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Feinberg School of Medicine

Mouse Models of Genetic Epilepsies

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Genomic Medicine Institute
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Disclosures

- None directly related to this presentation

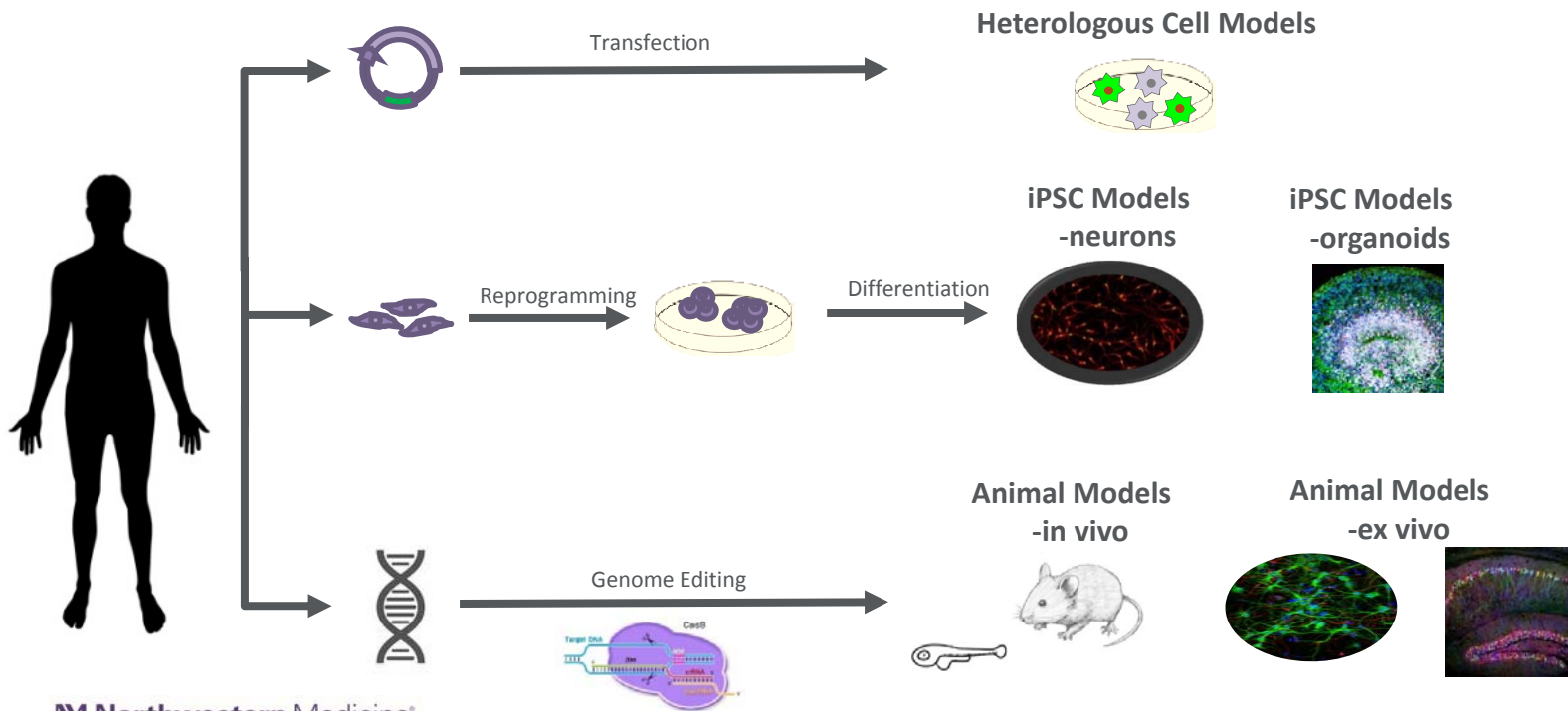
Learning Objectives

At the conclusion of this session, participants will be able to understand that

- Functional effects of epilepsy-associated genetic variants can be ascertained in a variety of model systems
- Characterizing the effects of genetic variants in animal models can elucidate pathogenic mechanisms and suggest targeted therapeutic strategies

