Comparing 4-phenylbutyrate and other small molecules in GABA transporter 1-encoding SLC6A1 mutations

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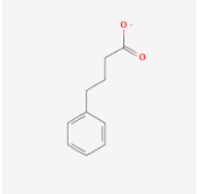
SLC6A1 Connect Scientific Symposium
Atlanta
Dec 4-25

This is the 7th talk in SLC6A1 symposium from my lab:

What have we accomplished?

we brought 4-phenylbutyrate (Ravicti) to patients



4-PBA

Gene Liau

Terry Jo Bichell

Zachery Grinspan,
then Scott,
Many others….

Amber Freed

We have published 12 manuscripts on SLC6A1 +

1 manuscript in press, 3 under revision/review

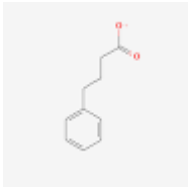
What does this mean to patients and parents who supported us and put their hope on our work?

Updates on 3 areas:

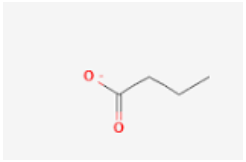
- Comparing 4 phenylbutyrate with other small molecules
  - in 32 SLC6A1 variants
- More in-depth study on PBA in cell and mouse models of representative mutations
- Characterizing a splice site mutation to determine if splice site mutation can benefit from Ravicti or gene therapy (NM-003042.3(SLC6A1) c.1426+1G>A

We have previously tested ~20 molecules but will only focus on the 5 below for today:

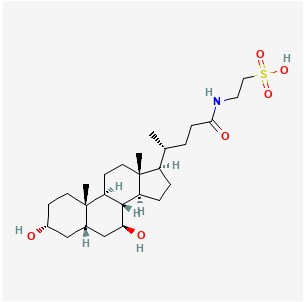
4 phenylbutyrate



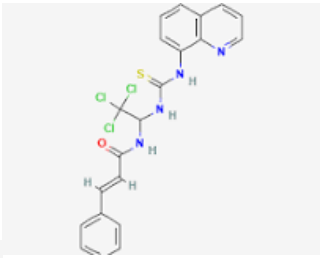
Butyrate



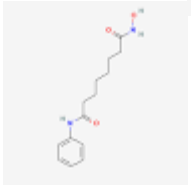
TUDCA (Taurourodeoxycholic acid)



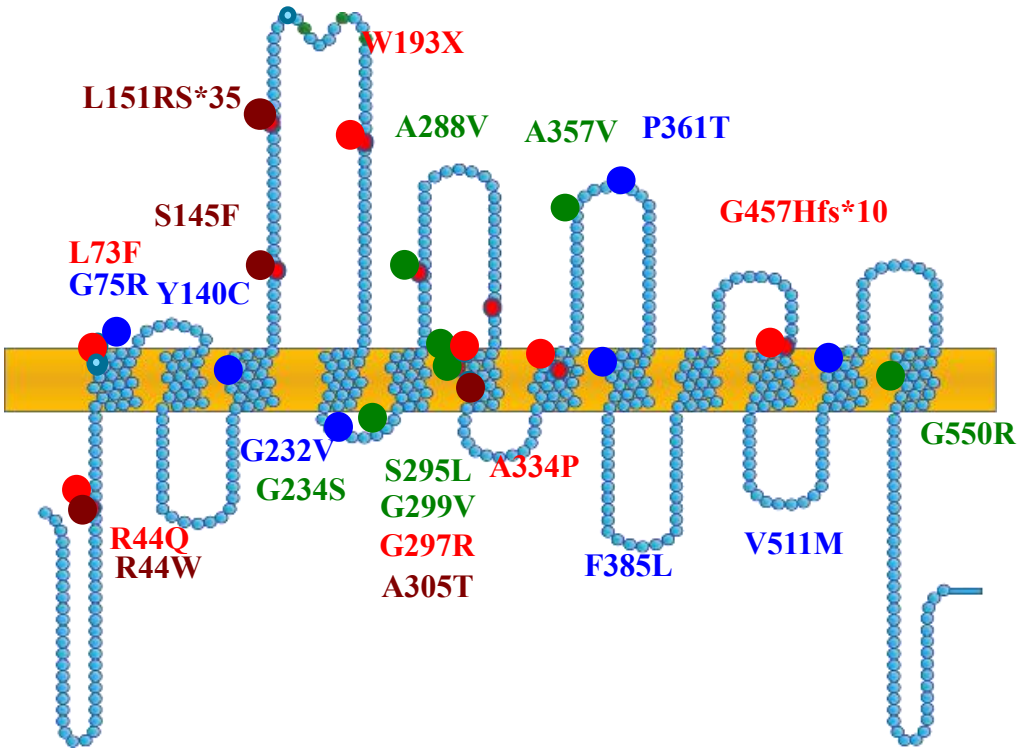
Salubrial



SAHA



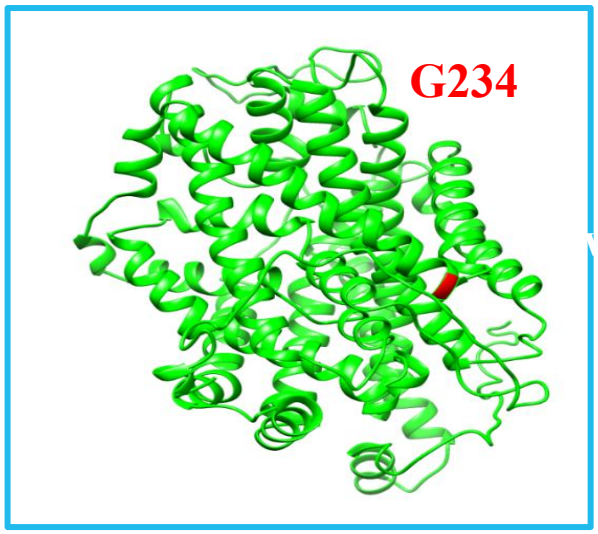
Many SLC6A1 variants are associated with DEEs (>1000 (1073))

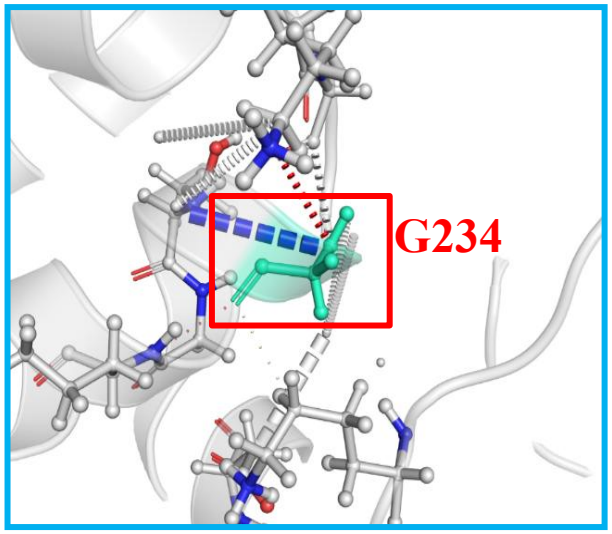


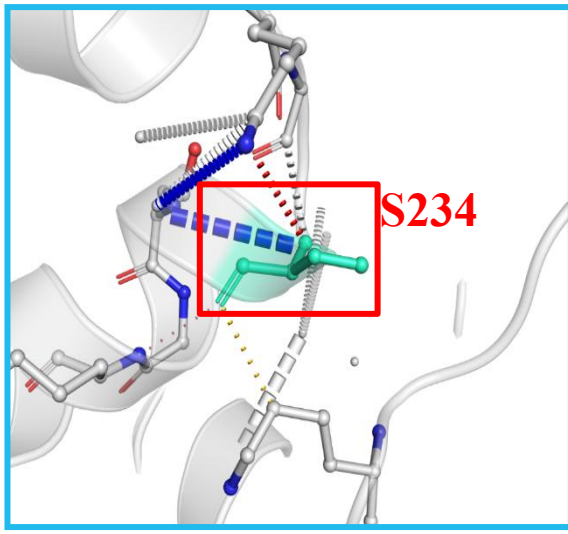
R44Q#: epilepsy with myoclonic-atonic seizures
R44W: developmental disorder
L73F: epilepsy
G75R#: generalized epilepsy, intellectual disability
Y140C#: epilepsy with myoclonic-atonic seizures, mild to moderate intellectual disability
S145F#: mild intellectual disability
L151RS*35#: intellectual disability
W193X#: epilepsy with myoclonic-atonic seizures
G232V#: epilepsy with myoclonic-atonic seizures, mild to moderate intellectual disability
G234S: Lennox-Gastaut Syndrome
A288V#: autism spectrum disorder
S295L: SLC6A1-related disorder, hypotonia
G297R#: epilepsy with myoclonic-atonic seizures
G299V: autism spectrum disorder
A305T: developmental disorder
A334P#: epilepsy with myoclonic-atonic seizures
A357V#: Rett-like syndrome
P361T: autism spectrum disorder, epilepsy with absence and atonic seizures
F385L#: epilepsy with myoclonic-atonic seizures, mild to moderate intellectual disability
G457Hfs*10#: epilepsy with myoclonic-atonic seizures
V511M#: Generalized epilepsy, mild intellectual disability
G550R#: autism spectrum disorder

Mermer .. Kang Brain 2021

We started AI prediction of the the impact of mutations since 2018

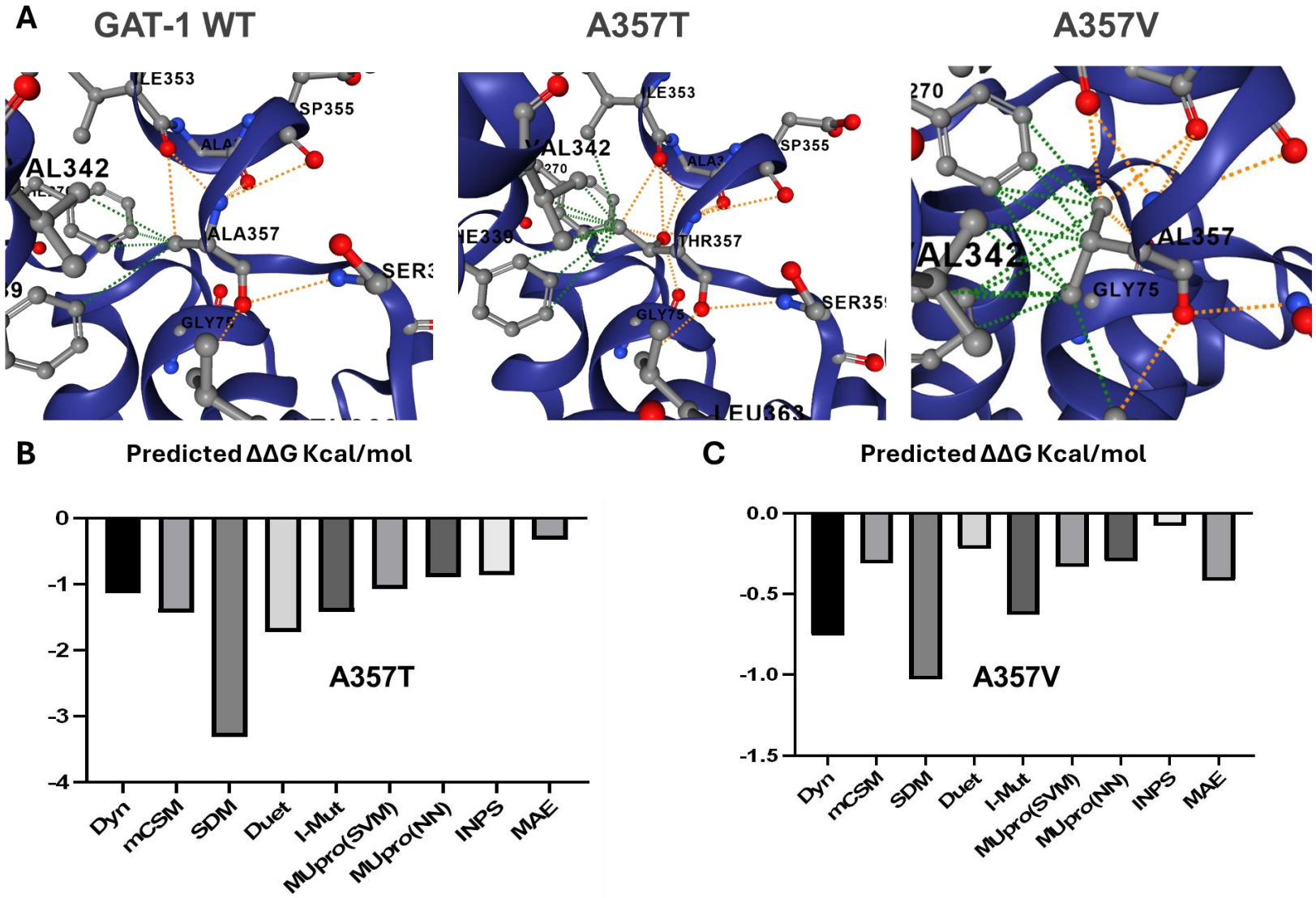






Cai & Kang, Experimental Neurology, 2019

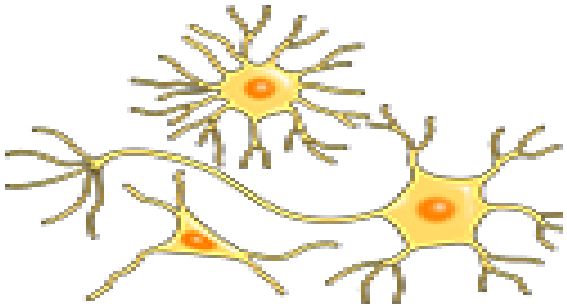
AI prediction of the impact of mutations and followed by experimental validation in cell models

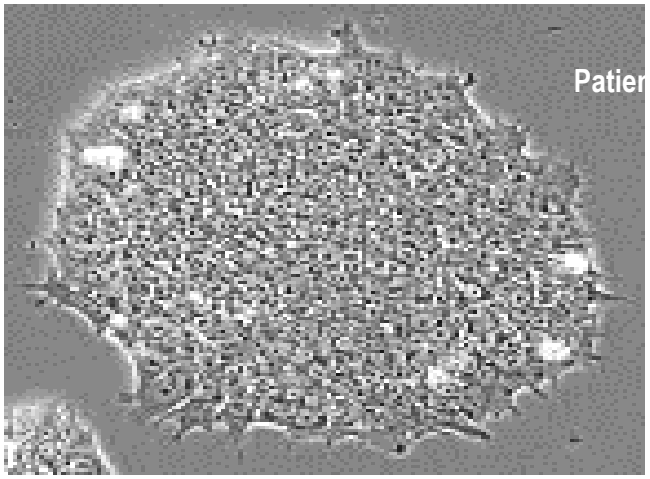


We study the trafficking and function of the wildtype and mutant GAT-1 in HEK293T, iPSCs, neurons and astrocytes

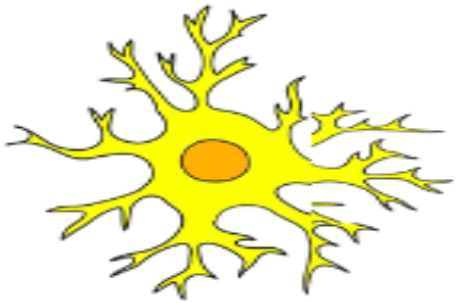
HEK293

Neurons



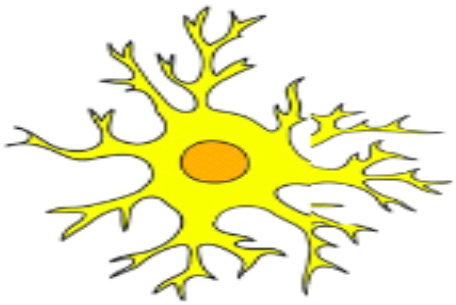


iPSCs
(induced pluripotent stem cells)



astrocytes

Previous studies of PBA on SLC6A1 variants demonstrated PBA restored GABA uptake function.

