous hypotonic dyskinetic motor behavior was detectable to a blinded observer in any of the transgenic lines in this study. Therefore, we sought to elicit episodes by utilizing known triggers for PNKD3. Since individuals harboring N999S and H444Q variants are mostly children without any reported alcohol exposures, a PNKD trigger specifically reported for D434G (Du et al., 2005), and no calibratable emotional responses are validated in mice, we focused on the standardizable stress experienced during physical restraint. Stress provocation is the closest stimulus to the natural triggers observed in PNKD3-affected individuals (Miller et al., 2021). Restraint stress provoked dyskinesia in most (85%) toting mice (Fureman et al., 2002), and PNKD mutant mice also showed dyskinesia after stressful handling when placed in a beaker (Lee et al., 2012).

To test whether restraint stress would produce paroxysmal dyskinesia, mice were subjected to an acute stereotypical manual restraint protocol by an experienced handler. After restraint, mice were placed in a beaker, a novel-constrained environment proposed to enhance stress (Lee et al., 2012). Mice with PNKD-like characteristics were predicted to show restraint-triggered hypokinetic episodes. Mice were scored for abnormal movement (time immobile, circling/hyperactivity, twisting/chorea or limb-clasping, tonic-clonic movement, flattened/dystonic posturing, tremor, listing and falling) in the beaker under video observation. Stereotypical behaviors such as grooming were also recorded. WT mice from all groups showed normal exploratory behavior including sniffing, grooming, and rearing with coordinated movements.

\(\text{Kcnma}^{\text{N999S/WT}}\) mice and WT littermates placed in the beaker without prior restraint did not show any dyskinetic movements or collapsing behavior (paws no longer touching the ground). There was no significant difference in the time spent immobile between these groups (Figure 5A). Next, restrained mice were placed in the beaker. Since mice increase grooming when released from stress Shirley et al., 2008, Jones and Mormède, 2000, this behavior was used as a control to indicate the presence of stress. \(\text{Kcnma}^{\text{N999S/WT}}\) mice and WT littermates both showed an increase in grooming events after restraint compared to their non-restraint controls (Figure 5B), confirming both genotypes responded to stress with an increase in stereotypical behavior.

After stress, \(\text{Kcnma}^{\text{WT/WT}}\) mice had exploratory behavior and spent less than a minute immobile in the beaker (51±10 s). Although the range was wider, their time spent immobile did not differ significantly from the unrestrained baseline. In contrast, \(\text{Kcnma}^{\text{N999S/WT}}\) mice were immobile for more than twice as long after stress (120±12 s) (Figure 5A, Figure 5 vide 1). After episodes of immobility, both genotypes resumed normal exploratory behavior or grooming.

In qualitative assessment, three \(\text{Kcnma}^{\text{N999S/WT}}\) mice had extended myoclonic ‘hiccups’ throughout the immobility that were not associated with respiratory rate. One mouse also showed listing, and three had a flattened posture. Evaluation of other dyskinetic behaviors (dystonia, chorea, clasping, etc.) in non-restraint controls and after stress revealed grossly normal movements for \(\text{Kcnma}^{\text{N999S/WT}}\) mice, with the exception of the notable immobility. In direct comparison, \(\text{Kcnma}^{\text{WT/WT}}\) littermates had raised heads and less hunched postures during their briefer immobility, suggesting the maintenance of normal axial tone. Brief hiccups were observed in one WT control, at shorter duration than the \(\text{Kcnma}^{\text{N999S/WT}}\) mice, and one mouse had a brief flattened posture during the first bin. Use of a fitted tube restraint, which may produce a stronger stress response, increased the ability of a blinded observer to predict genotype differences in immobility (n=11 mice, data not shown).
Six PNKD3-affected individuals harboring the N999S variant have been documented to experience nearly full resolution of immobilizing episodes with lisdexamfetamine, a prodrug of dextroamphetamine (d-amp; Keros et al., 2022). D-amp treatment is also highly effective against PNKD3 associated with another GOF KCNMA1 variant (N536H; Zhang et al., 2020). Acute daily d-amp administration reduces the number of PNKD3 episodes during the therapeutic window from >100 to 0 in some cases (Keros et al., 2022). If the stress-induced immobile state in Kcnma1N999S/WT mice is consistent with PNKD3, administering d-amp would be predicted to reduce immobility time.

To test for rescue effects, Kcnma1N999S/WT mice and WT littermates were injected with low dose d-amp (0.5 mg/kg), similar to PNKD3-affected patients taking lisdexamfetamine or d-amp (Keros et al., 2022; Zhang et al., 2020). Mice were then subjected to 5 min of restraint stress, followed...
with observation of mobility state in the beaker assay (Figure 5C). Experimental and separate control assays were conducted during the peak time window reported for d-amp effect from open field locomotion (30 min post-injection; Fu et al., 2021; Gould et al., 2007). As a control, d-amp administration without restraint stress does not affect immobility in either Kcnma<sup>N999S/WT</sup> mice or WT littermates (Figure 5—figure supplement 1A). This corroborates that after an acute injection, the restraint stress stimulus is still required to produce immobility in Kcnma<sup>N999S/WT</sup> mice (Figure 5C). Furthermore, d-amp does not increase wheel running or increase grooming in either genotype (Figure 5—figure supplement 1B-C), similar to prior C57BL/6J studies (Cytryn, 1980). These control data further indicate that general locomotor activity and the stress response are not markedly increased under the d-amp dosing conditions.

Under the restraint stress paradigm, saline-injected Kcnma<sup>WT/WT</sup> mice showed normal exploratory behavior with little immobility (<10 s), with one exception of a single mouse immobilized for 121 s exhibiting a hunched posture. D-amp treatment was not different from saline control for Kcnma<sup>WT/WT</sup> mice (Figure 5C). In contrast, restrained Kcnma<sup>N999S/WT</sup> mice produced a large increase in immobility after saline injection (>4-fold average), corroborating un-injected animals subjected to restraint stress (Figure 5A and C). The majority of saline-injected Kcnma<sup>N999S/WT</sup> mice spent 1–2 min immobile and assumed a hunched posture with the head lowered.

D-amp-injected Kcnma<sup>N999S/WT</sup> exhibited little immobility compared to saline-injected Kcnma<sup>WT/WT</sup> mice (3-fold less). These mice had normal exploratory behavior with shorter, more frequent episodes of grooming. However, when present, the shorter immobility bouts were associated with hunched posture. After d-amp treatment, there was no statistically significant difference in immobility between Kcnma<sup>N999S/WT</sup> mice and WT littermates (Figure 5C). Automated analysis of the assays by an additional blinded experimenter replicated the finding of abrogation of immobility with d-amp treatment in Kcnma<sup>N999S/WT</sup> mice (Figure 5—figure supplement 1D). In conclusion, these data show that d-amp treatment of Kcnma<sup>N999S/WT</sup> mice rescues stress-induced immobility at therapeutically relevant doses.

