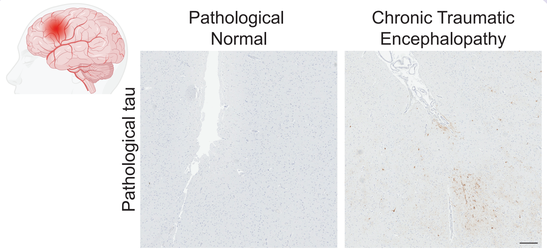
Repetitive Head Injury in *Drosophila Melanogaster*: a Novel Model of Chronic Traumatic Encephalopathy

Elizabeth Ochoa Ph.D.[1], Alia Hernandez[1], Zeliah Torres[1], Elena Ruff[1], Teniade Adetona[1], Mallory Keating HT(ASCP)[1], and Kevin F. Bieniek Ph.D.[1,2]

[1]Glenn Biggs Institute for Alzheimer's and Neurodegenerative Diseases, South Texas ADRC | [2]Department of Pathology and Laboratory Medicine
UT Health San Antonio

CTE is an understudied tauopathy



Evidence consistently links chronic traumatic encephalopathy (CTE) with exposure to repetitive head injury (RHI).

Current animal models recapitulate actue head injury and related cellular features, though CTE is often found decades after incidence.

A model that encompasses both repetitve head injury exposure, chronic neurodegeneration, and tau pathology is needed.

Transgenic fruit flies are well suited for studying neurodegeneration and chronic disease.

Therefore, we created a model of repetitive head injury in transgenic *Drosophila melanogaster* harboring human tau and evaluated survival, pathology, and neurological function.

A paradigm for repetitive head injury in *Drosophila melanogaster*

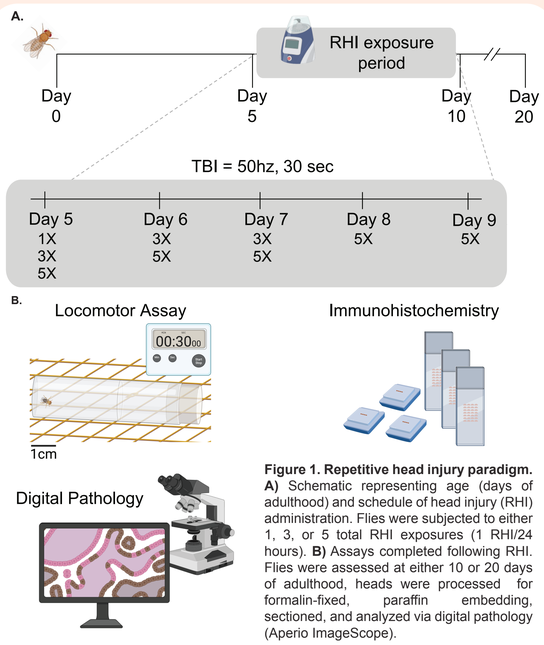


Figure 1. Repetitive head injury paradigm. A) Schematic representing age (days of adulthood) and schedule of head injury (RHI) administration. Flies were subjected to either 1, 3, or 5 total RHI exposures (1 RHI/24 hours). **B)** Assays completed following RHI. Flies were assessed at either 10 or 20 days of adulthood, heads were processed for formalin-fixed, paraffin embedding, sectioned, and analyzed via digital pathology (Aperio ImageScope).

Repetitive head injury has an incidence-associated effect on survival and locomotion

