**TABLE: *Drosophila* Models for TBI-related Research**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Model | Head-specific injury | Throughput potential | CO2 use for injury induction | Experimental design | Main findings |
| High-Impact Trauma (HIT) device 1,2 | no | High | no | * Used multiple age groups to characterize acute mortality, long-term survival and climbing deficits, as well as neuropathological evaluation of vacuoles to assess neurodegeneration
* Used both male and female flies
* Delivered repetitive injuries with lethal potential
 | * Single and repetitive injuries elicit acute mortality within 24h (~5% mortality following single exposure and ~20% following 4 repetitive exposures)
* Acute activation of innate immune response seen following injury
* Injury exacerbated age-related neurodegeneration seen 14d post-injury
* performed high-throughput genetic screen to compare mortality outcomes in mutant lines for innate immunity
 |
| Tissue Homogenizer model 3 | no | Low | yes | * 7d old male or female flies received multiple injuries; different sexes were used for different assays and not used for comparative analyses
 | * Flies receiving multiple sublethal injuries showed increases in innate immunity and autophagy within 24h until 1 week following injury
* Injured flies also exhibited acute climbing deficits, long-term circadian rhythm abnormalities and decreased overall survival
 |
| CO2 powered impactor model 4 | yes | Low | yes | * 2d old female flies received 1 or more head injuries from 4 different severities, including acutely sublethal (100% survival within 24h) to highly lethal (<10% survival)
 | * Following a single sublethal injury, flies exhibited a decreased locomotive response for 2d post-injury
* Following repetitive sublethal injuries, flies exhibited a persistent locomotive deficient through at least 20d post injury and a decreased overall survival
 |
| *Drosophila* Closed Head Injury (dCHI) 5 | No | Low | No | * 3-7d old male flies received either 1, 5, or 10 injuries inflicted by the pin of a pull-type solenoid
* Behavioral, pathological, and transcriptional analyses up to 7 days post injury.
 | * Injured flies exhibit immediate dose-dependent motor deficits, apoptotic cell death, fragmentation of sleep and reduction of lifespan.
* Acute activation of the innate immune system (increase of AMPs up to 3d post injury) but returns to baseline by 7d.
 |
| *Drosophila* TBI (dTBI) or piezo-electric actuator model 6-8 | yes | High | yes | * 3d old males received a single injury of one of three injury severities
* Behavioral and histological analysis was chronologically performed through 10d post-injury following severe injuries
* Transcriptional changes measured until 28d post-injury
 | * Dose dependent deficits in locomotion, reduced acute and chronic survival and vacuole formation following severe injury
* Recapitulated glial cellular reactivity following trauma
* Measured transcriptional changes following injury, including glial AP1 which has a biphasic role in tau pathology following injury
 |
| HIFLI model 9 | yes | High-throughput | no | * 2-5d old male and female flies received multiple mild head injuries and were assessed for behavioral and histological examination throughout lifespan
 | * Female flies demonstrate worse persistent locomotive deficits following repetitive injury
* Early injury-induced neuronal activity potentiates chronic neurodegeneration
 |

References cited:

1 Katzenberger, R. J. *et al.* A Method to Inflict Closed Head Traumatic Brain Injury in Drosophila. *Journal of visualized experiments : JoVE*, e52905, doi:10.3791/52905 (2015).

2 Katzenberger, R. J. *et al.* A Drosophila model of closed head traumatic brain injury. *Proceedings of the National Academy of Sciences* **110**, E4152-E4159 (2013).

3 Barekat, A. *et al.* Using Drosophila as an integrated model to study mild repetitive traumatic brain injury. *Scientific reports* **6**, 25252 (2016).

4 Sun, M. & Chen, L. L. A Novel Method to Model Chronic Traumatic Encephalopathy in Drosophila. *Journal of visualized experiments: JoVE* (2017).

5 van Alphen, B. *et al.* Glial immune-related pathways mediate effects of closed head traumatic brain injury on behavior and lethality in Drosophila. *PLoS Biol* **20**, e3001456, doi:10.1371/journal.pbio.3001456 (2022).

6 Byrns, C. N., Saikumar, J. & Bonini, N. M. Glial AP1 is activated with aging and accelerated by traumatic brain injury. *Nat Aging* **1**, 585-597, doi:10.1038/s43587-021-00072-0 (2021).

7 Saikumar, J. *et al.* Inducing different severities of traumatic brain injury in Drosophila using a piezoelectric actuator. *Nature Protocols*, 1-20 (2020).

8 Saikumar, J., Byrns, C. N., Hemphill, M., Meaney, D. F. & Bonini, N. M. Dynamic neural and glial responses of a head-specific model for traumatic brain injury in Drosophila. *Proc Natl Acad Sci U S A* **117**, 17269-17277, doi:10.1073/pnas.2003909117 (2020).

9 Behnke, J. A., Ye, C., Setty, A., Moberg, K. H. & Zheng, J. Q. Repetitive mild head trauma induces activity mediated lifelong brain deficits in a novel Drosophila model. *Sci Rep* **11**, 9738, doi:10.1038/s41598-021-89121-7 (2021).