Taken together, these data suggest that the presence of a stressor (restraint) produces a new behavioral state in Kcnma<sup>N999S/WT</sup> mice (immobility) that was not observed in the absence of the trigger or in WT littermates. If the immobility behavior resulted from stress-induced atomic or absence seizures, these events would likely have been observed during baseline EEG recordings given the number of occurrences in the 5 min beaker assay. However, seizure would not be expected to resolve with low-dose d-amp. Alternatively, if stress-induced immobility resulted from general hypooactvity or altered fear response, open field testing might show a difference in motor exploratory behavior between Kcnma<sup>N999S/WT</sup> and WT littermates. This was not observed (Figure 6B). Kcnma<sup>N999S/WT</sup> mice were also able to achieve the same peak speed as WT littermates during voluntary wheel running (Figure 6Ai). In addition, there is no evidence from patients for correlation of PNKD3 with increased anxiety, depression, or hypoactivity (Miller et al., 2021). We conclude that stress-induced immobility, which occurs in brief episodes that are instantaneously recovered, is responsive to d-amp, and occurs without other hyperkinetic or tonic-clonic manifestations, is consistent with the reversible triggered hypokinetic behavioral state in PNKD3-affected individuals (Heim et al., 2020; Keros et al., 2022).

To further characterize this mouse model for PNKD3, additional motor assays were conducted. Besides stress, PNKD3 episodes can be triggered by positive emotions or excitement, similar to cataplexy in patients with narcolepsy (Dauvilliers et al., 2014; Miller et al., 2021; Kelley, 2001 ; Sun et al., 2019a). Related to the reward and arousal effects in mice, cataplexy can be provoked in narcoleptic orexin-deficient (Hcr<sup>−/−</sup>) mice by wheel running (España et al., 2007; Mahoney et al., 2017; Novak et al., 2012). We also assessed this positive trigger to determine if voluntary wheel running could produce a PNKD-like behavior in the setting of a more complex motor task. In this assay, Kcnma<sup>N999S/WT</sup> mice covered a shorter distance compared to their WT littermates (Figure 6Aii). The reduction in running distance was not due to a decrease in the maximum speed the mice could achieve, but instead occurred as a result of increased gaps in activity (Figure 6Aii–iv). These gaps could be consistent with, but not exclusively attributable to, cessation of running during a PNKD-like immobility. However, we cannot rule out the reduced distance as a result of a baseline dyskinesia or muscle weakness, since Kcnma<sup>N999S/WT</sup> mice also show decreased time to fall in the rotorod and hanging wire assays (Figure 6C–D).

PNKD3 is also exhibited in individuals harboring heterozygous D434G variants and is provoked by additional triggers besides stress, such as alcohol (Du et al., 2005). However, without diagnostic
standardization, it is not clear whether this constitutes a different type of PNKD episode in individuals harboring D434G compared to N999S. In the stress assay, Kcnma1D434G/WT mice and WT littermates had similar immobility lasting 53±9 and 63±6 s, respectively (Figure 5D). However, in homozygous Kcnma1D434G/D434G mice, immobility time was similar to N999S heterozygotes (101±11 s). Thus, stress-induced dyskinesia is present in the D434G mouse model. It remains possible that alcohol would also be capable of triggering these episodes, but it is difficult to assess given the bi-directional motor effects of alcohol in mice (Jones and Mormède, 2000). Kcnma1D434G/D434G mice have aspects of reduced motor coordination on the rotarod and running wheels but are not hypokinetic under basal conditions (Figure 6—figure supplements 1 and 2).
In contrast, homozygous LOF manipulations showed a different directionality in stress-triggered dyskinetic behavior, with less immobility after restraint than either WT or heterozygous littermates. No phenotypic differences were detected in heterozygous mice of either line. However, immobility in Kcnma1H444Q/H444Q mice was reduced to 16±6 s (Figure 5E), and in Kcnma1+/− mice, it was reduced to 27±7 s (Figure 5F). Three Kcnma1+/− mice also showed hyperactive circling and rapid limb movements notable to the blinded observer, and one had notable tremor during the brief non-active periods. Kcnma1H444Q/H444Q and Kcnma1+/− mice had reduced latencies to fall from the hanging wire under basal conditions, but only Kcnma1+/− mice are profoundly impaired on the rotarod and running wheels due to ataxia (Figure 6—figure supplements 1 and 2). The reduced motor performance in Kcnma1H444Q/H444Q and Kcnma1+/− mice stands in contrast to the reduction in immobility observed after stress (Figure 5E–F). These data reveal that the H444Q variant and KCNMA1 null genotypes are not associated with PNKD immobility under the same triggers that provoke GOF variants. The results raise the possibility that stress-induced dyskinesia manifestation is influenced by mutation type, with GOF producing hypokinetic and LOF producing hyperkinetic responses.

Discussion

We have characterized the channel properties, neuronal activity, neurobehavioral phenotypes, and relative severity of three KCNMA1-linked channelopathy variants under equivalent conditions. Pathogenic potential was established using four criteria (MacArthur et al., 2014): (1) low variant frequency in the human population, classifying as a mutation (Miller et al., 2021), (2) variant alters BK channel gating properties, (3) variant alters neuronal BK currents and firing, since the channelopathy is a neurological disorder, and (4) variant produces phenotypes similar to the central patient diagnoses—seizure susceptibility and PNKD. The findings support the conclusion that KCNMA1-linked channelopathy, although symptomatically heterogenous and comprised predominantly of de novo variants, has the potential to be categorized as a monogenic disorder. The results substantiate hyperexcitability, increased seizure propensity, and PNKD as collective phenotypes replicated in two hypermorphic GOF KCNMA1 alleles. Moreover, our data for N999S and D434G corroborate mounting evidence, both in patients (Du et al., 2005; Keros et al., 2022; Miller et al., 2021; Zhang et al., 2020) and in animal models (Dong et al., 2021; Kratschmer et al., 2021), that PNKD can be considered the most consistent symptom for KCNMA1 GOF channelopathy.

N999S produced the strongest GOF effect on BK channel properties under these study conditions. Although N999S is the most commonly reported KCNMA1 variant, direct evidence that it caused channelopathy was lacking because it arose de novo in all known cases (Keros et al., 2022; Miller et al., 2021). In mice, our data validate its dominant inheritance and pathogenic potential as a GOF mutation able to increase BK current and neuronal activity in the heterozygous configuration in mice. Neurobehavioral validation further identified increased PTZ-induced seizure propensity and stress-triggered dyskinesia episodes resembling PNKD-like immobility, broadly consistent with the phenotypic occurrence in patients. The lethality of homozygous Kcnma1N999S/N999S (and hemizygous Kcnma1N999S+/-) genotypes, which have not been found in any patient, underscores the severity of this variant. It is not known which cell type the lethality stems from, but N999S channels can produce both increased peak action potential-evoked and subthreshold BK current (Moldenhauer et al., 2020b). Thus it is possible that N999S could increase firing in some cells, exemplified by the effect in dentate granule neurons, or prevent firing, depending on the cellular context.

D434G, a less severe GOF mutation than N999S at the BK channel level, had dominant inheritance for a subset of traits in our model, partially paralleling the familial pedigree (Du et al., 2005). The increased BK current, excitability, and PTZ-induced seizure propensity in the heterozygous configuration validated D434G pathogenicity. However, homozygosity was required to produce the PNKD-like immobility attacks, ranking D434G less pathogenic than N999S. While Kcnma1D434GD434G mice would produce obligate homotetrameric mutant BK channels, additional work is needed to understand whether the decreased severity in Kcnma1D434GD434G mice is indicative of heterotetrameric (WT:D434G) BK channel formation. Interestingly, three other D434G models have been reported, with some phenotypic variation. BAC-loxP-D434G mice exhibited GTC seizures in the absence of motor dysfunction (Ling et al., 2016). Another Cre/lox-D434G mouse line was comparatively more severe with complete penetrance of absence seizures and dyskinesia in the heterozygous configuration (Dong et al., 2021). This variability, observed in both mouse models and incomplete penetrance...
in patients, raises the possibility that additional genetic or environmental factors can influence symptomatic severity. Nevertheless, an analogous mutation in flies also alters neuronal activity and baseline motor behavior (Kratschmer et al., 2021).

