Investigating the Neuroprotective Effects of Insulin Signaling Modulation in an Alzheimer's Model

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A **strengthened insulin signaling pathway** in ***Drosophila melanogaster*** has a **positive effect** on Alzheimer's Disease and Traumatic Brain Injury. Overexpressed Insulin Receptor leads to **improved locomotion**, **learning**, and **memory**.

Research Question

How does manipulating the insulin signaling pathway in *Drosophila melanogaster* influence markers associated with neurodegenerative effects in an AD model?

Hypothesis

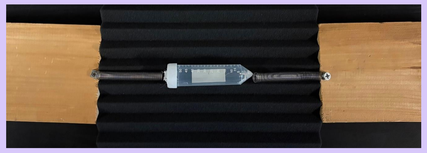
Enhancing the insulin signaling pathway in *Drosophila* will reduce neurodegeneration through markers such as locomotive ability and memory in an AD model.

Background

- Alzheimer's Disease (AD)
  - AD is a progressive **neurodegenerative disorder**
  - Caused by the buildup of **Amyloid β plaques** and **tau tangles**
- The **insulin signaling pathway (ISP)**
  - Facilitates **learning**, **memory** consolidation, and **neuronal plasticity**
  - Plays a large yet **contradictory role** in **neurodegeneration**
- *Drosophila melanogaster*
  - **Conserved** pathways
  - **Quantifiable** neurodegeneration
  - Easy genetic manipulation
    - **InR overexpression** suppressed by UAS-GAL80
- Approach to inducing AD-like phenotypes in flies:
  - **Traumatic Brain Injury (TBI)**

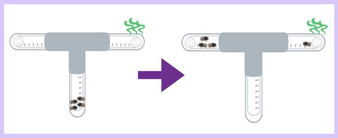
Methodology

Novel High Impact Trauma Device



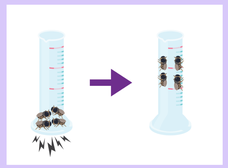
Impact velocity of **~3.0 m/s** and an average force of **2.5 N**

T-Maze Assay



- Comparing naïve and exposed flies
- Preference Index (PI) = $\frac{Olfactory - Distilled}{Total\ Flies}$
- Quantifies learning and memory

Climbing Assay



- Negative Geotaxis
- Success Benchmark at **3 inches**
- Quantifies locomotive ability

Results

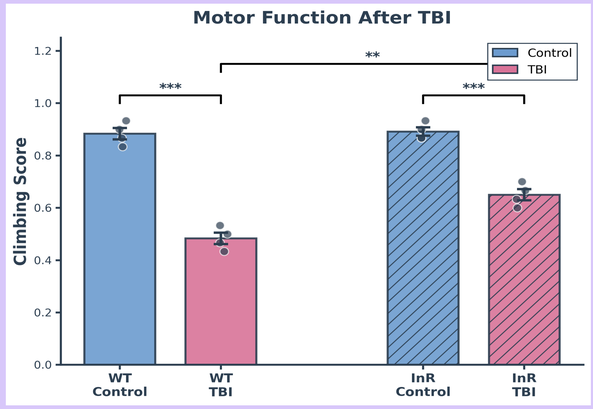


Figure 1. Motor function after TBI in wild-type and InR-overexpressing flies. TBI significantly reduced climbing ability in both wild-type (WT) and InR flies compared to their respective controls (***p < 0.001). InR Control flies performed comparably to WT Control (**p < 0.01), while InR TBI flies retained significantly better motor function than WT TBI flies (***p < 0.001). Error bars represent SEM; n = 4 trials per group with 30 flies per trial.

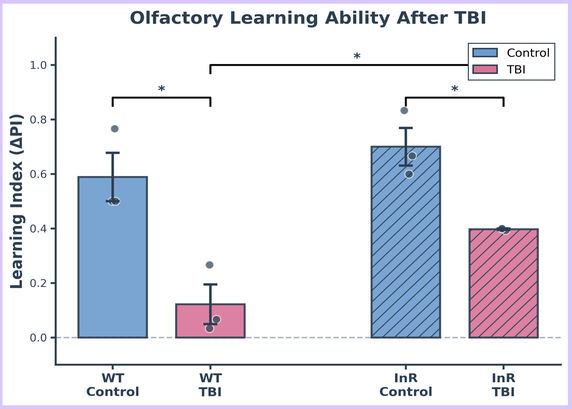


Figure 2. Olfactory learning ability after TBI in wild-type and InR-overexpressing flies. Learning index (ΔPI) represents the change in olfactory preference following conditioning. TBI significantly impaired learning in both wild-type (*p < 0.05) and InR flies (*p < 0.05) compared to their respective controls. InR Control flies showed enhanced baseline learning compared to WT Control (*p < 0.05). InR TBI flies retained significantly better learning ability than WT TBI flies (*p < 0.05). Error bars represent SEM; n = 3 trials per group with 30 flies per trial.

