Neuroprotective Anti-Inflammatory Properties of Psychedelic DOI for Closed Head Traumatic Brain Injuries in *Drosophila melanogaster*

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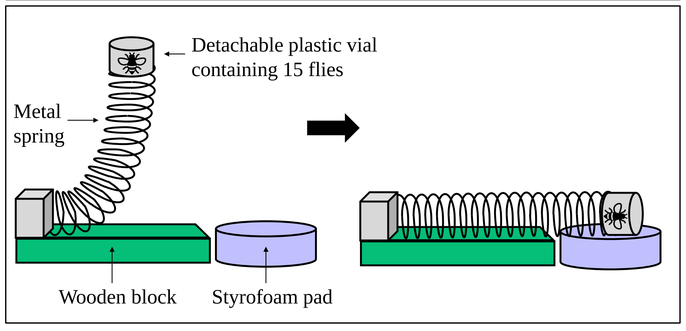
BACKGROUND

- *Drosophila melanogaster* and humans share common primary features of closed head traumatic brain injuries (TBIs) suggesting underlying mechanisms are conserved.[1]
- The High Impact Trauma (HIT) device delivers reliable and reproducible closed head TBIs to *D. melanogaster* by rapid acceleration and deceleration.[1] (Figure 1)

Figure 1: High Impact Trauma (HIT) device operation schematic



- Neuroinflammation caused by the HIT device was validated in our lab through qPCR analysis of pro inflammatory cytokine genes *Attacin C* (*AttC*) and *Diptericin B* (*DptB*) 24 hours following impact.[2, 3]
- Serotonin 2A receptor (5-HT-2AR) agonists, such as *R*-2,5-Dimethoxy-4-iodoamphetamine (*R*-DOI), have shown promise as powerful anti-inflammatory agents in mammalian models.[4]

We hypothesize that the application of *R*-DOI as preventative anti-inflammatory treatment for closed head TBIs in *Drosophila melanogaster* will reduce the degree of subsequent neuroinflammation.

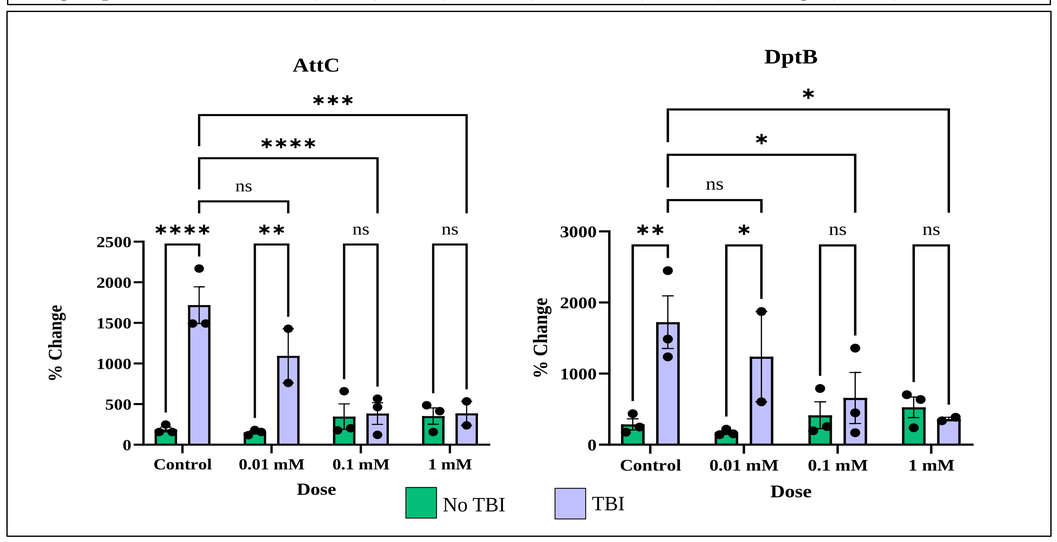
METHODS

- Place 0-4 day old *D. melanogaster* on experimental food (10% sucrose + 1% agarose gel containing 0 mM, 0.001 mM, 0.01 mM, or 1.0 mM *R*-DOI) for 48 hours at 25℃.
- Using the HIT device set to 90° impact, deliver TBI to *D. melanogaster* in groups of 15.
- Allow time for flies to recover from impact, and place on control food (10% sucrose + 1% agarose gel) for 24 hours at 25℃.
- Perform negative geotaxis assay to assess locomotion of surviving subjects prior to head extraction.
- Remove heads and extract RNA for analysis by qPCR using *RPL32* as the housekeeping gene and *AttC* and *DptB* as genes of interest.

