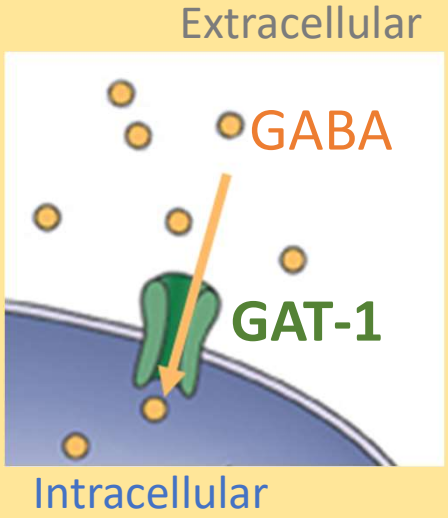
GABAergic System and the Role of GAT-1

GABA
(gamma-aminobutyric acid)

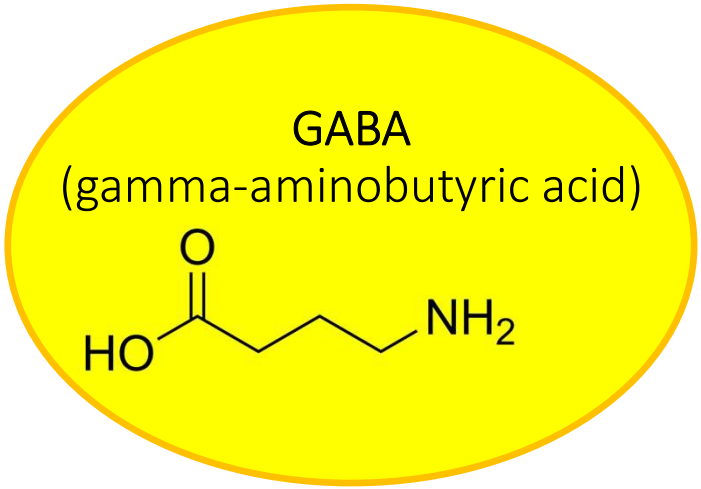
Kirill Zavalin, PhD

Dr. Jingqiong (Katty) Kang Laboratory
Department of Neurology
Vanderbilt University Medical Center

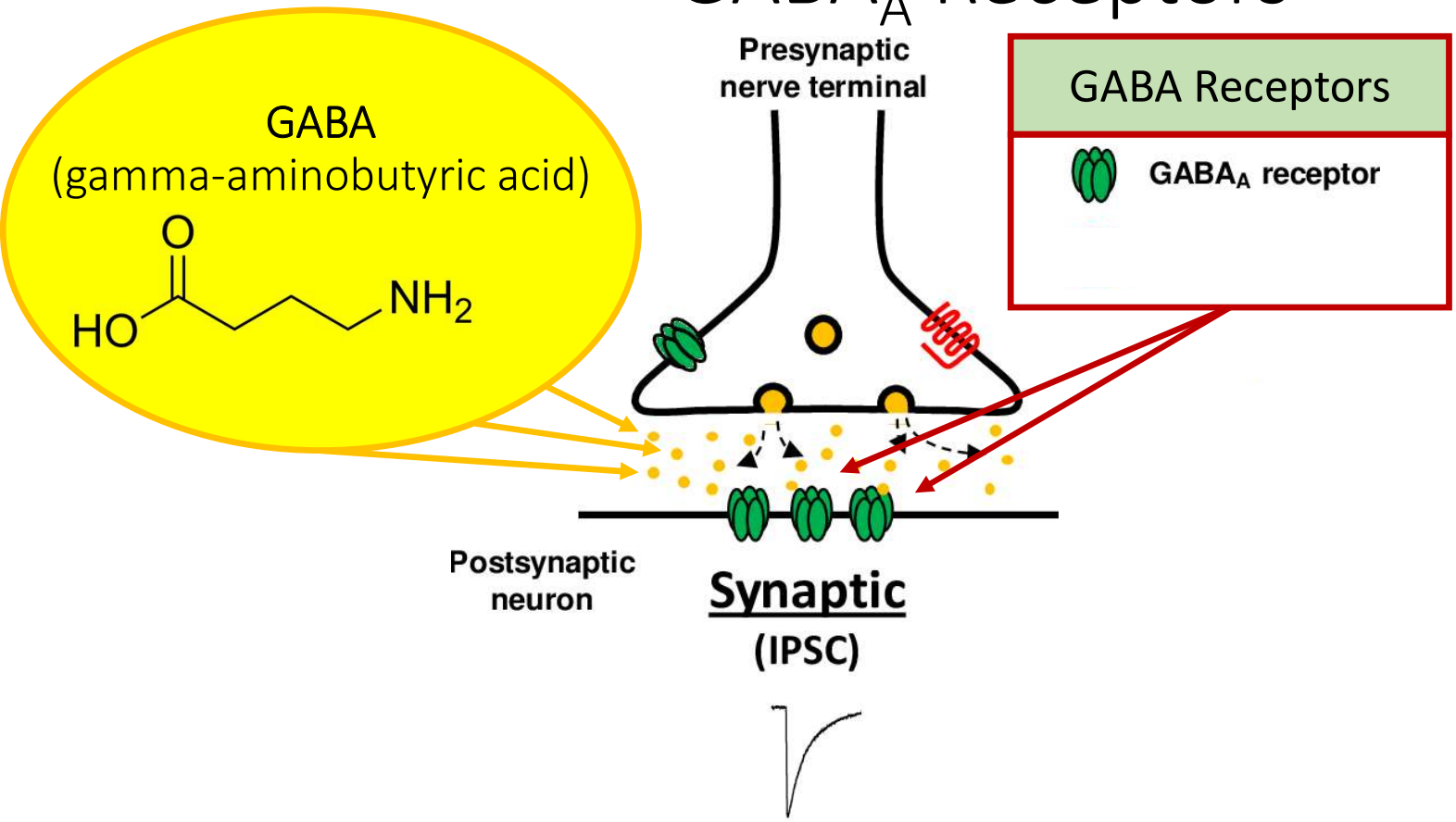


GABAergic Signaling Mediates Most Inhibitory Neurotransmission in the Adult Brain

- Target of many drugs with anxiolytic, anti-seizure, anesthetic, and sleep-inducing effects
- GABAergic deficits underlie neurological and neurodevelopmental disorders, such as epilepsy
- Brief overview of GABAergic system with focus on GAT-1