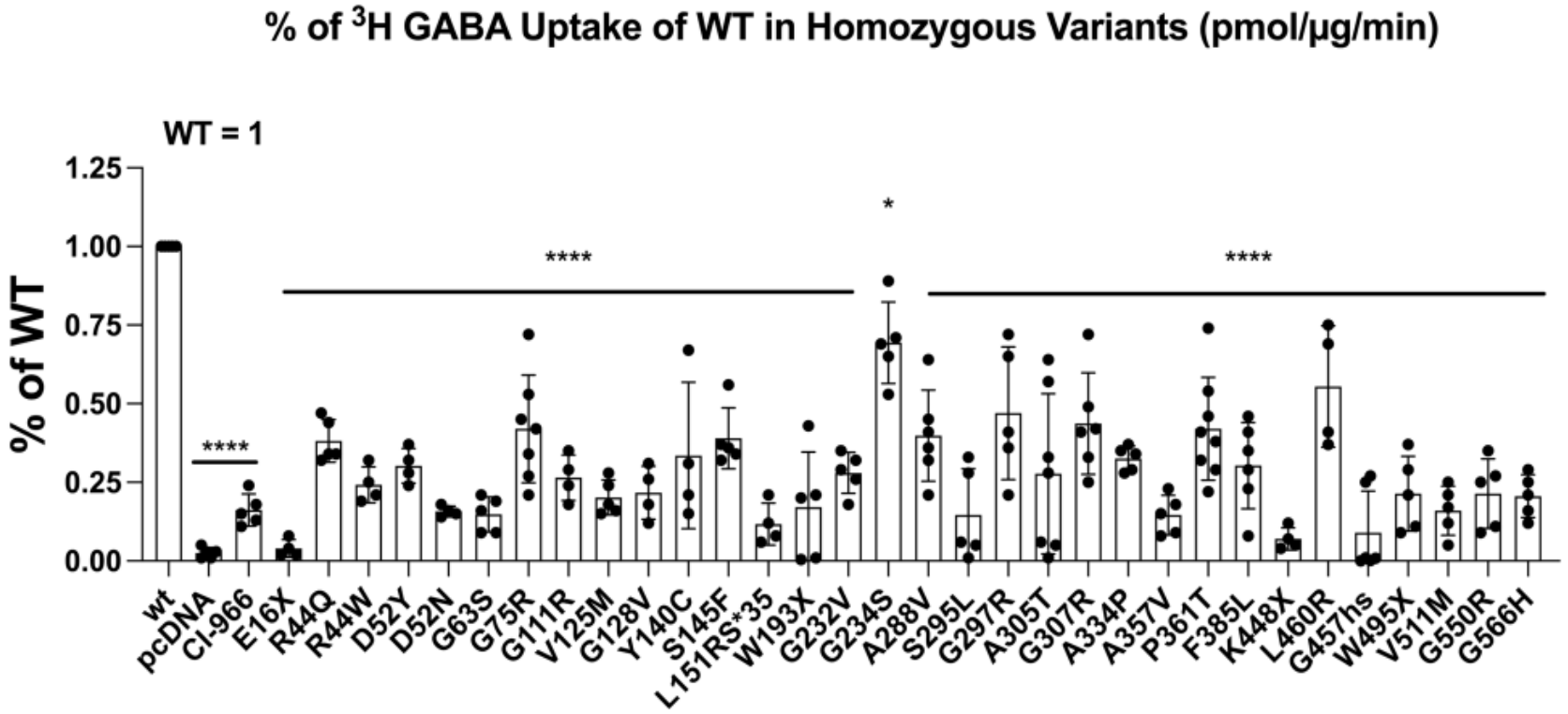


1. PBA increased GABA uptake of the mutant protein in HEK 293T cells.
2. PBA increased GABA uptake of the mutant protein in mouse/human neurons.
3. PBA increased GABA uptake of the mutant protein in mouse/human astrocytes.

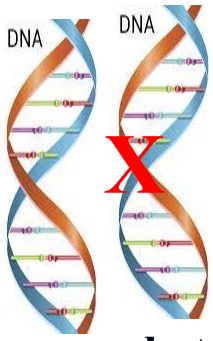
1. Comparing the effect of PBA and other molecules in 32 variants

SLC6A1 mutations differentially decreased GABA uptake of the mutant GAT-1 (32 variants)

mut alone

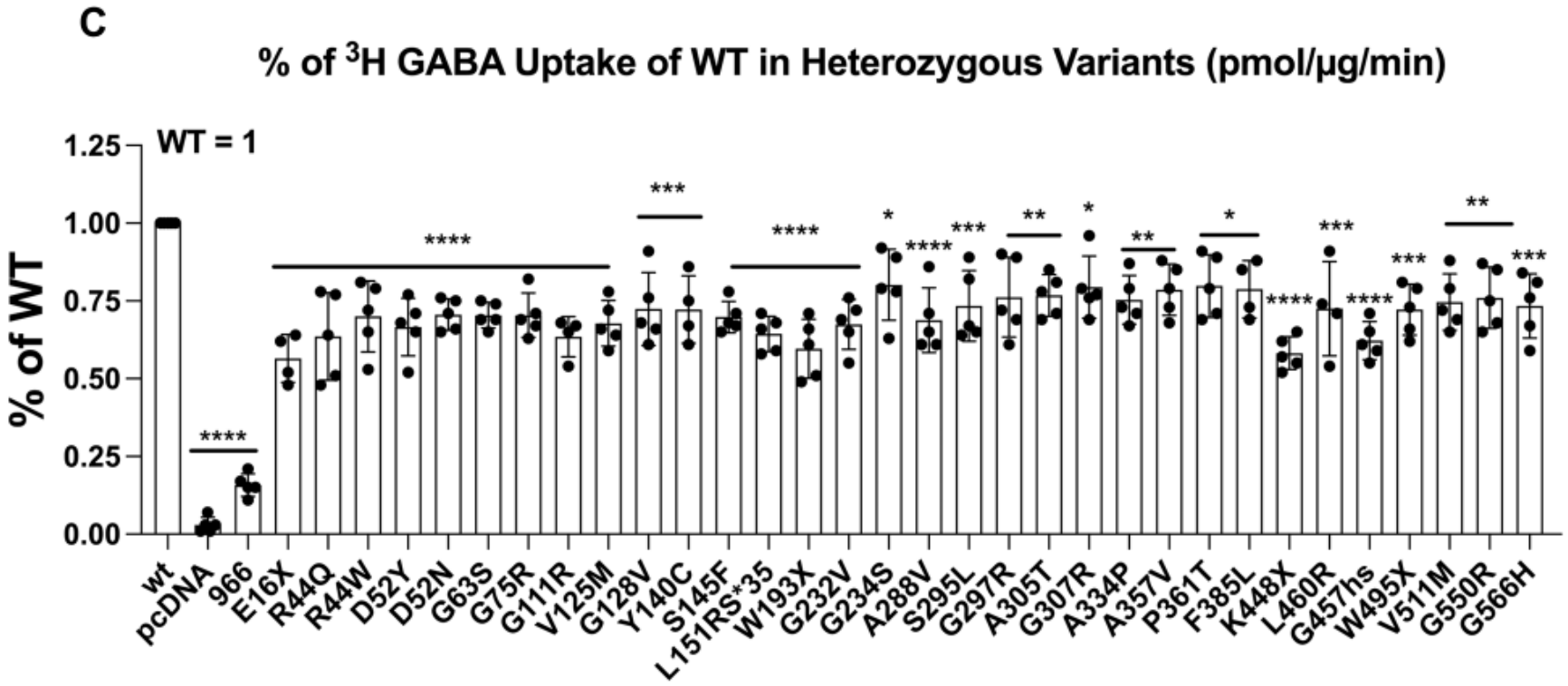


Bassett & Kang, (under review)



SLC6A1 mutations differentially decreased GABA uptake of the mutant GAT-1 (32 variants)

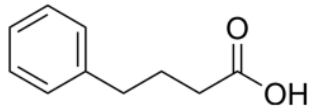
heterozygous patient condition



Bassett & Kang,
(under review)

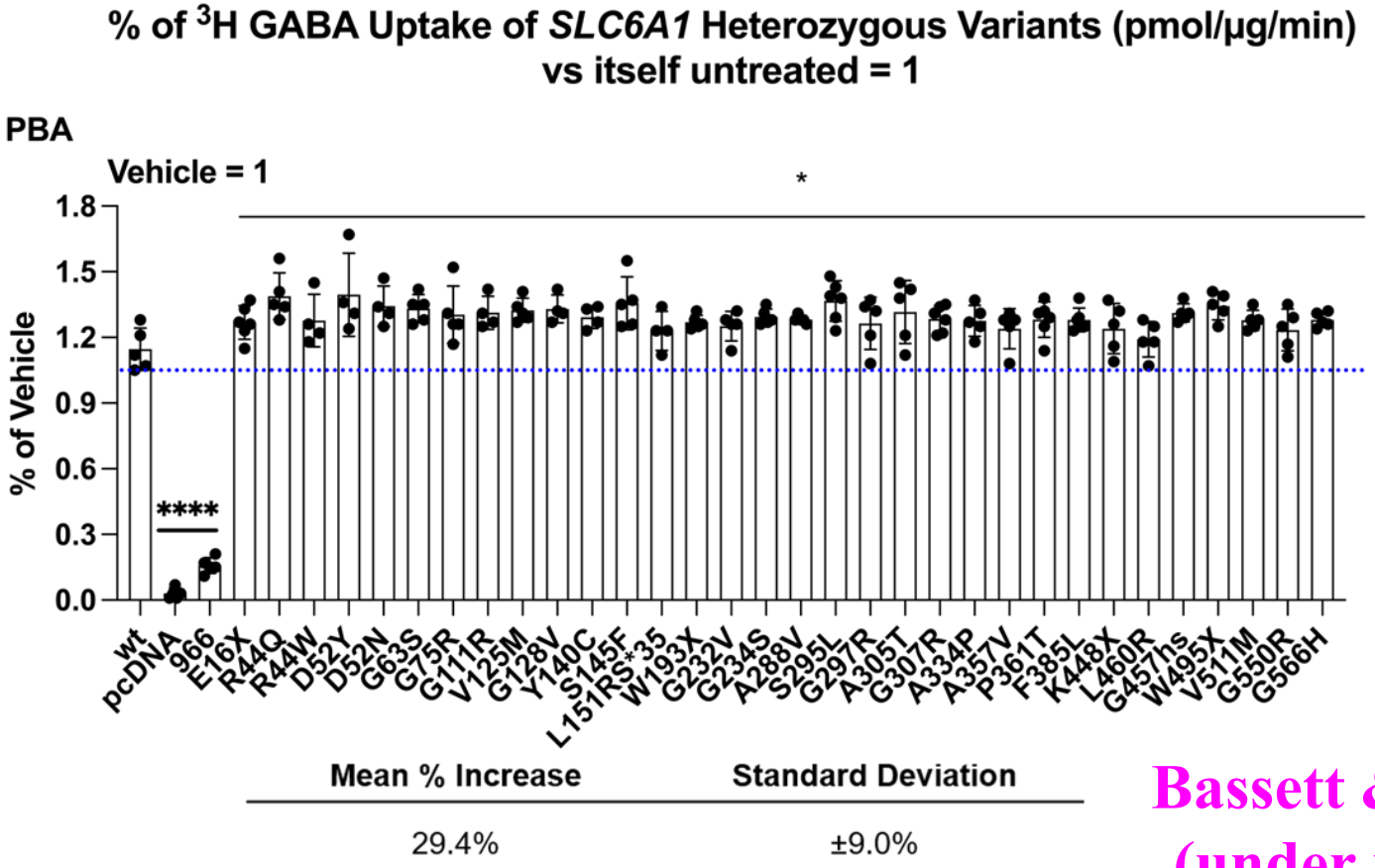
4-phenylbutyrate differentially increased GABA uptake of the mutant GAT-1 (32 variants)

A

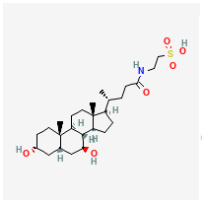


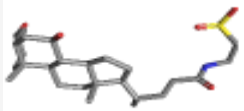
4-Phenylbutyrate (PBA)

B



Bassett & Kang, (under review)





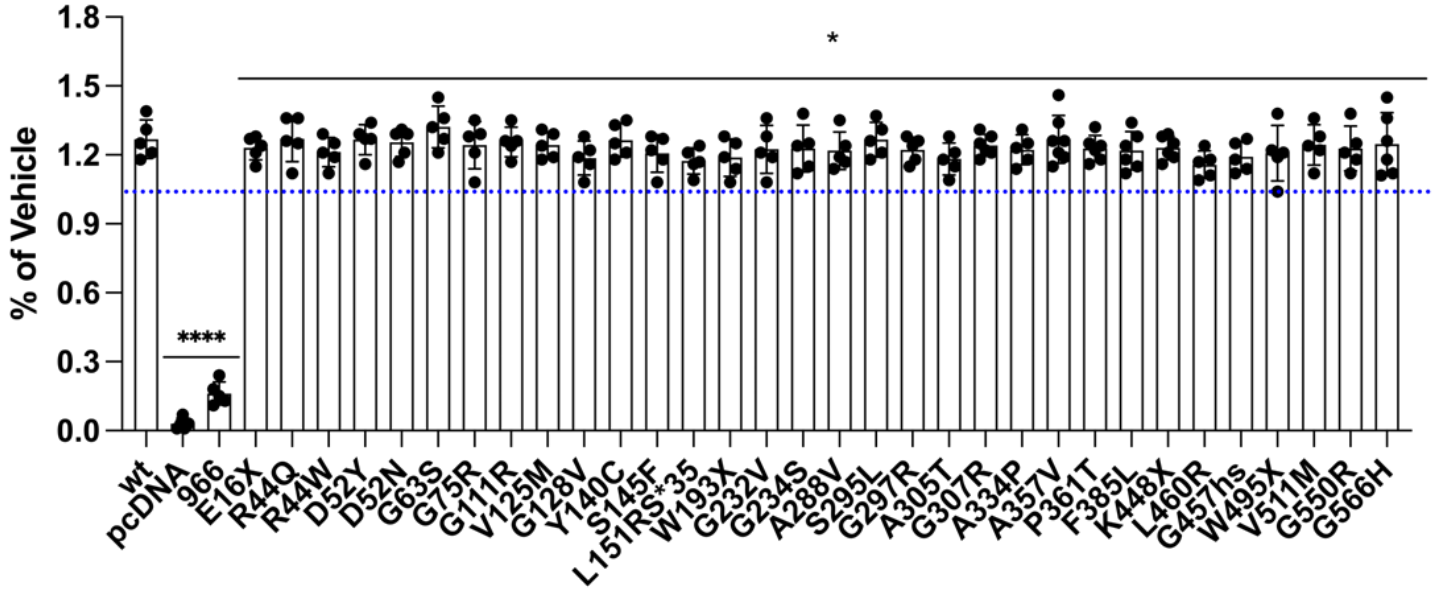
TUDCA differentially increased GABA uptake of the mutant GAT-1 (32 variants)

