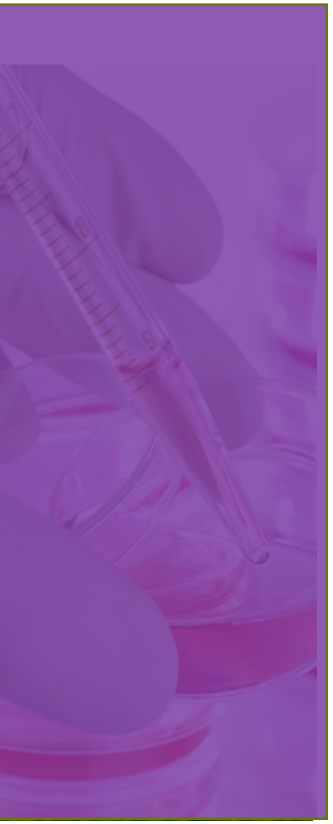
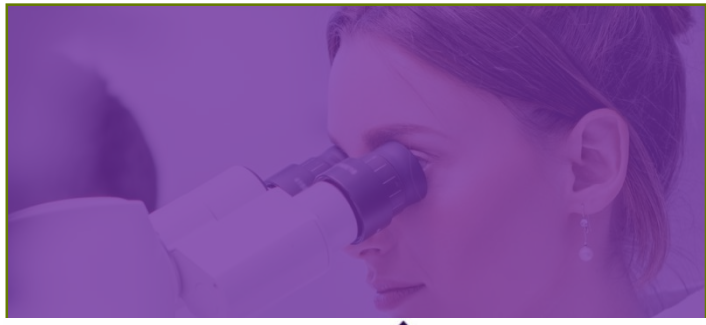
Collaborations Pharmaceuticals, Inc.



Potential drug targets for SLC6A1

Ana C. Puhl, PhD



SLC6A1 Connect

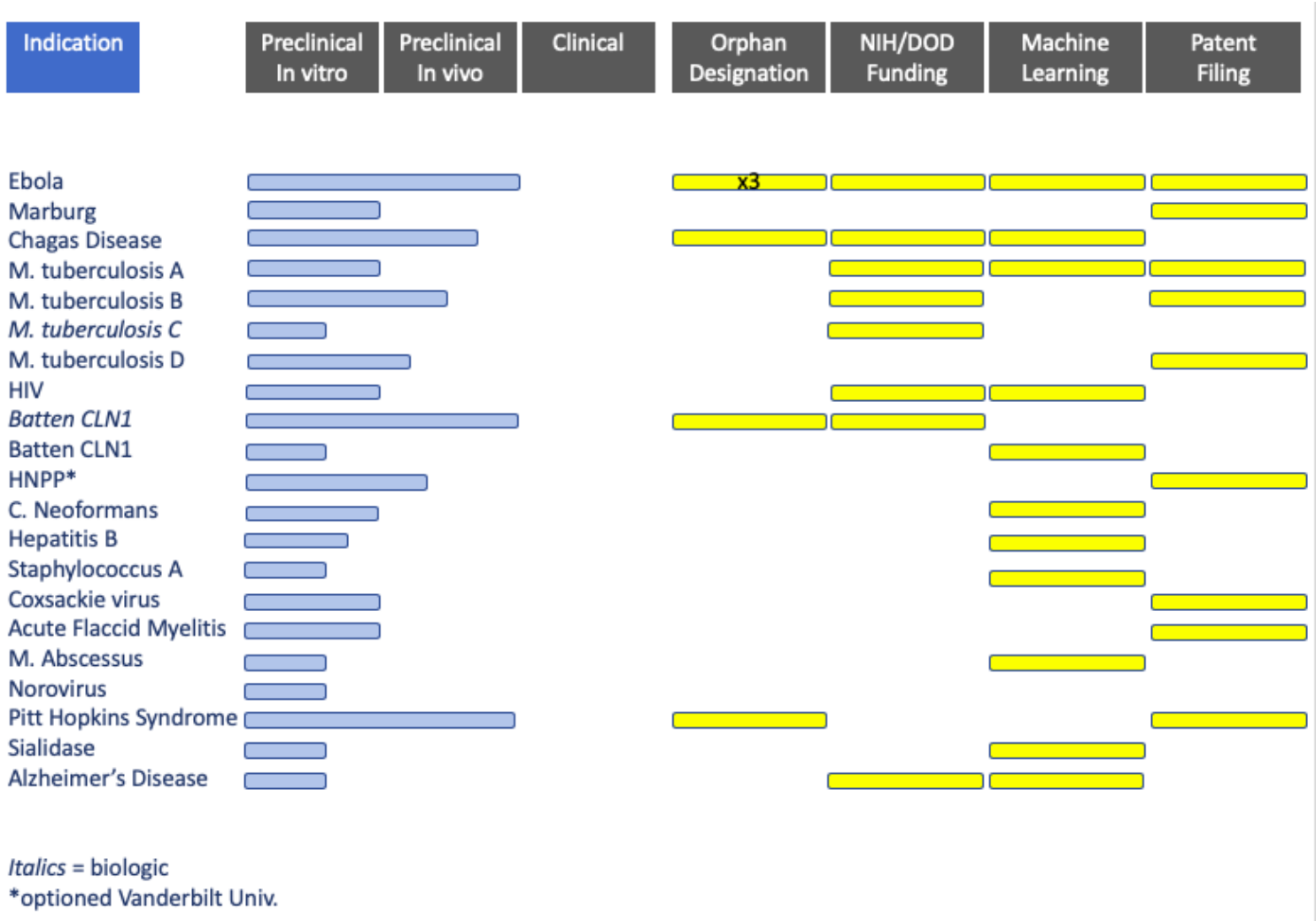
SLC6A1 Research & Support

Collaborations Pharmaceuticals

We bring novel and repurposed therapies from early research to the clinic with collaborators / partners in a quasi-virtual model

- **Founded in 2015. 9 employees**
- **6 FDA Orphan Drug Designations**
- **> $7M of funding to date on 16 NIH, DOD and DTRA -funded projects on developing drugs for neglected and rare diseases as well as software technologies**

