

Mouse Models for SLC6A1 Disorder

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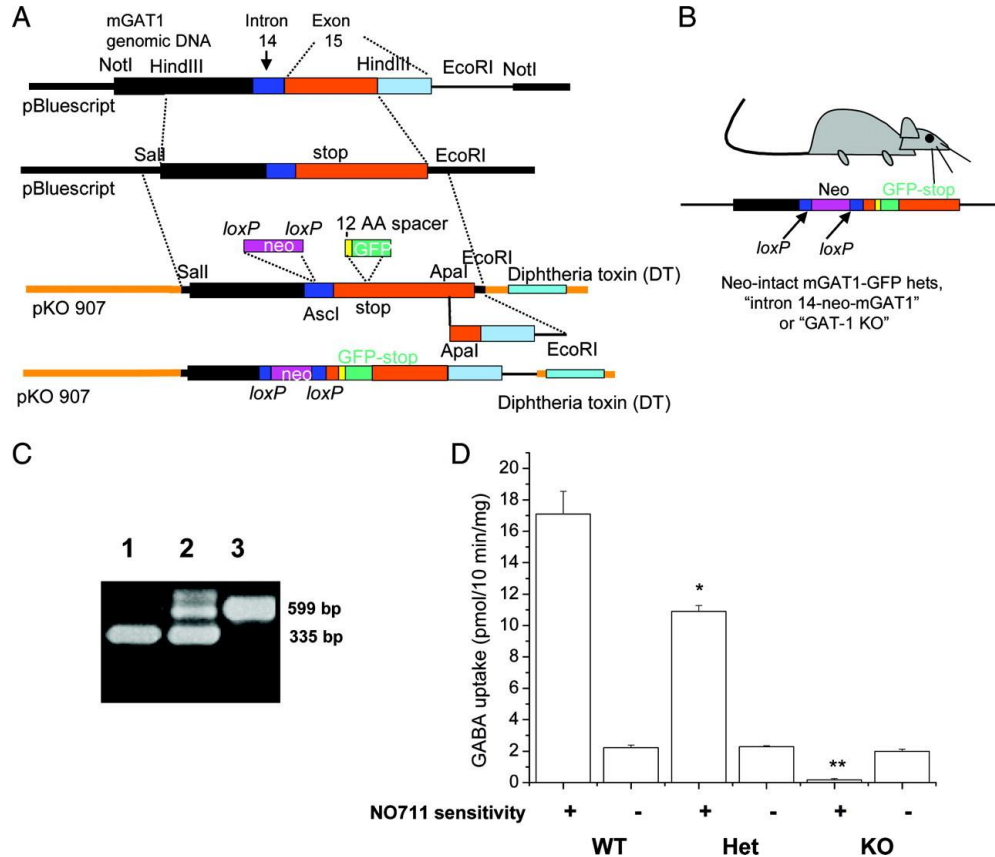
SLC6A1 Connect International Conference
Baltimore, December 5, 2019

