Drosophila models of *SLC6A1*-neurodevelopmental disorder

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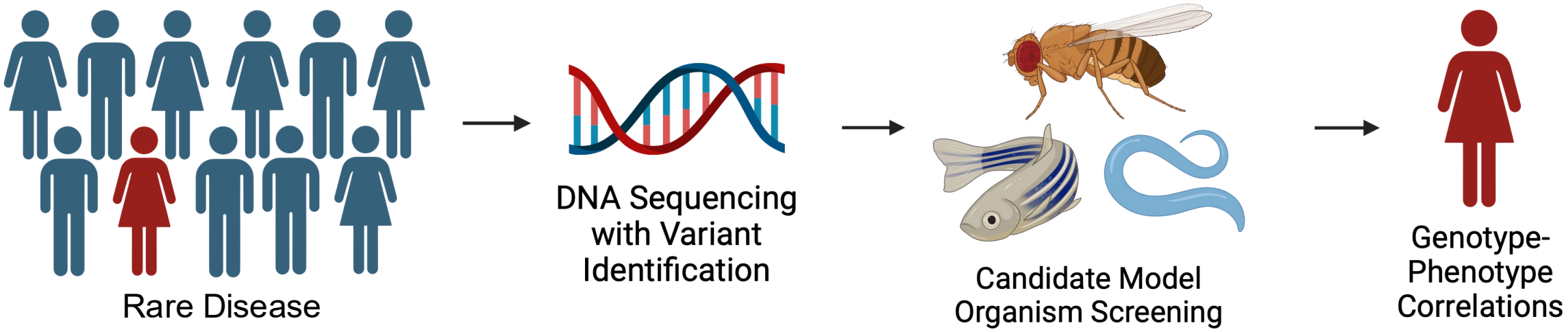
UDN's Model Organism Screening Center (MOSC) has led to several diagnoses

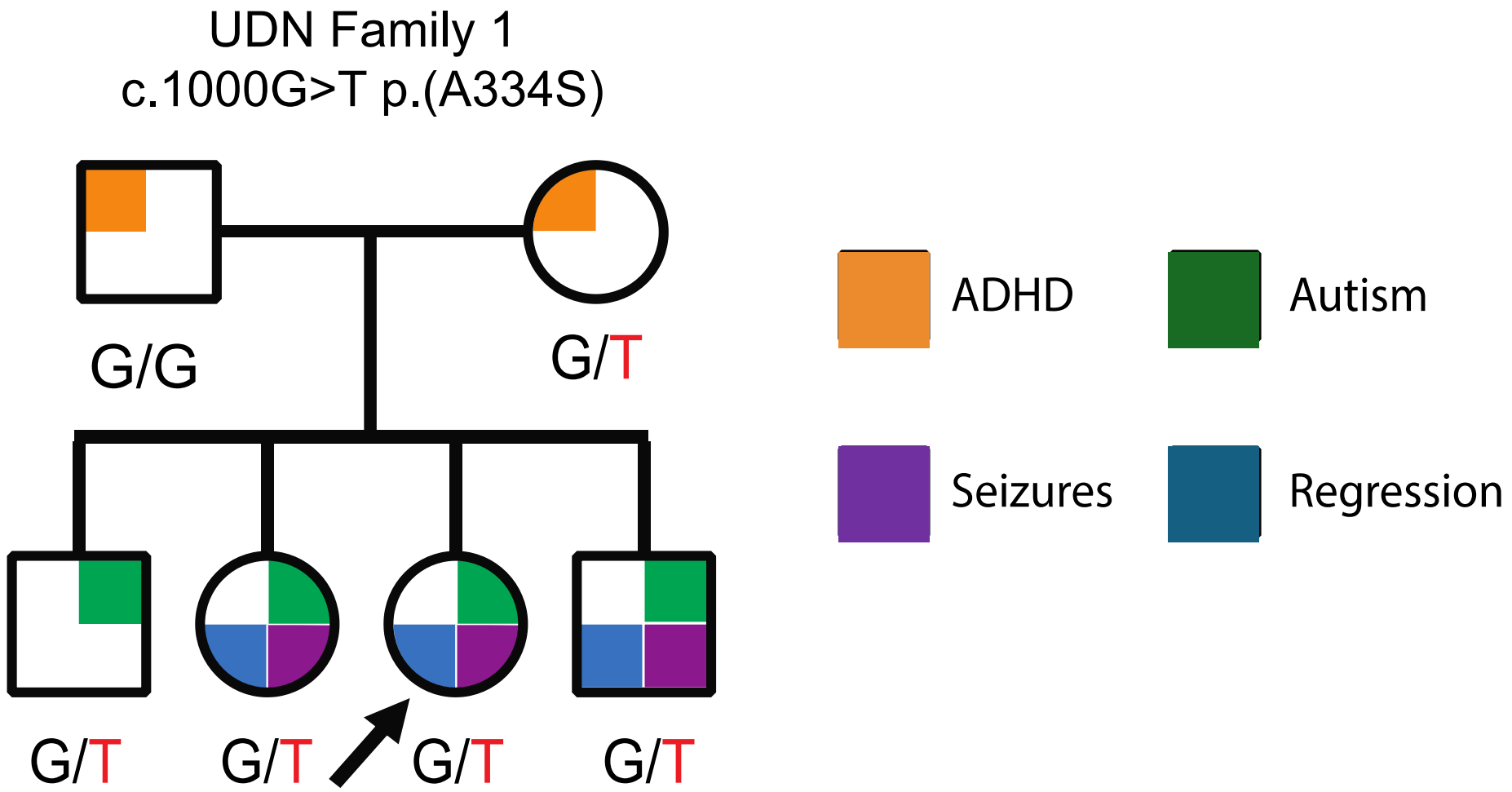


ABCA2	*BICRA*	*DMXL1*	*GPC4*	*MRTFB*	*NACC1*	*NRBP1*	*RYBP*	*TANC2*	*USP7*
ACOX1	*CLCN7*	*EBF3*	*GRB10*	*MRPS12*	*NCOA2*	*PHB*	*RBBP5*	*TBX2*	*WDR37*
ACSM5	*COL5A1*	*EZH1*	*LZTR1*	*MTA3*	*NOTCH3*	*PHACTR1*	*SCN4A*	*TBX3*	*WWP1*
APEX1	*CUL1*	*GNAO1*	*MADD*	*MYH11*	*NR5A1*	*RNF2*	*SETD5*	*TNS3*	*XRN1*

… and more!

Baldridge et al., *Orph. J. Rare Dis.*, 2021
Yamamoto et al., *Nat. Rev. Gen.*, 2023

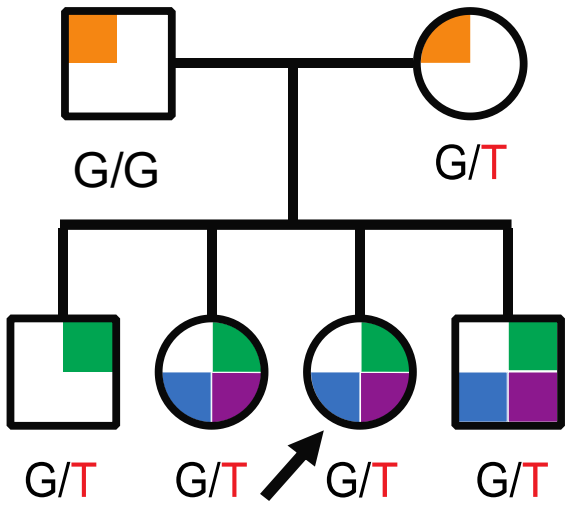
UDN identified VUS p.A334S in *SLC6A1*

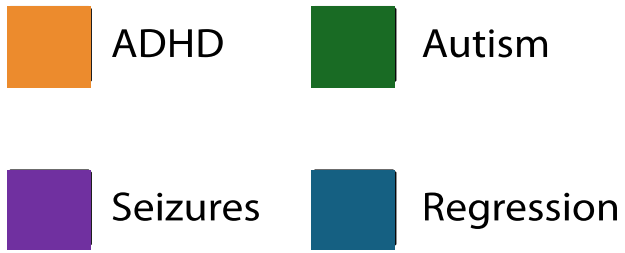


Jay, Gogate, and Hall *et al., HGG Advances,* In Press

An allelic series analysis can be used to determine pathogenicity

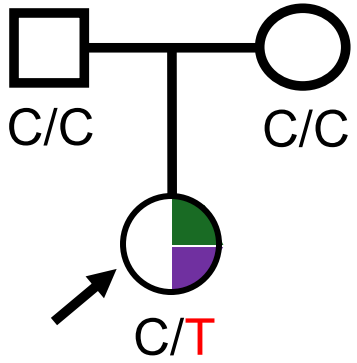
UDN Family 1
c.1000G>T p.(A334S)



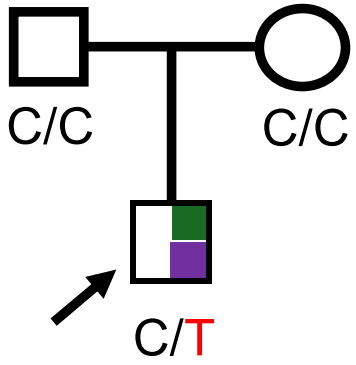


Allelic Series Analysis

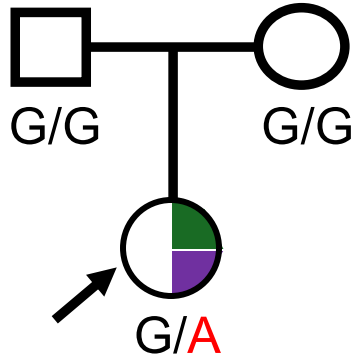
Brain Gene Registry
c.863C>T p.(A288V)