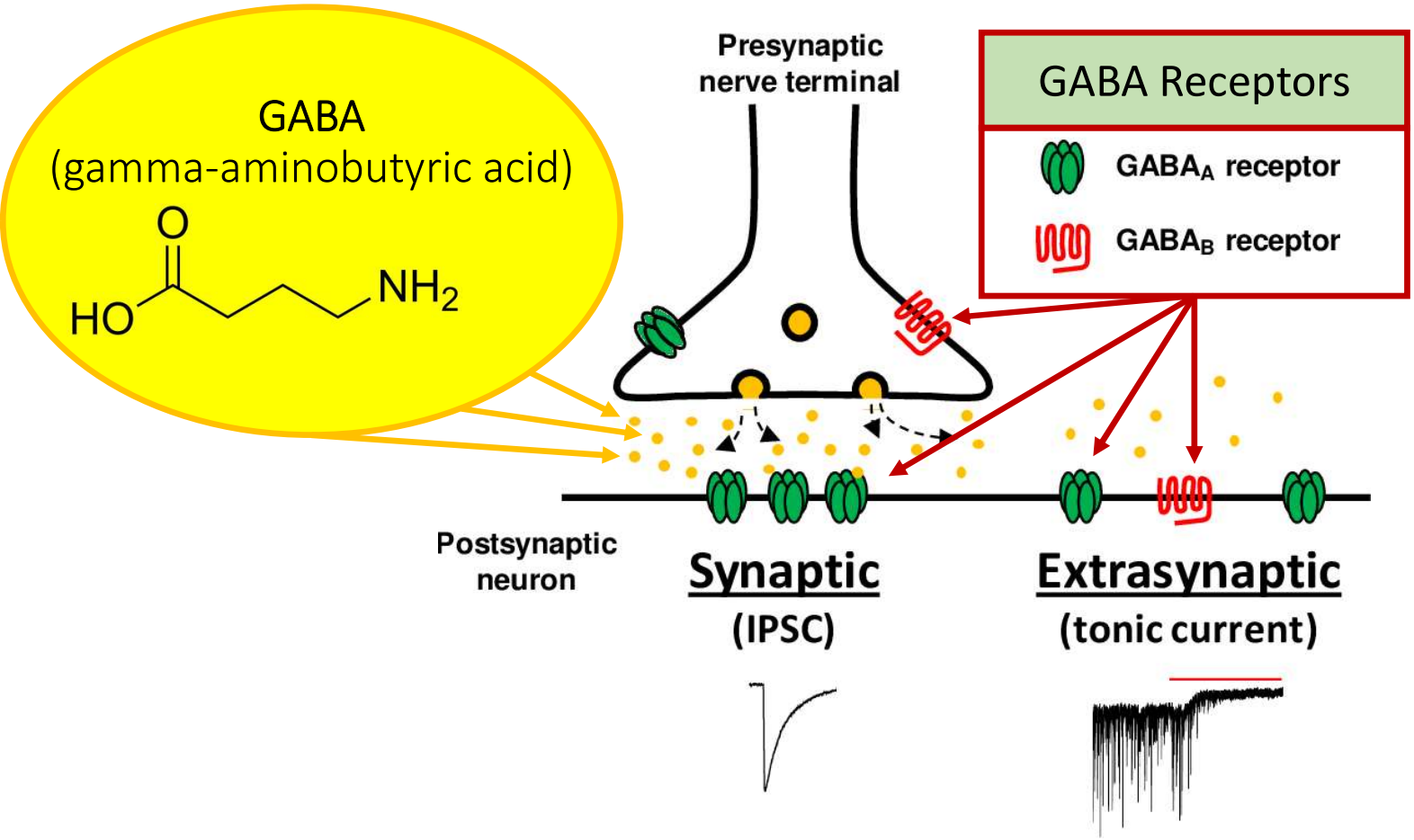


Synaptic GABAergic Responses Are Mediated by $GABA_A$ Receptors



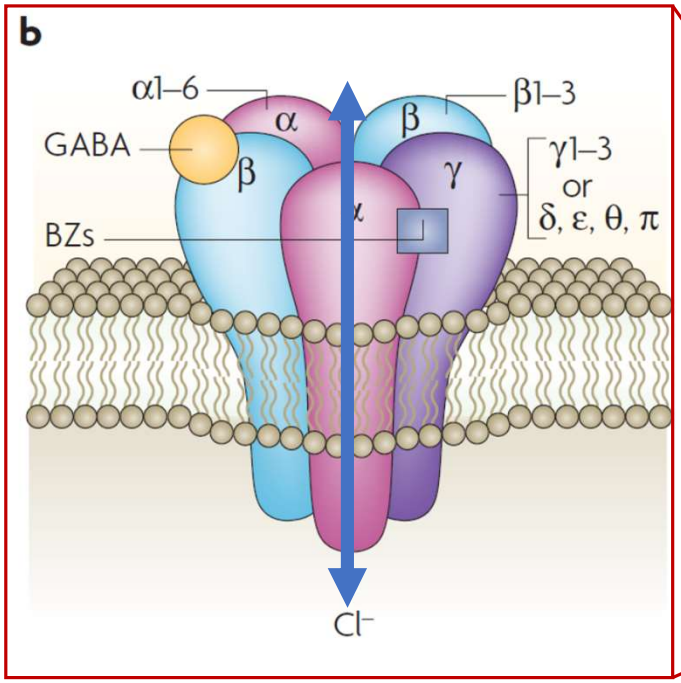
Bg Patel, 2015

GABA Currents Can Be Synaptic and Extrasynaptic

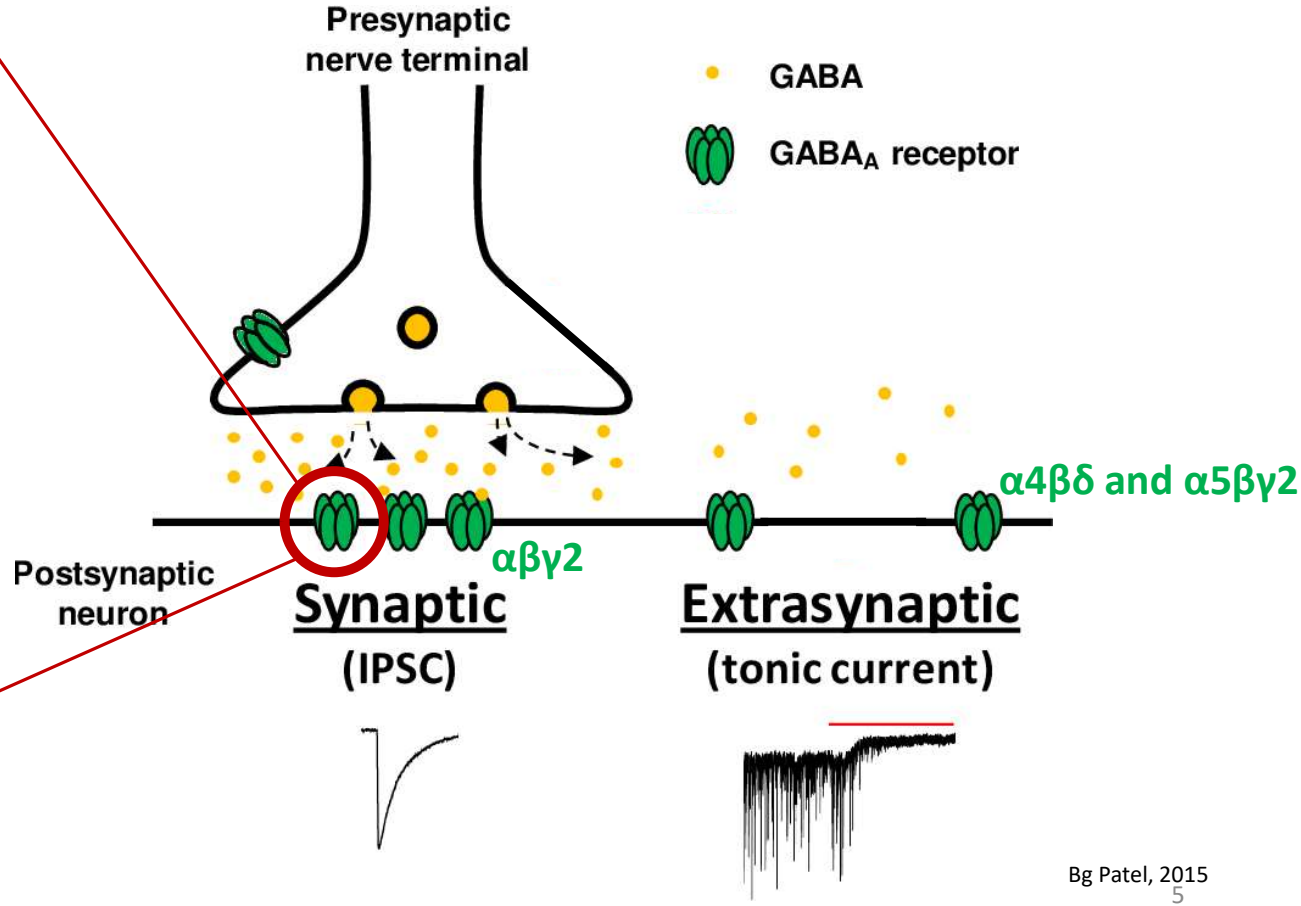


B.G. Patel, 2015

$GABA_A$ Receptors Mediate Quick Inhibitory Signals



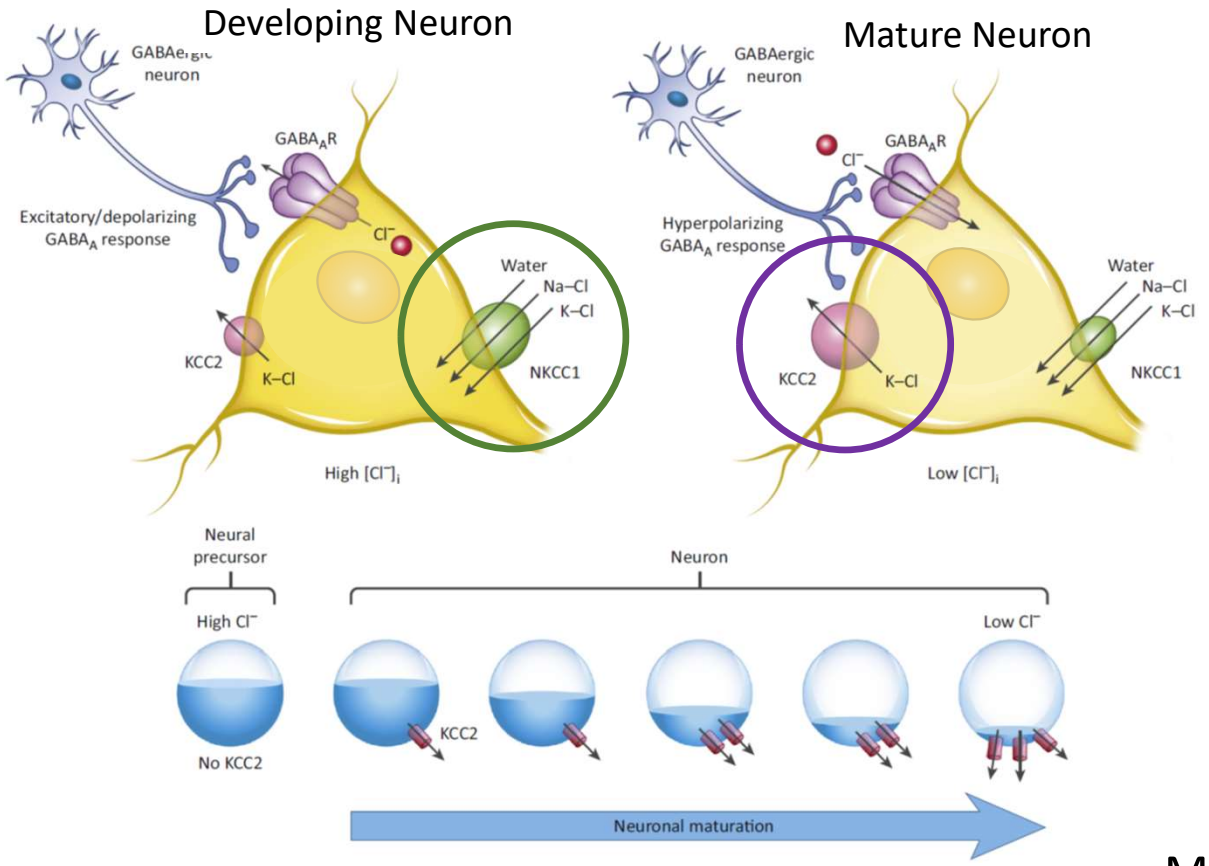
Jacob et al, 2008



Bg Patel, 2015

Neuronal Chloride Determines if GABAA Receptor Responses Are Inhibitory or Excitatory

NKCC1



KCC2

Moore et al, 2017

GAT-1 Mediates Re-Uptake of Extracellular GABA

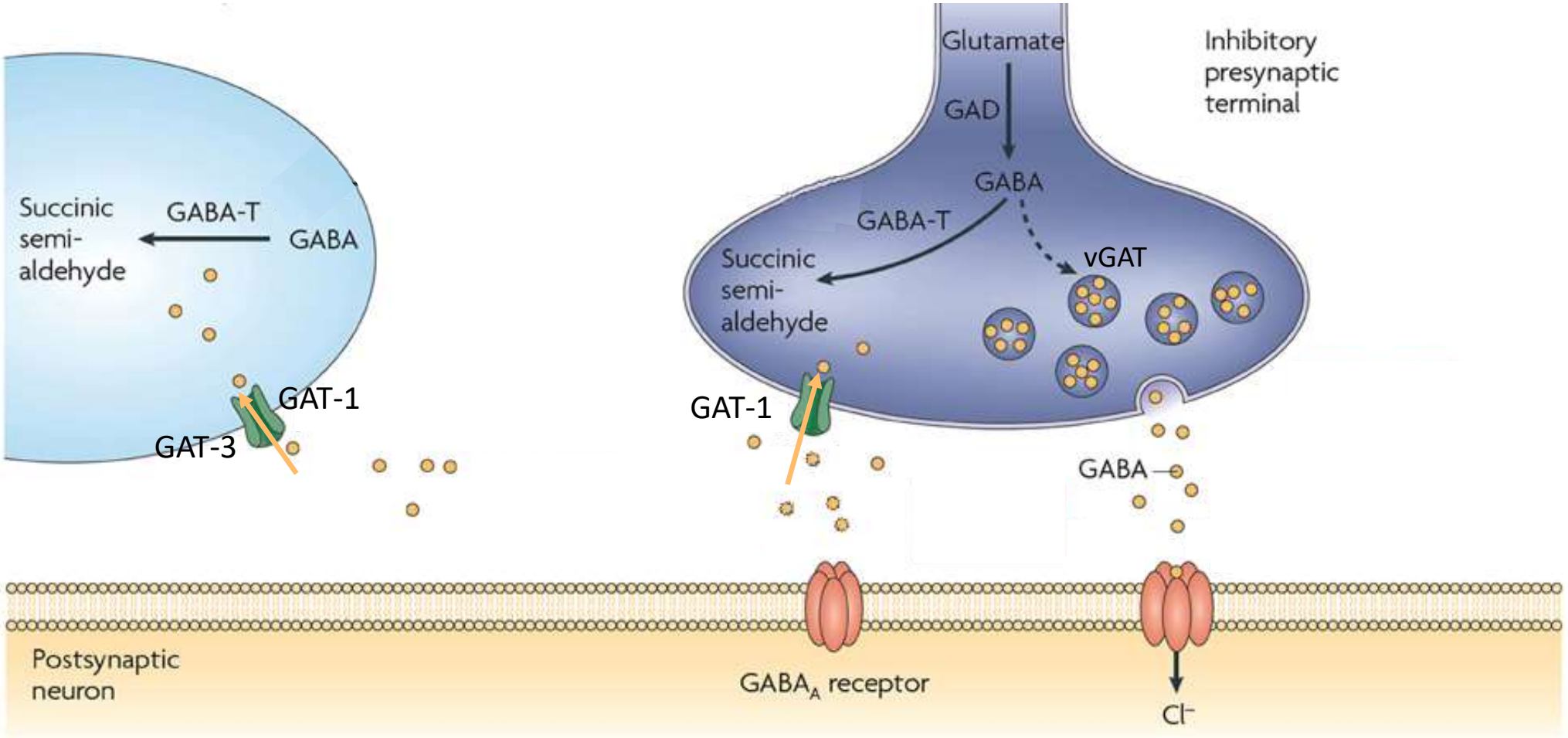


Figure adapted from: Liefferinge, J. V., Massie, A., Portelli, J., Giovanni, G. D. & Smolders, I. Are vesicular neurotransmitter transporters potential treatment targets for temporal lobe epilepsy? *Front Cell Neurosci* **7**, 139 (2013).

Many Anti-Seizure Drugs Act the GABAergic System

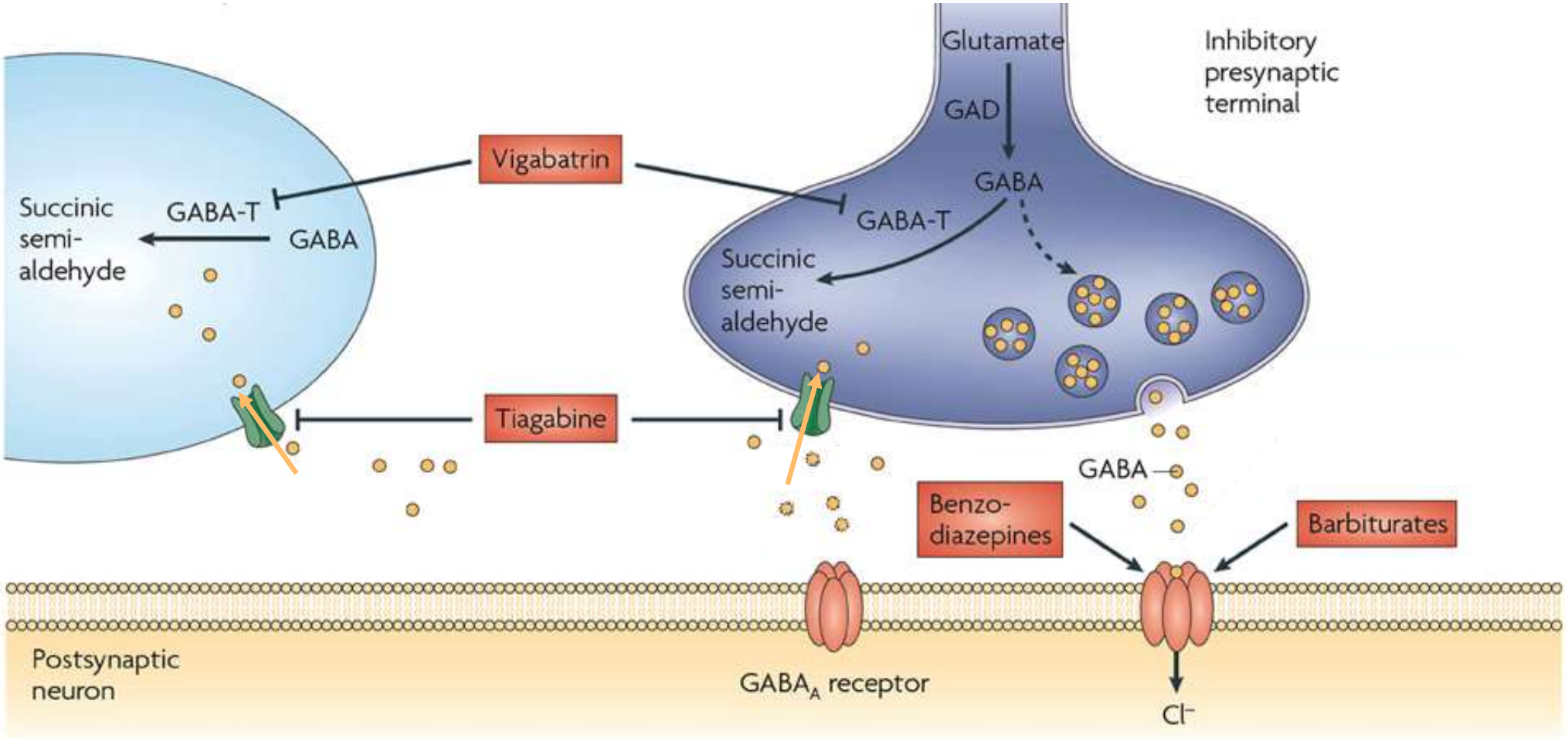


Figure adapted from: Liefferinge, J. V., Massie, A., Portelli, J., Giovanni, G. D. & Smolders, I. Are vesicular neurotransmitter transporters potential treatment targets for temporal lobe epilepsy? *Front Cell Neurosci* **7**, 139 (2013).