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

Decrease in frequency of quantal GABA release

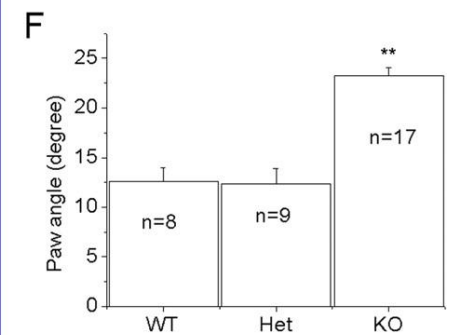
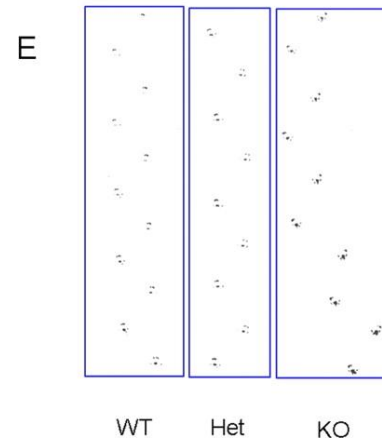
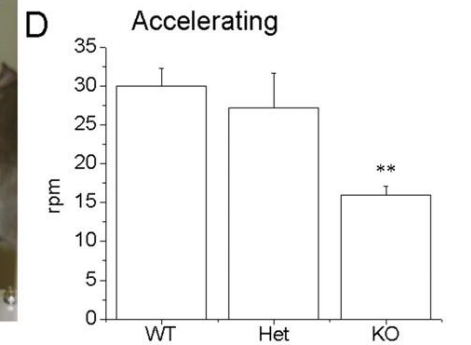
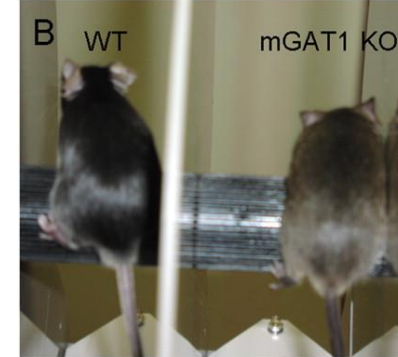
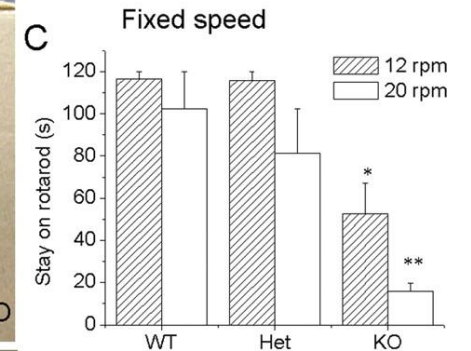
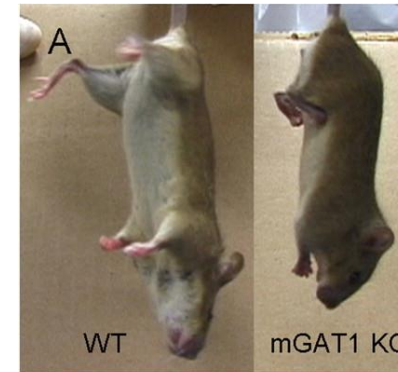
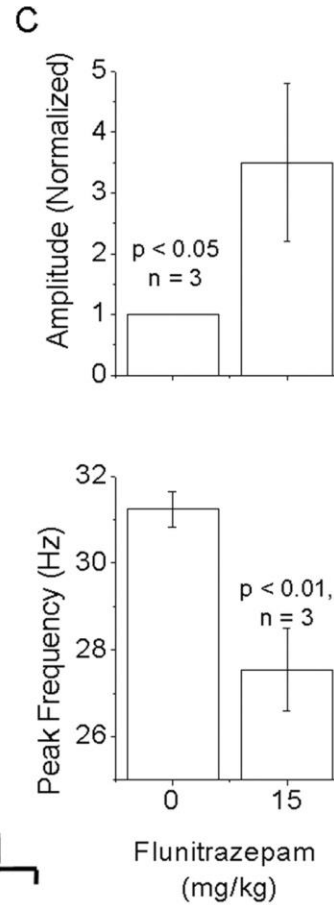
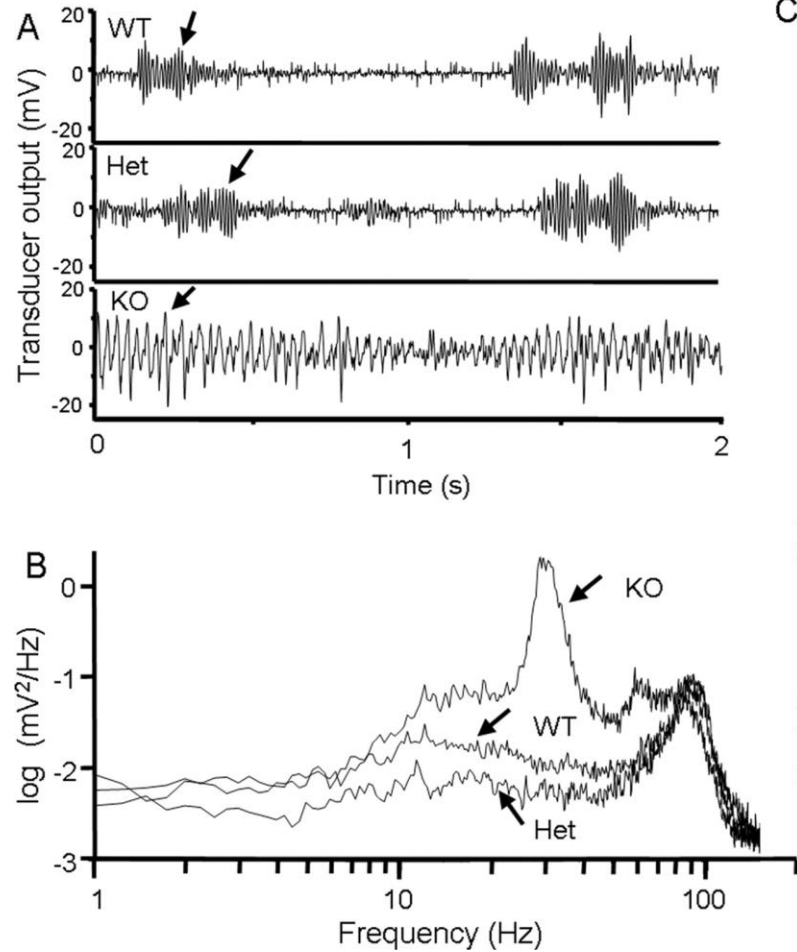
Lack of pre-synaptic GABA_B tone