The LOF variant, H444Q, demonstrated limited pathogenicity by decreasing BK channel activity but was not validated as a hypomorphic or haploinsufficient allele in neurons or mice. Homozygous Kcnma1^{H444Q/H444Q} mice showed a neurobehavioral phenotype distinct from PNKD: stress-induced hyperkinetic motor responses similar to Kcnma1^{-/-} null mice. Given multiple genetic findings and symptomatic ambiguity in the patient carrying this variant, the different dyskinetic responses compared to the two GOF models may suggest a basis to improve the diagnostic investigations for this and other KCNMA1 variants classified as LOF or VUS. At present, there are no patients with homozygous KCNMA1 alleles validated as functionally null for channel activity (Miller et al., 2021), but the ataxia, tremor, decreased strength, and hyperactivity in Kcnma1^{-/-} mice (Imlach et al., 2008; Meredith et al., 2004; Meredith et al., 2006; Sausbier et al., 2004; Tysz et al., 2013; Wang et al., 2020) are symptoms observed at lower incidence among patients. Lastly, our experimental conditions failed to corroborate the influence of LOF alleles on seizure propensity predicted from several animal studies (Ermolinsky et al., 2008; Kuebler et al., 2001; Pacheco Otalora et al., 2008; Sheehan et al., 2009; Shruti et al., 2008; Yao et al., 2021). KCNMA1 LOF channelopathy has also been proposed to carry a broader set of non-overlapping features associated with a subset of de novo LOF variants, referred to as Liang-Wang syndrome (Liang et al., 2019). However, none of the observable patient correlates were present in the LOF H444Q model studied here. H444Q mice also had little overlap with Ryegrass Staggers, a toxicity syndrome of livestock involving BK channel inhibition that is phenotypically similar to Kcnma1^{-/-} mice (Imlach et al., 2008).

Genotype-phenotype relationships are important for understanding KCNMA1 channelopathy disease mechanisms as well as potential therapeutics. The allelic series established from N999S, D434G, and H444Q BK channels in vitro is outwardly congruent with the relative severity in mice. Such allelic series have been pivotal in understanding other complex channelopathies, especially those delineating distinct disorders within the same gene association (Noebels, 2003; Pietrobon, 2005; Zwingman et al., 2001). However, it is not yet clear whether all KCNMA1 variants discovered in the setting of neurological diagnoses carry the same pathogenic potential. Because this KCNMA1 allelic series was derived from a limited set of conditions, designs of phenotypic severity could be further influenced by additional factors.

At the DNA level, it is likely that all three variant-containing alleles are expressed because the homozygous phenotypes are dissimilar to Kcnma1^{-/-}: Kcnma1^{N999S/N999S} and Kcnma1^{D434G/D434G} have unique phenotypes not found in Kcnma1^{-/-} (lethality and stress-induced immobility, respectively), and Kcnma1^{H444Q/H444Q} had fewer and less severe phenotypes than Kcnma1^{-/-} (e.g., rotarod). However, the phenotypic severity for the human variants could be mitigated by their context within the mouse gene, which was not humanized through additional rounds of gene editing. Mouse and human BK channels differ at eight constitutive coding residues and have minor differences in BK current properties in heterologous cells (Lai, 2015). D434G produces a larger GOF effect on the G-V relationship in the context of a human BK channel compared to mouse (Wang et al., 2009). Similarly, as the least potent variant, it is possible that H444Q has a further reduced effect on BK channel properties in mouse, potentially contributing to the lack of BK current differences.

At the channel level, unresolved questions concerning functional mechanisms and subunit composition illustrate the influence of additional factors besides variant genotype on the phenotypic severity. For example, BK current levels were relatively similar between Kcnma1^{N999S/WT} and Kcnma1^{D434G/WT} in one type of neuron (dentate granule), yet these two heterozygous genotypes exhibited differences in excitability. Instead, homozygous Kcnma1^{D434G/D434G} firing curves were more comparable to Kcnma1^{N999S/WT}. Underlying this, the ratio of expression from WT and mutant alleles, stoichiometry and properties of heterotetrameric channels, alternative splice variation, and the composition of αβCa2+ macrocomplexes in the loci responsible for neuropathology in heterozygous transgenic mice all still remain to be resolved. Additional experiments to probe the voltage and Ca2+-dependent bases for N999S and D434G gating defects (Diez-Sampedro et al., 2006; Du et al., 2005; Li et al., 2018; Moldenhauer et al., 2020b; Yang et al., 2010), and the manifestation in the presence of the highly expressed β4 subunit (Wang et al., 2009; Berkefeld and Fakler, 2013; Wang et al., 2016), may also further explain these relative differences in BK currents and pathogenicity. Interestingly, no KCNM84
variants associated with seizure have been described yet, even as the number of seizure-associated BKα variants has increased (Miller et al., 2021). This could suggest that loss of β4 regulation would have more severe consequences.

At the neurobehavioral level, increased dentate granule cell excitability may contribute directly or indirectly to the reduced seizure thresholds, but other areas of the brain are additionally involved in the generalized PTZ-evoked seizures. The BK channel inhibitor paxilline can block chemovconvulsant-induced seizures, associated with changes in cortical excitability (Sheehan et al., 2009; Shruti et al., 2008). These data suggest additional neurons to investigate for links between GOF BK channels and hyperexcitability. It is also not clear yet how PNKD-like symptomology is produced. BK channels regulate excitability in several motor-associated regions including the cerebellum, striatum, neuromuscular junction, and skeletal muscle (Abrahao et al., 2017; Goldberg and Wilson, 2005; Sausbier et al., 2005; Tricarico et al., 1997; Vatanpour and Harvey, 1995; Wang et al., 2020). Cre/lox-D434G and PRRT2 mice show changes in cerebellar excitability and morphology (Calame et al., 2020; Dong et al., 2021) but do not show hypokinetic PNKD described in this study. In other PNKD animal models, as in patients, the brain mechanisms are not well understood. Some PNKDs respond to antiepileptic medications, a few respond to deep-brain stimulation, but most necessitate trigger avoidance (Manso-Calderón, 2019). In PNKD3, stimulants (lisdexamfetamine and dextroamphetamine) are highly effective in reducing attacks described as both dystonic and hypotonic (Keros et al., 2022; Zhang et al., 2020), but neither drug has a direct effect on BKWT channel activity (Figure 1—figure supplement 2 and Zhang et al., 2020) or BK<sup>N999S</sup> (Figure 1—figure supplement 2) in heterologous cells, leaving the target for their actions on PNKD3 an open question. The significant reduction in stress-induced immobility with d-amp treatment in Kcnma1<sup>N999S/WT</sup> mice substantiates the utility of this model in future studies dissecting the cell and circuit basis for the PNKD3. Clinical observations and d-amp responsiveness in patients predict a central neurological dysfunction in producing the debilitating hypokinetic state (Keros et al., 2022; Zhang et al., 2020), but whether other factors such as altered neuromuscular transmission or episodic muscle hypotonia make some contribution during attacks remains to be systematically tested.

