The Effect of Traumatic Brain Injury on Aversive Taste Learning in CS and FMR1 Mutant *Drosophila melanogaster*

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Abstract

Traumatic brain injury (TBI), often caused by blunt force trauma to the head, results in 64,000 annual United States deaths. Recovery has been linked to neuroplasticity, the brain's ability to rearrange and form new neural pathways. This is a key function behind learning and memory retention. *Drosophila melanogaster* are ideal TBI models. The relationship between genetic vulnerability in TBI has not been extensively studied. Fragile-X Syndrome (FXS) is an inherited intellectual disability seen in 1:7,000 males and 1:11,000 females, caused by a lack of a functioning FMRP protein needed for brain development. FMR1 *Drosophila* mutants mimic FXS. The purpose of this study was to compare TBI flies to normal flies' ability to learn a noxious stimulus and its repetition. The second aim was to compare TBI effects on FMR1 mutants and wild-type flies. TBI was implemented via the High Impact Trauma device. Aversive taste testing paired fructose with a toxin to reverse natural attraction to sugar. Learning and memory were quantified as the number of proboscis (primary feeding organ) extensions after each fructose-quinine pairing. A 2-way ANOVA test was used for analysis. Between healthy and TBI flies, TBI caused a significant decline in learning and memory retention ($p=.01$). TBI FMR1 mutants had impaired cognition (mean proboscis extension difference=1.54) compared to TBI wild-type flies (mean proboscis extension difference=1.063). Additional research should identify strategies that promote learning in cognitively impaired and genetically vulnerable individuals. Widespread genetic testing may identify at risk individuals who should exercise caution in contact sports and military services.

Review of Literature

Traumatic brain injury (TBI) is a leading cause of death and disability in the world. There are an approximate 223,135 TBI-related hospitalizations and 64,000 TBI-related deaths annually in the United States. TBI is typically caused by a strong jolt or blunt force to the head resulting in

neurological damage or death. TBI's effects can range from physical to cognitive to emotional, and those effects can be short or long-term (Centers for Disease Control and Prevention, 2022). TBI is not a genetic condition and is frequently seen in athletes and active service members. It is also seen as a result of falls, collisions, and motor vehicle accidents. 57 million Americans live with long-term disabilities from TBI. Though both sexes are prone to TBI, TBI occurs 1.5 more times in males than in females (Pavlik). The annual financial expense of TBI to global society exceeds $400 billion (CENTER-TBI).

Neuroplasticity is the brain's ability to form neural pathways and memories allowing the human brain to respond to injuries with striking functional reorganization. Neuroplasticity is also a key mechanism behind learning and memory (Zuger, 2007).

Fragile-X Syndrome (FXS) is a genetic condition caused by changes in the Fragile X Messenger Ribonucleoprotein 1 (FMR1) gene that codes for a protein which is essential and responsible for regulating synaptic plasticity and brain development. FXS causes mild to severe intellectual abilities and physical differences; it is seen in 1 out of 7,000 males and 1 out of 11,000 females, with males tending to have more severe symptoms of the disorder (Centers for Disease Control and Prevention, 2022). Dfmr1 is the *Drosophila* homolog of the gene responsible for FXS (Wan, 2000).

In *Drosophila,* classical conditioning mimics neuroplasticity through its relation to learning and memory. Some popular forms of training are olfactory, visual, and aversive taste learning. Aversive taste learning uses two substances, one that is naturally repelling and another that is naturally attractive, to form a negative association between the two. This form of training utilizes the proboscis extension reflex (PER) which is based on the two primary feeding organs of *Drosophila:* the proboscis, where food ingestion occurs, and the tarsi, which have taste receptors

on them. The positive stimulus is placed on the fly's tarsi and as the fly extends it proboscis, expecting to be met with the positive stimulus, the noxious stimulus is quickly placed on the fly's proboscis. This form of conditioning teaches *Drosophila* to suppress their natural urge to extend their proboscis for sweet substances as it is now associated with some form of punishment. Learning and memory formation can be quantified through the number of proboscis extensions. Through classical conditioning, researchers are able to establish short and long-term memory in *Drosophila* (Masek and Keene, 2016).

There are many different models used to implement TBI in *Drosophila.* The High Impact Trauma device (HIT device) is an efficient closed head TBI model that consists of a vial containing flies attached to a metal spring which is clamped onto a wooden block. The side opposite where the spring is clamped has a polyurethane pad. The severity of brain damage inflicted can be altered depending on the angle at which the strike is hit, the number of strikes, and the time between each strike (Katzenberger) (see Figure 1).