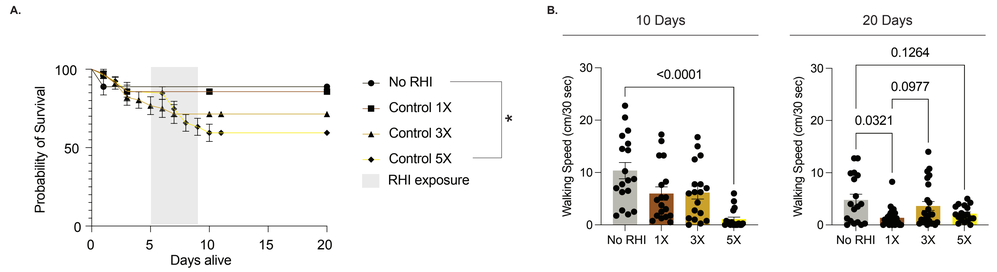


Figure 2. Effect of repetitive head injury in non-transgenic *Drosophila*. A) Kaplan-Meier survival curve for control flies exposed to either no, 1, 3 or 5 repetitive head injuries, each RHI administered for 30 seconds at 50Hz (RHI window in grey). *p<0.05, Holm-Sidak multiple comparisons test. Logrank test for all curves p=0.0072. n=200 biological replicates. **B**) Locomotor analysis of walking speed (cm/30 sec) across four trials at 10 days (left) or 20 days (right) of adulthood folllowing RHI exposure paradigm. n=18 biological replicates, trending and p<0.05 reported above comparisons, Welch one-way ANOVA with Dunnette's T3 multiple comparisons test.

Transgenic tau flies feature reduced survival and locomotion following repetitive head injury

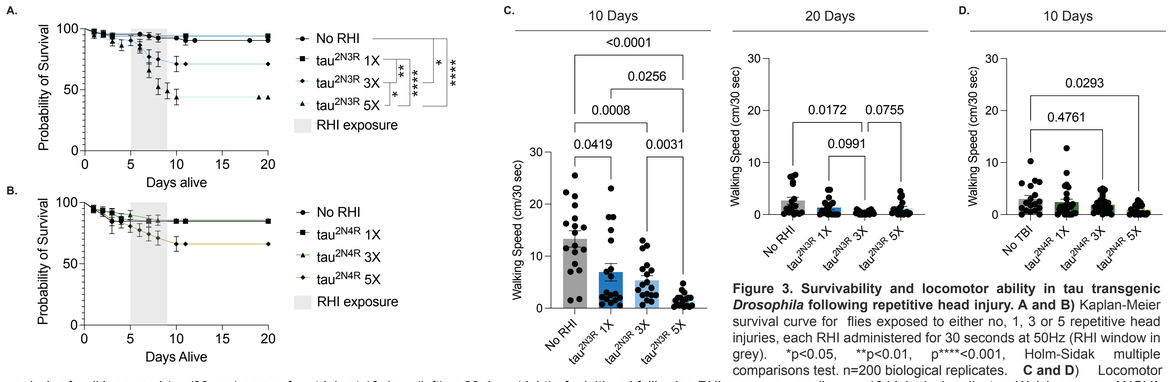
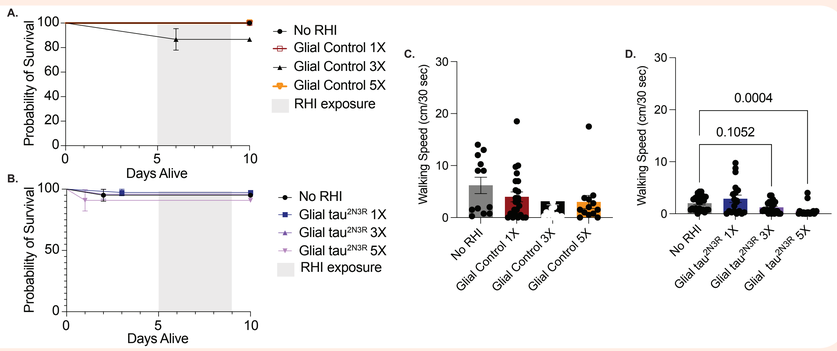


Figure 3. Survivability and locomotor ability in tau transgenic *Drosophila* following repetitive head injury. A and B) Kaplan-Meier survival curve for flies exposed to either no, 1, 3 or 5 repetitive head injuries, each RHI administered for 30 seconds at 50Hz (RHI window in grey). *p<0.05, **p<0.01, p****<0.001, Holm-Sidak multiple comparisons test. n=200 biological replicates. **C and D**) Locomotor analysis of walking speed (cm/30 sec) across four trials at 10 days (left) or 20 days (right) of adulthood folllowing RHI exposure paradigm. n=18 biological replicates, Welch one-way ANOVA with Dunnette's T3 multiple comparisons test.