C

% of ^{3}H GABA Uptake of *SLC6A1* Heterozygous Variants (pmol/µg/min)
vs itself untreated = 1

TUDCA

Vehicle = 1

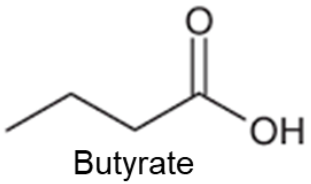


Mean % Increase	Standard Deviation
23.1%	±7.9%

Bassett & Kang, (under review)

The effect of Butyrate on GABA uptake of the mutant GAT-1 (32 variants)

A



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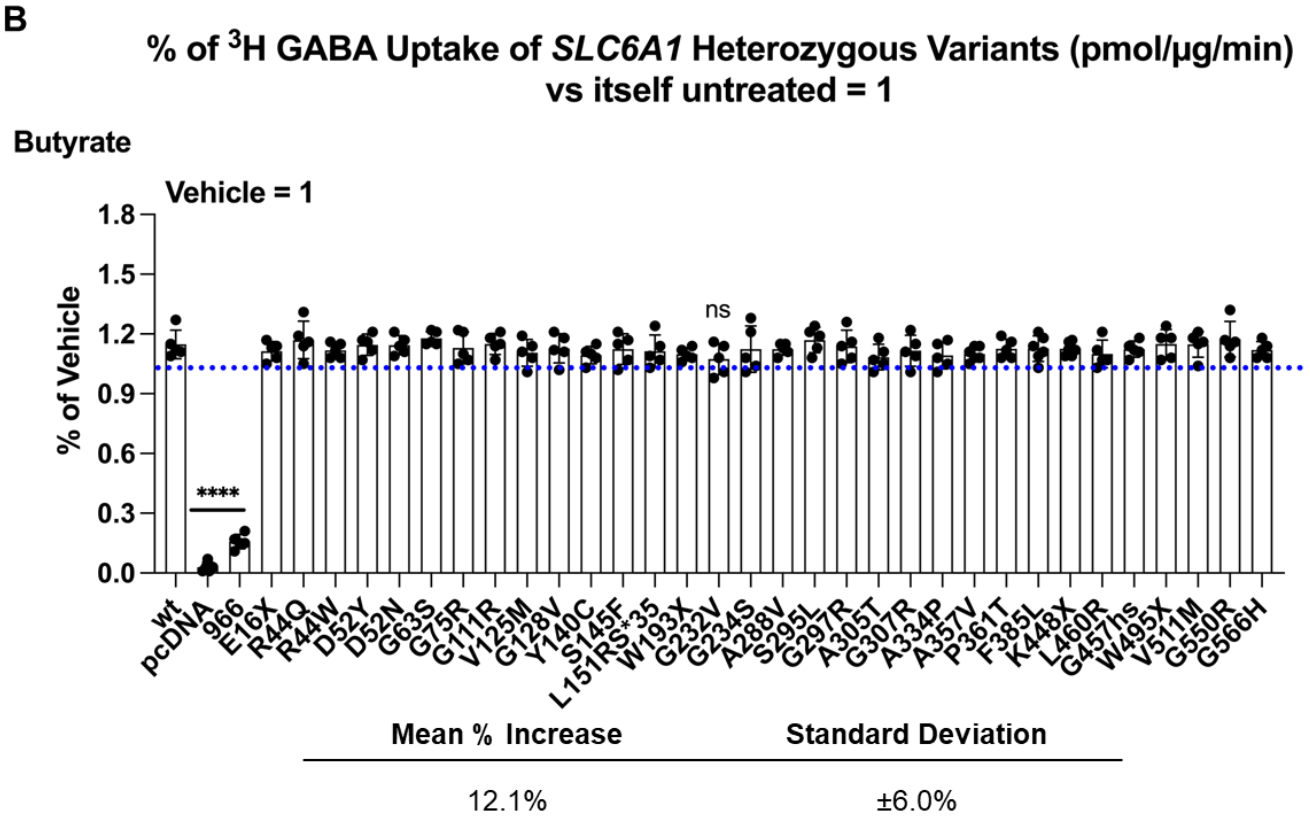
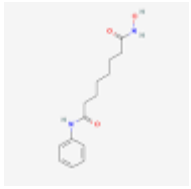


Bassett & Kang, (under review)

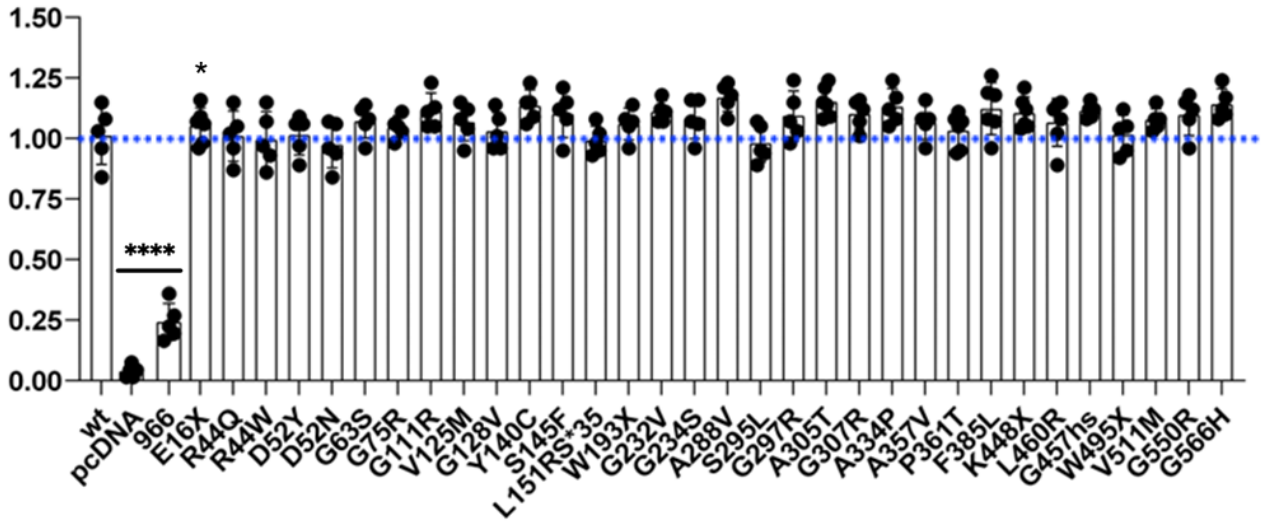


The effect of SAHA on GABA uptake of the mutant GAT-1 (32 variants)

C

% of ^{3}H GABA Uptake of *SLC6A1* Heterozygous Variants (pmol/µg/min)
vs itself untreated = 1

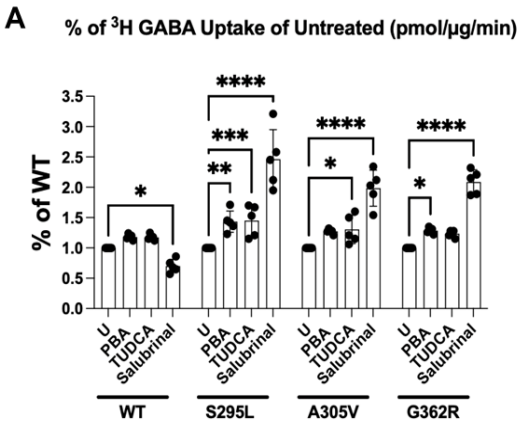
SAHA

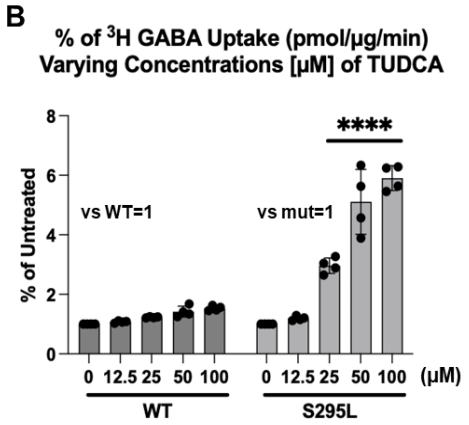


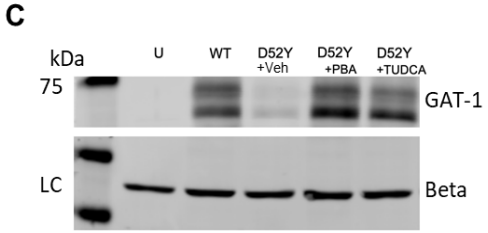
Mean % Increase	Standard Deviation
2.0%	±9.2%

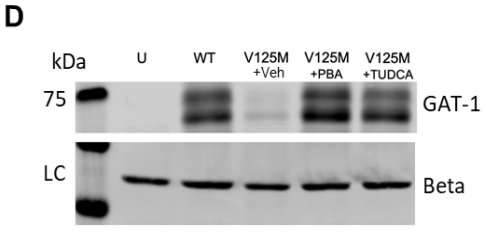
Bassett & Kang, (under review)

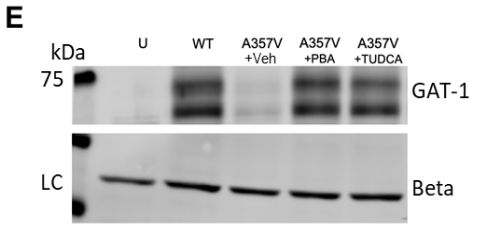
Both PBA and TUDCA increased the total protein of the GAT-1