Enhanced tonic and diminished phasic inhibition

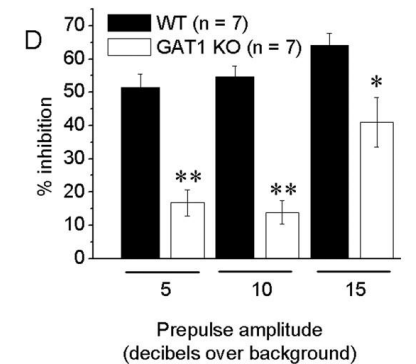
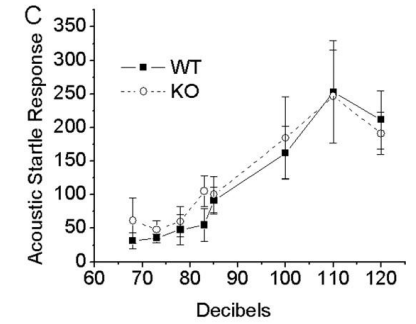
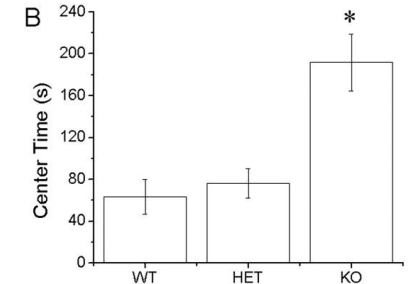
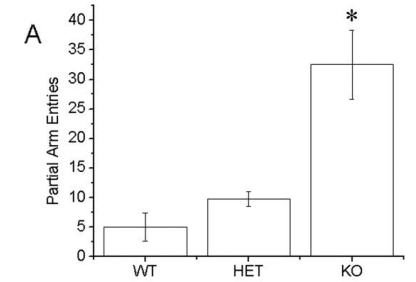
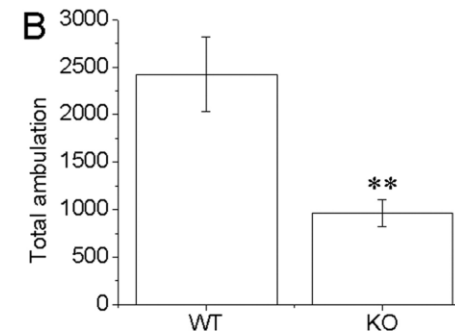
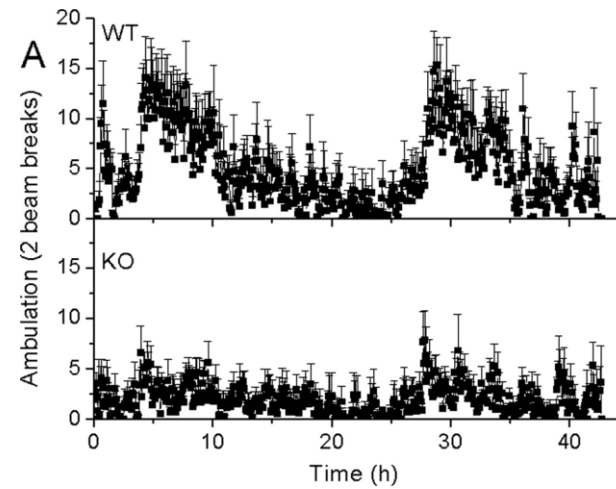
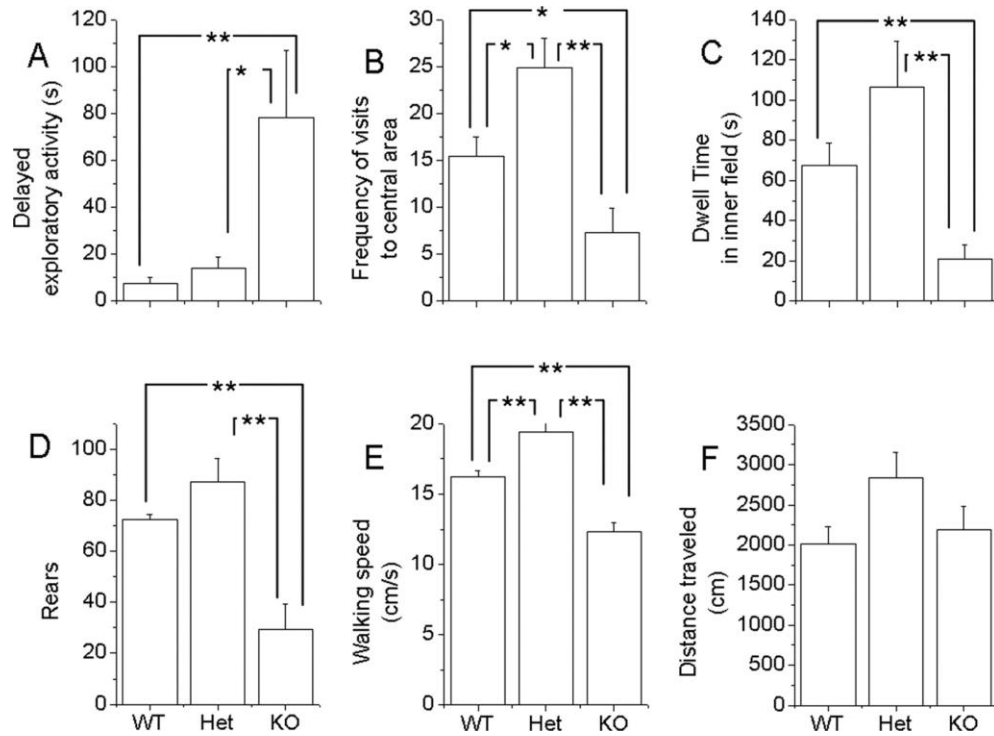
Mice present with ataxia, tremor, hindlimb claspings, and anxious behavior