We focus on finding treatments for rare and neglected diseases



Machine Learning

Data

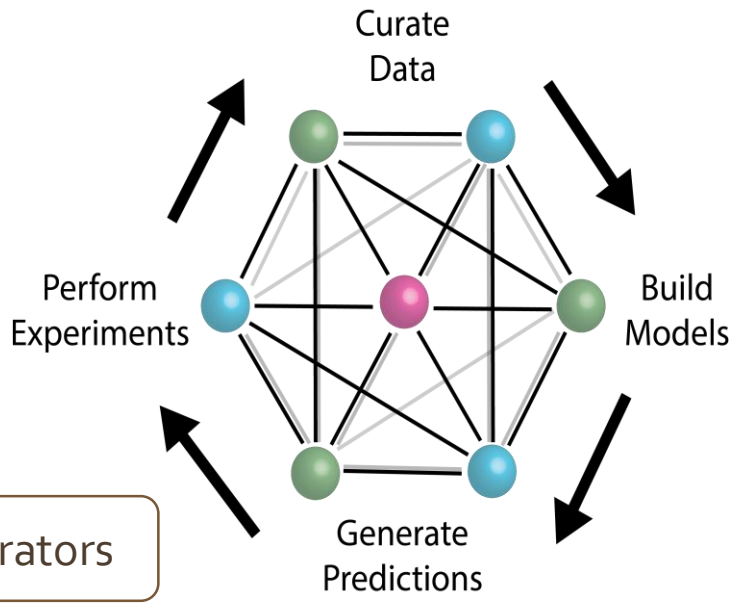
ChEMBL

PubChem

In house

BindingDB

Collaborators



Screen/Filter

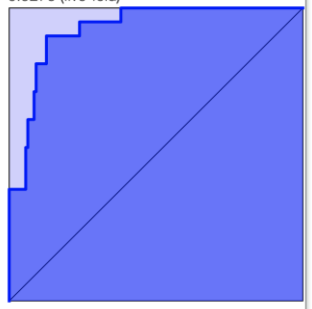
FDA approved drugs and other libraries

Do these molecules cross BBB?

Cytotoxicity?

Cathepsin D (NoHomoPro)

Origin:	Assay Central
Field:	human/cathepsin/D/nohomopro
Comments:	Threshold: > 7.437747887582859
Training Actives:	21 / 163
ROC:	0.9276 (five-fold)
Curve:	



Truth Table:

		Predicted Yes	Predicted No
Actual	**Yes**	19	2
Actual	**No**	18	124

Precision:	0.5135
Recall:	0.9048
Specificity:	0.8732
F1 score:	0.6552
Kappa:	0.5873
MCC:	0.6222

Build machine learning models using Assay Central

Cherry pick compounds Assay Central

Test: using melting temperature

Confirm hits: enzyme activity and binding assays

Test chaperones in patient cell lines

Ekins et al., Nature Materials, 2019, 18(5):435-441.

What do we know about SLC6A1 ?

GAT-1 KNOCKOUT MICE EXHIBIT SPONTANEOUS SPIKE-WAVE DISCHARGES (SWDS) AND ABSENCE SEIZURES

Cope et al.. Nat Med. 2009;15(12):1392-8

DE NOVO INACTIVATING VARIANTS IN SLC6A1 WERE REPORTED IN UP TO 4% OF PATIENTS WITH MYOCLONIC ATONIC EPILEPSY (MAE), SUGGESTING THAT PATHOGENIC SLC6A1 VARIANTS MIGHT BE SPECIFIC FOR MAE

Carvill et al. Am J Hum Genet. 2015;96(5):808-15.

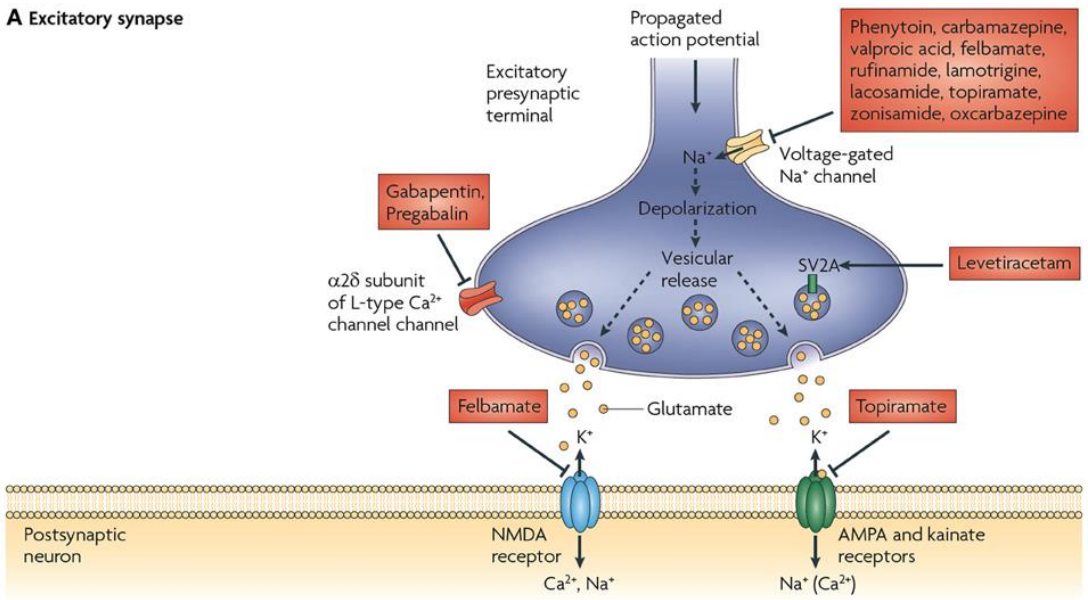
MAE IS A SYNDROME CHARACTERIZED BY THE PRESENCE OF MYOCLONIC–ATONIC SEIZURES, USUALLY IN AN OTHERWISE NORMAL CHILD, WHICH MAY TYPICALLY DEVELOP COGNITIVE IMPAIRMENT AFTER SEIZURE ONSET