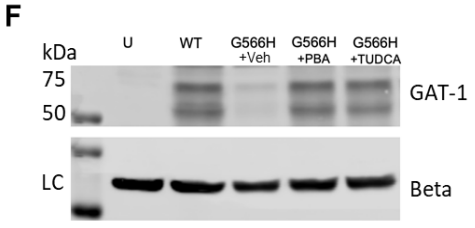


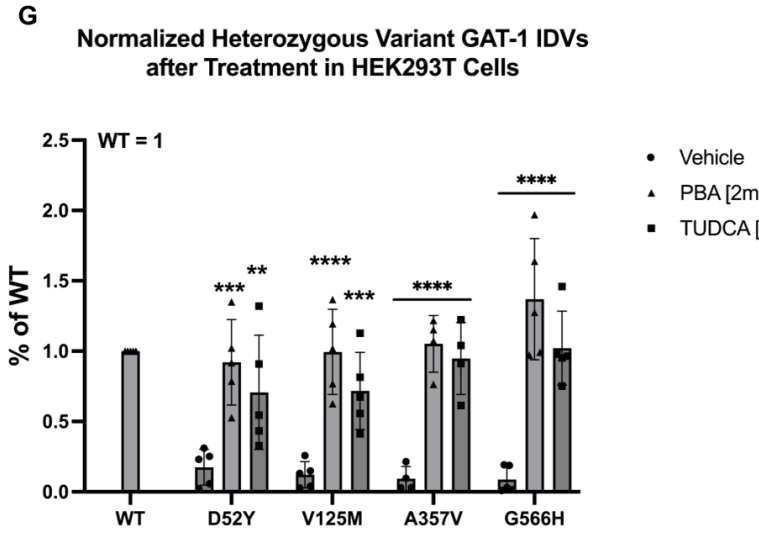






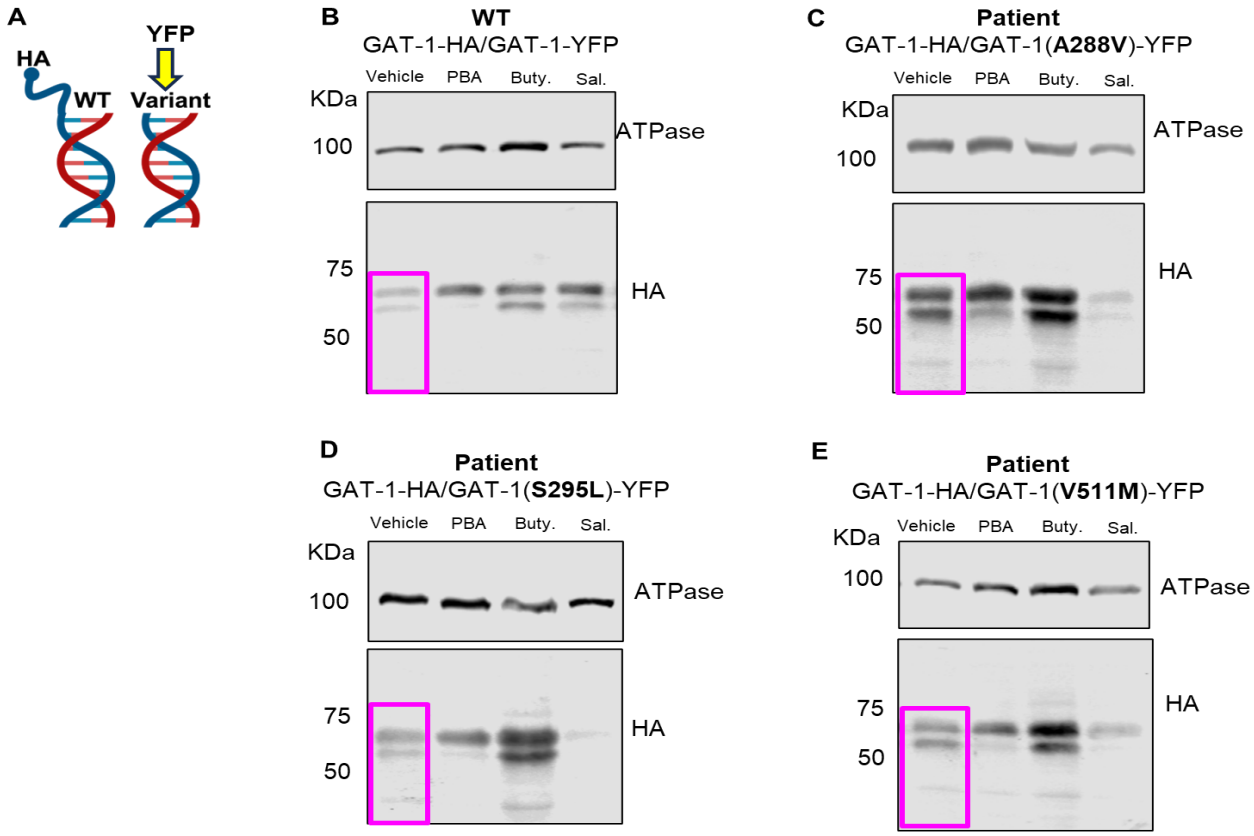


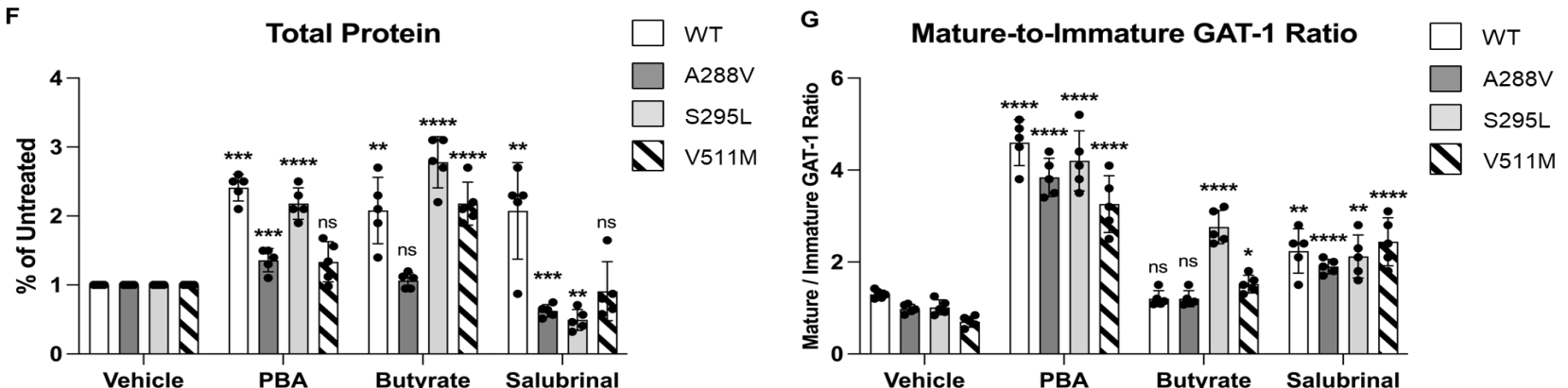




Bassett & Kang, (under review)

PBA and butyrate differentially increased the total protein of the wildtype GAT-1





2. In-depth study of PBA effect in SLC6A1 variant knockin mouse models:

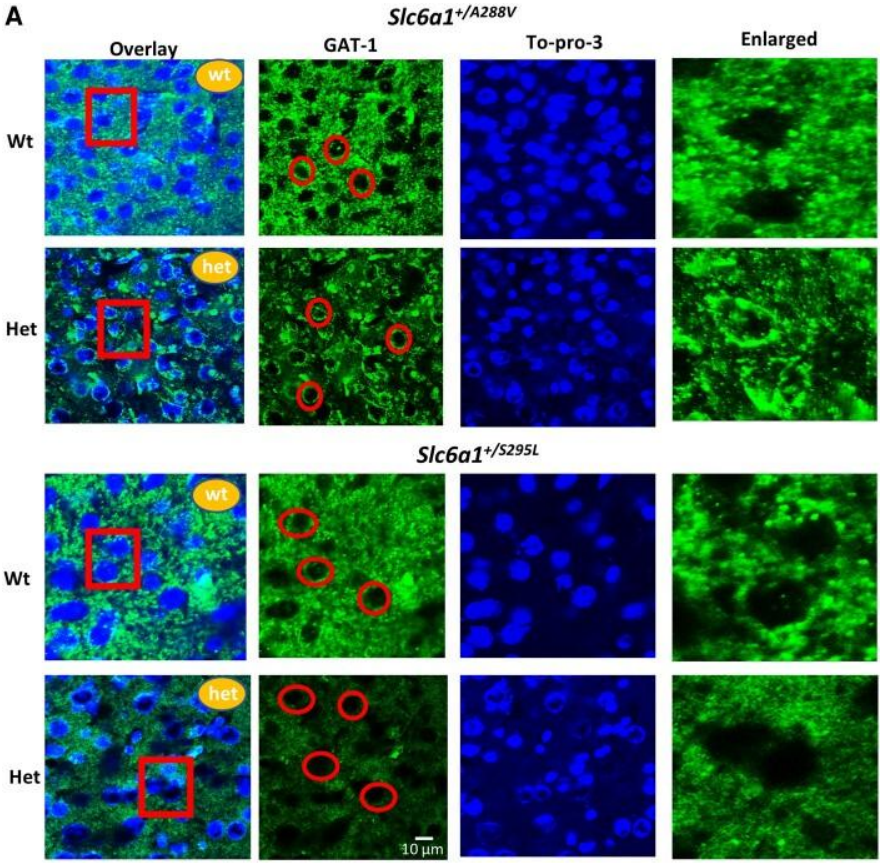
***Slc6a1*$^{+/S295L}$**

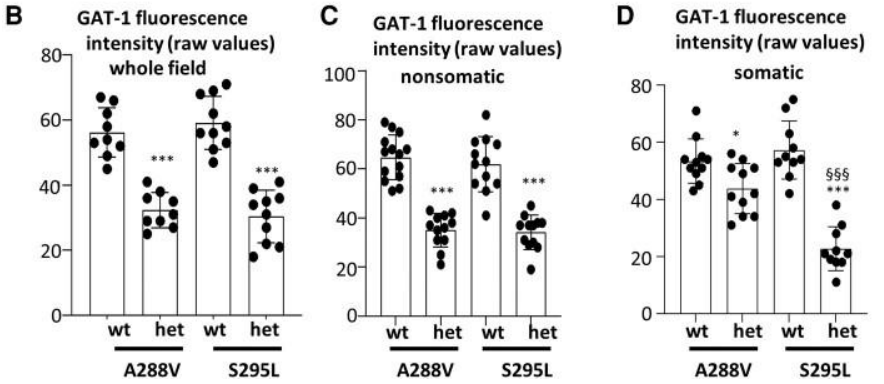
***Slc6a1*$^{+/A288V}$**

Study on PBA in SLC6A1 variants in mouse models:

Slc6a1$^{+/S295L}$

Slc6a1$^{+/A288V}$

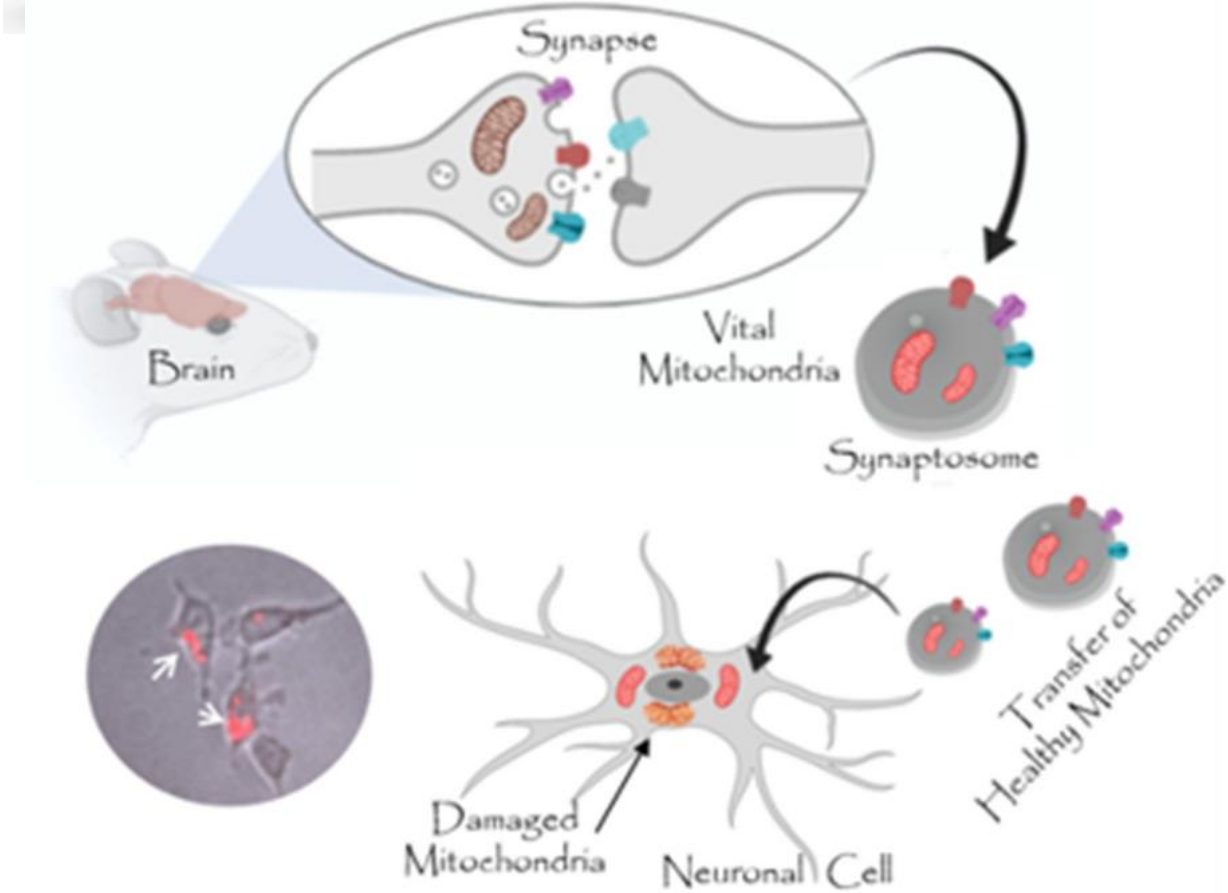




We evaluated GABA uptake in synaptosome of SLC6A1 variant knockin mouse models:

***Slc6a1*$^{+/S295L}$** ***Slc6a1*$^{+/A288V}$**

synaptosome: neuronal terminal acquired from homogenized brain tissue

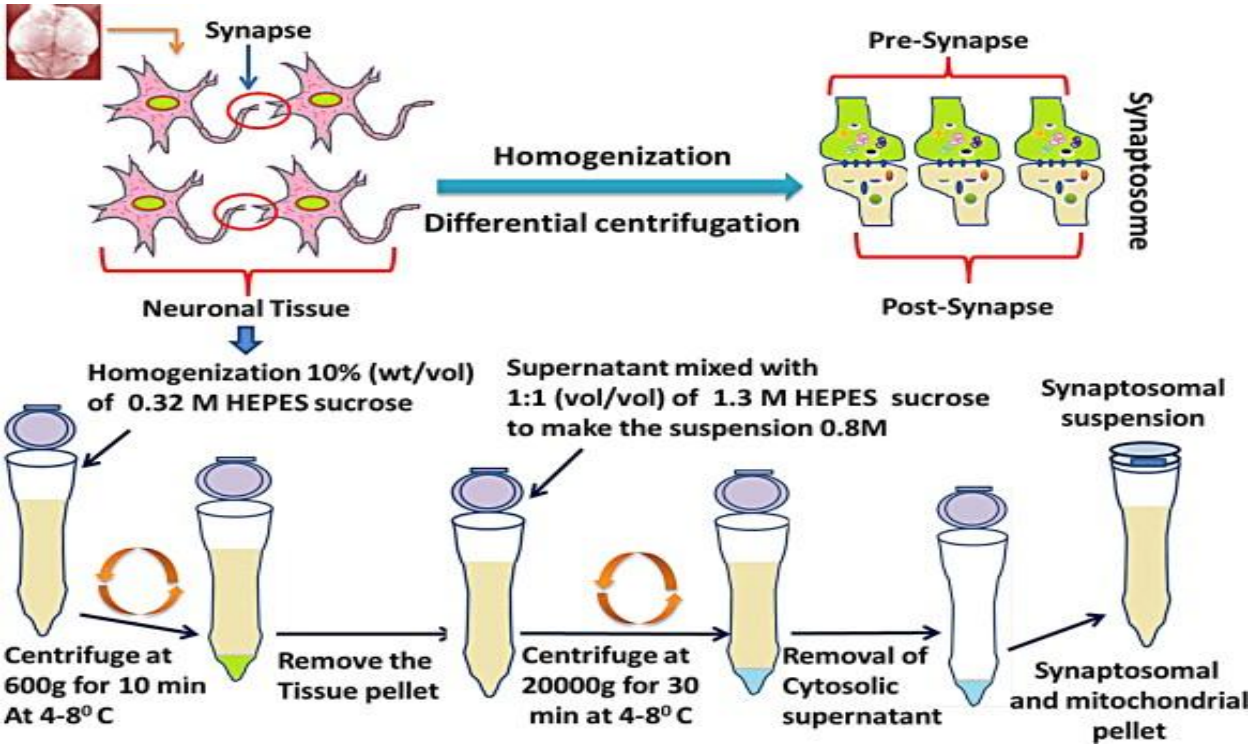


We evaluated GABA uptake in gliosome of SLC6A1 variant knockin mouse models:

***Slc6a1*$^{+/S295L}$**

gliosome: subcellular preparation originated from homogenized brain tissue

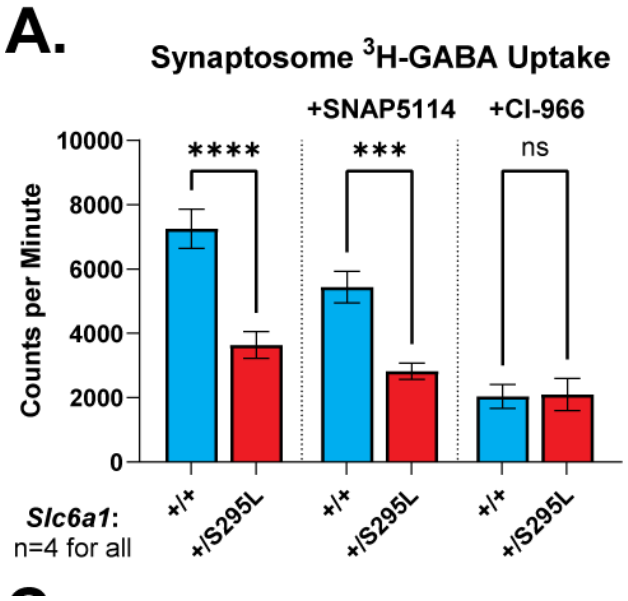
***Slc6a1*$^{+/A288V}$**

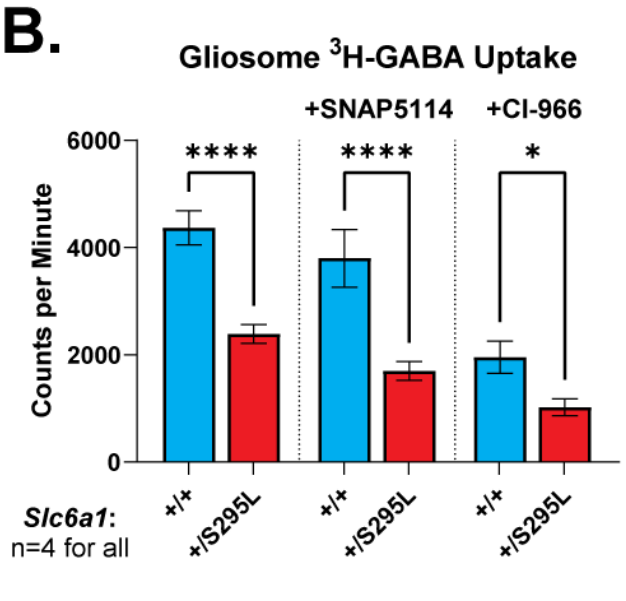


Reduced GABA uptake in the synaptosome and gliosome of SLC6A1 variant mouse models:

***Slc6a1*$^{+/S295L}$**

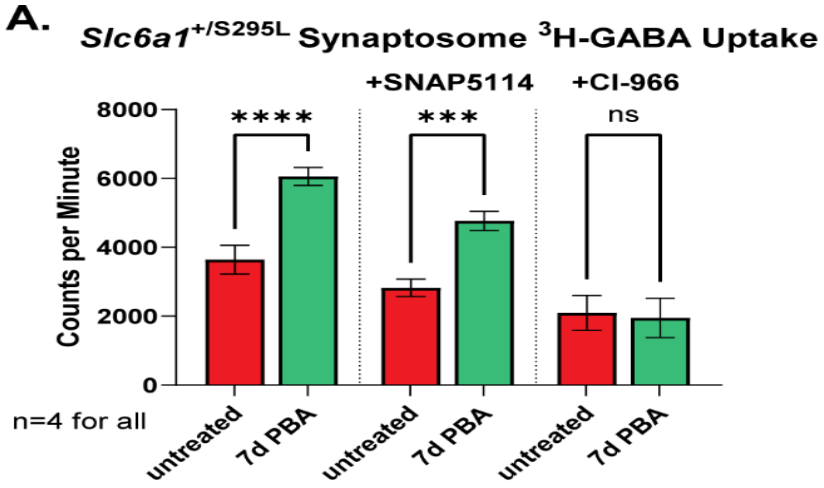
***Slc6a1*$^{+/A288V}$**

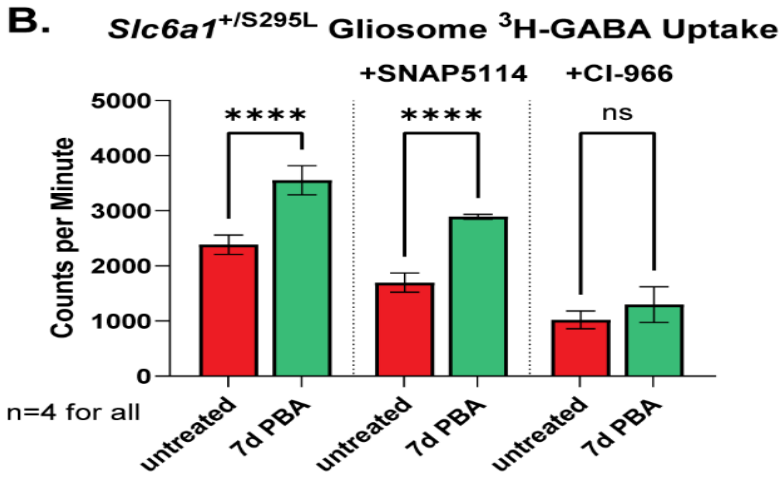




PBA effect in the synaptosome and gliosome of SLC6A1 variant mouse models:

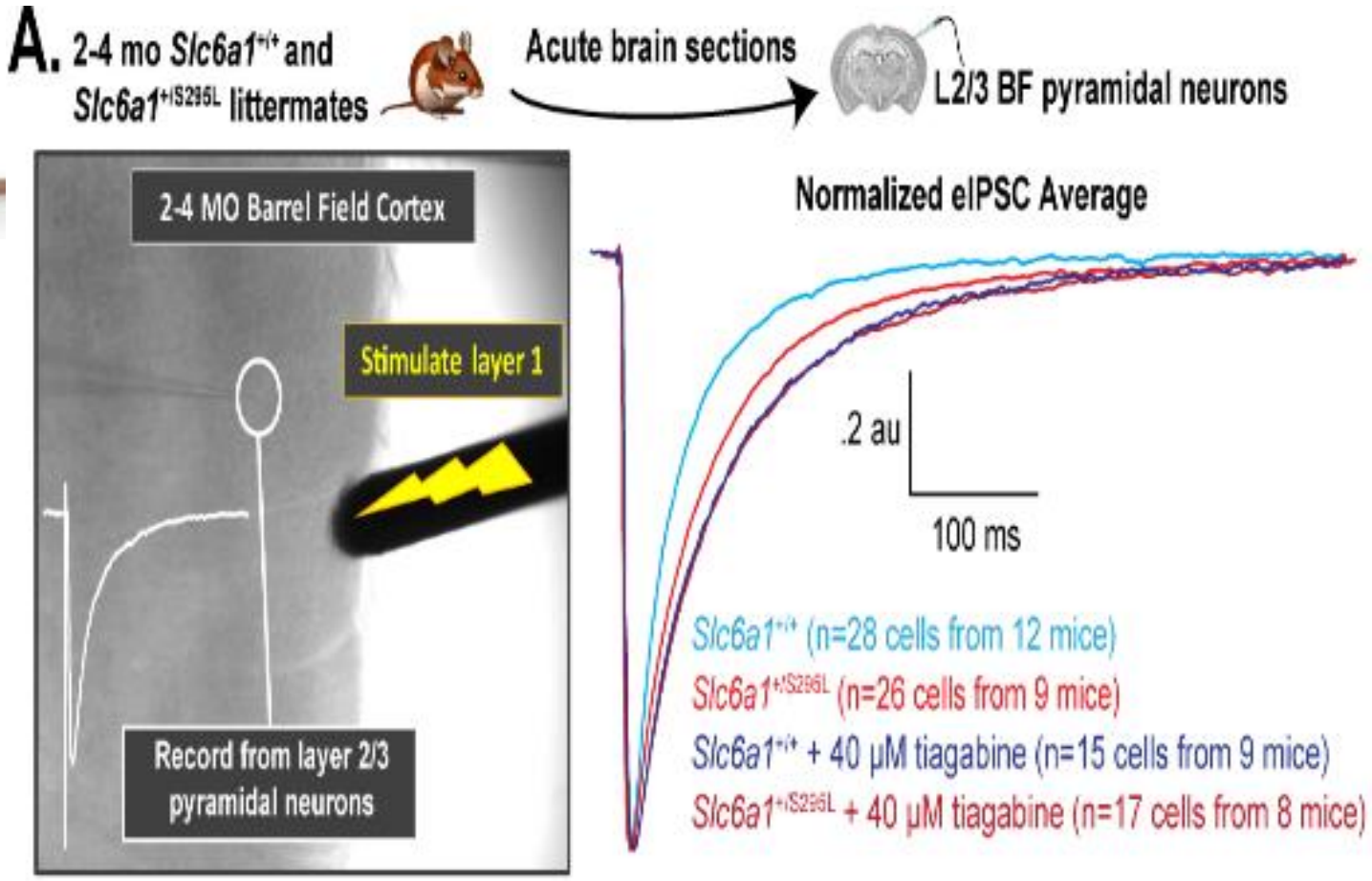
***Slc6a1*$^{+/S295L}$**





The mutant heterozygous mice had prolonged decay tau of eIPSC

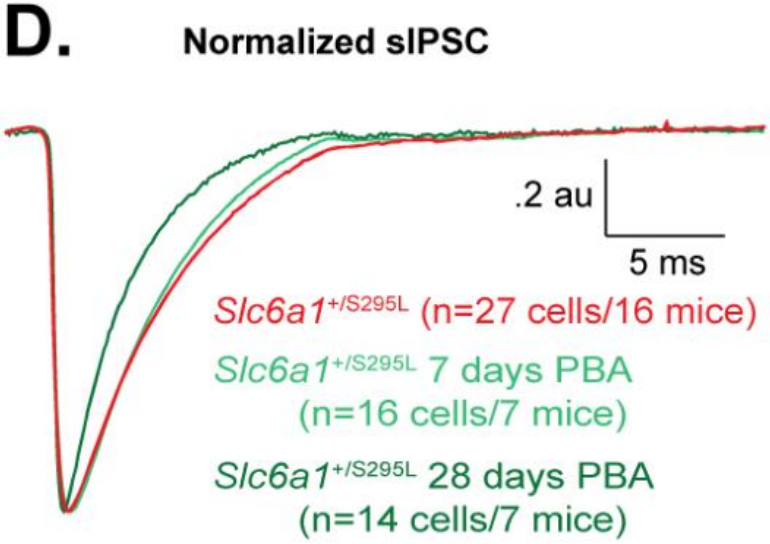
Slc6a1$^{+/S295L}$

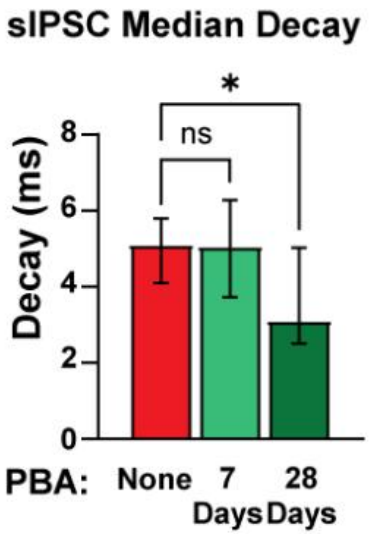


Zavalin & Kang, (in submission)

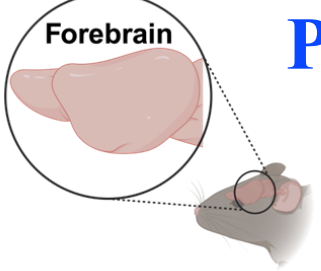
PBA effect (chronic treatment) normalized the eIPSC decay tau

***Slc6a1*$^{+/S295L}$**

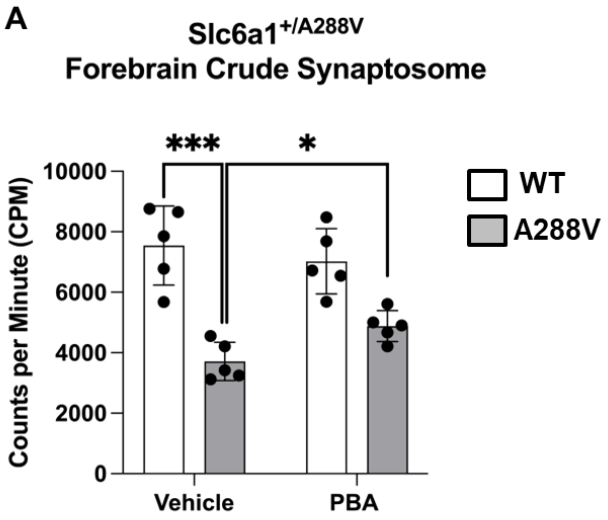


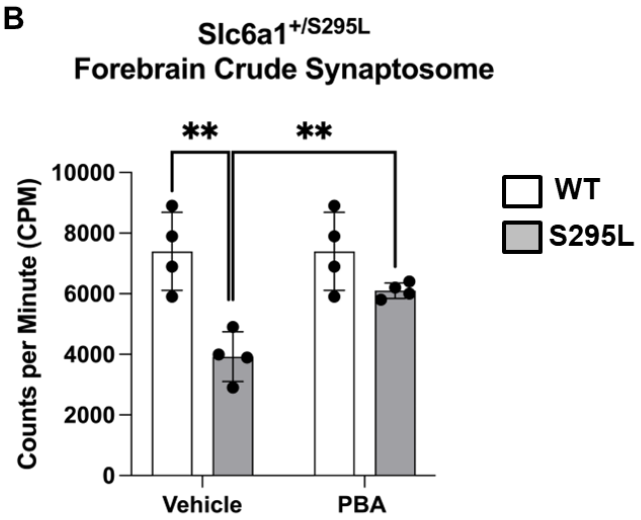


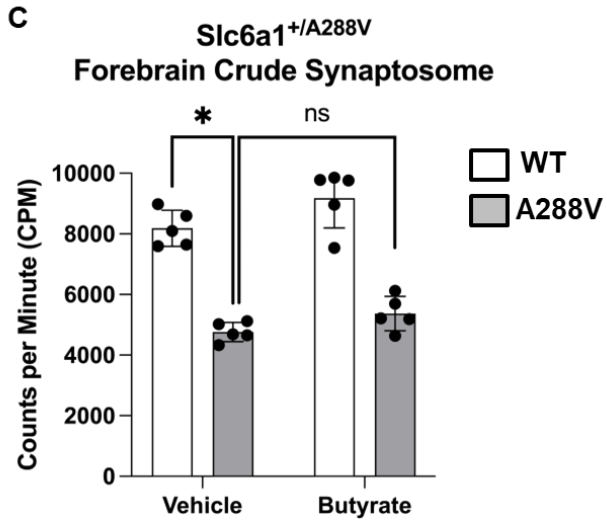
Zavalin & Kang, (in submission)