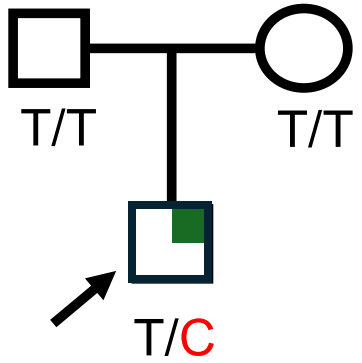
Brain Gene Registry
c.884C>T p.(S295L)



Brain Gene Registry
c.889G>A p.(G297R)



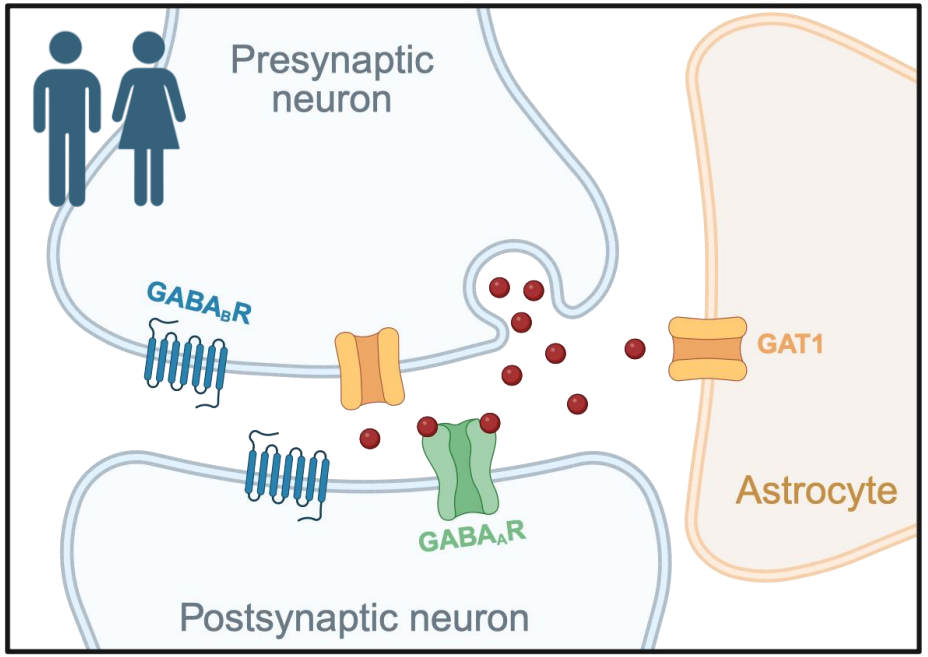
Autism-Associated
c.1017T>C p.(F339L)

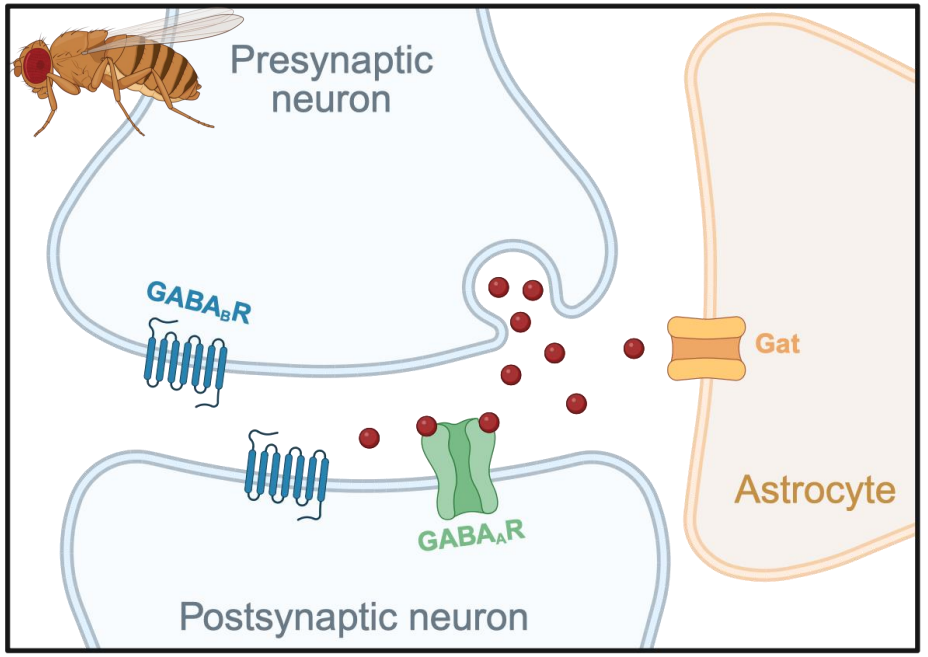


Yuen *et al.*, *Nat. Neuro.*, 2017

Drosophila (the fruit fly) are an ideal model organism to study neurodevelopmental disorders

- ~75% of human disease genes have a fly ortholog
- Rapid lifespan (10 days)
- Relatively inexpensive to create and maintain lines
- Highly conserved GABA biology