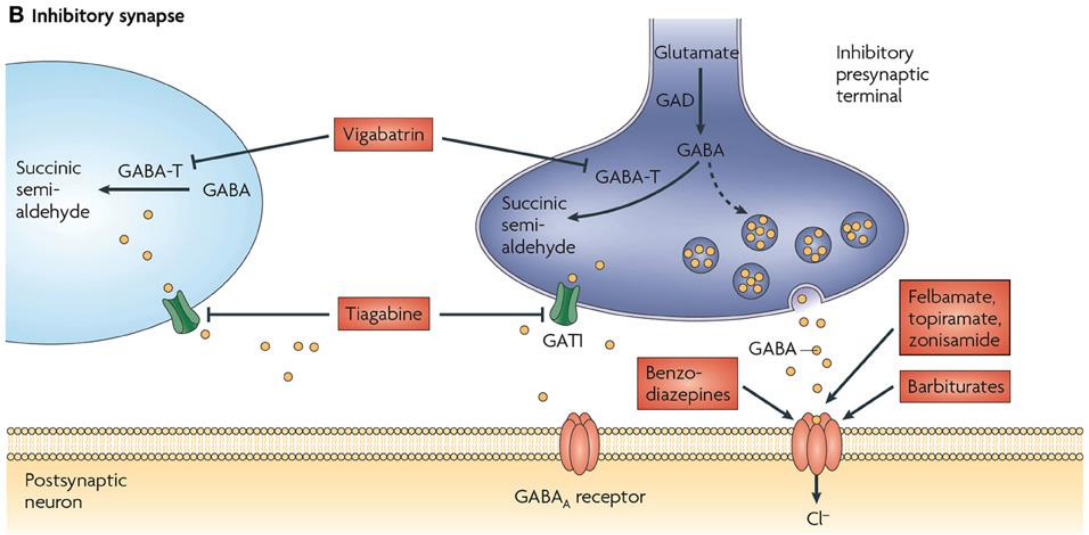
Guerrini and Aicardi. J Clin Neurophysiol. 2003;20(6):449-61.

GAT-1 dysfunction

- **GAT-1 dysfunction is expected to reduce GABA clearance, leading to increased GABA levels, both at the synapse and extrasynaptically**

- **Could lead to the overstimulation of extra synaptic $GABA_A$ and $GABA_B$ receptors**

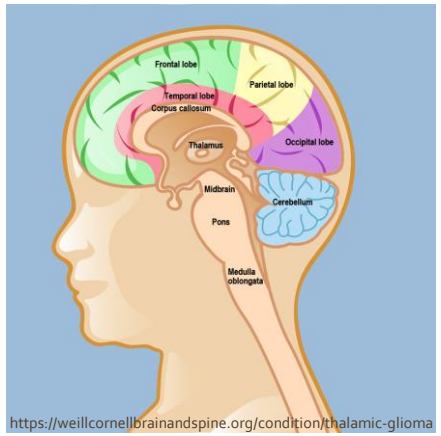




Van Liefferinge et al. Frontiersg 30;7:139. 2013.

Jensen et al . J Neurophysiol. 2003;90(4):2690-701.

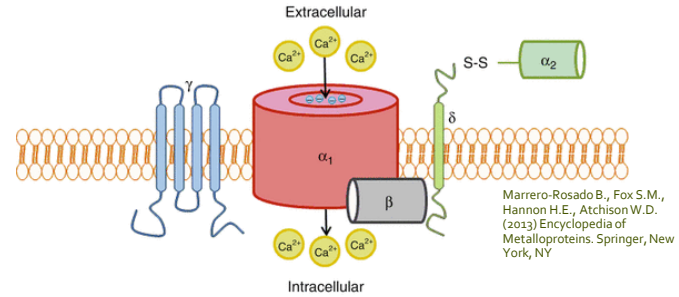
Hypothesis about the disease mechanism



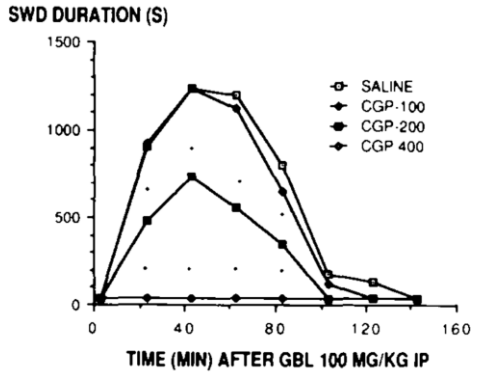
Pavlov and Walker. Neuropharmacology. 2013;69:55-61.

Increased $GABA_A$-mediated tonic inhibition can lead to neuronal hyperpolarization and burst pattern firing in thalamocortical neurons, which can promote the generation of spike-wave discharges

Similarly, prolonged activation of $GABA_B$ receptors is known to stimulate low voltage-activated (T-type) Ca^{2+} channels, which can cause recurrent excitation within the thalamocortical system through successive Na^+ spikes



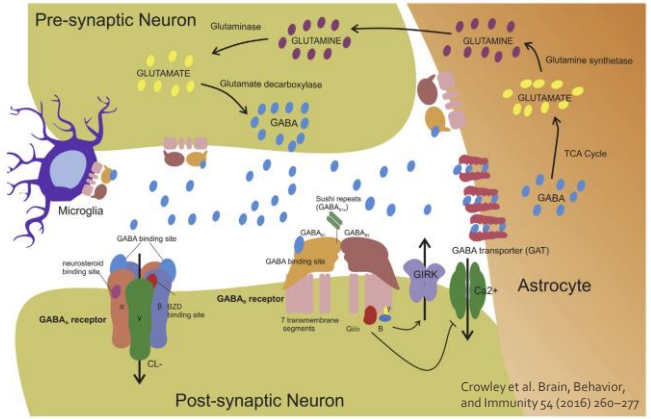
Khosravani H, Zamponi GW. Physiol Rev. 2006;86(3):941-66.



Snead OC. Eur J Pharmacol. 1992;213(3):343-9.

$GABA_B$ receptor activation causes absence seizures in mice and rats and that pretreatment with a $GABA_B$ antagonist can decrease the duration of chemically induced absence seizures