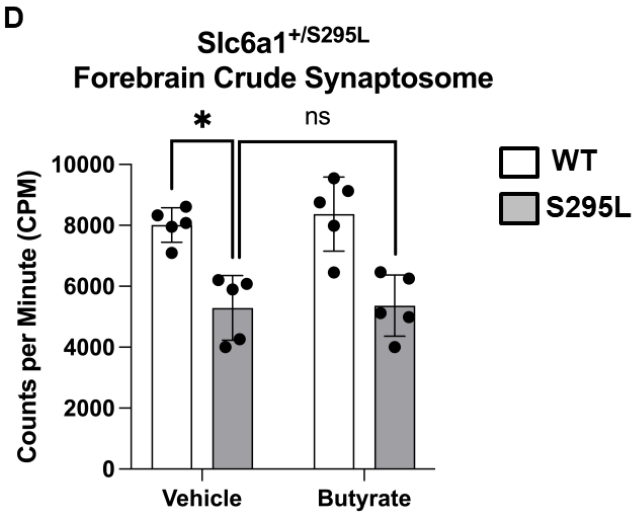


PBA is more efficacious than butyrate in restoring GABA uptake in synaptosome

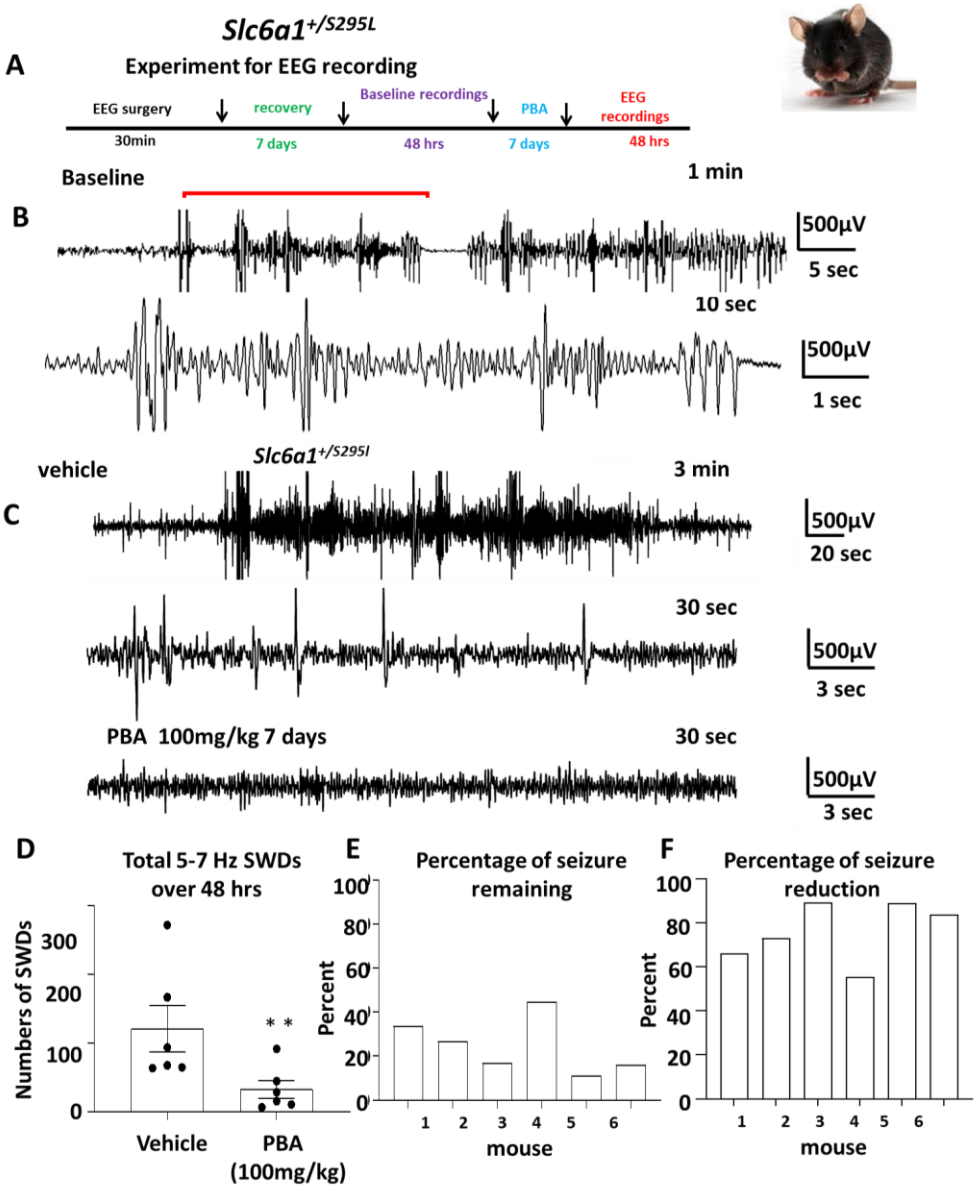






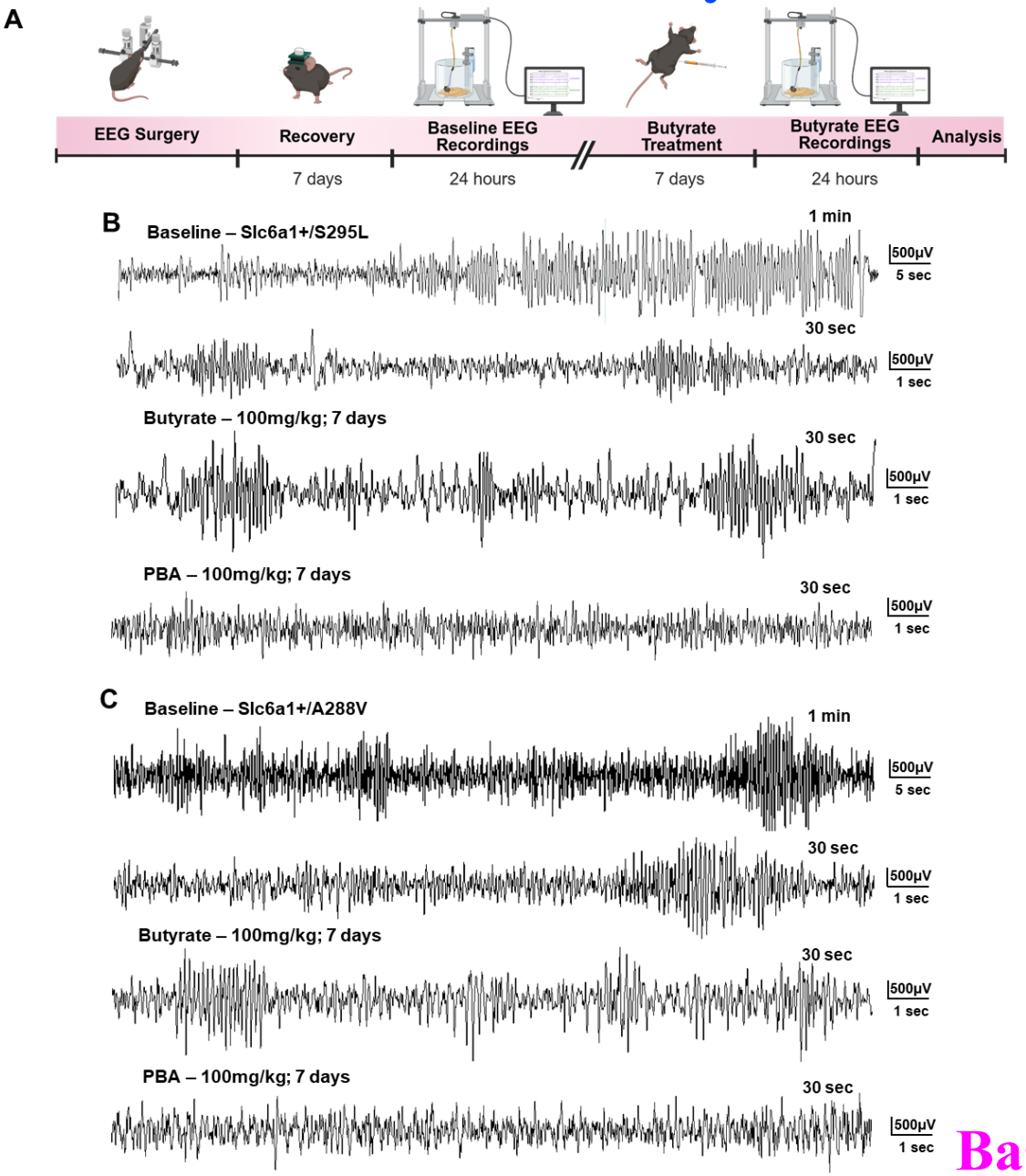


PBA mitigated seizures in SLC6A1 variants in mouse models:



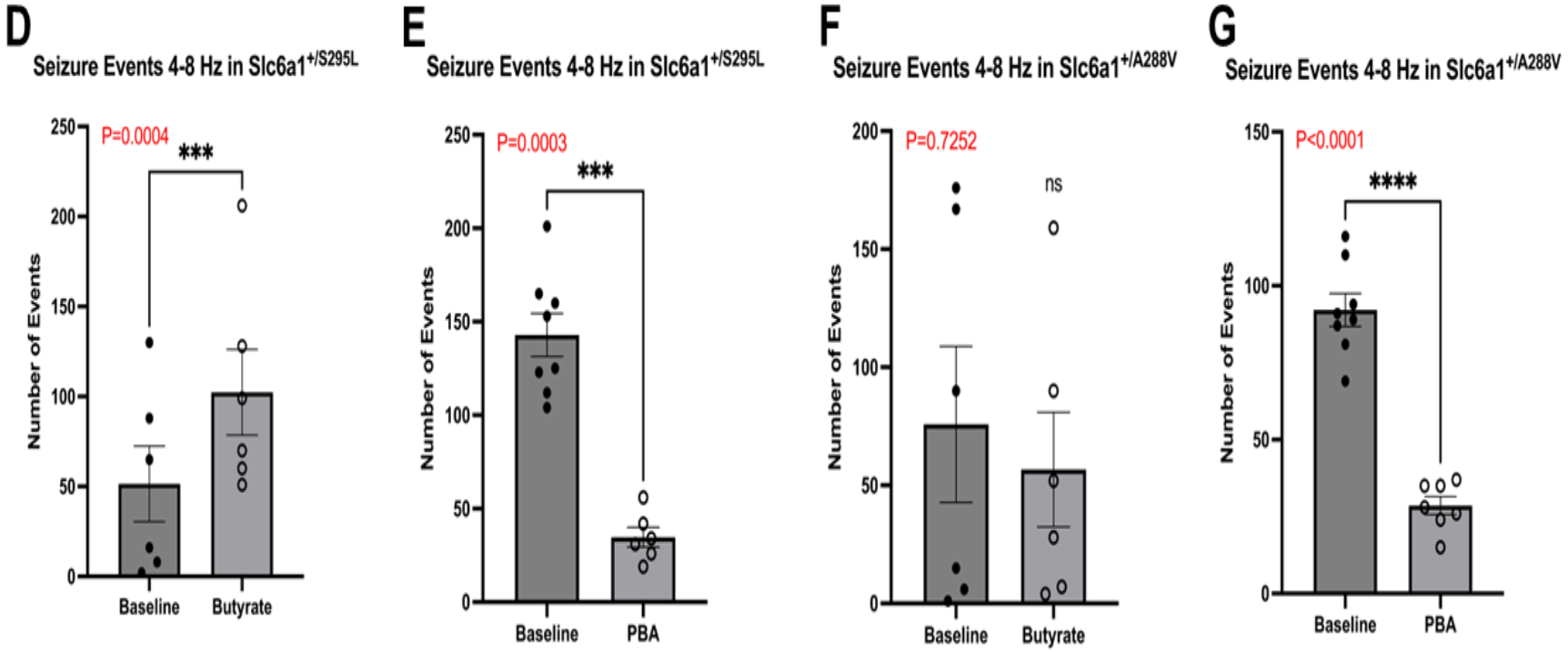
Nwosu et al., 2022 Brain Communications

PBA is more efficacious than butyrate in seizure reduction



Bassett & Kang, (under review)

PBA is more efficacious than butyrate in seizure reduction



PBA is more efficacious than butyrate in reducing seizures in Slc6a1$^{+/S295L}$ mice

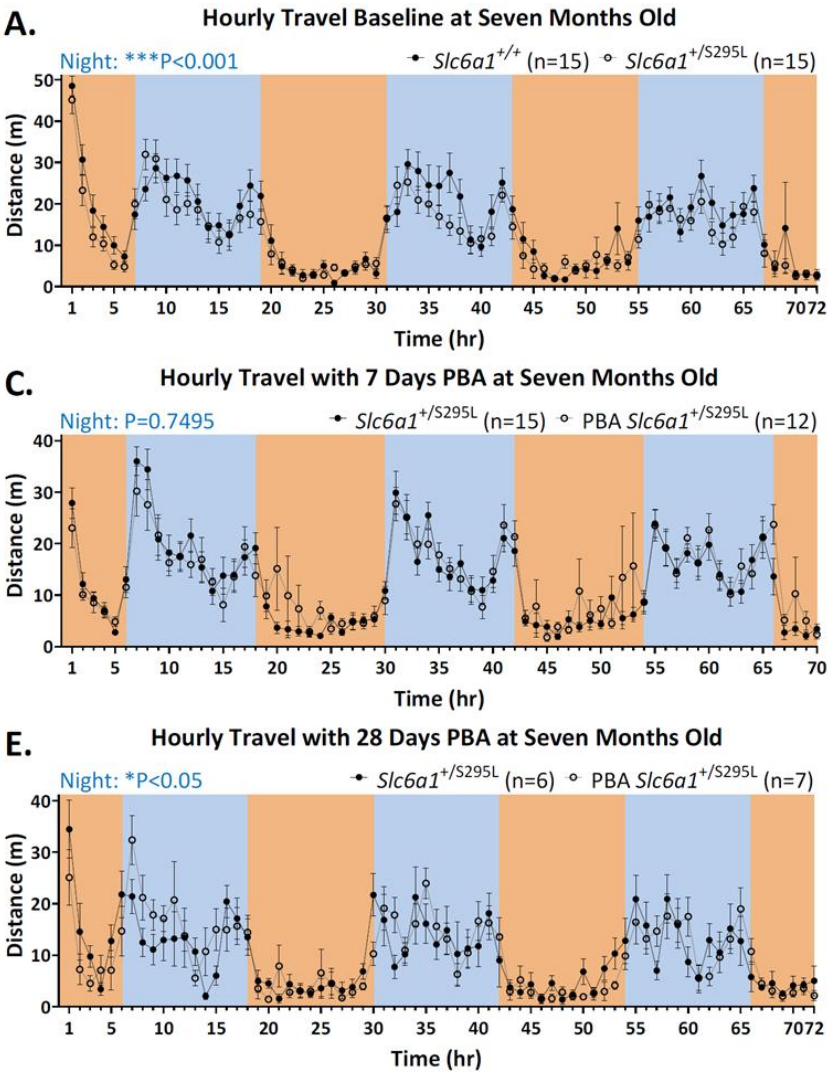
PBA rescued neurobehaviors in SLC6A1 variants in mouse models: the metrics we evaluated

Mouse traveling

Mouse hanging

Mouse sleeping

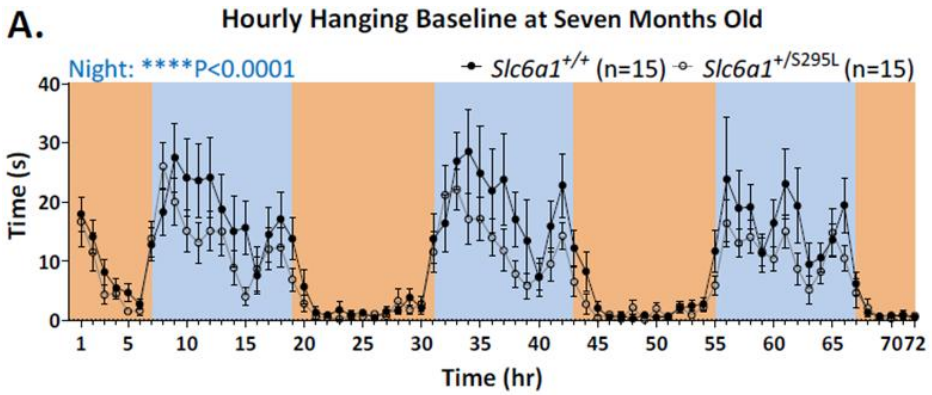
PBA rescued neurobehaviors in SLC6A1 variants in mouse models: Travelled distance