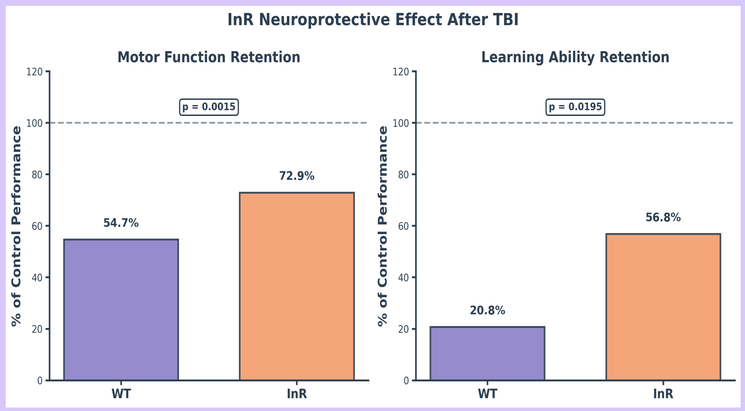


Figure 3. Neuroprotective effect of InR overexpression after TBI. Retention percentages were calculated as (TBI performance / Control performance) × 100% for each genotype. Left panel: Motor function retention showed InR TBI flies retained 72.9% of control climbing ability versus 54.7% in WT TBI flies (**p = 0.0015). Right panel: Learning ability retention showed InR TBI flies retained 56.8% of control learning capacity versus 20.8% in WT TBI flies (*p = 0.0195). Both metrics demonstrate significant neuroprotective effects of InR overexpression.

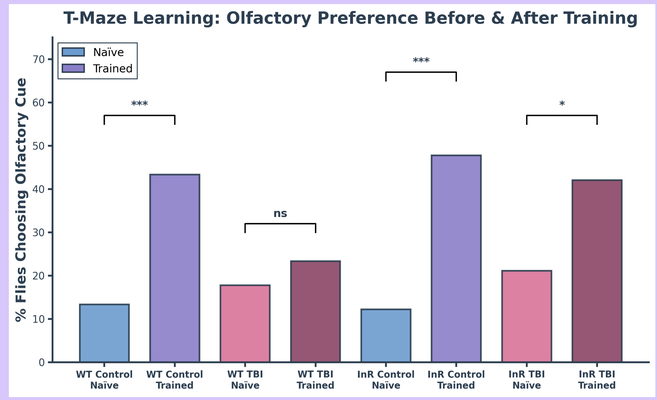


Figure 4. Olfactory preference shift in individual flies before and after training. Bars represent the percentage of flies choosing the olfactory cue in naïve (light colors) versus trained (darker colors) conditions. Wild-type control flies showed significant learning (13% to 43%, ***p < 0.001), while WT TBI flies showed minimal change (18% to 24%, ns). InR control flies demonstrated strong learning (12% to 48%, ***p < 0.001). Critically, InR TBI flies retained substantial learning capacity (21% to 42%, *p < 0.05), with post-training performance comparable to WT controls. n = 90 flies per group.

Statistical Testing

1. Two sample t-tests were performed to compare climbing scores between control and TBI groups in WT and InR flies. Both tests yielded p-values less than 0.001, confirming highly significant TBI-induced motor deficits
2. A one-way ANOVA test was performed to assess overall differences in motor function across all four experimental groups. The F-statistic was 94.803 with $p < 0.001$, confirming highly significant differences
3. Two sample t-tests were performed to compare wildtype TBI and InR TBI revealing significant neuroprotection. Motor function showed $p = 0.0015$ and learning showed $p = 0.019$, confirming that enhanced insulin signaling protects against both motor and cognitive deficits
4. Chi-square tests of independence analyzed T-maze choice distributions comparing naïve versus trained. Wildtype TBI flies showed no learning ($\chi^2 = 1.039$, $p = 0.595$), while InR TBI flies retained learning capacity ($\chi^2 = 9.057$, $p = 0.011$)

Conclusions

- TBI causes severe deficits in motor function and learning in WT flies (all $p < 0.05$)
- Increased INR significantly reduces motor deficits ($p = 0.0015$)
- WT TBI flies completely lose the ability to learn ($p = 0.595$) while InR TBI flies retain learning ability ($p = 0.011$)

Relevance

- Resolves conflicting evidence about the **role of insulin signaling** in neurodegeneration
- Enhanced insulin signaling **can protect** against neurodegenerative effects following brain trauma, highlighting **metabolic signaling pathways** as potential **therapeutic targets** for AD and TBI
- Novel HIT device

Future Work

- Investigate the neurodegenerative effects of weakening the ISP through knocking down the *chico* gene
- Expand experiments to include higher model organisms such as mice for translational relevance
- Create a CAD model for the HIT device and publish for use in TBI related research