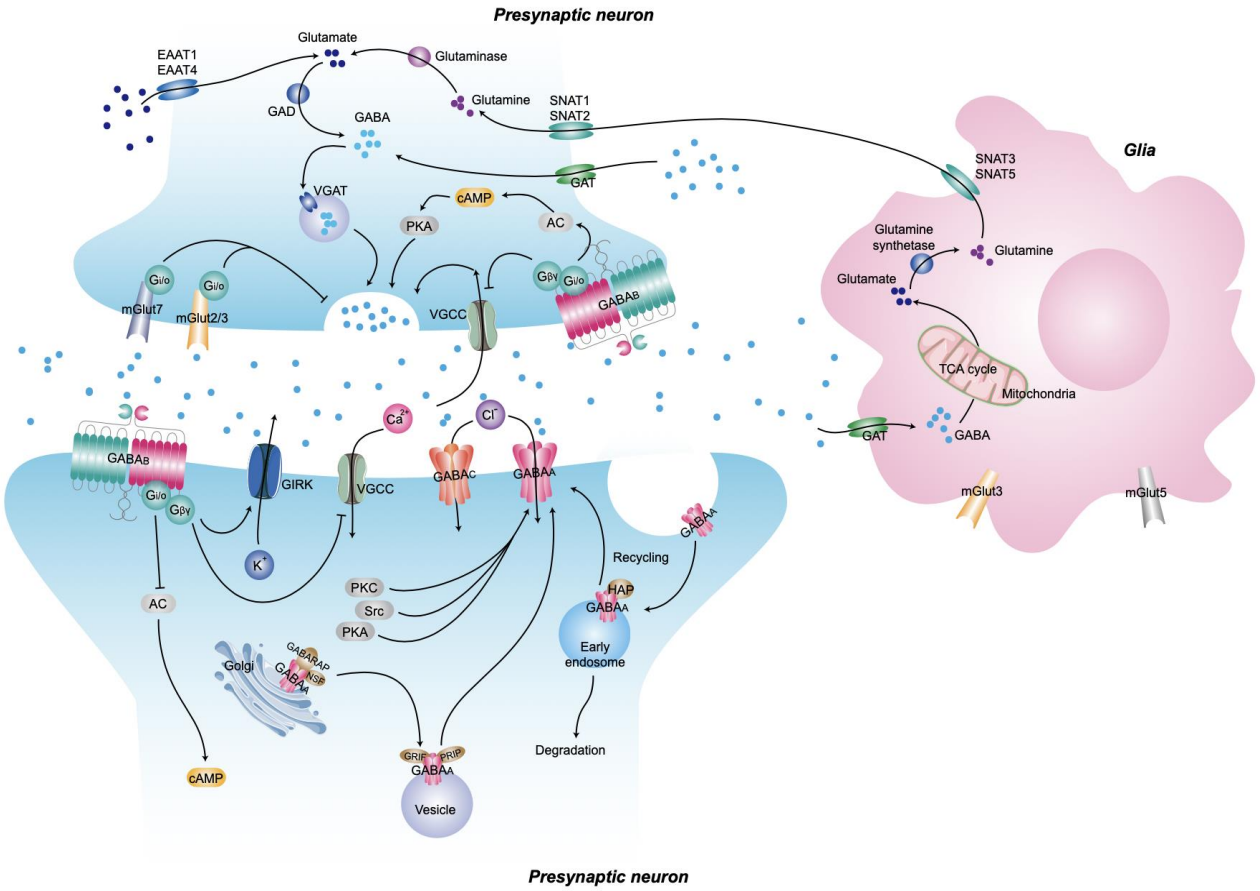
Reduced GAT-1 function could also decrease the amount of intracellular GABA available for release to activate $GABA_A$ mediated synaptic (phasic) signaling. Decreased $GABA_A$ mediated synaptic signaling is already associated with variants in several $GABA_A$ receptor subunits in genetic epilepsies



Jensen et al. J Neurophysiol 90: 2690–2701, 2003.

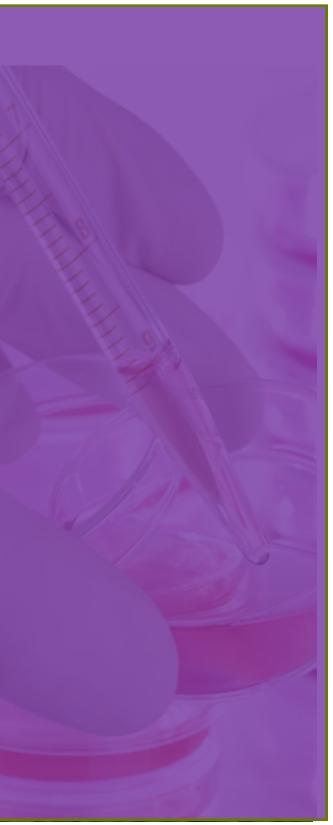
Macdonald, Jing-Qiong and Gallagher. *Physiol* 588.11 (2010) pp 1861–1869.

Hypothesis about the disease mechanism

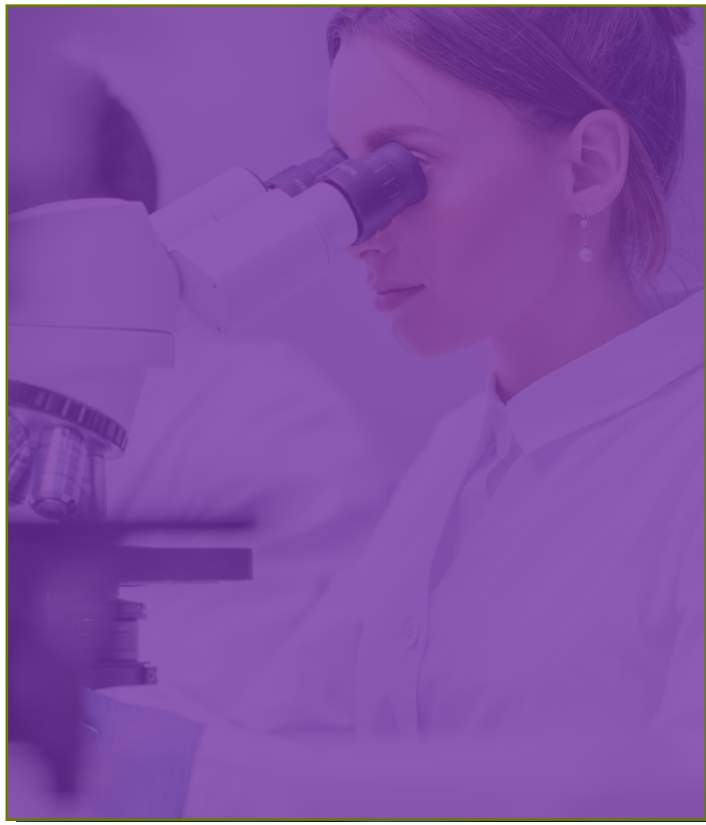


Together, these results suggest that reduced GAT-1 function might lead to epilepsy through overactivation of extra synaptic $GABA_A$ and $GABA_B$ receptors, and reduction in $GABA_A$ synaptic signaling.