R-DOI SUPPRESSES NEUROINFLAMMATION

- A two-way ANOVA was performed for each gene to graph the percent change ΔΔCt compared to non TBI control. (Figure 2)

Figure 2: Percent change in ΔΔCt of pro-inflammatory cytokine genes, *AttC* and *DptB*, across TBI and non TBI groups at 4 doses: control (0 mM), low (0.01 mM), medium (0.1 mM), and high (1 mM)

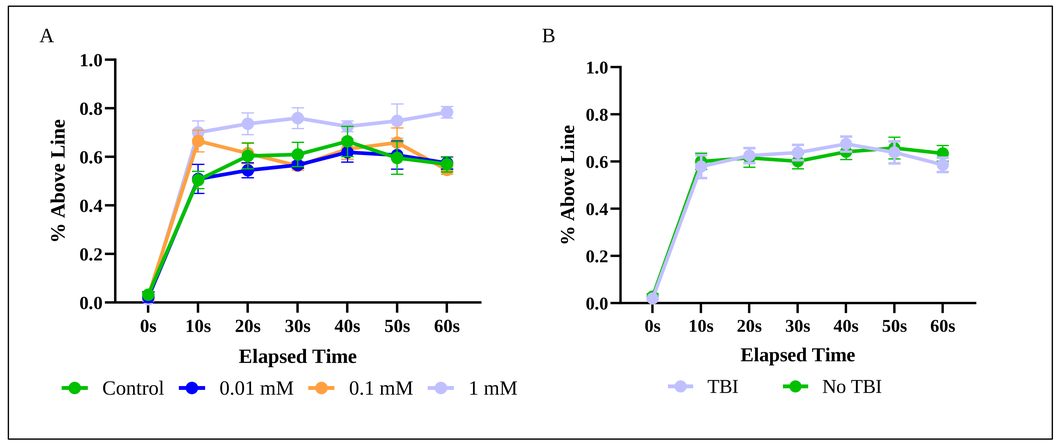


- There was a statistically significant interaction between the effects of *R*-DOI dose and the presence or absence of a TBI for *AttC* ($F(3, 14) = 11.92$, $p = 0.0004$) and *DptB* ($F(3, 14) = 3.656$, $p = 0.0390$).
- Main effects analysis showed that *R*-DOI dose had a statistically significant effect on *AttC* gene expression ($p = 0.0042$) but not *DptB* gene expression ($p = 0.2073$).
- TBI controls show significantly higher levels of inflammation compared to TBI medium (0.01 mM) and TBI high (1 mM) dose groups for *AttC* ($p < 0.0001$, $p = 0.0002$) and *DptB* ($p = 0.0481$, $p = 0.0220$) respectively.
- There was no significant difference between TBI and non TBI groups treated with medium and high dose *R*-DOI for both genes.

GEOTAXIS ASSAY

- There was no significant interaction between the presence or absence of a TBI and geotaxis assay performance, but there was a significant interaction between the doses of *R*-DOI and geotaxis assay performance ($F(1, 21) = 0.0052$, $p = 0.9432$). (Figure 3)
- At 60s, high dose groups showed significantly more flies above the marked line compared to control ($p = 0.0022$), low ($p = 0.0008$), and medium ($p = 0.0002$) dose groups at the same time point.

Figure 3: Geotaxis assay; A- Comparison of drug doses; B- Comparison of TBI vs non TBI groups



CONCLUSIONS

- Reduced inflammatory response: Groups treated with *R*-DOI showed decremental change in average expression of pro-inflammatory cytokines. Both medium and high dose groups showed comparable levels of inflammation between TBI and non TBI groups indicating that pharmacological treatment prevented the neuroinflammatory reaction experienced by control and low dose TBI groups.
- Pharmacological effect on locomotion: TBI and non TBI groups displayed no difference in performance on geotaxis assay while groups receiving high dose *R*-DOI displayed increased climbing locomotion compared to other drug dose groups. This indicates that *R*-DOI may have impact on *Drosophila* locomotion, but optimization of the geotaxis assay is needed before further conclusions can be made.
- Gene variability: Both genes showed the same trends across the experiment, but with varying levels of significance. *DptB* showed more variability within groups than *AttC* and thus the results pertaining to *DptB* were less statistically significant than those results for *AttC*.

FUTURE RESEARCH

Further gene expression research will explore the application of other 5-HT-2AR agonists, such as psilocybin, for both pretreatment and recovery from TBI related neuroinflammation. We will also assess morphological changes in *D. melanogaster* whole brain samples 14-21 days following closed head TBI using fluorescent microscopic analysis to assess the impact of pharmacological pretreatment and recovery treatment.

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