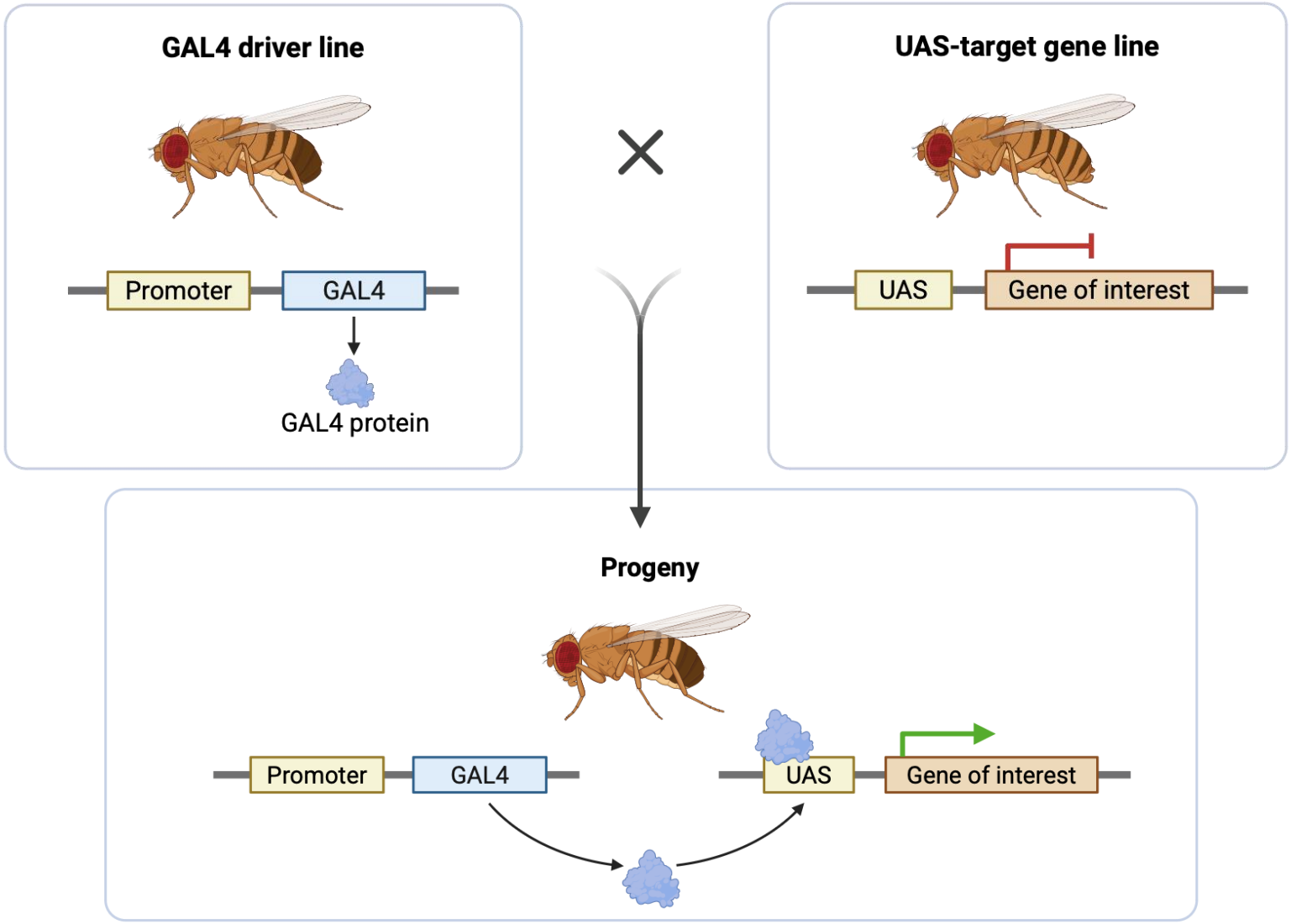




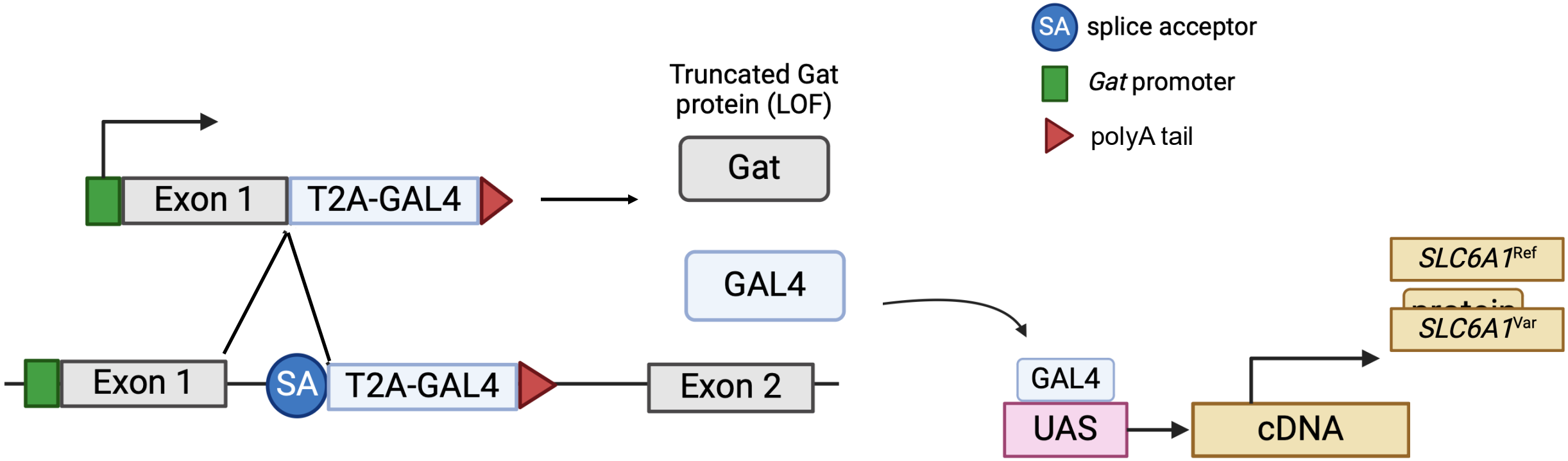
All variants of interest are highly conserved