GAT-1 and GAT-3 Mediate Re-Uptake of Extracellular GABA

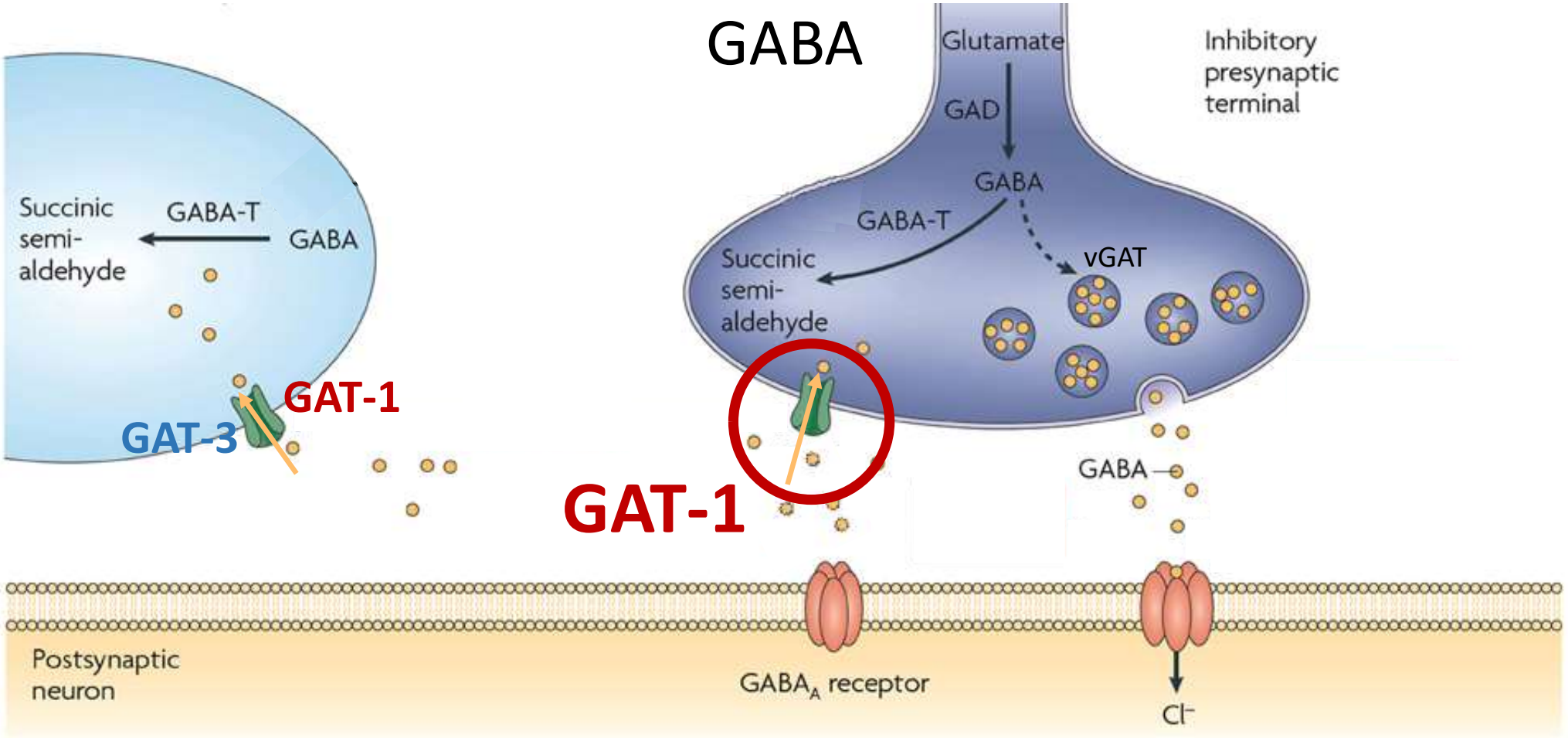
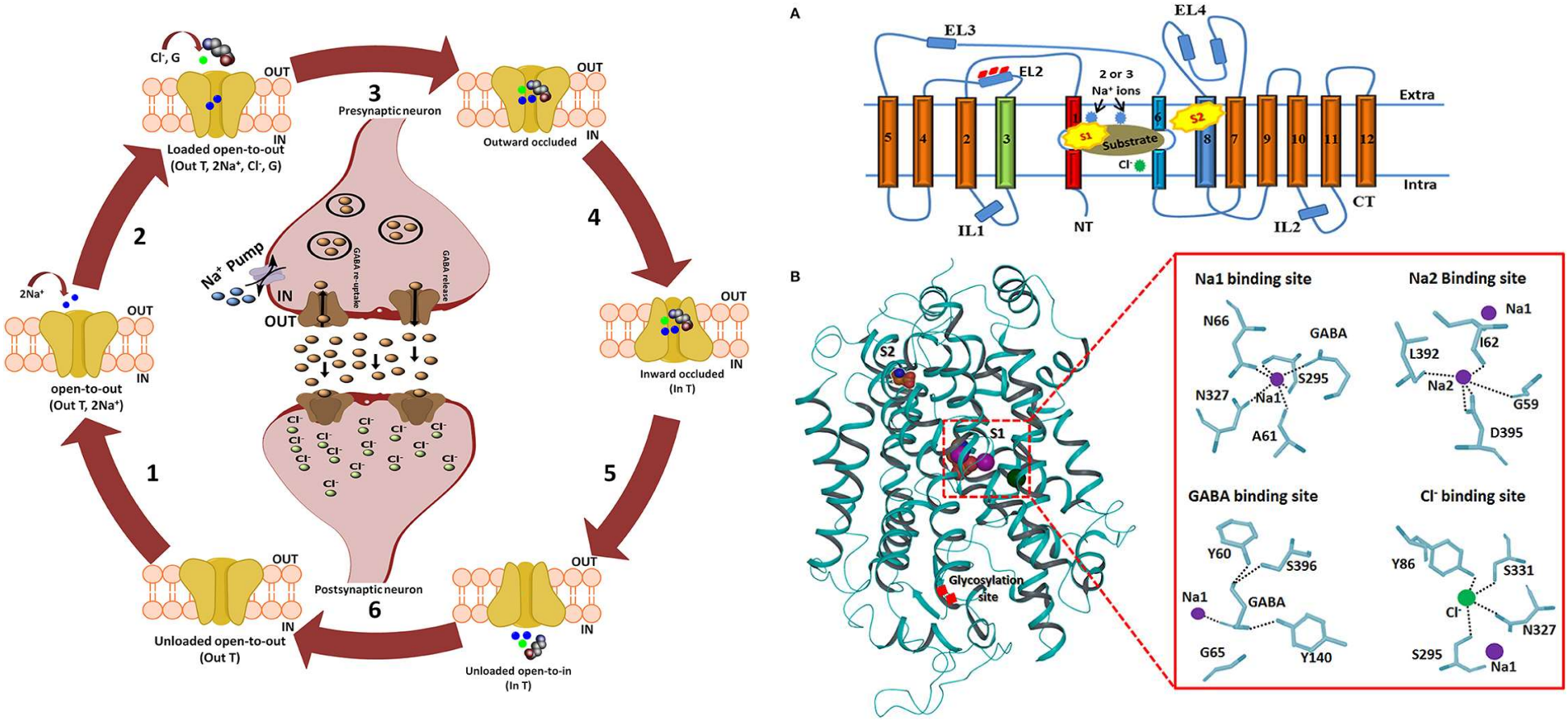


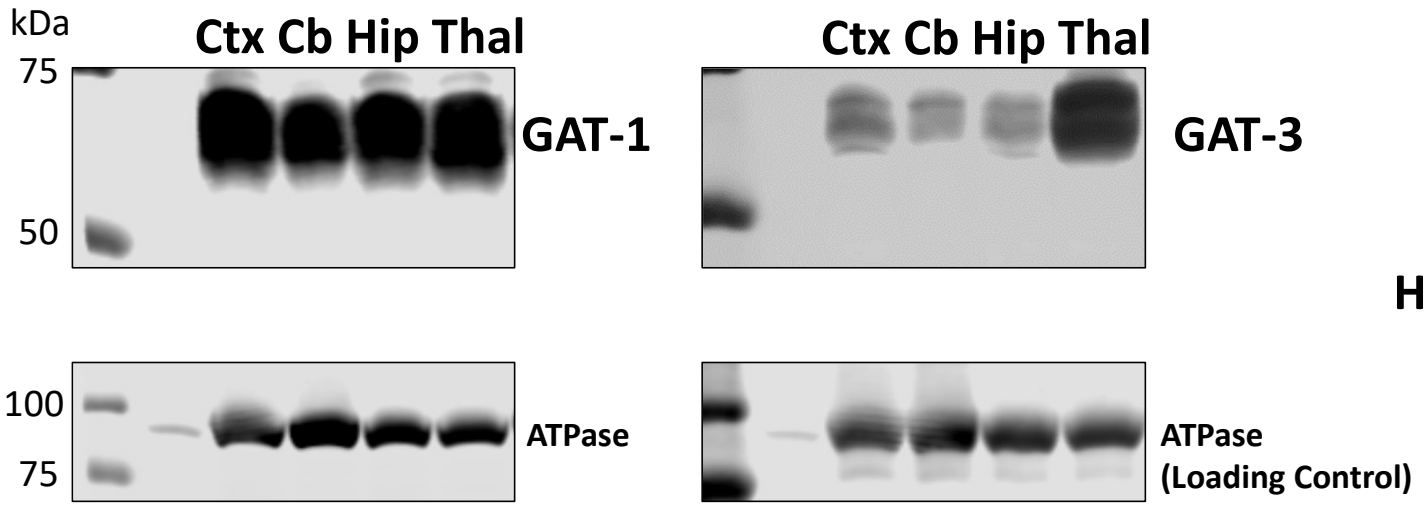
Figure adapted from: Liefferinge, J. V., Massie, A., Portelli, J., Giovanni, G. D. & Smolders, I. Are vesicular neurotransmitter transporters potential treatment targets for temporal lobe epilepsy? *Front Cell Neurosci* **7**, 139 (2013).

GAT-1 Transport Cycle and Structure



Zafar, S. & Jabeen, I. Structure, Function, and Modulation of γ-Aminobutyric Acid Transporter 1 (GAT1) in Neurological Disorders: A Pharmacoinformatic Prospective. Front Chem 6, 397 (2018).