Changes in BK channel function and/or KCNMA1 expression are associated with a growing number of neurodevelopmental disorders including epilepsy, dyskinesia, autism, Angelman’s syndrome, Fragile X syndrome, and brain and skeletal malformations (Cheng et al., 2021; Deng and Klyachko, 2016; Du et al., 2020; Kessi et al., 2020; Kshatri et al., 2020; Laumonnier et al., 2006; Liang et al., 2019; Miller et al., 2021; N’Gouemo, 2014; Sun et al., 2019a; Sun et al., 2019b). Neuropathology in these disorders is associated with changes in BK channel activity in both directions. Yet it has been challenging to distill KCNMA1-linked channelopathy into a cohesive GOF versus LOF symptomology because the existing patient data lack genetic pedigrees and diagnostic cross-comparability. Looking ahead, the phenotypic penetrance and heterogeneity investigated here validate only a few of the 40+ patient-associated KCNMA1 variants, but it will not be possible to make transgenic models for every case. There is less symptomatic consistency among non-GOF alleles (LOF or VUS), identifying this as a potentially fruitful area for future investigations.

### Materials and methods

| Key resources table | Designation | Source or reference | Identifiers | Additional information |
|---------------------|-------------|---------------------|-------------|------------------------|
| Recombinant DNA reagent | BK<sup>N999S</sup> | This paper | MG279689 with rs886039469 | BK channel expression construct in pcDNA3.1+ (Figure 1) |
| Recombinant DNA reagent | BK<sup>D434G</sup> | This paper | MG279689 with rs137853333 | BK channel expression construct in pcDNA3.1+ (Figure 1) |
| Recombinant DNA reagent | BK<sup>H444Q</sup> | This paper | MG279689 with c.1332C>G, p.H444Q | BK channel expression construct in pcDNA3.1+ (Figure 1) |
| Recombinant DNA reagent | BKWT | Genbank | hBKQEERL MG279689 | BK channel expression construct in pcDNA3.1+ (Figure 1) |

**Materials and methods**

Continued on next page
| Reagent type (species) or resource | Designation | Source or reference | Identifiers | Additional information |
|-----------------------------------|-------------|---------------------|-------------|------------------------|
| Chemical compound, drug           | DMEM        | Gibco, Life Technologies Corp | Cat. #11995-065 | Mouse line maintained in A. Meredith’s lab (Figure 1—figure supplement 1) |
| Chemical compound, drug           | Fetal bovine serum | Sigma-Aldrich | Cat. #4135 | Mouse line maintained in A. Meredith’s lab (Figure 1—figure supplement 1) |
| Chemical compound, drug           | Penicillin/streptomycin | Mediatech Inc | Cat. #30-002 Cl | Mouse line maintained in A. Meredith’s lab (Figure 1—figure supplement 1) |
| Chemical compound, drug           | L-glutamine | Mediatech Inc | Cat. #25-005 Cl | Mouse line maintained in A. Meredith’s lab (Figure 1—figure supplement 1) |
| Chemical compound, drug           | Trans-IT LT1 | Mirius Biological | | |
| Chemical compound, drug           | Poly-L-lysine | Sigma-Aldrich | Cat. #P4832 | |
| Chemical compound, drug           | Dextroamphetamine sulfate | Sigma-Aldrich | Cat. #1180004 | |
| Gene (Mus musculus)               | Kcnma1     | Gene Bank; Ensembl | ID: 16531, ENSMUSG00000063142 | |
| Strain (Mus musculus)             | C57BL/6J   | Jackson Laboratories | Stock #000664 | |
| Genetic reagent (Mus musculus)    | Kcnma1N999S | This paper | Gene ID:16531 with rs886039469 | |
| Genetic reagent (Mus musculus)    | Kcnma1D434G | This paper | Gene ID:16531 with rs137853333 | |
| Genetic reagent (Mus musculus)    | Kcnma1H444Q | This paper | Gene ID:16531 with c.1332C>G, p. H444Q | |
| Sequence-based reagent (oligonucleotides) | N999S gRNA | Integrated DNA Technologies | | |
| Sequence-based reagent (oligonucleotides) | D434G/H444Q gRNA | Integrated DNA Technologies | | |
| Sequence-based reagent (oligonucleotides) | N999S donor | Integrated DNA Technologies | | |
| Sequence-based reagent (oligonucleotides) | D434G donor | Integrated DNA Technologies | | |
| Sequence-based reagent (oligonucleotides) | H444Q donor | Integrated DNA Technologies | | |
| Sequence-based reagent (oligonucleotides) | N999S genotyping primer (F) | Transnetyx, Inc | TCGGTTGTTGCTTA AGAATGCTT Kcnma1N999S | |
| Sequence-based reagent (oligonucleotides) | N999S genotyping primer (R) | Transnetyx, Inc | CCTCAGCTTATGAG CCTGAGCTTC Kcnma1N999S | |
| Sequence-based reagent (oligonucleotides) | WT genotyping reporter | Transnetyx, Inc | CAGACATATTTCAAT GACAATAT Kcnma1N999S | |
| Sequence-based reagent (oligonucleotides) | N999S genotyping reporter | Transnetyx, Inc | CAGACATATTTCAAT GACAATAT Kcnma1N999S | |

