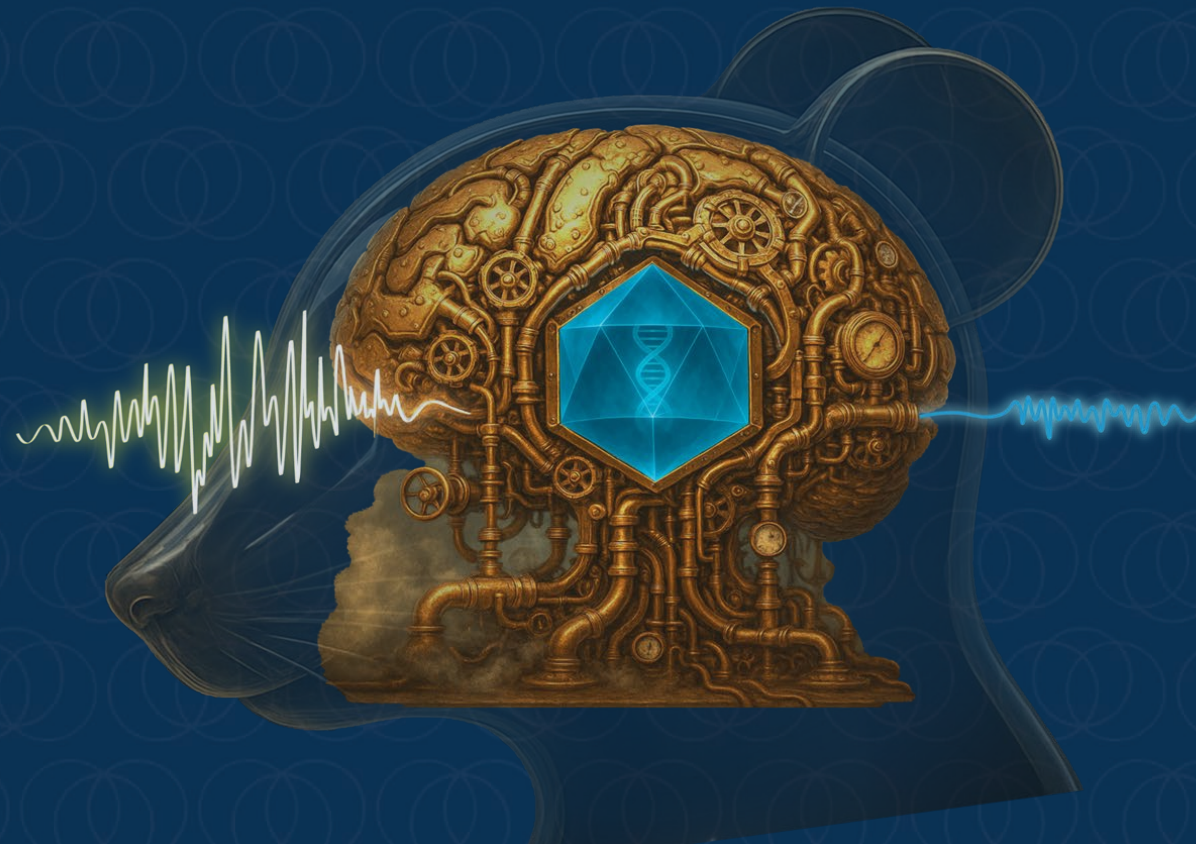
ALLEN INSTITUTE for BRAIN SCIENCE

Cell Type-Specific AAV Gene Therapy for *SLC6A1*-Related Disorder



Rong Guo PhD

Scientist III

Allen Institute for Brain Science

2025.12

Disclosures & Acknowledgements

- **Disclosures**
  - This project was funded in part through a sponsored research agreement with BioMarin Pharmaceutical.
  - Authors are inventors on related patent(s).

- **Acknowledgements**

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- **Other Gene Therapy Members:** Jonathan Ting; Tanya Daigle; Michal Fortuna; Avery Hunker; Naz Taskin; Andrew Clark; and other team members.
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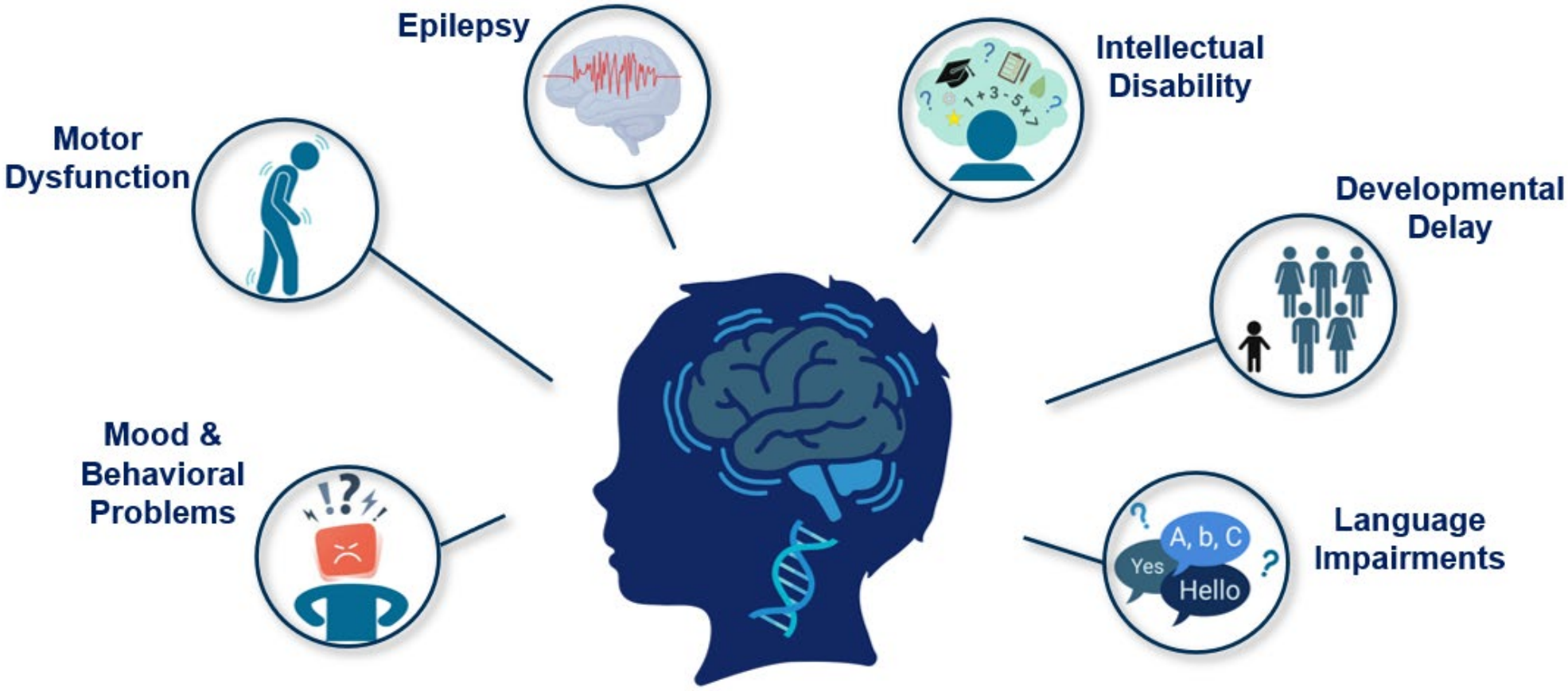
- **Legal:** Myra Imanaka; Scott Smith.

BIOMARIN

- Manuel Lopez
- Justin Ichida

SLC6A1-Related Disorder (SRD)