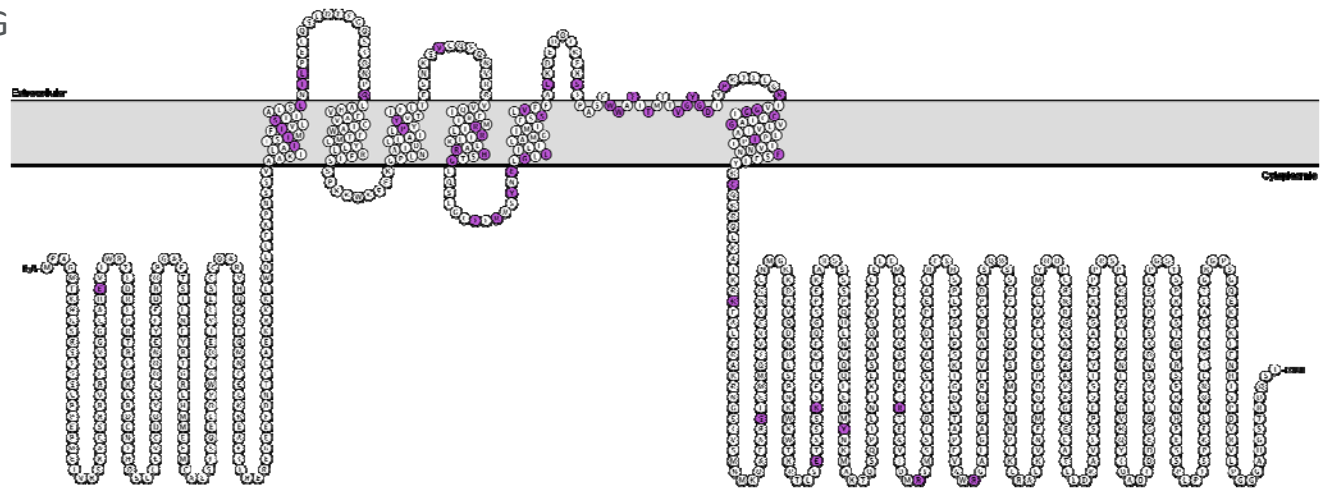
Preclinical Models for Precision Medicine

Genetic Epilepsies



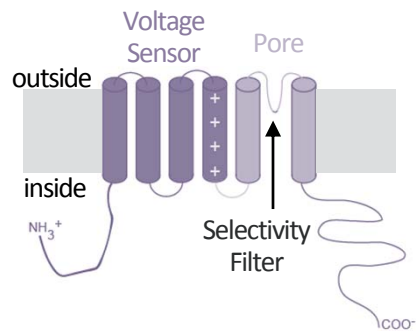
KCNB1 – Developmental & Epileptic Encephalopathy (DEE)

- *De novo* pathogenic variants in *KCNB1*
 - Developmental delay / ID
 - Infant/early childhood onset epilepsy in most patients
 - Multiple seizure types
 - Often drug-refractory
 - Abnormal interictal EEG
 - Features of ASD
 - Behavioral issues
 - Motor dysfunction

