Impaired M-current in KCNQ2 Epileptic Encephalopathy Evokes Dyshomeostatic Modulation of Excitability in Patient-Derived Neurons

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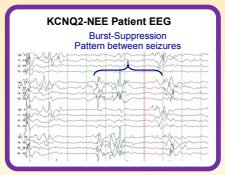
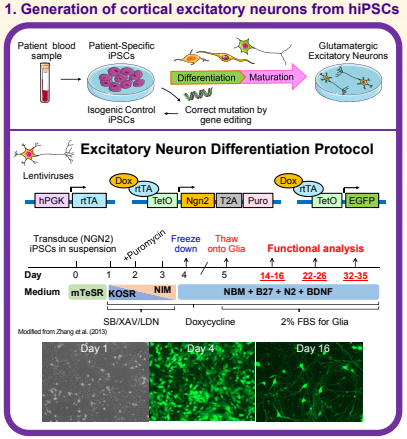
Introduction

Mutations in *KCNQ2*, which encodes a pore-forming K^+ channel subunit responsible for neuronal M-current, have been associated with neonatal epileptic encephalopathy (NEE). This complex disorder manifests as severe early-onset seizures and impaired neurodevelopment due to an imbalance in neuronal circuit activity in the brain. While the effects of *KCNQ2* mutations have been studied extensively in heterologous expression systems, their effects on the inherent properties of human neurons have not. Specifically, what remains unclear is how the likely defects in M-current affect the electrophysiological properties of human neurons during a critical period of neuronal maturation.

Here, we use KCNQ2-NEE patient-specific and isogenic control iPSC-derived excitatory neurons to elucidate the dynamic functional effects of a prototypical *KCNQ2* mutation. We have found that while patient-derived excitatory neurons exhibit reduced M-current early, they develop intrinsic and network hyperexcitability progressively over time in culture. This hyperexcitability is associated with faster action potential repolarization, larger afterhyperpolarization, and a functional enhancement of large conductance Ca^{2+}-activated K^+ (BK) channels. These properties facilitate a burst-suppression firing pattern that is reminiscent of the interictal electroencephalography pattern in patients. Importantly, we were able to phenocopy these excitability features in control neurons only by chronic but not acute pharmacological inhibition of M-current. Our findings suggest that dyshomeostatic mechanisms compound KCNQ2 loss-of-function and lead to alterations in the neurodevelopmental trajectory of patient-derived neurons. Our work has therapeutic implications in explaining why KCNQ2 agonists are not beneficial unless started at an early stage of the disease.

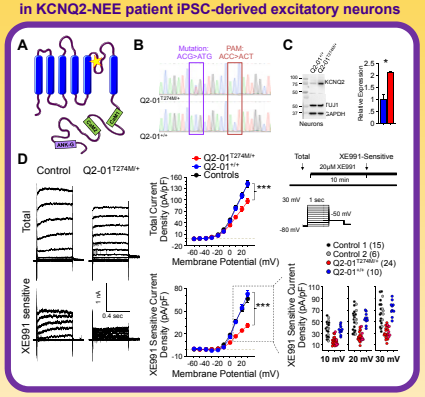


1. Generation of cortical excitatory neurons from hiPSCs

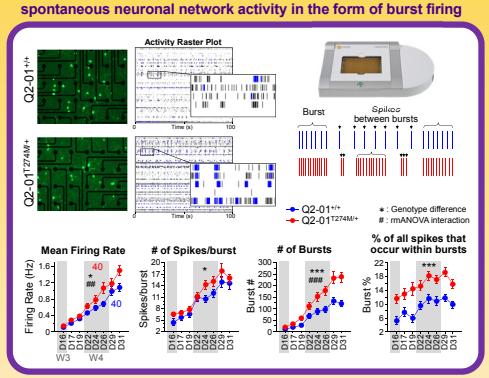


Results

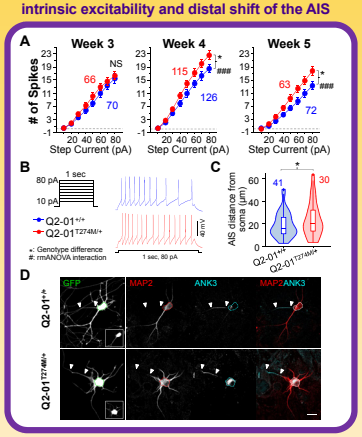
2. Reversing KCNQ2 T274M mutation rescues M-currents in KCNQ2-NEE patient iPSC-derived excitatory neurons



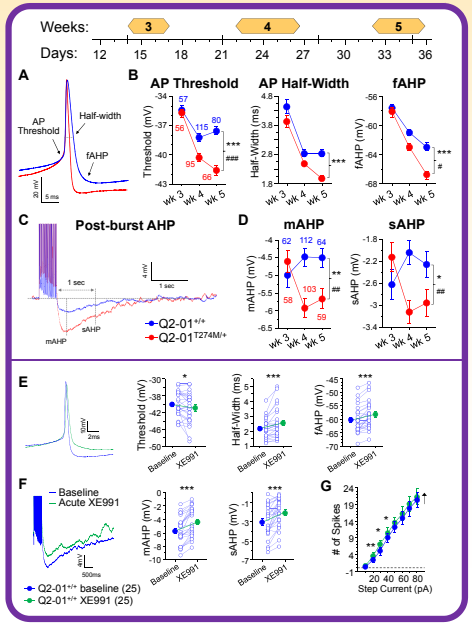
3. KCNQ2-NEE patient neurons exhibit progressive enhancement of spontaneous neuronal network activity in the form of burst firing