Continued on next page
### Continued

| Reagent type (species or resource) | Designation | Source or reference | Identifiers | Additional information |
|-----------------------------------|-------------|---------------------|-------------|------------------------|
| Sequence-based reagent (oligonucleotides) | D434G genotyping primer (F) | Transnetyx, Inc | CTCTAACTTCTCGAAA GGAATTCTCGACA | Kcnma<sup>D434G</sup> |
| Sequence-based reagent (oligonucleotides) | D434G genotyping primer (R) | Transnetyx, Inc | CAGAGAGAAGCATG AGTTAGGTTGGCA | Kcnma<sup>D434G</sup> |
| Sequence-based reagent (oligonucleotides) | WT genotyping reporter | Transnetyx, Inc | ACCGGGATGATGTCA | Kcnma<sup>D434G</sup> |
| Sequence-based reagent (oligonucleotides) | D434G genotyping reporter | Transnetyx, Inc | ACCGTGGTGATGTCAA | Kcnma<sup>D434G</sup> |
| Sequence-based reagent (oligonucleotides) | H444Q genotyping primer (F) | Transnetyx, Inc | CTGTGGACACATTAC TCTGGAGAGTG | Kcnma<sup>H444Q</sup> |
| Sequence-based reagent (oligonucleotides) | H444Q genotyping primer (R) | Transnetyx, Inc | GGGTCTGGTGGCAGCA | Kcnma<sup>H444Q</sup> |
| Sequence-based reagent (oligonucleotides) | WT genotyping reporter | Transnetyx, Inc | TCTTACTTGTGAAGAAAG | Kcnma<sup>H444Q</sup> |
| Sequence-based reagent (oligonucleotides) | H444Q genotyping reporter | Transnetyx, Inc | CTCTTACTTCTGCAGAAAG | Kcnma<sup>H444Q</sup> |
| Genetic reagent (Mus musculus) | Kcnma<sup>T</sup>+/+ | PMID:15184377 DOI: 10.1074/jbc.M405621200 or available from the Jackson Laboratories | S<sup>lo</sup>+/+ (Meredith Lab) or Stock #035902 (B6.129(FVB)-Kcnma<sup>Tm1Rwa/J</sup>, Jackson Laboratories) | Breeder to generate Kcnma<sup>T</sup>+/+ |
| Sequence-based reagent (oligonucleotides) | WT genotyping primer (F) | Transnetyx, Inc | CATCATAACCGTGACCAGGA | Kcnma<sup>T</sup>+/+ |
| Sequence-based reagent (oligonucleotides) | WT genotyping primer (R) | Transnetyx, Inc | CCAAGAAGCCCACCACATG | Kcnma<sup>T</sup>+/+ |
| Sequence-based reagent (oligonucleotides) | WT genotyping reporter | Transnetyx, Inc | CCCGGCTGTCGCAGC | Kcnma<sup>T</sup>+/+ |
| Sequence-based reagent (oligonucleotides) | Neomycin genotyping primer (F) | Transnetyx, Inc | GGGCGCCCGTCTTTT | Kcnma<sup>T</sup>+/+ |
| Sequence-based reagent (oligonucleotides) | Neomycin genotyping reporter | Transnetyx, Inc | CCTCCTGCTACCTTCA | Kcnma<sup>T</sup>+/+ |
| Sequence-based reagent (oligonucleotides) | Neomycin genotyping primer (R) | Transnetyx, Inc | ACCTGTCCGGTGCCC | Kcnma<sup>T</sup>+/+ |
| Commercial assay, kit | miRNeasy Mini Kit | Qiagen | Cat. #217004 |
| Commercial assay, kit | Mouse Clariom D Assay | Applied Biosystems | Cat. #902514 |
| Chemical compound, drug | Paxilline | alomone labs | Cat. #P-450 |
| Chemical compound, drug | 4-Aminopyridine | Sigma-Aldrich | Cat. #275875 |
| Chemical compound, drug | TTX | alomone labs | Cat. #T-550 |
| Chemical compound, drug | Pentylenetetrazol | Sigma-Aldrich | Cat. #P6500 |
| Software, algorithm | pClamp10.7 | Molecular Devices |
| Software, algorithm | Transcriptome Analysis Console Software | ThermoFisher Scientific | TAC version 4.0.1 |
| Software, algorithm | Sirenia Acquisition software | Pinnacle Technology Inc | Version 2.2.4 |
| Software, algorithm | Sirenia Seizure Pro software | Pinnacle Technology Inc | Cat. #9037 |
| Software, algorithm | Prism Software | GraphPad (Dotmatics) | Prism version 9.02 |
| Software, algorithm | Ethovision Software | Noldus Information Technology | Ethovision XT version 11.5 |

**HEK cell patch-clamp electrophysiology**

The N999S (rs886039469; also numbered as N995S, N1036S, and N1053S in other reference sequences), D434G(rs137853333), and H444Q mutations were introduced into wild-type (WT) human...
hBKc 

cDNA sequence (MG279689) in the pcDNA3.1+ mammalian expression vector. Mutations were verified by sequencing. Channel constructs contained an N-terminal Myc tag and an EYFP tag in regulators of conductance of potassium 2 (RCK2) domain.

HEK293T cells (CRL-11268, ATCC, Manassas, VA) were cultured in media containing Dulbecco’s modified Eagle medium (Cat. #11995-065, Gibco, Life Technologies Corp., Grand Island, NY), 10% fetal bovine serum (Cat. #4135, Sigma-Aldrich, St. Louis, MO), 1% penicillin/streptomycin (Cat. #30-002 CI, Mediatech Inc, Manassas, VA), and 1% L-glutamine (Cat. #25-005 Cl, Mediatech Inc, Manassas, VA) and incubated with 5% carbon dioxide at 37°C. HEK cells were transfected with WT or mutant constructs using Trans-IT LT1 (Mirus Biological, Madison, WI) at 1:2 ratio of DNA and the reagent. After 4–12 hr, cells were re-plated onto glass coverslips pre-coated with poly-L-lysine (Cat. #P4832, Sigma-Aldrich, St Louis, MO). After 14–24 hr, recordings were performed. Constructs were allocated into the same cell transfections on the same days without blinding. HEK cells were authenticated at 17 STR loci with 94% profile match; Mycoplasma negative (ATCC Authentication Service). 

BK currents were recorded using inside-out patch-clamp at room temperature in symmetrical K+. One μM intracellular Ca²⁺ was used, a physiologically relevant Ca²⁺ condition near concentrations where altered gating behavior was manifested in prior studies (Li et al., 2018; Moldenhauer et al., 2020b; Moldenhauer et al., 2020a; Wang et al., 2009; Yang et al., 2010). Thin-walled borosilicate glass pipettes with resistances of 1–3 MΩ were filled with (in mM): 140 KMeSO₃, 2 KCl, 2 MgCl₂, and 20 HEPES. The internal (bath) solution contained (in mM): 140 KMeSO₃, 2 KCl, 2 MgCl₂, and 5 HEDTA with CaCl₂ added to achieve 1 μM free CaCl₂, pH adjusted to 7.2 with KOH. Free Ca²⁺ concentrations were calculated with WebMaxC: (https://somapp.ucdmc.ucdavis.edu/pharmacology/bers/maxchelator/webmaxc/webmaxcS.htm).

Macroscopic currents were recorded with a Multiclamp 700B amplifier, and signals were filtered at 10 kHz and digitized at 50 kHz using Digidata1440A and pCLAMP v10 software (Molecular Devices, Sunnyvale, CA). BK currents were activated with a voltage protocol stepped from a holding potential of −100 mV stepping to +250 mV with +10 mV increments for 30 ms and back to −100 mV for 15 ms to generate tail currents. G-V curves were generated from the tail currents 150–200 μs after the peak normalized to the maximum conductance (G/Gmax) and plotted against the activating voltage step (V). V₀.5 values were calculated from a Boltzmann fit of the G-V curves (Prism v9 GraphPad Software, San Diego, CA). Leak currents were compensated using a P/5 protocol with a subsweep holding potential of −120 mV.

Activation time constants were obtained from the same patches in Figure 1. τ was obtained by fitting the rising phase of the outward K⁺ current to single exponential function. For the deactivation kinetics, BK currents were elicited by +200 mV voltage command for 20 ms from a holding potential of −100 mV followed by 15 ms voltage steps from −200 to −10 mV with +10 mV increments. Deactivation time constants were obtained by fitting tail currents with single exponential functions. Leak currents were compensated using a P/5 protocol with a subsweep holding potential of −120 mV.

For experiments with dextroamphetamine and lisdexamfetamine, BK currents were recorded in inside-out patches in physiological K⁺ and 10 μM intracellular Ca²⁺ as described in Moldenhauer et al., 2020a. In voltage-clamp mode, patches were held at −150 mV, stepped from −150 to +150 mV for 30 ms (10 mV increments), and stepped back to −150 mV. Lisdexamfetamine dimesylate (catalog L-026, Supelco Millipore-Sigma) and dextroamphetamine (catalog 1180004, Millipore Sigma) were applied at 155 ng/ml, and paxilline (#2006; Tocris, Bristol, UK) was applied at 100 ng/ml. Current levels were assessed at baseline and 5 min after drug application and were normalized to control current levels for each patch.