GAT-1 Predominantly Expressed in Neocortex, GAT-3 Expression High in Thalamus

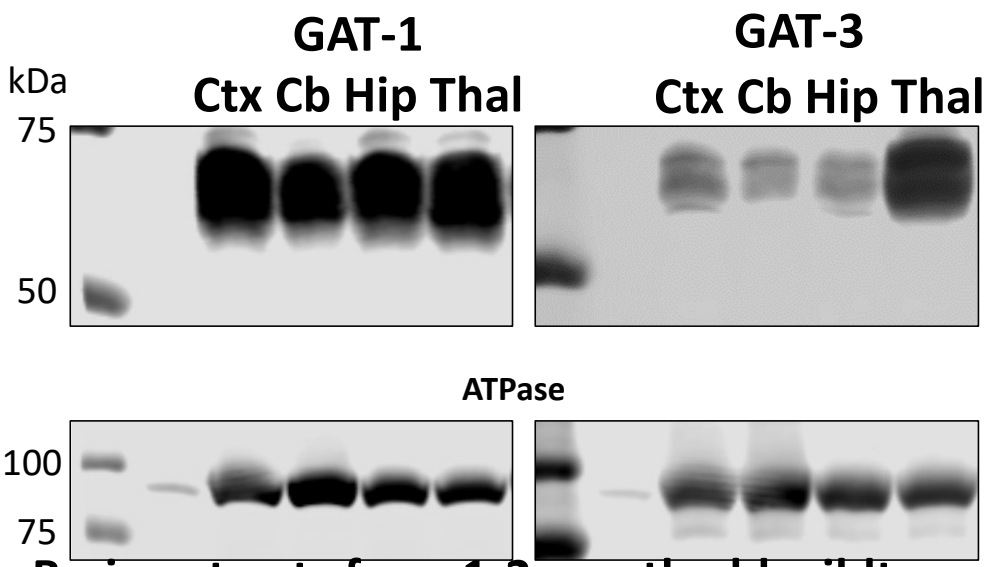


Ctx: Cortex
Cb: Cerebellum
Hip: Hippocampus
Thal: Thalamus

Brain extracts from 1-2 month old wildtype mice

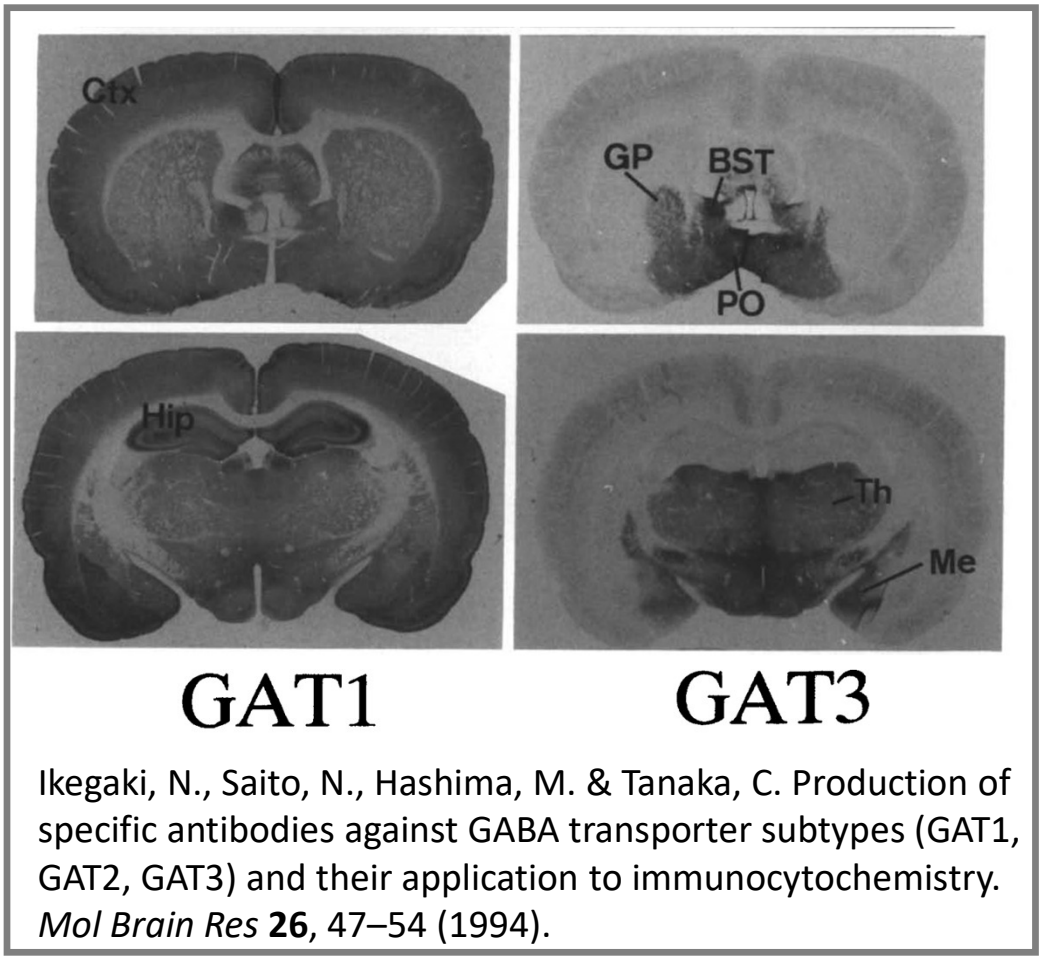
Work of Dr. Wangzhen Shen

GAT-1 Predominantly Expressed in Neocortex, GAT-3 Expression High in Thalamus



Brain extracts from 1-2 month old wildtype mice

Work of Dr. Wangzhen Shen



Ikegaki, N., Saito, N., Hashima, M. & Tanaka, C. Production of specific antibodies against GABA transporter subtypes (GAT1, GAT2, GAT3) and their application to immunocytochemistry. *Mol Brain Res* **26**, 47–54 (1994).

GAT-1 Knockout Mouse Has Seizures and Other Disorders

- GAT-1 knockout (homozygote, complete GAT-1 loss) generated by Istvan Mody's research group (Jensen et al, 2003)
- Spontaneous absence seizures with spontaneous spike-wave discharges (Cope et al, 2009)
- Sensitivity to PTZ-induced seizures (Chiu et al, 2005)
- Motor disorders, including gait abnormality, tremor, reduced locomotor activity (Jensen et al, 2003)
- Behavioral abnormalities, including altered anxiety (Chiu et al, 2005; Yu et al, 2013; Gong et al, 2015; Lie et al, 2007; Yu et al, 2013)
- Cognitive deficits (Yu et al, 2013)

Increased Tonic GABAergic Current in GAT1 KO

Hippocampal CA1 pyramidal neurons in P15-25 Mice

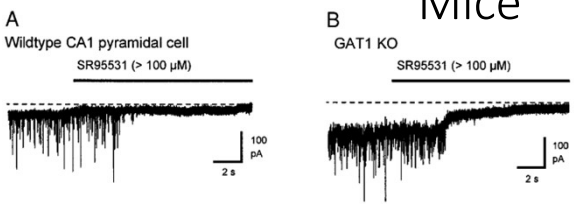


TABLE 1. *Tonic $GABA_A$ receptor-mediated currents in CA1 pyramidal cells in wild-type (WT) and in GABA transporter 1 (GAT1) knockout (KO) mice under various treatments*

Treatment	SR95531-Sensitive Tonic Current, pA	No. of Cells
No treatment	5.7 ± 1.0	20
WT	4.9 ± 2.3	4
WT + 10 μM NO-711	25.2 ± 6.0	5
WT + 50 μM NO-711	12.6 ± 3.5	4
WT + 10 μM NO-711 + 0.8 μM GABA	45.4 ± 21	4
GAT1 KO	45.8 ± 11	5
GAT1 KO + 10 μM NO-711	38.1 ± 14	7
GAT1 KO + 1 μM TTX	17.0 ± 4.9	5
GAT1 KO + 0.6 mM furosemide	18.9 ± 3.9	4
GAT1 KO + 1 mM furosemide	17.9 ± 6.3	4

Values are means ± SE. No treatment: spontaneous holding current fluctuations during 20 s with no applied SR95531.

Jensen et al, 2003 (Mody)

Increased Tonic GABAergic Current in GAT1 KO

Hippocampal CA1 pyramidal neurons in P15-25 Mice

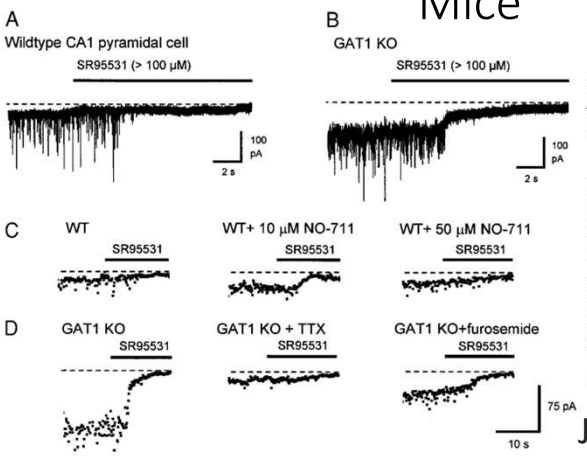


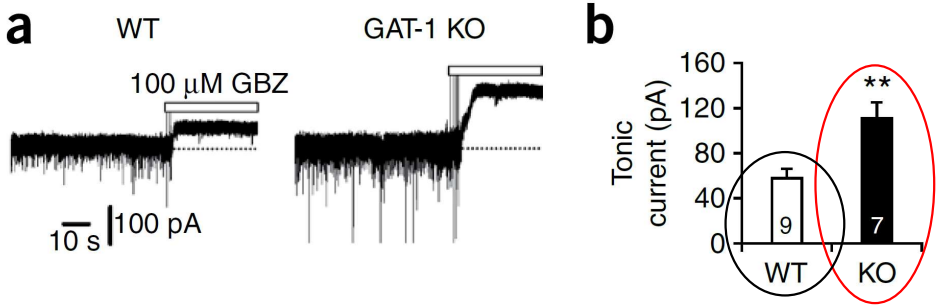
TABLE 1. *Tonic $GABA_A$ receptor-mediated currents in CA1 pyramidal cells in wild-type (WT) and in GABA transporter 1 (GAT1) knockout (KO) mice under various treatments*

Treatment	SR95531-Sensitive Tonic Current, pA	No. of Cells
No treatment	5.7 ± 1.0	20
WT	4.9 ± 2.3	4
WT + 10 μM NO-711	25.2 ± 6.0	5
WT + 50 μM NO-711	12.6 ± 3.5	4
WT + 10 μM NO-711 + 0.8 μM GABA	45.4 ± 21	4
GAT1 KO	45.8 ± 11	5
GAT1 KO + 10 μM NO-711	38.1 ± 14	7
GAT1 KO + 1 μM TTX	17.0 ± 4.9	5
GAT1 KO + 0.6 mM furosemide	18.9 ± 3.9	4
GAT1 KO + 1 mM furosemide	17.9 ± 6.3	4

Values are means ± SE. No treatment: spontaneous holding current fluctuations during 20 s with no applied SR95531.

Jensen et al, 2003 (Mody)

Neurons in ventrobasal thalamus in P68-74 mice



Cope et al, 2009 (Crunelli)

Increased Tonic GABAergic Current in GAT1 KO

Hippocampal CA1 pyramidal neurons in P15-25 Mice

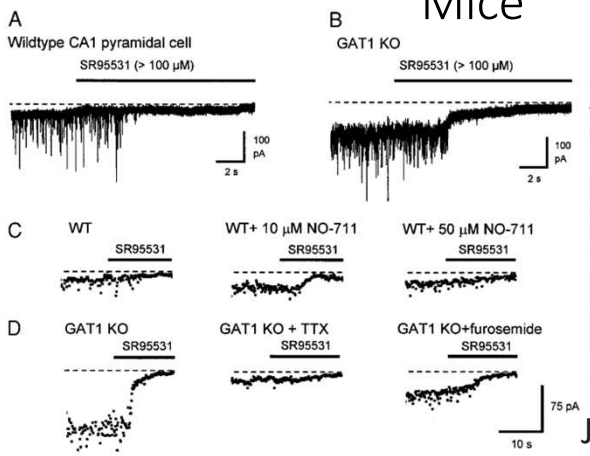


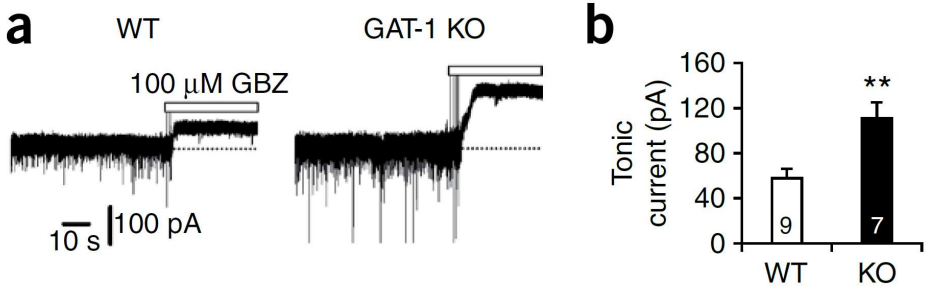
TABLE 1. *Tonic $GABA_A$ receptor-mediated currents in CA1 pyramidal cells in wild-type (WT) and in GABA transporter 1 (GAT1) knockout (KO) mice under various treatments*

Treatment	SR95531-Sensitive Tonic Current, pA	No. of Cells
No treatment	5.7 ± 1.0	20
WT	4.9 ± 2.3	4
WT + 10 μM NO-711	25.2 ± 6.0	5
WT + 50 μM NO-711	12.6 ± 3.5	4
WT + 10 μM NO-711 + 0.8 μM GABA	45.4 ± 21	4
GAT1 KO	45.8 ± 11	5
GAT1 KO + 10 μM NO-711	38.1 ± 14	7
GAT1 KO + 1 μM TTX	17.0 ± 4.9	5
GAT1 KO + 0.6 mM furosemide	18.9 ± 3.9	4
GAT1 KO + 1 mM furosemide	17.9 ± 6.3	4

Values are means ± SE. No treatment: spontaneous holding current fluctuations during 20 s with no applied SR95531.

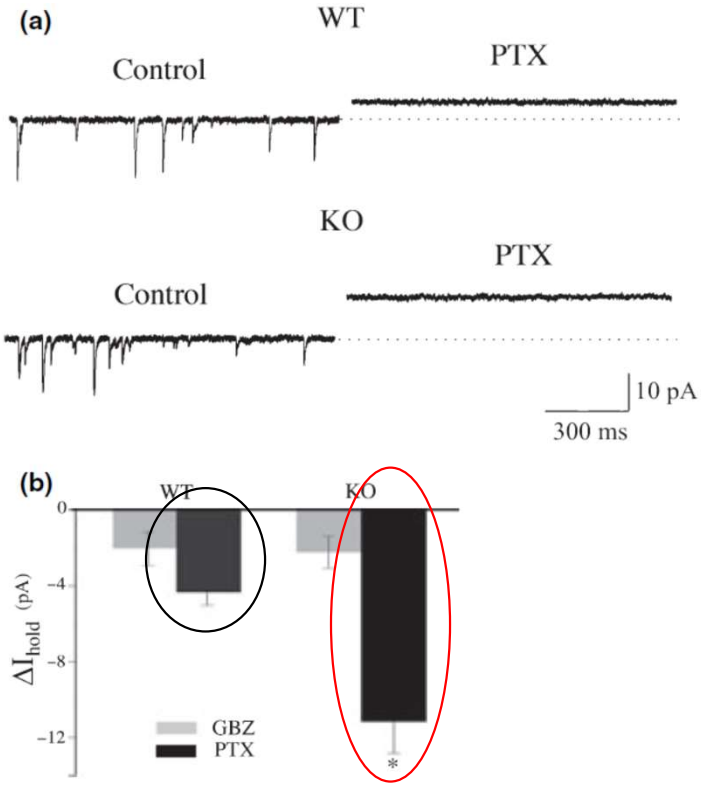
Jensen et al, 2003 (Mody)

Neurons in ventrobasal thalamus in P68-74 mice



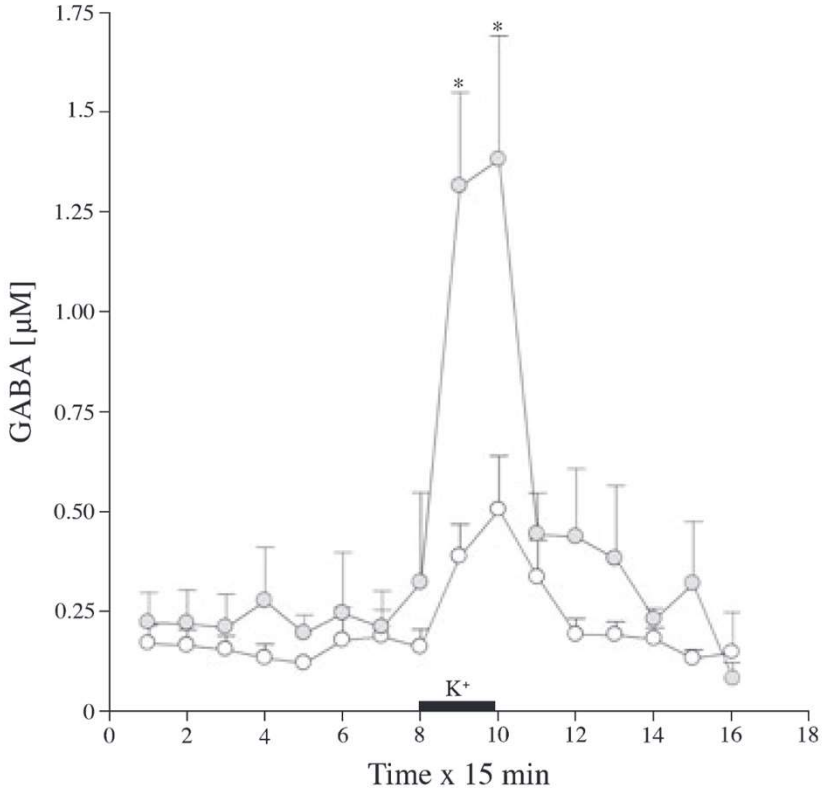
Cope et al, 2009 (Crunelli)