Research Questions

The goal of this study was to determine the effect of traumatic brain injury on aversive taste learning in Standard (CS wild-type) *Drosophila melanogaster* and learn if FMR1 Mutant *Drosophila* suffered from more severe consequences from TBI in learning and memory.

1. Does traumatic brain injury affect aversive taste learning in *Drosophila melanogaster?*
2. Does traumatic brain injury affect aversive taste learning in FMR1 Mutant *Drosophila melanogaster* more severely than in Standard (CS wild-type) *Drosophila?*

The expected outcomes for this experiment were that TBI would lead to a decline in learning and memory in standard *Drosophila* and TBI would have a stronger negative effect on cognition in FMR1 Mutant *Drosophila* than in standard *Drosophila.*

Methodology

This study consisted of four groups: Control CS, HIT CS, Control FMR1, and HIT FMR1. The methodology can be broken down into five major steps: Preparation, Pre-Test, Training Trials I, II, III, Test, and Negative Control.

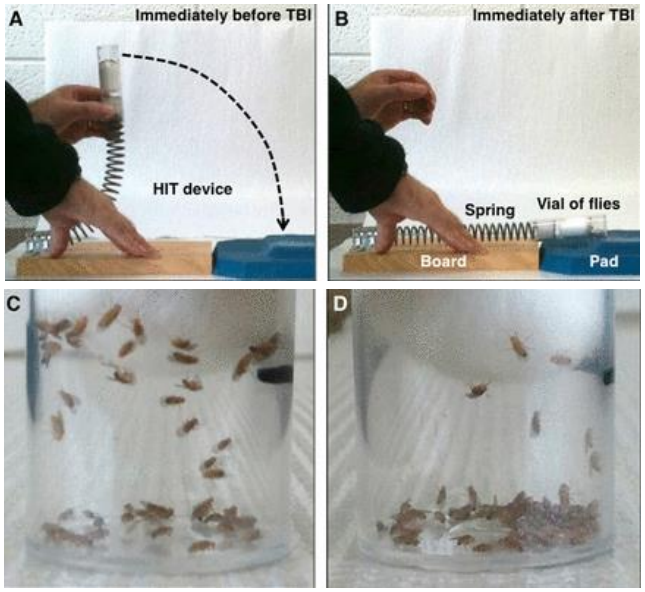


Figure 1: a) A plastic vial of flies is pulled back to a 90-degree angle b) The spring is released, and the vial hit the pad c) There are approximately 20 flies in the vial and there is a cotton ball on the top of the vial to act as a lid d) The mortality of the flies is shown

Preparation: The initial step was to sex and group the flies into vials consisting of 20 flies. This study only used female flies. TBI was implemented into the TBI CS and TBI FMR1 groups via the HIT device. Flies were put into empty vials with a cotton ball as a lid and struck 3 times at a 90° angle with 5 minutes between each strike. To strike the flies at an accurate angle, a protractor was placed behind the HIT device. At this point, all four groups were synced. All groups were then placed onto fresh food for 24 hours to adjust to their new environment and, for the corresponding groups, recover from TBI. The flies were then starved for 24 hours to elicit the proboscis extension reflex. After the starvation period, the flies were anesthetized using a CO_2 pad and glued onto microscope slides in groups of 10 with Elmer's glue. The flies were then put into a humidified box to recover for 1 hour. During this

period, 10mM Quinine, 100mM Fructose, and 1M Sucrose solutions were prepared. After the recovery time, the Pre-Test occurred.

Pre-Test: The primary purpose of the Pre-Test was to ensure that the flies were able to extend their proboscis. This stage was especially important to the flies that received TBI as it guaranteed that the HIT device did not injure them in a way that prevented them from extending their proboscises. The microscope slides, with glued on flies, were mounted at an approximate angle of 15° under a standard dissection microscope. Next came satiation, where water was placed on each fly's tarsi for 2-3 seconds and flies were able to drink until satisfied. This step ensured that when the flies later extended their proboscis for the fructose, it was purely because of fructose's sugary taste and was unrelated to thirst. After the satiation period, fructose was placed on each fly's tarsi for 2-3 seconds with a 10 second intertrial interval (ITI) and the amount of proboscis extensions were recorded. For this step, it was mandatory that all flies completely extended their proboscises for all 3 presentations of fructose because as of now, there were no pairings presented, so the flies' biological instincts should be to extend their proboscises for the sugar. Any flies that did not pass this step were excluded from the study. After the Pre-Test, the Training Trials occurred.