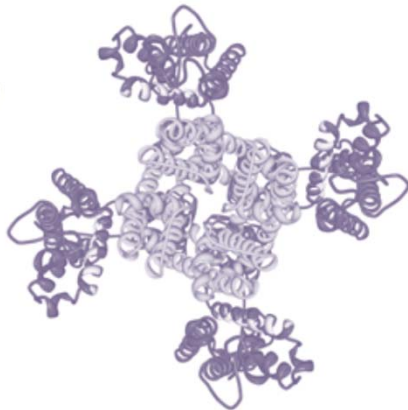


KCNB1 - Kv2.1 Voltage-Gated K⁺ Channel α subunit

Tetrameric Channel



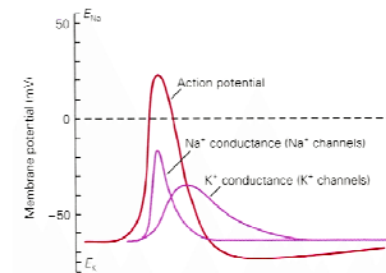
Top View



Multi-Functional Protein

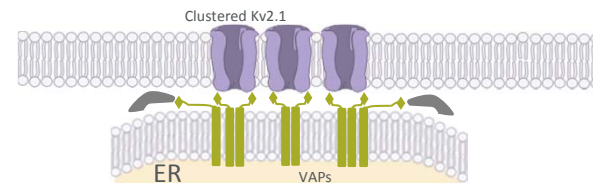
- **Conducting**

- Delayed rectified K⁺ currents



- **Non-conducting – dense clusters**

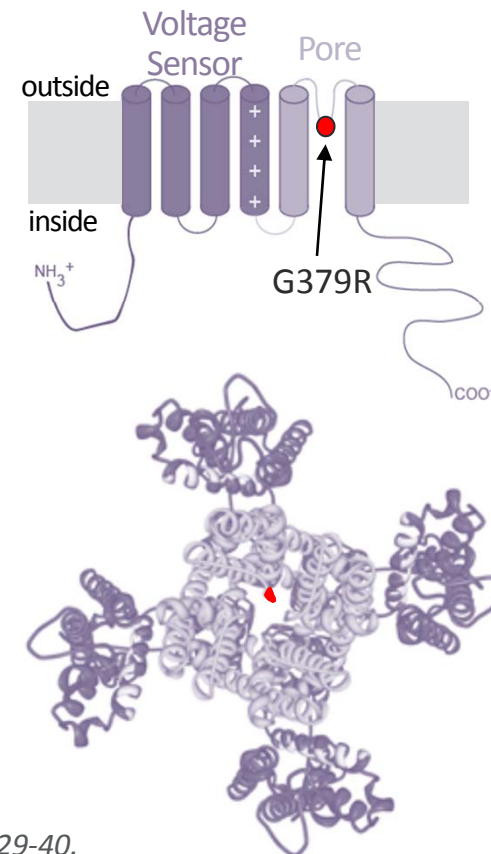
- Ca⁺⁺ homeostasis
- Vesicular exocytosis
- Membrane protein trafficking



KCNB1 Developmental and Epileptic Encephalopathy

p.G379R

- 4 yo male
- Seizure onset at 8 months
 - Infantile spasms w/hypsarrhythmia
 - Multiple seizure types – pharmacoresistant
- Severe global DD / ID
- ASD / atypical Rett syndrome
- Truncal hypotonia
- Ataxia



KCNB1 DEE – p.G379R

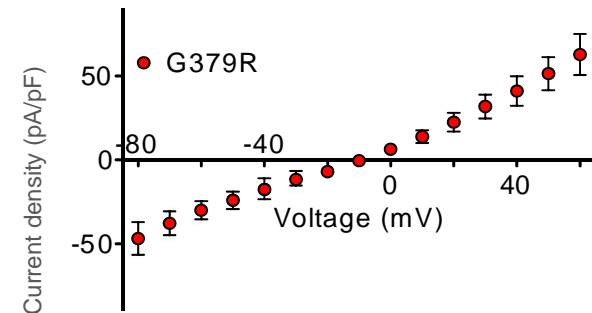
- Critical for determining K⁺ selectivity
- Evolutionarily invariant residue

	Pre-pore Transmembrane	Turret	Pore Helix	Select.	Extend.	Post-pore Transmembrane
Human	LLILFLAMGIMIFSSLVFFAEKDED--DTKPKSIPASFWWATITMTTVGYGDIYPKTL			RI 379		LGKIVGGLCCIAGVLVI
Gorilla
Dog
Mouse
Chicken
Platypus
Fugu
Marine_Worm	•M•.....•AY•.....P--N••N•.....DT•••G•.....•V•E•I•.....V•SV••C•.....					
Drosophila	•M•.....VL••AY•.....K---V•.....ET••G•.....•T•A•.....VI•TV••C•.....					
Sea_Squirt	•M•.....V••AY•.....M•N--AEM•N•.....•V•.....L•AT••T•.....					
c.Elegans	•VF•IL•V•AA••YY••M•ANPNQ•Q•.....LGL••IC•.....MT•H•SF•RL•S•AVM••T•.....					
KcsA	•VIV•LA---G•Y•AVL•R--GAPGAQLITY•RAL•SVE•A•.....L•V••W•RL•AVVVMV•ITSF					

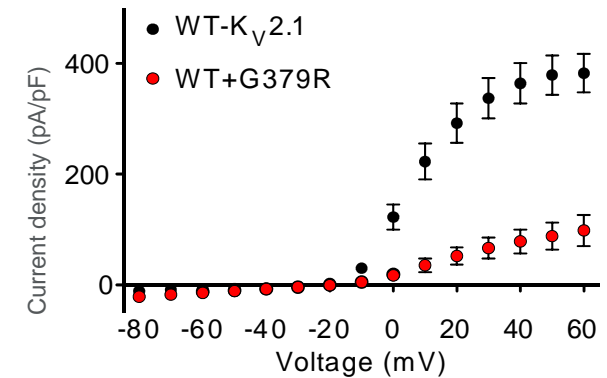
- Voltage clamp recording in CHO-K1 cells



