Mouse Models for SLC6A1 Disorder

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SLC6A1 Connect International Conference
Baltimore, December 5, 2019
SLC6A1 Disorder

- Patients present with developmental delay, varying degrees of intellectual disability, abnormal EEG, and seizures
- Few available models for studying disease progression and therapeutic intervention
- Possibly haploinsufficiency disorder; genetic knock-out model could be useful
GAT1-KO Mouse Model

- Created as intermediate to GAT1-GFP mouse
- Large insertion in intron 14 results in highly hypomorphic GAT-1, resulting in functional KO
- KO has <2% GABA uptake activity compared to WT; Hets have ~64% WT activity
- Mice present with ataxia, tremor, and anxious behavior

**GAT1-KO Mouse Phenotype**

<table>
<thead>
<tr>
<th>Phenomenon</th>
<th>Description</th>
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<tr>
<td>Decrease in frequency of quantal GABA release</td>
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<td>Lack of pre-synaptic GABA(_B) tone</td>
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<td>Enhanced tonic and diminished phasic inhibition</td>
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<tr>
<td>Mice present with ataxia, tremor, hindlimb clasping, and anxious behavior</td>
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<tr>
<td>Hets have mostly normal phenotype, despite having diminished GABA uptake</td>
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Tremor, Ataxia and Coordination

Chiu et al. J Neurosci, 2005
Activity and Anxious Behavior

Chiu et al. J Neurosci, 2005
GAT1 KO Behavioral Battery

- Locomotion
- Open Field
- Elevated Plus Maze
- Marble Burying*
- Nesting*
- 3-Choice Social Interaction*
- Accelerating Rotarod
- Pre-Pulse Inhibition
- Digigait*
- Hotplate*
- Fear Conditioning*
- Wire-hang

*Previously undescribed behavior
Accelerating Rotarod

GAT1 KO mice perform poorly on rotarod

** WT
* Het
** KO
Open Field

GAT-1 KO mice have decreased movement in 5-10 min interval, and increased time in periphery/decreased time in center during both 0-5 and 5-10 min intervals
Nest Building

GAT1 KO mice are poor nest builders

Fear Conditioning

GAT1 KO mice are poor learners in contextual fear conditioning, but do not have a deficit in cued fear conditioning.
GAT1 KO Behavior Summary

- Decreased body weight
- Tremor
- Hindlimb clasping
  - Developed scoring system (0-5)
- Wire-hang
  - Decreased latency to fall*
- Elevated-Plus Maze
  - More time spent in open arms; inconsistent with OF data
- No significant Pre-pulse inhibition*
- No changes in hot-pate sensitivity
- Unable to score 3-choice social interaction

- Rotarod
  - Decreased latency to fall
- Open Field
  - Decreased movement in 5-10 min interval
  - Increased time in periphery during both 0-5 and 5-10 min intervals
- Nest building
  - Decreased nest building score
- Context fear conditioning
  - Decreased freezing time, indicating poor contextual learning
  - No deficit in cue fear conditioning

*different from previously reported
• Compared to tethered system
  • Less invasive
  • Can measure more parameters
  • Shorter total recoding time

• DSI system/ sensors for this project:
  • Record up to 16 animals simultaneously (more if alternating)
  • Sensor records EEG, EMG, activity and temperature simultaneously
EEG Recordings

24 hr recordings with automated spike detection
• Threshold ratio 5
• Max interval: 1.5s
• Min # spikes: 3

<table>
<thead>
<tr>
<th>Subject</th>
<th>Genotype</th>
<th># Spikes</th>
<th># Spike Trains</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Het</td>
<td>872</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>KO</td>
<td>2,788</td>
<td>69</td>
</tr>
<tr>
<td>3</td>
<td>WT</td>
<td>235</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>KO</td>
<td>5,103</td>
<td>151</td>
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GAT1 KO Mouse as a Model for SLC6A1 Disorder

- KO recapitulates patient-like phenotypes
  - Motor deficits
  - Cognitive deficits
  - Abnormal EEG
- Genotype/phenotype does not match patient population
  - Haploinsufficiency or dominant negative disorder?

- Patient-specific mutations models may provide additional insights
  - A288V (Kang Lab, Vanderbilt)
  - S295L
Acknowledgments

**Gray Lab**

PI: Steven Gray, PhD  
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Sarah Sinnett, PhD  
Thomas Dong, PhD  
Xin Chen, MD, PhD  
Nanda Regmi, PhD  
Juan Rodriguez, PhD  
Yuhui Hu  
Widler Casy, PhD  
Qinglan Ling, PhD  
Matthew Rioux  
Kathryn McMillan  
Emily Boyle  
Chris Lyons

**Behavior Core**

Shari Birnbaum, PhD  
Priscilla Saenz

**Neuro Models Core**

Erik Plautz, PhD

Berge Minassian, MD, PhD  
Kimberly Goodspeed, MD  
Neil Hackett, PhD  
Amber & Mark Freed