Training Trials I, II, III: The 100mM fructose solution was applied to each fly's tarsi for 2-3 seconds and the 10mM quinine solution was quickly applied to the fly's proboscis for no more than 2 seconds. This was repeated for a total of 3 times to each fly with a 10 second ITI. The flies were then given water for a minimum of 2 seconds to rinse their tarsi. If the flies extended their proboscises for the water, then they were allowed to drink until satisfied. The 3 presentations of the fructose-quinine pairing followed by satiation were considered 1 Training Trial. There was a total of 3 Training Trials for each fly in this study.

Test: The test occurred 1 hour after the 3 Training Trials. An hour was selected as it gave the flies enough time to recover from the bitter poison quinine and also ensured that memory was

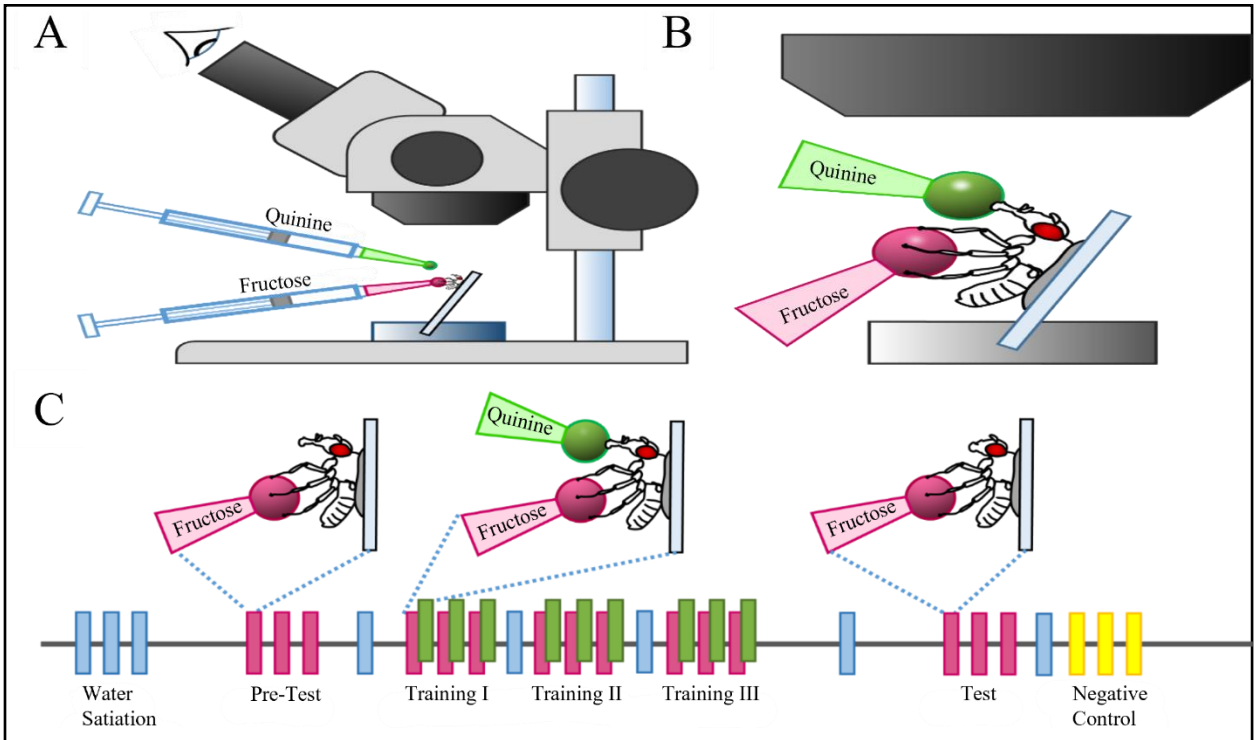


Figure 2: a) Fructose is put on the fly's tarsi and quinine is ready to touch the fly's proboscis b) The fructose is on the fly's tarsi and the quinine is on the fly's proboscis c) A brief overview of the timing of each presentation of solutions (water, fructose, quinine, and sucrose)