Mattison et al. Epilepsia. 2018 Sep;59(9):e135-e141./ Image:https://www.creative-diagnostics.com/gabaergic-synapse-pathway.htm



Potential drug targets for SLC6A1



Antagonists and $GABA_B$ receptors

- GABAB receptors can produce a late hyperpolarization in response to synaptically released GABA by enhancing K+ permeability through G-protein-coupled inwardly rectifying potassium channels (GIRK/Kir3.x)
- They can activate low voltage-activated (T-type) Ca^{2+} channels, which can cause recurrent excitation within the thalamocortical system

GABAergic Synapse Pathway

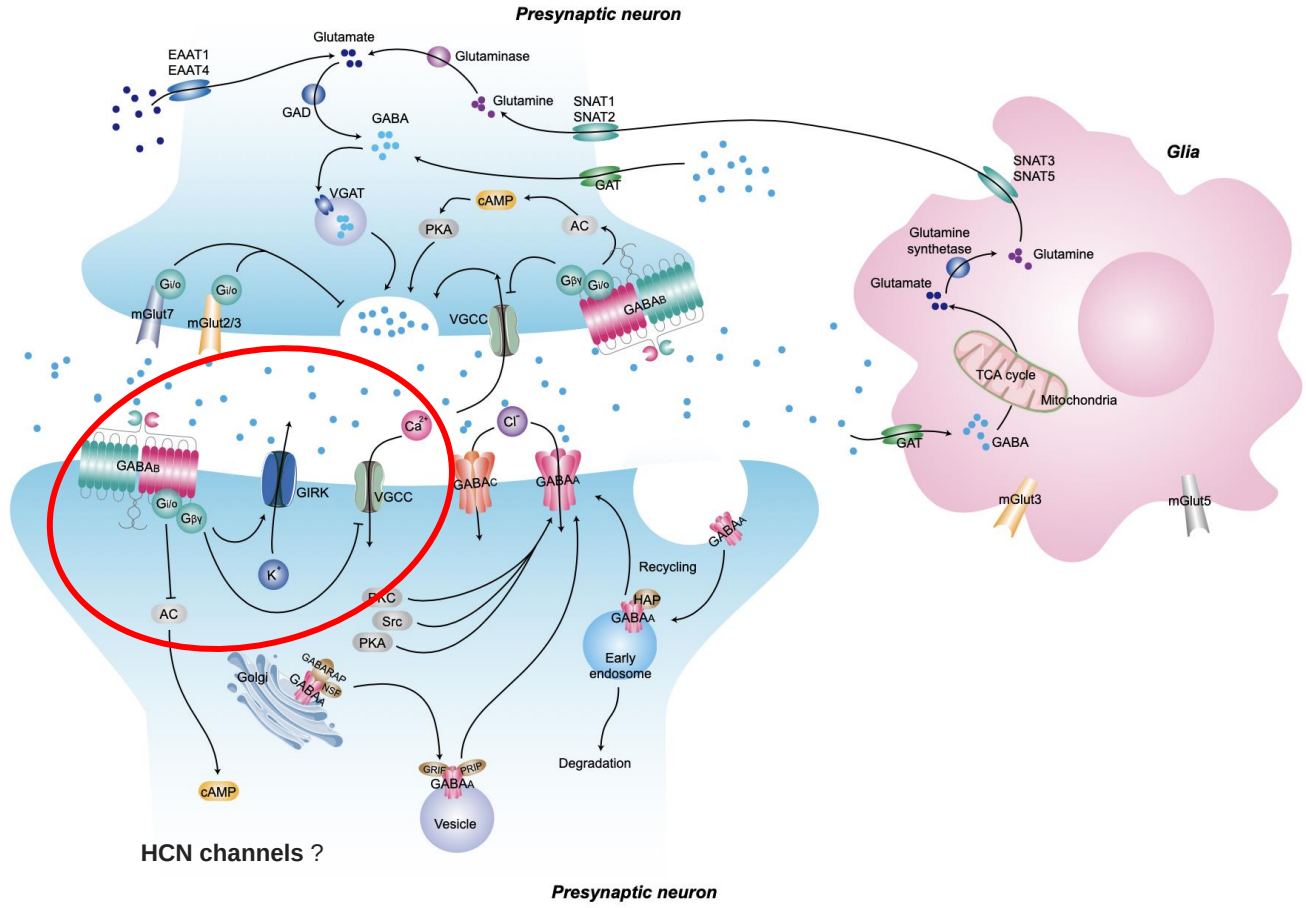


Image:https://www.creative-diagnostics.com/gabaergic-synapse-pathway.htm

A Role for Diminished GABA Transporter Activity in the Cortical Discharge Phenotype of MeCP2-Deficient Mice

Liang Zhang[1,2,3], Robert G Wither[4,5], Min Lang[2,4,5], Chiping Wu[1,4], Elena Sidorova-Darmos[4,5], Hristo Netchev[4], Catherine B Matolcsy[4], Orlando Carter Snead[2,3] and James H Eubanks[*,2,4,5,6]

[1]Division of Fundamental Neurobiology, Toronto Western Research Institute, University Health Network, Toronto, ON, Canada; [2]University of Toronto Epilepsy Research Program, University of Toronto, Toronto, ON, Canada; [3]Department of Medicine (Neurology), University of Toronto, Toronto, ON, Canada; [4]Division of Genetics and Development, Toronto Western Research Institute, University Health Network, Toronto, ON, Canada; [5]Department of Physiology, University of Toronto, Toronto, ON, Canada; [6]Department of Surgery (Neurosurgery), University of Toronto, Toronto, ON, Canada

- Loss-of-function mutations of methyl-CPG-binding protein 2 (MECP2) can cause Rett syndrome
- **GAT-1 knockout mice and MeCP2- deficient mice display overlapping phenotype**
- GAT-1 Protein Levels, but not GABAB or Extra-Synaptic GABAA Receptor Subunits, are Diminished in the MeCP2-Null Cortex
- Enhancing GABAB Receptor Activity Increases Cortical Epileptiform Discharges in Mecp2+/ – Mice
- **Attenuating GABAB Receptor Activity Decreases Cortical Epileptiform Discharges in Mecp2+/ – Mice**

$GABA_B$ receptor antagonism abolishes the learning impairments in rats with chronic atypical absence seizures

