Epigenetic Mediated Neuroprotection by Tip60 in a Drosophila Model of ALS

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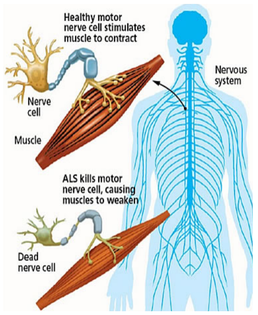
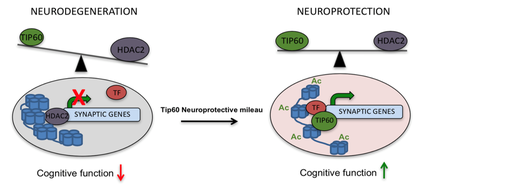
Introduction



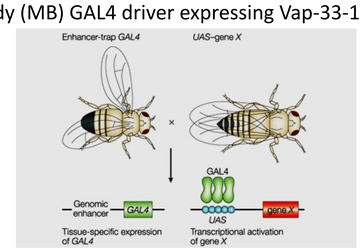
Histone acetylation is a type of epigenetic modification that is regulated by histone acetyltransferases (HAT) and histone deacetylases (HDAC). Disruption of HAT/HDAC balance in the brain contributes to synaptic and cognitive deficits found in Alzheimer's disease (AD). Although HDAC inhibitors are used as therapeutic targets, targeting HAT activity remains to be explored. Our lab has demonstrated that HAT Tip60 is implicated in AD and plays critical roles in cognition, sleep-wake cycle, synaptic plasticity, and axonal transport. Increasing Tip60 levels in AD model rescued cognitive impairments and our recent focus on other neurodegenerative diseases show that it also protects against cognitive deficits in PD and HD models.

ALS is characterized by motor neuron degeneration in the CNS, leading to paralysis, locomotive, and cognitive deficits. The ALS model used overexpresses Vap-33-1, a vesicle-associated membrane protein B (VAPB) protein homolog that regulates NMJ growth, protein trafficking, and ER homeostasis. This study an insight on Tip60 imbalance as a general early feature in ALS and its neuroprotective role in protecting against ALS associated deficits.



Methods

The GAL4/UAS system was used to target the expression of transgene. 201Y is a mushroom body (MB) GAL4 driver expressing Vap-33-1 in MB of Drosophila.



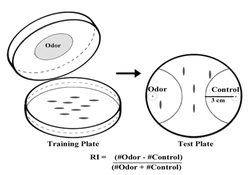
201Y	x	w^{1118}
201Y	x	Vap-33-1
201Y;Tip60	x	Vap-33-1

Larva Motor Function Assay and Speed Test

Third instar larvae were placed on a petri dish containing 2.5% agarose and allowed to equilibrate for 5min. In the line crossing assay, the number of lines crossed by the head of a larva in 30sec on a grid 0.4 cm × 0.4 cm was recorded. Tracker software was used to calculate average velocity (mm/s).

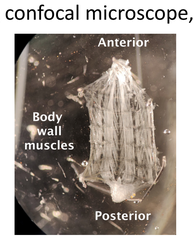
Learning and Memory Assay

Olfactory and gustatory reflexes were measured using same test plate for three and five minutes, respectively. Third instar larvae were trained for 30min on 2.5% agar plates coated with 1M SUC or DW with odorant (LIN) spotted on the lid. After 30min of training, 50-100 larvae were transferred to the test plate. After 3min, the number of larvae that moved in the semi-circular area was counted to determine the responsive index (RI).



Imaging and Analysis

Larval NMJs located between muscles 6/7 in the segment A3 were imaged using Olympus FV1000 Fluoview laser scanning confocal microscope, sectioned as a Z-stack, and analyzed using Image J software.



Results

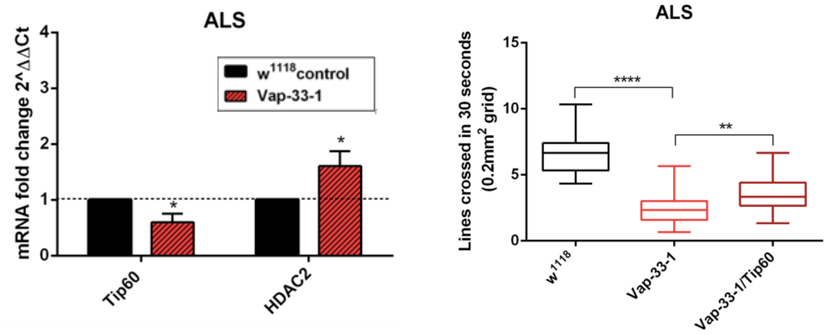


Figure 1: (A) Early disruption of Tip60 HAT/HDAC2 balance. (B) Tip60 partially restores locomotor defects in ALS determined by larva motor function assay. *p<0.05, **p<0.01, ***p<0.001. Error bar represent SEM.

Table 1: Olfactory & Gustatory Reflexes and Speed in ALS. Speed is impaired while olfactory and gustatory reflexes are not impaired.