- Both GAT1 and Gat share a 12 transmembrane domain structure

Gal4-UAS system is essential for *Drosophila* experiments

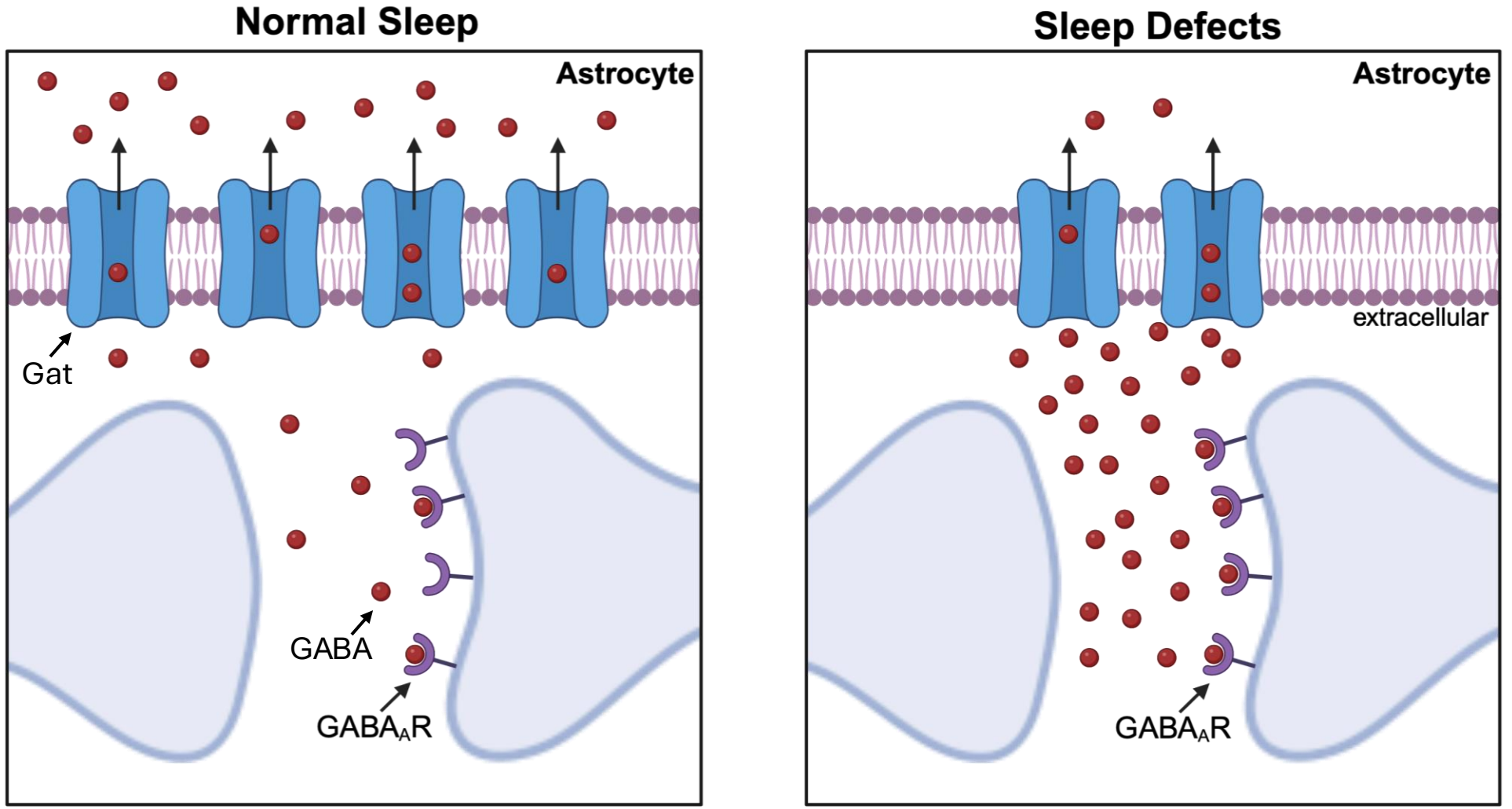


Gat^{T2A}-Gal4 can be used for a humanization approach



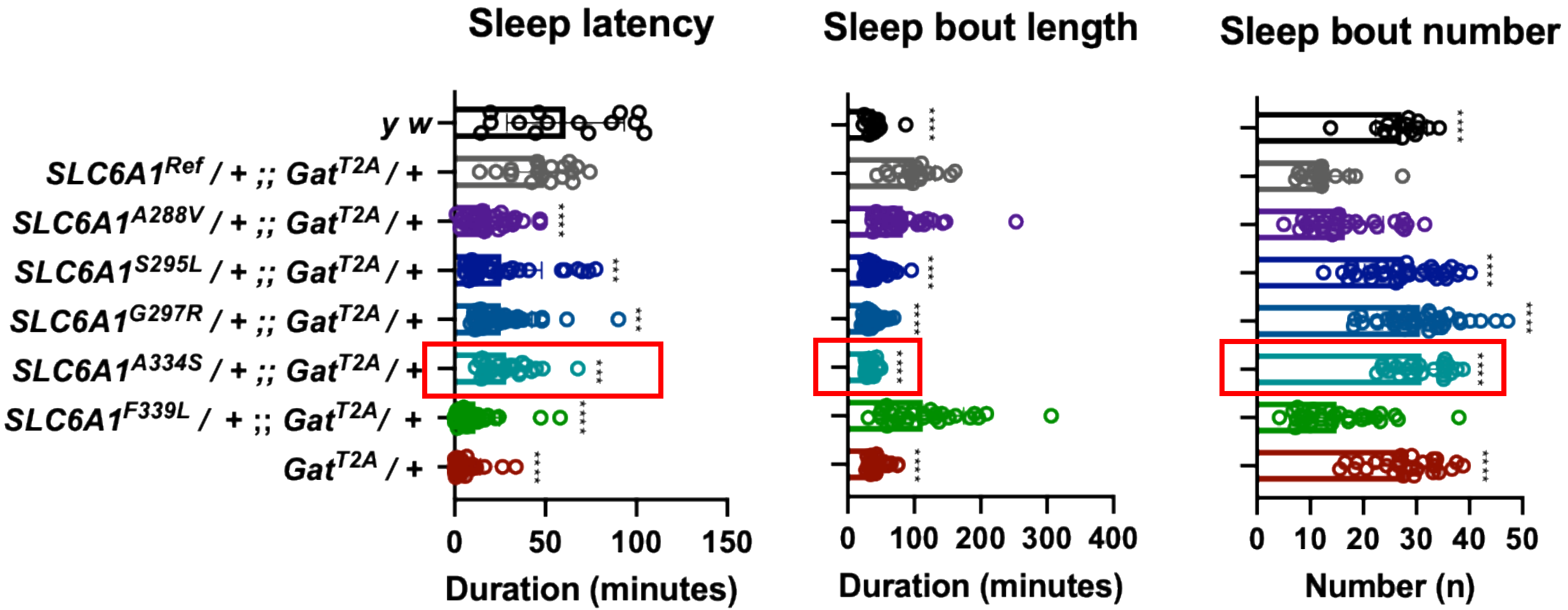
Bellen *et al. Hum Mol Genet.* 2019

Drosophila sleep is highly regulated by Gat



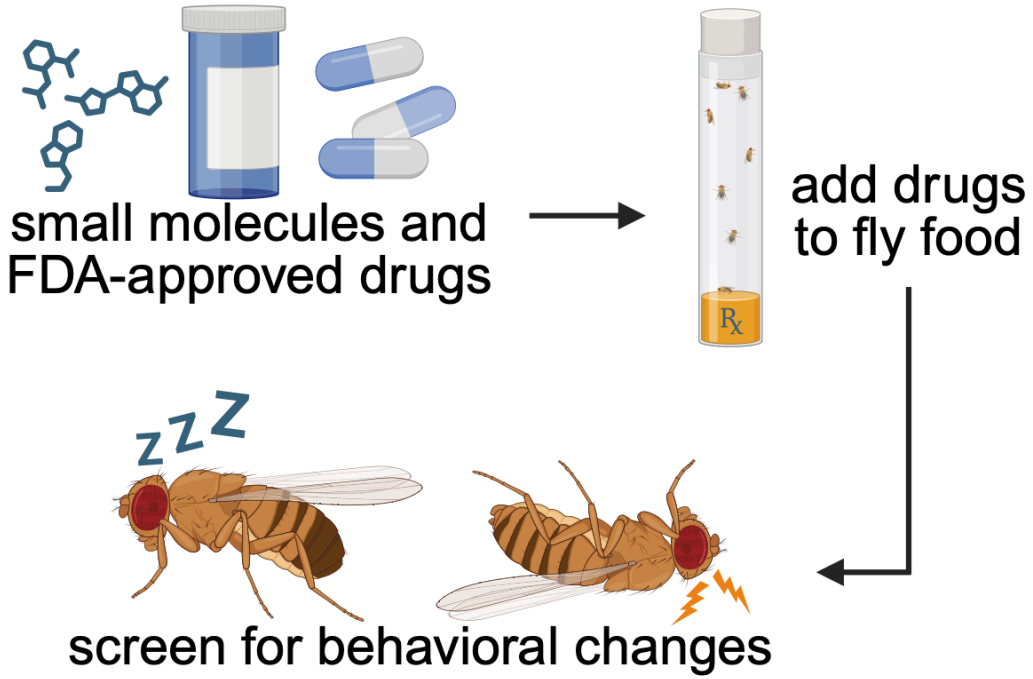
Adapted from Chaturvedi et al., *Curr. Biol.*, 2022

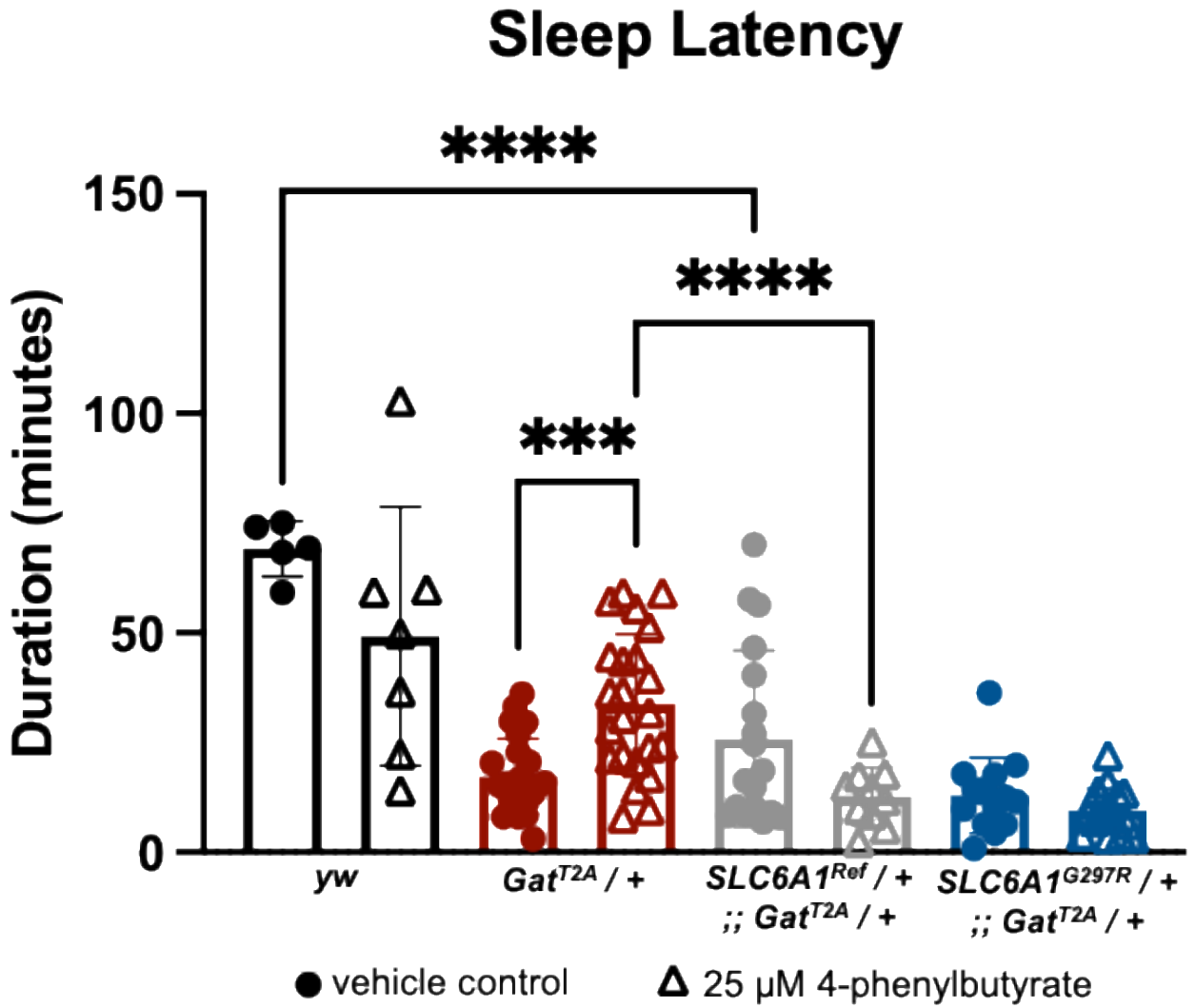
Variant-expressing flies show significant sleep defects



Brown-Forsythe and Welch ANOVA, n.s. p>0.05, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 compared to reference