Katherine F.Y. Chan [a,b,c], W. McIntyre Burnham [b,c], Zhengping Jia [a], Miguel A. Cortez [a,b,d], O. Carter Snead III [a,b,c,d,*]

[a] *Division of Neurology, The Brain and Behavior Program, Hospital for Sick Children, Toronto, ON, Canada M5G 1X8*
[b] *The University of Toronto Epilepsy Research Program, Faculty of Medicine, University of Toronto, Toronto, ON, Canada M5S 1A8*
[c] *Department of Pharmacology, Faculty of Medicine, University of Toronto, Toronto, ON, Canada M5S 1A8*
[d] *The Department of Pediatrics, Faculty of Medicine, University of Toronto, Toronto, ON, Canada M5S 1A8*

Received 7 November 2005; received in revised form 27 March 2006; accepted 10 April 2006
Available online 26 April 2006

Abstract

Chronic atypical absence seizures are a component of the Lennox–Gastaut syndrome, a disorder invariably associated with severe cognitive impairment in children. However, the cause of this intellectual delay remains unclear. The AY9944 model of chronic atypical absence seizures in rats reliably reproduces the electrographic, behavioral, pharmacological and cognitive features of clinical atypical absence. Using this model, we tested the hypothesis that the cognitive impairment associated with this disorder involves a γ-aminobutyric acid B ($GABA_B$) receptor-mediated mechanism. Therefore, we examined the effect of a specific, high affinity $GABA_B$ receptor antagonist, CGP35348, on the atypical absence seizures, the working memory deficits, and the altered long-term potentiation that we have observed in the AY9944 model. CGP35348 blocked atypical absence seizures, restored long-term potentiation to normal level, and reversed the cognitive deficit in the AY9944-treated animals. However, dose–response studies showed that lower doses of CGP35348 that failed to influence atypical absence seizure activity, completely reversed the spatial working memory deficit. These data suggest that $GABA_B$ receptor-mediated mechanisms are responsible for the cognitive dysfunction in the AY9944 model of chronic atypical absence seizures and further, that their cognitive impairment is independent of the seizure activity. The data raise the possibility that $GABA_B$ receptor antagonists may have therapeutic potential for the treatment of cognitive impairment in epilepsy syndromes where atypical absence seizures are a component.

Keywords: $GABA_B$ receptor; CGP35348; Atypical absence seizure; Cognitive deficit; Radial arm maze

Modulators of GABA A receptor

Tonic $GABA_A$ Receptor-Mediated Signaling in Epilepsy

Matthew C Walker and Dimitri M Kullmann*.[1]

- GABAA receptors can mediate a "tonic" form of signaling that is not time-locked to presynaptic action potentials, and which depends upon detection of ambient GABA by extrasynaptic receptors.
- Tonic currents can have a paradoxical excitatory role
- Tonic currents hyperpolarize thalamocortical neurons and so modulate their firing pattern from regular to burst firing.
- Tonic currents are increased in animal models of absence epilepsy, and promote the generation of spike-wave discharges

Enhance GAT1 and GAT3 activity

Previous

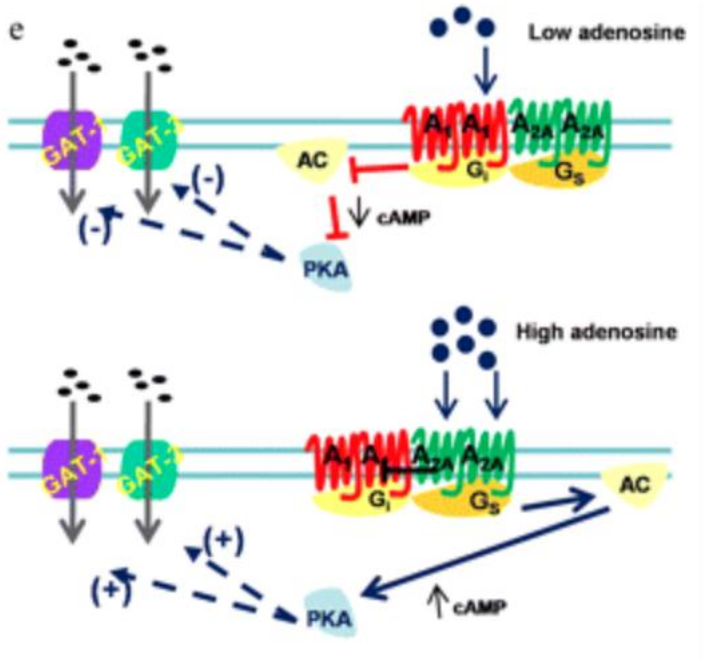
Featured Article | Articles, Cellular/Molecular

Modulation of GABA Transport by Adenosine A_1R–$A_{2A}R$ Heteromers, Which Are Coupled to Both G_s- and $G_{i/o}$-Proteins

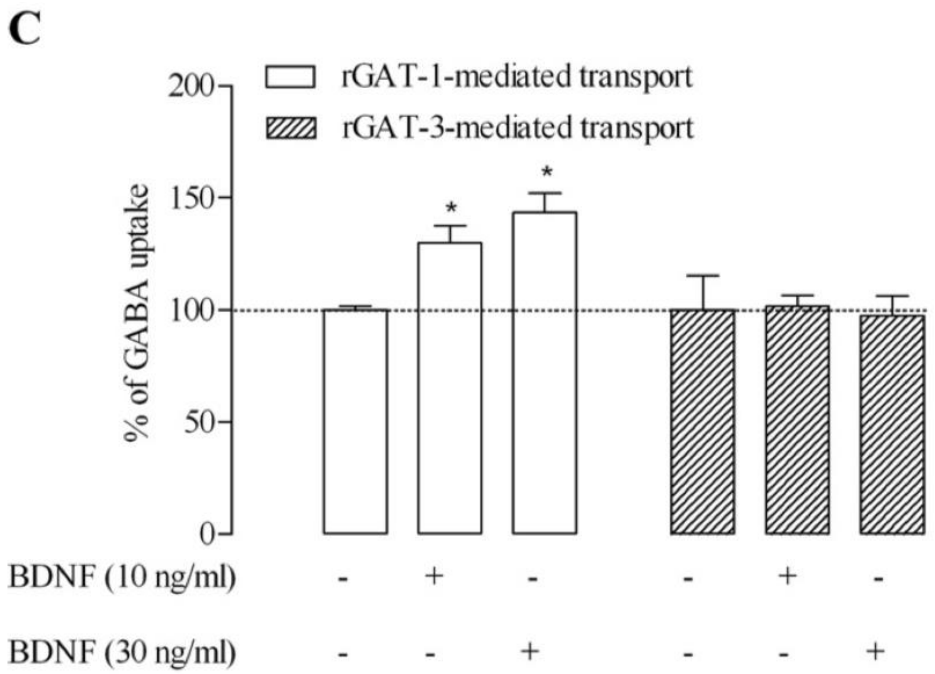
Sofia Cristóvão-Ferreira, Gemma Navarro, Marc Brugarolas, Kamil Pérez-Capote, Sandra H. Vaz, Giorgia Fattorini, Fiorenzo Conti, Carmen Lluis, Joaquim A. Ribeiro, Peter J. McCormick, Vicent Casadó, Rafael Franco, and Ana M. Sebastião