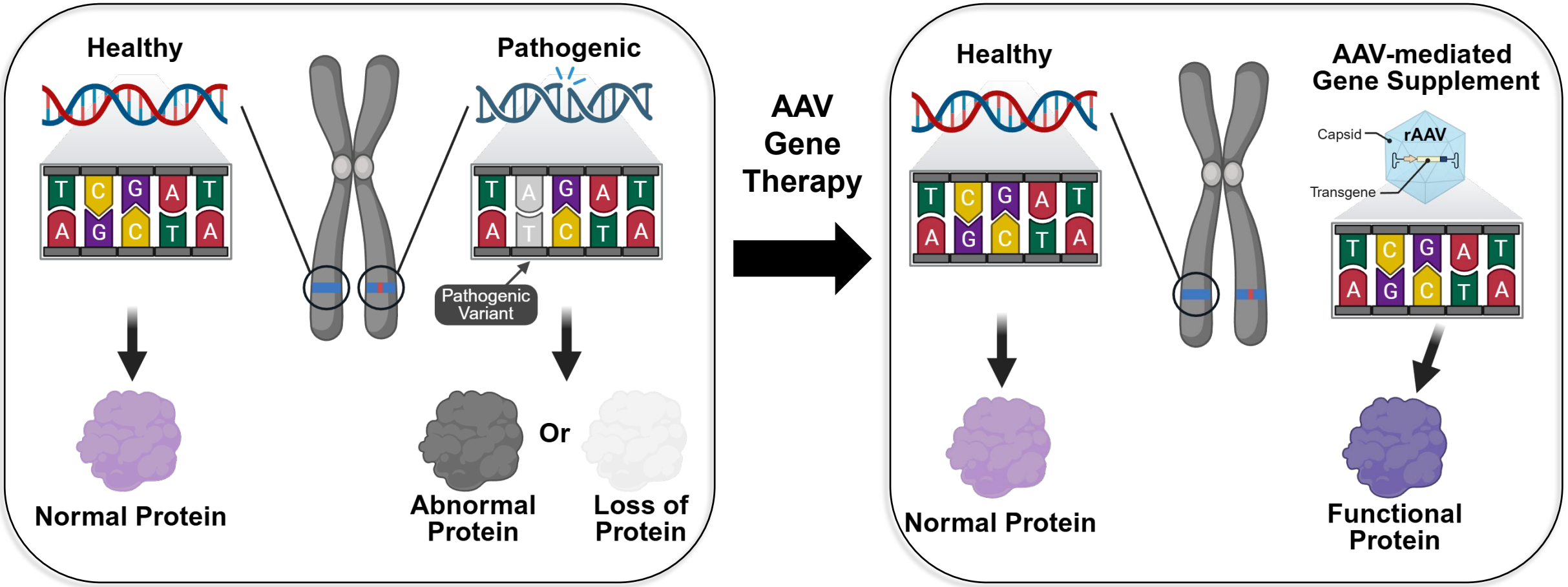


~ 1 in 38,000 births

No known anatomical changes, cell loss or widespread neurodegeneration

→ suitable candidate for gene replacement therapy

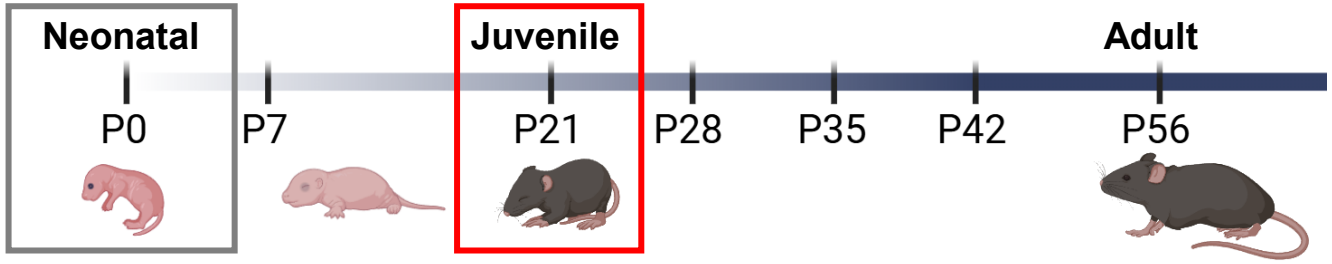
AAV Gene Therapy for *SLC6A1*-Related Disorder (SRD)



Non-Selective AAV Gene Therapy for SRD Can Cause Side Effects

- **Neonatal treatment in mice:**
  - Ubiquitous or pan-neuronal;
  - Improved EEG and behavioral measurements;
  - High *mortality* and *convulsive seizures*.

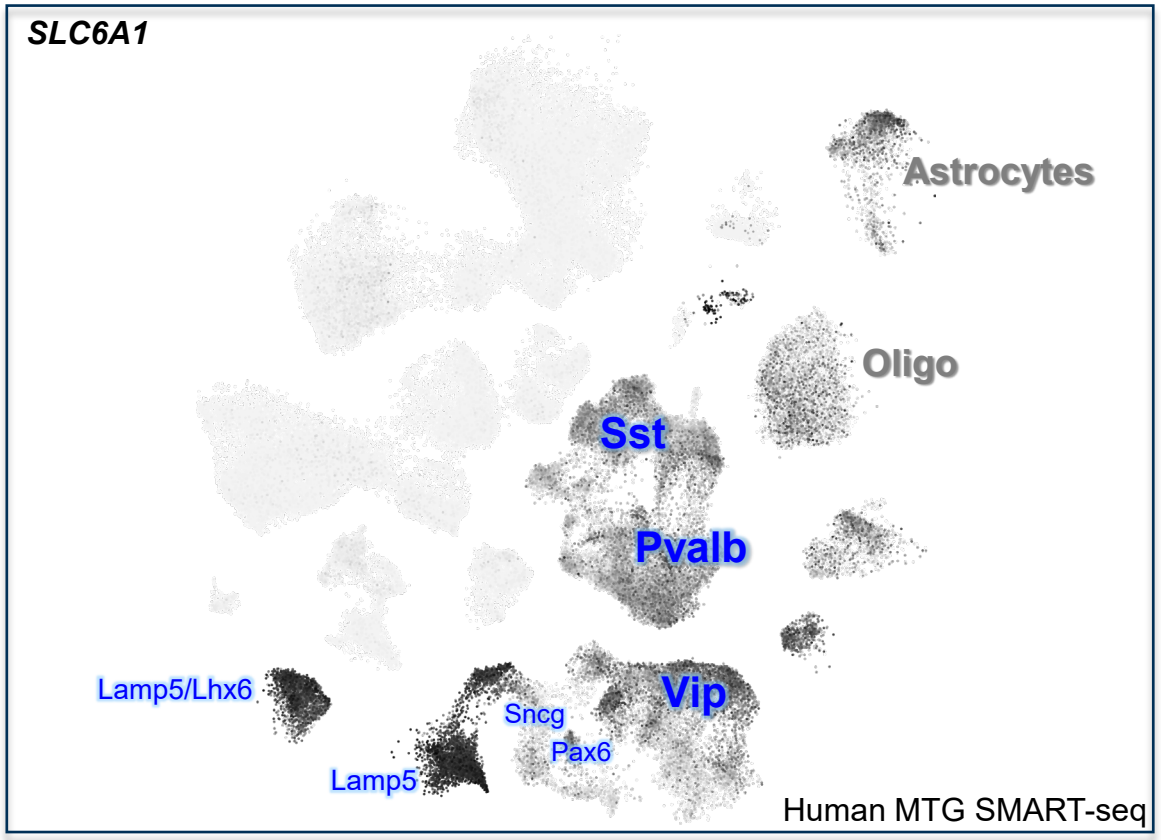
Guo, Weirui, et al. The Journal of Clinical Investigation (2025).



- **Juvenile treatment in mice:**
  - Pan-neuronal;
  - High dose → high *mortality* across genotypes;
  - Mid-dose → spikes and *convulsive seizures*;
  - Low dose → no rescue of key phenotypes.

Cell Type-Specific AAV Gene Therapy for SRD: Restricted Expression in the Right Cell Type(s)

- *SLC6A1* expression in the brain is enriched in **inhibitory neurons** and glial cells, e.g., astrocytes.

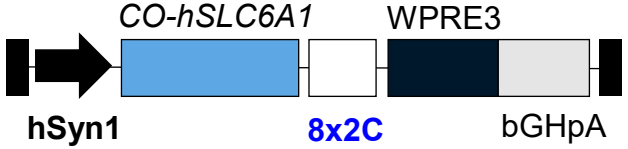


Jorstad, Nikolas L., et al. Science (2023).

Cell Type-Specific AAV Gene Therapy for SRD: Restricted Expression in the Right Cell Type(s)

- *SLC6A1* expression in the brain is enriched in **inhibitory neurons** and glial cells, e.g., astrocytes.
- miRNA binding sites can restrict *SLC6A1* expression to inhibitory neurons.

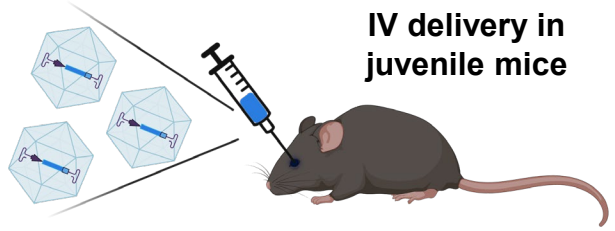
Pan-inhibitory:



hSyn1 Pan-neuronal promoter

8x2C miRNA binding sites that mediate transgene degradation in excitatory neurons (GABA-Selective)

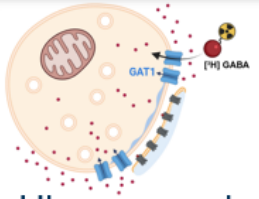
BBB-crossing PHP.eB capsid



IV delivery in juvenile mice

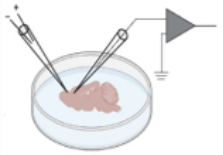
ex vivo

Function Validation



Hippocampal synaptosome ^{3}H-GABA uptake

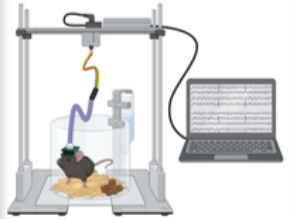
Circuit Dynamics



Acute slice recording: evoked IPSCs

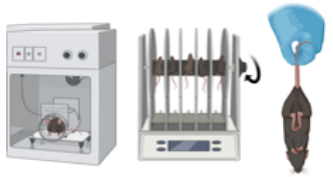
in vivo

Epilepsy



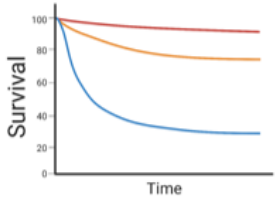
Epileptiform (EEG)

Motor Dysfunction



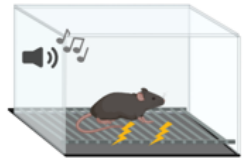
Motor Deficits (e.g., tremor, rotarod, hindlimb clasping)

Developmental Delay



Failure-to-thrive (survival, body weight)