Layer 3 cortical pyramidal neurons, 1-4 month old mice



Bragina et al, 2008 (Cherubini and Conti)

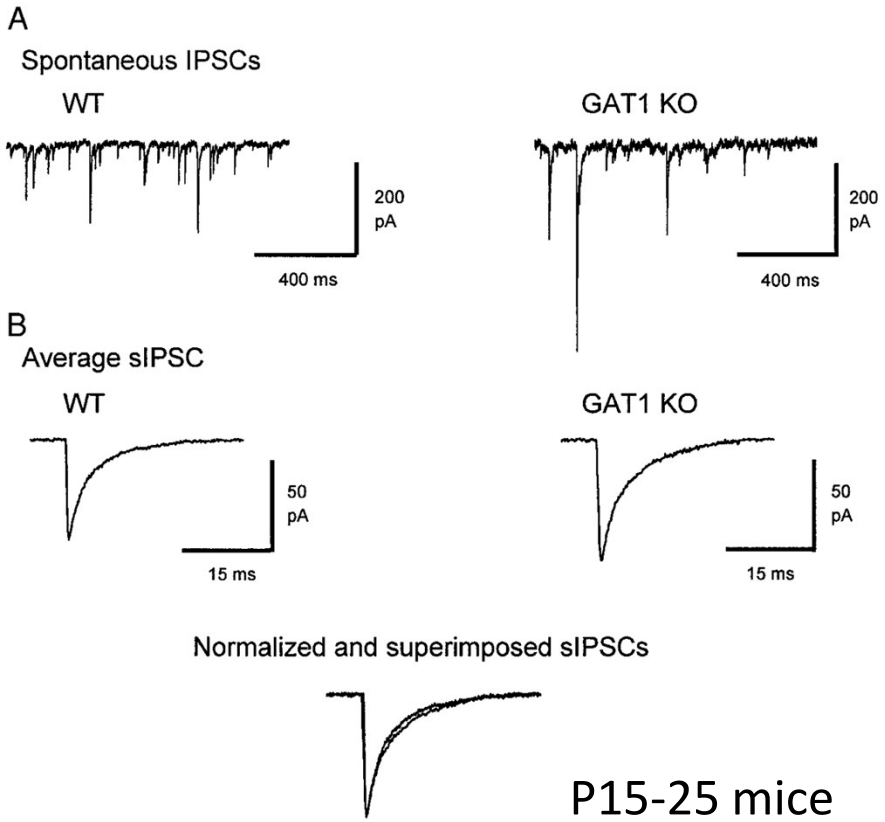
Increased Cortical GABA Levels with KCl Stimulation in GAT-1 KO



Bragina et al, 2008 (Cherubini and Conti)

Normal Spontaneous Synaptic Currents in Hippocampal Pyramidal Neurons

sIPSC in CA1 pyramidal neurons

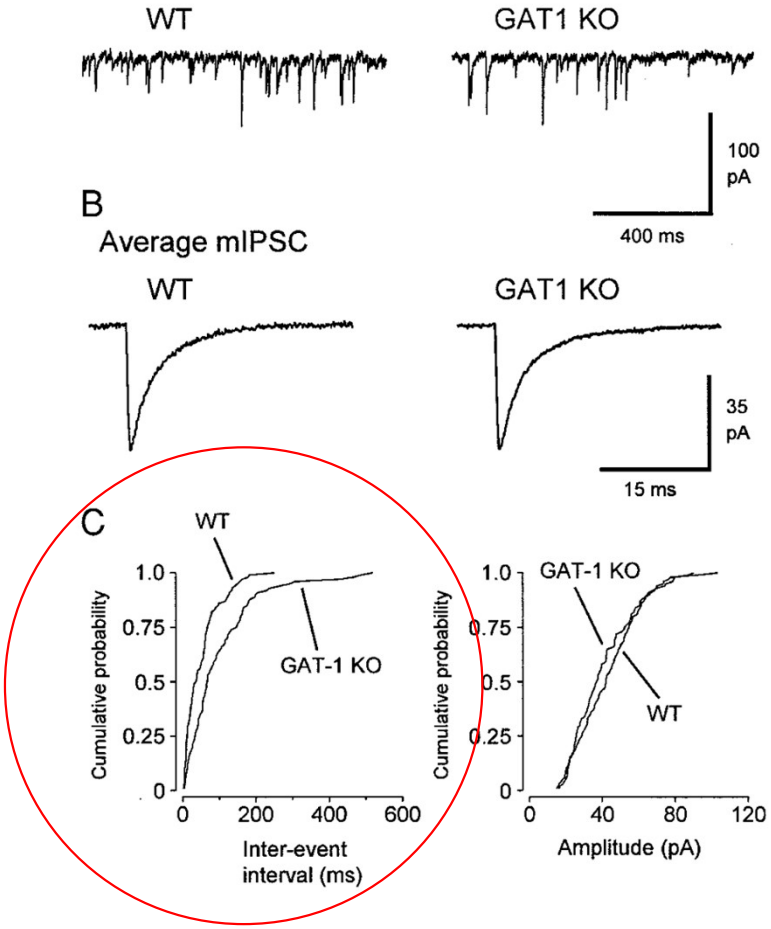


P15-25 mice

Jensen et al, 2003 (Mody)

Increased Frequency of Miniature IPSCs in Hippocampus

mIPSC in CA1 pyramidal neurons

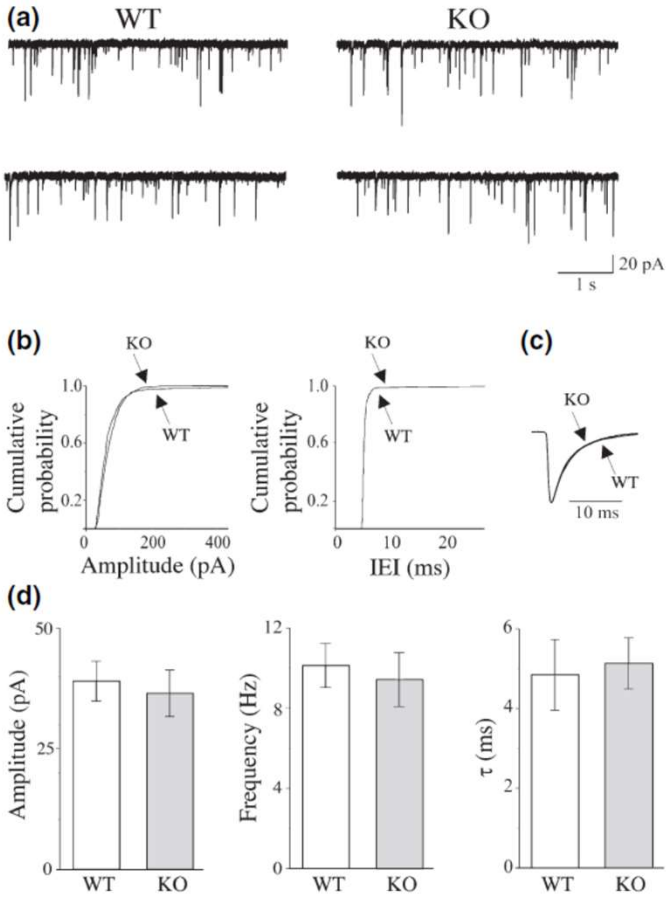


P15-25 mice

Jensen et al, 2003 (Mody)

Normal spontaneous synaptic events in GAT-1 KO

sIPSCs in layer 2-3 pyramidal neurons



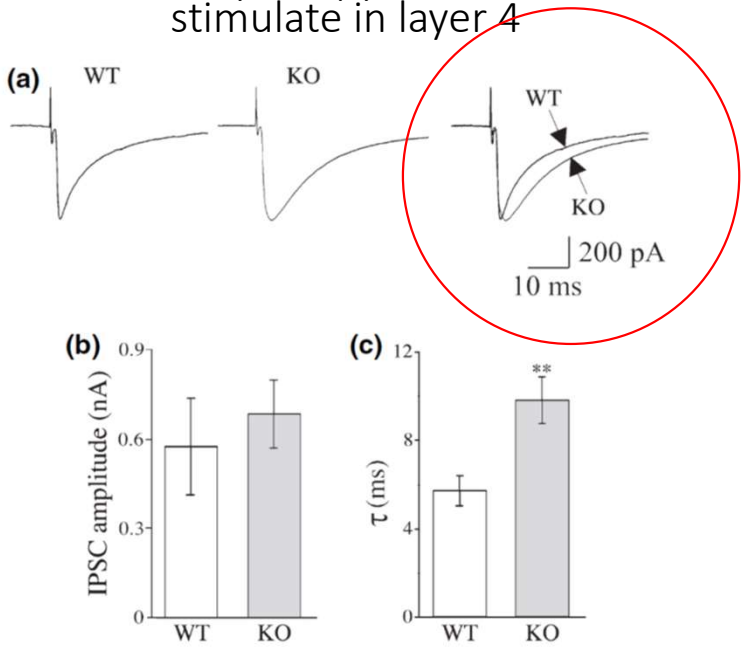
1-4 month old mice

Bragina et al, 2008
(Cherubini and Conti)

Evoked GABAergic Currents Take Longer to Recover in Cortex

eIPSCs

Record in layer 2 pyramidal neuron, stimulate in layer 4

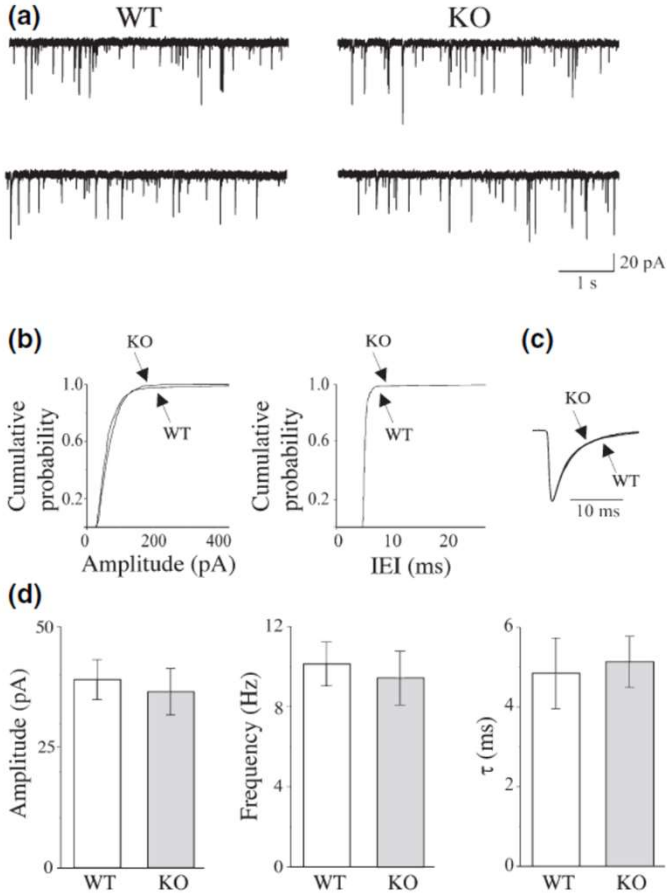


1-4 month old mice

Bragina et al, 2008
(Cherubini and Conti)