Hets have mostly normal phenotype, despite having diminished GABA uptake

Tremor, Ataxia and Coordination



Activity and Anxious Behavior



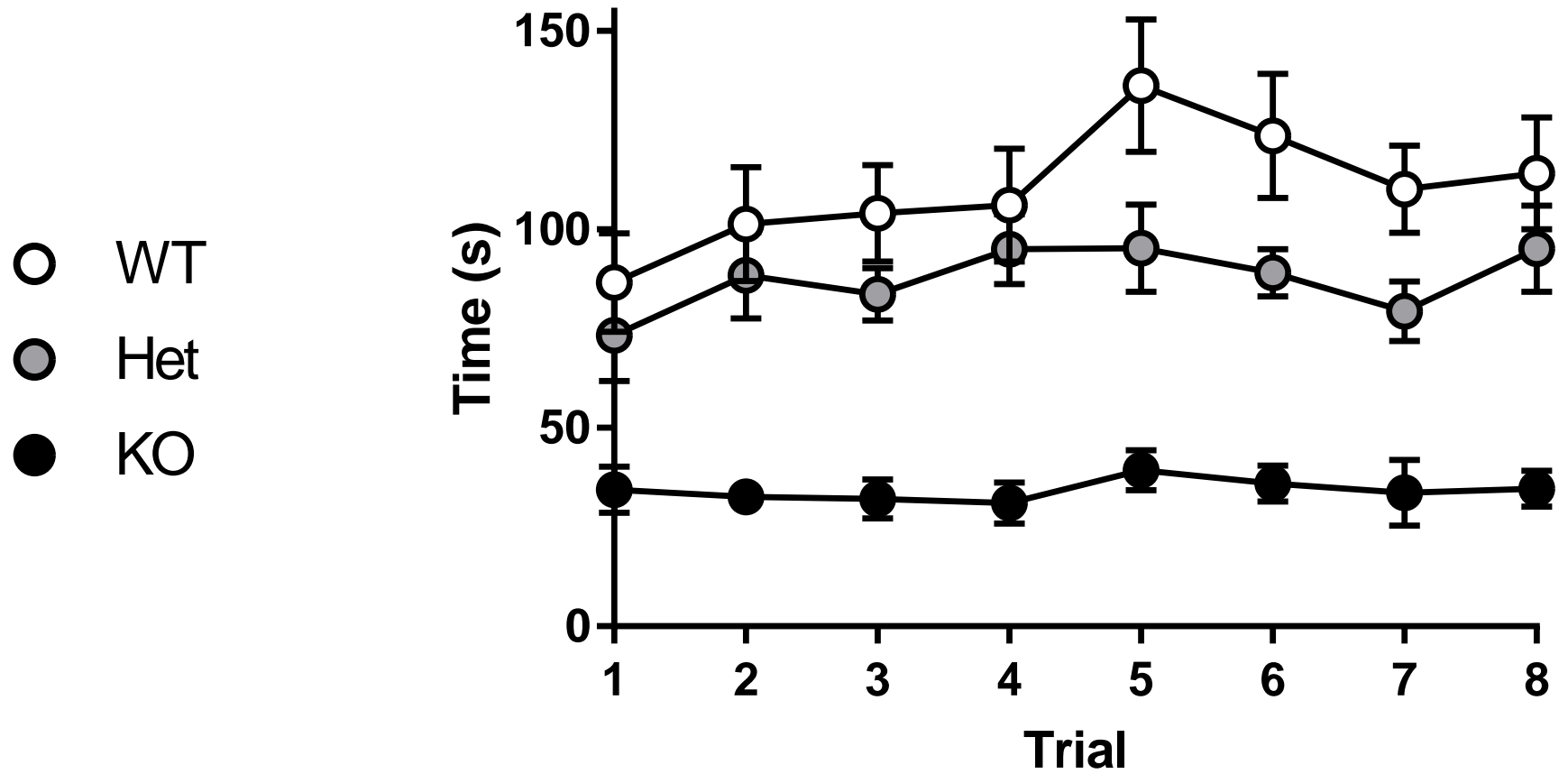
GAT1 KO Behavioral Battery

- 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