Homomer

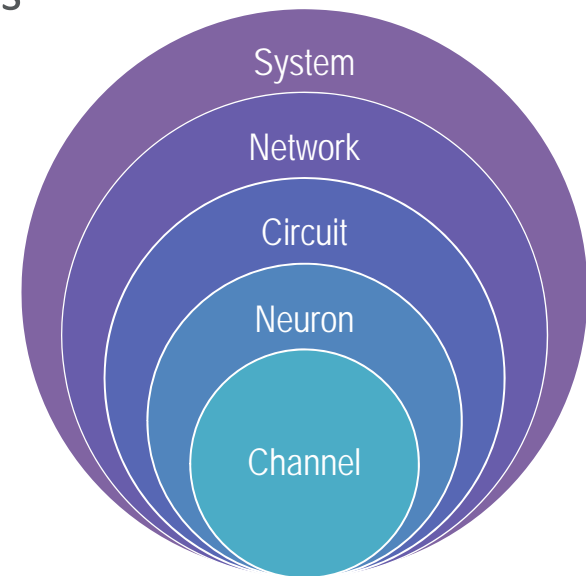


Heteromer



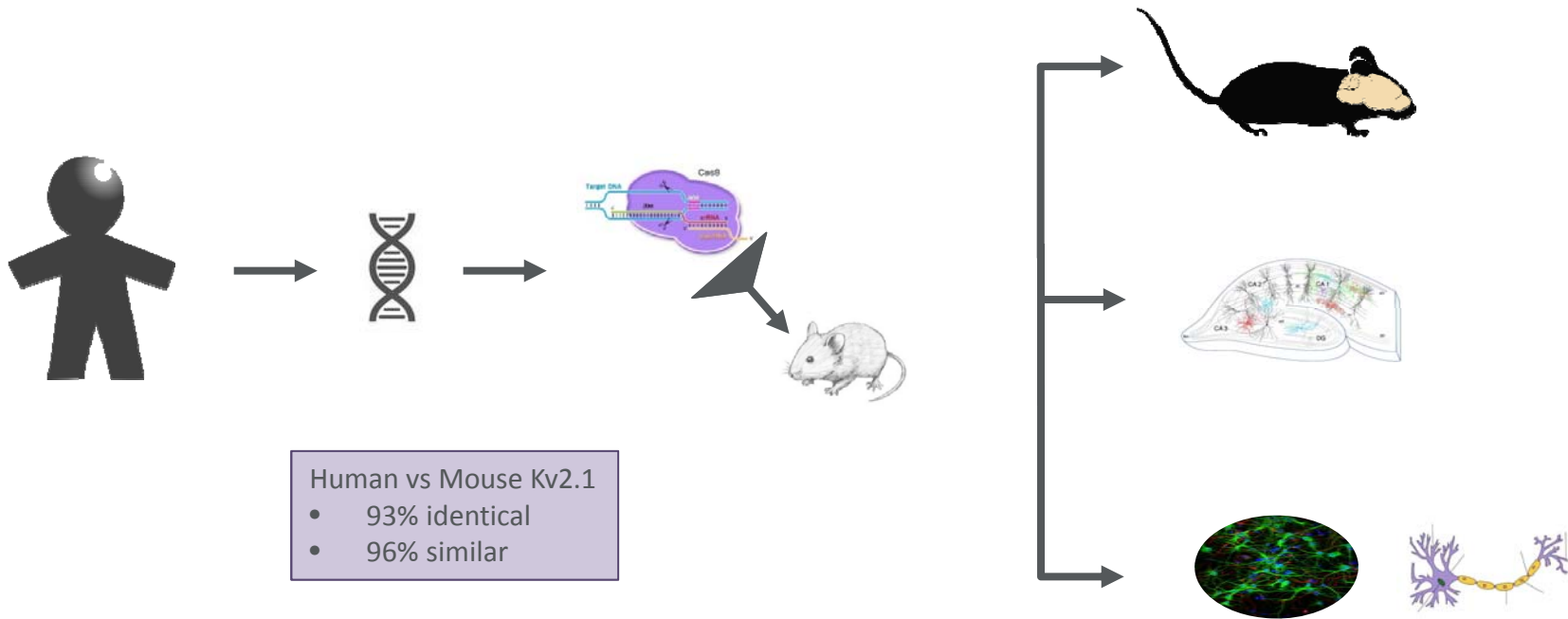
Challenges to interpreting the effect of variants

- Effect of variant on channel \neq effect at higher levels of analysis
- Complex molecular composition of neurons
- Plasticity of nervous system
- Neurodevelopmental evolution
- Pleiotropic effects