4-PBA drug repurposing shows sleep improvements



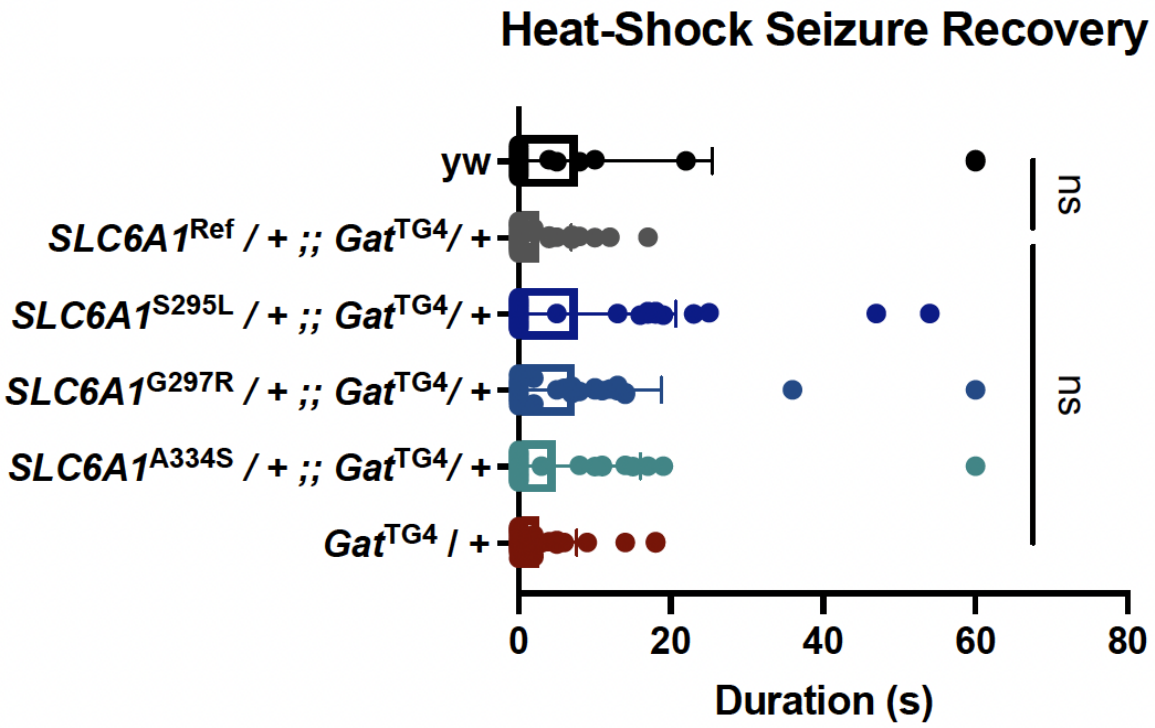


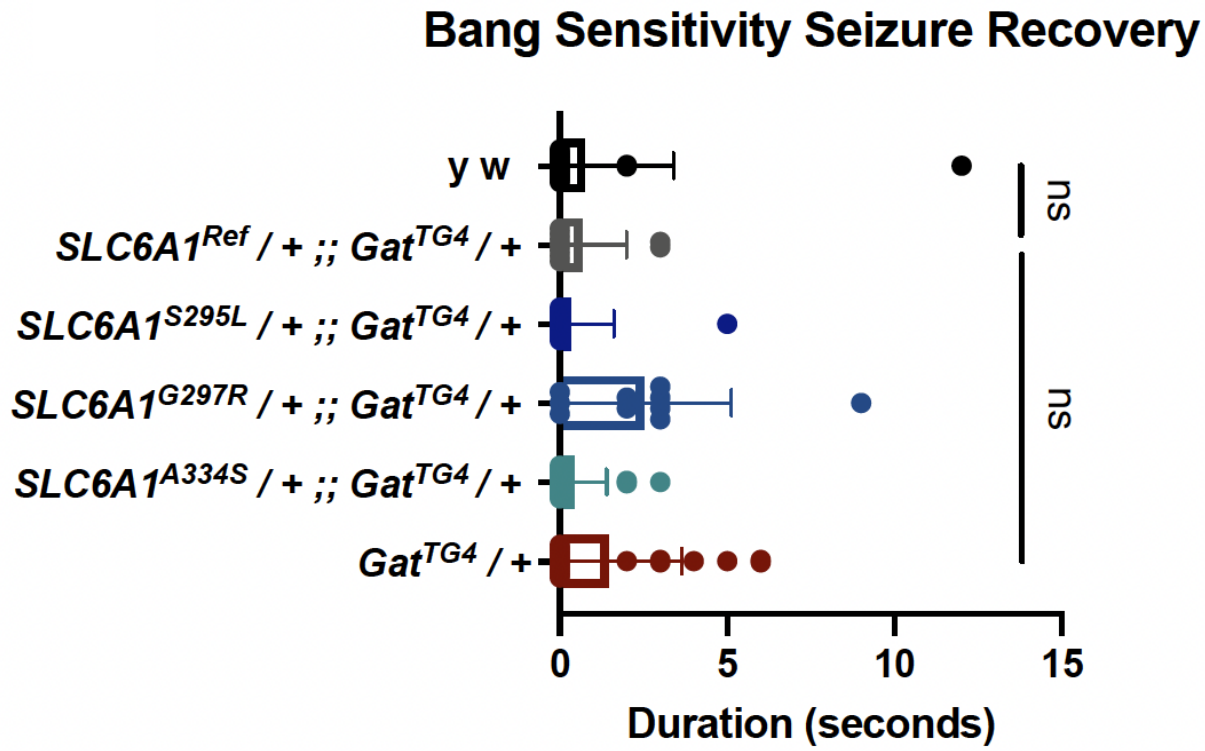
Conclusions

- A334S variant shows consistent phenotypes with known pathogenic variants
- Variant-expressing flies show significant sleep defects, suggesting the presence of excess GABA in the synapse
- Variant-specific effects are observed
  - F339L variant more closely mimics reference in sleep phenotypes
- 4-PBA has promising results for rescuing sleep defects due to *Gat* LOF

Automated Quantification of Behavioral Phenotypes Across *SLC6A1* Variants In Drosophila

Observation-based seizure assays not sensitive enough for screening

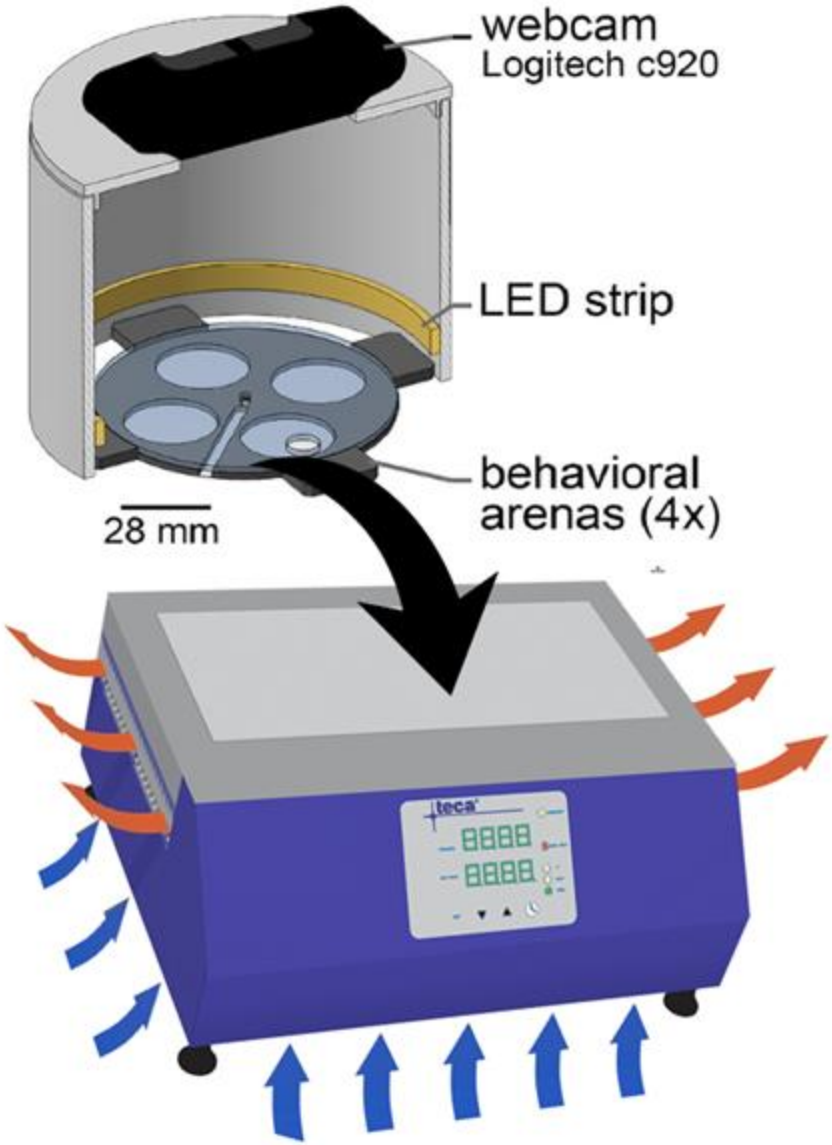




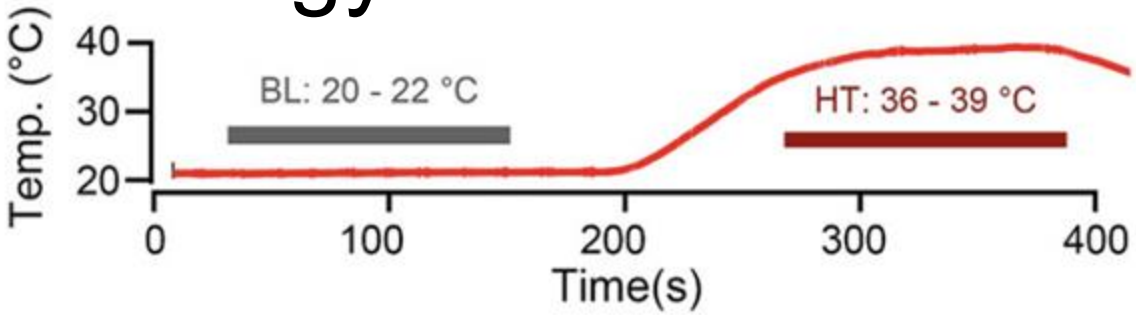
Jay *et. al*. 2025. *HGG Adv.* 2025 October: 7(1):100541 . PMID: 41174879

Automated Fly Tracking Methodology

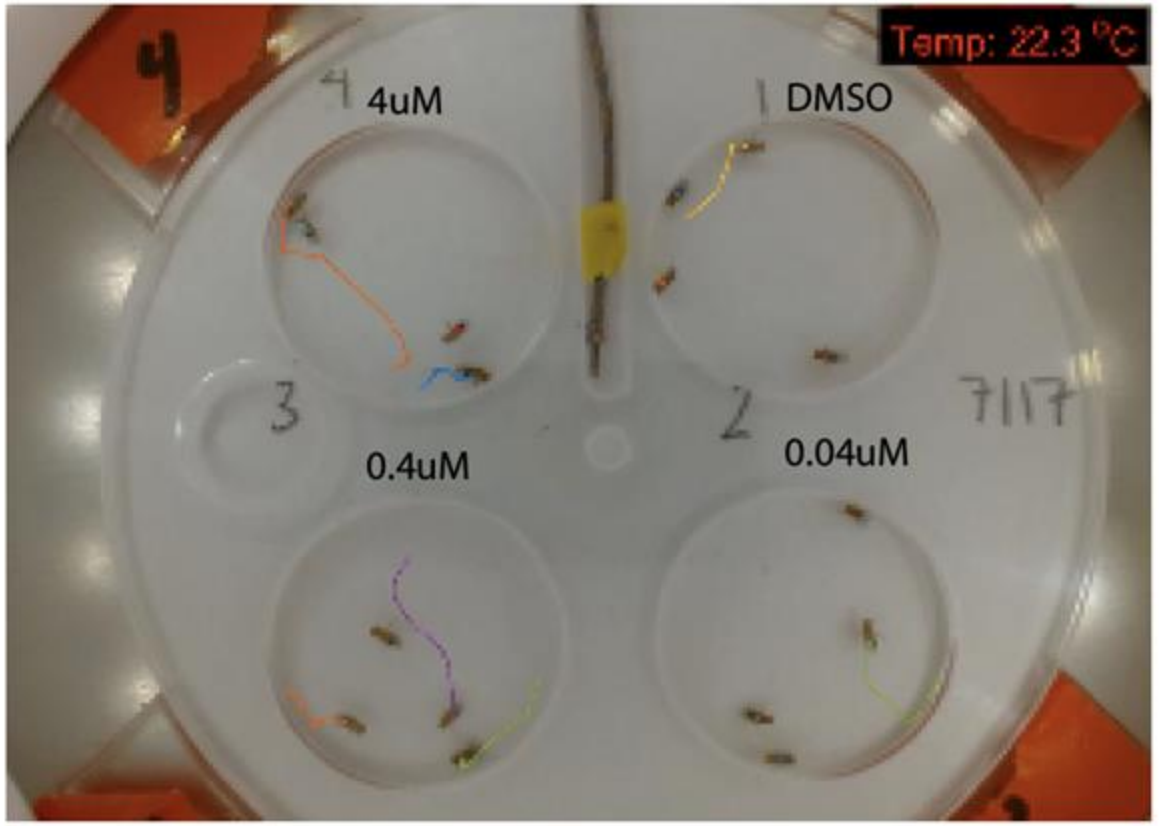
A.