Generation of Kcnma1N999S, Kcnma1D434G, and Kcnma1H444Q mouse lines

Heterozygous founders introducing N999S (AAT → AGT, exon 25), D434G (GAT → GGT, exon 10), and H444Q (CAC → CAG, exon 10) mutations into the mouse Kcnma1 gene (Gene ID:16531) were generated with CRISPR/Cas9 homologous recombination methods in the C57BL/6J strain (Figure 1—figure supplement 1). Kcnma1D434GWt and Kcnma1H444QWT mice were generated at the Transgenic Mouse Core at John Hopkins University (Baltimore, MD). Kcnma1N999SWT mice were generated at the Jackson Laboratory (Bar Harbor, ME). Transgenic mice were validated with Kcnma1 sequencing, and founders without additional non-synonymous mutations were bred with C57BL/6J for N1 progeny at The University of Maryland School of Medicine. Genotyping was performed at Transnetyx, Inc.
(Cordova, TN) using primers described in ‘Genotyping of Kcnma1^{N999S}, Kcnma1^{D434G}, Kcnma1^{H444Q}, and Kcnma1^{−/−} mouse lines’ section. Transgenic lines were backcrossed with C57BL/6J up to four generations (N4) for experimental cohorts with heterozygous progeny.

N2-N4 heterozygous mice were intercrossed to produce homozygous progeny. Transgenic heterozygous Kcnma1^{N999S/WT}, Kcnma1^{D434G/WT}, Kcnma1^{H444Q/WT} (as well as homozygous Kcnma1^{D434G/D434G} and Kcnma1^{H444Q/H444Q}) mice showed no gross differences in home cage behavior, body weights or gross morphology, or breeding. Kcnma1^{N999S/WT} × Kcnma1^{N999S/WT} heterozygous crosses produced either no viable pups (n=5/10 breedings) or no homozygous pups (n=56 pups). Additional crosses with Kcnma1^{−/−} heterozygous dams mated to Kcnma1^{N999S/WT} males (n=15 viable pups from five breedings) also produced no Kcnma1^{N999S} pups.

Kcnma1^{−/−} (Slo KO) mice were maintained on a C57BL/6J background (>N12 generation). Litter-mates of each genotype were produced from heterozygous Kcnma1^{+/−} × Kcnma1^{+/−} breeding pairs, as described previously (Meredith et al., 2004) using primer sequences described in ‘Genotyping of Kcnma1^{N999S}, Kcnma1^{D434G}, Kcnma1^{H444Q}, and Kcnma1^{−/−} mouse lines’ section. For all lines, male and female mice were separated by sex and group housed on a 12 hr light/12 hr dark cycle. Chow and water were provided ad libitum. Kcnma1^{N999S/WT} breeders were fed with high fat chow supplement to enhance breeding. All experiments were conducted in accordance with the University of Maryland School of Medicine Animal Care and Use Guidelines and approved by the Institutional Animal Care and Use Committee (Protocol #1120011). Sex-matched WT and transgenic littermates of both sexes were used for experimental procedures at the indicated ages. Experimenters were blinded to animal genotype at data collection and analysis.

**Genotyping of Kcnma1^{N999S}, Kcnma1^{D434G}, Kcnma1^{H444Q}, and Kcnma1^{−/−} mouse lines**

Genotyping was performed tail snips by TaqMan real-time PCR at Transnetyx, Inc (Cordova, TN) using the following: Kcnma1^{N999S} (Kcnma1-9 MUT probe set: (F) TCGGTTGCTTTAAGAATGCTT; (R) CCTCAGCTATTAGAGGCCTCGAGCTCT; WT reporter: CAGACATACTTCAATGACAATAT; N999S reporter: CAGACATATTCTAGTCAATAT), Kcnma1^{D434G} (Kcnma1-8 MUT probe set: (F) CAGACATACTTCAATGACAATAT; (R) CCTCAACTTTCTGGCAACA; D434G reporter: ACCGGATGTCAG), and Kcnma1^{H444Q} (Kcnma1-7 MUT probe set: (F) CCTTACTTTCTGGGAGAGT; (R) GGTTCTGTGGCCGAGCA; H444Q reporter: CTCTACTCTTCAGAAAG).

Kcnma1^{−/−} (Slo KO) mice were genotyped using the WT primer set (F) CATCATTCGCTTGACCATGGAG (ATGG; R) CCAAGAAAACCGCCACCACATG; WT Reporter: CCCGGCTGTGCCAG and Neomycin primer set (F) GGCGCGCGGTGTCT; (R) CCTCTGCTGACTCCATCTC; Neo Reporter: ACCGGTTGCCGCAGCCC).

**Patient data**

Patient phenotype and genetics data cited as ‘data not shown’ was exempt under University of Maryland School of Medicine Institutional Review Board (IRB) Non-Human Subjects Research (NHSR) Protocols HP-00083221, HP-00086440, and HP-00092434.

**Gene expression**

Unilateral hippocampus and the medial portion of cerebellum were extracted from 4-month-old mice, and directly put them in 1.0 mm diameter zirconium beads with 750 µl of trizol for bead homogenization. RNA was extracted using the miRNeasy Mini Kit (Qiagen, Valencia, CA) following the manufacturer’s protocol. To assess the RNA quality, RNA was quantified via RNA-40 nanodrop and OD 260/280 ratio of all samples were between 1.94 and 2.05. Integrity of RNA was examined via Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). RNA integrity number of all samples were >9. For transcriptome analysis, mouse Clariom D Assay (Applied Biosystems, Waltham, MA) was used following the manufacturer’s instruction. RNA extraction and array processing were done at the Genomics Core Facility, University of Maryland, Baltimore, MD. The raw microarray profiling data was preprocessed and quartile-normalized using the Transcriptome Analysis Console Software (version 4.0.1) (accessed on December 1, 2021). All samples passed
array quality control evaluation. Data normalization, differential expression, and hierarchical clustering analysis was performed with default parameters by an experimenter blinded to genotype and brain region.

**Hippocampal slice electrophysiology**

Three- to 4-week-old mice were anesthetized with isoflurane, and brains were removed and placed into ice-cold sucrose dissection solution (in mM): 10 MgCl₂, 26 NaHCO₃, 1.25 Na₃HPO₄, 3.5 KCl, 0.05 CaCl₂, 10 glucose, 200 sucrose, 1.2 sodium pyruvate, and 0.4 vitamin C, bubbled with 95% O₂ and 5% CO₂. The brain slices were cut coronally at 300 μm on a VT1000S vibratome (Leica Microsystems, Wetzlar, Germany) at 3–4°C. Slices containing hippocampus were incubated at 32°C for 30 min and kept at the room temperature in oxygenated artificial cerebrospinal fluid (ACSF) containing (in mM): 125 NaCl, 1.7 MgCl₂, 26 NaHCO₃, 1.25 Na₃HPO₄, 3.5 KCl, 2 CaCl₂, 10 glucose, 1.2 sodium pyruvate, and 0.4 vitamin C.

Whole-cell patch clamp recordings were performed in the dentate gyrus granule cells of the hippocampus using borosilicate glass electrodes pulled at 3–5 MΩ filled with (in mM): 123 K-MeSO₃, 9 NaCl, 0.9 EGTA, 9 HEPES, 14 Tris-phosphocreatine, 2 Mg-ATP, 0.3 Tris-GTP, and 2 Na₂ATP, pH adjusted to 7.3 with KOH, 290–300 mOsm. The low EGTA intracellular solution allows endogenous Ca²⁺ influx to activate BK channels (Fakler and Adelman, 2008; Jackson et al., 2004; Müller et al., 2007; Whitt et al., 2016; Whitt et al., 2018). The slices were perfused with oxygenated ACSF at room temperature during the recordings. Granule cells in the DG were visualized with a Luca-R DL-604 EMCCD camera (Andor, Belfast, UK) under IR-DIC illumination on an FN1 upright microscope (Nikon, Melville, NY). Current- and voltage-clamp recordings were made with a Multiclamp 700B amplifier, and signals were filtered at 10 kHz and digitized at 50 kHz using Digidata1440A and pCLAMP v10 software (Molecular Devices, Sunnyvale, CA). All data were corrected for liquid junctional potential (10 mV).