Genotype	Speed (mm/s)	Olfactory Response	Gustatory Response
w^{1118} x 201Y	0.84 ± 0.06	0.51 ± 0.08	0.67 ± 0.17
Vap-33-1 x 201Y	0.48 ± 0.05	0.43 ± 0.09	0.60 ± 0.03
Vap-33-1 x 201Y;Tip60	0.61 ± 0.04	0.38 ± 0.12	0.53 ± 0.09

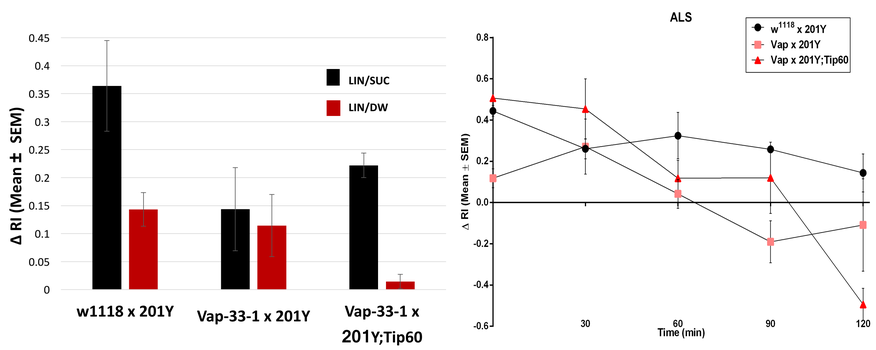
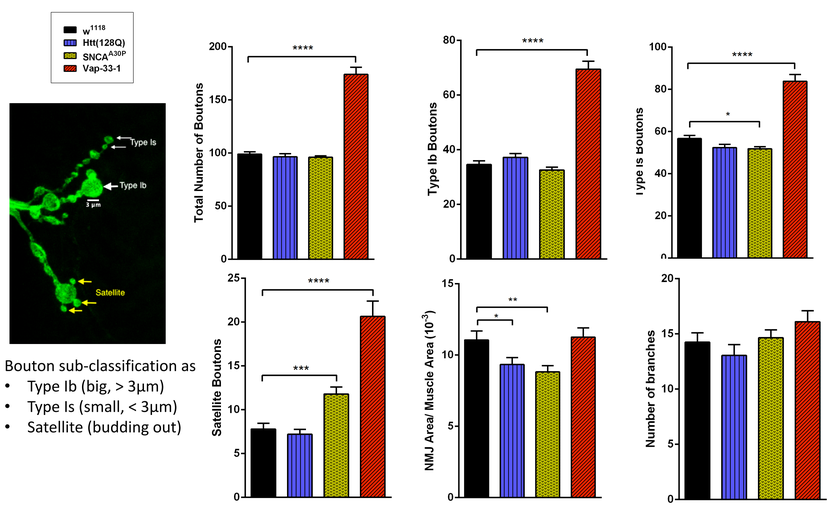


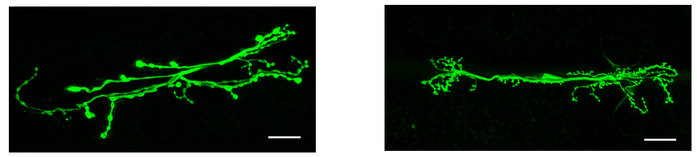
Figure 2: (C) Associative Learning with LIN. (D) Memory Retention. *p<0.05, ****p<0.0001. Error bar represent SEM.



Bouton sub-classification as
- Type Ib (big, > 3µm)
- Type Is (small, < 3µm)
- Satellite (budding out)

Quantitative analysis of the synaptic morphology, including the total number of boutons, bouton sub-classification, NMJ size and branching (n=20)

Wild type w^{1118} | Amyotrophic Lateral Sclerosis Vap-33-1



Confocal imaging of larval NMJ stained for presynaptic neuronal membrane (anti-HRP). Scale bar represents 20µm.

Conclusion

- The cognitive decline was recapitulated in ALS larvae through motor function assay and learning and memory assay. This cognitive decline may be due to a misregulation of synaptic plasticity genes in early stages of ALS.
- Defects in synaptic morphology were observed
  - ALS: ↑ Total boutons, ↑ Type Ib, ↑ Type Is, ↑ satellite
- Increasing HAT Tip60 levels shows a partial rescue in locomotor defects, learning, and memory defects in ALS larvae compared to wild type control larvae.
- This suggests a neuroprotective role of Tip60 in restoring locomotor and cognitive defects in ALS larvae.

Future Work

- Test other genes linked to ALS
- Larval and adult MB imaging to assess for defective alpha lobes
- Analysis of neuromuscular junction (NMJ) to observe any defects and rescue in boutons, branches, and area

Acknowledgements

Special thanks to Gayathri Vijayakumar, and Niteesha Betini for their assistance. This work was supported by NIH grant R01HD057939 to F.E.

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