B.

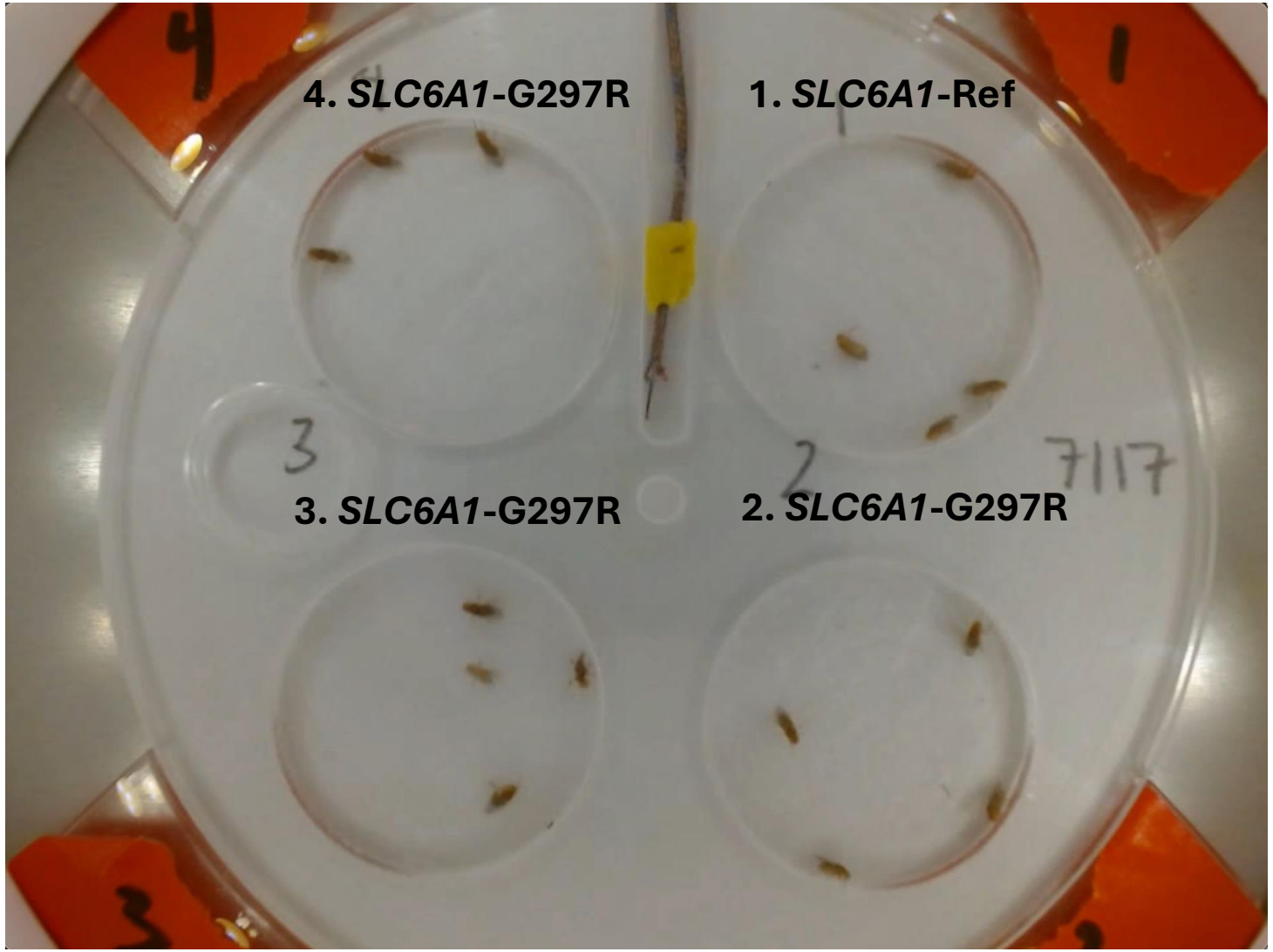


C.

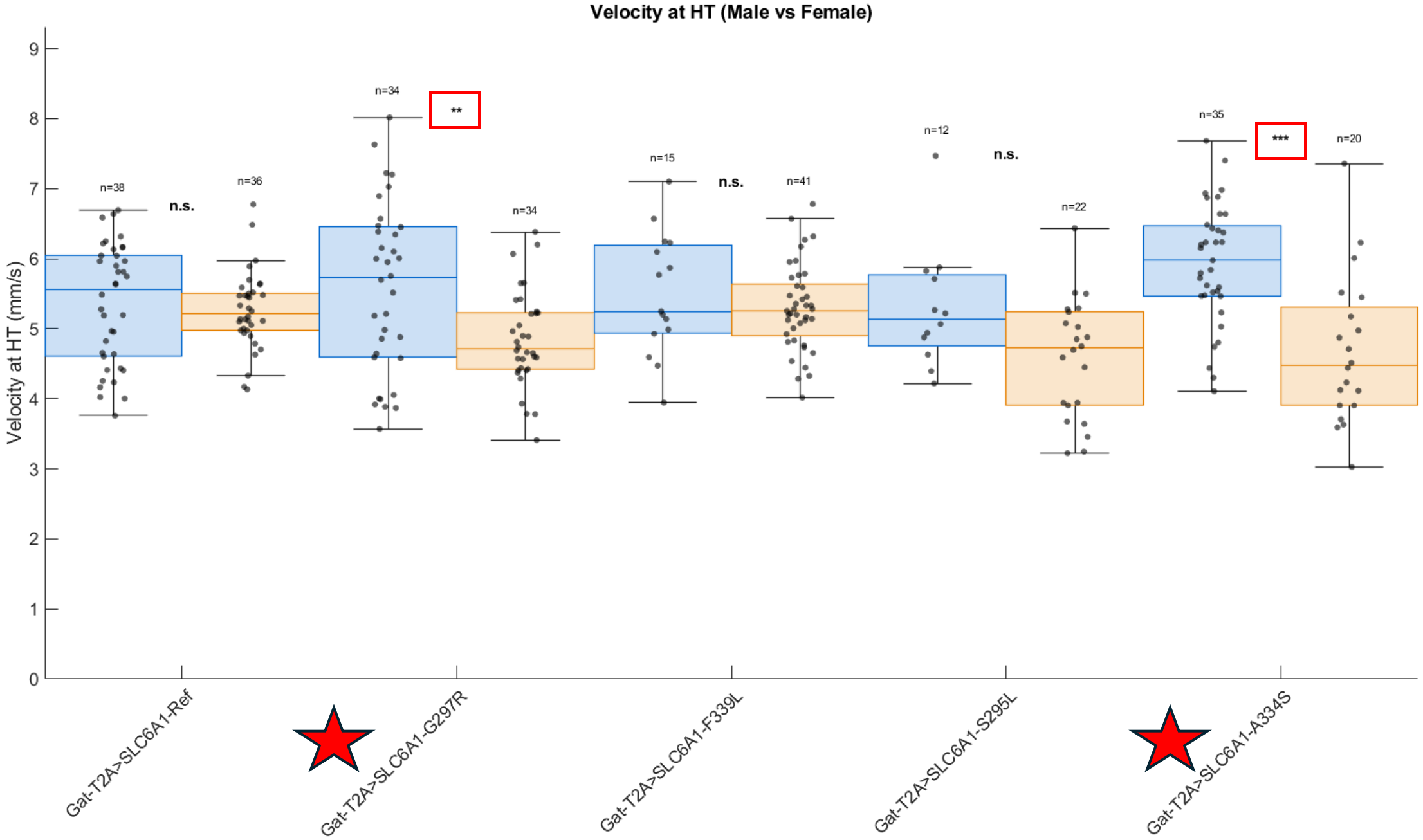


Can this optimized methodology determine *SLC6A1* variant-specific severity?