Journal of Neuroscience 2 November 2011, 31 (44) 15629-15639; DOI: https://doi.org/10.1523/JNEUROSCI.2526-11.2011

- Cortical astrocytes express GAT-1 and GAT-3 subtypes, and it has been estimated that ~20% of extracellular GABA may be taken up into astrocytes
- We found A_1R–$A_{2A}R$ receptor heteromers in astrocytes.
- $A_{2A}R$ protomer mediating facilitation of GABA transport into astrocytes.



Enhance GAT1 GABA uptake



BDNF enhances GAT-1 GABA transport in astrocyte primary cultures.

Brain-derived Neurotrophic Factor (BDNF) Enhances GABA Transport by Modulating the Trafficking of GABA Transporter-1 (GAT-1) from the Plasma Membrane of Rat Cortical Astrocytes*

Sandra H. Vaz[‡§], Trine N. Jørgensen[¶], Sofia Cristóvão-Ferreira[‡§], Sylvie Duflot[‡§], Joaquim A. Ribeiro[‡§], Ulrik Gether[¶] and Ana M. Sebastião[‡§,1]

+ Author Affiliations

↵[1] To whom correspondence should be addressed: Instituto de Farmacologia e Neurociências, Faculdade de Medicina e Instituto de Medicina Molecular, Universidade de Lisboa, Av. Professor Egas Moniz, Edíficio Egas Moniz, Piso 1B, 1649–028 Lisbon, Portugal. Tel.: 351-21-7985183; Fax: 351-21-7999454; E-mail: anaseb@fm.ul.pt.

Capsule

Background: Transport of GABA into astrocytes is crucial for excitability control.

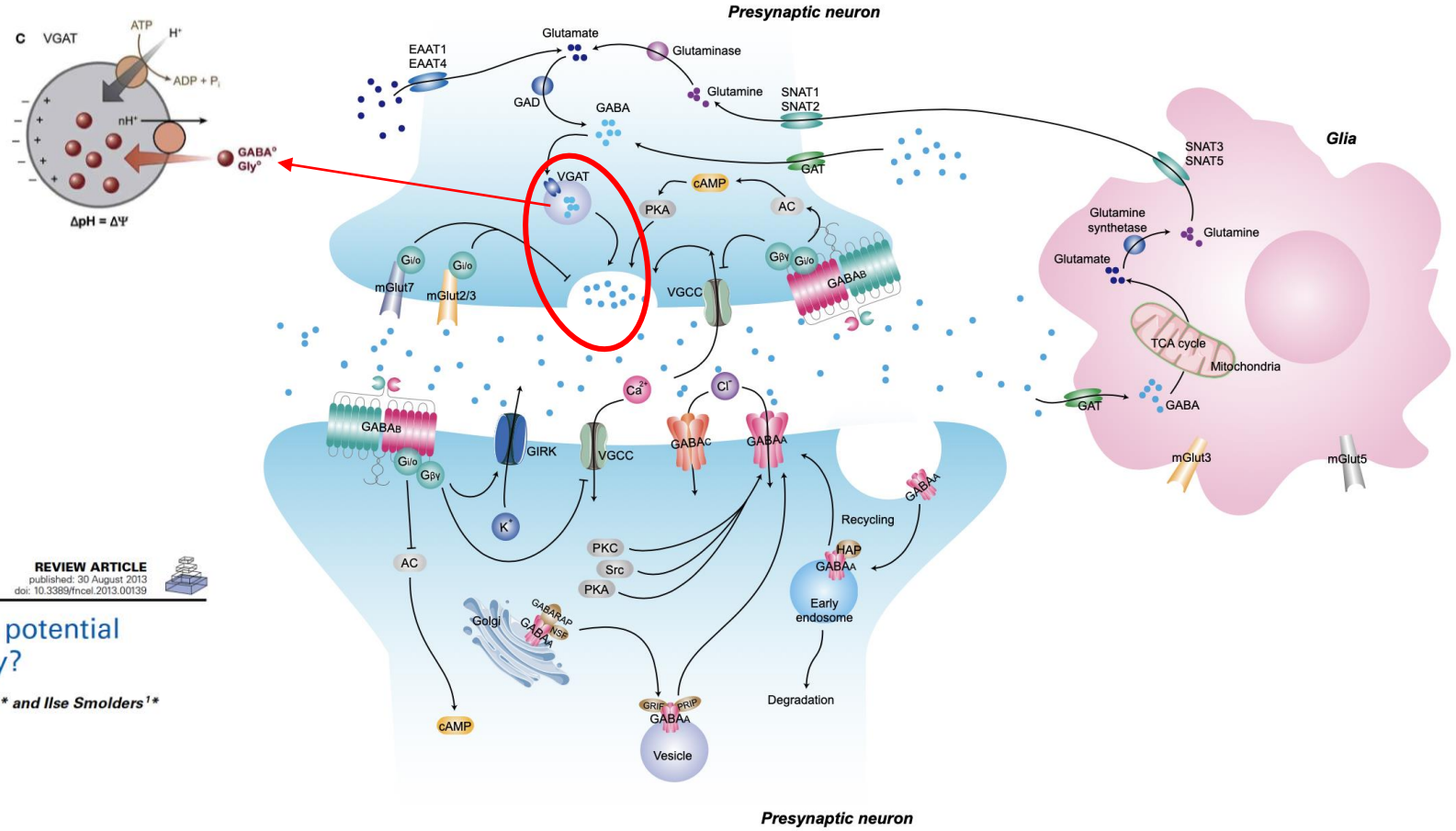
Results: The neurotrophin BDNF, through TrkB-t receptor activation, enhances GABA transport into astrocytes, which requires adenosine A_{2A} receptor signaling.