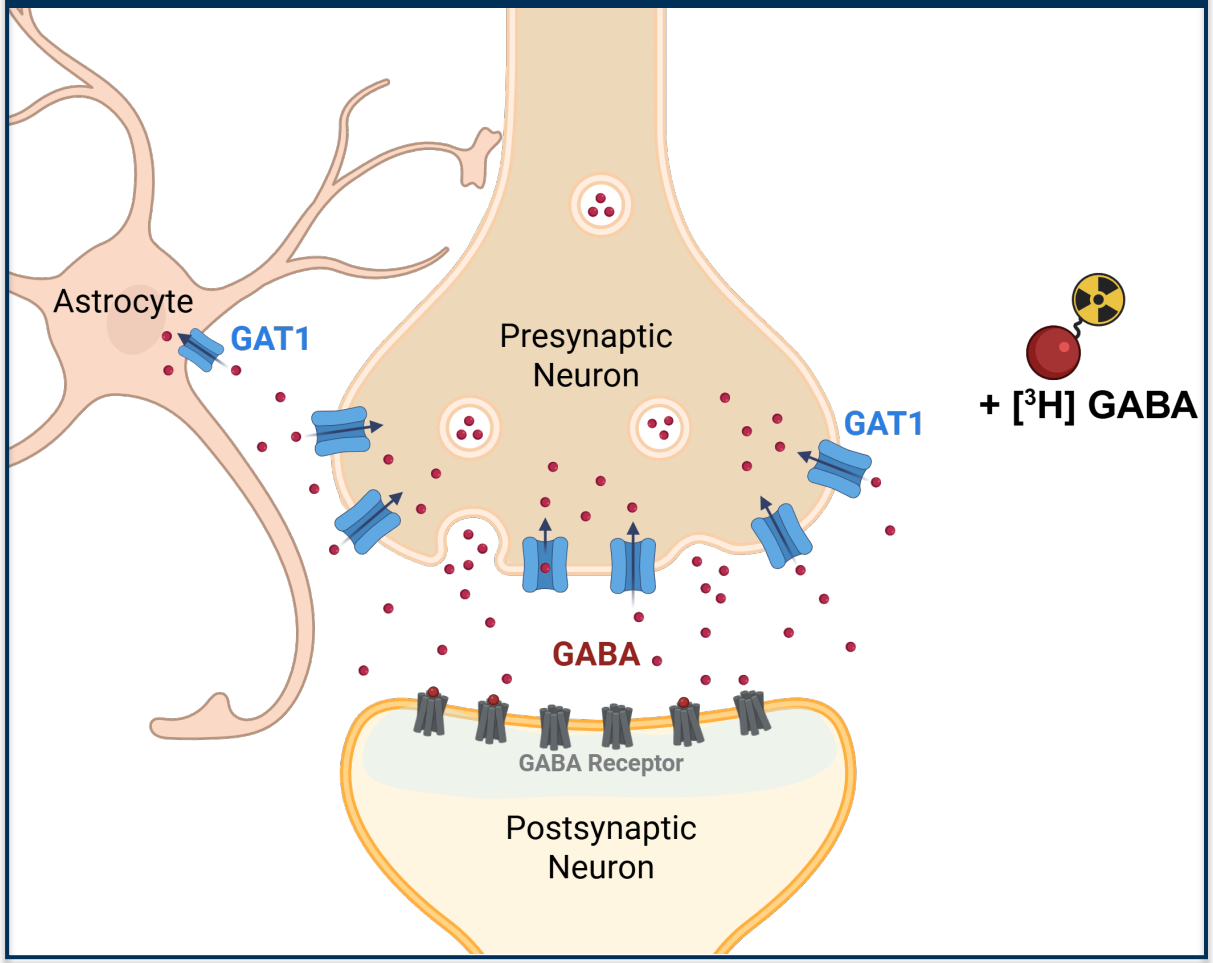
Intellectual Disability



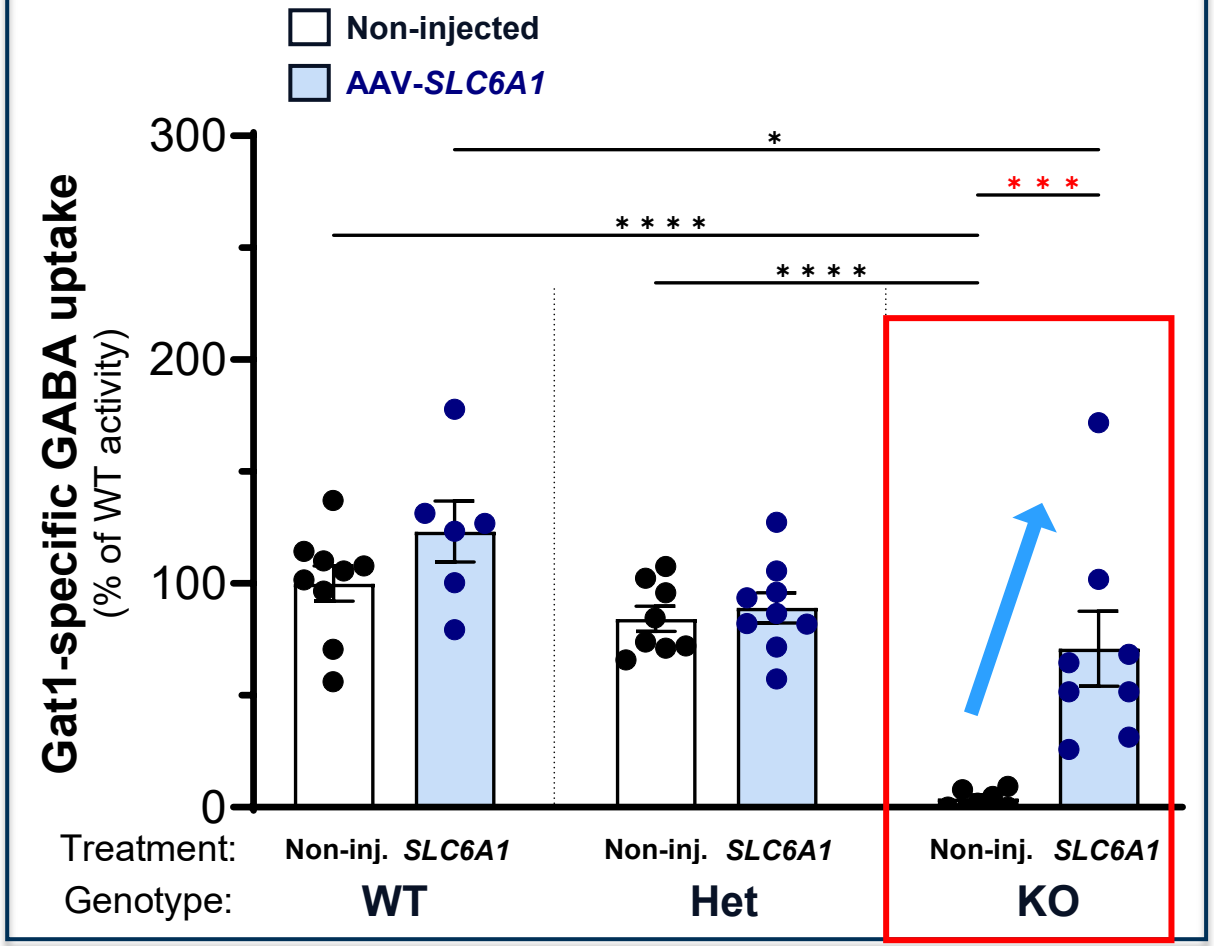
Memory Deficits (fear conditioning)

Brain-wide GABAergic *SLC6A1* Expression Restores GABA Uptake Function

GAT1: the Primary GABA Transporter

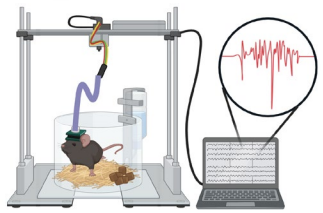


Ex Vivo: Hippocampal Synaptosome [^{3}H] GABA Uptake



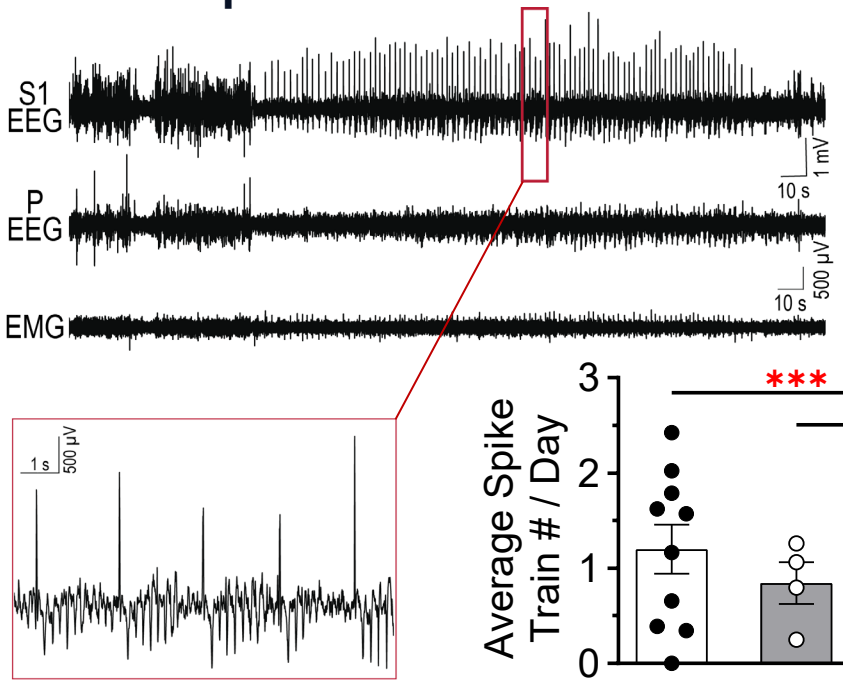
GABAergic *SLC6A1* Reduces Epileptiform Activity