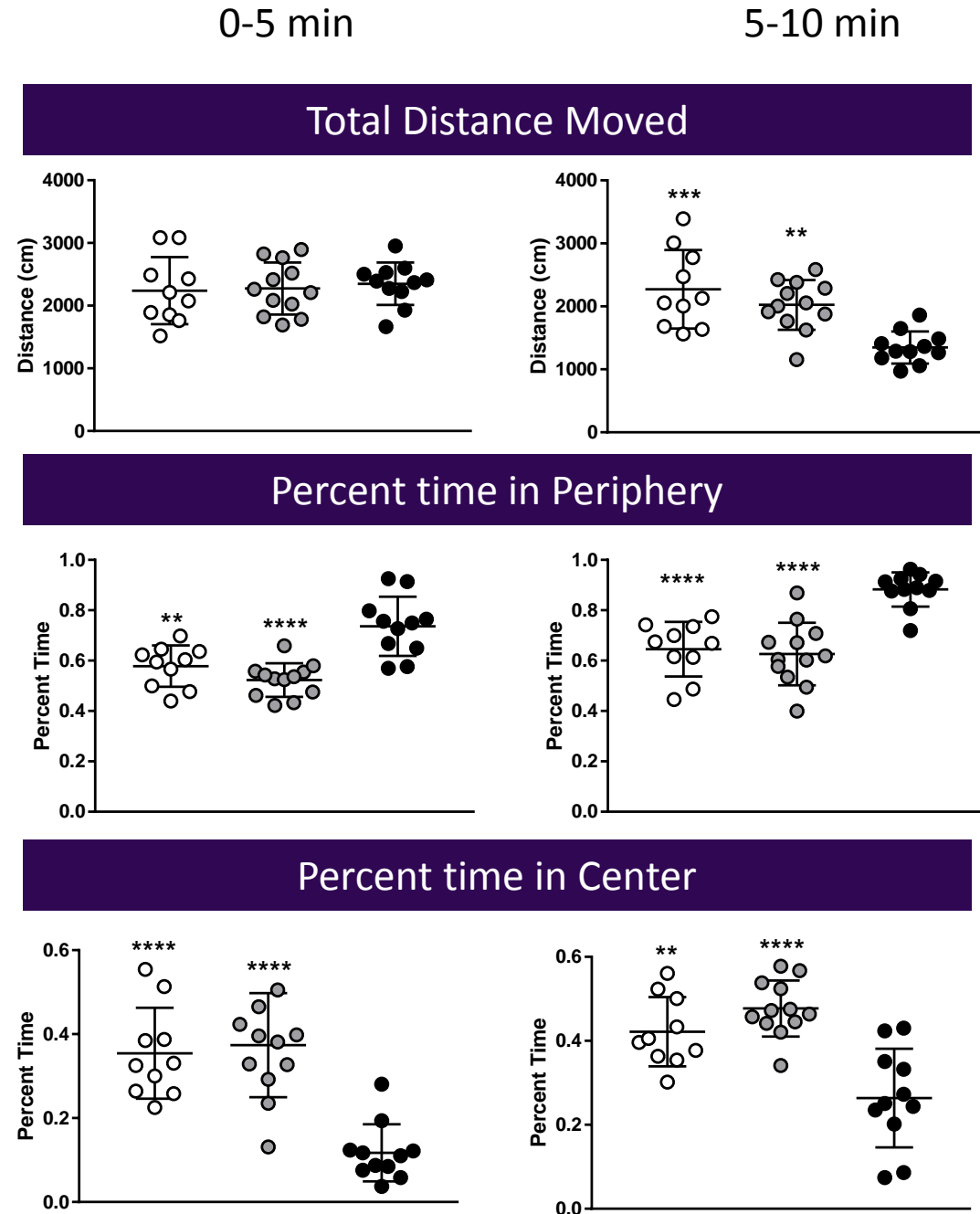
GAT1 KO mice perform poorly on rotarod



Open Field

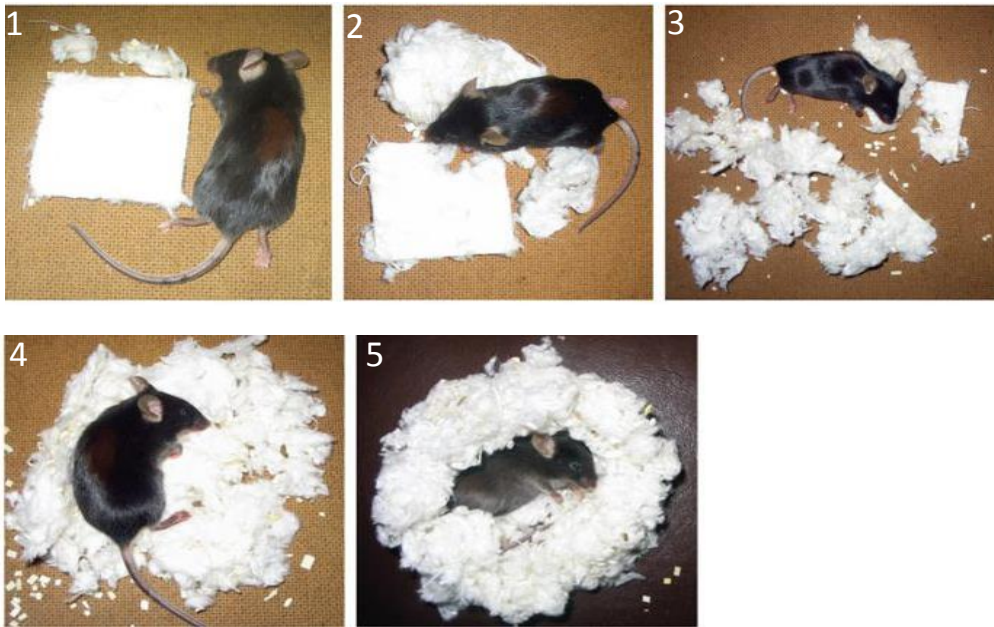
GAT-1 KO mice have decreased movement in 5-10 min interval, and increased time in periphery/ decreased time in center during both 0-5 and 5-10 min intervals