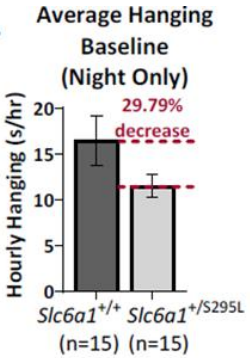


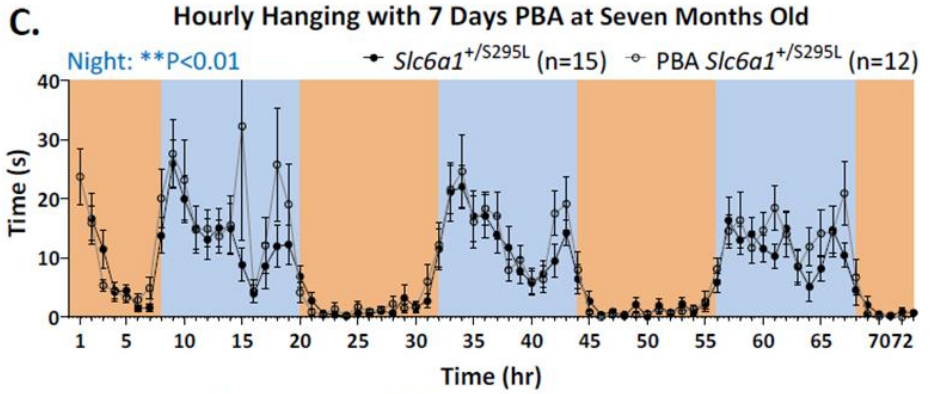
The effect of chronic treatment is better than acute treatment for travelled distance

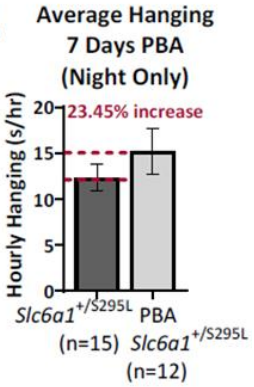
Randhave et al., 2025 Epilepsy Research

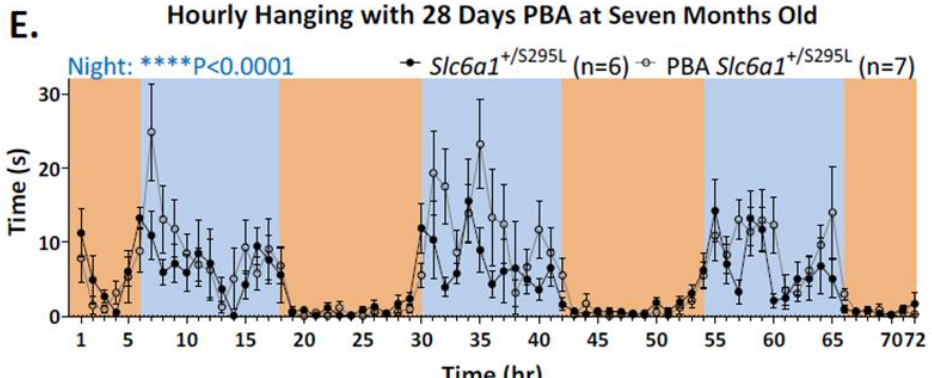
PBA rescued neurobehaviors in SLC6A1 variants in mouse models: hanging

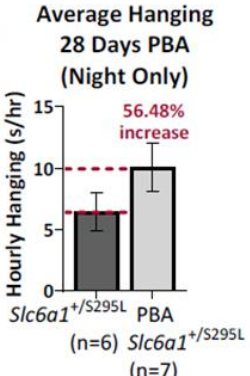








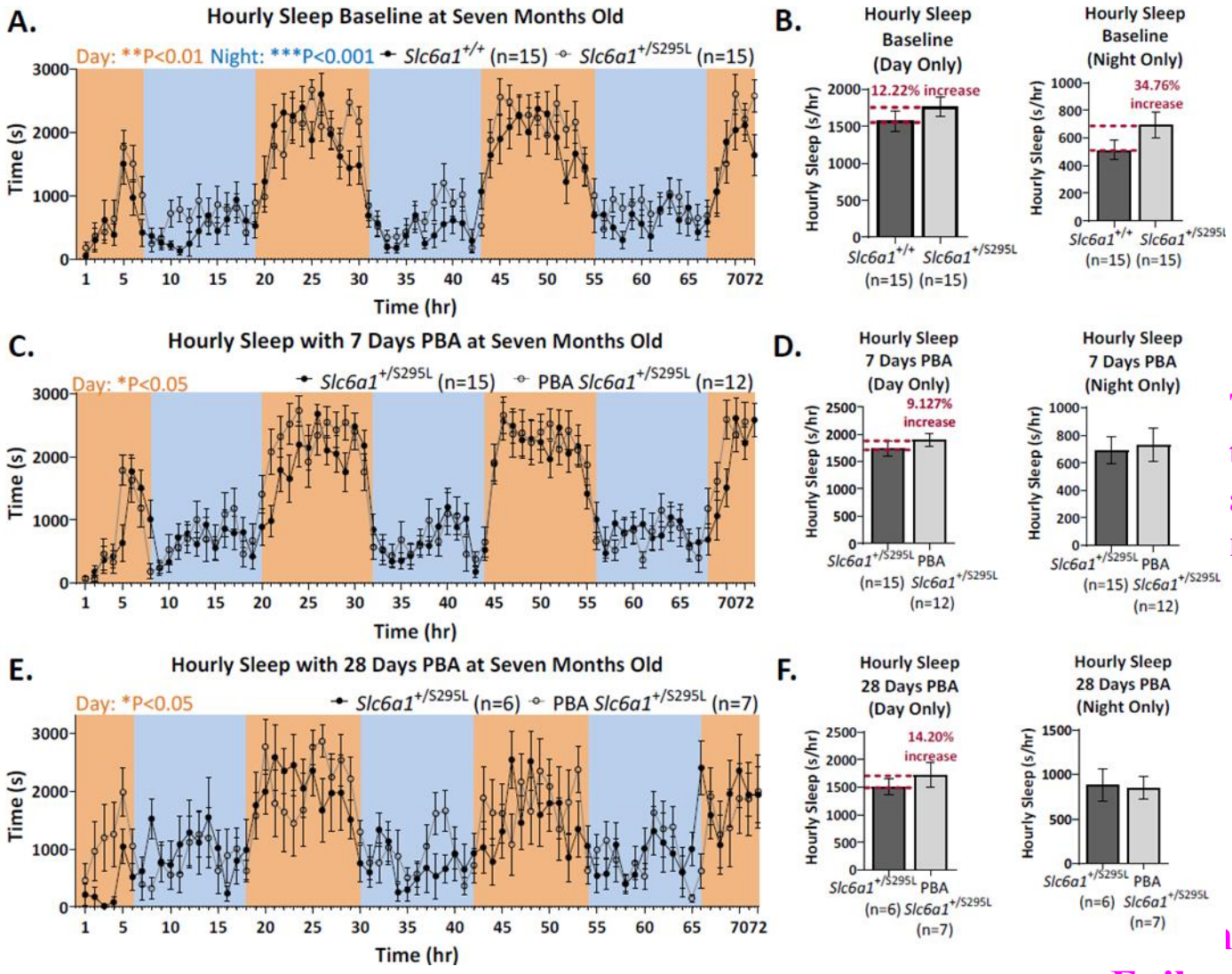




The effect of chronic treatment is similar to acute treatment for hanging

Randhave et al., 2025 Epilepsy Research

PBA rescued neurobehaviors in SLC6A1 variants in mouse models: sleep

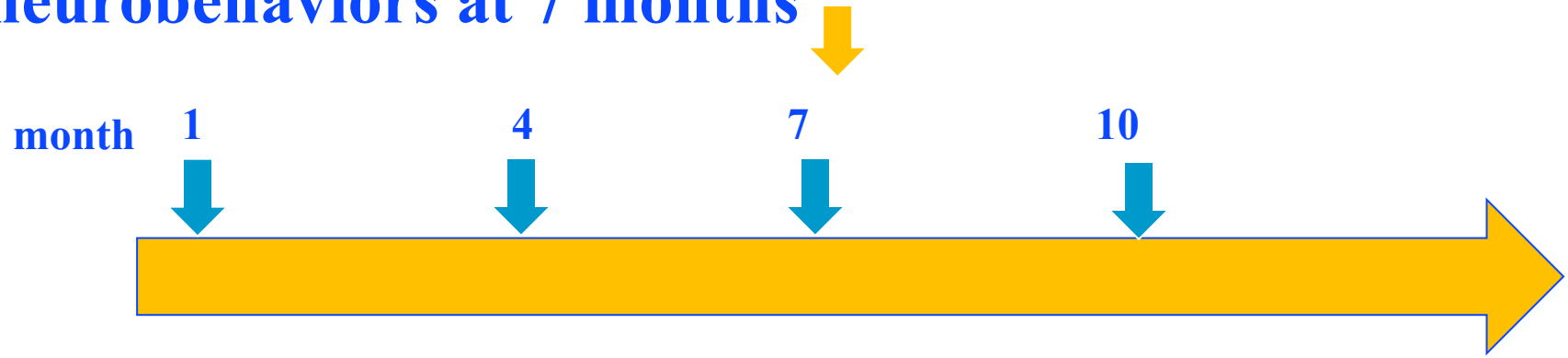


The effect of chronic treatment is similar to acute treatment for restoring sleep

ıave et al., 2025
Epilepsy Research

The data from the SLC6A1 mutation knockin mice suggest PBA/ravicti may be disease-modifying:

We normalized the extracellular GABA level and neurobehaviors at 7 months

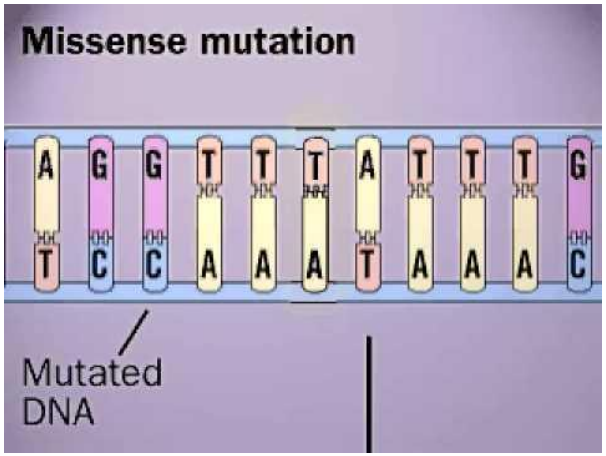


3. Update on minigene project/ a massive endeavor

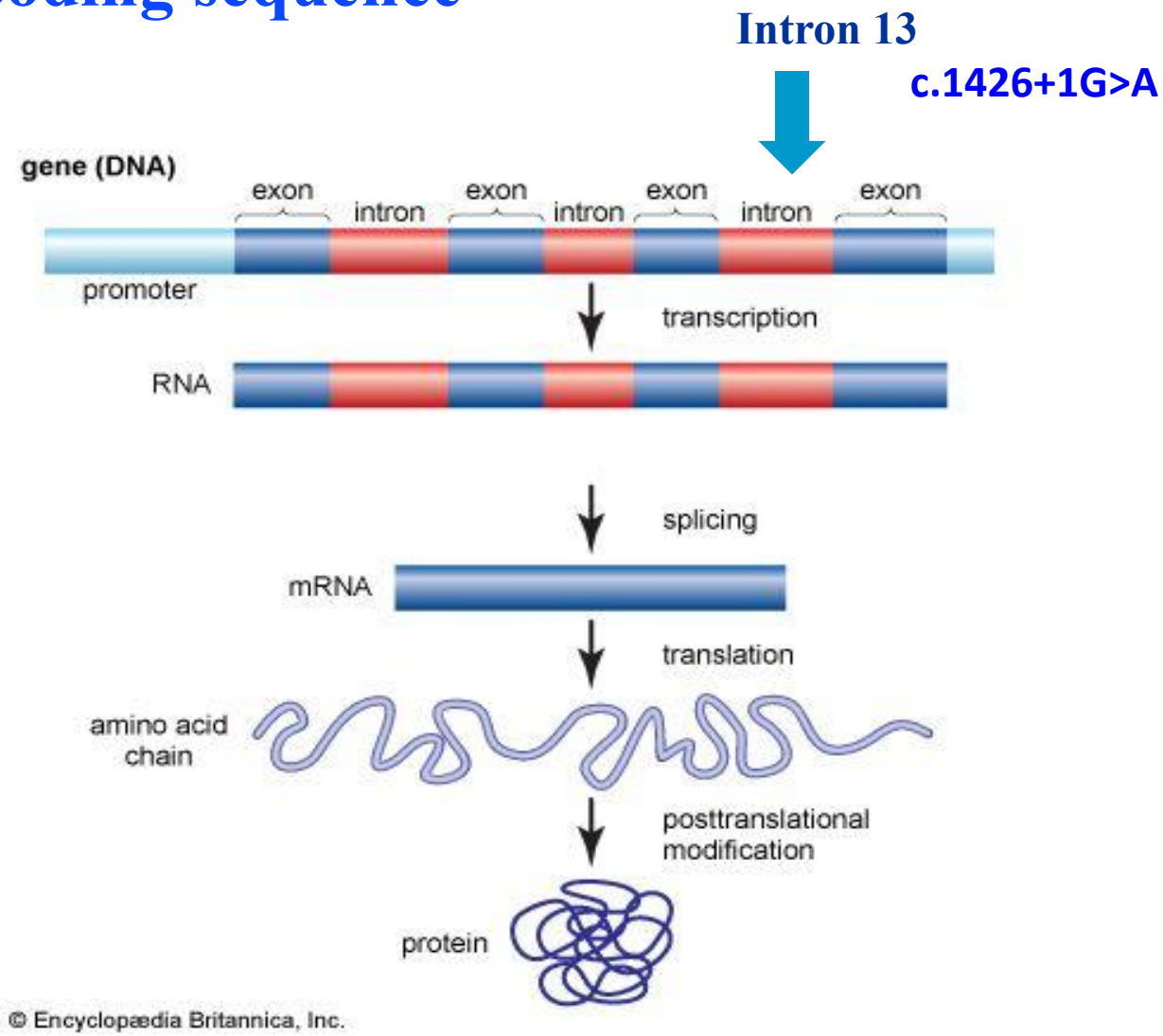
We have previously characterized mutations including missense, nonsense, frameshift and CNV etc.