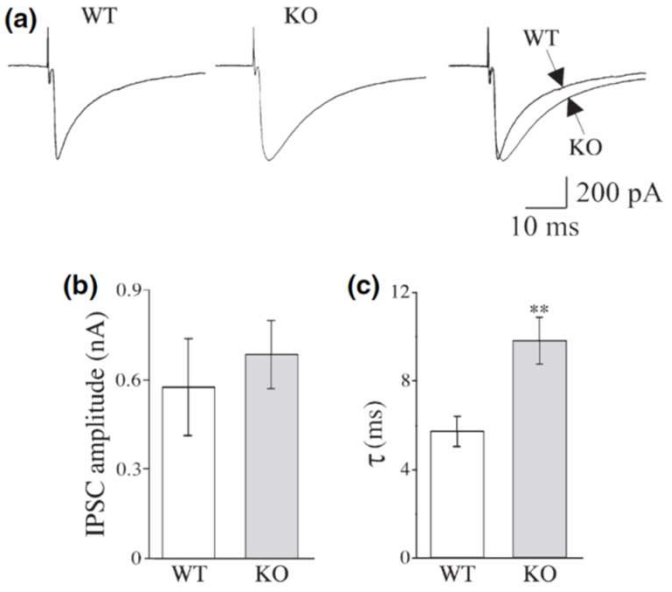
Evoked GABAergic Currents Take Longer to Recover

sIPSCs in layer2-3 pyramidal neurons



eIPSCs – longer decay

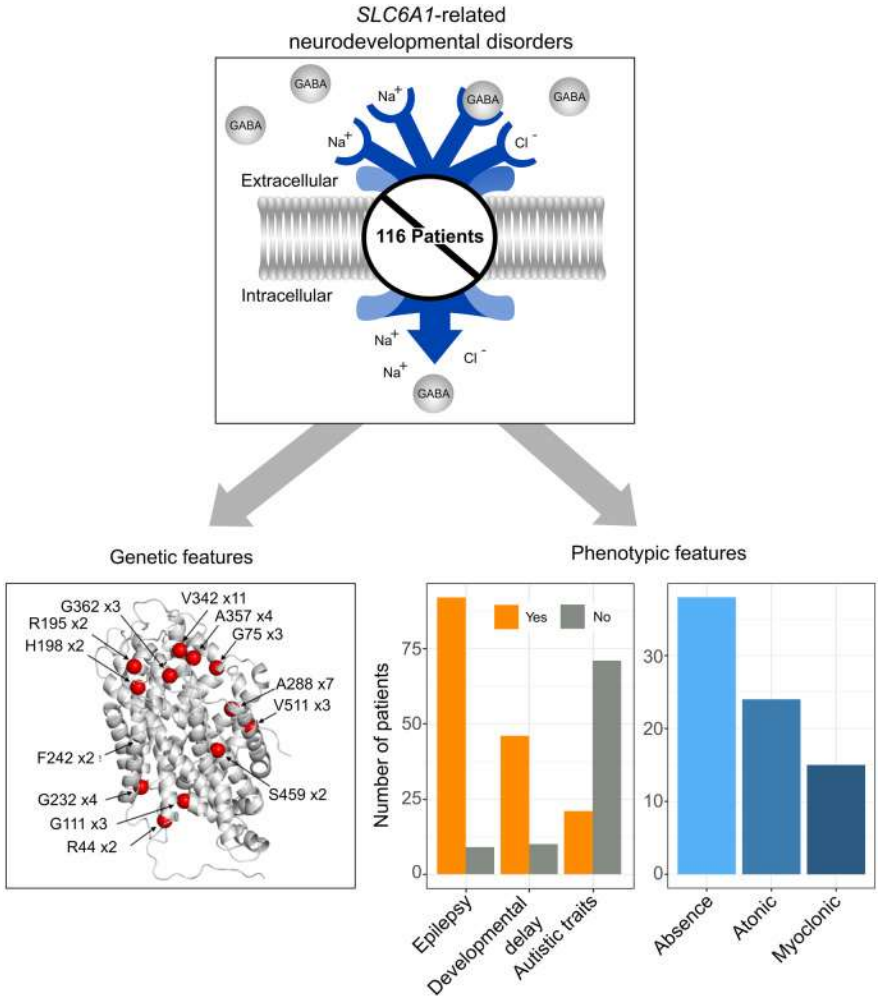
Record in layer 2 pyramidal neuron, stimulate in layer 4



Cortical pyramidal neurons in 1-4 month old mice

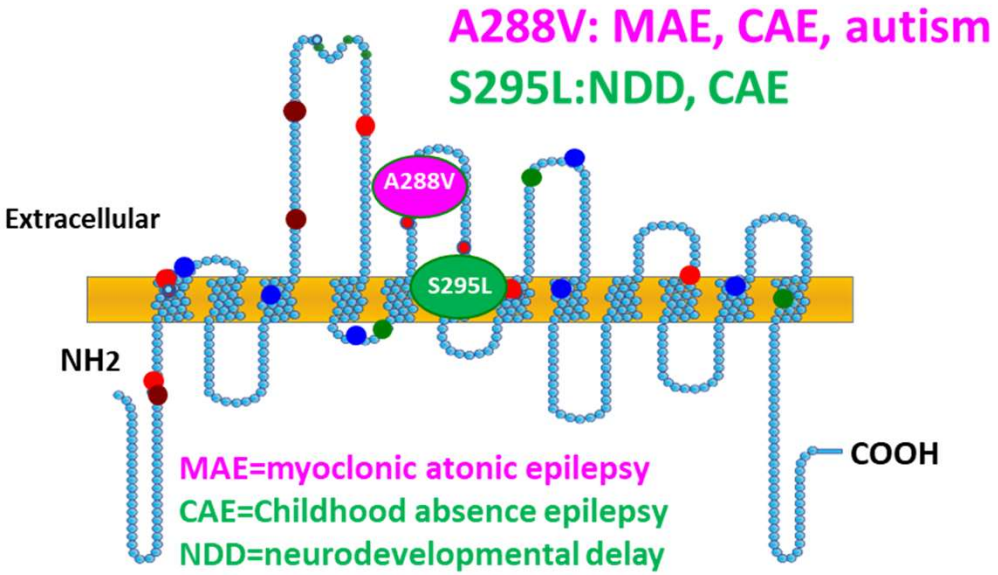
Bragina et al, 2008
(Cherubini and Conti)

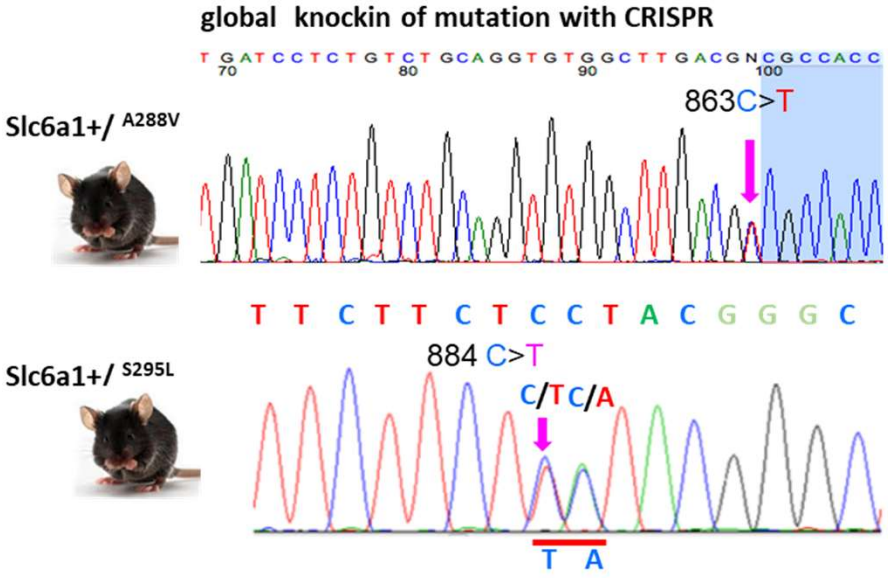
Mutant Variants of SLC6A1 Are Pathogenic



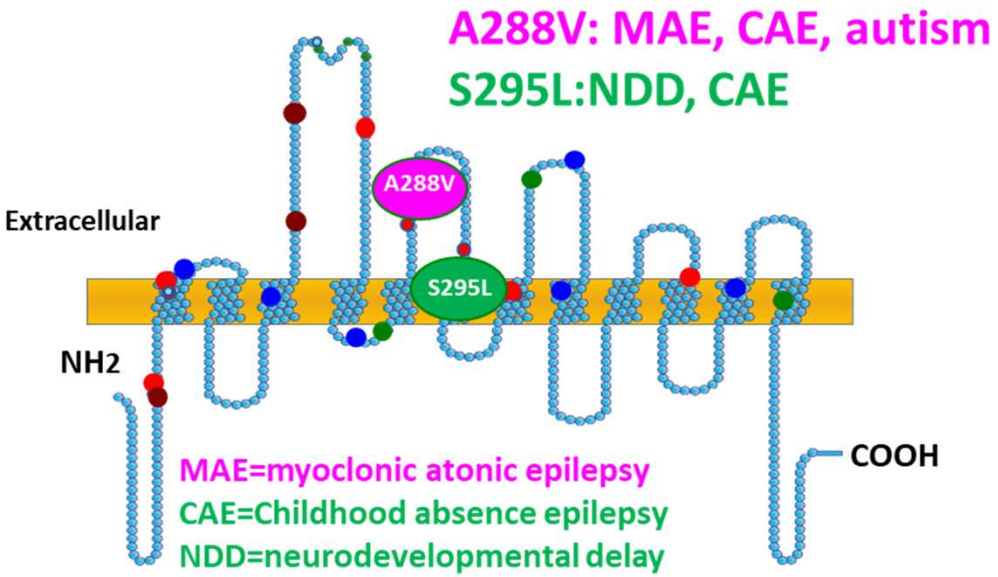
Goodspeed, K. et al. Current knowledge of SLC6A1-related neurodevelopmental disorders. Brain Commun 2 (2020).

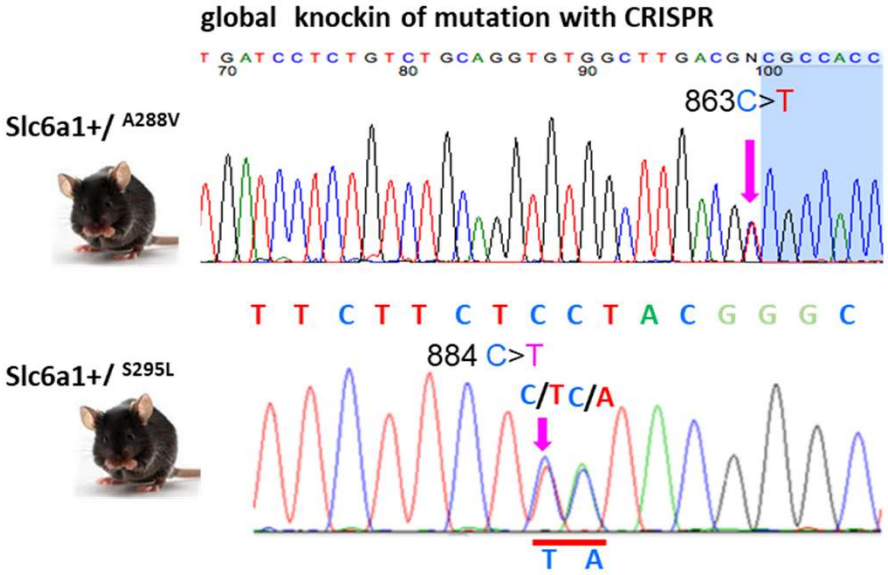
Knock-In Mice Used to Study Slc6a1^{A288V} and Slc6a1^{S295L} Patient Mutations





Knock-In Mice Used to Study Slc6a1^{A288V} and Slc6a1^{S295L} Patient Mutations



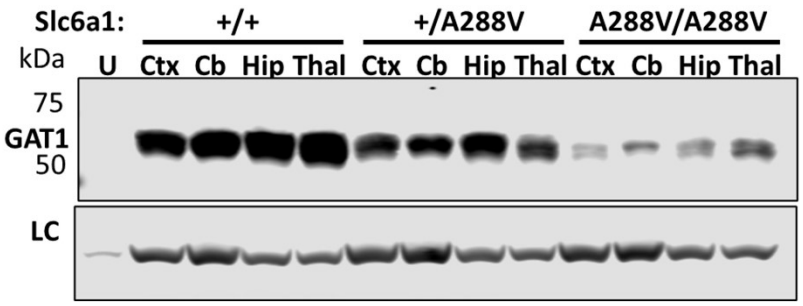


In Heterozygotes:

- Absence seizures
- Reduced motor activity

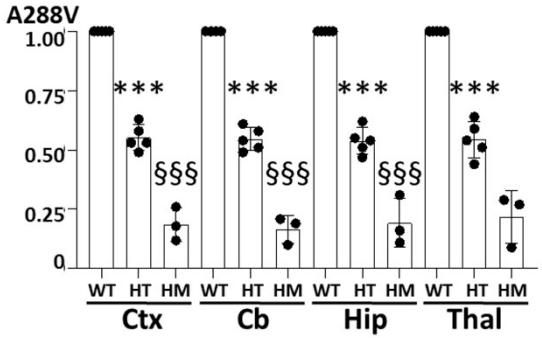
Reduced GAT-1 Expression in *Slc6a1*$^{+/A288V}$ and *Slc6a1*$^{+/S295L}$ Mice

Reduced GAT-1 Expression in Slc6a1^{A288V} Mice



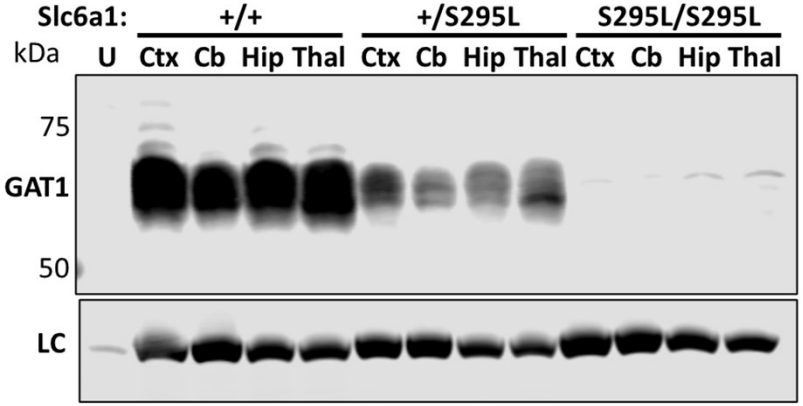
Partial LOF

Normalized GAT-1 Protein in Slc6a1^{A288V} Mice



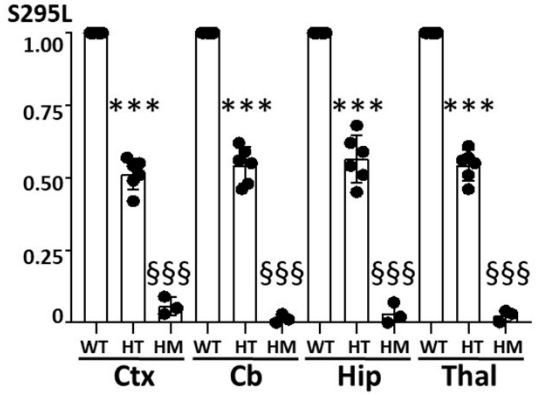
WT: Slc6a1$^{+/+}$, HT: Slc6a1$^{+/A288V}$, HM: Slc6a1$^{A288V/A288V}$