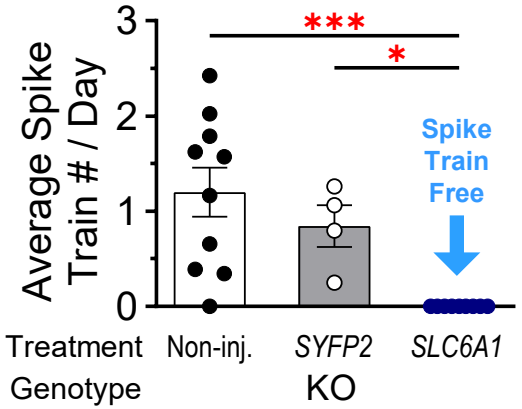
Epilepsy

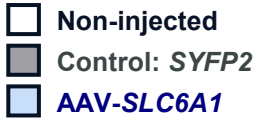


- **Spike Trains** ..... *Electrographic Status Epilepticus* ✓
- **Spike-and-wave Discharges (SWDs)** ..... *Absence Seizures* ✓
- **Parietal Spikes** ..... *Interictal Spikes* ✓
- **FFT Power Spectrum Features** ..... *EEG Biomarker; Network Oscillations* ✓

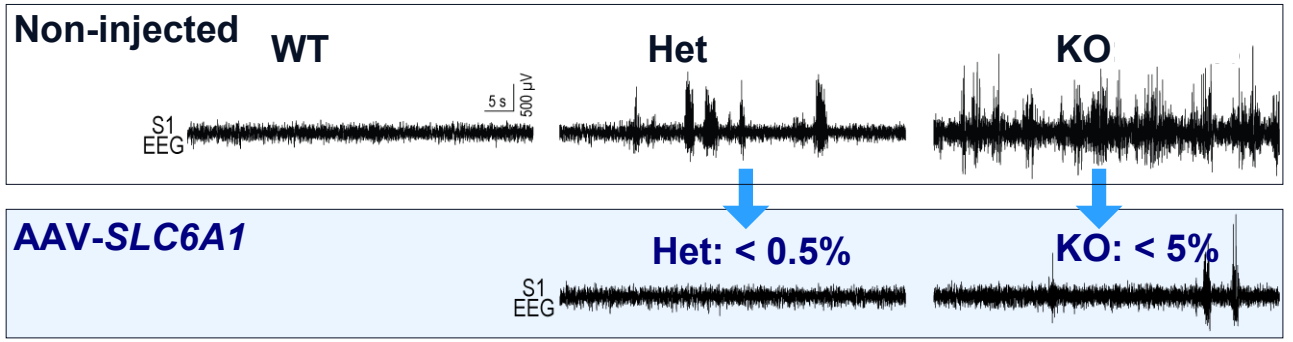
Spike Train in KO Mice

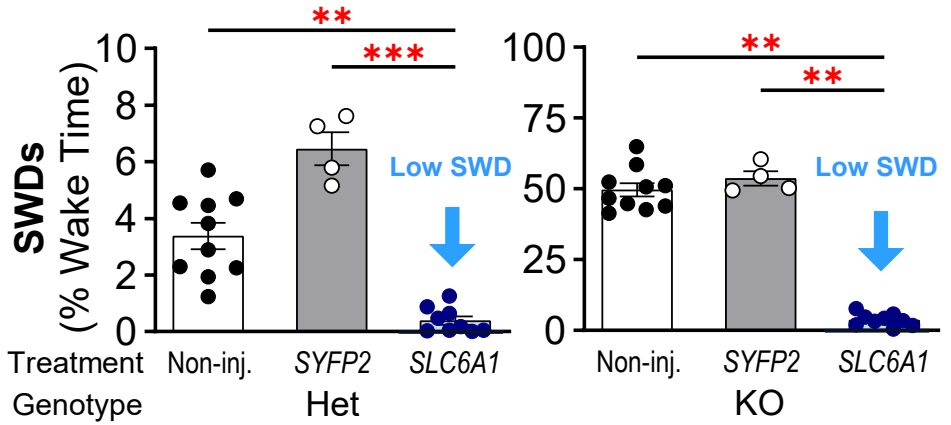




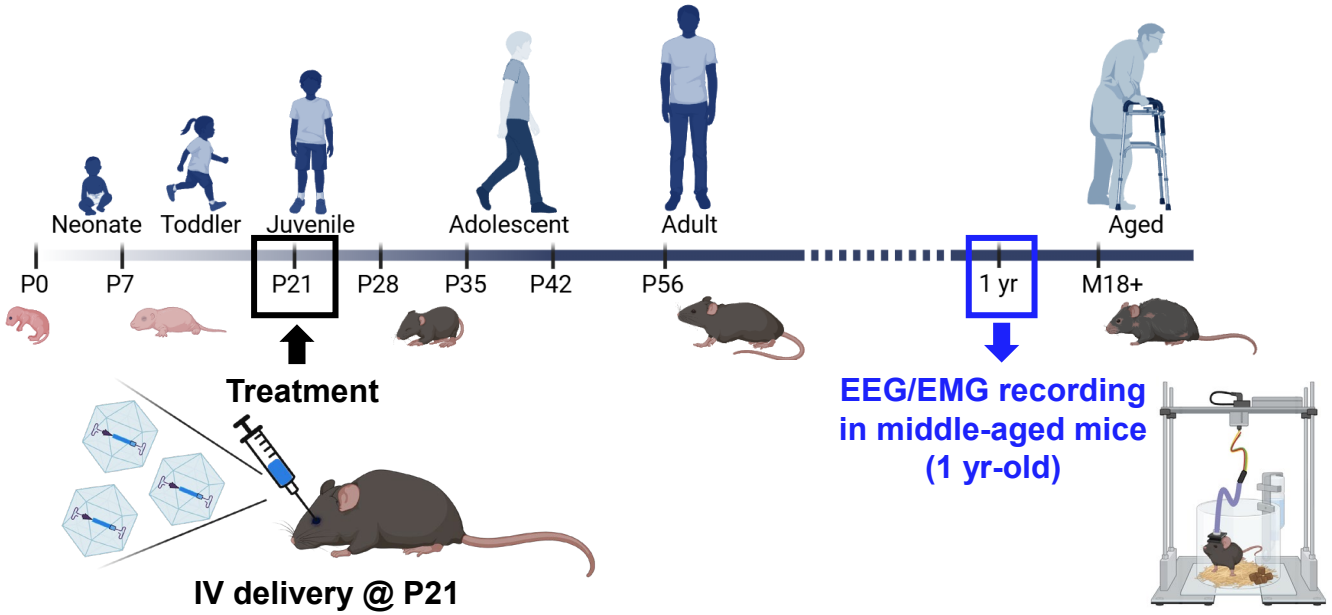


SWDs

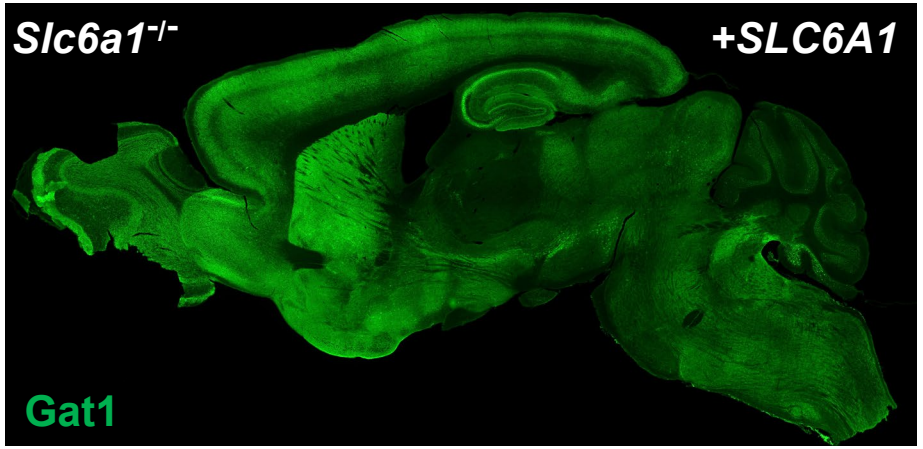




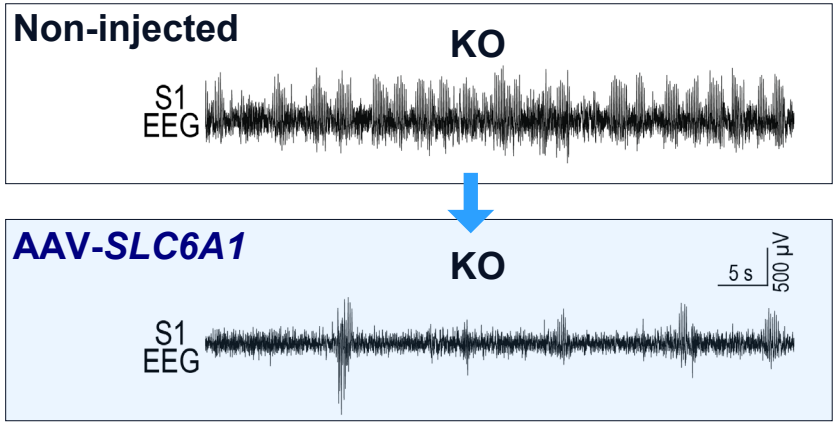
Persistent Reduction of Absence Seizures