In current-clamp recordings, the resting membrane potential was measured without any current input after a whole-cell mode was made. If the initial resting membrane potential was more depolarized than −70 mV or a series resistance was larger than 20 MΩ, the recording was not proceeded. The membrane potential was maintained at around −80 mV by injecting positive currents. Firing frequency was obtained from running a single protocol containing 1 s step current injections (0 to +400 pA, 20 pA increment, 10 s ISI). Bridge balance was used. The input resistance (Rᵢ) was measured with a linear regression of voltage changes from 400 ms hyperpolarizing current injections (−40 to −10 pA in 10 pA increments). The membrane time constant was calculated from the averaged traces to 20 consecutive hyperpolarizing current pulses (−20 pA; 400 ms) with a single exponential function (Lopez et al., 2012). If the series resistance (Rs) or membrane capacitance (Cᵣ) was changed more than 20% over the recording, the cell was not further analyzed.

In voltage-clamp recordings, Rs was compensated at least 60%. BK currents were measured by subtracting currents with 10 μM paxilline from the total current in the presence of 1 μM tetrodotoxin (TTX) and 2 mM 4-aminopyridine (Montgomery and Meredith, 2012). Cells were held at −90 mV, 150 ms voltage step of −100 to +30 mV in 10 mV increments was applied and stepped back to −90 mV for 130 ms. Three current traces were averaged for analysis, and leak currents were subtracted using the P/4 method with a subsweep holding potential of −90 mV. Paxilline was applied to the slice using a local perfusion pencil for at least 10 min prior to the second recording. BK current levels were obtained from the peak and normalized to cell capacitance. No paxilline-sensitive current was present in Kcnma1−/− dentate granule neurons (n=2).

**Action potential waveform analysis**

Action potential amplitude was defined as the difference between the peak and threshold. Half width (tw1/2) was the width of action potential at 50% of the peak amplitude from the action potential threshold. The amplitude of fAHP was defined as the voltage change from the action potential threshold to the most negative voltage (AHP anti-peak) after repolarization. The fAHP decay was measured as the depolarization rate from the AHP anti-peak over the first 3 ms (ΔV/3 ms). Action potential thresholds, defined as the membrane potential where the first derivative reached 10 mV/ms, were not different between genotypes for any mouse line (data not shown).
EEG and EMG recordings

Behavioral observations, surgeries, EEG and EMG recordings and data analyses were performed blinded to experimental condition and genotype. After daily monitoring of behavioral signs of seizures, 2- to 4-month-old mice were implanted with dural electrodes, with or without EMG lead implantation at the dorsal clavotrapezious neck muscles behind the base of the skull Pinnacle Technology 4 channel EEG system, Lawrence, KS (Iffland et al., 2020). Following a minimum of 72 hr of recovery period, video-EEG recordings were obtained using Pinnacle Technology Sirenia Acquisition software for 24 hr at a sampling rate of 2 kHz. Mice were visually monitored during seizures and behavioral responses were scored using modified Racine criteria: (1) raised tail and/or abnormal posturing; (2) myoclonic movement of a limb, favoring one side; (3) brief tonic-clonic convulsive episodes (approx. 1–5 s); (4) tonic-clonic seizures associated with rearing or jumping; and (5) status epilepticus (Lüttjohann et al., 2009; Van Erum et al., 2019). Continuous EEGs were manually reviewed for interictal epileptiform discharges and/or spontaneous seizures. Interictal epileptiform discharges were defined as discrete and sharply contoured discharges (e.g., spike and wave). Seizures were defined as at least 10 s of sharply contoured and rhythmic bursts of activity.

Seizure thresholds were defined in response to a single chemoconvulsant challenge in naïve animals. A baseline 15 min video-EEG recording was obtained, followed by injection of 40 mg/kg of PTZ (Sigma, Cat. #P6500, 10 mg/ml stock in sterile saline) and an additional 30 min recording after injection. Thirty minutes post-PTZ injection, the experiment was terminated, and mice were euthanized by CO₂ asphyxiation and thoracotomy. Video-EEG and EMG data were analyzed using Sirenia Seizure Pro (Pinnacle Technology, Lawrence, KS). Experimenters were blinded to experimental group during PTZ-induced seizure, observation, and data analysis. Seizure threshold was defined as the latency to first seizure after PTZ injection. Total EEG power was reported as the sum of all frequency bands. EEG traces were examined visually for significant artifacts, and EEGs resulting in anomalous power data were excluded from the analysis, defined as EEGs with high amplitude movement artifacts (>16,000 µV²/Hz) or low amplitude signals (<750 µV²/Hz). EMG data were analyzed by manual review and the longest durations of attenuated EMG activity were scored. Attenuated EMG activity was defined as at least 1 s of EMG activity that was lower in amplitude than pre-PTZ injection baseline.

Stress-induced dyskinesia assays

Two- to 3-month-old mice were used in all assays for N999S and D434G cohorts. Two- to 8-month-old mice were used for Kcnma1°/° cohorts due to breeding difficulties (Meredith et al., 2004). For acute stress-induced dyskinesia evaluation, the total restraint time was 5 min. Mice were restrained for 2.5 min by hand, clasping the dorsal cervical aspect between the index finger and thumb and the tail with the pinky finger, with the mouse dorsal side flat against the palm in a vertical upright position. Afterward, the tail was released, leaving only the upper body restrained for 2.5 min. In separate experiments, dextroamphetamine (d-amp) was administered via intraperitoneal (IP) injection at 0.5 mg/kg, or an equivalent volume of saline, to a final volume of 5 ml/kg. Syringes were prepared by a second blinded experimenter. Thirty minutes after injection, mice were restrained in a plexiglass tube (MH-100, IBI Scientific, Dubuque, IA) for 5 min.

After restraint, mice were placed in a transparent 1000 ml beaker under video recording for 5 min. Behavioral parameters were modified from stereotypic behavioral scoring (Kelley, 2001) and prior dyskinesia mouse models (Khan et al., 2004; Khan and Jinnah, 2002; Sebastianutto et al., 2016; Shirley et al., 2008) and were manually scored from side-view videos totaled for grooming time, or the number of occurrences of rearing, sniffing, circling/hyperactivity, twisting/choreiform movement, tonic-clonic movement, flattened/dystonic posturing, trembling, listing, and falling. Immobility/behavioral arrest was defined as lack of positional and translational movement from the body, limbs, and head, excluding involuntary movement from respiration. Episodes were cumulatively timed to obtain the total immobility in 5 min. Videos from d-amp-injected mice were additionally subjected to automated analysis (EthoVision XT 11.5, Noldus) by a third blinded experimenter. Movement tracking was calibrated from side-view video to beaker dimensions: rim = 11.110 cm, base = 10.795 cm, and height = 15.875 cm. Immobility time was obtained from the center point movement parameter with automated body detection. Mice were considered mobile with center point velocities exceeding
0.151 cm/s, and immobility time was obtained from intervals where the center point velocity was below 0.050 cm/s.