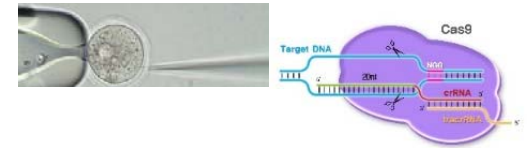


Mouse Models for Genetic Epilepsies

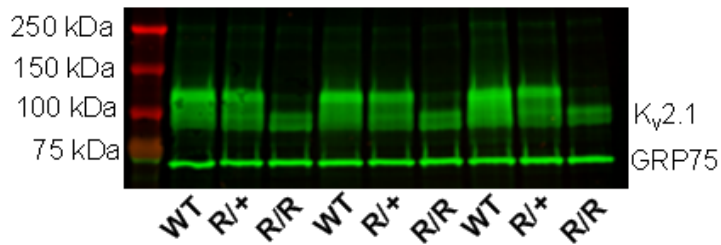
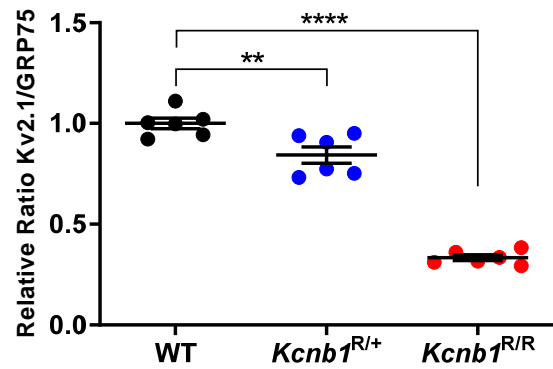
CRISPR/Cas-Mediated Genome Engineering



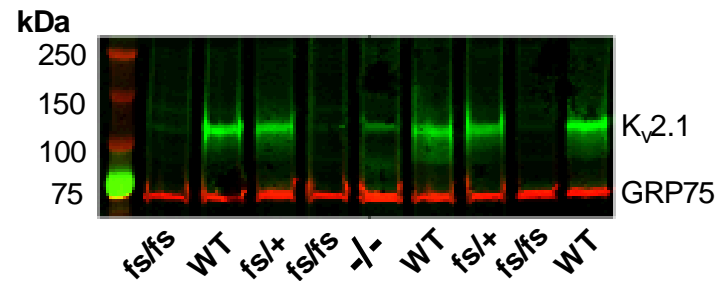
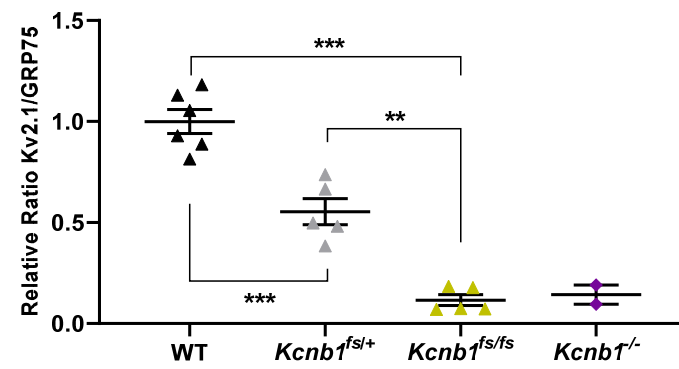
Kcnb1 Mouse Models



Kcnb1^{G379R} Knock-in



Kcnb1^{G379VfsX6} Frameshift

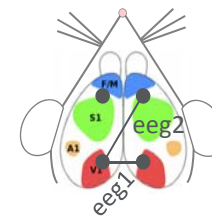
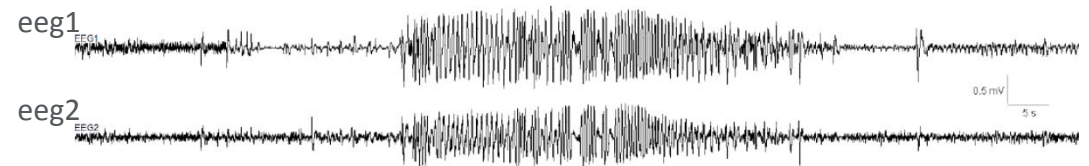