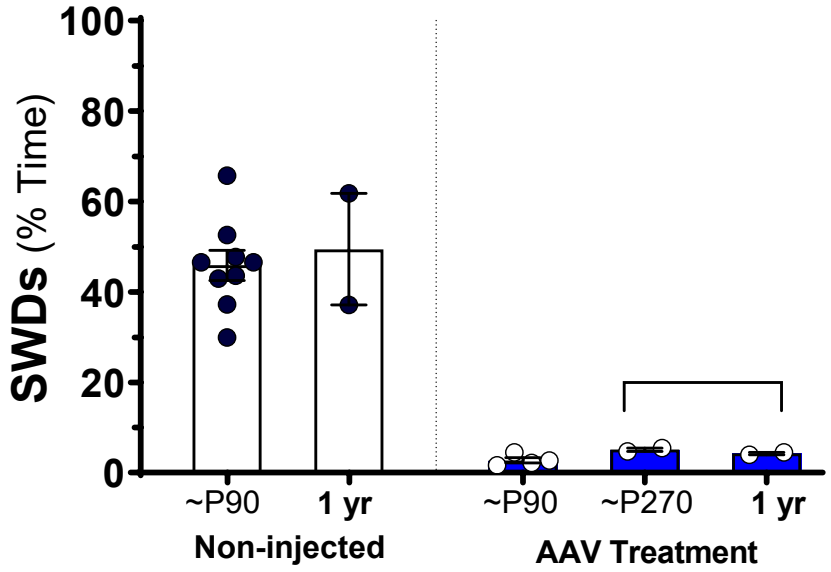


IHC: Persistent Expression of GAT1



SWDs in Middle-aged KO Mice



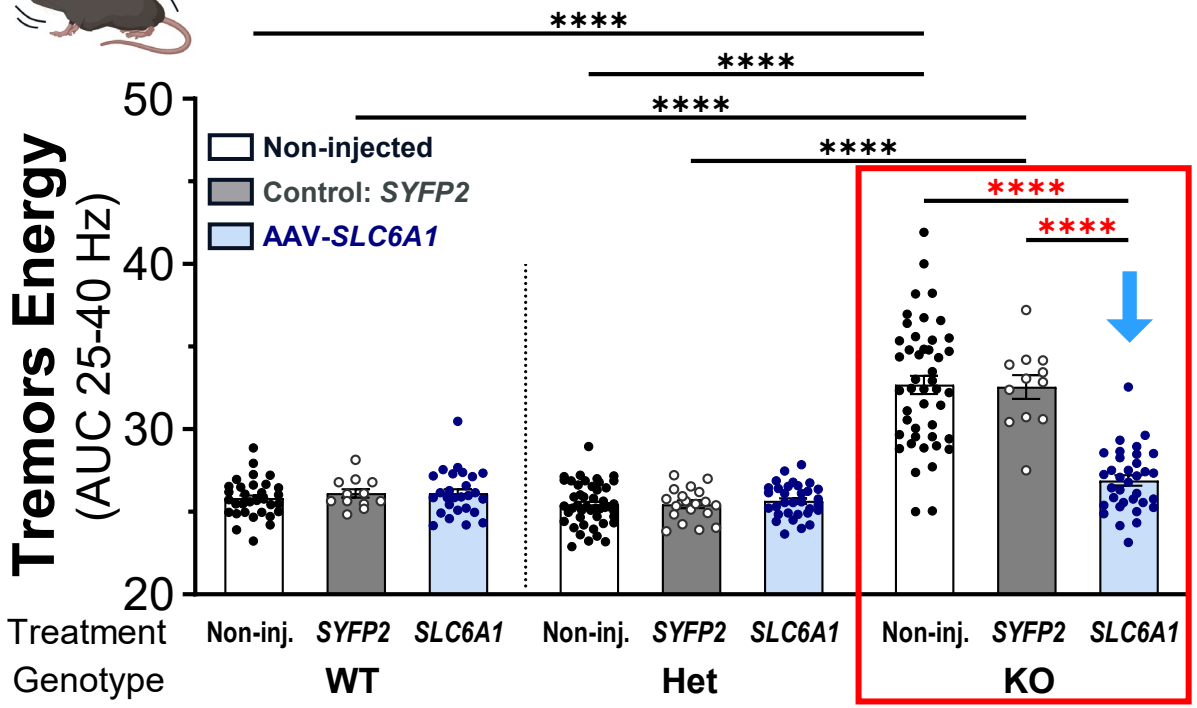


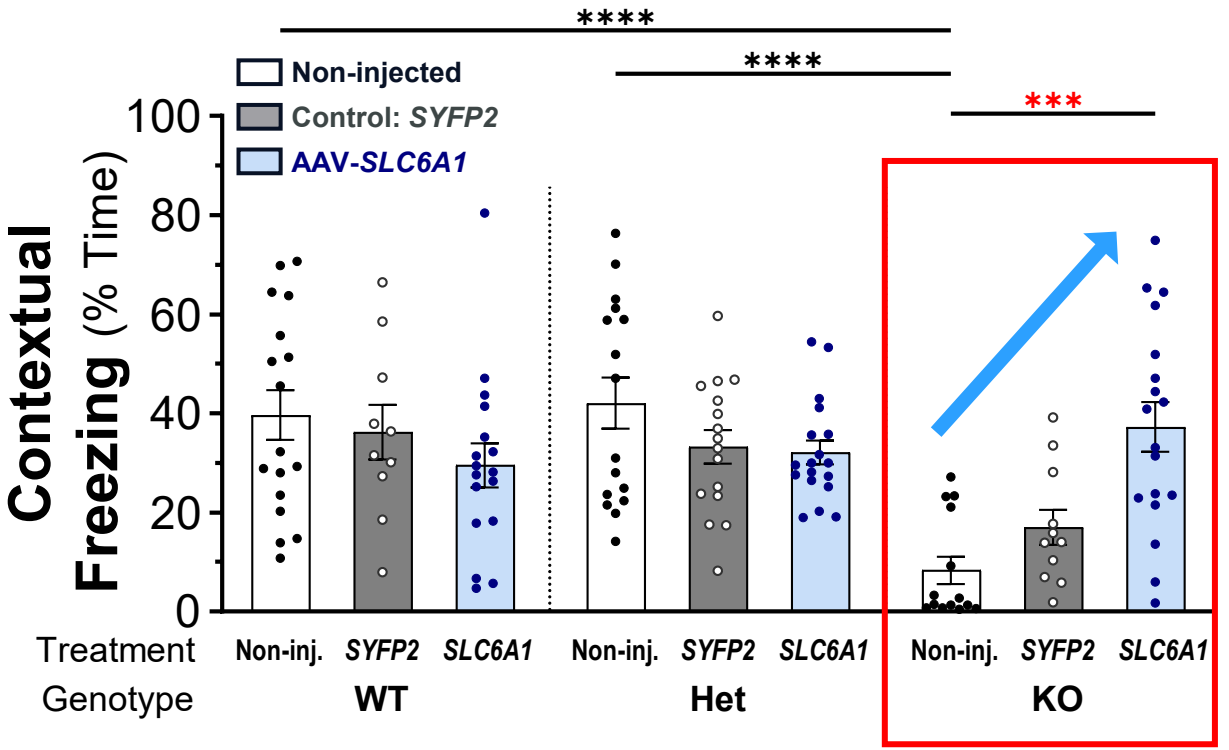
GABAergic *SLC6A1* Improves Motor & Cognitive Functions

Motor Dysfunction
- Resting Body Tremor ✓
- Rotarod ····· *Coordination & Balance* ✓
- Hindlimb Clasping ✓

Developmental Delay
- Body Weight ····· *Failure-to-thrive* ✓

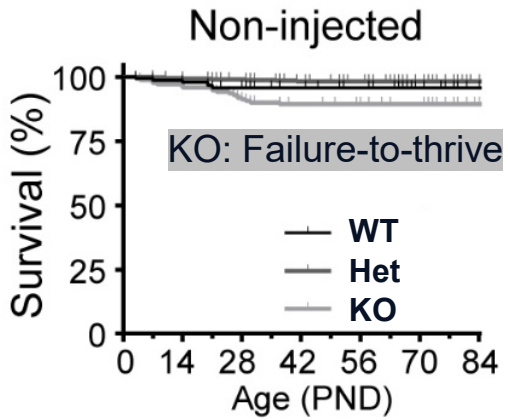
Intellectual Disability — Fear Conditioning Test
- Startle Response ····· *Sensory Sensitivity* ✓
- Contextual Freezing ····· *Short-term Learning & Memory* ✓
- Auditory Cued Freezing ····· *Short-term Learning & Memory* ✓

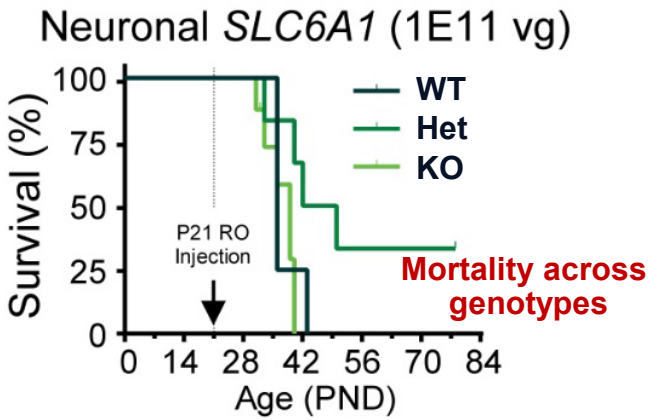


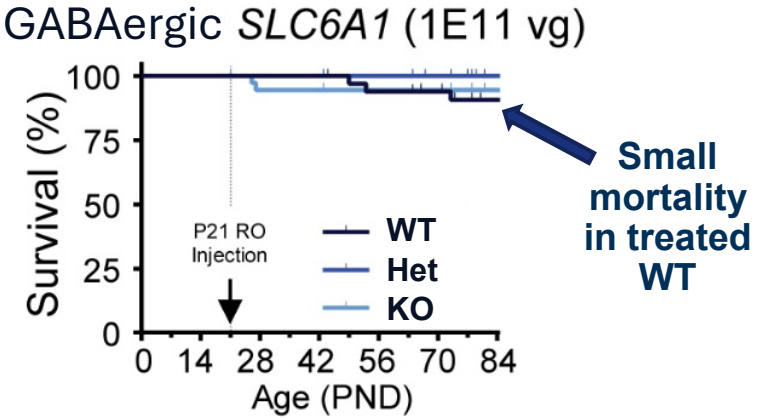


Overexpression of *SLC6A1* in WT Littermates Causes Side Effects

- GABAergic *SLC6A1* treatment is tolerated in diseased mice but shows mortality in WT littermates.