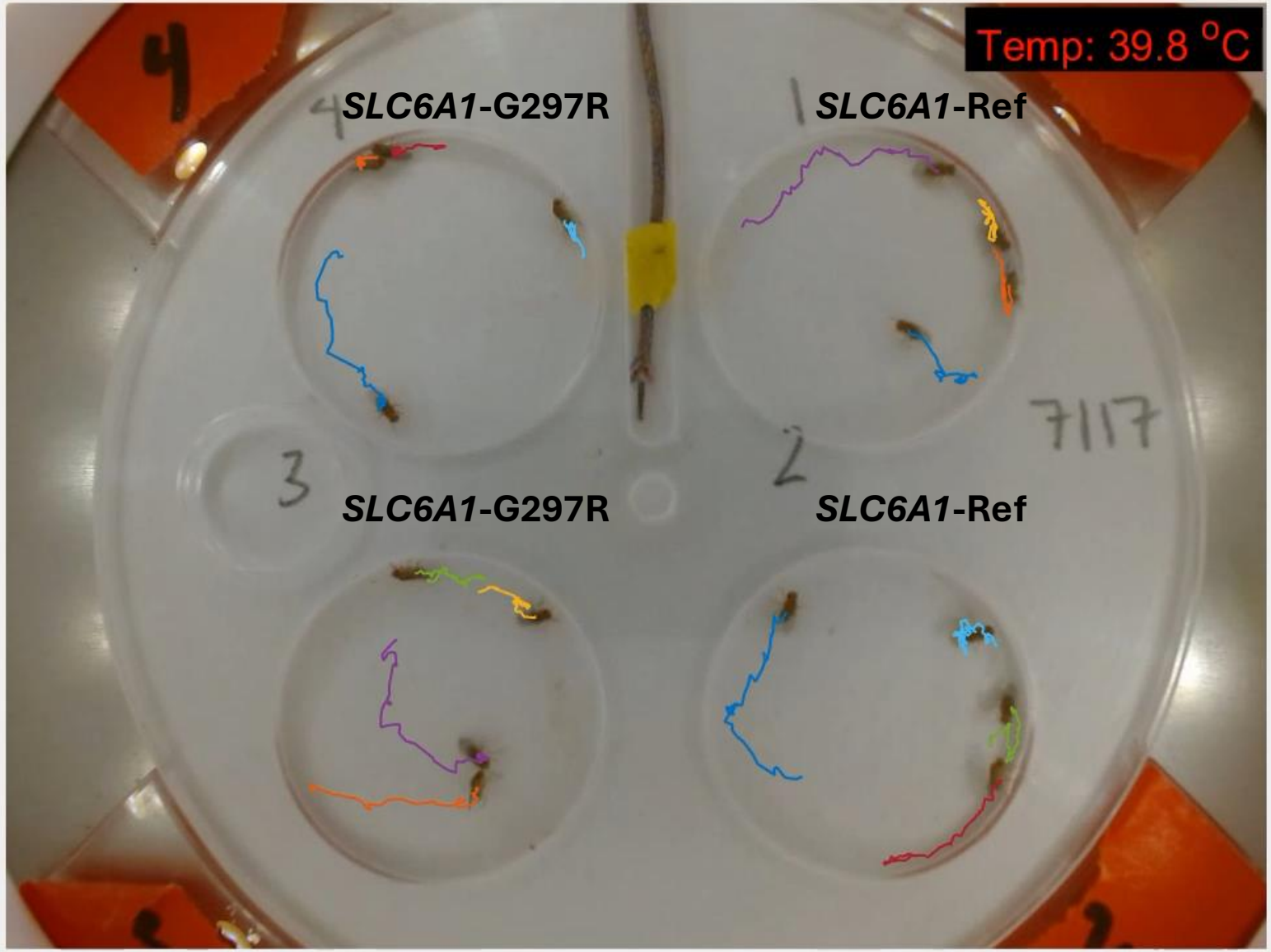
Gat-T2A-GAL4 > UAS-*SLC6A1*



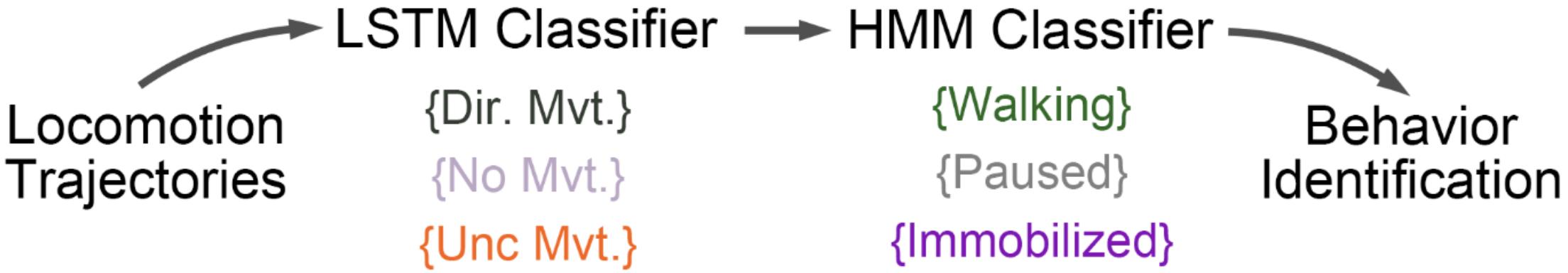
Sex-differences in velocity with *Gat*-driven flies