Reduced GAT-1 Expression in Slc6a1^{S295L} Mice



Complete LOF

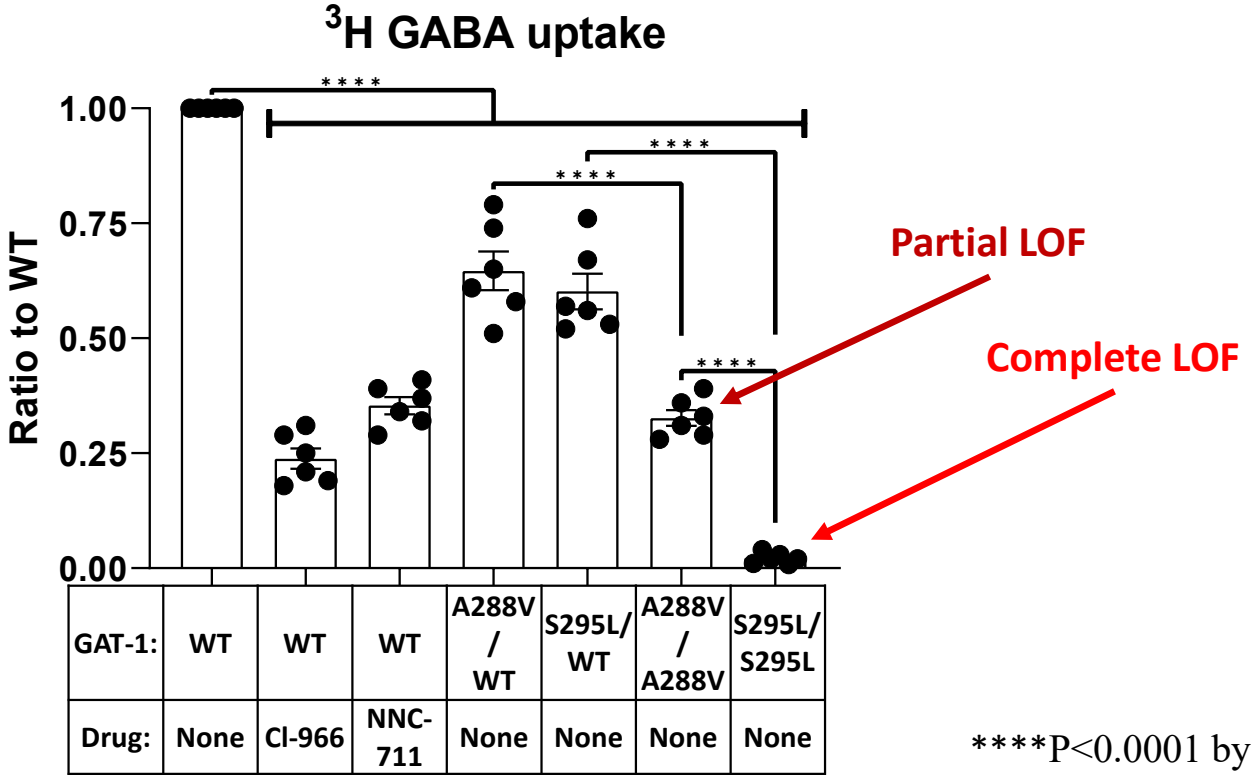
Normalized GAT-1 Protein in Slc6a1^{S295L} Mice



WT: Slc6a1$^{+/+}$, HT: Slc6a1$^{+/S295L}$, HM: Slc6a1$^{S295L/S295L}$

Work of Dr. Wangzhen Shen

Reduced GABA Uptake of GAT-1(A288V) and GAT-1(S295L) in HEK Cells



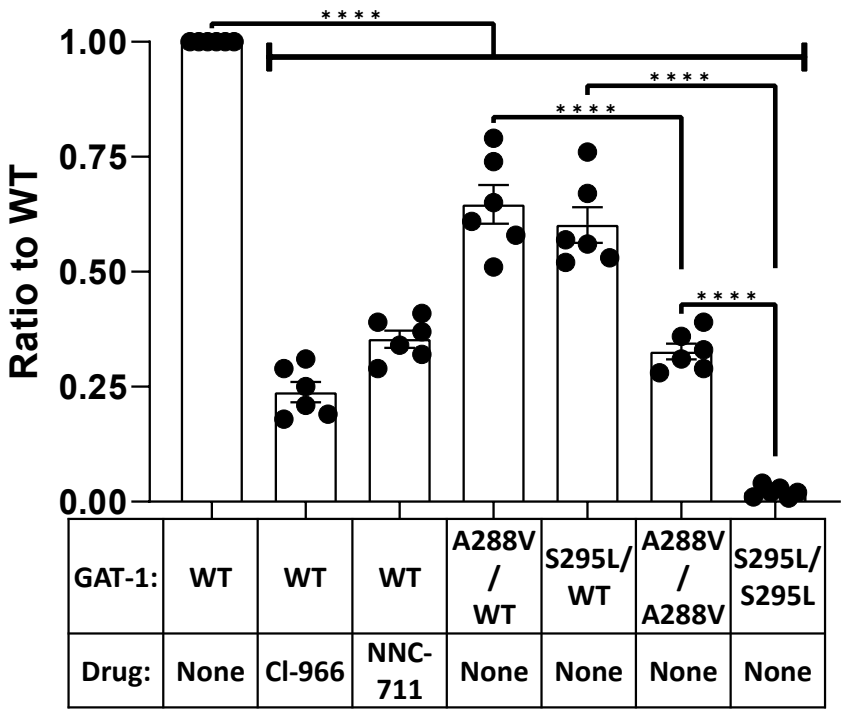
Work of Felicia Mermer and Dr. Jingqiong Kang

****P<0.0001 by one-way ANOVA with Sidak's multiple comparisons. Values expressed as mean ± S.E.M.

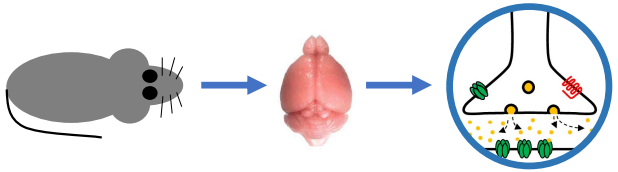
Reduced GABA Uptake in GAT-1 Variants

HEK Cells

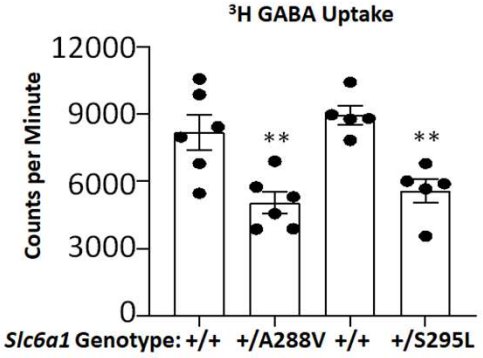
H^3 GABA Uptake

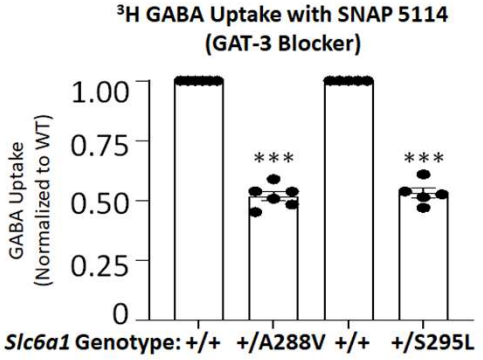


Work of Felicia Mermer and Dr. Jingqiong Kang

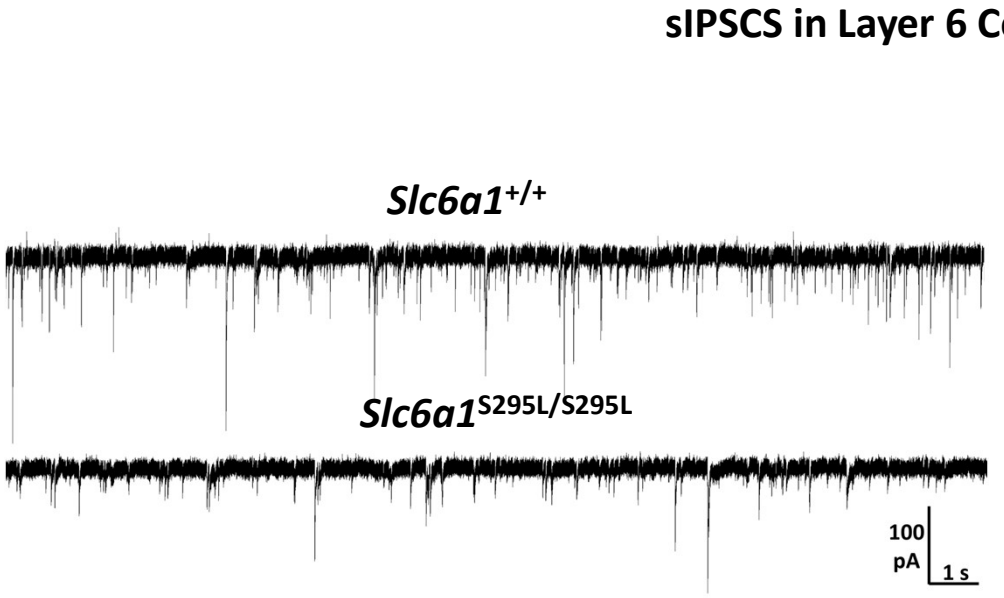


Synaptosomes
from 1-2 month old mice

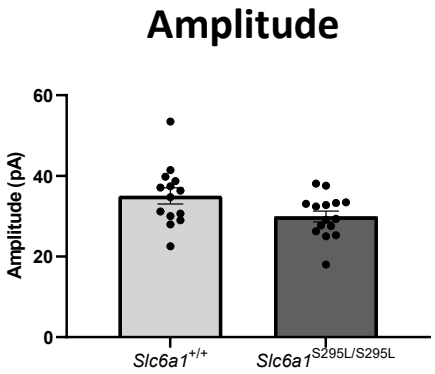


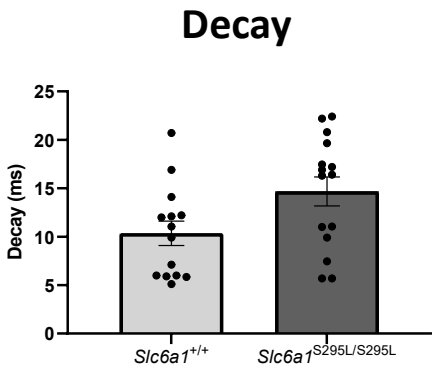


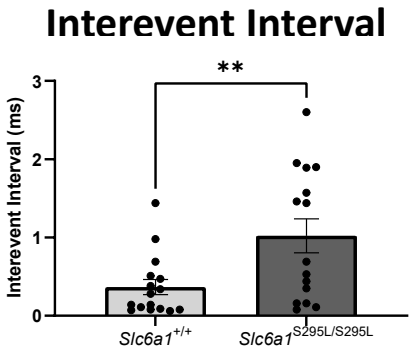
Spontaneous Synaptic Events in Slc6a1$^{S295L/S295L}$ Mouse Cortex: Decreased Event Frequency