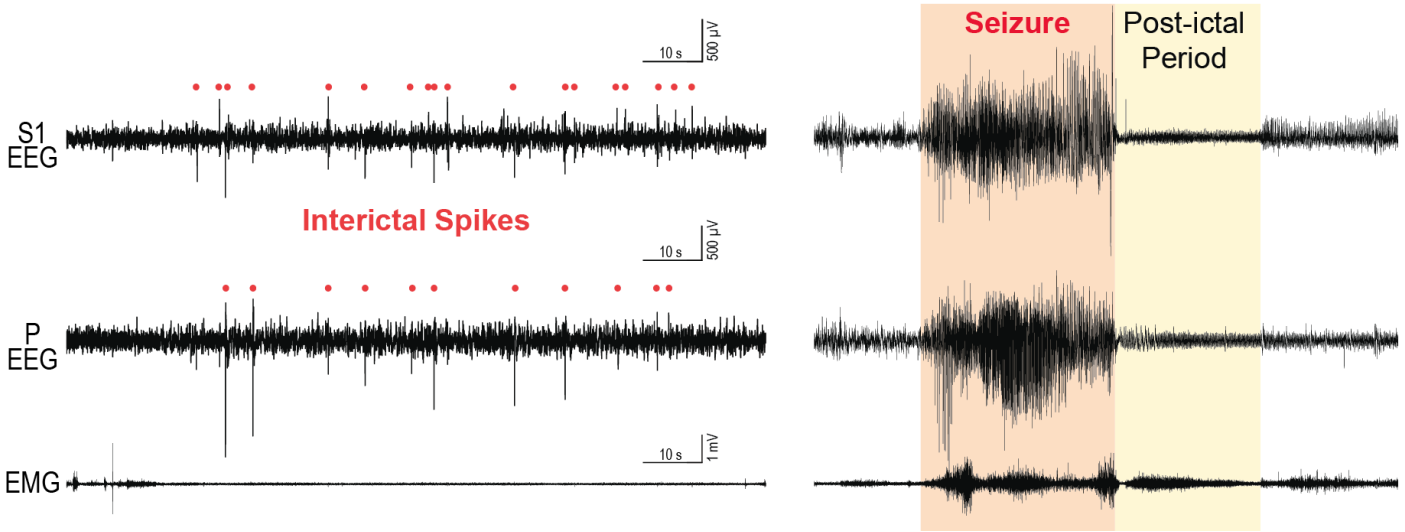




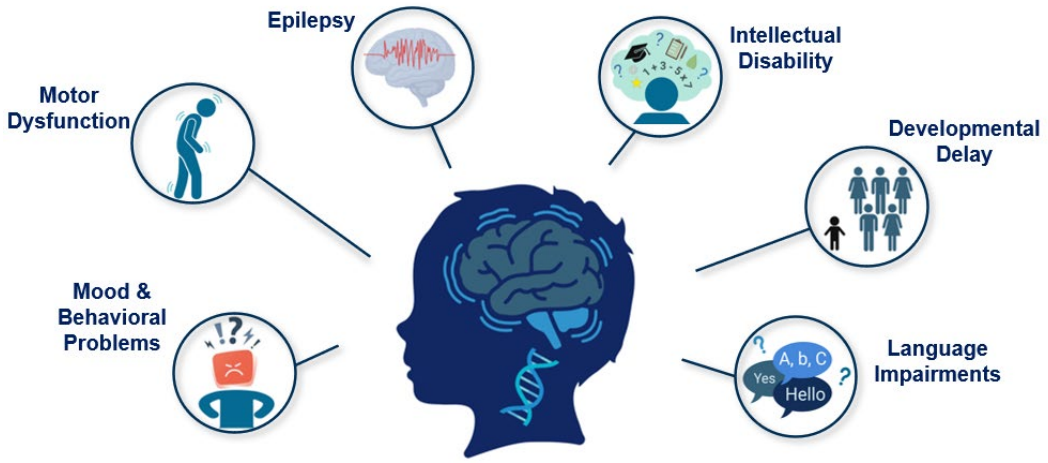


- Epileptiform side effects are observed in injected wildtype littermate mice.

Example EEG/EMG traces from a treated WT littermate



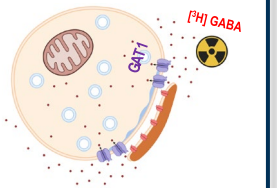
Cell-type Specificity Is Key to Safer & More Effective Gene Therapy



- Non-selective: side effects; low efficacy.
- **Brain-wide GABAergic**: multifaceted improvements and rescue in juvenile mice.
- GABAergic treatment: tolerated in diseased mice but causes side effects in WT littermates.

ex vivo

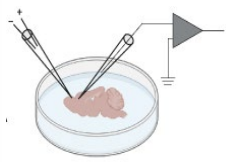
Function Validation



Hippocampal synaptosome ^{3}H-GABA uptake

✓

Circuit Dynamics

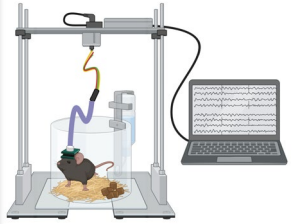


Acute slice recording: evoked IPSCs

✓

in vivo

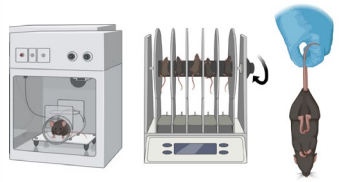
Epilepsy



Epileptiform (EEG)

✓

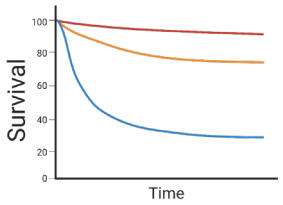
Motor Dysfunction



Motor Deficits (e.g., tremor, rotarod, hindlimb clasping)

✓

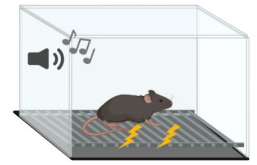
Developmental Delay



Failure-to-thrive (survival, body weight)

✓

Intellectual Disability

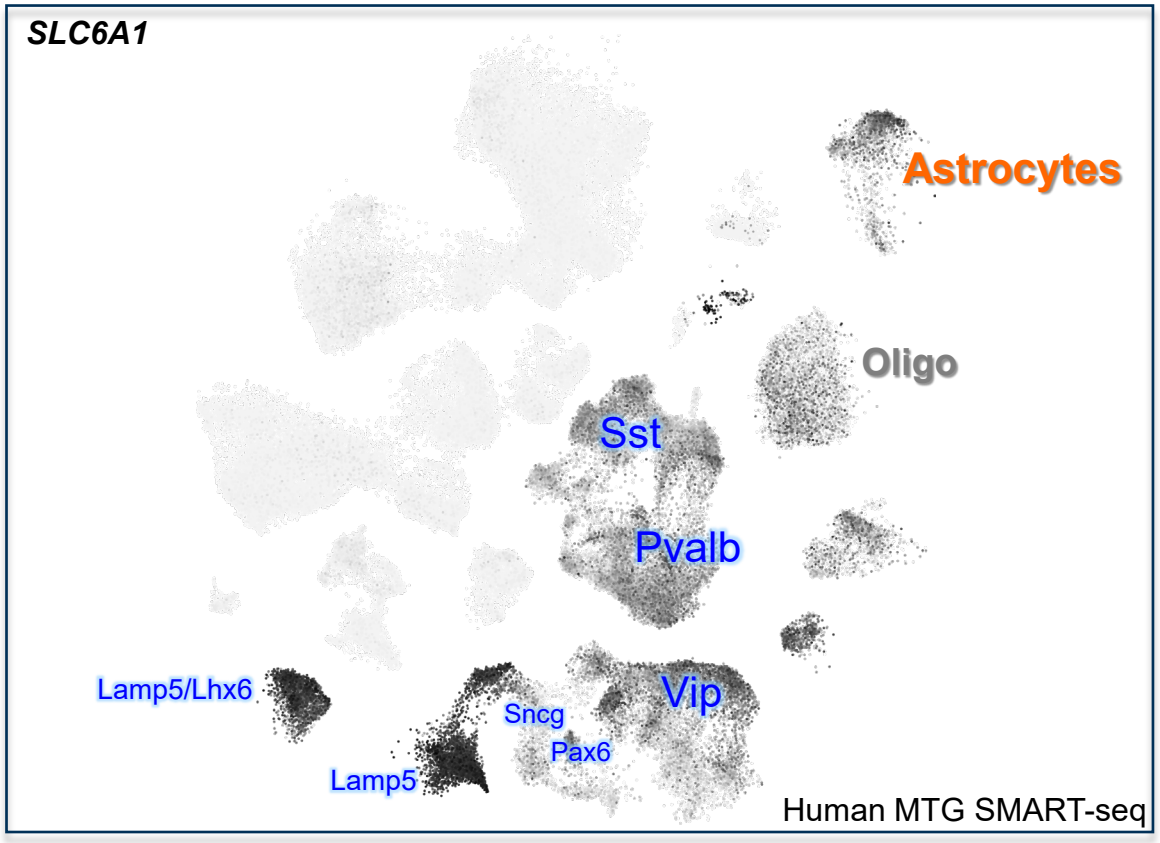


Memory Deficits (fear conditioning)

✓

SLC6A1 Is also Enriched in Astrocytes.