Kcnc1^{G379R} Mice – Seizures



Nicole Hawkins

- Spontaneous and Handling-Induced Generalized Tonic-Clonic Seizures



Kcnc1^{G379R} Mice - EEG

Abnormal background EEG

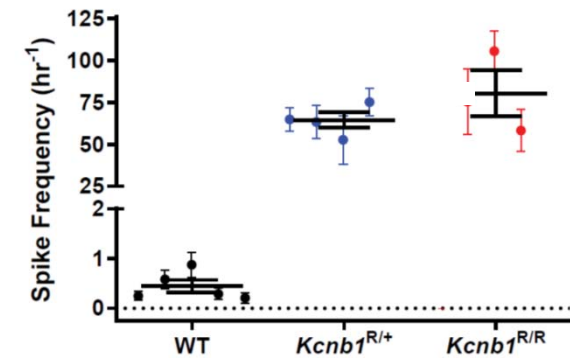
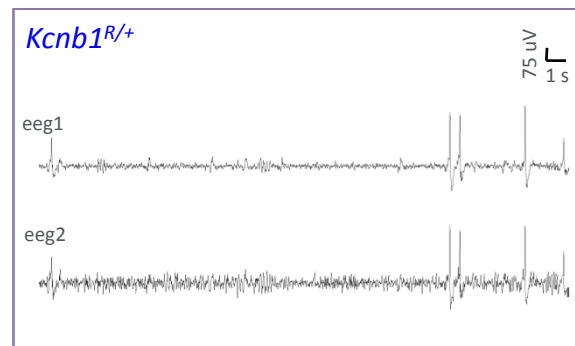
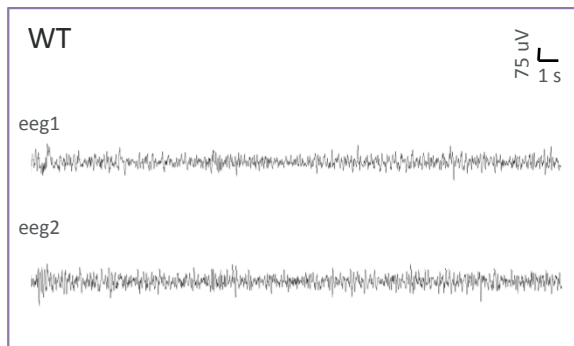


Nicole Hawkins



Sunita Misra

- R/+ - Isolated spike and slow wave complexes
- R/R – Isolated spike and slow wave complexes + recurrent runs (1-2 Hz)



Kcnc1^{G379R} Mice – Seizure Susceptibility

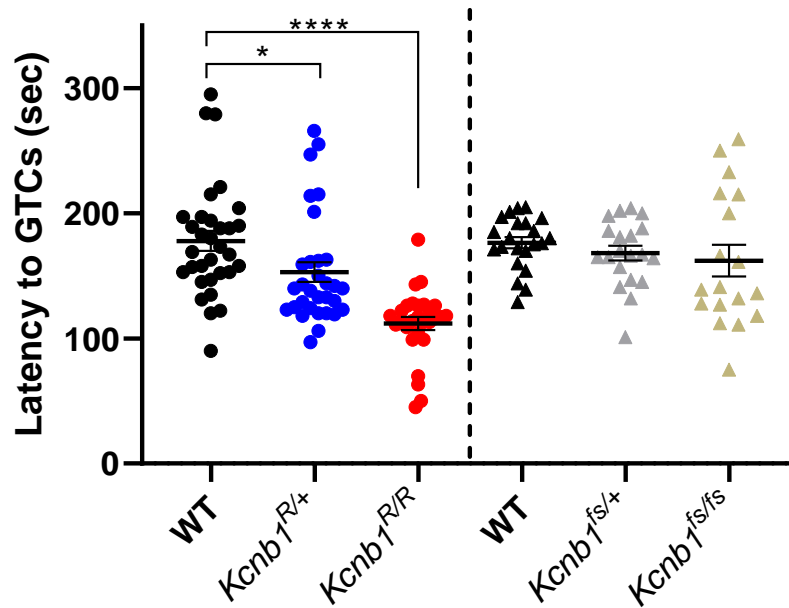
Flurothyl – GABA antagonist



Alex Huffman



Nicole Hawkins



- Lower threshold vs WT
 - *Kcnc1*^{R/+}
 - *Kcnc1*^{R/R}
- No difference vs WT
 - *Kcnc1*^{fs/+}
 - *Kcnc1*^{fs/fs}