Conclusion: BDNF plays an active role in the synaptic clearance of GABA.

Significance: This new regulatory role for TrkB-t receptors discloses their relevance for excitability control at the tripartite synapse.

Inhibitors of Vesicular GABA transporter (VGAT)

- VGAT fuse with the presynaptic membrane of the cell to release GABA from the presynaptic cell to the synaptic cleft




frontiers in
CELLULAR NEUROSCIENCE

REVIEW ARTICLE
published: 30 August 2013
doi: 10.3389/fncel.2013.00139

Are vesicular neurotransmitter transporters potential treatment targets for temporal lobe epilepsy?

Joeri Van Liefferinge[1], Ann Massie[1], Jeanelle Portelli[1,2], Giuseppe Di Giovanni[3] and Ilse Smolders[1]**

[1] Center for Neurosciences, Vrije Universiteit Brussel, Brussels, Belgium
[2] Institute for Neuroscience, Ghent University Hospital, Ghent, Belgium
[3] Department of Physiology and Biochemistry, University of Malta, Msida, Malta

Image: https://www.creative-diagnostics.com/gabaergic-synapse-pathway.htm

Questions to address

- **Amount of GABA in synaptic cleft compared to WT**

- **Which receptors are upregulated in this disease?**
  - Evaluate which GABA targets are upregulated or downregulated by the excess of GABA in the synaptic cleft.
  - Which receptor has been upregulated: $GABA_A$, $GABA_B$?

    This information might be useful to define better targets.

- **Study the pharmacological mechanism of seizures**
- Not enough inhibitory signal, since there is less GABA phasic release?
- Low voltage-activated (T-type) Ca^{2+} channel, activated by GABA B receptor?
- Activation of other receptors?

Ongoing research

- 2 compounds:
- CPI1204
- CPI1205

- Therapeutic effect in cultured neurons, astrocytes
- GABA levels in lysates
- GABA receptors expression
- Mice Model: SLc6a1$^{+/A288V}$
- Thermal induction - EEG recording

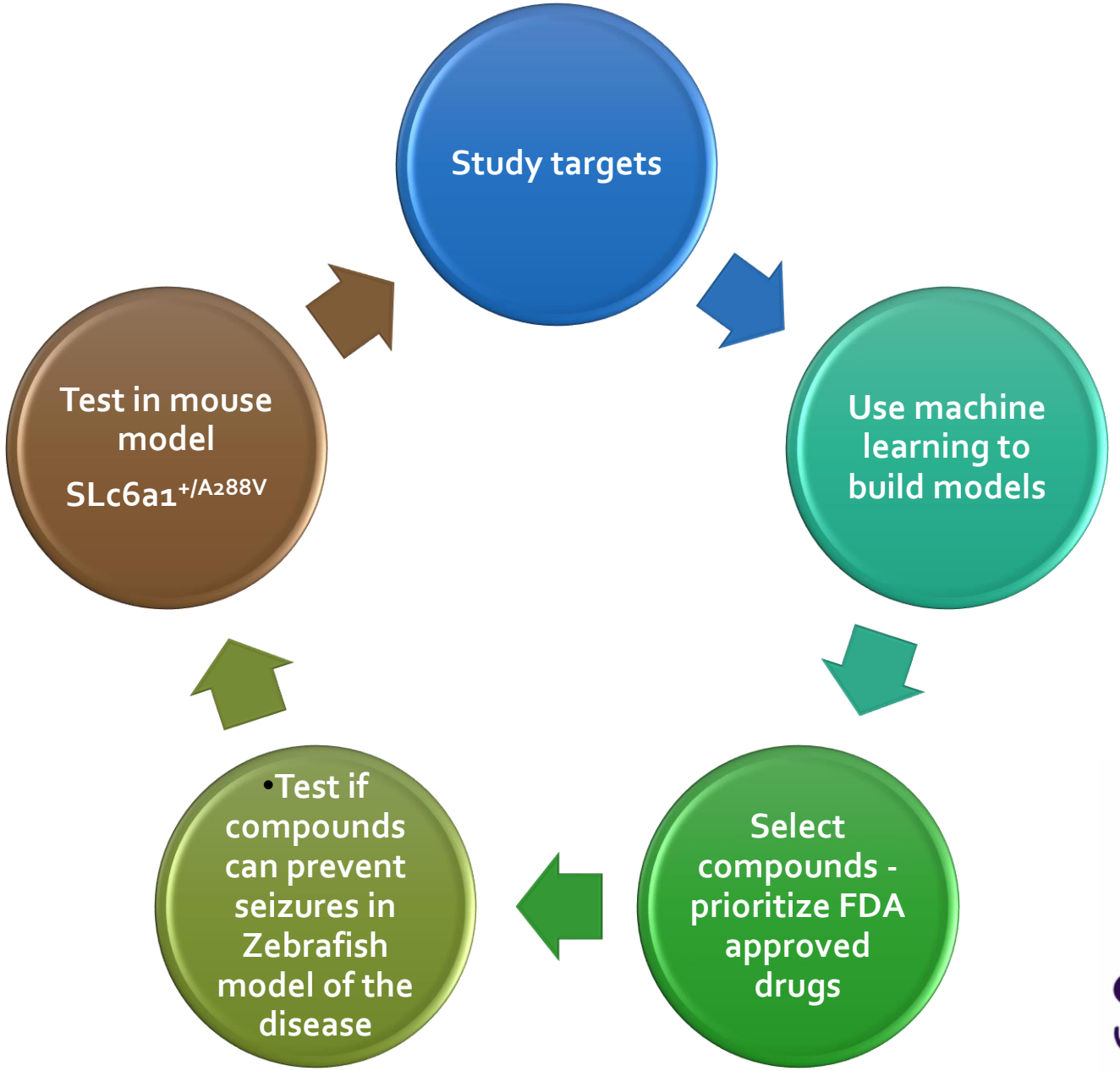
Jing-Qiong Kang, M.D., Ph.D.

Assistant Professor of Neurology

Investigator

VANDERBILT UNIVERSITY MEDICAL CENTER

Next steps



SLC6A1 Connect
SLC6A1 Research & Support

Thank you!

Collaborations Pharmaceuticals, Inc.

No disease is too small

ana@collaborationspharma.com