- *SLC6A1* expression in the brain is enriched in inhibitory neurons and glial cells, e.g., **astrocytes**.



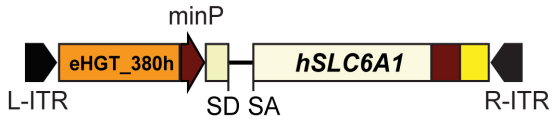
Jorstad, Nikolas L., et al. Science (2023).

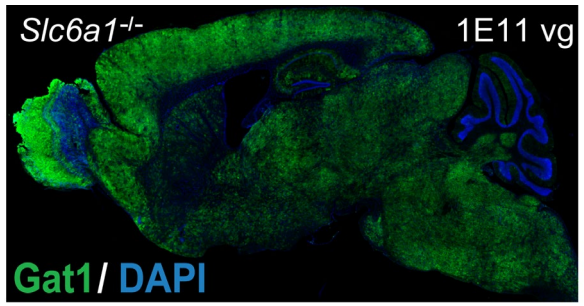
✓ **Inhibitory neurons.**

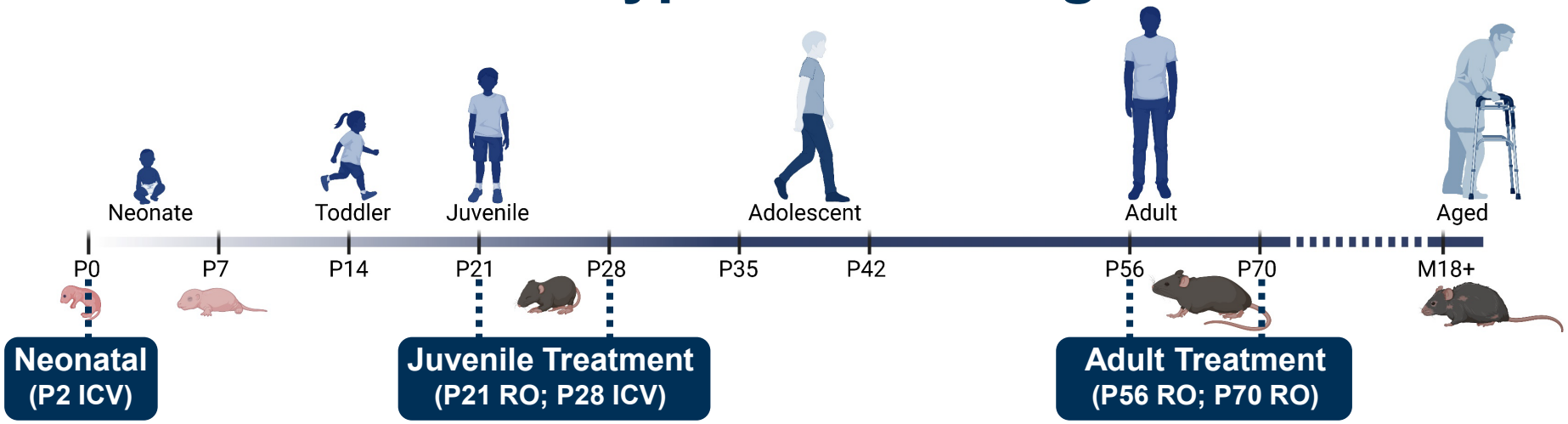
❑ **What about astrocytes?**

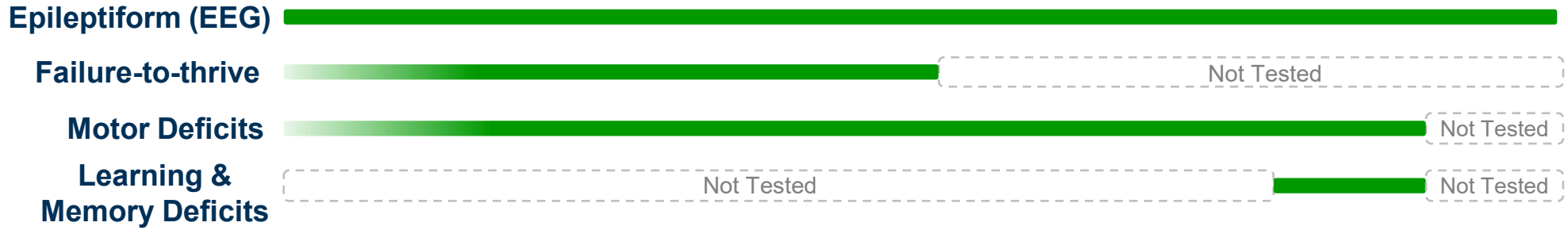
Brain-wide Expression of *SLC6A1* in Astrocytes Rescues Disease Phenotypes Across Ages

Astrocytic:





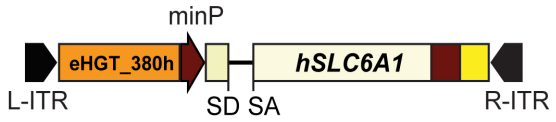


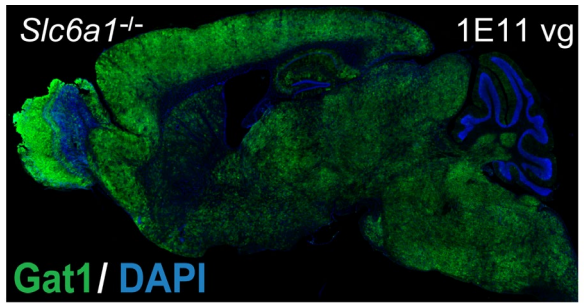


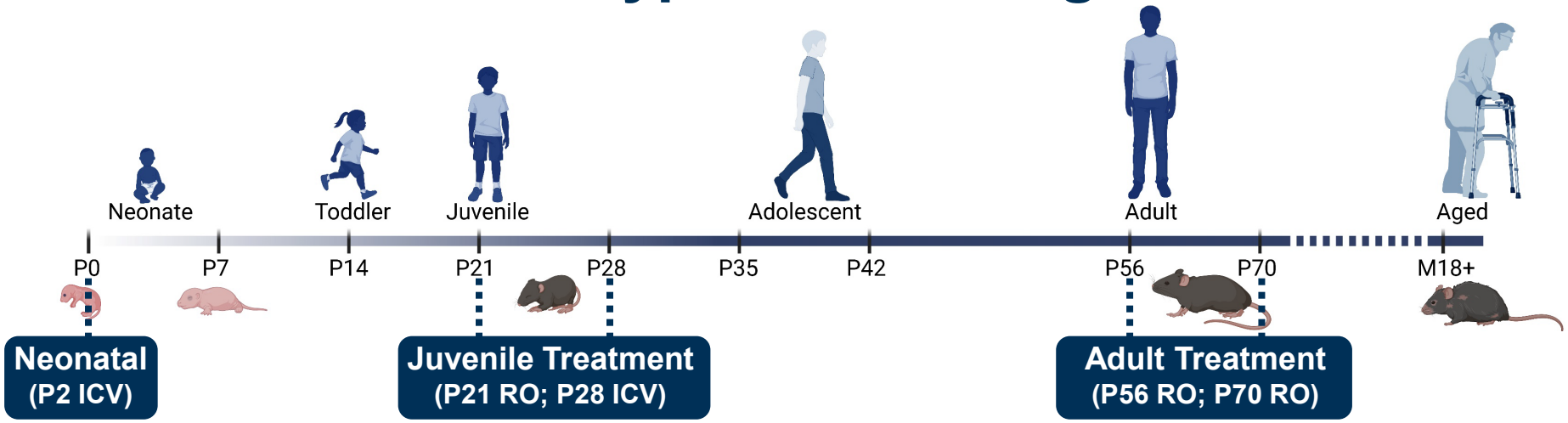
- Multiple Ages: P2, P21, P28, P56, P70
- Multiple Routes of Administration: ICV, RO, direct intra-thalamic injection.
- Multiple Doses: low dose, mid-dose, high dose;

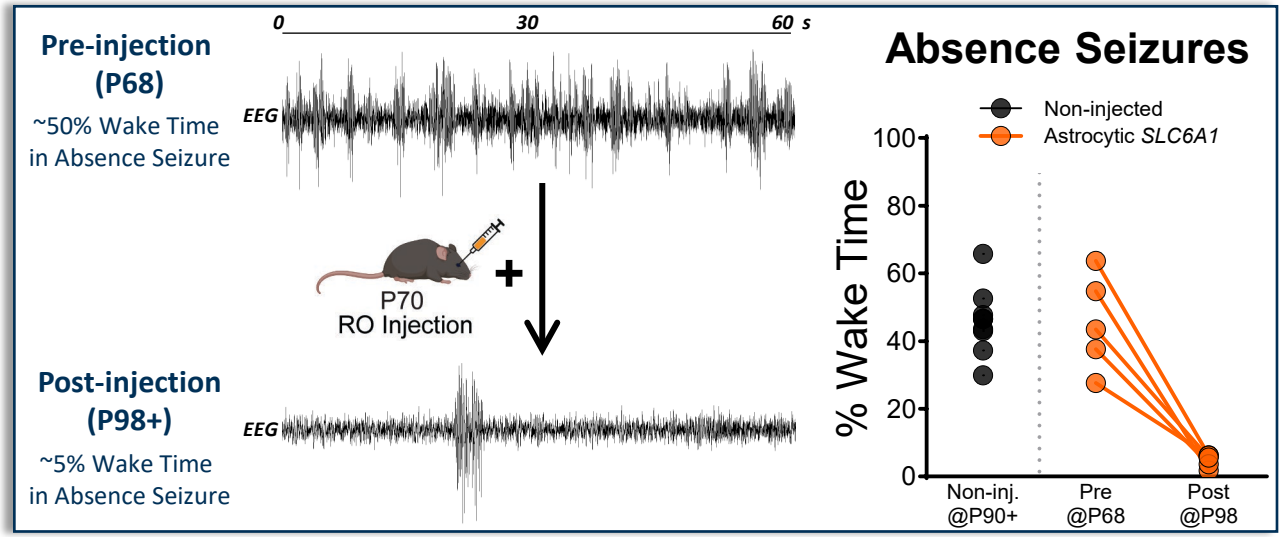
Brain-wide Expression of *SLC6A1* in Astrocytes Rescues Disease Phenotypes Across Ages