Kcnb1^{G379R} Mice - Behavioral Phenotyping

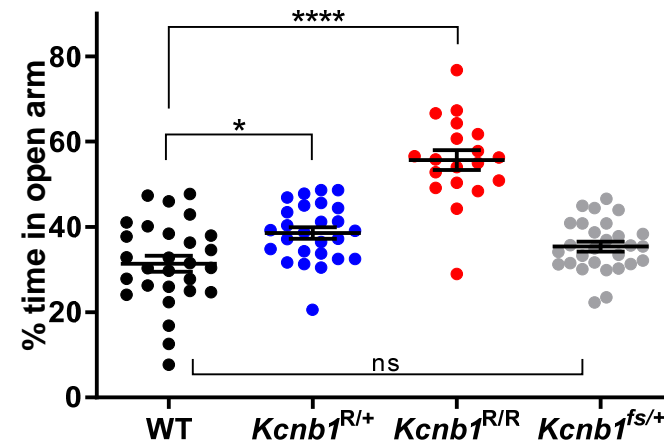
Zero Maze Assay

- Unconditioned approach/avoidance
- Anxiety-like behavior



Manny Jurado

Zero Maze - Open Arm Time



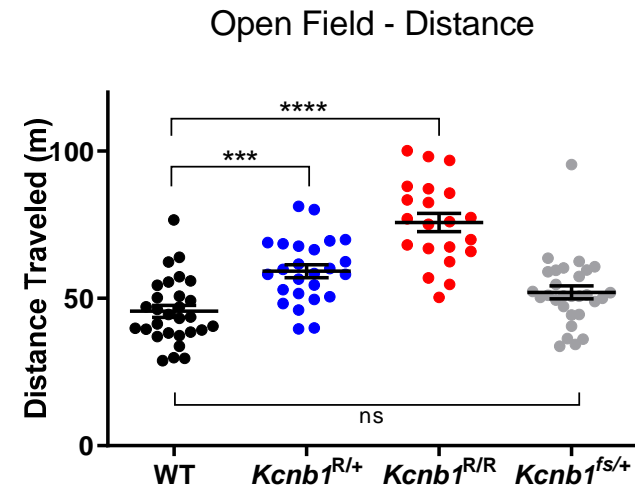
Kcnb1^{G379R} Mice - Behavioral Phenotyping

Open Field Test



Manny Jurado

- General motor function/activity

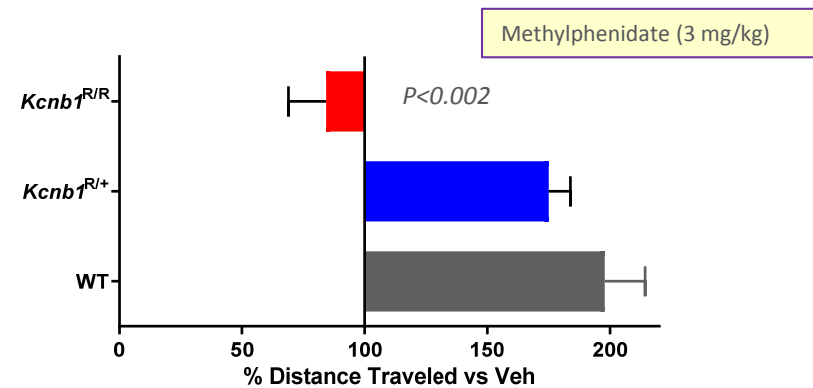
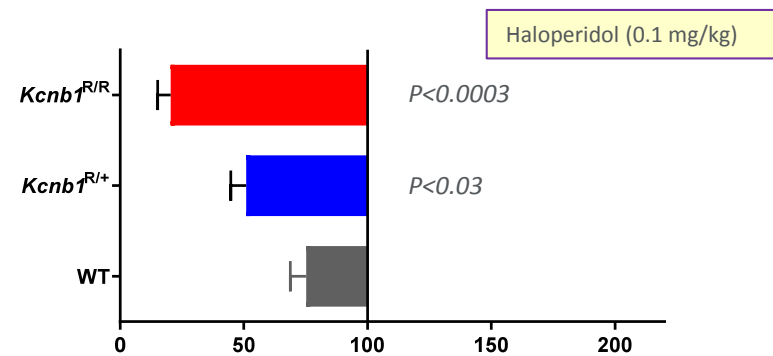
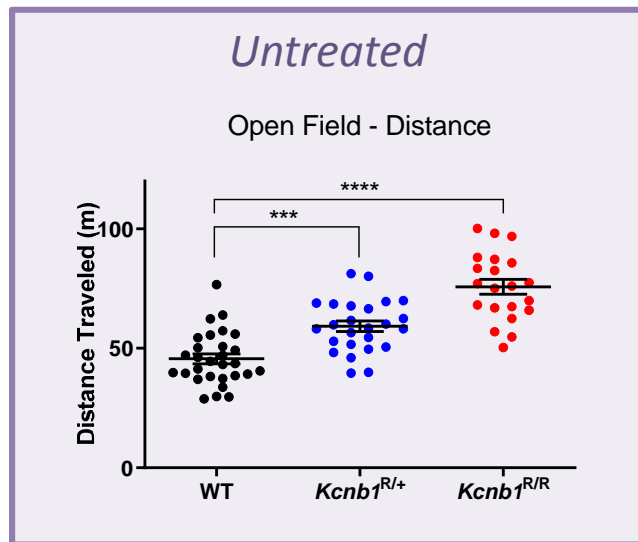


Kcnb1^{G379R} Mice – Profound Hyperactivity

Dopamine-Mediated?