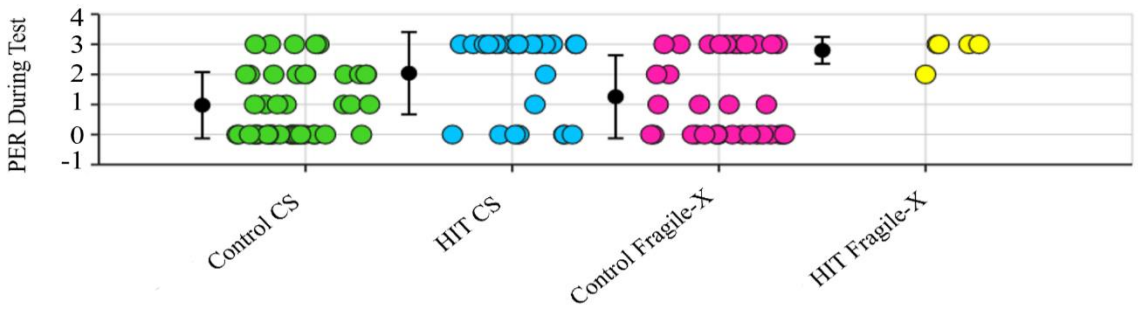
properly retained. The 100mM fructose solution was put on each fly' tarsi for 2-3 seconds and the number of full proboscis extensions were recorded. This was repeated for a total of 3 times with a 10 second ITI for all flies.

Negative Control: The negative control ensured that the flies, throughout this entire process, never lost their abilities to physically extend their proboscises. The 1M sucrose solution was presented to the flies 3 times with a 10 second ITI. Despite both sucrose and fructose being sugars, they are chemically different compounds. Since the sucrose had not been paired with any noxious substances, the flies' biological instincts should be to extend their proboscises. Any flies that did not extend their proboscises for the sucrose were not included in the data analysis.

Results

The inclusion criteria for this study were that all flies had to completely extend their proboscises for all 3 presentations of fructose in the Pre-Test and all 3 presentations of sucrose in the Negative Control. An average of 0 extensions during the Test meant that learning and memory occurred as the fly formed a negative connection between fructose and quinine and learned to stop extending its proboscis for the fructose. On the other hand, an average of 3 extensions would signify that no learning or memory took place. The mean average extensions during the test for the Control CS, HIT CS, Control FMR1, and HIT FMR1 groups were .977, 2.04, 1.26, and 2.8

Figure 3



Note: Dot shows the mean. Error bars above and below show Standard deviation.

Group	Mean	Std. dev	SEM	ci95lo	ci95hi	Median	Min	Max	q25	q75
Control CS	0.977	1.1	0.168	0.636	1.32	1	0	3	0	2
HIT CS	2.04	1.37	0.274	1.48	2.6	3	0	3	0	3
Control FMR1	1.26	1.38	0.233	0.785	1.73	1	0	3	0	3
HIT FMR1	2.8	0.4472	0.2	2.243	3.357	3	2	3	2	3

Figure 4

Effect	Degrees of Freedom (df)	F-statistic (MS / MS residual)	P-value
Flies (X)	1	2.4	0.13
Treatment (Z)	1	15	<0.01
Interaction (X * Z)	1	0.51	0.48
Error or Residual			

Results of post-hoc Tukey's Range Test:

Pair	Mean diff	P-value
(CS, HIT) - (CS, Control)	1.06	0.01
(Fragile-X, Control) - (CS, Control)	0.28	0.76

respectively (see Figure 3). A 2-way ANOVA test was used to calculate p-values (x-axis: Flies [CS or Fragile-X]; y-axis: PER During Test; z-axis: Treatment [Control or HIT]) (see Figure 4).

Figure 4 cont.

(Fragile-X, HIT) - (CS, Control)	1.82	0.01
(Fragile-X, Control) - (CS, HIT)	-0.783	0.08
(Fragile-X, HIT) - (CS, HIT)	0.76	0.60
(Fragile-X, HIT) - (Fragile-X, Control)	1.54	0.05

Discussion

This study supported the first hypothesis stating that TBI would lead to a decline in learning/memory in Standard (CS wild-type) *Drosophila*. Through use of the close head HIT device, the location of each fly in the vial affected the TBI implemented. Though this factor was taken into account by restricting the available space in each vial to approximately .25" with a cotton ball, it is still important to acknowledge the likely differences in TBI location and severity throughout the sample. In regard to the second hypothesis stating that FMR1 Mutant *Drosophila* would have more severe consequences on their cognition after TBI, there was a data trend that supported this hypothesis; however, the p-value was insignificant. This was most likely due to the small sample size of the HIT Fragile-X group. This study used female flies since TBI in females is not as heavily researched as it is in males.