- WT
- Het
- KO



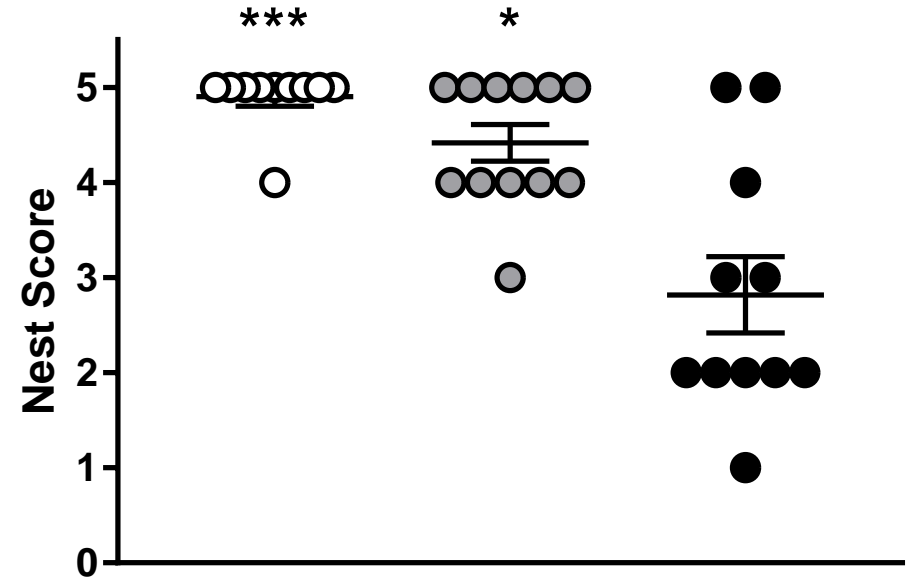
Nest Building

GAT1 KO mice are poor nest builders



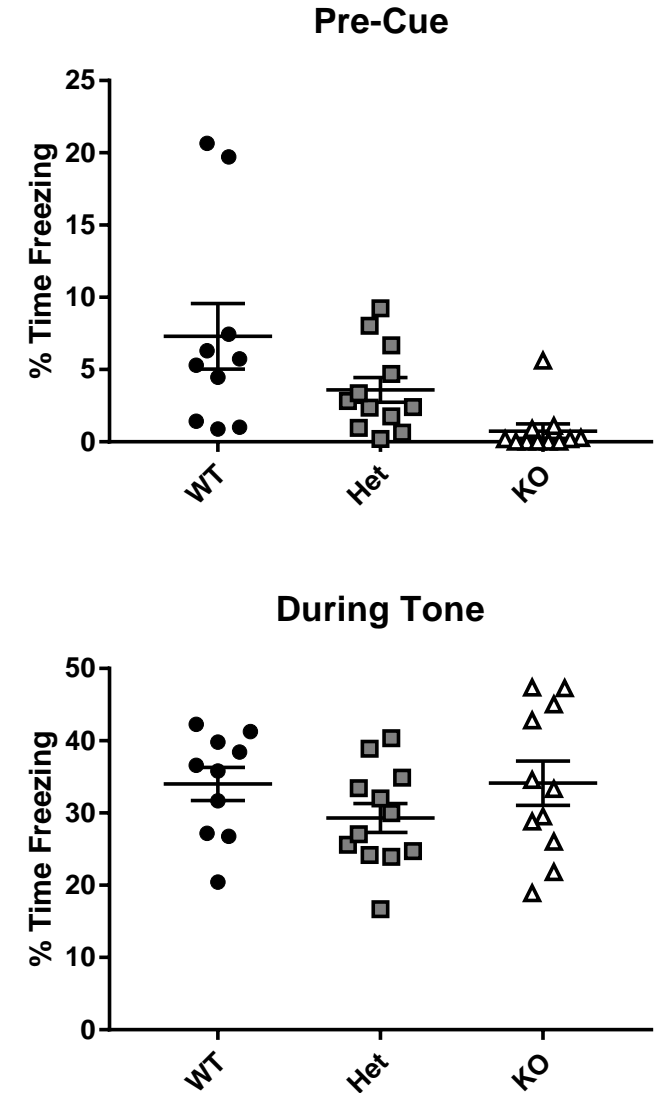
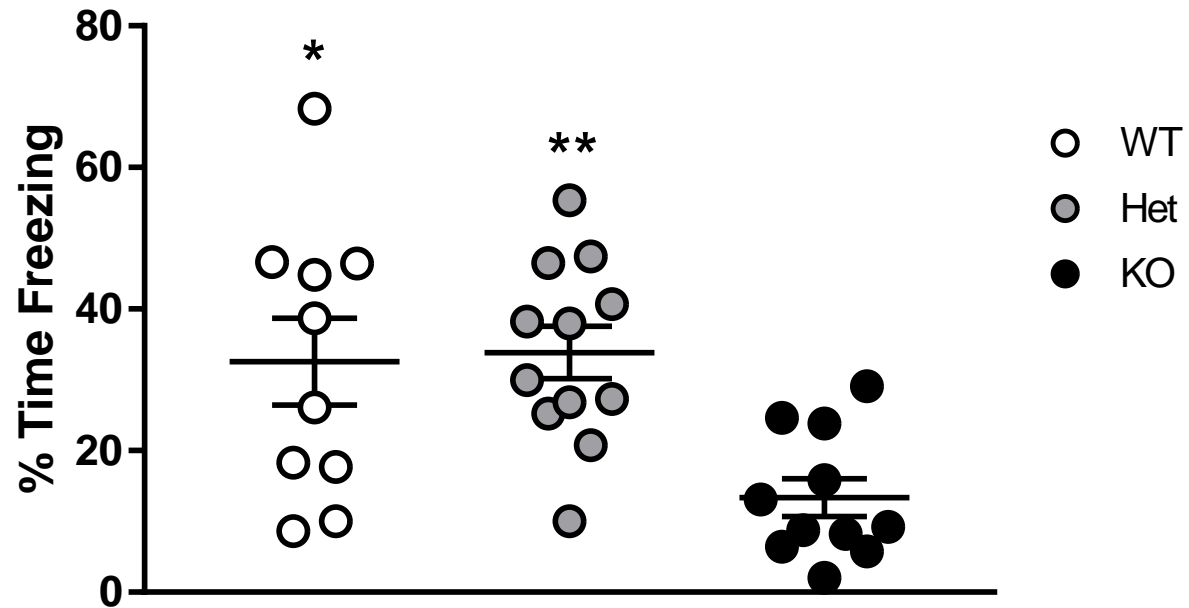
Deacon, *Nature Protocols*, 2006