missense **nonsense** **Deletion/insertion frameshift**

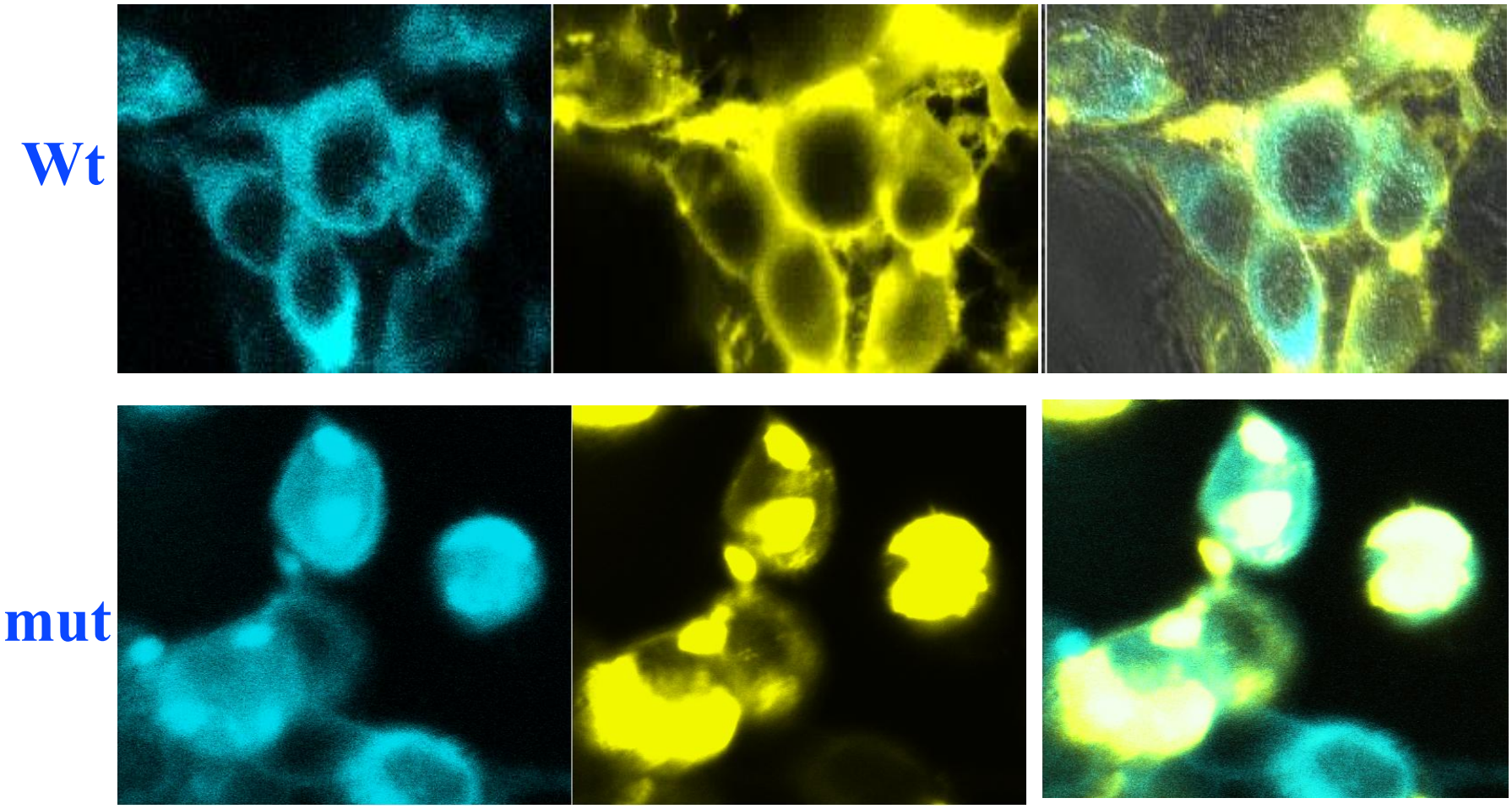
missense



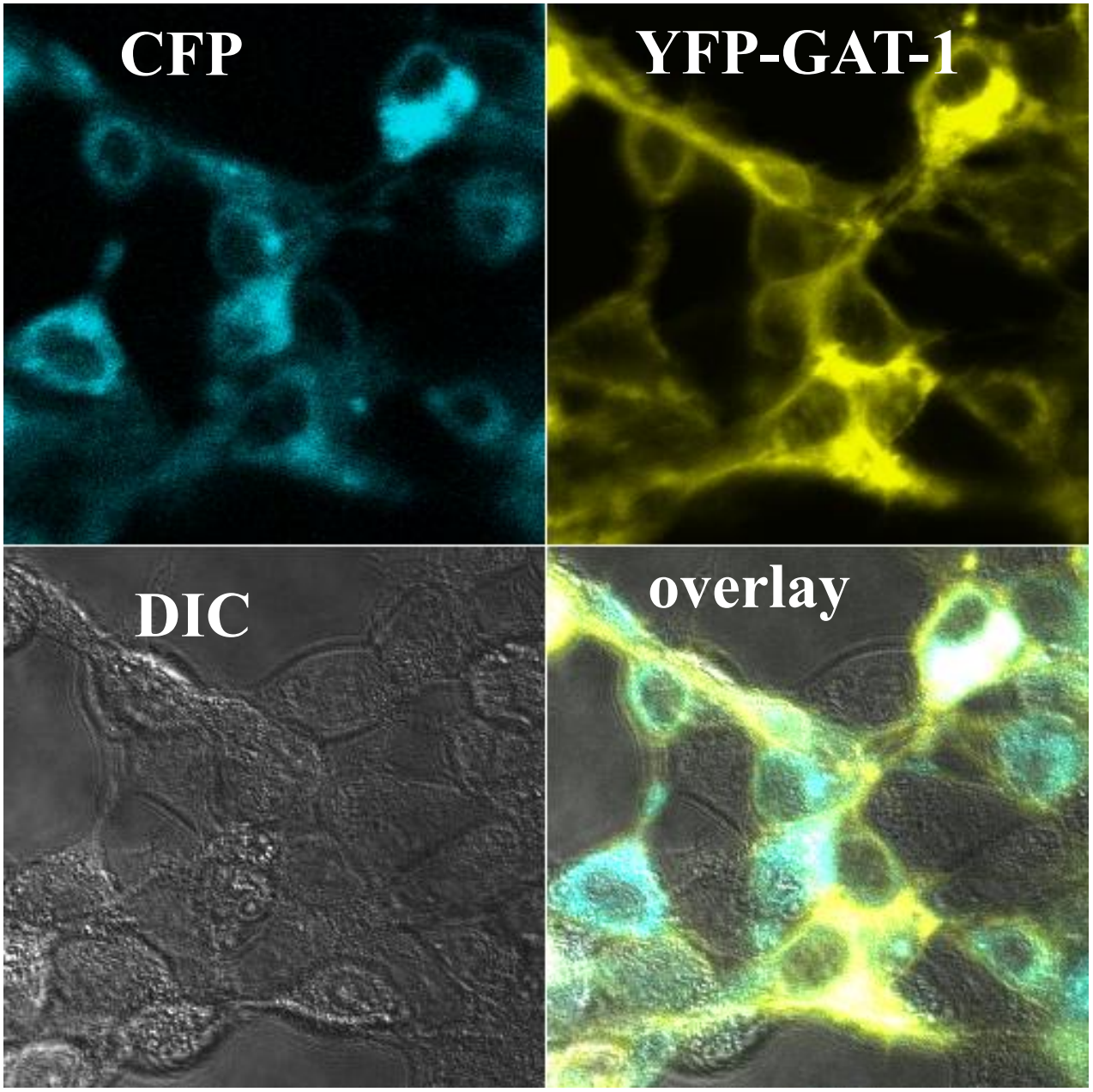
We have now characterized a splice site mutation with a minigene approach by engineering the intron into the coding sequence



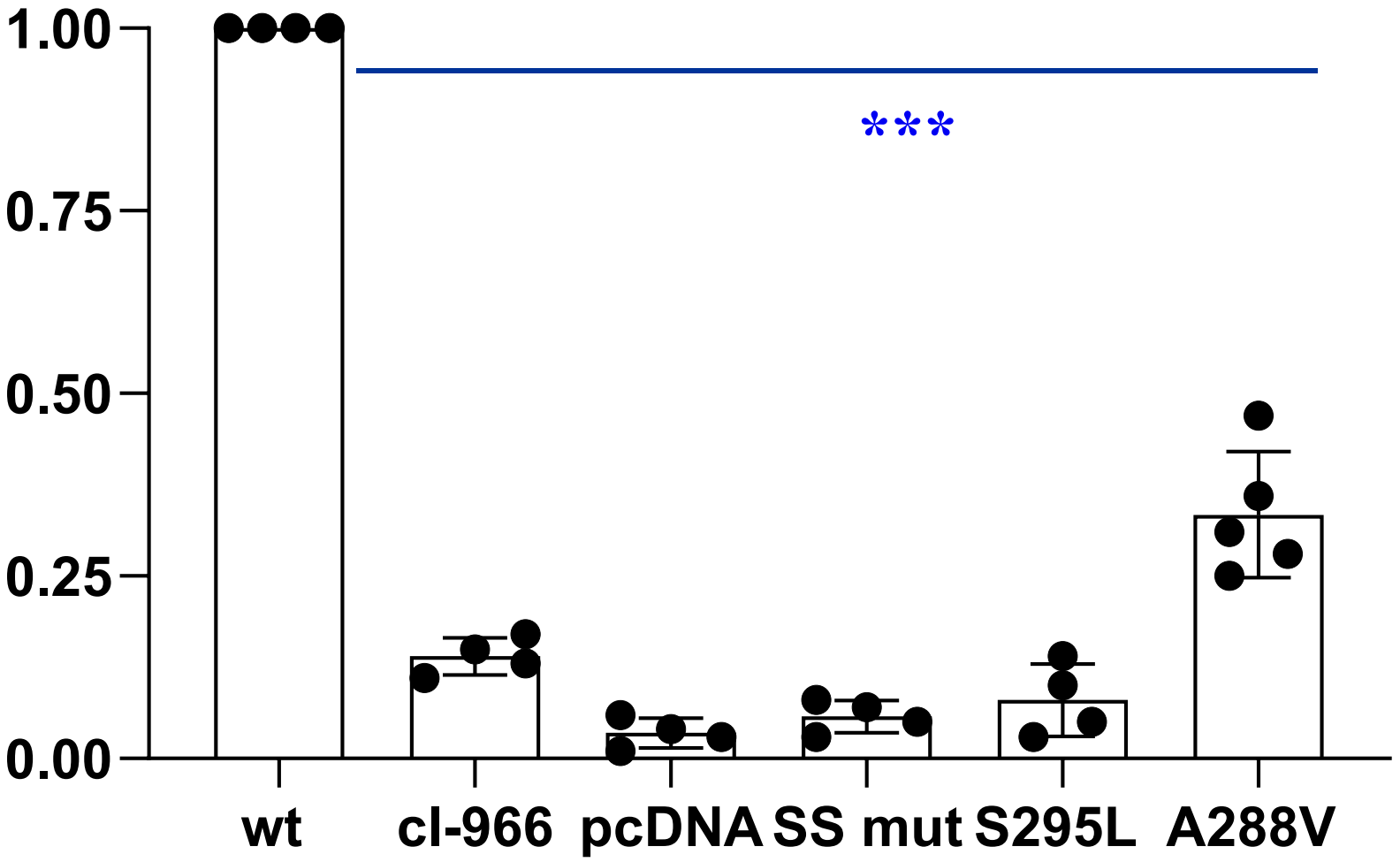
The splice site mutation had mutant protein ER retention



heterozygous



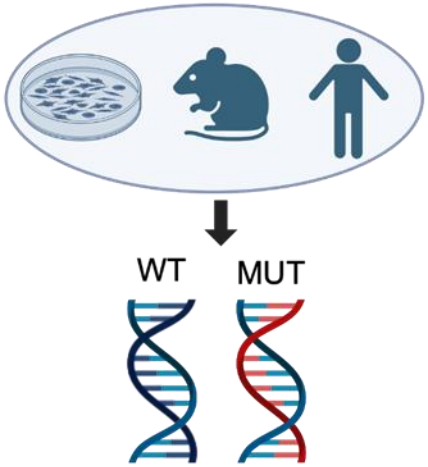
The splice site mutation had reduced GABA uptake

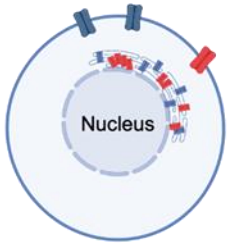


Ongoing work is evaluating the effect of PBA/Ravicti/butyrate on the splice site mutation

Pathogenic Mechanism of *SLC6A1* Variants

SLC6A1 Variants Cause ER Retention and Loss of Function



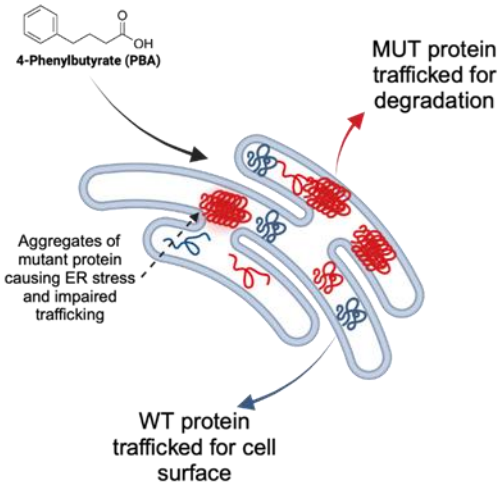


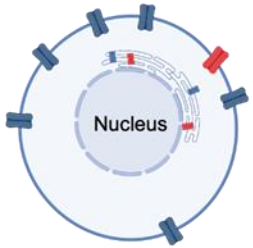
- Less functional WT on cell surface
- WT trapped in ER

- Aggregates of nonfunctional MUT retained in ER
- ER stress impairs WT trafficking
- MUT on cell surface nonfunctional

Impact of Pharmacochaperones *in vitro*

PBA Restores GAT-1 Trafficking and Function through Pharmacochaperoning Activity in ER





End Points: *in vivo* Effects of PBA on Seizure

PBA Treatment Reduces Seizures in Slc6a1 Knock-in Mice

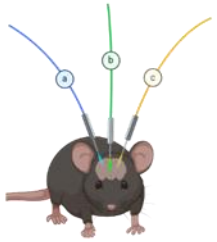
PBA Reduces # Seizure Events

PBA Reduces Avg. Duration of Seizure Events

Butyrate Increases # Seizure Events in S295L Mice

Butyrate Does Not Reduce Duration

Butyrate Shows No Significant Difference in # Seizure Events in A288V Mice



Taking home message from the updates today:

1) PBA is likely disease-modifying

2) Slc6a1 spice site mutation may benefit from PBA/Ravicti

3). PBA is likely more efficacious than butyrate in seizure control for SLC6A1 variants

I got SLC6A1(S295L) mutation, let's fight!!!