Conclusions

The most significant result obtained from this study was that TBI, at the level implemented via the HIT device, led to a decline in aversive taste learning in Standard (CS wild-type) *Drosophila melanogaster.* Additional research is needed to employ a larger sample size to produce statistically significant results and test varying levels of TBI's effects on aversive taste learning.

Gender's role should also be investigated as gender seems to play an unidentified role in many aspects of neuroscience; FXS is more prevalent and prominent in males and TBI is more common in males. More research on learning in *Drosophila* can hint towards more short and long-term effects of TBI on neuroplasticity and can advance scientific knowledge of the relationship between genetic vulnerabilities and TBI.

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References

[1] "Get the Facts about TBI." *Centers for Disease Control and Prevention*, Centers for Disease Control and Prevention, 21 Mar. 2022, https://www.cdc.gov/traumaticbraininjury/get_the_facts.html.

[2] Haddadi, Mohammad. "Which Protocol Reflects More Reliable Facts about Learning and Memory in Fruit Flies?" *EXCLI Journal*, Leibniz Research Centre for Working Environment and Human Factors, 12 July 2019, https://www.ncbi.nlm.nih.gov/pubmed/31423131.

[3] Katzenberger, Rebeccah J., et al. “A Drosophila Model of Closed Head Traumatic Brain Injury.” *PNAS*, National Academy of Sciences, 29 Oct. 2013, https://www.pnas.org/content/110/44/E4152.

[4] Liu;Chen;Zeng, Jia;Xiao;Xin. “Classical Olfactory Conditioning in the Oriental Fruit Fly, Bactrocera Dorsalis.” *PloS One*, U.S. National Library of Medicine, https://pubmed.ncbi.nlm.nih.gov/25837420/.

[5] Malik, Bilal R, and James J L Hodge. “Drosophila Adult Olfactory Shock Learning.” *Journal of Visualized Experiments : JoVE*, MyJove Corporation, 7 Aug. 2014, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4672959/.

[6] Masek, Pavel. “Measuring Aversive Taste Memory in Drosophila.” *CSHS DrosNeuroProtocols2019*, 2019.

[7] Masek, Pavel, and Alex C Keene. “Gustatory Processing and Taste Memory in Drosophila.” *Journal of Neurogenetics*, U.S. National Library of Medicine, June 2016, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5911158/.

[8] Pavlik, R. “Statistics and Prevalence.” *Cambridge Center for Behavioral Studies*, https://behavior.org/statistics-and-prevalence/.

[9] “TBI Data.” *Centers for Disease Control and Prevention*, Centers for Disease Control and Prevention, 21 Mar. 2022, https://www.cdc.gov/TraumaticBrainInjury/data/index.html.

[10] “Traumatic Brain Injury Fact Sheets and Policy Brief.” *CENTER-TBI*, European Brain Injury Consortium, https://www.center-tbi.eu/files/news/21571f81-20b8-4860-a3dd-1f6e27d02b3d.pdf.

[11] “Traumatic Brain Injury.” *Mayo Clinic*, Mayo Foundation for Medical Education and Research, 4 Feb. 2021, https://www.mayoclinic.org/diseases-conditions/traumatic-brain-injury/symptoms-causes/syc-20378557.

[12] Wan, L, et al. “Characterization of DFMR1, a Drosophila Melanogaster Homolog of the Fragile x Mental Retardation Protein.” *Molecular and Cellular Biology*, American Society for Microbiology, Nov. 2000, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC102159/.

[13] Widmann A;Eichler K;Selcho M;Thum AS;Pauls D; “Odor-Taste Learning in Drosophila Larvae.” *Journal of Insect Physiology*, U.S. National Library of Medicine, https://pubmed.ncbi.nlm.nih.gov/28823531/.

[14] “What Is Fragile X Syndrome (FXS)?” *Centers for Disease Control and Prevention*, Centers for Disease Control and Prevention, 3 June 2022, https://www.cdc.gov/ncbddd/fxs/facts.html.

[15] Zuger, "The Brain: Malleable, Capable, Vulnerable (Published 2007)". Nytimes.Com, 2007, https://www.nytimes.com/2007/05/29/health/29book.html.