- WT
- Het
- KO



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 claspings
 - 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

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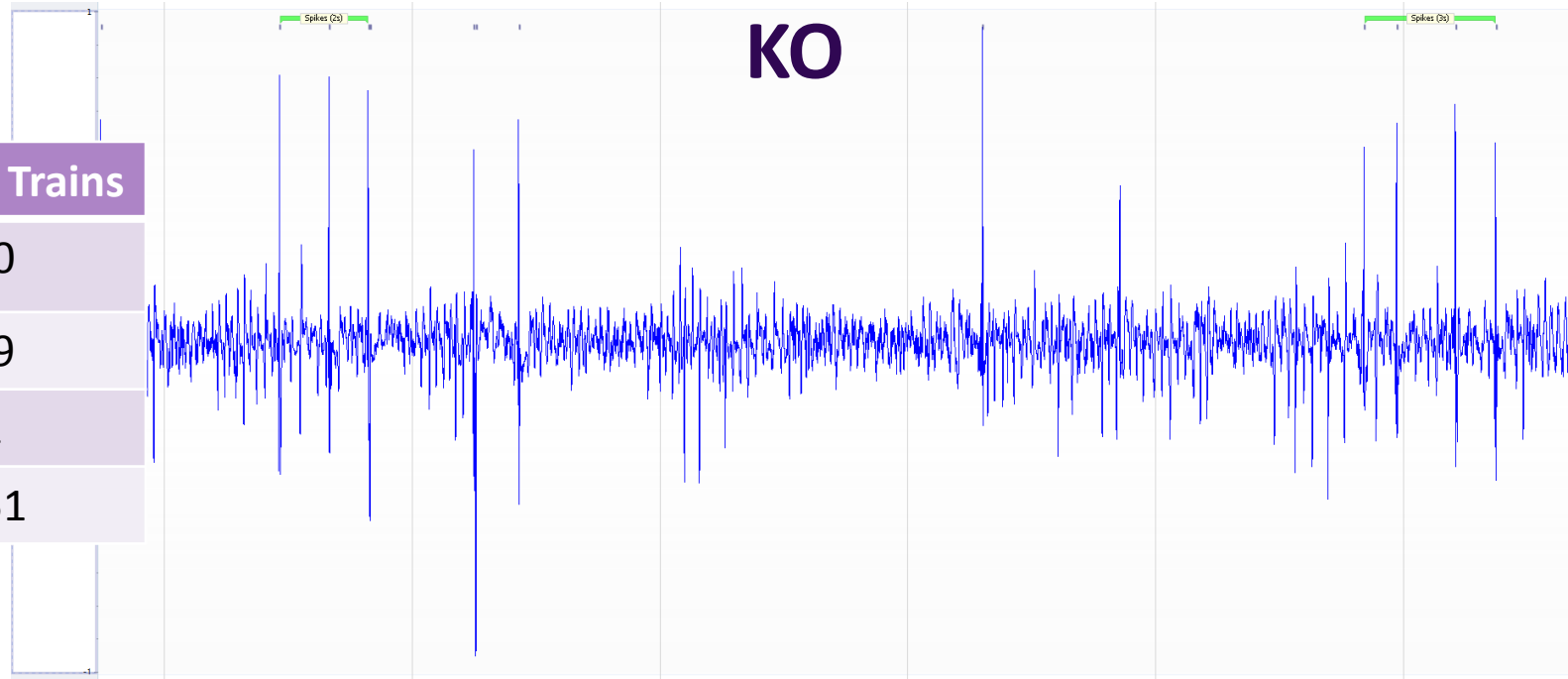
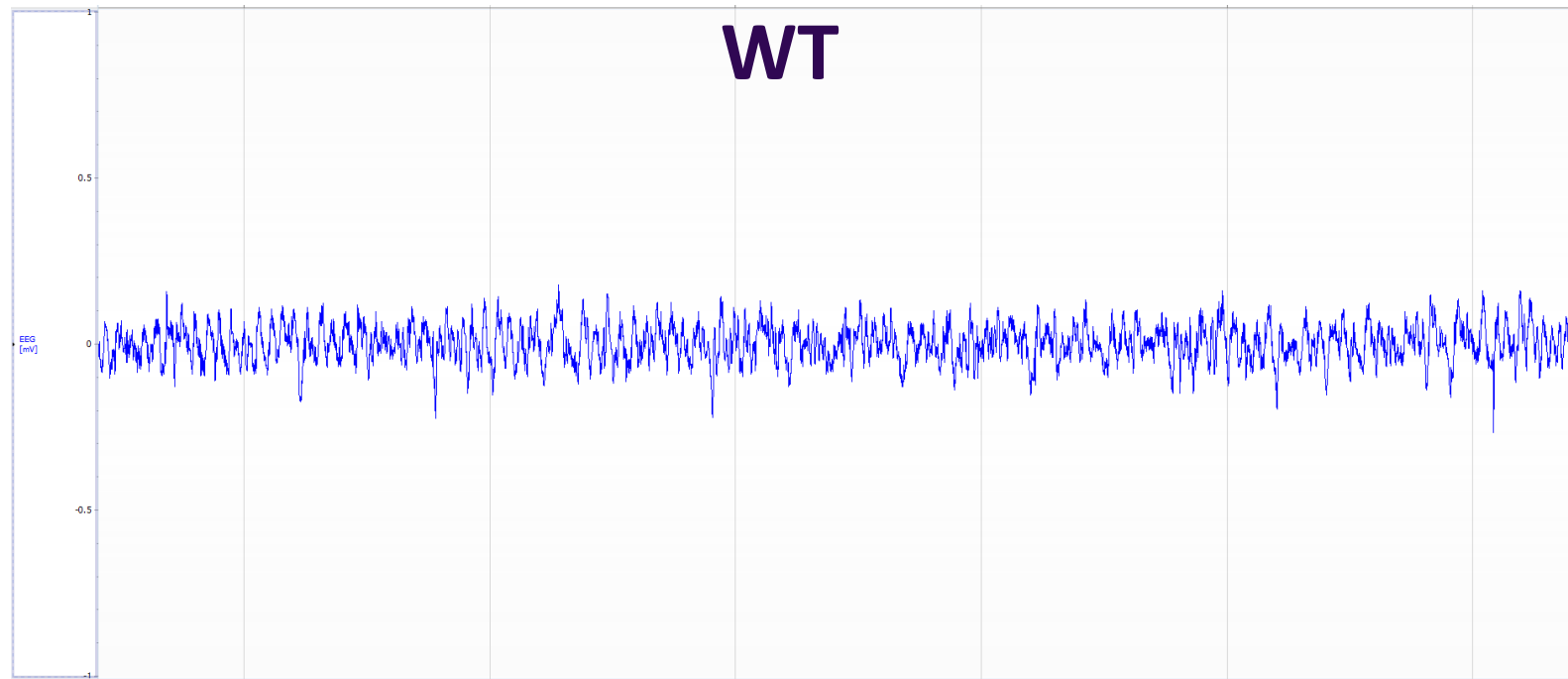
- Compared to tethered system
 - Less invasive
 - Can measure more parameters
 - Shorter total recoding time
- DSI system/ sensors for this project:
 - Record up to 16 animals simultaneously (more if alternating)
 - Sensor records EEG, EMG, activity and temperature simultaneously

EEG Recordings

24 hr recordings with automated spike detection

- Threshold ratio 5
- Max interval: 1.5s
- Min # spikes: 3

Subject	Genotype	# Spikes	# Spike Trains
1	Het	872	10
2	KO	2,788	69
3	WT	235	1
4	KO	5,103	151



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

LM: Erik Lykken, PhD

Administrator: Lacey Stearman

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



UT Southwestern
Medical Center

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





Maxwell



Arthur



Lolly



Chase



Tyson



Michael