**Open field activity**
Mice were acclimated in the testing room 1 hr prior to assays. Each mouse was placed in the open arena (70 × 30 × 25 cm³, *Cover et al., 2019* for 15 min. Mouse movement and total distance was analyzed in EthoVision XT (Noldus).

**Wheel running activity**
Mice were placed in housing cages with running wheels (Coulbourn Instruments) on a standard 12:12 hr light-dark cycle for 48 hr with ad libitum access to food and water. Wheel activity was measured via magnetic switches and recorded using ClockLab software (Actimetrics). Individual mouse wheel rotation counts were then quantified in 1 min bins in ClockLab software running in Matlab v6.1 (Mathworks). The following parameters were calculated for the 12 hr dark phase as average measurements: speed, maximum speed, number of activity gaps (defined as consecutive 1 min bins registering 0 rpm), activity gap duration, and maximum activity gap duration. All parameters were calculated by a custom python script (code provided as a source data file).

In separate mice, baseline wheel activity recording was collected (30 min), followed by 0.5 mg/kg d-amp or saline injections as described in the previous section. Thirty minutes after injection, each mouse’s post-treatment activity count (30 min) was normalized to the baseline.

**Rotarod**
Motor coordination was tested by rotarod over 7 days of trials on an accelerating rod. Mice were acclimated to the testing environment in their normal housing cages for 1 hr prior to testing. Mice were trialed three times a day for 7 consecutive days under video capture. Body weight was measured on days 1 and 7. After placement on the rotarod (IITC Life Science Inc, Rat Mouse Rotarod), mice were acclimated on the apparatus for 30 s prior to first trial. During the acclimation, mice were allowed to fall off up to two times. Rod acceleration was 4–40 rpm over 5 min for each trial. Mice were given a 2 min inter-trial interval. ‘Fall’ was called when the mice fell off from the rod or made a 360 degree revolution around the rod. Three trials per day were averaged for each mouse.

Hypotonia and PNKD-like paroxysms would be expected to produce extremely short latencies to fall, since a single major loss of tone would be catastrophic for coordination. Alternately, baseline coordination could be impaired in the absence of immobility. Among the individual mice, there was no explicit evidence for individual trials with extremely short fall latencies that would be consistent with the triggering of sudden hypotonic events. However, partial loss of tone might be compensated for by the high level of attention induced in this assay. Compared to the voluntary running wheel activity, successful navigation of the rod’s surface and rotation requires a higher degree of attention to motor coordination than the wider home cage wheel. Thus, mice fall off the rotarod at lower speeds than the maximum speeds achieved on the voluntary running wheel.

**Hanging wire**
Acute muscle strength was tested by hanging mice using their limbs. Mice were acclimated to the testing environment in their home cages for 1 hr prior, and body weights were measured prior to the start of testing. Three consecutive trials were then performed in 1 day. Mice were placed right-side-up on a standard cage lid with parallel metal bars, the lid was gently shaken three times to provoke grasping the bars, then the lid was slowly inverted to put mice in the hanging position. Trial duration maximum was 120 s. A 10 s inter-trial interval was given after falling. Mice remaining hanging up to the maximum time were inverted to right-side-up on the lid and given a 10 s interval before the next trial. Most WT mice can hang on for several minutes before losing grip and falling (*Jones and Mormède, 2000*), although each WT control cohort exhibited a wide range of latency to fall values. No sudden hypotonia was observed, and there was a spread in the range of *Kcnma¹¹¹⁹⁹⁹⁵/WT* values. This observation suggests it unlikely that the hanging wire assay was a significant trigger for PNKD-like immobility in *Kcnma¹¹¹⁹⁹⁹⁵/WT* mice, potentially due to the short duration compared to the full restraint stress used in *Figure 5*.
Statistics
Sample sizes were determined based on prior datasets, except the stress-induced behavioral assay where power calculation based on pilot data indicated sample sizes of 5 animals per group (effect size d=2.29 at 0.8 power with 0.05 α; G*power 3.1). Electrophysiology and behavioral data were tested for normality with Shapiro-Wilk normality test and either parametric or non-parametric statistics were analyzed in GraphPad Prism v9.02 (San Diego, CA). Outliers were determined by the ROUT method and were included in all datasets. Data are plotted as either mean ± SEM, or individual data points with median and inter-quartile range, as indicated in figure legends. The statistical test used for each dataset is indicated in the figure legend, and p<0.05 was considered significant. p-Values in figure legends are reported for post hoc tests when the main effect was p<0.05, or reported for the main effect, if p>0.05. Groups with three or fewer data points were not included in statistical analysis, as noted in legends. For parametric data, two-tailed, unpaired t-tests were performed with Welch’s correction for unequal variance. For multiple comparisons, one-way ANOVAs were performed with Welch’s correction followed by Dunnett’s T3 post hoc test. Two-way repeated measures ANOVAs were performed with Geisser-Greenhouse correction followed by Bonferroni post hoc test for multiple comparisons (comparisons between genotypes across voltages). Mann-Whitney or Kruskal-Wallis followed by Dunn’s multiple comparisons were used for non-parametric data.

In gene microarray studies, differential mRNA transcript expression was determined at a 2-fold change cutoff, with p>0.05 and false discovery rate, FDR = 0.99 using an ANOVA with an eBayes test was used (Ritchie et al., 2015) (Applied Biosystems Transcriptome Analysis Console (TAC) Software v4.0.1).

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Su Mi Park, Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, established the transgenic lines, performed HEK cell recordings, and neuronal recordings., Supervision, Validation, Visualization, Writing – original draft, Writing – review and editing; Cooper E Roache, performed locomotor assays, data analysis, and animal husbandry., Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Validation, Visualization, Writing – original draft; Philip H Iffland, Conceptualization, Data curation, Formal analysis, Investigation, Methodology, performed EEG recordings., Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review and editing; Hans J Moldenhauer, Conceptualization, Data curation, Formal analysis, performed HEK cell recordings with d-amp and lis, animal husbandry, and provided electrophysiology analytical methodology., Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – review and editing; Katia K Matychak, Conceptualization, Data curation, Formal analysis, Investigation, assisted with EEG recordings and animal husbandry. Validation, Visualization, Writing – review and editing; Amber E Plante, established the transgenic lines and performed animal husbandry, Investigation, Methodology, Project administration, Validation, Writing – review and editing; Abby G Lieberman, performed open field assays and Ethovision analysis., Investigation, Methodology, Project administration, Validation, Writing – review and editing; Peter B Crino, Funding acquisition, Investigation, provided EEG recording set ups., Project administration, Resources, Supervision, Writing – review and editing; Andrea Meredith, provided KCNMA1 sequences, patient data, designed the transgenic models, and provide analytical methods in electrophysiology and motor function., Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review and editing

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Ethics
All experiments were conducted in accordance with the University of Maryland School of Medicine Animal Care and Use Guidelines and approved by the Institutional Animal Care and Use Committee (Protocol #1120011).

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Additional files

Supplementary files
• Transparent reporting form
• Source code 1. Locomotor wheel activity script. Python code to calculate wheel speed and the number and duration of activity gaps.

Data availability
All data generated and analyzed during this study are included in the manuscript, or provided as source data files. Python code is provided as Source Code File 1.
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