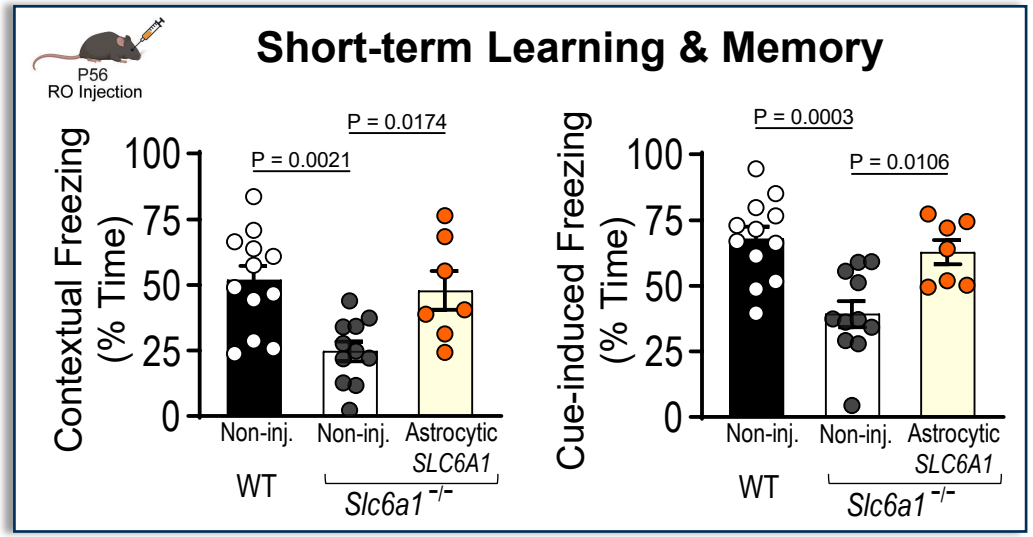
Astrocytic:





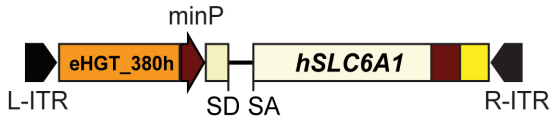


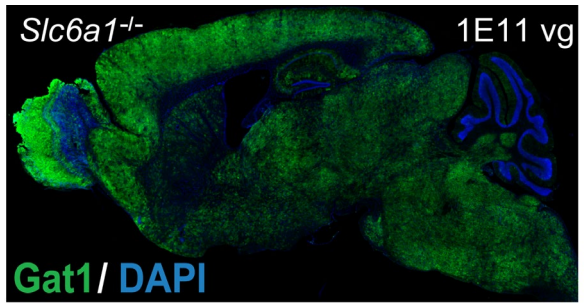


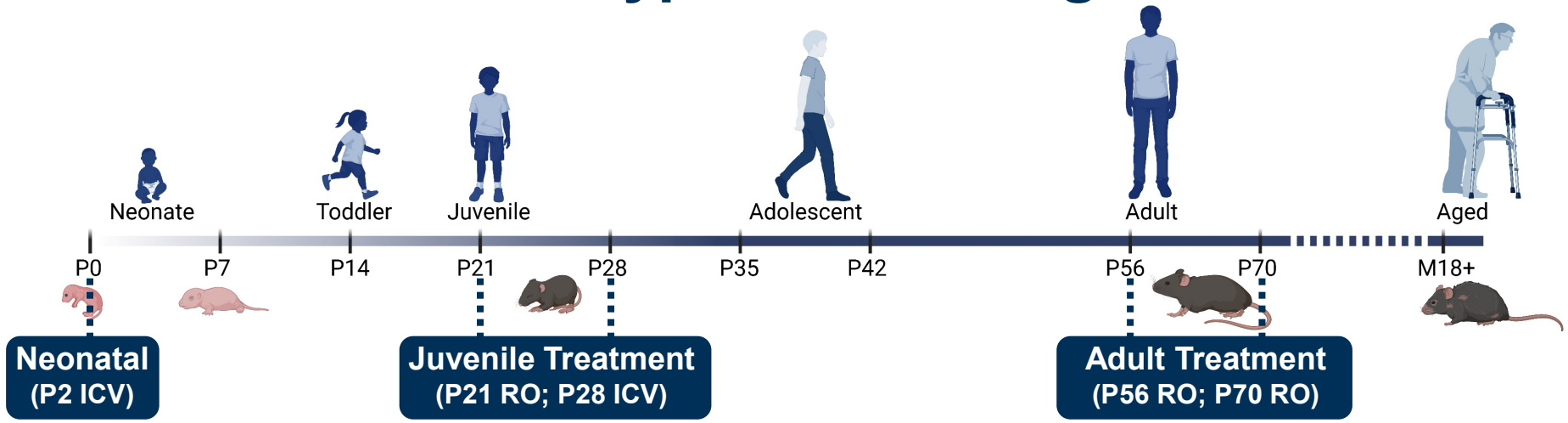


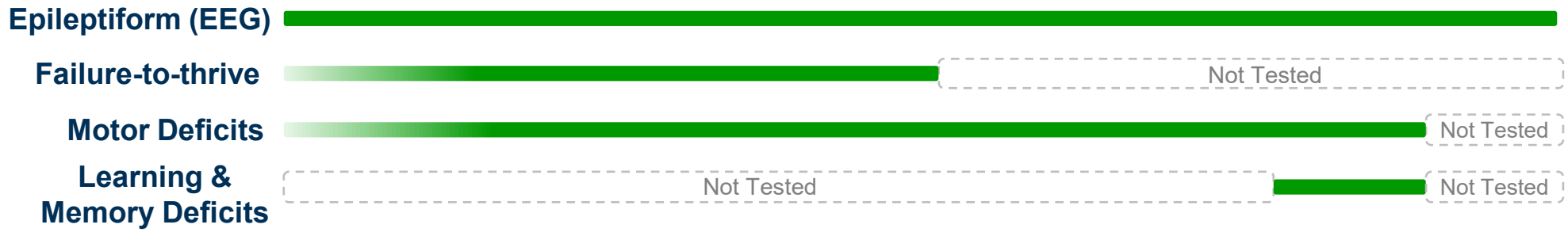
Brain-wide Expression of *SLC6A1* in Astrocytes Rescues Disease Phenotypes Across Ages

Astrocytic:









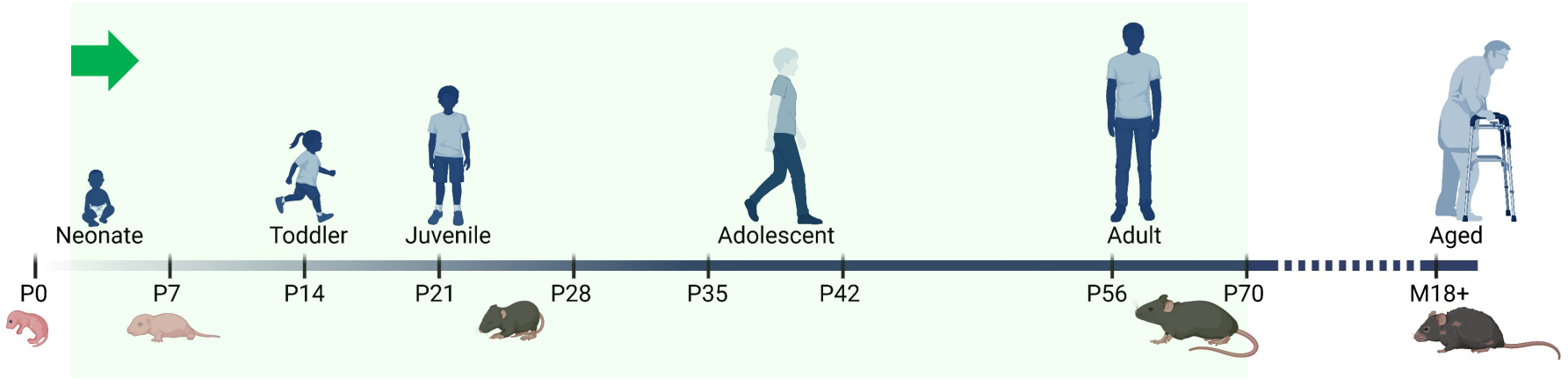
- Age and the route of administration are not the limiting factors.
- Efficacy comparable to pan-inhibitory vector (*except for parietal spikes).
- Overexpression, even in KO (Slc6a1$^{-/-}$) mice, can lead to side effects (e.g., mortality, spikes, and convulsive seizures).

Cell-type Specificity Is Key to Safer & More Effective Gene Therapy

- ❑ Non-selective: side effects & toxicity; low therapeutic efficacy.
- ❑ Cell type-specific approach

  enhances **therapeutic efficacy and safety**.

extends the developmental **therapeutic window** beyond the neonatal period.



- ❑ Brain-wide expression: multifaceted improvements and rescue.
- ❑ Main challenge: translation to a viable human therapeutic.

Thank you

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