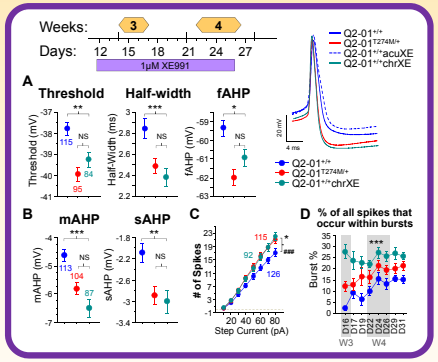
4. Patient neurons exhibit progressive increase of intrinsic excitability and distal shift of the AIS



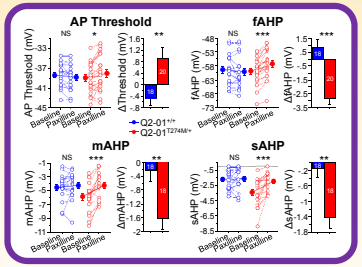
5. Patient neurons have enhanced AP repolarization and post-burst AHP, opposite of the effect of acute M-current inhibition



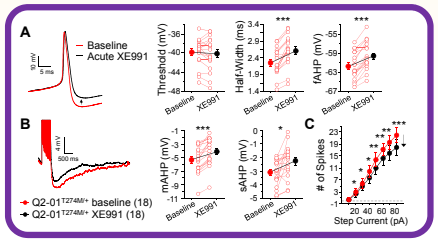
6. Chronic M-current block phenocopies patient excitability



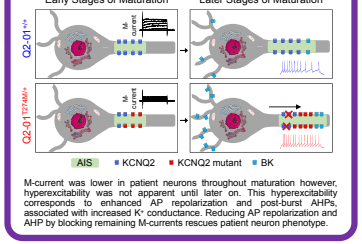
7. KCNQ2-NEE neurons exhibit a dyshomeostatic increase in BK channel function



8. Acute M-current inhibition rescues patient derived-neuron excitability phenotype



KCNQ2 dysfunction evokes dyshomeostatic enhancement of other K^+ channels over time in culture, contributing to hyperexcitability in KCNQ2-NEE patient neurons



M-current was lower in patient neurons throughout maturation however, hyperexcitability was not apparent until later on. This hyperexcitability corresponds to enhanced AP repolarization and post-burst AHPs, associated with increased K^+ conductance. Reducing AP repolarization and AHP by blocking remaining M-currents rescues patient neuron phenotype.

Methods

Subjects: KCNQ2-NEE patient with loss of *KCNQ2* function mutation (T274M), CRISPR/Cas9 generated isogenic control and 2 healthy control iPSC lines differentiated to cortical excitatory neurons (Fig. 1). All iPSC lines exhibited normal male karyotype, typical stem cell morphology, and expressed pluripotency markers.

Electrophysiology: Whole-cell current- and voltage-clamp recordings in neurons, bath perfused with bubbled aCSF at 34°C; K-MeSO$_4$ pipette solution.

Patch protocols: XE991 sensitive current density was subtracted from total current density to obtain M-current density measures. Current-clamp: V_h=-65mV; fAHP and AP half-width measures taken at half AP amplitude from baseline during a current injection ramp. AHPs induced by 25 suprathreshold 2 ms current pulses at 50 Hz. No significant difference in resting membrane potential, input resistance, series resistance, AP amplitude.

MEA recordings: Spontaneous activity recorded 5 min/day in culture using Maestro MEA system (Axion BioSystems).

Data Analysis: Custom written MATLAB scripts; Statview for statistical analyses for patch clamp data; Axion biosystems software for MEA analysis.

Conclusions

M-current

- Excitatory cortical neurons differentiated from KCNQ2 epileptic encephalopathy patient iPSCs exhibited reduced M-currents compared to healthy controls. This was rescued by CRISPR/Cas9 gene editing to correct the T274M KCNQ2 mutation.

Excitability

- Patient neurons exhibit progressive enhancement of spontaneous activity restricted to phasic burst firing on MEAs.
- Patient-derived neurons exhibit enhanced AP firing frequency/I_{step} during week 4 and 5 but not week 3.

Morphology

- The axon initial segment (AIS) in patient neurons was distally shifted from the soma, a potential response to hyperexcitability.

Intrinsic AP properties

- Patient neurons exhibit reduced AP thresholds, faster AP repolarization and enhanced post-burst AHPs over time in culture, indicative of an enhancement of K^+ conductance.

Pharmacology

- Acute block of M-currents with XE991 slowed AP repolarization, reduced post-burst AHPs and increased excitability in control neurons.
- Chronic but not acute inhibition of M-current, in control neurons, phenocopied patient neuron intrinsic and network excitability properties.
- Larger effect of paxilline on AP properties in patient neurons suggests enhanced BK channel function.
- Acute inhibition of remaining M-currents in patient neurons reduced AP repolarization and AHPs and rescued hyperexcitability.

While the defect is in KCNQ2, these channels are not directly responsible for the functional defect producing the type of hyperexcitability exhibited by patient neurons. Dyshomeostatic mechanisms compound KCNQ2 channel dysfunction leading to hyper-excitability and morphological changes.

Acknowledgments: Shoai Hattori for MATLAB scripts.

Supported by: NU Dixon Translational Award, Dravet foundation (EK), NIH grant NS032387 (ALG), Axion Biosystems travel award (DS).