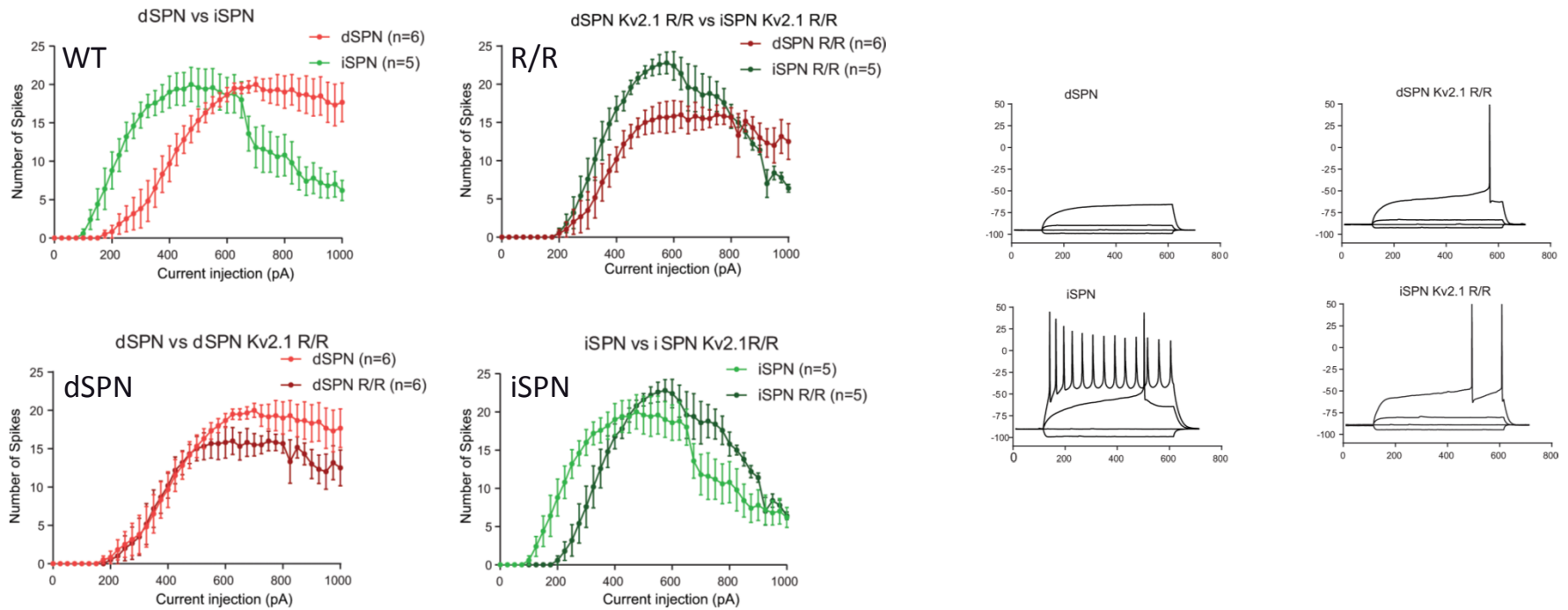


Manny Jurado



Kcnb1^{G379R} Mice – Imbalance in Striatal Projection Neuron Output

Hypoexcitability of indirect pathway SPNs (motor suppressing)



Summary

- DEE associated *KCNB1*-p.G379R variant affects channel function
 - Lower K⁺ conductance, altered ion selectivity, dominant-negative effects with WT co-expression
 - Altered expression/localization of Kv2 channel complexes
- *KCNB1*-p.G379R modeled in mice results in a DEE-like phenotype
 - Elevated seizure susceptibility, epilepsy and abnormal interictal EEG
 - Neurobehavioral abnormalities (hyperactivity, lower anxiety)
 - Imbalance in striatal output => Excitatory/Inhibitory network imbalance
- *Kcnb1*^{G379R} >> *Kcnb1*^{fs} supporting that *KCNB1*-p.G379R is not simply due to loss-of-function
- *Kcnb1*^{G379R} model will serve as a useful platform for understanding disease pathophysiology and testing therapeutics

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