RHI in flies harboring glial human tau^{2N3R} does not reduce survivability or locomotion

Figure 4. Survivability and locomotor ability in *Drosophila* harboring transgenic tau in glia. A and B) Kaplan-Meier survival curve for flies exposed to either no, 1, 3 or 5 repetitive head injuries, each RHI administered for 30 seconds at 50Hz (RHI window in grey). Holm-Sidak multiple comparisons test, Logrank test for all curves nonsignificant. n=200 biological replicates. **C and D**) Locomotor analysis of walking speed (cm/30 sec) across four trials at 10 days of adulthood folllowing RHI exposure paradigm in glial control and glial tau^{2N3R} flies. n=18 biological replicates, p values trending or < 0.05 reported above comparisons, Welch one-way ANOVA with Dunnette's T3 multiple comparisons test.



Increased pathological tau in RHI tau transgenic flies

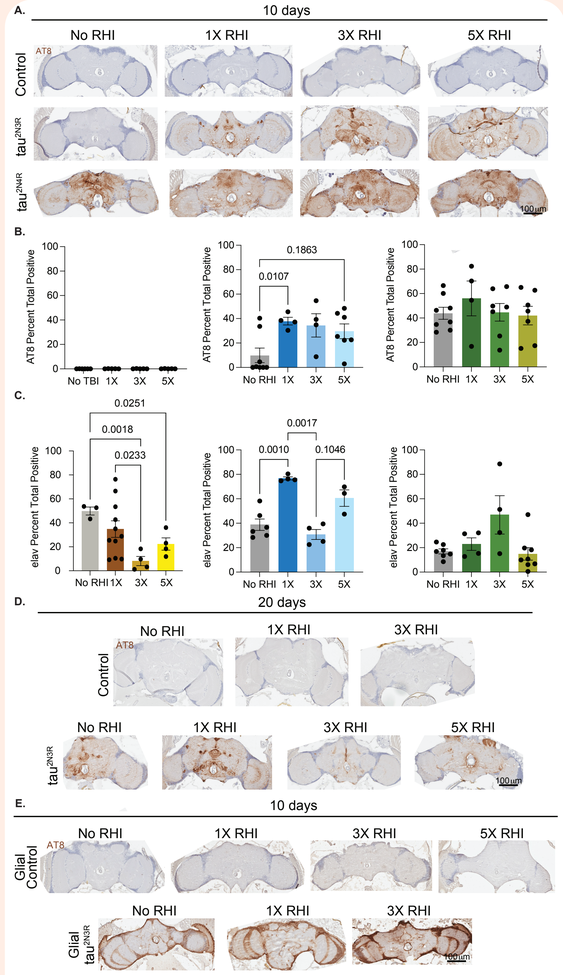
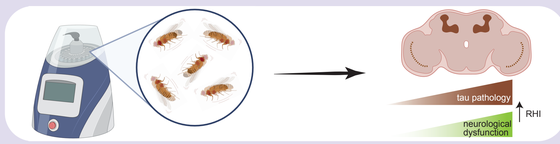


Figure 5. Tau pathology burden and neuronal levels in RHI flies. A and B). Representative brightfield images (**A**) of AT8-positive tau in control and tau transgenic flies (blue & green, panneuronal) at 10 days of adulthood following RHI exposure, quantified via digital pathology (**B**, Aperio ImageScope Color Deconvolution v9). **C)** Quantification of elav-positive neurons. one-way ANOVA, p values > 0.05 reported above comparisons, n=4-8 biological replicates. **D and E)** Representative brightfield images of AT8-positive tau burden in control and tau transgenic flies (panneuronal 20 days **D**, panglial 10 days **E**) following RHI exposure. Scale bar: 100μm.

A working model of repetitive head injury to study CTE



NIA P30AG066546 (KFB)
Bill and Rebecca Reed Prescision Medicine Center, Bartell & Mollie Zachry Endowment for Alzheimer Research and Patient Care (KFB)

ochoae@uthscsa.edu
neuropathlab.org

no photos please

Texas Alzheimer's Research and Care Consortium (EO)
NIGMS IRACDA K12 GM111726 (EO)