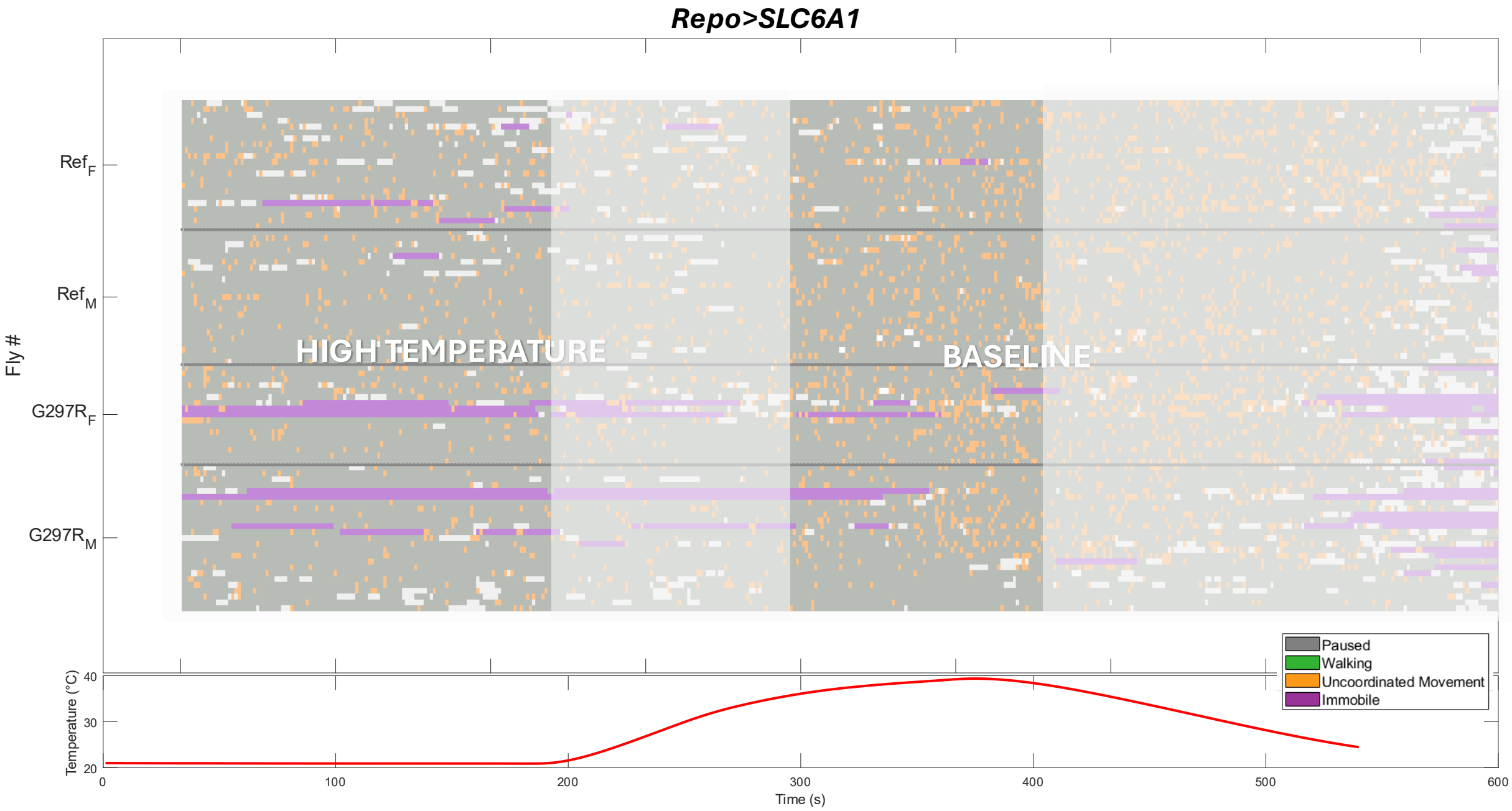


Repo-driven female flies at high temperature

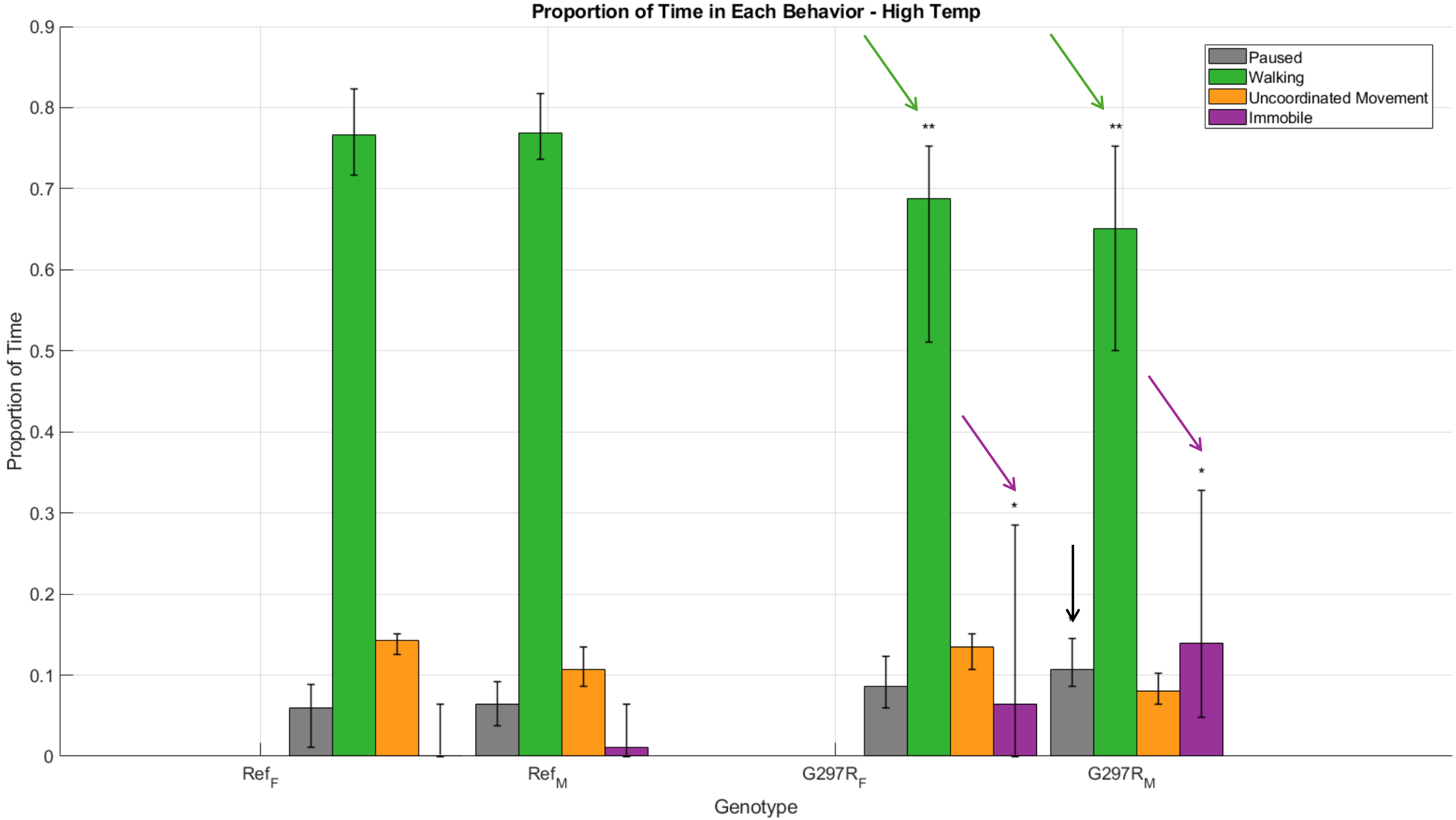


Hidden Markov Model (HMM) classifies behavior

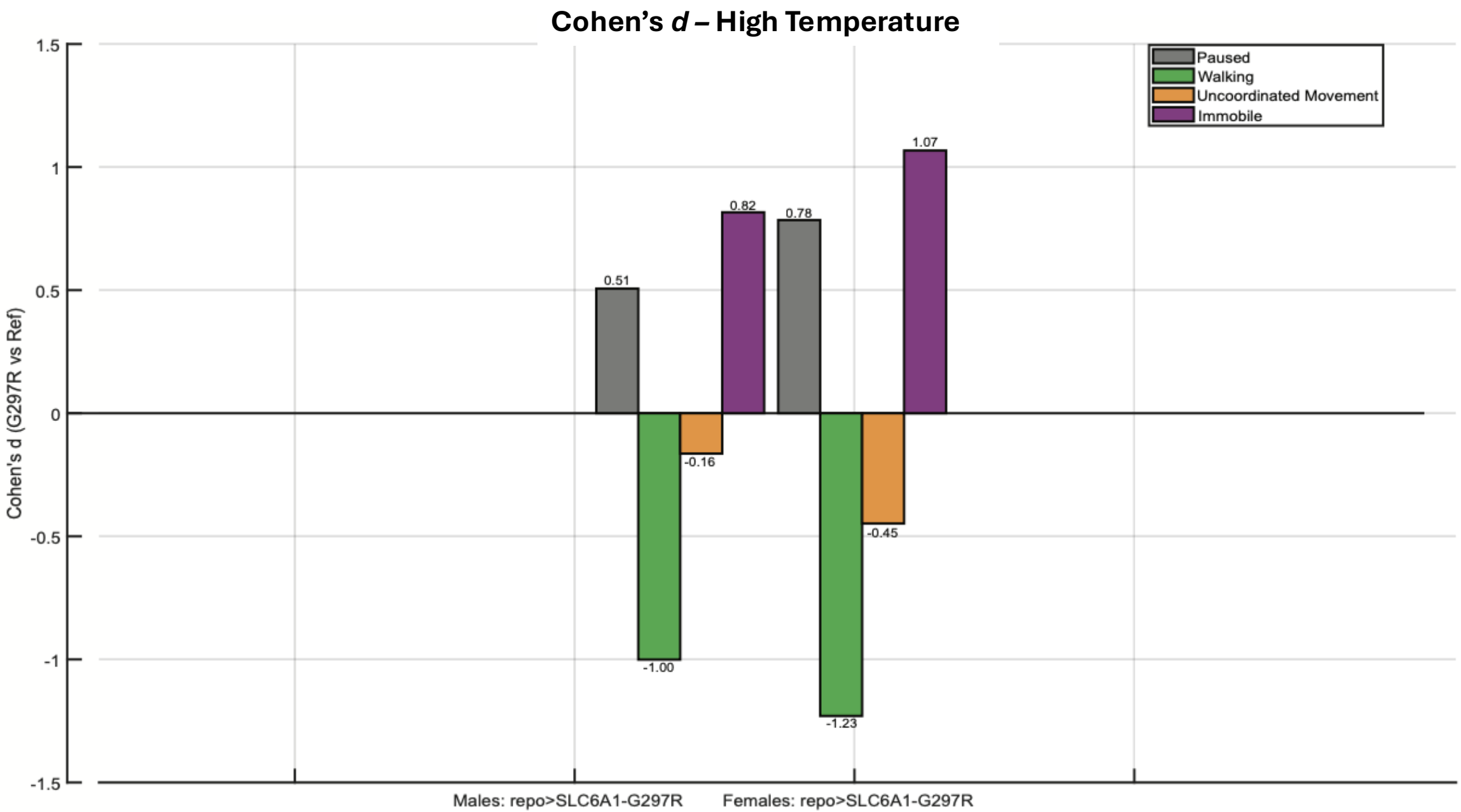



Repo>SLC6A1
Ref$_F$
Ref$_M$
G297R$_F$
G297R$_M$
Fly #
HIGH TEMPERATURE
BASELINE
Paused
Walking
Uncoordinated Movement
Immobile
Temperature (°C)
40
30
20
0
100
200
300
400
500
600
Time (s)

Significant increase in seizure-associated behaviors in *repo>SLC6A1*-G297R compared to *repo>SLC6A1*-Ref



Large effect sizes in both sexes as measured by Cohen's *d*



Future Directions

-Continue increasing sample sizes in primary experiment

-Begin drug screening experiment to see if phenotypes can be rescued

Drug	Mechanism of Action
Bicuculline	$GABA_A$ receptor antagonist
Allylglycine	GAD inhibitor
4-Phenylbutyric Acid (4-PBA)	pharmacochaperone
CGP 55845	$GABA_B$ receptor antagonist
Baclofen	$GABA_B$ receptor agonist
Tiagabine	GAT1 inhibitor
Valproic Acid	acts on GABA levels in the brain, blocks voltage gated ion channels and also acts as HDAC inhibitor

Acknowledgements

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Undiagnosed Diseases Network

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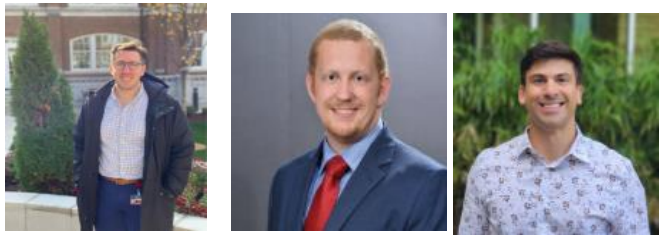
Baylor College of Medicine BRASS Formula For a Cure Research and Funding The Formula For a Cure

UPLIFTING ATHLETES

Read the Wangler Lab paper here!

Project contributors: L-R: Julie Cohen, Saul Landaverde, Dr. Lawrence Reiter, Dr. Atulya Iyengar



The Reiter Lab!: L-R: Ben Geier, Tayler Hedgecock, Dr. Tyler Rodriguez

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