sIPSCS in Layer 6 Cortical Pyramidal Neurons

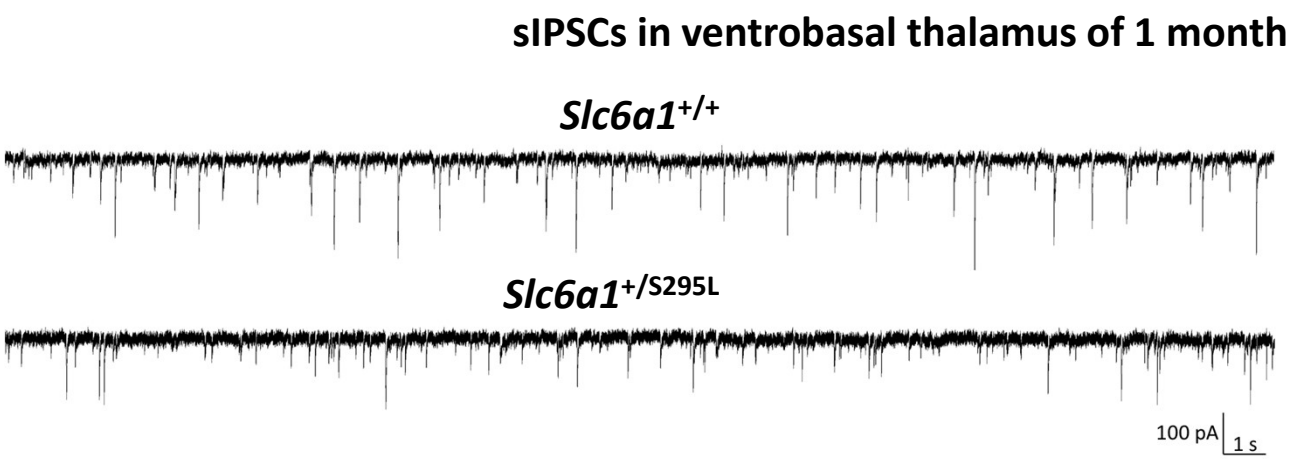


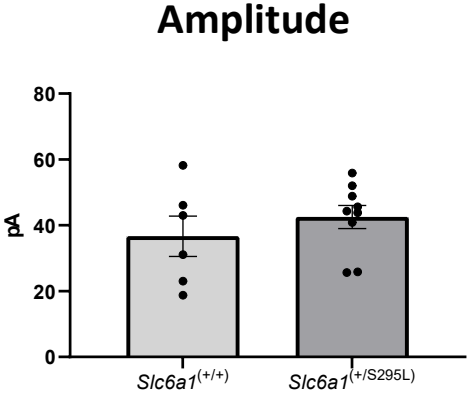


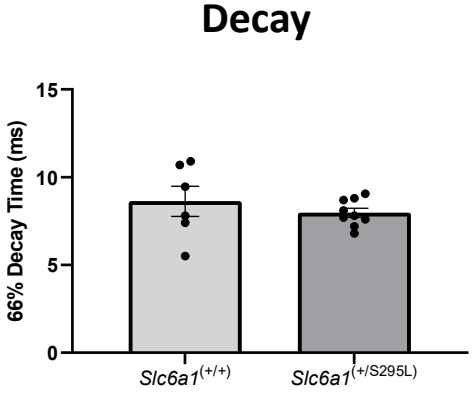


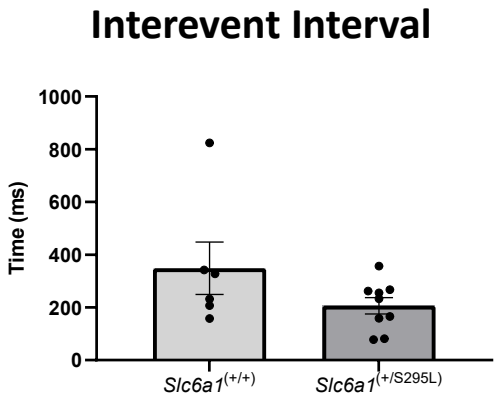
**P<.01 by t-test

Normal sIPSCs in Thalamus of Slc6a1$^{+/S295L}$

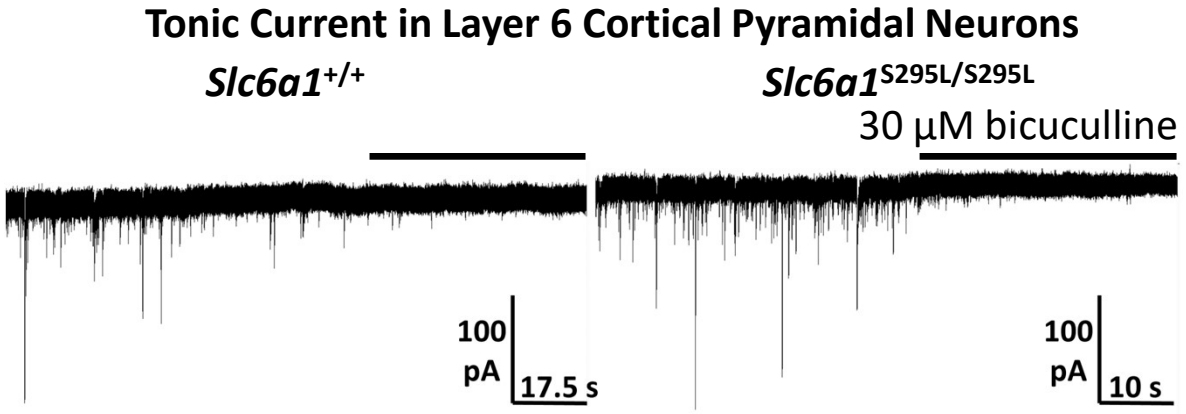


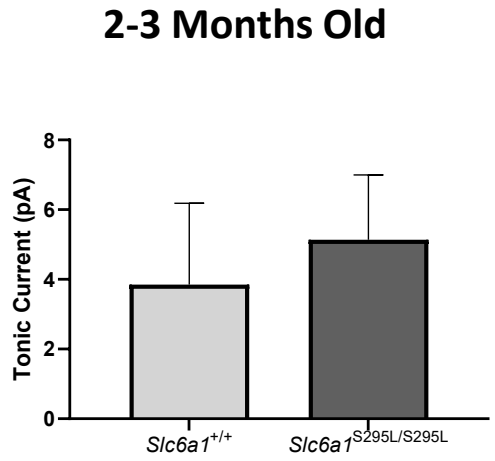




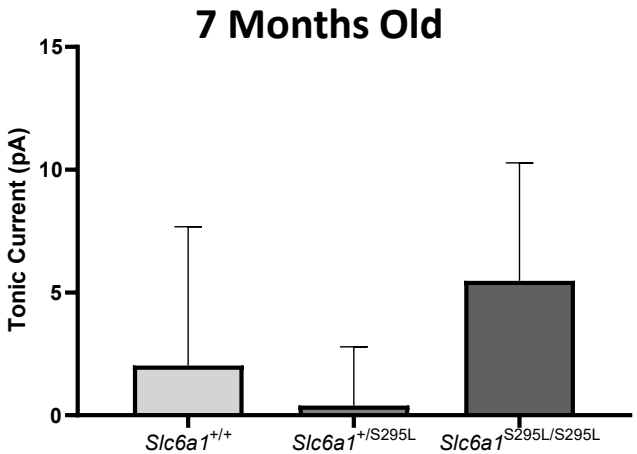


Tonic Current in Slc6a1$^{S295L/S295L}$ Mouse Cortex



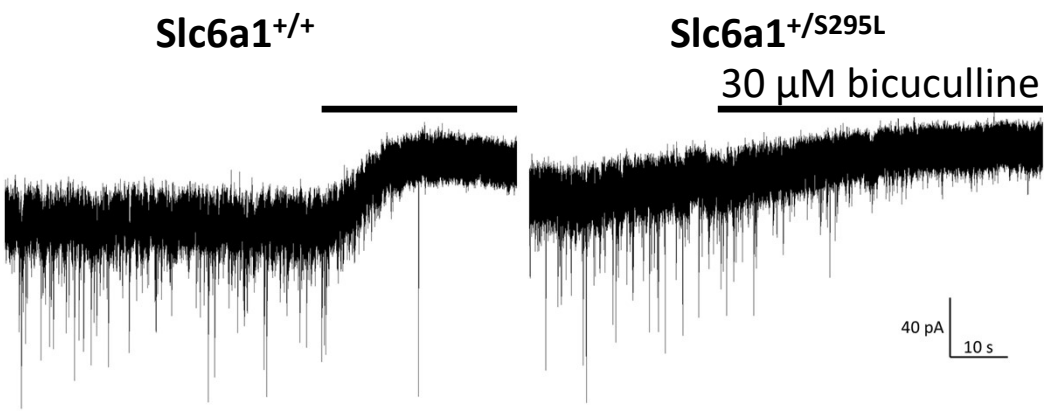


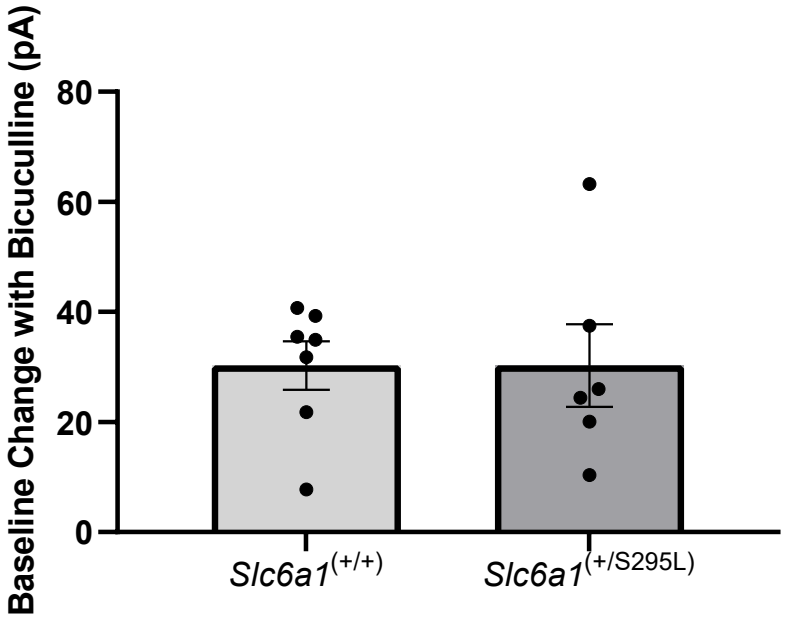
n = 10 *Slc6a1*$^{+/+}$, 9 *Slc6a1*$^{+/S295L}$ cells



n = 2 *Slc6a1*$^{+/+}$, 7 *Slc6a1*$^{+/S295L}$, 4 *Slc6a1*$^{S295L/S295L}$ cells

Tonic Current in *Slc6a1*$^{+/S295L}$ Mouse Ventrobasal Thalamus





Summary

- GAT-1 is one of the primary GABA transporters in CNS
- GAT-1 KO mice:
  - Spontaneous absence seizures
  - Increased tonic GABAergic current
  - Some alterations in synaptic events
- *Slc6a1*^{A288V} and *Slc6a1*^{S295L} knock-in mice:
  - Based on dominant DEE patient variants
  - Spontaneous absence seizures in heterozygotes
  - Reduced GAT-1 expression
  - Reduced GABA uptake
  - Normal tonic current in VB thalamus and cortex (L6)
  - Normal amplitude of thalamic and cortical synaptic events
  - Reduced frequency of sIPSCs in cortex (L6)

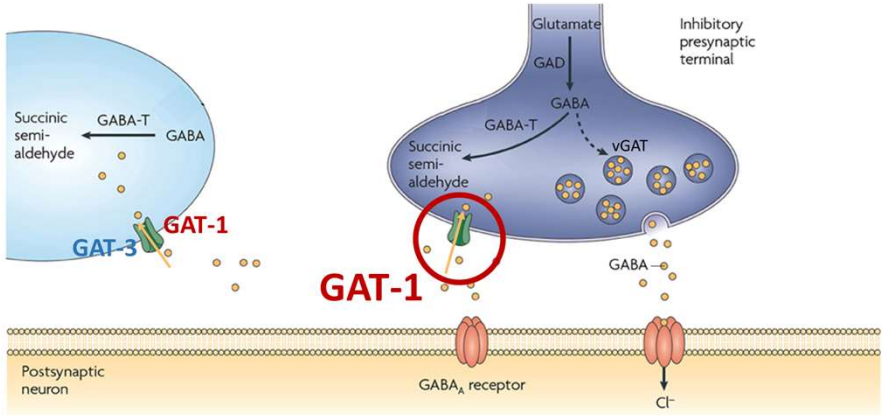
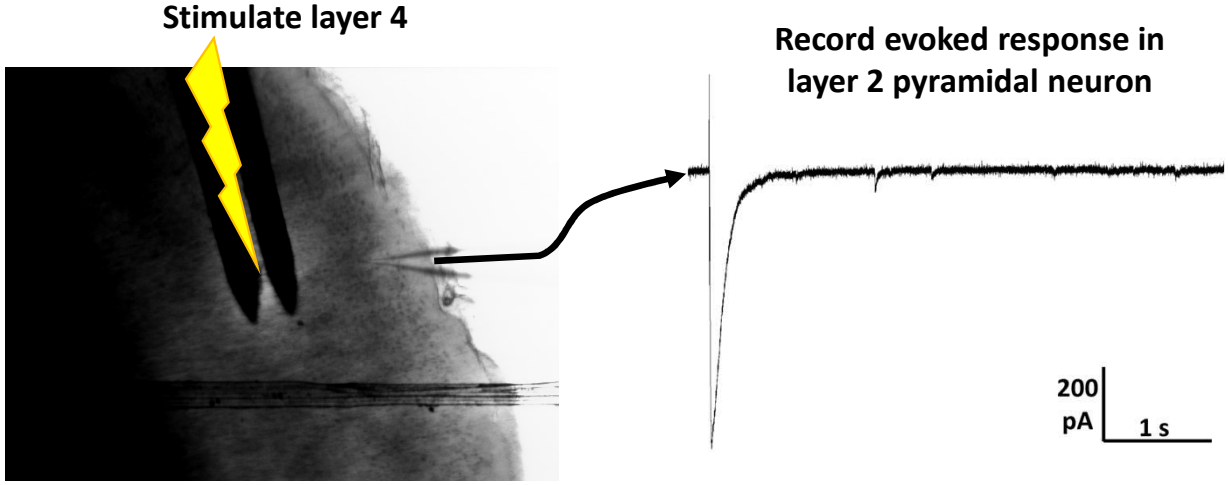


Figure adapted from: Liefferinge, J. V., Massie, A., Portelli, J., Giovanni, G. D. & Smolders, I. Are vesicular neurotransmitter transporters potential treatment targets for temporal lobe epilepsy? *Front Cell Neurosci* **7**, 139 (2013).

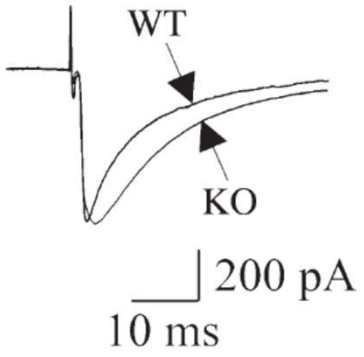
Future Directions: How is GABAergic neurotransmission altered in *Slc6a1*^{A288V} and *Slc6a1*^{S295L} mice?

- Conditions in which GABAergic uptake deficiency is detected
- eIPSCs/mIPSCs, animal age, brain region

Measure changes in evoked IPSC kinetics in *Slc6a1*$^{+/S295L}$ mice



Work by Bragina et al, 2008 suggests that GAT-1 loss in GAT-1 knockout mouse increases decay of evoked events in cortex.



Thank you! Questions?

- Kang Lab
  - Carson Flamm
  - Melissa Bassett
  - Sarah Poliquin
  - Gerald Nwosu
  - Karishma Randhave
  - Dr. Muhamman Khan
  - Dr. Wangzhen Shen
  - Katherine Langer
  - Ziang Song
  - Nicholas Troy

Thank you for research grant support by SLC6A1 Connect, UCB, and NIH NINDS NS121817 to KJQ.